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Distribution, Movement, and Ecology of Etheostoma spilotum (Gilbert), the Kentucky Arrow Darter, in Gilberts Big Creek and Elisha Creek, Red Bird River Basin, Clay and Leslie Counties, Kentucky

Jonathan Bradley Baxter
Eastern Kentucky University

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By

Jonathan Baxter

Thesis Approved:

Chair, Advisory Committee

Member, Advisory Committee

Member, Advisory Committee

Dean, Graduate School

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By:
Jonathan Baxter

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Dedication

This thesis and love for fish is dedicated to my grandparents Willard ("Granddaddy") and Sadie Igleheart. Without them and more specifically Granddaddy, I am not sure I would have been introduced to, or developed my passion for, the outdoors. Additionally, I would like to dedicate this thesis to my loving wife, Priscilla, and two beautiful kids, Logan and Isabella, who not only sacrificed their time and patience with this process but also offered endless love and support.

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Abstract

The Kentucky Arrow Darter (KAD), *Etheostoma spilotum*, is an endemic species to the Upper Kentucky River Basin and is currently proposed for listing as threatened under the Endangered Species Act. The ecology and population status of this benthic species is poorly understood, so this study was designed to investigate the species' movement capabilities, population dynamics, and overall ecology in two streams (Gilberts Big and Elisha Creek) in the Red Bird Ranger District, Daniel Boone National Forest, Kentucky. Project objectives included quantification of movement patterns, identification of microhabitat use, and estimation of population size in both streams. Sampling was conducted during three seasons (spring summer, and fall) in 2013 utilizing a probabilistic sampling design, with a total of 752 microhabitat plots being sampled from 23 reaches across those seasons. Utilizing passive integrated transponder (PIT) tags for continuous tracking, movements of 121 KADs ranged from 28-4,078m in both up and downstream directions. Population estimates ranged from 80-1498 individuals but varied depending on stream and season, with the spring season yielding the lowest estimate. Habitat associations between occupied and unoccupied reaches and plots were compared both seasonally and across all seasons. Results suggested that pool habitats with cobble, higher mean depths, and lower composition of sand, gravel, and boulders were more commonly associated with KAD presence.

Key Words: Etheostoma; Benthic fish; Fish movement; Habitat association; Darter; Microhabitat

INTRODUCTION

Conservation efforts are important for endemic fishes because these species are more vulnerable to population declines caused by habitat destruction or modification, stochastic events, or low genetic diversity. Within the United States, Kentucky ranks third in fish diversity, supporting approximately 236 native species and 24 introduced species (Thomas 2011; KDFWR 2013). Even with a high number of native species, only eight are endemic to Kentucky, including five described species and three more awaiting formal description (M. Thomas, M. Compton pers. comm. 2012). Fifty-nine of Kentucky's species have been identified as Species of Greatest Conservation Need (SGCN) by the Kentucky Department of Fish and Wildlife Resources (KDFWR 2013), and six of these species have been listed as federally threatened or endangered by the United States Fish and Wildlife Service (USFWS). The Kentucky River Basin is limited to one endemic fish species, the Kentucky Arrow Darter (KAD), *Etheostoma spilotum* (Gilbert), which has been proposed for federal listing under the Endangered Species Act (ESA) (USFWS 2015).

The KAD is a relatively large darter reaching lengths of up to 125mm. The species has a long, slender body; elongated, pointed snout; and relatively large mouth (Kuehne and Barbour 1983, Etnier and Starnes 1993). Base color in males is often a pale yellow to green. Females retain the pale yellow color year round. The head, breast, and opercular flaps are naked, and the infraorbital canal is fully developed. The breast and nape are often fully scaled. The dorso-lateral line consists of five to seven weak bands that often blend with eight to eleven lateral U-shaped bars, which often become indistinct in larger fish. Often there is a vertical bar at the caudal peduncle caused by the fusion of two caudal spots. The mean lateral scale count is less than 59, and dorsal fin ray and pectoral fin ray counts are 13 and 14, respectively. Breeding males have a bluish appearance with bright orange bars. The first dorsal fin is outlined in a reddish orange color with a bluish base, while the second soft dorsal fin is dark blue to black with orange speckling. The pelvic and anal fins are dark blue to black (Etnier and Starnes 1993).

The KAD is most commonly found in first and second order streams; however, a few historical records are available for larger streams, and recent surveys by KSNPC, USFWS, and KDFWR documented KADs in several third order streams. Lotrich (1973) also observed KADs in his study of first, second and third- order streams. Interestingly, those individuals were only found in third-order streams during summer months and during periods of drought.

The KAD was just recently elevated to species status; the species' taxonomic history was summarized by USFWS (2015):

> "The Kentucky arrow darter belongs to the Class Actinopterygii (ray-finned fishes), Order Perciformes, and Family Percidae (perches) (Etnier and Starnes 1993; Page and Burr 2011). The species was described from the Kentucky River basin (Sturgeon Creek, Owsley County) as *Etheostoma nianguae spilotum* (Gilbert 1887) but was later recognized and accepted as one of two subspecies of the arrow darter, *E. sagitta* (Jordan and Swain): *E. s. sagitta* (Cumberland arrow darter) and *E. s. spilotum* (Kentucky arrow darter) (Bailey 1948; Kuehne and Bailey 1961; Kuehne and Barbour 1983; Burr and Warren 1986). Thomas and Johansen (2008) questioned the subspecies status of *E. sagitta* by arguing

that (1) the two subspecies, *E. sagitta sagitta* and *E. sagitta spilotum*, were distinguishable based on scale size and development of the lateral line; (2) the two subspecies existed in allopatry (separate ranges with no overlap); (3) the two subspecies lacked intergrades (intermediate forms); and (4) unpublished genetic data (mitochondrial DNA) suggested evolutionary independence of Kentucky and Cumberland basin populations (with no recent genetic exchange). Based on these analyses, the two arrow darter subspecies have been elevated to species rank (Page and Burr 2011; Eschmeyer 2014)."

This resulting speciation between *E. sagitta* and *E. spilotum* most likely occurred through cladogenesis when the species was divided into two geographically separated populations. This was thought to have occurred when the headwaters of Collins Fork, which initially fed into Little Richland Creek (Cumberland watershed), changed direction, most likely caused by rapid erosion, and drained into Hammons Fork in the Kentucky River Basin (Kuehne and Bailey 1961).

Assessing habitat characteristics and identifying factors that affect fish movements are integral to determining the potential response of fishes to environmental perturbations. Although, there has been considerable research on movement capabilities of sport fishes, there have been relatively few in-depth studies on the movement of small nongame species (Schumann et al. 2015). Detar and Mattingly (2013) found the federally threatened blackside dace, *Chrosomus cumberlandensis*, made frequent in-stream movements, including a maximum movement of about 4 km. Additionally, the first documented intertributary movement of the species, a dispersal that included a crossing of an embayment of Lake Cumberland in Pulaski County. Albanese et al. (2004) found the probability of emigrating from a reach for Blacknose Dace (*Rhinichthyes atratulus*) and Torrent Sucker (*Thoburnia rhothoeca*) was related to the position of the reach in the drainage, habitat complexity, reach intermittency, and fish body size. Probability of movement decreased with increased distance from the mainstem; in addition, intermittency in stream flow was an important determinant of movement for the Bluehead Chub (*Nocomis leptocephalus)*, where 82.4% of fishes marked in intermittent reaches were later captured in perennial reaches.

The KAD is of special concern to federal and state resource agencies because of a recent USFWS proposal to add it to the federal list of endangered and threatened wildlife (USFWS 2015). The species has suffered numerous extirpations and has decreased in overall abundance across its range. Although it is still present in all five sub-basins in which it was found historically, recent surveys by the KSNPC, KDFWR, and USFWS (2007-2010) revealed the species was present in only 34 of 68 historical streams (50%) and 45 of 100 historical sites (46%; Figure 1; USFWS 2009; USFWS 2015). Additional surveys by these agencies demonstrated that the KADs current distribution includes 47 streams; however, 45 percent of these occur on private lands that are more vulnerable to anthropogenic disturbance.

The major reasons for the species' range reduction and individual population declines include a variety of impacts associated with anthropogenic activities within the upper Kentucky River basin. These include, but are not limited to, surface coal mining, logging, agriculture, oil and gas development, stream channel reconfiguration, and land development. The most significant source of threat to the KAD is surface coal mining, more specifically, strip mining and mountain top removal. Mountain top removal alters the stream's chemical and physical characteristics and often creates water quality conditions that are unfavorable for sensitive species. Overburden from these types of mining operations can leach minerals such as pyrite, calcium, sulfur, magnesium, manganese, sodium, and potassium (Tiwary 2001) into surface streams, causing increases in stream conductivity and sulfates.

During a study conducted by Dyer and Curtis (1977) from 1968 to 1975 (pre- and post- mining), conductivity levels in several Kentucky River tributaries increased as much as 650 microsiemens (μS)/cm as a direct result of mining practices. This is concerning because the KAD is thought to have a conductivity tolerance threshold level of around 250-300 μS/cm (M. Floyd pers. comm.). Elevated conductivity and poor water quality conditions can also affect egg and larval development in some fishes by altering the water hardening process of the chorion layer of the egg (Helfman et al. 2009); the effects of elevated conductivity on egg and larval development of KADs are unknown.

Loss of riparian vegetation and the resulting erosion and siltation of streams is a threat to the spawning success of KAD. In silt-free streams, the male will fan out a small depression in gravel substrates (2-15mm) and begin courtship with the female. The female buries herself in the substrate and is subsequently mounted by the male during spawning (Etnier and Starnes 1993). Increased sedimentation in streams can result in fewer spawning events for fishes and fewer eggs laid during those events (Burkhead and Jelks 2001), causing shifts in entire stream fish assemblages (Jones et al. 1999).

There have been no studies directed at localized spatial scales specific to KAD movement behavior and habitat selection. Additionally, an attempt to compare and establish baseline population dynamics within and between streams is lacking. The data generated by this study will help identify critical populations and will contribute to the establishment of critical habitat and key conservation areas for the protection and enhancement of this species. These data will also establish baseline information for comparison and extrapolation of variables (habitat, populations, and associations) throughout the species' range. Specific objectives of this study included the following: 1) identify micro-habitat use of KADs in order to identify key conservation areas for the species; 2) estimate KAD population size in two tributaries of the Red Bird River, Gilberts Big Creek and Elisha Creek, through three seasons of sampling; and 3) utilize pit-tagging and stationary antenna systems to conduct movement surveys to identify key migration/movement patterns of KADs in Elisha Creek and Gilberts Big Creek.

Study Area

The Red Bird River encompasses approximately 195 mi^2 and is located in eastern Clay, western Leslie, and northern Bell counties, Kentucky. It originates in southern Kentucky and flows north where it joins Bullskin and Goose Creeks near Oneida, Kentucky to form the South Fork Kentucky River. The South Fork Kentucky River is one of three major forks of the Kentucky River located in the Eastern Kentucky Coal Field region with geographic features consisting of mountains with sandstone, limestone, coal, and shale Kentucky River Assessment Report (2000).

This region is subject to many impacts from anthropogenic activities such as mining, logging, and channel alteration for agriculture. However, there are other threats that contribute to degradation of stream systems in the Kentucky River watershed. Non-point source pollution, such as nearly 7,000 straight pipes and/or failing septic systems, have also been documented within the Kentucky River watershed (Kentucky River Keeper 2002).

Gilberts Big Creek and Elisha Creek were chosen based on previously high documented capture rates, the two stream's close proximity, and recent records of KADs, as well as the high degree of protection provided by the DBNF (Figure 2). Although several private inholdings occur on both streams, the majority of both watersheds are undisturbed and in public ownership (DBNF). The protection provided by the DBNF's forested watersheds and densely vegetated riparian buffers ensures less anthropogenic input and disturbance. Both streams flow from east to west and are located approximately 2 km apart in eastern Clay and western Leslie counties (Figure 2).

Study Design

The study design was modeled after the United States Environmental Protection Agency's Probability-Based Random Sampling Design (USEPA 2006). Geographic Information systems (GIS; Version 10.2) was used to measure the length of each stream and its tributaries from its confluence with the Red Bird River to an upstream 0.5 mi^2 watershed boundary or known barrier to fish movement (e.g., waterfall) (Figure 3). Initially, a total of 170 120-m reaches were established in Elisha Creek (100 reaches) and Gilberts Big Creek (70 reaches). A 10 percent sub-sample from each tributary (17 total sites, 10 for Elisha Creek and 7 for Gilberts Big Creek) was randomly selected for inclusion in the study. Then reaches where then surveyed through a series of randomly chosen plots (5m long X 2m wide) sampled three separate occasions during 2013 (spring, 17 May- 8 June; summer, 23 July- 4 August; fall, 12-19 October). After spring sampling efforts yielded low capture rates of KAD within designated reaches, an additional 3 sites (reaches) per stream were incorporated into subsequent summer and fall samplings.

Reach level measurements

Reach-specific habitat information was derived from averaging microhabitat measurements for each specific reach. Prior to sampling microhabitat plots, physicochemical parameters were measured at the downstream end of the reach utilizing an YSI meter (Yellow Springs Instruments, Yellow Springs, Ohio). Field parameters included water temperature (^{o}C), pH, dissolved oxygen (mg/L), and conductivity (microSiemens/cm).

Microhabitat Plots

Biotic and microhabitat data were collected using a modified sampling technique described by Compton and Taylor (2013). During the initial spring sample, 10 microhabitat plots (2m X 5m) were sampled in each reach. Plots where chosen randomly prior to sampling using a random number generator (1=left bank, 2= center stream, 3=right bank), and a 5-m buffer was maintained between each plot to ensure independence of plots sampled (Figure 4). Due to low capture rates observed during initial spring surveys, the number of sample plots was increased from 10 to 12. This level of effort was continued during summer and fall sampling.

Fish sampling

Fishes were collected from each microhabitat plot using a backpack electro-shocker (Smith-Root, Vancouver, Washington) and a dip net. Sampling effort ranged averaged between 47-62 seconds; however, sampling times varied due to differences in seasons, habitat type and complexity, water depth, capture efficiency, and number of fishes observed. Fishes collected in a plot were placed in an aerated bucket for identification and enumeration after the entire plot had been sampled. KADs were measured (mm), weighed (gm), and PIT (passive integrated transponder) tagged, and collection location documented using a hand-held GPS.

Microhabitat measurements

Microhabitat parameters, including water depth (cm), substrate type, flow velocity, and presence of large woody debris, were recorded within each 2-m X 5-m plot (Compton and Taylor 2013). Each of the four corners and the center of each individual plot were measured for water depth, and substrate (Figure 5). Depth was measured using a top-set wading rod. The dominant substrate particle was measured at each point and categorically classified using a modified Wentworth scale: fines/sediment <0.06 mm; sand 0.06–2mm; gravel 2–15 mm; pebble 16–63 mm; cobble 64–256 mm; boulder >256 mm; bedrock (Bain and Stevenson 1999). In addition, maximum water depth (m), presence of large woody debris (categorized as stable, >200mm in diameter and >1m in length), and largest substrate particle within the plot were recorded.

Movement Survey

PIT tags were implanted in KAD individuals (only KADs > 50 mm TL) to monitor their intra- and inter-tributary movement patterns among seasons. This method of tagging was preferred for this study because PIT tags, in combination with antenna systems equipped with transceivers for auto-detection, allow continual monitoring of upstream and downstream movements of marked KAD individuals. Other methods of tagging would limit collection of KAD movement data, and mark-and-recapture methods would most likely provide few recaptures of initially marked individuals. Hoger (2012) marked ten thousand individuals of four darter species with visual implant elastomer (VIE) markers. However, only 800 individuals were recaptured. Such a low recapture rate (8 percent) was a cause for concern for the KAD, an imperiled species with naturally lower abundances. Additionally, the typical retention rate of PIT tags is over 95% and also is not considered to hinder growth, movement, or behavior of small benthic fishes (Knaepkens et al. 2007).

KADs captured from May 2013-February 2014 were implanted with a Biomark HPT8 minichip^{TM} (8.4mm X 1.4mm 134.2KHz) PIT tag. Intratributary movement of KADs was monitored throughout Elisha Creek with antenna transceiver systems (Biomark, Boise, Idaho). Seven antenna systems were placed in the streambed in areas with natural channel constrictions dispersed throughout the tributary (Figures 2 and 6) and checked on a bi-weekly basis. Stations were monitored over a 12-month period (May 2013- May 2014). Additionally, intertributary movement between Gilberts and Elisha Creek was monitored with antenna systems placed at the confluence of each stream with the Red Bird River. All antenna systems were equipped with a transceiver, housing for equipment, and data port for collection of data. During each capture event, KADs were sedated by submersion in 40-60p.p.m of clove oil (eugenol) in order to reduce stress. Clove oil was found to rapidly sedate juvenile Rainbow Trout (*oncorhynchus mykiss*; Keene et. al 1998) thus this concentration was used for this study. Detar and Mattingly (2004) also found clove oil to be the preferred method of rapid anesthesia for southern red-belly dace. Once specimens were fully sedated, PIT tags were injected subcutaneously into the abdominal cavity using a MK165 implanter equipped with a 2” 16-gauge needle. Pit tags were inserted into the body cavity, anterior of the vent (Figure 7), following the mid-ventral line at an approximate 45^{o} angle. Once the abdominal muscle wall had been pierced, the tag was pushed into the cavity following methods similar to Ruetz et al. (2006). After individuals were measured, weighed, and tagged, they were placed into aerated buckets and monitored for recovery from effects of anesthesia and PIT tag insertion. Once each tagged fish recovered, they were released at their original capture location.

Procedures related to anesthesia, capture, and handling were reviewed by Eastern Kentucky University’s Institutional Animal Care and Use Committee and approved as Protocol #02-2013.

DATA ANALYSIS

Population Estimates

Population estimates were computed using number of KADs captured, wetted channel width at each plot, and an extrapolation coefficient. Reach scale population estimates were calculated by first determining the area of each reach. The area was determined by multiplying average stream width by total reach length (100m for spring, 120m for summer and fall). An extrapolation coefficient was calculated by dividing total reach area by the actual sampling area (plots). The number of KADs captured in plots was then multiplied by the extrapolation coefficient to determine each reach estimate. The KAD population in each stream was estimated by multiplying the mean population estimates from all reaches by the total possible number of potential reaches in the watershed.

Movement Analysis

Movement was analyzed using Antenna stations with onboard computer systems to record PIT tags as individual KADs passed over the antenna. ArcGIS version 10.2 (http://www.esri.com/software/arcgis) was used to calculate distances traveled by individual KADs by plotting original capture locations and last observation from either antenna or recapture event. Movements of KADs were analyzed relative to seasonality, sex, and fish size. All original capture points and gathered information from the antenna systems were plotted using ArcMap to calculate distances and aid in correlating patterns.

<u>Habitat association</u>

For each habitat variable (% Riffle, % Run, % Pool, Stream Width (m), Large Woody Debris (LWD), Substrate Count, % Fines, % Sand, % Gravel, % Pebble, % Cobble, % Boulder, % Bedrock, Mean Depth (mm), Depth MAX (mm), and Large (LRG) Boulder (m)) for occupied and unoccupied reaches and plots, a mean value within and across seasons was calculated. Kruskal-Wallis two-sample tests were used to compare differences in habitat between occupied and unoccupied plots and reaches within and across seasons using the NPAR1WAY procedure in SAS version 9.3 (http://www.sas.com/en_us/software/sas9.html). Statistical significance was evaluated at $\alpha=0.05$.

RESULTS

A total of 7,593 fishes representing 6 families were captured in 752 plots during this study (Table 1). The four most commonly observed species where Creek Chubs (*Semotilus atromaculatus*; n=4,695), Rainbow Darter (*Etheostoma caeruleum*; n= 751), Southern Redbelly Dace (*Chrosomus erythrogaster*; n=619), and Central Stoneroller (*Campostoma anomalum;* n= 570). The Kentucky Arrow Darter was the seventh most abundant fish comprising one percent of all fishes observed. KAD was preceded only by Creek Chubs (62%), Rainbow Darter (10%), Southern Redbelly Dace (*Chrosomus erythrogaster)* (8%), Central Stoneroller (*Campostoma anomalum*) (8%), Faintail Darter (Etheostoma flabellare; 1%), Greenside Darter (Etheostoma blennioides; 1%), Freckelbelly Darter (Percina stictogaster; 1%), Striped Shinner (Luxilus chrysocephalus; 1%). An additional 8,200 fishes were captured during qualitative surveys (about 35,000 shocking seconds), but no new species were documented. KADs were caught during all seasons in both study streams. However, the number of occupied plots per sample event increased from spring to fall (Table 2). Only 58 KADs were captured during quantitative efforts from a total of 38 plots (spring, n=5; summer, n=11; fall, n=22; Table 2).

KAD population estimates varied seasonally across both streams. Population sizes for spring, summer, and fall ranged between 80-208 (95% CI), 175 -533, and 393-776 for Gilberts Big Creek and 319 -724, 592-1175, 661-1498 for Elisha Creek, respectively. Mean densities observed in spring, summer, and fall were 0.002 KAD/m^2 (N=2), 0.007 KAD/m^2 (N=8), and 0.015 KAD/m^2 (N=18) for Gilberts Big Creek (Figure 8) and 0.004 KAD/m^2 (N=4), 0.006 KAD/m^2 (N=10), and 0.01 KAD/m^2 (N=16) for Elisha Creek (Figure 9), respectively. Mean reach densities within watersheds ranged from 0.0-0.04 KAD/m^2 and 0.0-0.05 KAD/m^2 at Gilberts Big Creek and Elisha Creek, respectively.

Only 121 of the 145 captured KADs were large enough (TL≥ 50mm) to be fitted with PIT tags. A length frequency histogram of captured KADs (Figure 10) suggested four age classes represented by males (N=79), females (N=60), and unknown (N=6). A total of 18 (15%) KADs moved from their initial capture location. Travel distances ranged from 40m to 4,078m (2.54 miles; Table 3) from May 2013 through April 2014, with both up and downstream movements being recorded. Additional movement data was obtained through eight recaptures of previously tagged KADs (Table 3). No patterns or correlations between seasonality, sex, or size (age class) were detected for individual movements.

Physicochemical parameters for both streams ranged throughout the seasons (Gilberts Big Creek: temperature, 17.8-22.4^{o}C; pH, 7.6-8.3; dissolved oxygen, 5.7-13.4 mg/L; and conductivity 71-145 µS/cm; Elisha Creek: temperature, 16.7-20.8^{o}C; pH, 7.7-8.72; dissolved oxygen, 6.31-8.31 mg/L; and conductivity, 35-78 µS/cm).

Habitat comparisons for occupied (n=5; n=8; n=16), and unoccupied reaches (n=15; n=15; n=7) for spring, summer, and fall yielded only one significant finding (Table 4). Occupied reaches in fall had significantly lower stream widths (Kruskal-Wallis $\chi^2 \geq 4.43$; $P \leq 0.04$). When comparisons were made across seasons (occupied n=29; unoccupied n=37;Table 5) occupied reaches had significant less percent riffles (Kruskal-Wallis $\chi^2 \geq 4.08$; $P \leq 0.04$;) runs (Kruskal-Wallis $\chi^2 \geq 5.25$; $P \leq 0.02$) and significantly more pool habitat (Kruskal-Wallis $\chi^2 \geq 5.91$; $P \leq 0.02$). Additionally, occupied reaches had significantly less percent composition of boulders (Kruskal-Wallis

$\chi^2 \geq 5.06$; $P \leq 0.02$). Plot scale comparisons within seasons yielded several significant findings (Table 5). Spring comparisons (occupied n=5; unoccupied n=20) showed significantly lower percent riffle habitat (Kruskal-Wallis $\chi^2 \geq 9.89$; $P \leq 0.001$), and percent composition of gravel (Kruskal-Wallis $\chi^2 \geq 11.72$; $P \leq 0.001$). Summer (occupied n=5; unoccupied n=23) analysis revealed occupied plots had significantly less percent riffle and run habitat and higher percent pool habitat (Kruskal-Wallis $\chi^2 \geq 17.68$; $P \leq 0.001$; $\chi^2 \geq 8.93$; $P \leq 0.003$; $\chi^2 \geq 9.87$; $P \leq 0.002$), as well as significantly higher maximum depth (Kruskal-Wallis $\chi^2 \geq 13.71$; $P \leq 0.0002$) than unoccupied plots (Table 6). Comparisons for fall (occupied n=12; unoccupied n=23) showed occupied plots had a significantly higher percent of pools and less riffles (Kruskal-Wallis $\chi^2 \geq 20.57$; $P \leq 0.001$; $\chi^2 \geq 20.57$; $P \leq 0.001$), lower percent composition of pebbles (Kruskal-Wallis $\chi^2 \geq 7.59$; $P \leq 0.01$), and higher mean depths (Kruskal-Wallis $\chi^2 \geq 7.73$; $P \leq 0.01$) than unoccupied plots. When occupied (n=25) plots were compared to unoccupied (n=66) plots across seasons (Table 7), occupied plots had significantly higher percentage of pools, mean depth, maximum depth, and less percent riffle, run, large woody debris, gravel, pebble, and boulder (Kruskal-Wallis $\chi^2 \geq 29.65$; $P \leq 0.0001$; $\chi^2 \geq 5.76$; $P \leq 0.02$; $\chi^2 \geq 7.10$; $P \leq 0.01$; $\chi^2 \geq 49.21$; $P \leq 0.0001$; $\chi^2 \geq 12.07$; $P \leq 0.001$; $\chi^2 \geq 6.92$; $P \leq 0.01$; $\chi^2 \geq 11.65$; $P \leq 0.001$; $\chi^2 \geq 7.96$; $P \leq 0.005$; $\chi^2 \geq 5.06$; $P \leq 0.02$).

DISCUSSION

The increasing trend in occupied plots from spring to fall could have been attributed to a substantial rain event prior to initiation of spring sampling. Increased stream flow likely caused greater dispersal of fishes throughout each stream. Additionally, water levels during fall sampling were very low and fish were most likely confined to available pool habitats. Thus sampling efficiency was higher yielding an increase in occupied plots. This decrease in water availability and increase in available pool habitat is consistent with what Lotrich (1973) observed in Clemons Fork.

Prior to this study, population densities of KADs in streams located on the Red Bird River basin (particularly Gilberts Big Creek and Elisha Creek) were thought to be some of the highest within the species' range (Michael Floyd, pers. comm. 2013). Following the same increasing seasonal trend observed for occupied plots, population estimates also showed an increase from spring to fall. The flood event prior to sampling in the spring could explain the lower numbers for that season. Taylor et al. (1996) found that spatial and temporal changes in fish assemblage structure and individual species abundances were greatest in spring with increased discharge resulting from flood events and natural population dynamics. The increased number for fall could be attributed to the capture of young-of-year (YOY) within plots that I previously did not observe in prior sample events (spring and summer). Eleven YOY were captured in plots in the fall while the only other juvenile (< 50mm) individuals observed in plots came from the spring. Mean densities and the number of occupied plots were higher in Gilberts Big Creek than Elisha Creek for all sample seasons. However, KAD abundance in plots was higher in Elisha Creek, resulting in a higher population estimate.

Ample sampling efforts were made above the waterfall on Gilberts Big Creek to further the length of occupied stream, however, there was no success of capturing KAD. KAD likely historically occupied the stream above this waterfall; however, stochastic events could have extirpated this upper portion and confined KAD to lower stream reaches when seeking favorable conditions during low water conditions. The waterfall on Gilberts Big Creek likely acts as a biogeographic barrier and limits the upper portion of the watershed for KAD dispersion, re-colonization, and reproduction. Upstream movements of fishes can be inhibited or prevented by high gradient habitats such as waterfalls or extensive cascades or by man-made structures such as culverts (Rahel 2007). Similar results were observed for Blackside Dace on Lick Fork, Bell County, KY, when populations above a perched culvert slowly declined until presumed extirpated above the culvert while populations continued to flourish below the culvert (Eisenhour and Floyd 2013).

In August of 2013, EKU partnered with USFWS, KSNPC, and the University of Kentucky (UK) to conduct a population estimate in Clemons Fork, Breathitt County Kentucky. This watershed is 99% owned by UK and managed for the preservation of all wildlife and plant communities found within its boundaries. Population estimates for Clemons Fork revealed a robust population estimate (95% CI) of 986 ±1,127 (Baxter et. al. unpublished data). This study produced similar estimates, but populations in many other streams across the species' range are considered to be less stable (USFWS 2015). In comparison, another federally endangered species, the Fountain Darter (*Etheostoma fonticola*), was estimated at 102,966 individuals within an 8.4-km section of the San Marcos River (Schenck and Whiteside 1976). A combined summer total population estimate of 1,756 individuals across three streams (Giberts Big Creek, Elisha Creek, and Clemons Fork) that are not as constrained by habitat, water quality or anthropogenic limitation as other streams throughout KADs range, demonstrates the vulnerable state and low abundance of this species. Additionally, a study estimating densities of Orangebelly Darters (*Etheostoma radiosum cyanorum*) yielded an estimate of 818 adult darters in a 307m^2 stretch of the Blue River (Scalet 1973). This equated to a density of approximately 2.66 darters per m^2 compared to a maximum KAD reach density of 0.05 darters/m^2.

Movement studies of benthic fishes are very limited, and mostly conducted in a controlled method that involves chance recapture of tagged or marked individuals in confined reaches. Although this method provides evidence on dispersal and movement of individuals, it is limited by the sampling efforts of investigators. Throughout all KAD sampling occasions, an accumulative recapture rate of tagged individuals was 5%. This is consistent with similar studies involving mark recapture type studies with benthic species and more specifically the genus *Etheostoma*. Common reports of 8-16 percent recapture rates (Hoeger ,Roghair et. al 2014, Dammeyer et. al 2013) have occurred in studies that used visual implant elastomers (VIE), dyes or other methods of tagging (i.e. fin clipping). I suspect that methods such as these are prone to underestimating most if not all long-range dispersal events. In this study, all but one observed movement greater than 100m was recorded using VIA antenna methods.

Eighty percent of movement captured via antenna stations was greater than 100m. These movements most likely represented individuals that were actively dispersing from their original capture location or assumed home range. The most notable recorded movement was an individual on Gilberts Big Creek that moved a minimum distance of 4,078m (2.53 miles) downstream to the confluence with the Red Bird River. This 66mm (TL) female traveled this distance over a 10-month period (June-March) and is assumed to have entered the Red Bird River and disbursed up or downstream. Utilizing antennas has a limitation of certainty that the tagged fish continued the direction of travel over the antenna (unless recorded on an additional antenna in direction of travel). However, this dispersal shows the capability and probability of intertributary movement by this species. In a study of the Niangua Darter, a related species belonging to the same subgenus, investigators observed the species utilizing 3^{rd}-6^{th} order streams more commonly than smaller order streams (Mattingly and Galat 2002). Use of larger streams by KADs was supported during a KAD reintroduction study in Long Fork, a tributary to Hector Branch, Clay County, Kentucky (M. Thomas, pers. comm. 2015). Matt Thomas (KDFWR unpublished data) recorded movements ranging from 0.42-1.1 mi. Recent USFWS surveys produced four individuals between two independent sampling events in a previously unoccupied stream, Bear Branch, Breathitt County. These individuals were considered to be transient individuals from Clemons or Coles Fork, Breathitt County, located approximately 2 miles upstream of Bear Branch (Michael Floyd, pers. comm. 2015)

These documented movements and recorded dispersal abilities could enable conservation agencies to focus recovery efforts to watersheds or areas that have source populations but contain significant dispersal barriers (i.e perched culverts, in-stream road crossings, etc.). Removal of these barriers could allow KADs to naturally reoccupy new streams. These naturally occurring sinks may be more sustainable and practical from a management perspective. Although many species do not have dispersal capabilities or are limited to only portions of their historical range (KADs included), initial recovery efforts should be focused on connectivity and restoration of habitat rather than propagation of fishes. Additionally, propagated fishes can possibly diverge from their wild phenotype, limiting their success, and breeding capabilities. Mass propagation of KADs without new brood stock or using captive individuals for brood stock could lead to the hindrance of reared supplemental populations (Fleming, 1994).

Habitat use by KADs was evaluated on two scales (reach and plot) by season and across seasons. Reaches within individual seasons showed KADs associated with narrower stream widths for fall. This could be attributed to two main factors. First, base stream flow was lowest during fall, with reduced stream widths compared to spring and summer. However, this would not account for why occupied reaches differed from unoccupied. Secondly, occupied reaches for the fall sampling event fell in the mid to upper sampled reaches located within the watersheds (Gilberts Big and Elisha's Creek). Because base flow is typically lower during this season, it is likely that KADs became confined to pool habitats and could not migrate to downstream portions/reaches of the watershed as easily when compared to spring or summer during higher flows. This would support Lotrich's (1973) observations that KADs were confined to isolated pools in the fall in Clemons Fork.

When reaches were analyzed across seasons it became evident that KADs were more commonly associated with reaches that had a higher percentage of pools and were rarely found in riffles or runs. Interestingly, another variable that was significant was percent boulder composition. Boulder composition was less in occupied reaches than in unoccupied reaches, indicating that KADs seek areas with smaller substrates. Mattingly and Galat (2002) found that the Niangua darter was commonly observed with substrate particles ranging from 30-50mm (pebble). This could be due to smaller interstitial spaces between substrate and the active feeding habits of KADs. Because the mouth is situated terminally, they could actively seek prey that could be taking refuge in the smaller spaces.

During spring, occupied plots had less composition of gravel and riffles compared to other habitat and substrate categories. This contradicts previous observations on the KADs reproductive ecology that involve the use of gravel substrates by the female and male (Etnier and Starnes 1993). One explanation for this finding would be the large spate event prior to sampling. This high flow event could easily move these smaller substrates to erosional areas (riffles during high flow). Additionally, darters may have already abandoned riffle habitats when my surveys were conducted (May). Male darters establish territories over riffles from March to May (Kuehne and Barbour 1983, p. 71; Lotrich), so spawning could have concluded prior to onset of sampling, thereby explaining why gravel substrates and riffles were not associated with occupied plots.

Summer and fall sampling periods followed similar trends. When KADs where present, pool habitat was the dominant habitat characterization, with composition of riffle and run being obsolete. Reaches with riffle habitat in fall sampling actually yielded no observations of KADs. The other variables that stood out as significant were maximum depth (summer) and mean depth (fall). This was expected considering that occupied reaches consisted of significantly more pool habitat. If KADs can migrate to other reaches/stream habitats, it is reasonable to assume they are commonly associated with reaches with pool habitats in order to seek refugia from high temperatures and low dissolved oxygen and to seek areas with habitat complexity. An additional explanation could be the availability of water. As mentioned earlier, the base flow of streams is typically lower during summer and fall. These low flow conditions could not only confine KADs to pool type habitats, but they could also cause stressful conditions (elevated temperature, lower dissolved oxygen, etc.) that would cause KADs to actively seek pool type habitat with greater water depths. These deeper water habitats could support cooler water temperatures and more available oxygen.

When occupied plots (n=25) were substantiated with unoccupied plots (n=66) on a smaller scale, associations really became evident, and observed KAD in-stream distributions could be explained. Once again, pool habitat became the most significant habitat characterization, which explains the significant mean depth and maximum depth findings. Substrate associations also became clear by analyzing habitat use at a smaller scale that encompasses all sampling plots. Percent pebble, gravel, and boulder showed a negative relation between occupied and unoccupied plots. However, proportion of cobble, although not significant (P=0.056), appears to play a role in plots occupied by KADs. These results from plot comparisons are consistent with the KAD surveys conducted in 2007-2009 and 2012-2013 (Michael Floyd, USFWS; Mike Compton, KSNPC pers. comm. 2014). During these surveys, KADs were observed in pools containing a high composition of cobble, and a mixture of boulder, gravel, and pebbles.

Even though significant results of habitat associations to KADs are indicative of observed habitat (both myself and other agency initiated surveys), it is apparent that the low frequency of KAD occurrence and the species' naturally low abundance limit our complete understanding of this imperiled species. My understanding of the species' ecology is limited to observations completed at Gilberts Big Creek and Elisha Creek. However, general inferences for the species as a whole are possible based on the quality and effort of this study. To better understand if KADs associate with the habitat identified in this study, a third stream (or more) could be studied. Ideally, a historically occupied stream where the species is now considered to be extirpated - a factor USFWS outlined as key to KADs occupancy and distribution (USFWS 2015). This could aid in determining whether habitat differed between currently occupied streams and those where the species is now extirpated. This data could give insight into management implications when looking at habitat compositions. Additionally, it would allow for restoration resources to be utilized more efficiently. Thus, instead of large scale restoration and propagation of KADs in streams that are in proximity of KADs, allow the species' natural dispersal ability to recolonize these streams and aid in recovery of the species. It would allow resource agencies to better utilize resources to enhance possibilities of dispersal (i.e. culvert replacements, low water ford rehab, and protection of corridors). Not only would this allow for KADs to recolonize naturally, but it would also promote greater genetic variation compared to propagated fishes. Although inferences can only be made about Gilberts Big Creek and Elisha Creek, the results from this study can be used to evaluate potential impacts to development and resource extraction projects on streams to be disturbed that could support KADs. Understanding the habitat use and dispersal ability of KADs allows for more focused survey efforts, as well as a better use of resources. Additionally, it allows managers to quickly access areas to sample and focus their efforts.

It is important to realize that this study was not intended to be all-inclusive and only represents findings from two occupied streams within the KADs current range. Although findings are consistent with other limited field observations, it cannot be concluded that the Kentucky Arrow Darter exhibits these behaviors throughout its range. More intensive range-wide population surveys are needed to improve our understanding of the species' ecology and determine how best to manage and conserve the species.

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APPENDIX A:

Tables

***Table 1*.** Fish abundance during quantitative sampling spring, summer and fall 2013 in Gilberts Big Creek and Elisha Creek.

Family	Species	TNI Spring 2013		TNI Summer 2013		TNI Fall 2013	
		Gilberts Big Creek	Elisha Creek	Gilberts Big Creek	Elisha Creek	Gilberts Big Creek	Elisha Creek
Petromyzontidae	*Ichthyomyzon fossor*	0	0	2	0	0	0
	Lampetra aepyptera	0	0	0	0	3	0
Cyprinidae	*Campostoma anomalum*	19	23	65	99	157	207
	Chrosomus erythrogaster	57	13	93	73	238	145
	Cyprinella whipplei	0	0	1	0	0	0
	Luxilus chrysocephalus	4	10	15	13	9	20
	Notropis ariommus	0	0	1	0	0	0
	Notropis buccatus	0	0	0	0	5	0
	Notropis rubellus	0	0	1	4	0	1
	Pimephales notatus	2	1	6	2	3	3
	Semotilus atromaculatus	155	198	379	1230	1026	1707
Catostomidae	*Catostomus commersonii*	5	0	2	8	6	1
	Hypentelium nigricans	2	1	4	4	26	17
	Moxostoma duquesnei	0	0	1	1	0	0
Cottidae	*Cottus bairdi*	0	0	3	0	5	0

***Table 1*. (continued).**

Family	Species	TNI Spring 2013		TNI Summer 2013		TNI Fall 2013	
		Gilberts Big Creek	Elisha Creek	Gilberts Big Creek	Elisha Creek	Gilberts Big Creek	Elisha Creek
Centrarchidae	*Lepomis cyanellus*	0	0	2	3	0	0
	Lepomis macrochirus	0	0	4	1	2	0
	Micropterus dolomieu	0	0	0	0	0	3
Percidae	*Etheostoma baileyi*	0	0	1	4	6	20
	Etheostoma blennioides	8	10	19	21	16	30
	Etheostoma caeruleum	48	31	129	198	158	187
	Etheostoma flabellare	43	32	118	75	56	53
	Etheostoma nigrum	13	1	3	1	27	9
	Etheostoma spilotum	2	4	8	7	19	16
	Etheostoma variatum	0	2	0	6	1	12
	Percina copelandi	3	0	0	0	0	0
	Percina maculata	1	8	4	3	2	2
	Percina stictogaster	6	1	18	16	28	16
	SEASON TOTALS	368	335	879	1769	1793	2449
		Total Fishes	7593				

***Table 2*.** Number of plots occupied by Kentucky Arrow Darter across seasons at Gilberts Big and Elisha Creek.

	Total # occupied plots	Plots sampled
Spring		
Elisha Creek	2	100
Gilberts Big Creek	3	100
Summer		
Elisha Creek	4	156
Gilberts Big Creek	7	120
Fall		
Elisha Creek	9	156
Gilberts Big Creek	13	120
TOTALS	38	752

***Table 3*.** Movement data of Kentucky Arrow Darter in Gilberts Big and Elisha Creek 2013-2014.

Latitude	**Longitude**	**Label**	**Date**	**Sex**	Total Length	Distance moved	**Direction Moved**
37.07295	-83.50641	IC	24-May-13	M	86mm	105m	↓
37.07284	-83.50760	LO	12-Apr-14				
37.07432	-83.51111	IC	12-Oct-13	M	77mm	1,280m	↓
37.08273	-83.5194	LO	15-Mar-14				
37.07292	-83.50833	IC	30-Jul-13	F	77mm	66m	↑
37.07284	-83.50760	LO	30-Mar-14				
37.07421	-83.51111	IC	29-Jul-13	F	68mm	355m	↑
37.07284	-83.50760	LO	3-Apr-14				
37.08248	-83.51884	IC	27-Jul-13	M	67mm	58m	↓
37.08273	-83.5194	LO	17-Apr-14				
37.06965	-83.49789	IC	30-Jul-13	M	98mm	933m	↓
37.07284	-83.50760	LO	7-Apr-14				
37.10637	-83.51899	IC	8-Jun-13	F	66mm	4,078m	↓
37.1078	-83.5559	LO	29-Mar-14				
37.08852	-83.52704	IC	22-May-13	F	58mm	332m	↓
37.08568	-83.5265	LO	28-Feb-14				

Table 3. (continued).

37.08206	-83.51834	IC	28-Jul-13	F	70mm	896m	↓→
37.08568	-83.5265	LO	9-Apr-14				
37.09536	-83.51857	IC	12-Oct-13	M	107mm	170m	↑
37.09639	-83.5173	LO	21-Feb-14				
37.07292	-83.50833	IC	30-Jul-13	M	94mm	40m	↓*
37.07311	-83.50869	LO	19-Oct-13				
37.10293	-83.52695	IC	24-Jul-13	M	98mm	91m	↓*
37.10286	-83.52786	LO	19-Oct-13				
37.07292	-83.50833	IC	30-Jul-13	F	73mm	40m	↑ *
37.07311	83.50869	LO	17-Oct-13				
37.10276	-83.5273	IC	24-Jul-13	M	93mm	28m	↑*
37.10297	-83.52718	LO	14-Oct-13				
37.09633	-83.517	IC	23-May-13	M	111mm	40m	↑ *
37.09578	-83.51812	LO	29-Jul-13				
37.07292	-83.50833	IC	30-Jul-13	F	76mm	40m	↓ *
37.07311	83.50869	LO	16-Oct-13				
37.10595	-83.52143	IC	7-Jun-13	F	68mm	418m	↓ *
37.10521	83.51713	LO	18-Oct-13				

IC= Initial capture location
LO= Last observation location
↓= Down stream movement
↑= Up stream movement
→= Right turn
*= Movement obtained via recapture

Table 4. Kruskal-Wallis two-sample comparison of occupied (O) and unoccupied (U) reaches within seasons within Gilberts Big Creek and Elisha Creek. Significant p-values ($p<0.05$) are in bold.

	Mean		Spring		Means		Summer		Means		Fall	
Variable	O	U	X^2	P	O	U	X^2	P	O	U	X^2	P
% Riffle =	0.22	0.35	2.89	0.09	0.14	0.21	2.61	0.11	0.17	0.11	2.24	0.13
% Run =	0.50	0.35	0.78	0.38	0.32	0.32	0.00	0.97	0.00	0.00	0.00	1.00
% Pool =	0.26	0.30	0.10	0.76	0.52	0.44	0.57	0.45	0.83	0.89	2.24	0.13
Stream Width (m)	6.22	5.61	0.84	0.36	6.04	4.81	0.07	0.80	4.28	5.76	4.43	**<u>0.04</u>**
LWD	0.26	0.21	0.29	0.59	0.03	0.07	1.88	0.17	0.05	0.00	2.63	0.10
Substrate Count	2.60	2.79	0.94	0.33	2.61	2.64	0.00	1.00	2.48	2.64	0.41	0.52
% Fines	0.00	0.00	0.00	1.00	0.01	0.01	0.08	0.78	0.00	0.00	0.44	0.51
% Sand	0.16	0.16	0.00	0.97	0.25	0.27	0.03	0.87	0.36	0.35	0.16	0.69
% Gravel	0.07	0.11	1.42	0.23	0.07	0.06	0.03	0.87	0.10	0.10	0.06	0.81
% Pebble	0.20	0.18	0.01	0.93	0.16	0.12	1.60	0.21	0.08	0.12	1.16	0.28
% Cobble	0.22	0.26	0.12	0.73	0.20	0.21	0.07	0.80	0.15	0.12	0.66	0.42
% Boulder	0.07	0.09	0.24	0.63	0.01	0.00	0.21	0.64	0.00	0.02	2.29	0.13
% Bedrock	0.26	0.20	0.00	0.96	0.30	0.31	0.02	0.90	0.30	0.29	0.01	0.92
Mean Depth (mm)	127.5	152.7	2.60	0.11	112.4	94.6	0.05	0.82	104.0	92.8	0.11	0.74
Depth MAX (mm)	274.4	289.3	1.00	0.32	270.3	250.8	1.25	0.26	214.7	193.1	0.22	0.64
LRG Boulder (m)	11.22	8.06	0.09	0.76	0.69	0.62	0.00	1.00	0.52	0.47	0.07	0.79

Table 5. Kruskal-Wallis two-sample comparison of occupied and unoccupied reaches across seasons within Gilberts Big Creek and Elisha Creek. Significant p-values ($p<0.05$) are in bold.

	Means			
Variable	Occupied	Unoccupied	X^2	P
% Riffle =	0.17	0.25	4.08	**0.04**
% Run =	0.18	0.27	5.25	**0.02**
% Pool =	0.65	0.47	5.91	**0.02**
Stream Width (m)	5.10	5.32	1.47	0.22
LWD	0.08	0.11	1.10	0.29
Substrate Count	2.54	2.70	2.45	0.12
% Fines	0.00	0.00	0.13	0.72
% Sand	0.30	0.24	3.04	0.08
% Gravel	0.09	0.09	0.10	0.76
% Pebble	0.12	0.15	1.53	0.22
% Cobble	0.17	0.22	1.93	0.16
% Boulder	0.01	0.04	5.06	**0.02**
% Bedrock	0.29	0.26	0.39	0.53
Mean Depth (mm)	110.34	117.77	2.02	0.15
Depth MAX (mm)	240.34	255.48	2.41	0.12
LRG Boulder (m)	2.41	3.61	1.00	0.32

Table 6. Kruskal-Wallis two-sample comparison of habitat variables assessed in occupied and unoccupied plots within seasons Gilberts Big Creek and Elisha Creek. Significant p-values (p<0.05) are in bold.

	Mean		Spring		Mean		Summer		Mean		Fall	
Variable	O	U	X²	P	O	U	X²	P	O	U	X²	P
% Riffle	0.00	0.32	9.89	**0.00**	0.00	0.19	17.68	**<.0001**	0.00	0.17	20.57	**<.0001**
% Run	0.40	0.39	0.56	0.45	0.09	0.33	8.93	**0.003**	0.00	0.00	0.00	1.00
% Pool	0.60	0.28	0.57	0.45	0.91	0.46	9.87	**0.002**	1.00	0.83	20.57	**<.0001**
Stream Width (m)	5.90	5.77	0.37	0.54	4.50	5.23	0.43	0.51	3.97	4.82	1.24	0.27
LWD	0.40	0.22	0.00	0.94	0.00	0.18	3.54	0.06	0.00	0.04	2.94	0.09
Substrate Count	3.20	2.74	2.26	0.13	2.55	2.66	0.02	0.89	2.37	2.54	0.31	0.58
% Fines	0.00	0.00	0.00	1.00	0.02	0.01	0.58	0.44	0.00	0.00	0.52	0.47
% Sand	0.32	0.15	1.34	0.25	0.33	0.26	0.00	1.00	0.40	0.36	1.32	0.25
% Gravel	0.00	0.10	11.72	**0.001**	0.04	0.07	0.47	0.30	0.07	0.10	1.03	0.31
% Pebble	0.24	0.18	0.91	0.34	0.07	0.14	3.12	0.08	0.04	0.09	7.59	**0.01**
% Cobble	0.20	0.25	0.51	0.47	0.16	0.21	2.17	0.14	0.17	0.14	0.68	0.41
% Boulder	0.12	0.08	0.51	0.47	0.00	0.00	0.72	0.40	0.00	0.01	0.52	0.47
% Bedrock	0.12	0.22	1.96	0.16	0.33	0.31	0.82	0.37	0.28	0.30	0.33	0.57
Mean Depth (mm)	173.8	146.4	0.0	0.9	130.3	87.9	2.8	0.1	197.6	94.3	7.7	**0.01**
Depth MAX (mm)	345.4	284.0	0.2	0.7	385.8	211.5	13.7	**0.001**	326.3	197.9	3.7	0.1
LRG Boulder (m)	0.5	0.7	1.2	0.3	1.1	1.7	0.9	0.3	0.5	0.5	0.3	0.6

***Table 7*.** Kruskal-Wallis two-sample comparison of habitat variables assessed in occupied and unoccupied plots across seasons Gilberts Big Creek and Elisha Creek. Significant p-values (p<0.05) are in bold.

	Means			
Variable	Occupied	Unoccupied	X^2	P
% Riffle =	0.00	0.22	49.21	**<.0001**
% Run =	0.09	0.23	12.07	**0.001**
% Pool =	0.91	0.55	29.65	**<.0001**
Stream Width (m)	4.44	5.23	1.20	0.27
LWD	0.06	0.14	6.92	**0.01**
Substrate Count	2.54	2.64	0.00	0.96
% Fines	0.01	0.00	1.17	0.28
% Sand	0.37	0.27	2.99	0.08
% Gravel	0.05	0.09	11.65	**0.001**
% Pebble	0.08	0.13	7.96	**0.005**
% Cobble	0.17	0.20	3.64	0.06
% Boulder	0.02	0.03	5.06	**0.02**
% Bedrock	0.27	0.28	1.86	0.17
Mean Depth (mm)	173.03	106.18	5.76	**0.02**
Depth MAX (mm)	346.62	226.46	7.10	**0.01**
LRG Boulder (m)	0.74	1.00	0.31	0.58

APPENDIX B:

Figures

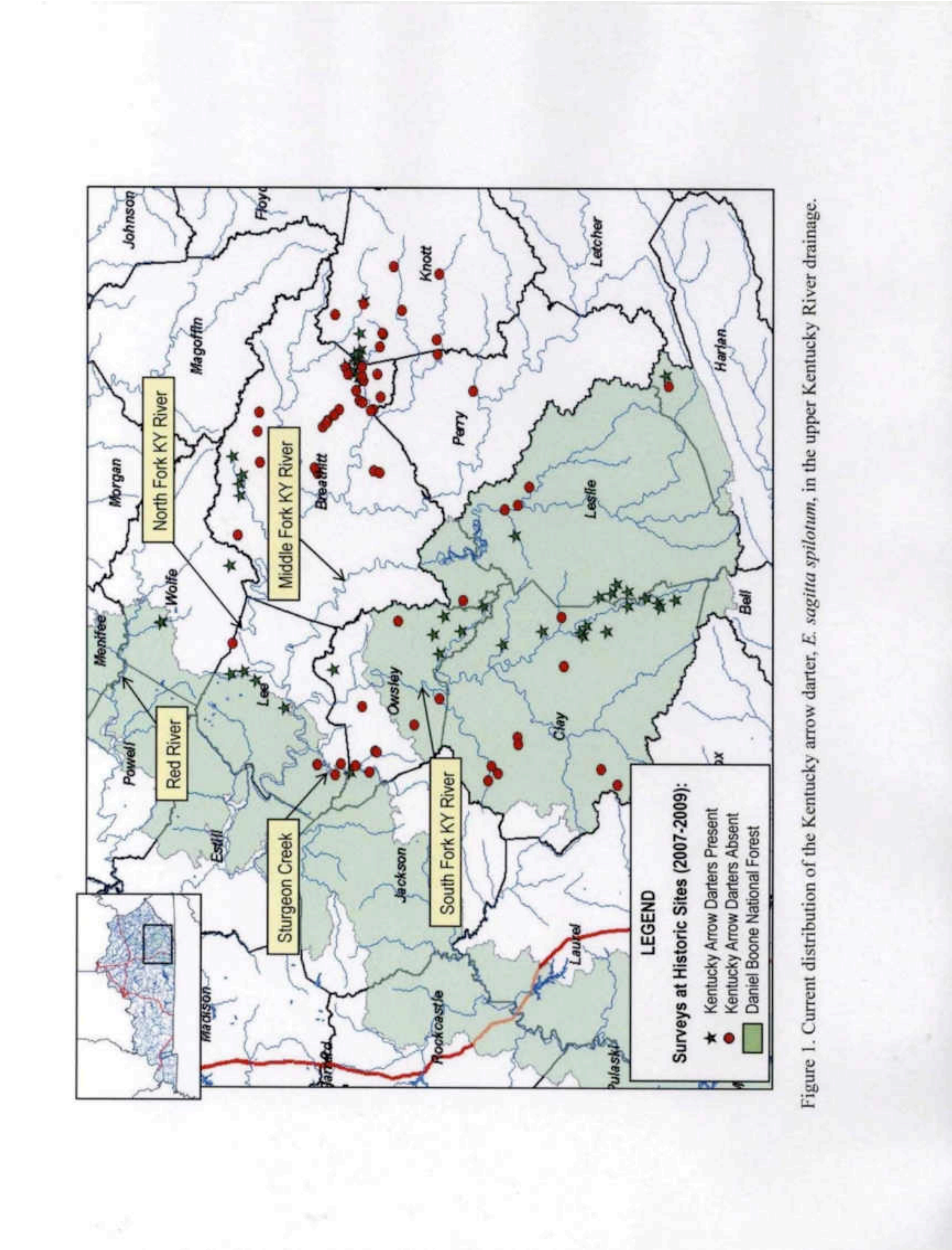

Figure 1. Distributional map of historical KAD locations showing current presence/absence during 2007-2009 sampling.

Source: United States Fish and Wildlife Service. 2009. Project report: Kentucky arrow darter surveys in eastern Kentucky. Kentucky Ecological Services Field Office. Frankfort, Kentucky. 4 pp.

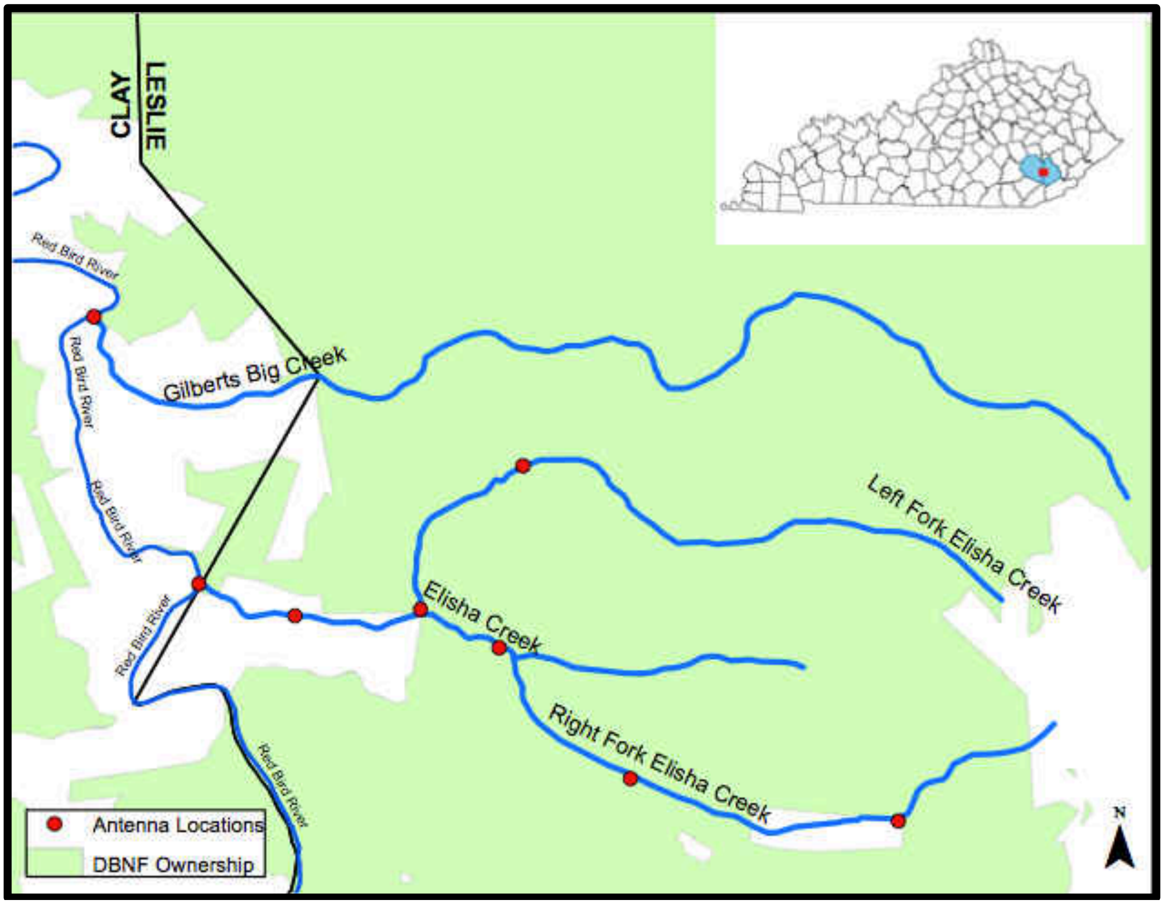

Figure 2. Study area with antenna locations for Gilberts Big and Elisha Creek within Red Bird District of the Daniel Boone National Forrest (highlighted in green).

Figure 3. Barrier on Gilberts Big Creek located ≈7000m from confluence of the Red Bird River.

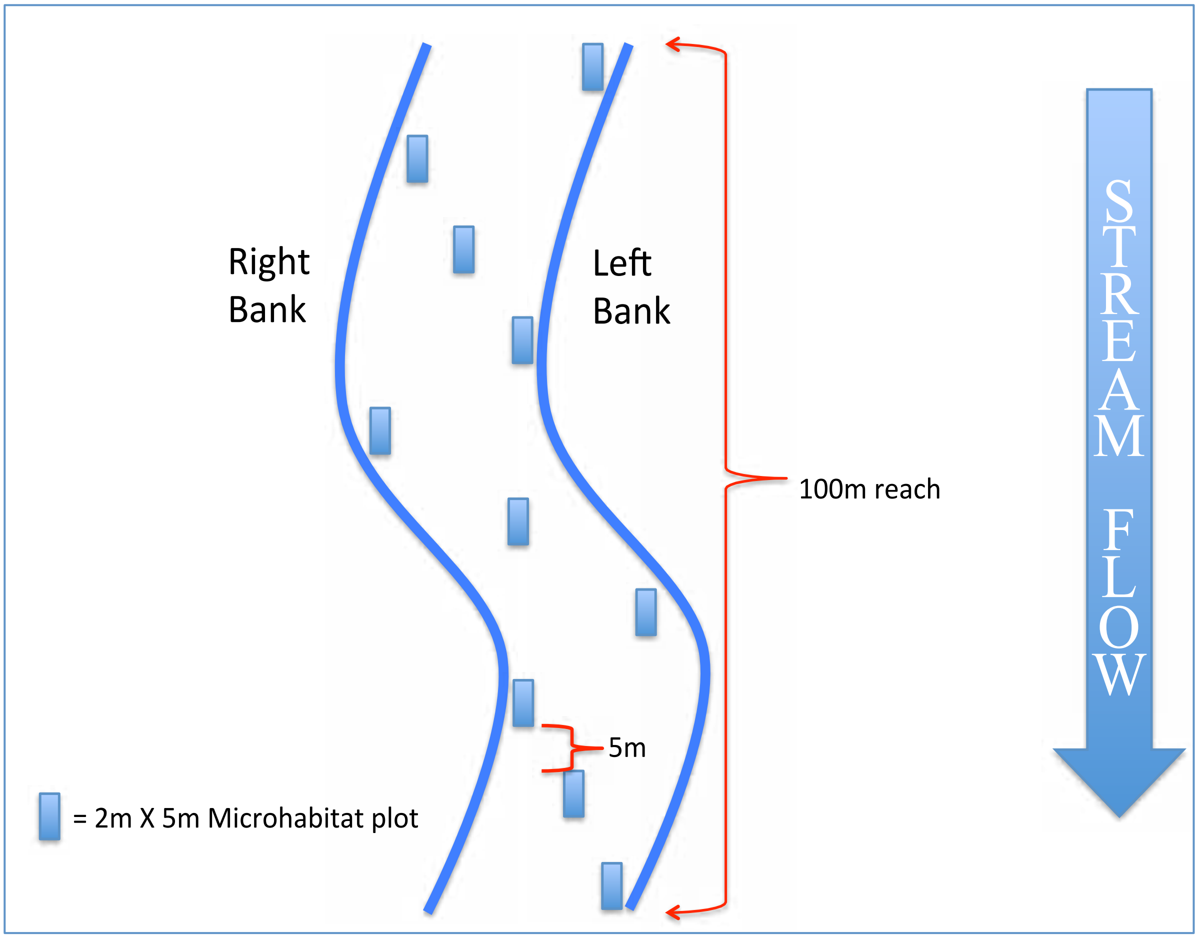

Figure 4. Diagram depicts microhabitat sampling plot locations for assessing Kentucky Arrow Darter habitat at the reach level.

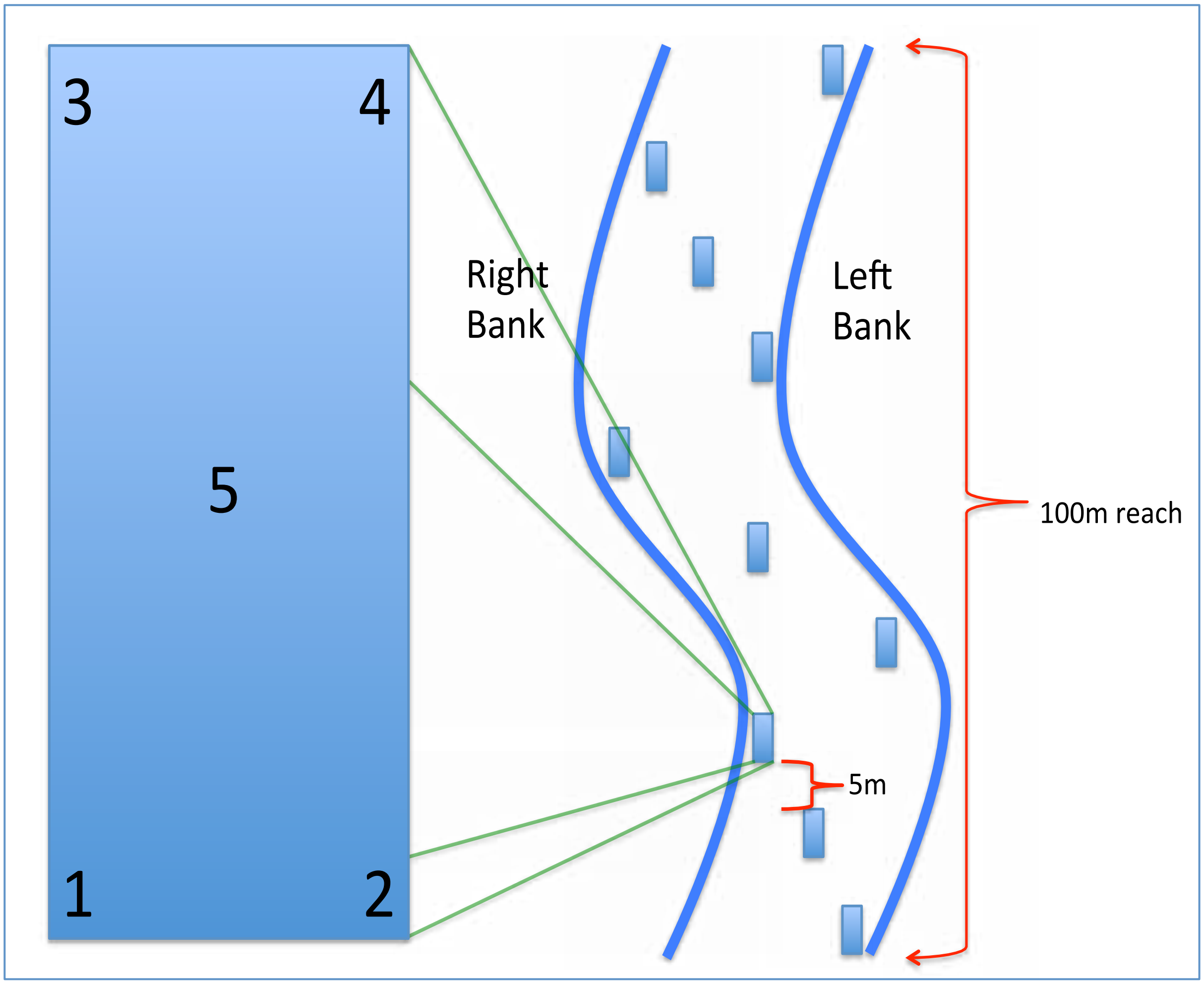

Figure 5. Diagram depicts microhabitat sampling locations for assessing Kentucky Arrow Darter habitat at the reach level.

Figure 6. Kentucky Arrow Darter antenna transceiver located on Elisha Creek (antenna E7), showing placement in a naturally constricted segment of the channel.

Figure 7. Ventral PIT-tagging procedure used on Kentucky Arrow Darters >50mm (TL); tags placed just anterior of the vent.

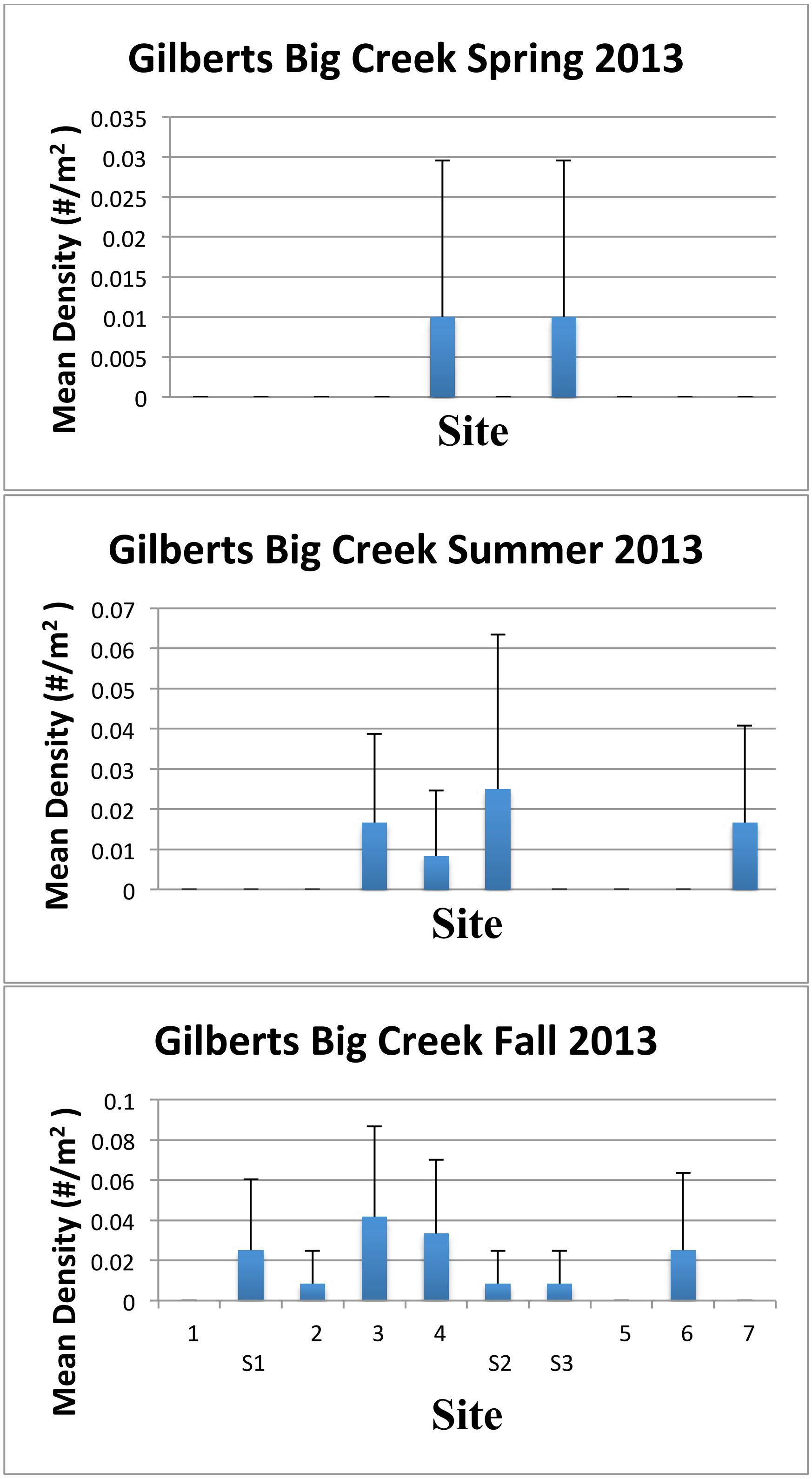

***Figure 8*.** Mean reach density (Kentucky Arrow Darters /m^2) for Gilberts Big Creek across seasons.

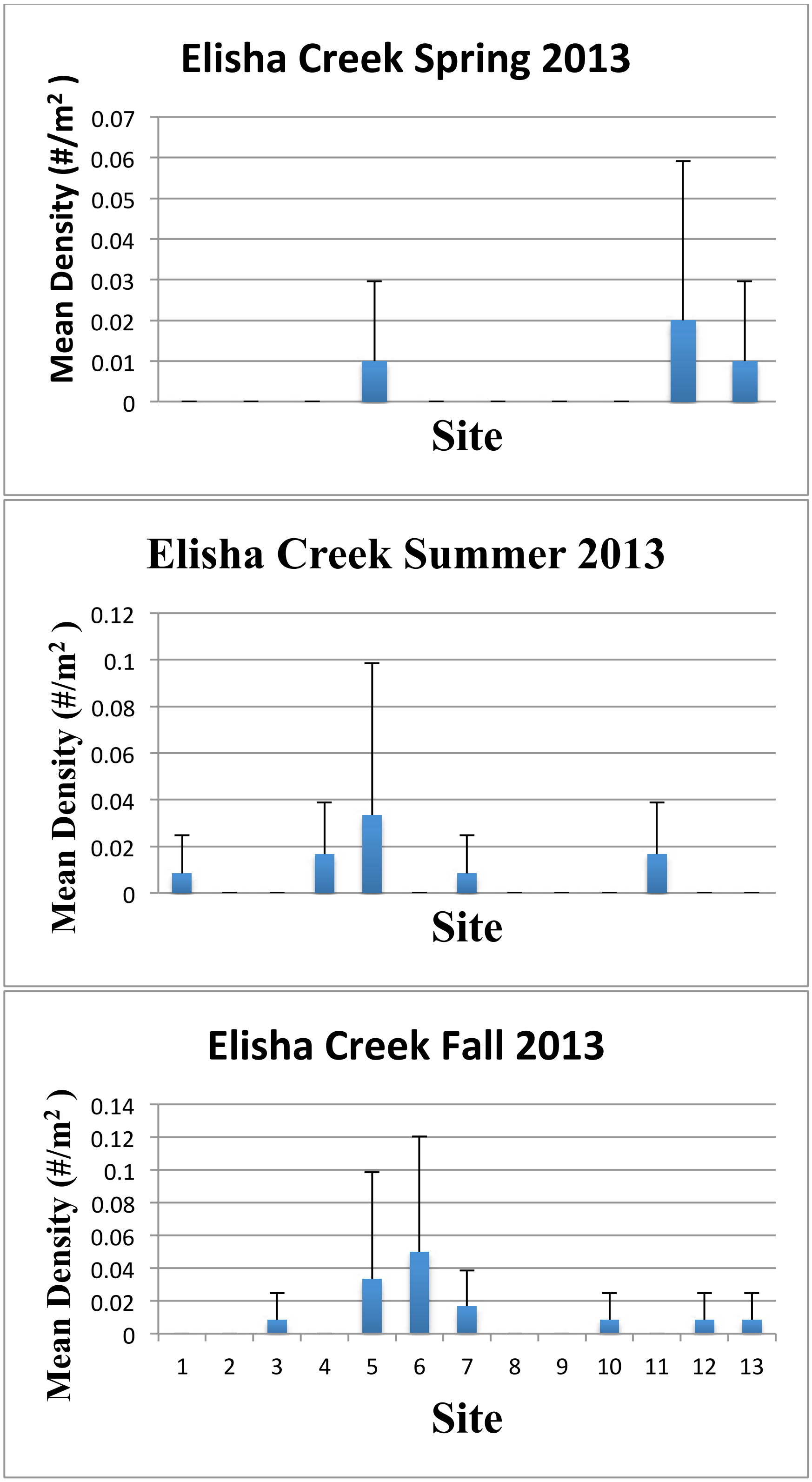

***Figure 9*.** Mean reach density of (Kentucky Arrow Darters /m2) for Elisha Creek across seasons.

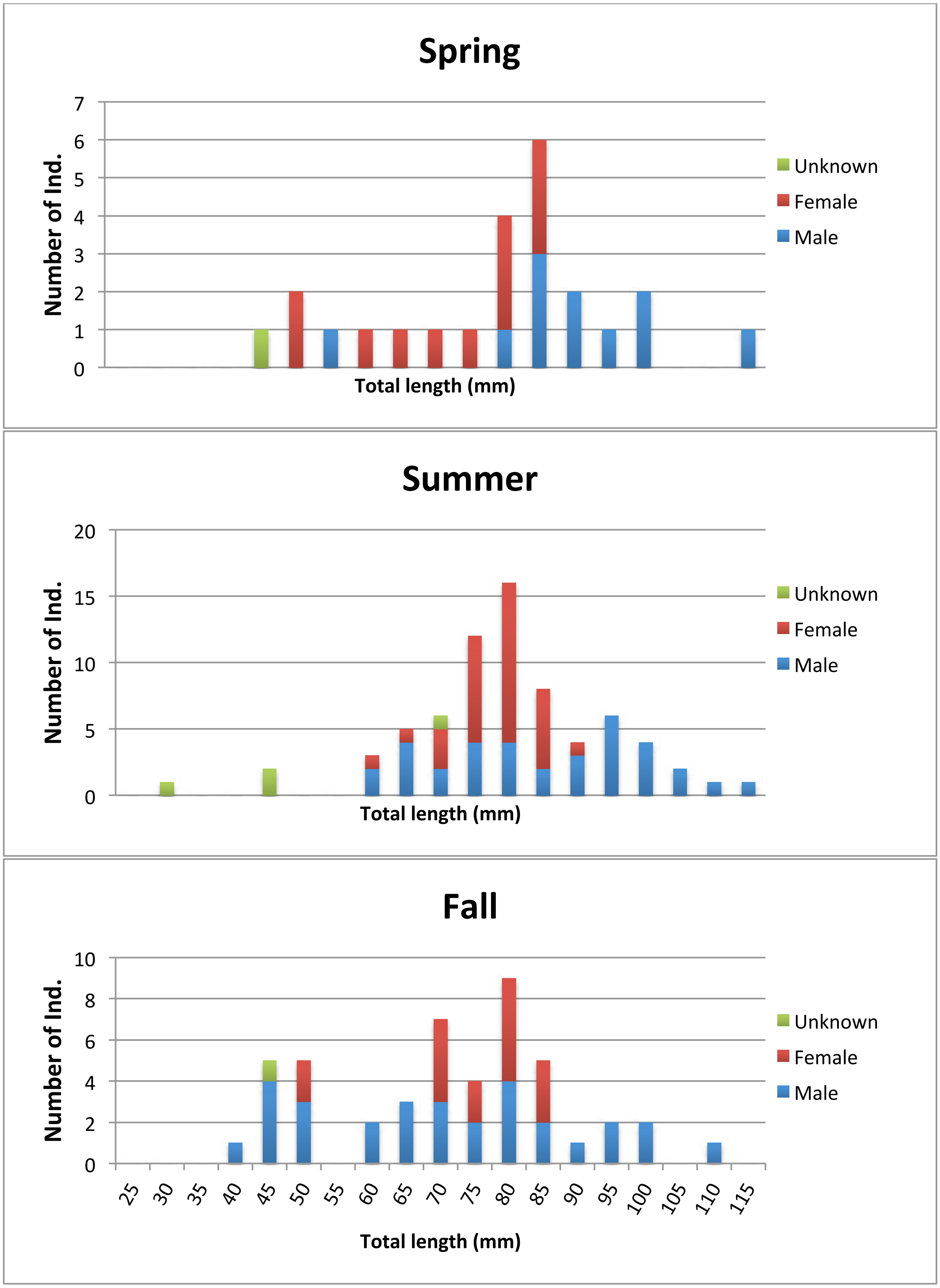

***Figure 10*.** Length frequency histogram of all Kentucky Arrow Darters (Male N=79, Female N= 60, Unknown N=6) captured at Gilberts and Elisha Creek across seasons during 2013.