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Population Size And Habitat Association Of Etheostoma Nebra, The Buck Darter, Using Abundance Modeling In The Buck Creek System, Cumberland River Drainage, Kentucky

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BY

HAROLD DAVIDSON BLACK

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ABSTRACT

Etheostoma nebra (Buck Darter) is a recently described fish species confined to the Buck Creek system, Cumberland River drainage, Kentucky. A 2010-2012 survey of Buck Creek by Kentucky Department of Fish and Wildlife Resources personnel observed *E. nebra* at 2 of 47 historical sites. Within the entire system, individuals were found only in Flat Lick Creek around the confluence of two spring-fed tributaries, Big Spring Branch and Stewart Branch. The objectives of this study were to: (1) determine population size, demographics, and habitat association of *E. nebra* in Big Spring Branch and Stewart Branch, (2) evaluate habitat conditions both within extant range and historical range, (3) complete fish surveys within the species' historical range, and (4) monitor and compare water quality at extant and historical localities within the system. Totals of 75 and 86 individuals of *E. nebra* were captured in Big Spring Branch and Stewart Branch, respectively; however, no individuals were observed in Buck Creek or other tributaries. In both extant populations, average total Length of males exceeded that of females (TL_{males} = 63.5 mm, $TL_{females}$ = 54.5 mm). Using N-Mixture models to predict abundance based on measured habitat variables and to account for imperfect detection, the total population in both streams was estimated to consist of approximately 17,000 individuals. Analyses of the two occupied streams yielded higher abundance estimates of *E. nebra* in Big Spring Branch. Important habitat variables associated with their abundance included a mixture of substrate size classes resulting in habitat complexity. Pebble and cobble showed positive relationships with predictions of increased *E. nebra* abundance. Comparison of occupied and unoccupied plots within Big Spring Branch and

Stewart Branch showed significant differences in most of the habitat variables (i.e. maximum depth, average depth, median substrate type, and presence of canopy). However, when unoccupied plots from historical streams were added and compared to occupied plots, few habitat variables showed significant differences. This indicated that unoccupied plots at historical streams had similar habitat conditions compared to occupied plots of Stewart and Big Spring Branch, and thus, habitat conditions were most likely not contributing to the disappearance of *E. nebra* at these streams. Average conductivity was higher in occupied streams than historical streams across seasons (Occupied: 342 µS/cm, Unoccupied: 146 µS/cm). Additionally, with more springs present, occupied streams exhibited warmer average winter temperatures and lower average summer temperatures than unoccupied streams (Range of monthly temperatures, Occupied: 11.6- 20.6 ℃, Unoccupied: 9.2- 23.9 ℃). Spawning activity of *E. nebra* was observed through July, and lower summer water temperatures could be a contributing factor to the species' persistence in these two streams. These results will aid cooperating natural resource agencies in making decisions toward management and conservation of this imperiled species.

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Chapter I: Introduction

The southeastern United States is home to some of the richest freshwater fish diversity in North America north of Mexico (Burr & Mayden, 1992). In a review by Warren et al. (2000), 28% of these fish taxa had their range restricted to one single drainage unit. Many species are realized to be imperiled or facing extinction by the time they are discovered or described (Warren et al., 2000). Ricciardi and Rasmussen (1999) noted that the projected extinction rates for freshwater fauna are five times that of terrestrial fauna. Darters are a species-rich group of fishes restricted to North America; represented by the subclade Etheostomatinae of the family Percidae (Near et al., 2011). Approximately one-quarter of the species in this group are imperiled to some degree (Warren et al., 2000).

Darters are benthic fishes, spending much of their time at or near the bottom of the water column, as many species lack a functional swim bladder. Sizes vary greatly across darter species, but the majority fall in the 5-7 cm range. Most are predaceous, feeding mainly on immature stages of aquatic insects and other invertebrates (Etnier & Starnes, 1993). Habitats are highly variable across darter species, and feeding ecology related to microhabitat contributes to how they partition themselves for co-occurrence, rather than prey selection (Carlson & Wainwright, 2010). Darters live within the same microhabitats as their prey and occupy a range of microhabitat types, contributing to such diverse speciation (Page, 1983).

Etheostoma nebra, the Buck Darter, is a recently described darter species within the clade *Oopareia* of the subgenus *Catonotus* (Near & Thomas, 2015). *Oopareia* is a clade of seven darter species commonly known as the barcheek darters, named for the iridescent bar on each cheek (Near et al., 2011). Currently, *E. nebra* is geographically confined to two tributaries of Flat Lick Creek in the Buck Creek system, Pulaski County, Kentucky; however, the species formerly occupied sites throughout the system in Lincoln, Pulaski, and Rockcastle counties. The species was originally thought to be a geographically isolated population of *E. virgatum*, the Striped Darter, until it was described as a separate species through morphological and genetic comparisons by Near and Thomas (2015).

There have been no life history studies conducted on *E. nebra*; however, Kornman (1980) studied the life history of *E. virgatum* in Clear Creek, a tributary within the Rockcastle River system, also part of the Cumberland River Drainage in Kentucky. Kornman (1980) noted that most individuals were found in shallow raceways and slack riffles generally less than 90 cm in depth, with individuals rarely being observed or taken in silty areas. Kornman (1980) observed upstream migrations and spawning as water temperatures increased to 12-15 ^{o}C. Spawning site selection included areas of washed sand and small gravel interspersed with flat rocks under which they could nest. These rocks were used as egg attachment sites, as described for other species in the subgenus *Catonotus* (Etnier & Starnes, 1993; Kornman, 1980).

Kopp (1985) studied the ecology and life history of *E. obeyense*, the Barcheek Darter, in Fishing Creek, another tributary to the Cumberland River Drainage in

Kentucky. *E. obeyense* is also included in the clade *Oopareia* and therefore may exhibit similar behaviors and habitat preferences to *E. nebra* (Near et al., 2011). Midge larvae (Family Chironomidae), mayfly nymphs (Order Ephemeroptera), and copepods (Order Copepoda) were the three most common taxa observed in gut analyses of *E. obeyense* (Kopp, 1985). Kopp (1985) noted that *E. obeyense* nests were almost always in a pool or run; however, they were found in depths ranging from 15-70 cm. Males were observed exhibiting territorial behavior when guarding nests, including chasing of potential egg predators (Kopp, 1985). Kopp (1985) collected *E. obeyense* from a variety of habitat types but noted that the majority came from habitats that provided places of refuge (e.g., flat rocks).

Historical records of *E. nebra* are known from throughout the Buck Creek system in Lincoln, Pulaski, and Rockcastle counties, including 22 of 39 sites (56%) visited in 1985 by Cicerello and Butler (1985). These sites included 7 major tributaries of Buck Creek and the Buck Creek mainstem (Cicerello & Butler, 1985). Thomas and Brandt (2013) observed *E. nebra* at only 2 of 47 sites (4%) during a comprehensive survey (2010-2012) of the Buck Creek system. *E. nebra* was observed in both Big Spring Branch and Stewart Branch, two spring-fed first-order tributaries of Flat Lick Creek, a second order tributary to Buck Creek (Near &Thomas 2015). In Flat Lick Creek, *E. nebra* was observed only near the confluence of both tributaries (Thomas & Brandt 2013).

Based on decreasing numbers of collection location records, declines in *E. nebra* populations appear to have begun in the 1980s (Near & Thomas, 2015). The direct cause of the species' decline is unknown; therefore, more information concerning the habitat requirements and population status of *E. nebra* is needed to help with management decisions. The goal of this study was to delineate factors that may be contributing to the decline of *E. nebra* in the Buck Creek system. Study objectives included the following: to (1) determine population size, demographics, and habitat associations of *E. nebra* in Big Spring Branch and Stewart, (2) evaluate habitat conditions both within extant range and historical range, (3) complete fish surveys within the species' historical range, and (4) monitor and compare water quality at extant and historical localities within the system.

Chapter II: Methods

Study Area

Buck Creek is a fifth order tributary to the Cumberland River in southeastern Kentucky with a watershed area of 767 km^2 (Figure 1). The watershed is split between two ecoregions; the upper portion falls within the Eastern Highland Rim subsection of the Interior Plateau Ecoregion and the lower section falls within the Plateau Escarpment subsection of the Southwestern Appalachians Ecoregion (Woods et al. 2002). This division occurs approximately where KY Highway 80 intersects the Flat Lick Creek system. The Eastern Highland Rim has more level terrain and is underlain by Mississippian limestone. The streams are characterized by moderate gradient and substrates are composed of a mixture of cobble, gravel, and bedrock. The Plateau Escarpment is lithologically different from the Eastern Highland Rim. It is underlain primarily by Pennsylvanian sandstones and coal, and the topography includes cliffs and deep valleys. The streams are characterized by higher gradients and boulder and bedrock substrates (Woods et al., 2002).

Big Spring Branch and Stewart Branch, tributaries within the Flat Lick Creek system; are cool, clear, headwater streams consisting mainly of shallow (<0.5m) pools and runs, and coarse substrates such as slab rocks (Near & Thomas, 2015). Relative to the rest of the Buck Creek system, Big Spring Branch and Stewart Branch have high numbers of springs within their watershed, receiving year-round groundwater

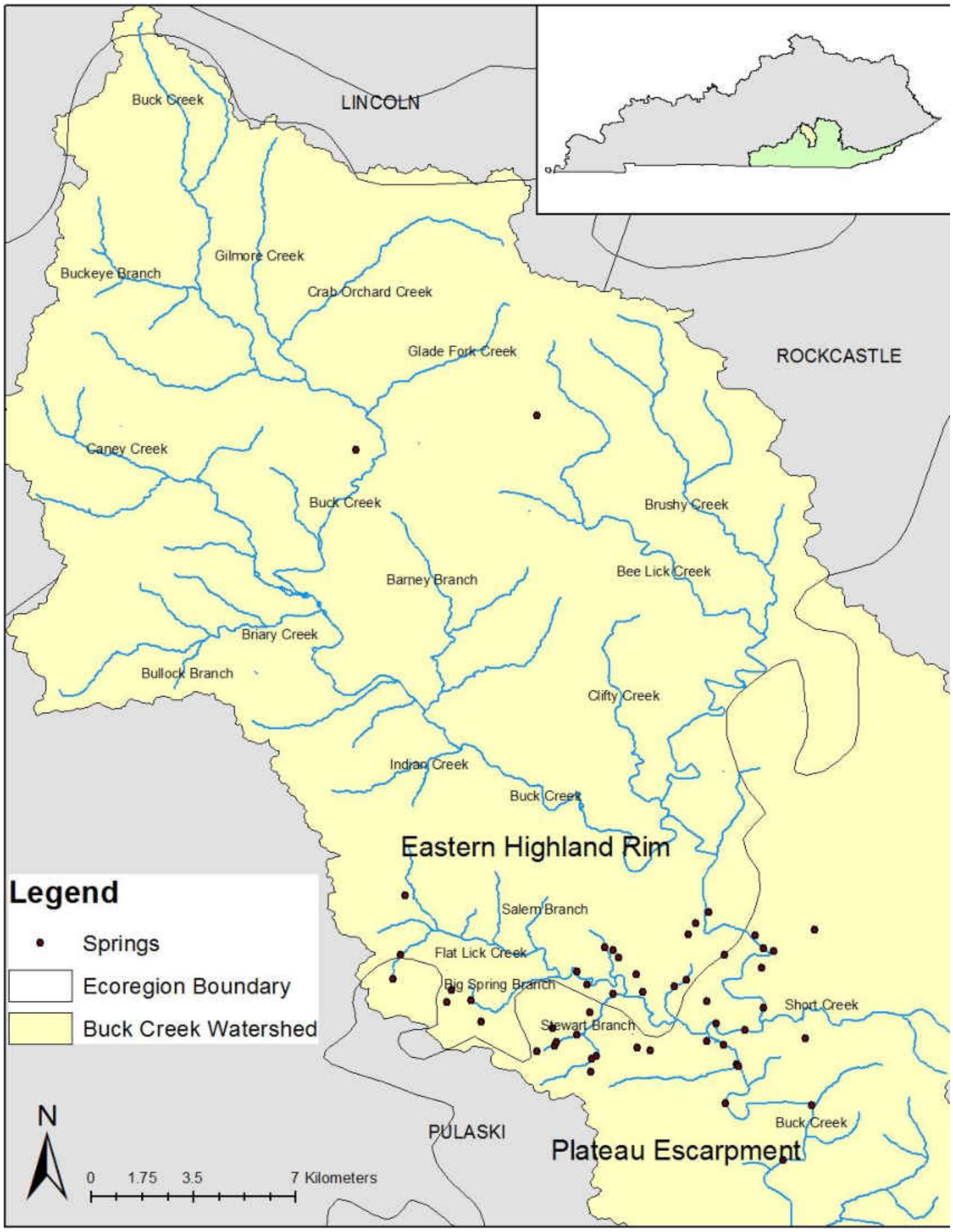

Figure 1. Map of the Buck Creek system in Pulaski, Lincoln, and Rockcastle counties of Kentucky; showing watershed area, ecoregion boundaries, and springs.

influence. Predominant land use in the surrounding area is agriculture, with small blocks of forested riparian areas found on some stream sections (Cicerello & Butler, 1985).

Study Design

The study design used in quantitative surveys of *E. nebra* in Stewart Branch, Big Spring Branch, and historical sites was modeled after methods outlined by Compton and Taylor (2013). Google Earth (https://www.google.com/earth/) was used to measure the length of Stewart Branch and Big Spring Branch from each stream's confluence with Flat Lick Creek upstream to the headwaters. Stewart Branch totaled 2.99 km (Twenty-five possible 120-m reaches), and Big Spring Branch totaled 3.41 km (Twenty-eight possible 120-m reaches) in length. Six randomly selected 120-m reaches were sampled in each stream during summer 2017 (Figure 2, Appendix A), thus, 24% of Stewart Branch and 21% of Big Spring Branch were sampled. At each 120-m reach, 12 randomly chosen microhabitat plots (5-m long X 2-m wide) were sampled with a 5-m buffer between each plot. Microhabitat plot placement within the survey reach was chosen prior to sampling (1 =left bank, 2 = center stream, 3 = right bank) using a random number generator. If stream width was 2-m or less, the entire width of the stream was sampled.

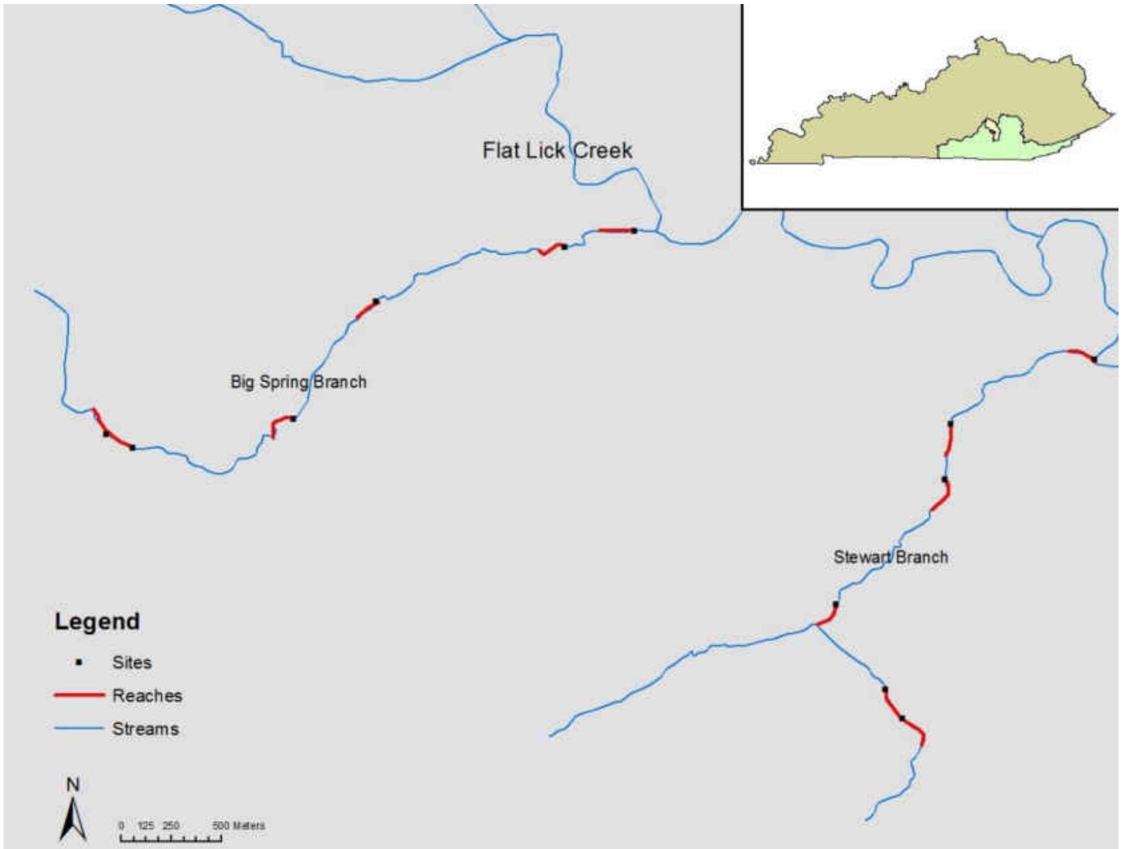

Figure 2. Map of Big Spring Branch and Stewart Branch within the Flat Lick Creek system, showing 2017 survey sites and reaches.

Physicochemical, Habitat, and Fish Sampling: Big Spring Branch and Stewart Branch

Surveys took place in late July 2017and continued into September 2017 to avoid the *E. nebra* spawning season which unexpectedly extended into July. Prior to sampling fish and measuring microhabitat within plots, water quality data was recorded at the downstream end of each reach using a YSI multi-probe (Yellow Spring Instruments, Yellow Springs, OH). Parameters included conductivity (µS/cm), water temperature (°C), and pH. Fish and microhabitat data were collected using a modified sampling technique described by Compton and Taylor (2013). Fishes were collected from each microhabitat plot using a backpack electro-shocker (Smith-Root, Vancouver, WA) and a dip net. All captured fishes were placed in an aerated bucket, identified to species, counted, and released. All *E. nebra* were measured (total length in mm) and weighed (g). Start and end points of each sampling reach were documented using a handheld GPS unit (Garmin USA). This project was reviewed and approved by Eastern Kentucky University's Institutional Animal Care and Use Committee as Protocol 10-2016.

Water depth (m) and substrate size (Wentworth Scale) were measured at each corner and the center of each plot. Substrate categories included fines/sediment, <0.06 mm; sand, 0.06–2 mm; gravel, 2–15 mm; pebble, 16–63 mm; cobble, 64–256 mm; boulder, >256 mm; and bedrock (Bain & Stevenson, 1999). Other plot measurements included flow velocity (m/s) presence of large woody debris (>10-cm in diameter and >1-m in length), maximum water depth, and dominant substrate particle (m). Flow velocity was measured with a Marsh McBirney Flo-Mate 2000 (Hach Company, Loveland, CO). At each microhabitat plot, other measurements included channel unit (riffle, run, or pool), wetted stream width, and canopy cover. Canopy cover was measured as present or absent from a single point in the center of each plot using a GRS Densitometer (Geographic Resource Solutions, Arcata, CA).

Physiochemical, Habitat, and Fish Sampling: Buck Creek System

In summer 2017, fishes were sampled quantitatively in seven tributaries located within the Buck Creek system and one Stewart Branch site not included as part of the population estimate. Biological and physiochemical field methods followed the same procedures outlined for Big Spring Branch and Stewart Branch. Beginning in fall of 2016 an additional 18 sites throughout the Buck Creek system were sampled qualitatively to search for unknown populations of the Buck Darter and to survey fish at historical collection locations (Figure 3, Appendix B). Depending on stream size, qualitative searches were completed in reaches ranging in size from 100-300-m. All available habitat types were sampled in each reach, and all captured fishes were identified. A species list was developed for each site.

Monitoring of Seasonal Stream Temperature and Conductivity

In early spring 2017, temperature loggers (Onset HOBO Data loggers, Cape Cod, MA) were placed at each of three locations in Stewart Branch and Big Spring Branch (upper, middle, and lower); a single logger was placed at each of nine randomly selected historical sites where *E. nebra* are now presumed to be absent. Temperature loggers provided water temperature data at 20-minute intervals across the seasons. In summer 2017, conductivity loggers were placed in Big Spring Branch, Stewart Branch, and two randomly selected historical localities: Bee Lick Creek, and Gilmore Creek. Conductivity measurements (µS/cm) were recorded at 20-minute intervals. Onset software (HOBOware Pro 3.3.1) was used to download the data on 45-day intervals. Location information for all the loggers is provided in Appendix C and positions within the Buck Creek system are shown on a map (Figure4).

Figure 3. Map showing the sites sampled in the 2016-2017 survey efforts of historical collection locations and other localities within the Buck Creek system, Kentucky.

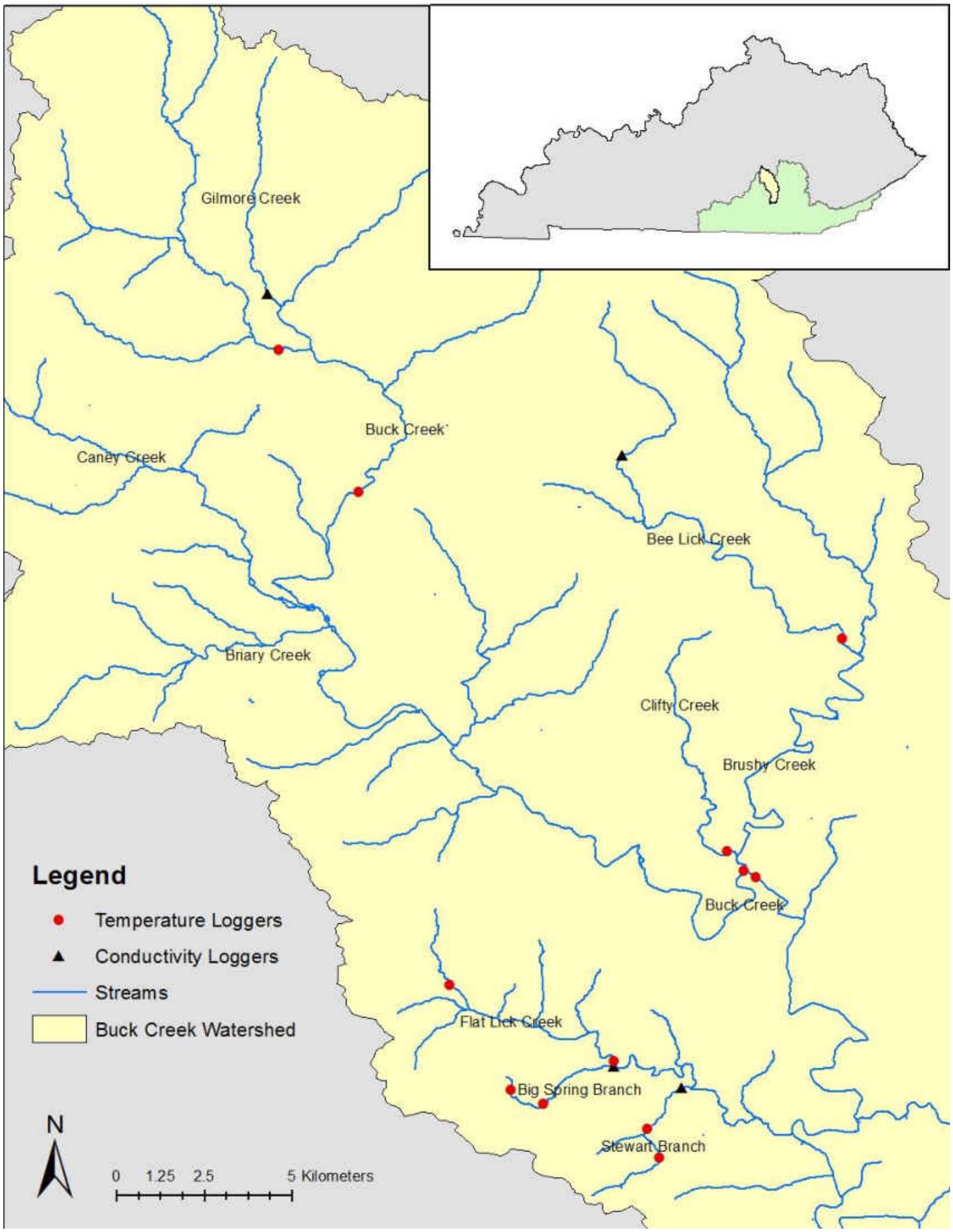

Figure 4. Map of temperature and conductivity data logger positions within the Buck Creek system, Kentucky.

Data Analysis: Population Structure

A length frequency histogram was created for all *E. nebra* captured in this study that could be sexed (based on coloration and morphology), with males and females plotted separately. A log regression of *E. nebra* lengths and weights was calculated and plotted for each stream, as the relationship between fish length and weight can be estimated on the logarithmic scale (Hubert & Quist, 2010). The average body size of each sex was calculated for each stream and then compared within each stream using a Tukey's honest significant difference (HSD) test. Analysis was completed within the R, and RStudio statistical programming environment (R Core Team, 2017; RStudio Team, 2016; Wickham, 2009).

Data Analysis: N-Mixture Modeling Population Estimate

N-Mixture models were used to estimate the population size of *E. nebra* (N), taking into account imperfect detection, the assumption that not all individuals available for capture were captured (Royle, 2004; Royle, Nichols, & Kery, 2005). Using the observed *E. nebra* abundances (n = number of individuals captured in each plot) and the measured habitat parameters as covariates, a set of candidate N-Mixture models were fitted to the data (Royle, 2004). All modeling was completed within R and the RStudio statistical programming environment (R Core Team, 2017; RStudio Team, 2016) using packages 'unmarked' (Fiske & Chandler, 2011) and 'AICcmodavg' (Mazerolle, 2017). All possible candidate models were developed from the covariates that were identified as having a true effect on the estimation parameters, defined by having a fully negative or fully positive confidence interval (i.e., CI did not intercept zero). The candidate models were then ranked using AICc (Mazerolle, 2006).

Coefficients were then averaged, and abundance predictions were calculated using the "predict()" function in package 'unmarked' to simultaneously model average and back-transform the estimates (Fiske & Chandler, 2011). This calculation produced an abundance estimate at the plot level (10-m^2) for each reach. Modifying methods outlined by Meyer et al. (2006) to account for stream area in addition to linear stream length, reach area was then determined by multiplying average stream width within the reach by total reach length. An extrapolation coefficient was calculated by dividing total reach area by the total area of microhabitat plots sampled within the reach. The estimated abundance in microhabitat plots (n/120-m^2) was then multiplied by the extrapolation coefficient (reach area/sample area) to estimate *E. nebra* population size per reach. The *E. nebra* population size in each stream (N) was then estimated by multiplying the mean estimate of all reaches in each stream by the total number of potential 120-m reaches (i.e., 25 reaches in Stewart Branch and 28 reaches in Big Spring Branch). Density was estimated by dividing the population estimate for each stream by the calculated total area of the stream (total stream length X average stream width).

Data Analysis: Habitat Association

By averaging all models containing a specific parameter, the overall effect (β, regression coefficient) of that parameter on the state function (λ, abundance) was estimated along with a confidence interval. This effect was estimated for each habitat variable that was assessed when selecting candidate models. These effect sizes were then ranked and plotted. Habitat variables that had a fully positive or negative β (i.e., a 95% confidence interval that did not intercept zero) were predictors of *E. nebra* abundance. Predicted *E. nebra* densities in relation to observed values of habitat covariates that showed a significant β-value were then plotted over a Kernel Density Estimation (KDE) using the "stat_density2d()" function in the package 'ggplot2' and a raster fill based on the density function (Wickham, 2009). KDE allows inferences of the relative likelihood for a drawn random variable along the axes to fall within any given interval when compared to another interval and can provide visual indication of data distribution (Silverman, 1986). Habitat variables collected at the plot level were compared between occupied and unoccupied plots, both within occupied streams and again including all quantitative sites using a Kruskal-Wallis test. Violin plots, which resemble box plots but instead show the kernel probability density for the data at different values, were created to visualize differences in median values for the occupied and unoccupied plots. The observed values were plotted as points within the violin plots and jittered to reduce overplotting. Analyses and plots were conducted within the R, and RStudio statistical programming environment (R Core Team, 2017; RStudio Team, 2016; Wickham, 2009).

Data Analysis: Temperature and Conductivity Data

The data retrieved from the HOBO loggers was compiled into one long data series for each logger location and paired with the dates and times of the logged measurements. Temperature and conductivity readings were then grouped by dates, so they could be averaged. Because there were so many data points and logger locations, temperatures are reported as monthly averages so that a seasonal trend could be plotted over the course of a year. Also, during the *E. nebra* spawning season, average weekly temperatures were calculated. Conductivity measurements were averaged to provide daily means, as there were fewer data points and fewer locations to be plotted. Averaged data sets were then plotted to show trends in the temperature and conductivity regimes of the different streams in the Buck Creek System.

Chapter III: Results

Etheostoma nebra were captured at 9 of 12 total reaches within Big Spring Branch and Stewart Branch, with a total number of 161 individuals ($n_{Big\ Spring\ Branch}$ = 75, $n_{Stewart\ Branch}$ = 86). Reach 11 of Big Spring Branch had the maximum number of individuals captured (n = 37), also the maximum number of individuals captured.in a plot (n = 9) which calculated to 3.08 individuals per plot. The average number captured per plot across all reaches was 1.34. A perched culvert was discovered on Big Spring Branch, and no *E. nebra* were observed upstream of it. Therefore, the two uppermost reaches sampled in Big Spring Branch were not considered in the population analysis. One site in Stewart Branch (Reach 1), immediately above the confluence with Flat Lick Creek, did not have any *E. nebra* captures.

Population Structure

Both sexes were well represented during surveys on Big Spring Branch and Steward Branch (n_{males} = 84, $n_{females}$ = 45, $n_{unknown}$ = 32). A length frequency analysis of captured individuals did not reveal any strong divisions for age class cohorts (Figure 5). Strong positive relationships existed between total length and weight of *E. nebra* in both streams (Figure 6). Tukey's HSD comparisons of mean total length and weight indicated that on average males grow larger than females (Figure 7).

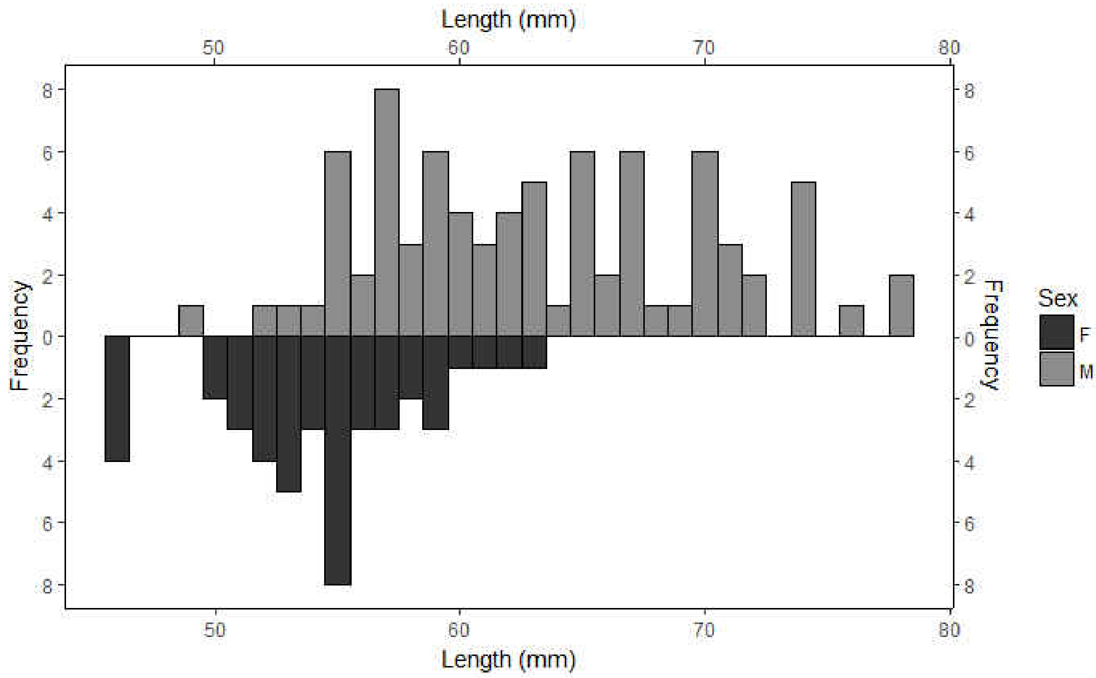

Figure 5. Length frequency histogram of female and male *Etheostoma nebra* captured in 2017 from Big Spring Branch and Stewart Branch, Buck Creek system, Kentucky.

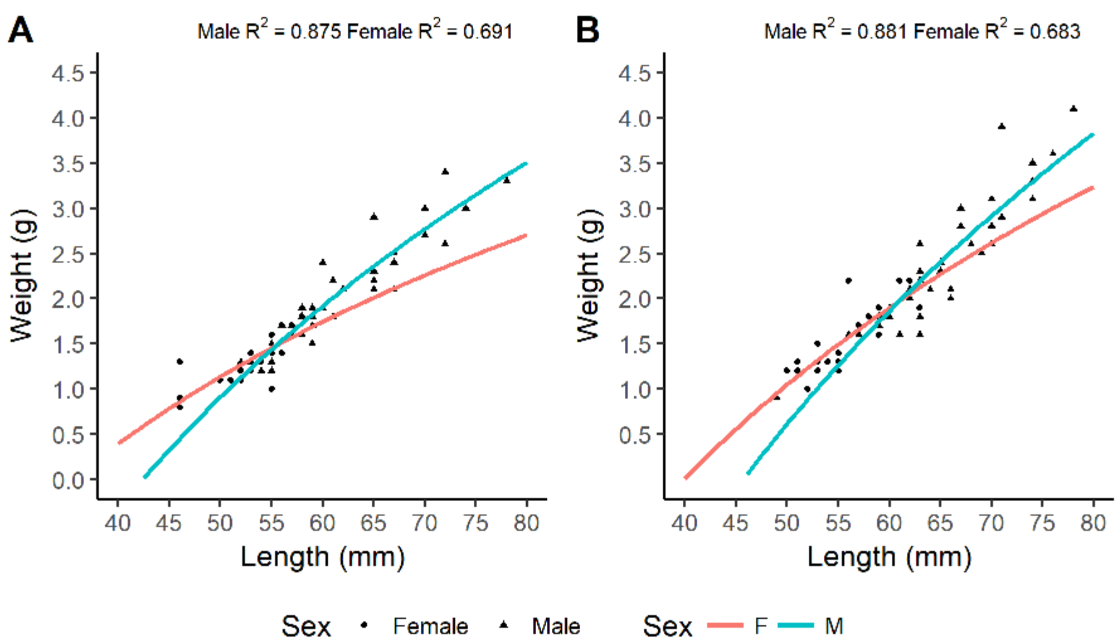

Figure 6. Length-weight relationships of male and female *Etheostoma nebra* in Big Spring Branch (A), and Stewart Branch (B), Buck Creek system, Kentucky.

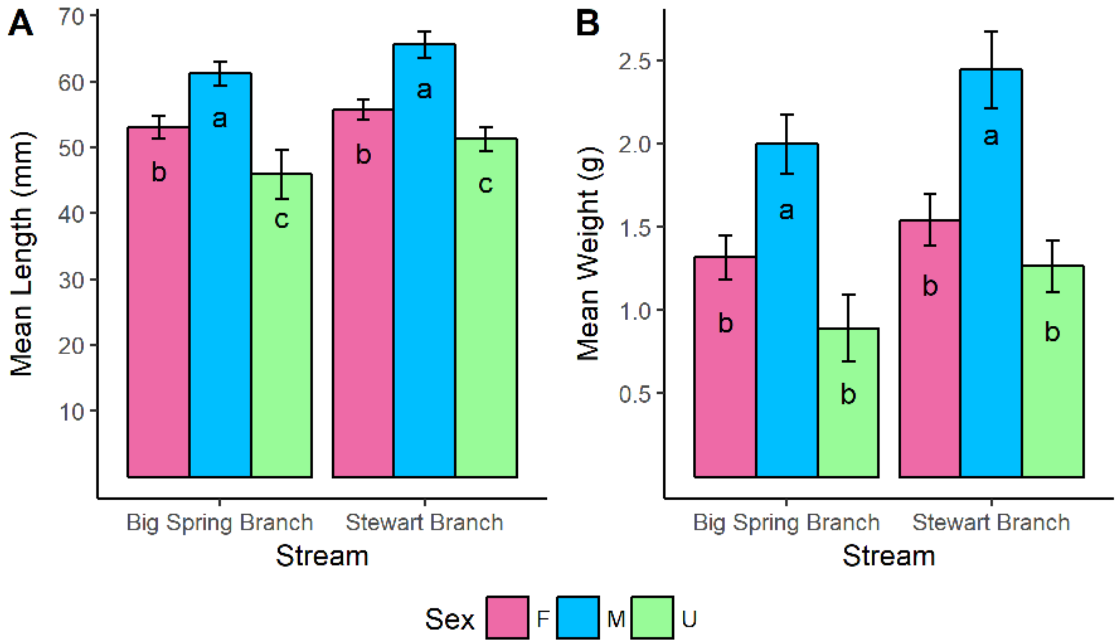

Figure 7. Comparisons of Mean Length (A) and Mean Weight (B) of *Etheostoma nebra* in Big Spring Branch and Stewart Branch, Buck Creek system, Kentucky. Error bars represent 1 standard deviation, within each stream shared letters signify no difference based on Tukey's HSD groupings.

Population Estimates

The parameterized candidate models used in this study and their AICc rankings can be found in Appendix D. Model averaged estimates of the *E. nebra* abundances are reported including an estimated 95% confidence interval and standard error, calculated as part of the maximum likelihood estimation prediction process within the predict function of the 'unmarked' package (Fiske & Chandler, 2011). Extrapolated to the stream level, the estimates were $N_{\text{Big Spring Branch}}$ = 10,391 (95% CI: 6,010-18,149 SE: 3,033) and $N_{\text{Stewart Branch}}$ = 6,792 (95% CI: 4,143-11,206 SE: 1,806). Using modeled abundance estimates in Big Spring Branch and Stewart Branch, density estimates for *E. nebra* were 1.73/m² and 0.98/m², respectively. Using the maximum observed number of *E. nebra* per plot of 9, the maximum observed density was 0.9/m². Detection probabilities (the probability of any given individual to be captured) were 12% (95% CI: 8-18%) and 11% (95% CI: 7-16%) in Big Spring Branch and Stewart Branch, respectively.

Habitat Association

The effect on the variation in predicted abundances of each covariate in the modeling process can be used show relative effect of covariates on the predicted value (Royle, 2004). The regression coefficient of each covariate was estimated and are reported as effect sizes (β, Beta value, Figure 8). The substrate proportions are the number of each classified substrate type out of the total number of substrate classifications within each reach. Areas with higher proportions of gravel (2-15 mm), pebble (16-63 mm), and cobble (64-256 mm) were predicted to have higher *E. nebra* abundance. Large woody debris was also predicted to have a strong positive effect on

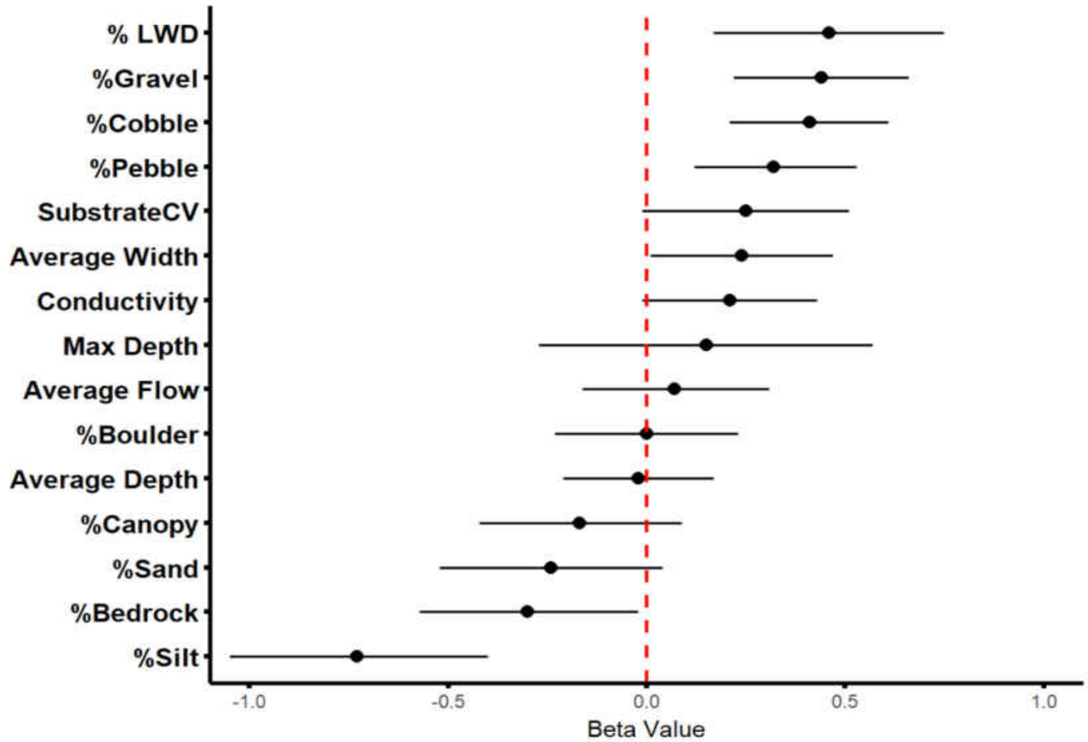

Figure 8. Effect sizes (Beta, with line representing 95% CI) of covariates used in candidate model creation for N-mixture modeling of *Etheostoma nebra* in the Buck Creek system, Kentucky. A red line denoting zero was added to better show which intervals intercepted zero. Abbreviations used are as follows: LWD = large woody debris and Substrate CV = substrate coefficient of variation.

E. nebra abundance. Although large woody debris was scarce in most of the sample locations, it likely contributed to the complexity of the habitat when present. Based on their β value, higher proportions of bedrock and silt were shown to have a negative effect on *E. nebra* abundance.

When plotted over a Kernel Density Estimation layer, the trends of several of these habitat variables to predicted *E. nebra* abundance became more apparent. Proportions of cobble, pebble, and gravel all appeared to show positive trends (Figure 9A-C), while bedrock showed a negative trend (Figure 9D). Proportion of silt was low across a wide range of predicted *E. nebra* abundances, but predicted abundance was relatively low at higher silt values (Figure 9E). Large woody debris was absent or at low percentages at most sites, giving it a wide range of predicted abundance values when low. However, with an increase in woody debris there generally was an increase in predicted *E. nebra* abundance (Figure 9F).

Habitat and water quality parameter measurements at quantitative sites in the Buck Creek system did not exhibit major differences between occupied sites and historical sites (Table 1). Conductivity was the exception; Big Spring Branch, Stewart Branch, and Flat Lick Creek did have higher in-situ conductivity measurements. However, when plot-level habitat measurements were compared between occupied and unoccupied plots within Big Spring Branch and Stewart Branch ($n_{occupied} = 66$, $n_{unoccupied} = 66$) using a Kruskal Wallis test, almost all habitat variables were statistically different between the two (Table 2). The only non-significant habitat variables were channel unit ($X^2 = 2.984$, $p = 0.084$), and wetted width ($X^2 = 0.277$, $p = 0.599$).

Runs were the most common channel unit of both occupied and unoccupied plots. Plots that were occupied by *E. nebra* appeared to be shallower than unoccupied plots overall; the range of maximum depth for occupied plots stayed < 50-cm (Figure 10A). This was also true for the average depth within the plot, where, again, occupied plots were shallower (Figure 10B). The median substrate size classification within the plots tended to be larger (pebble) in occupied plots, with smaller substrate (more sand and gravel) in unoccupied plots (Figure 10C). Canopy presence was found to be absent more often in occupied plots than unoccupied plots (Figure 10D).

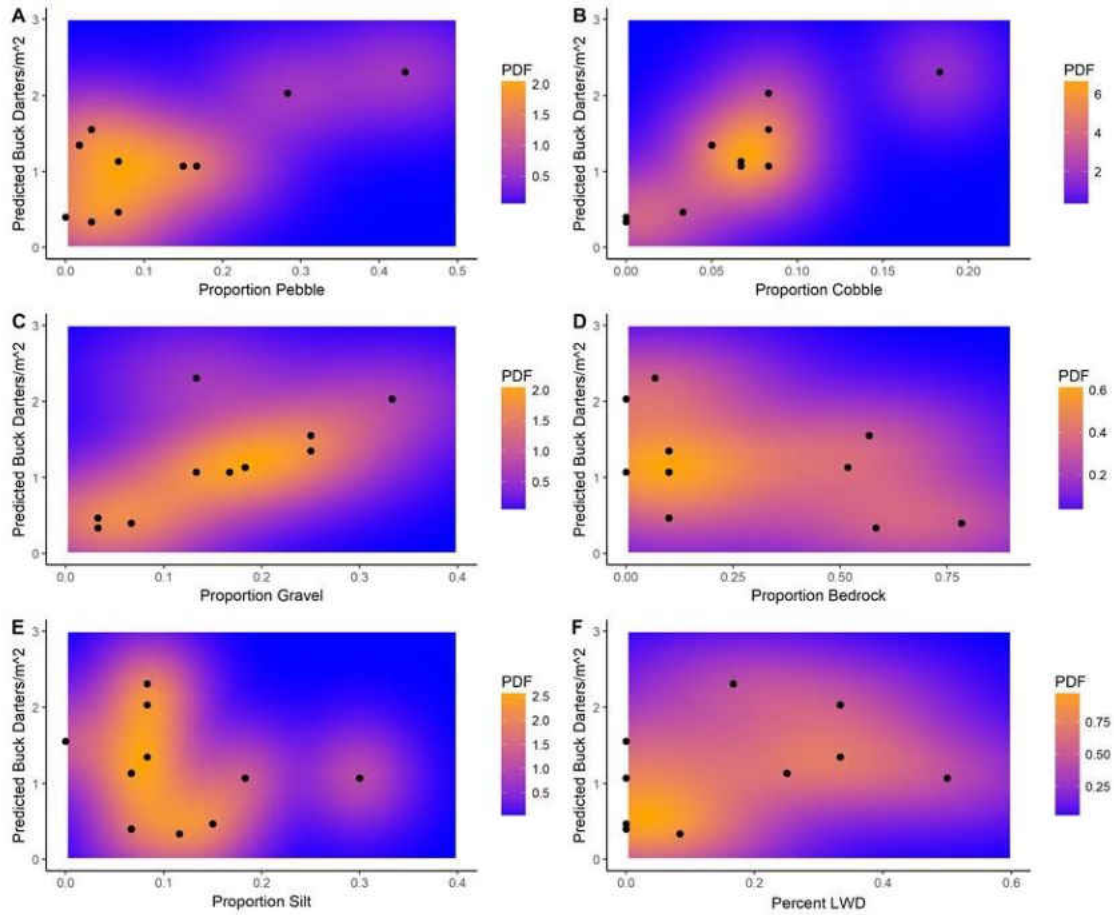

Figure 9. Relationship between habitat parameters (A: Pebble, B: Cobble, C: Gravel, D: Bedrock, E: Silt, and F: Large Woody Debris) and predicted *Etheostoma nebra* (Buck Darter) abundance (n/m²), shown as points plotted over a Kernel Density Estimation to allow inferences of relative likelihood using the probability density function (PDF).

Table 1. Summary of selected habitat variables measured at reach level or if measured at plot level averaged across all twelve plots within quantitative sites during the 2017 study in the Buck Creek system, Kentucky.

Stream	Reference Code	Date Visited	Water Temperature (°C)	Conductivity (μS/cm)	Average Depth (cm)	Average Width (m)	Average Flow (m/s)	Percent Canopy Cover
Big Spring Branch	BSB 2	9/9/2017	14.3	345.1	19.33	4.07	0.34	91.67
Big Spring Branch	BSB 6	9/9/2017	15.4	350.8	14.52	3.09	0.53	66.67
Big Spring Branch	BSB 11	8/28/2017	18.6	365.8	16.73	2.91	0.38	0.00
Big Spring Branch	BSB 16	8/28/2017	17.1	376.9	10.16	3.07	0.50	25.00
Stewart Branch	STB 1	7/10/2017	18.7	335.4	26.64	4.06	0.23	67.00
Stewart Branch	STB 7	7/24/2017	21.4	341.0	11.17	4.40	0.14	92.00
Stewart Branch	STB 9	7/24/2017	23.8	373.6	11.12	5.04	0.23	50.00
Stewart Branch	STB 16	7/25/2017	26.1	418.2	24.02	4.36	0.11	0.00
Stewart Branch	STB 19	7/25/2017	21.8	348.9	16.78	2.62	0.09	42.00
Stewart Branch	STB 20	7/25/2017	23.2	347.0	14.44	2.53	0.13	25.00
Buckeye Branch	BKB 1	6/12/2017	24.3	199.0	6.15	2.62	0.53	75.00
Gilmore Creek	GIC 2*	6/13/2017	20.3	131.5	20.45	6.50	0.17	83.33
Flat Lick Creek	FLC 2	6/14/2017	20.0	282.1	13.49	3.64	0.39	83.33
Clifty Creek	CLC 2	6/20/2017	23.2	218.1	17.10	3.77	0.13	66.67
Bee Lick Creek	BLC 2*	7/20/2017	24.0	221.2	17.50	6.79	0.52	83.33
Caney Creek	CAC 1	6/28/2017	23.3	252.4	12.45	6.58	0.25	100.00
Barney Branch	BAB 1	7/7/2017	25.0	171.5	25.16	2.03	0.66	0.00

* Denotes a historical (pre-1985) Etheostoma nebra (Buck Darter) collection location. Streams are abbreviated as follows: Barney Branch (BAB), Buckeye Branch (BKB), Bee Lick Creek (BLC), Big Spring Branch (BSB), Flat Lick Creek (FLC), Gilmore Creek (GIC), and Stewart Branch (STB).

Table 2. Summary of Kruskal Wallis test results comparing habitat variables between plots occupied and unoccupied by *Etheostoma nebra* (Buck Darter) in Big Spring Branch and Stewart Branch during the 2017 study in the Buck Creek System, Kentucky.

Habitat Variable	X^2	p
Average Depth	4.9536	0.0260
Average Flow	4.4547	0.0348
Maximum Depth	4.2819	0.0385
Median Substrate	4.2326	0.0396
Largest Substrate Type	10.463	0.0012
Large Woody Debris	3.9667	0.0464
Channel unit	2.9838	0.0841
Wetted Width	0.2766	0.5989
Canopy Cover	4.3345	0.0373

Results are based on a Kruskal Wallis test comparing occupied (n = 66) and unoccupied (n = 66) plots

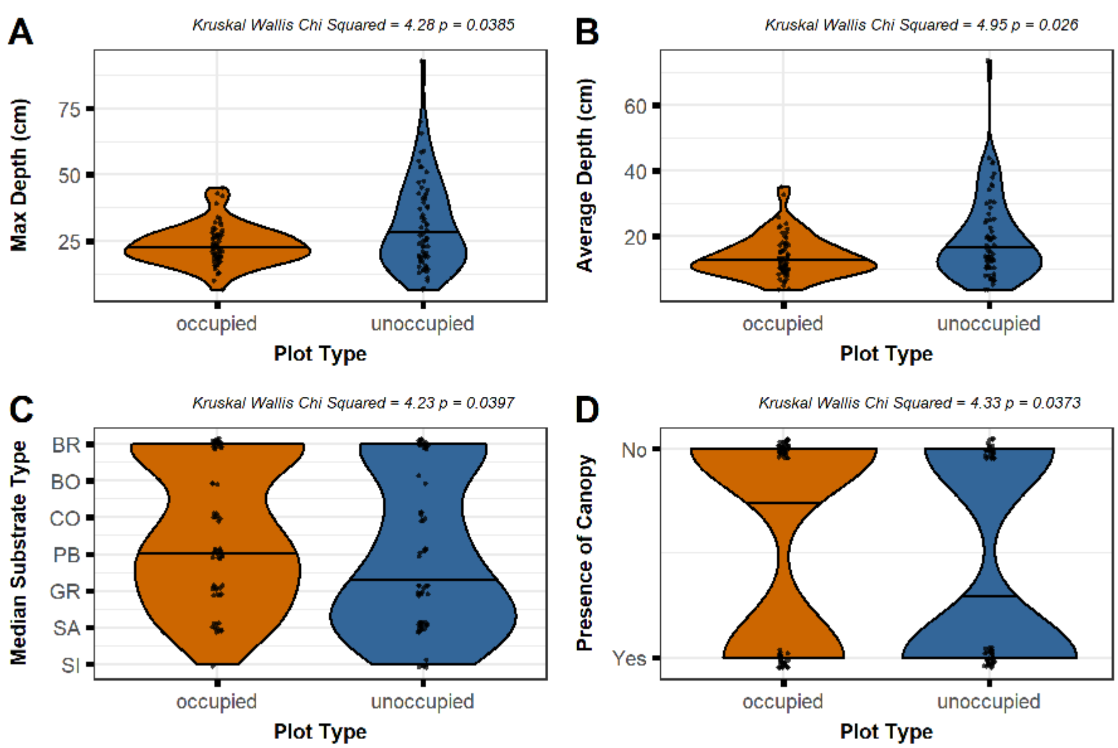

Figure 10. Comparison of habitat parameters (A: Maximum Water Depth, B: Average Depth, C: Median Substrate Type, and D: Presence of Canopy) between plots occupied and unoccupied by *Etheostoma nebra* within Big Spring Branch and Stewart Branch, sampled during the 2017 study in the Buck Creek system, Kentucky. Substrates are abbreviated as follows: SI = silt, SA = sand, GR = gravel, PB = pebble, CO = cobble, BO = boulder, BR = bedrock.

Comparison of plot-level habitat measurements between occupied and unoccupied plots, with the historical sites included ($n_{occupied} = 66$, $n_{unoccupied} = 162$), revealed less significant differences in habitat between occupied and unoccupied plots (Table 3). This indicates that the available habitat in presently occupied streams and historical streams is not that different. In the analysis that included plots in historical streams the largest substrate present was more often of a smaller substrate size class in unoccupied plots than occupied plots (Figure 11A). Pools occurred at a higher frequency in unoccupied plots than occupied plots. (Figure 11B). Canopy continued to appear to be absent more often in unoccupied plots than occupied plots (Figure 11C). Wetted width at the plot was larger in unoccupied plots than in occupied plots (Figure 11D). However, this is likely a result of some of the historical streams being larger than the presently occupied streams.

Table 3. Summary of Kruskal Wallis test results comparing habitat variables between plots occupied and unoccupied by *Etheostoma nebra* (Buck Darter) in in all sites sampled quantitatively during the 2017 study in the Buck Creek System, Kentucky.

Habitat Variable	X^2	p
Average Depth	2.2431	0.134
Average Flow	1.6668	0.196
Maximum Depth	3.0876	0.078
Largest Substrate Type	14.050	<0.001
Large Woody Debris	0.2807	0.596
Channel unit	4.3072	0.038
Wetted Width	11.463	<0.001
Canopy Cover	11.720	<0.001
Median Substrate	0.1811	0.670

Results are based on a Kruskal-Wallis test comparing occupied (n = 66) and unoccupied (n = 162) plots.

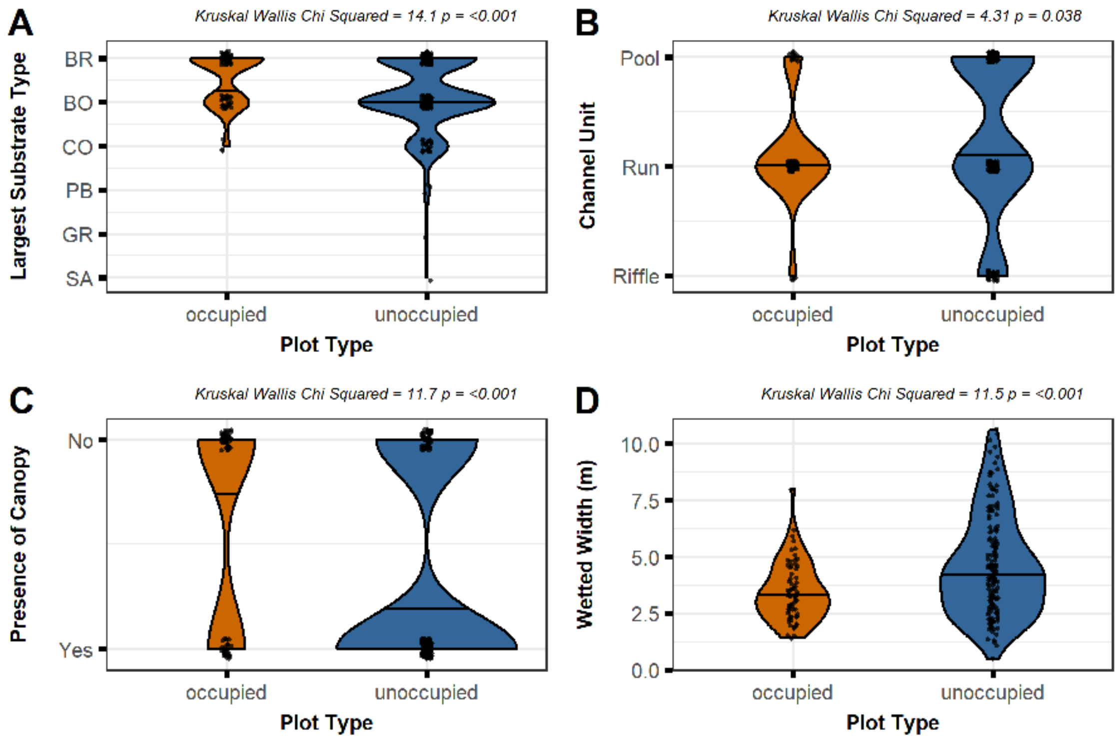

Figure 11. Comparison of habitat parameters (A: Largest Substrate Type, B: Channel Unit, C: Presence of Canopy, and D: Wetted Width) between plots occupied and unoccupied by *Etheostoma nebra*, in all sites quantitatively sampled during the 2017 study in the Buck Creek system, Kentucky. Substrates are abbreviated as follows: SI = silt, SA = sand, GR = gravel, PB = pebble, CO = cobble, BO = boulder, BR = bedrock.

Fish Surveys of Historical Range

Apart from Big Spring Branch, Stewart Branch, and Flat Lick Creek in close proximity to the Big Spring Branch confluence, there were no *E.nebra* captured at any other stream in the Buck Creek system during this study. The highest species richness (n=18) observed during this study was shared between a site on the main stem of Buck Creek (BUC3) and a site on Bee Lick Creek (BLC2); both were historical *E. nebra* collection locations. The most common species observed while sampling the Buck Creek system were *Semotilus atromaculatus* (Creek Chub, 25/26 sites), *Lepomis cyanellus* (Green Sunfish, 20/26 sites), and *Campostoma oligolepis* (Largescale Stoneroller, 19/26 sites). *E. caeruleum* (Rainbow Darter) was the most common darter species, found at 14 of the 26 sites sampled. A comprehensive table of all the species presence data at all sites sampled that were not included in the population estimate is found in Appendix E.

HOBO Logger Data

Big Spring Branch and Stewart Branch appeared to have a more stable water temperature regime than the rest of the Buck Creek system. These two streams did not reach as high sustained water temperatures during the summer as other streams (Figure 12). They appeared to warm up slower in the spring during what was observed as peak *E. nebra* spawning season (Figure 13). The maximum water temperature recorded (31.7 ℃) was in Stewart Branch at the middle logger location on July 14, 2017; however, average water temperatures ranged from 13.4℃ to 24.3℃ across the seasons

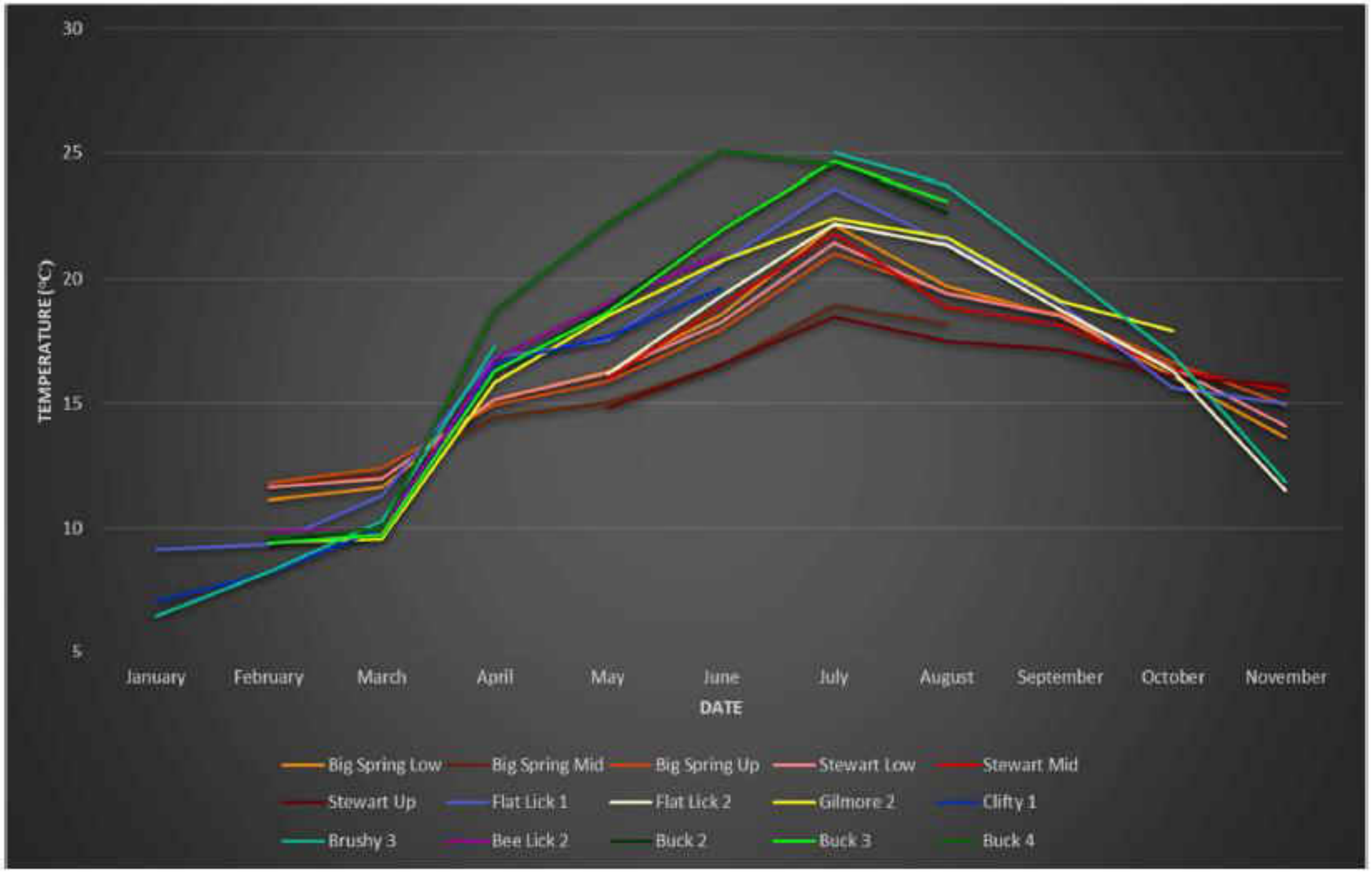

Figure 12. Monthly average temperatures around the Buck Creek system of Kentucky during the 2017 study. Incomplete lines represent periods when a logger was missing.

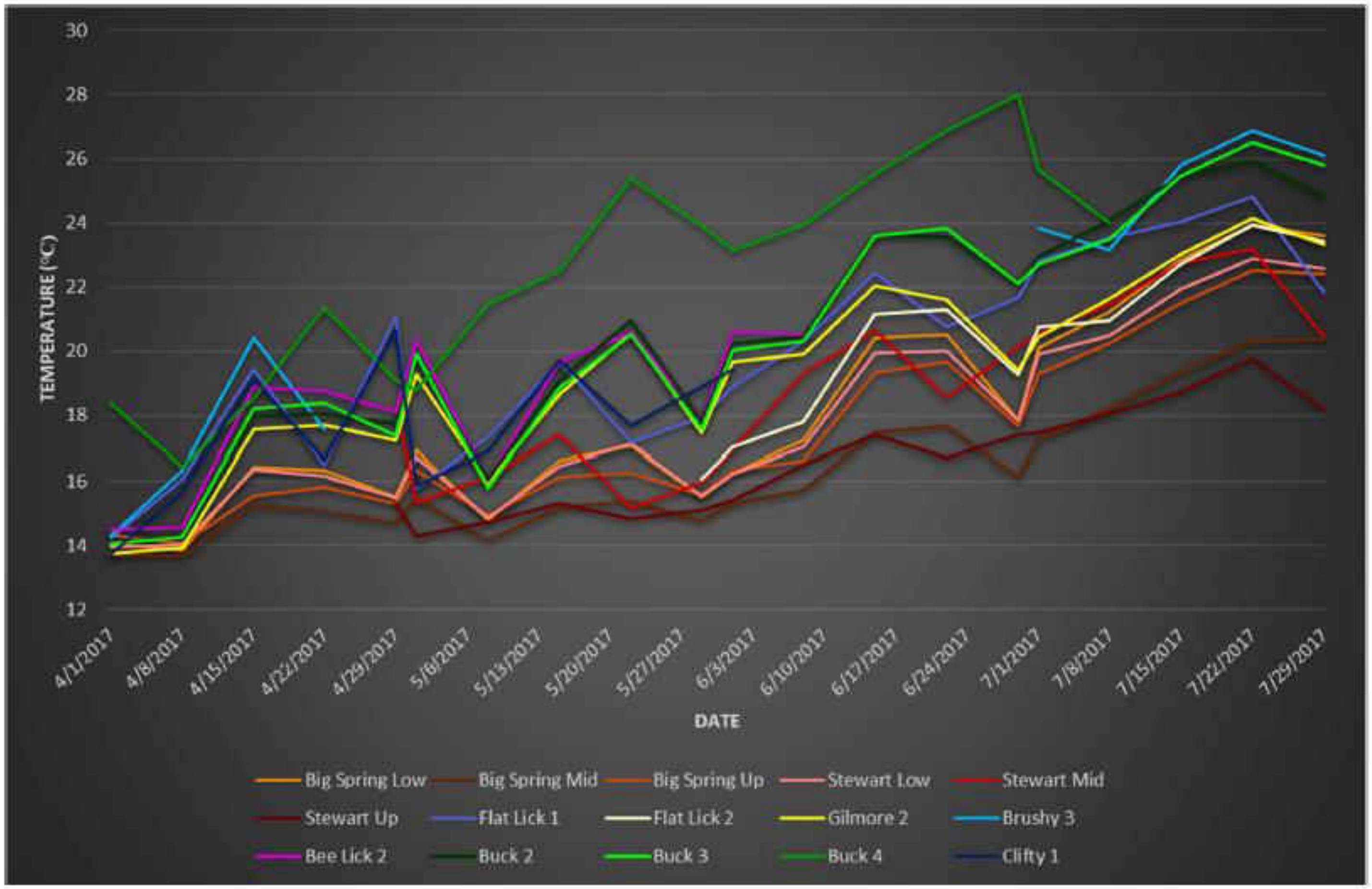

Figure 13. Weekly average temperatures during the observed spawning season (April-July) of *Etheostoma nebra* during the 2017 study in the Buck Creek system of Kentucky. Incomplete lines represent periods a logger went missing.

Conductivity trends showed that Big Spring Branch and Stewart Branch had consistently higher conductivity values than Gilmore Creek (Figure 14). Conductivity values in Gilmore Creek spiked to 3000 µS/cm in the early morning hours of July 18, 2017, causing the daily average to be above that of Big Spring Branch and Stewart Branch. This spike only lasted about an hour and returned back to normal within 2.5 hours of the initial increase. The conductivity logger that was placed in Bee Lick Creek went missing sometime between its initial deployment on June 14, 2017 and the first download date of August 9, 2017.

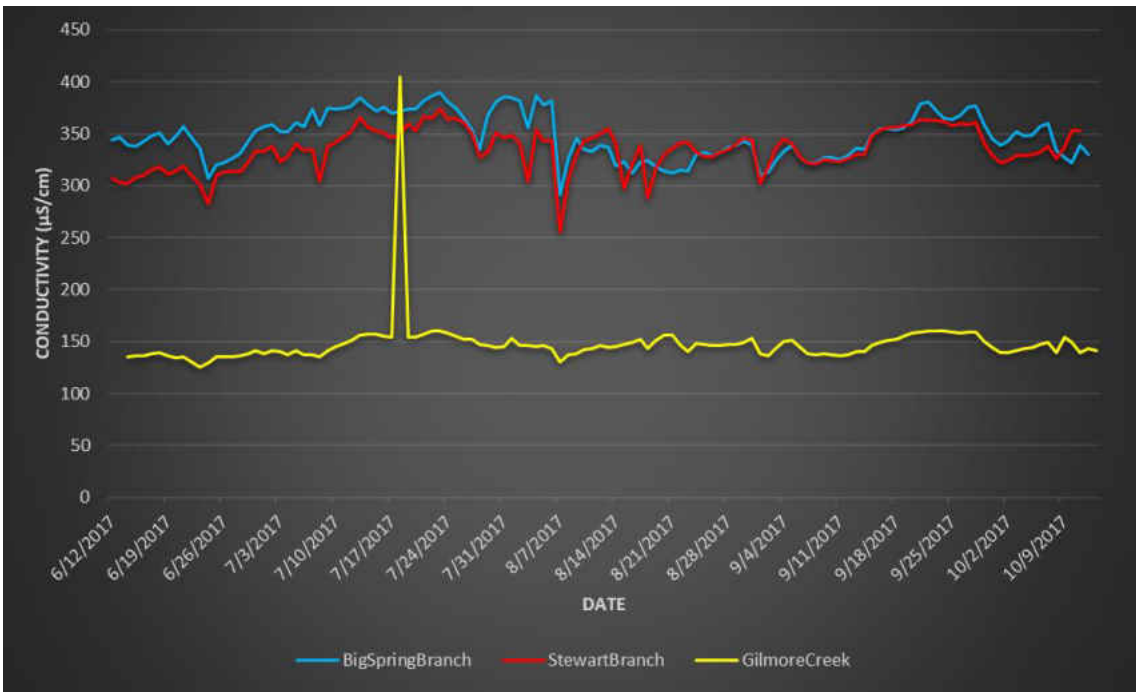

Figure 14. Conductivity measurements in Big Spring Branch, Stewart Branch, and Gilmore Creek during the 2017 study in the Buck Creek system, Kentucky.

Chapter IV: Discussion

An important objective of this study was to estimate the population size of *E. nebra* populations persisting in Big Spring Branch and Stewart Branch. The N-mixture modeling methods used to estimate population size for *E. nebra* were developed to account for imperfect detection, as well as other factors that might affect abundance (Royle, 2004; Royle et al., 2005). Based on these estimates, approximately 17,000 individuals are expected to persist within these two streams, although there may be as few as 10,000. Big Spring Branch is predicted to have a larger population than Stewart Branch. Even though Big Spring Branch likely has a larger population of *E. nebra*, it is worth considering that the estimate for Big Spring Branch may be inflated due to the lower number of sample sites used in the analysis. The higher counts in Big Spring Branch were weighted higher because of having only 4 sites in the analysis than the higher counts of the 6 sites in Stewart Branch which were weighted less due to larger sample size. Big Spring Branch is also longer than Stewart Branch so has more available habitat.

The predicted densities of *E. nebra* in both streams fell within published densities ($<1/m^2$ to $>5/m^2$) for other darter species (Ingersoll, Hlohowskyj, & Mundahl, 1984; Rakocinski, 1988; Scalet, 1973). Additionally, predicted densities were close to an estimate of $1.63/m^2$ in a related species, *E. obeyense* (Kopp, 1985). These previously reported density estimates lend further support to the accuracy of total abundance estimates predicted by the models within this study. While the maximum observed density did not reach the larger predicted density, a likely explanation is the range in densities over the habitat gradient and the fact that predicted density was a calculation based on total area. The low detection probabilities also may contribute to this decreased observational density, as only a portion of the available individuals were captured. There is also the likelihood that more juveniles were present than effectively sampled using electrofishing sampling techniques due to their small size; they have less surface area to be affected by the electrical current, and the decreased chances of being observed as they escape into interstitial space within the substrate.

Persisting populations of *E. nebra* in Big Spring Branch and Stewart Branch appear to be locally abundant, and exhibit trends apparent of a stable and reproducing population. The analysis of length and weight data showed similar body sizes between the streams. Near and Thomas (2015) reported a maximum size of 71.7 mm. During this study, multiple males were observed in the 75 mm range, with a maximum total length of 78 mm. The length frequency histogram showed some smaller individuals (<50 mm), but most of the individuals able to be sexed (based on coloration and morphology) were larger individuals (>50 mm), suggesting the presence of at least 2 cohorts. The smaller, under-represented portion were likely age-0 fish, which are hard to effectively capture by electrofishing methods used in this study. The following peak likely represented age-1 and age-2 fish, which were better represented in the sample. Having observed an extended spawning season in this species, there may not be clear divisions between age classes based on length alone. It should be suggested however, that *E. nebra* likely follows the 2- to 3-year longevity predictions for *Oopareia* noted in previous studies (Kopp, 1985; Kornman, 1980). As Kopp noted in his 1985 study, male *E. obeyense* reached larger sizes than females. This size difference appears to hold true for *E. nebra* as well.

Thomas and Brandt (2013) and Near and Thomas (2015) provided some qualitative information on habitat use by the species, but quantitative information was lacking. Based on the results of the habitat analysis portion of this study, areas with greater amounts of pebble and cobble were predicted to support higher abundances of *E. nebra*. Kopp (1980) noted that *E. obeyense* avoided areas of bedrock, sand, and silt. While *E. nebra* were captured from almost all available habitat types, including bedrock, higher numbers were observed in areas with a mixture of substrate types, and the species was detected more often in areas with larger substrates present. An absence of canopy cover was observed in more of the occupied plots than unoccupied plots; however, this is likely just a consequence of much of the landscape surrounding the sample area consisting of pasture. It appears that *E. nebra* is often found in a variable and complex habitat, with a preference for larger substrates.

The presence of woody debris and multiple substrate classes creates a variety of microhabitat types which provide abundant refuges for benthic fishes. *E. nebra* were seldom observed in open water; they usually were captured from under rocks or other debris when sampling. Similarly, Page and Burr (1976) noted use of slab rocks as refuges when studying the life history of *E. smithi* (Slabrock Darter). Based on the analysis performed during this study, habitat usage of *E. nebra* seems to be similar to that of *E. virgatum* (Kornman, 1980) and *E. obeyense* (Kopp, 1980), with the species often utilizing shallow (<0.5-m) pools and runs that contain mixed coarse substrate.

Habitat and fish community data also were recorded for seven qualitative sites. When included in the modeling for *E. nebra* abundance, many of these sites were similar enough to occupied sites, based on habitat covariates, that the models became unresponsive and did not converge, and would not produce estimates. This similarity meant that the models could not separate characteristics of the occupied plots from the unoccupied ones found in the other streams that had similar habitat characteristics. This habitat and community data should be used in a future study to analyze whether there have been any other shifts in the fish community (e.g., an increase in abundance for any other darter species in the absence of *E. nebra*) since completion of historical surveys

During this study, *E. nebra* was absent from all historical collection locations except Big Spring Branch and Stewart Branch (Cicerello & Butler, 1985; Near & Thomas, 2015; Thomas & Brandt, 2013). When historical sites were included, there was little significant difference in occupied and unoccupied plots, suggesting that suitable habitat for *E. nebra* is still present in those streams. Potential impacts from agricultural land use impacts are similar throughout the Buck Creek system, with no apparent differences between Big Spring Branch, Stewart Branch, and historical locations. This is supported by an analysis of land use and land cover in this system using remote sensing and Geographic Information Systems (GIS) that documented high agricultural use throughout the Buck Creek system, including Big Spring Branch and

Stewart Branch (Peter Grap, unpublished data). This also suggests that some factor, or combination of factors, other than solely agricultural land use has caused the decline of *E. nebra* in the Buck Creek system.

Temperature measurements at all historical locations seemed to follow similar seasonal trends and fell within known ranges for darter suitability (Etnier & Starnes, 1993; Kornman, 1980). Monthly average temperatures in Big Spring Branch and Stewart Branch did appear to stay a few degrees cooler on average than many of the historical locations. The highest recorded temperature was in Stewart Branch; however, riparian cover at that location was absent, receiving a lot of direct sunlight, and the water was less than 0.5 m in depth. When averaged and shown as a trend, that location did not appear to hold consistently higher temperatures than other locations. Several other historical locations had maximum temperatures around 30 ℃ and appeared to stay closer to those temperatures for longer periods.

Conductivity readings remained stable at most sites; however, there was one conductivity spike in Gilmore Creek that was likely caused by some anthropogenic disturbance. This logger location was only about 25-m downstream of a bridge crossing where some sort of contaminant could have been introduced. Conductivity was inherently higher in Big Spring Branch and Stewart Branch than other sites sampled in the Buck Creek system. This was seen both in the HOBO logger results and the in-situ measurements taken during quantitative sampling events throughout the system.

Due to the spring influences in Big Spring Branch and Stewart Branch, stream flow was likely more stable throughout the dryer months of the summer than in headwaters of other tributaries. Kornman (1980) postulated that temperatures over 34℃ could lead to population declines in *E. virgatum* when associated with low flows and disturbance. Lower water temperatures paired with stable hydrologic regimes likely leads to less stress on *E. nebra* populations and could be one reason behind the species' unexpected spawning patterns. Water temperatures in Big Spring Branch and Stewart Branch warmed more slowly in the spring and summer, allowing the darters to spawn for longer periods that extended later into the summer. The spawning seasons of other related darter species, *E. virgatum* and *E. obeyense*, were observed to peak in April-May and ended by June (Kopp, 1985; Kornman, 1980). *E. nebra* were observed with active nests into late July. This behavior may contribute to the species' persistence in Big Spring Branch and Stewart Branch.

Another notable behavior is that the presence of active nests at some prime nest locations persisted throughout the spawning season, and probably included clutches from multiple females. This behavior was reported for *E. obeyense* by Kopp (1985), who noted that many nests appeared to have more eggs than one female could produce. Kopp also postulated that female *E. obeyense* did not spawn until their second year. The extended spawning season observed for *E. nebra* in Big Spring Branch and Stewart Branch may be allowing age-1 females to become sexually mature and participate in the latter half of the spawning period. Based on these speculations about the spawning habits of *E. nebra* more study is needed on this species' reproductive life history.

The fish communities in Big Spring Branch and Stewart Branch were not exceptionally diverse. Typical associates of *E.* nebra were *S. atromaculatus* (Creek Chub), *Rhinichthys obtusus* (Western Blacknose Dace), and *Chrosomus erythrogaster* (Southern Redbelly Dace). *C. erythrogaster* is considered an intolerant species in the Kentucky Index of Biotic Integrity (M. C. Compton, Pond, & Brumley, 2003). Other species found in the same streams as *E. nebra* but not in high numbers were *C. oligolepis* (Largescale Stoneroller), *L. cyanellus* (Green Sunfish), and *Ambloplites rupestris* (Rock Bass). *E. nebra* was the only darter species collected within the Flat Lick Creek portion of the Buck Creek system; this was also the case during Thomas and Brandt's 2013 survey. This result suggests a lack of competition with the other darter species found within the Buck Creek system that may have historically competed with *E. nebra* for food, refuge, and possibly spawning sites from certain species such as *E. flabellare* (Fantail Darter).

During this study, multiple challenges and obstacles were encountered. One of these was the above-mentioned extended spawning season. Sampling that had been intended for the summer of 2017 had to be pushed back as active nests kept appearing during surveys. Another unexpected development was the discovery of a fish passage barrier prohibiting upstream movement by *E. nebra*., resulting in removal of two Big Spring Branch sites from the analysis. Fish passage barriers have been considered as one cause of declines observed in fish communities (O'Hanley &

Tomberlin, 2005). By the time the perched culvert was located, it was too late in the survey period to go back in and complete samples at additional sites. While there was also a site with no observed *E. nebra* in Stewart Branch, habitat at this site appeared poor, consisting mainly of pools and areas of fine sediment deposition, likely causing absence or a low local abundance.

Another problem that occurred during this study was the loss or disappearance of multiple temperature loggers and one conductivity logger. Some of these losses were attributed to shifting substrates caused by flooding, while other losses were attributed to anthropogenic influences, such as gravel dredging – an activity that was observed in several locations during this study. There was also at least one temperature logger disappearance attributed to theft, as the cable securing it in the stream was apparently cut. These types of anthropogenic disturbance are evidence of a changing physical habitat in these streams over the past 50 years, as has occurred in many of North America's flowing waters (Benke, 1990). Likewise, the missing conductivity logger also was assumed to be stolen, as the housing tube was found broken in half on the bank of the stream.

Compton and Taylor (2013) postulated that population declines of fishes are typically caused by degradation of available habitats . In the case of *E. nebra*, loss of habitat complexity in some streams over the years paired with stochastic events may have contributed to their declines and eventual extirpation from many historical sites. There was no single, obvious factor that could explain the species' decline within the system. Currently, it appears that *E. nebra* persists in two, locally abundant populations, Big Spring Branch and Stewart Branch, confined to the Flat Lick system.

Factors behind this species' persistence are speculated to be a combination of stable water temperatures, reduced competition with other darters, and increased reproductive potential associated with a longer spawning season. *Etheostoma nebra* populations appear to be stable, and suitable habitats are present for them to persist in these streams. However, due to the species' small range, the risk of catastrophic endangerment still exists. The lack of a single critical factor complicates the conservation of *E. nebra* into the future. The conservation of this species may require overall habitat restoration, preservation, and continued research into the species' behavior and life history to increase the likelihood of its persistence within the Buck Creek system.

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APPENDICES

Appendix A:

Summary of quantitative survey reaches within Big Spring Branch and Stewart Branch, Buck Creek system, Kentucky (2017).

Appendix A: Summary of quantitative survey reaches within Big Spring Branch and Stewart Branch, Buck Creek system, Kentucky (2017).

Stream	Reach	Location Description	Latitude/Longitude	Date Sampled	Reference Code
Big Spring Branch					
	2	~120 m upstream from Flat Lick Confluence	37.1678/-84.5015	9/9/2017	BSB2
	6	~600 m upstream from Flat Lick Confluence	37.1619/-84.5049	9/9/2017	BSB6
	11	Billy Vaught Property	37.15947/-84.51334	8/28/2017	BSB11
	16	William Vaught Property	37.15405/-84.51699	8/28/2017	BSB16
	22	Along soybean field	37. 15315/-84.52433	9/11/2017	BSB22
	23	Along soybean field	37.15353/-84.52542	9/11/2017	BSB23
Stewart Branch					
	1	From Flat Lick Creek confluence upstream 120 m	37.15687/-84.48118	7/10/2017	STB1
	7	Lower end of Steve Cook's property	37.15398/-84.48774	7/24/2017	STB7
	9	Below big curves in Grundy Rd, on Steve Cook's property	37.15150/-84.48789	7/24/2017	STB9
	16	Stewart Farm	37.14602/-84.49280	7/25/2017	STB16
	19	Stewart Farm	37.14230/-84.49052	7/25/2017	STB19
	20	Stewart Farm	37.14148/-84.49052	7/25/2017	STB20

Latitude and Longitude are based on a WGS84 datum.

Appendix B:

Summary of 2016-2017 qualitative survey reaches in the Buck Creek system, Kentucky. Location information is provided, and a site reference code is assigned to each site.

Appendix B: Summary of 2016-2017 qualitative survey reaches in the Buck Creek system, Kentucky. Location information is provided, and a site reference code is assigned to each site.

Stream	Location Description	Latitude/Longitude	Date Sampled	Reference Code
Stewart Branch	Shopville Community Park	37.157042°/-84.483506°	9/20/2016	STB3
Clifty Creek	~0.3 km above Confluence with Brushy Creek	37.217633°/-84.471767°	9/20/2016	CLC1*
Briary Creek	Estill Hackney Rd Crossing	37.273961°/-84.577637°	9/26/2016	BRC1
Briary Creek	Old Waynesburg Rd Crossing	37.267665°/-84.607964°	9/26/2016	BRC2
Bullock Branch	Above Confluence with Briary Creek	37.264998°/-84.610924°	9/26/2016	BLB1
Glade Fork Creek	Glade Fork Rd Crossing	37.345344°/-84.552193°	9/26/2016	GFC1
Bee Lick Creek	KY-3267 Crossing	37.319139°/-84.498819°	9/29/2016	BLC1
Brushy Creek	Upstream of KY-328 Crossing	37.327419°/-84.465618°	9/29/2016	BHC1*
Brushy Creek	Upstream of Edgar Cash Rd Crossing	37.355556°/-84.469583°	9/29/2016	BHC2
Crab Orchard Creek	Brad Petrey Rd Crossing	37.380658°/-84.559739°	10/10/2016	CRC1
Gilmore Creek	Allen Store Rd Crossing	37.395941°/-84.591269°	10/10/2016	GIC1
Buck Creek	Vic Greer Rd Crossing	37.364113°/-84.601292°	10/10/2016	BUC1
Buckeye Branch	OK Schuller Rd Crossing	37.374774°/-84.615241°	10/13/2016	BKB1
Buck Creek	Broughtentown Rd	37.346177°/-84.586708°	10/13/2016	BUC2*
Flat Lick Creek	From Big Spring Branch confluence upstream to Barnesburg Rd	37.163826°/-84.500818°	11/1/2016	FLC1
Salem Branch	Downstream from Coin Rd Crossing	37.180157°/-84.508476°	11/1/2016	SAB1
Salem Branch	Downstream from Harper Rd Crossing	37.173623°/-84.509369°	11/1/2016	SAB2
Brushy Creek	At Confluence with Buck Creek	37.212626°/-84.467453°	11/1/2016	BHC3*
Flat Lick Creek	Upstream from White Rd Crossing	37.183824°/-84.543102°	11/1/2016	FLC2
Gilmore Creek	Downstream from Ephesos School Rd Crossing	37.360452°/-84.589562°	6/13/2017	GIC2*
Clifty Creek	Downstream of Ocala Rd Crossing	37.242202°/-84.483333°	6/20/2017	CLC2
Caney Creek	Upstream of KY-328 Crossing	37.328527°/-84.650656°	6/28/2017	CAC1
Buck Creek	Goodhope-Goochtown Rd Crossing	37.309904°/-84.566444°	6/28/2017	BUC3*
Barney Branch	Upstream of Goodhope-Estes School Rd	37.293407°/-84.543438°	7/7/2017	BAB1
Flat Lick Creek	Upstream from Stewart Branch confluence	37.157074°/-84.480997°	7/10/2017	FLC3
Bee Lick Creek	Friendship Church Rd Crossing	37.272220°/-84.442325°	7/20/2017	BLC2*

* Denotes a historical (pre-1985) *Etheostoma nebra* (Buck Darter) collection location. Latitude and Longitude are based on a WGS84 datum.

Appendix C:

Summary of data logger locations within the Buck Creek system, Kentucky (2017).

Appendix C: Summary of data logger locations within the Buck Creek system, Kentucky (2017).

Stream	Location Description	Latitude/ Longitude	Logger Type	Reference Code
Historical Sites				
Bee Lick Creek	Downstream of Friendship Church Rd Crossing	37.272220°/-84.442325°	Temperature	BLC2
Brushy Creek	Upstream of confluence with Buck Creek	37.212626°/-84.467453°	Temperature	BHC3
Buck Creek	Upstream of Broughtentown Rd Crossing	37.346177°/-84.586708°	Temperature	BUC2
Buck Creek	Upstream of Goodhope-Goochtown Rd Crossing	37.309904°/-84.566444°	Temperature	BUC3
Buck Creek	Upstream of Old Mt. Vernon Rd Crossing	37.211018°/-84.464541°	Temperature	BUC4
Clifty Creek	~0.3 km above confluence with Brushy Creek	37.217633°/-84.471767°	Temperature	CLC1
Flat Lick Creek	Downstream of Barnesburg Rd Crossing	37.163826°/-84.500818°	Temperature	FLC1
Flat Lick Creek	Downstream from White Rd Crossing	37.183375°/-84.543010°	Temperature	FLC2
Gilmore Creek	Downstream of Ephesos School Rd Crossing	37.360452°/-84.589562°	Conductivity/ Temperature	GIC2
Occupied Sites				
Big Spring Branch	Downstream of KY-1317	37.156536°/-84.527248°	Temperature	BSBUP
Big Spring Branch	Off end of Barnesburg Spur Rd	37.152906°/-84.518946°	Temperature	BSBMID
Big Spring Branch	~25m upstream of confluence with Flat Lick Creek	37.162592°/-84.500818°	Conductivity/ Temperature	BSBLO
Stewart Branch	At upper farm road crossing on the Stewart Farm	37.139104°/-84.489235°	Temperature	STBUP
Stewart Branch	Off Grundy Rd at lower end of Stewart Property	37.146435°/-84.492431°	Temperature	STBMID
Stewart Branch	At Shopville Community Park	37.157042°/-84.483506°	Conductivity/ Temperature	STBLO

Latitude and Longitude are based on a WGS84 datum.

Appendix D:

AICc table of N-Mixture models for *Etheostoma nebra* abundance in Big Spring Branch and Stewart Branch, Buck Creek system, Pulaski County, KY (2017). All models were run with a zero-inflated Poisson mixture.

Appendix D: AICc table of N-Mixture models with a ΔAICc <10 used in estimation of *Etheostoma nebra* abundance in Big Spring Branch and Stewart Branch, Buck Creek system, Pulaski County, KY (2017). All models were run with a zero-inflated Poisson mixture.

Parameterization	K	AICc	ΔAICc	AICcWt
p(Average Stream Width @ Plot) λ(Percent Cobble)	5	387.33	0	0.64
p(Average Stream Width @ Plot) λ(Percent Gravel)	5	390.21	2.88	0.15
p(Average Stream Width @ Plot) λ(Percent Gravel + Percent Cobble)	6	392.34	5.01	0.05
p(Channel Unit) λ(Percent Cobble)	6	392.41	5.07	0.05
p(Average Stream Width @ Plot) λ(Percent Pebble)	5	393.28	5.95	0.03
p(.) λ(.)	3	395.19	7.85	0.01
p(Average Stream Width @ Plot) λ(Percent LWD)	5	395.38	8.04	0.01
p(Average Stream Width @ Plot) λ(Percent Bedrock)	5	395.74	8.41	0.01
p(Average Stream Width @ Plot) λ(Percent Silt)	5	395.78	8.45	0.01
p(Channel Unit) λ(.)	5	395.82	8.48	0.01
p(Average Stream Width @ Plot) λ(.)	4	396.32	8.99	0.01

p = detection, λ = abundance, K = number of parameters, AICc = corrected Akaike's Information Criteria, ΔAICc = $AICc_i - AICc_{TopModel}$, AICcWt = model weight

Appendix E:

Summary of fishes observed at all qualitative and quantitative (not included in the population estimate) reaches within the Buck Creek system, Kentucky (2016-2017).

Appendix E: Summary of fishes observed at all qualitative and quantitative (not included in the population estimate) survey reaches within the Buck Creek system, Kentucky (2016-2017)

Site Reference Code	BAB1	BHC1*	BHC2	BHC3*	BKB1	BLB1	BLC1	BLC2*	BRC1	BRC2	BUC1	BUC2*	BUC3*	CAC1	CLC1*	CLC2	CRC1	FLC1	FLC2	FLC3	GFC1	GIC1	GIC2*	SAB1	SAB2	STB3
Species																										
Ambloplites rupestris				x				x												x						x
Ameiurus natalis								x	x								x									
Campostoma oligolepis	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x	x	x				
Catostomus commersonii		x	x					x					x													
Chrosomus erythrogaster																								x		
Cyprinella galactura				x				x							x											
Etheostoma blennioides		x		x				x	x				x													
Etheostoma caeruleum		x		x	x		x	x	x	x	x	x	x	x		x						x	x			
Etheostoma camurum				x																						
Etheostoma flabellare				x	x			x	x	x	x	x	x				x						x			
Etheostoma lawrencei	x				x									x		x							x			
Etheostoma maydeni													x													
Etheostoma nebra																		x								x
Etheostoma sanguifluum																										
Etheostoma zonale				x				x					x													
Fundulus catenatus				x	x						x	x	x	x				x		x		x				
Gambusia affinis	x												x													
Hypentilium nigricans				x				x	x		x		x		x			x	x	x			x			x
Lepomis cyanellus	x	x	x		x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x			x	
Lepomis macrochirus	x	x	x					x			x	x	x	x			x	x		x		x				
Lepomis megalotis		x					x	x					x		x		x	x	x	x		x				
Luxilus chrysocephalus		x	x				x	x		x	x	x	x				x			x			x			
Lythrurus fasciolaris								x					x					x		x						
Micropterus dolomieu				x														x								
Micropterus punctulatus				x									x			x										
Micropterus salmoides											x						x									
Moxostoma erythrurum													x		x											
Notropis buccatus		x	x		x			x																		
Notropis photogenis		x		x	x							x			x											
Notropis spp.									x																	
Notropis telescopus				x																						
Percina caprodes				x																						
Pimephales notatus		x	x				x	x			x					x	x	x	x			x				
Rhinichthys obtusus		x												x					x					x	x	x
Semotilus atromaculatus	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Richness	6	13	8	15	9	3	7	18	9	6	11	9	18	7	6	7	9	11	6	10	3	8	6	3	3	5

* Denotes a historical (pre-1985) Etheostoma nebra (Buck Darter) collection location. Streams are abbreviated as follows: Barney Branch (BAB), Brushy Creek (BHC), Buckeye Branch (BKB), Bullock Branch (BLB), Bee Lick Creek (BLC), Briary Creek (BRC), Buck Creek (BUC), Caney Creek (CAC), Clifty Creek (CLC), Crab Orchard Creek (CRC), Flat Lick Creek (FLC), Glade Fork Creek (GFC), Gilmore Creek (GIC), Salem Branch (SAB), and Stewart Branch (STB).