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By

Colin Arnold

Thesis Approved:

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Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
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DEDICATION

This thesis is dedicated to my family,
For the love and support they have provided me.

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First, I would like to thank my major professor, Dr. Sherry Harrel, for her patience and guidance throughout the completion of this study. I would also like to thank my committee members, Dr. David Hayes and Dr. Charles Elliott, for their additional guidance throughout the process of completing this thesis. A special thanks goes to Eastern Kentucky University (EKU) for the use of equipment to complete the fieldwork and data analyses associated with this study. I would also like to thank everyone who provided support in the field: Dr. Michael Floyd, of the U.S. Fish and Wildlife Service (USFWS); Pam Martin and Sandie Kilkpatrick, of the U.S. Forest Service (USFS); fellow graduate students, Jonathan Baxter and Eric Smith; and EKU wildlife students, Andrew Ault and Jack Fetters. In addition, special thanks go out to the USFWS and the Kentucky Department of Fish and Wildlife Resources (KDFWR) for funding and financial support.

ABSTRACT

The movement patterns of small-bodied fishes in headwater streams are poorly understood. This study was designed to examine the movement patterns of the Kentucky Arrow Darter (*Etheosotma spilotum*), Frecklebelly Darter (*Percina stictogaster*, and Southern Redbelly Dace (*Chrosomus erythrogaster*) in two dynamic headwater streams, Elisha Creek and Gilbert's Big Creek, in the Red Bird River, Kentucky utilizing Passive Integrated Transponders and an antennae detection system. *Etheostoma spilotum* is listed as a threatened species under the Endangered Species Act. *Percina stictogaster* is acknowledged as a species of greatest conservation need in Kentucky by the Kentucky Department of Fish and Wildlife Resources. Over the duration of this study, a total of 182 fishes were PIT tagged and released in Elisha Creek and Gilbert's Big Creek. A total of 35 detected intra-raceway movements among 24 individuals were recorded from the summer of 2013 to the spring of 2016. Movement distances ranged from 41 m to 4,044 m, with an average detected movement distance of 795±147 m. The effects of length, weight, sex, season, temperature, light intensity, and depth on the distance moved were examined utilizing General Linear Models. The results suggested that length and weight were significant factors influencing the movement of *E. spilotum*, and season was a significant factor influencing the movement of *P. stictogaster*.

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1. Introduction

Understanding animal behavior has proven to be an important part of the conservation and management of species (Sanderson et al. 2002, Caro 2007). Movement and dispersal patterns are useful for understanding various aspects of the ecology of a variety of taxa, as well as providing useful insight in decision making and conservation efforts of such organisms (Dodd and Cade 1998, Sanderson et al. 2002, Banko et al. 2002, Bhattacharya et al. 2003, Schrank and Rahel 2004). Movement and dispersal are essential ecological processes of fishes residing within a dynamically variable stream environment (Schlosser and Angermeier 1995, Alldredge et al. 2011). Movement of stream fishes varies among individuals (Smithson and Johnston 1999), and across changes in complexity of habitat (Albanese et al. 2004). Most fish movement research has been directed towards fishes which are known to be highly migratory species such as salmonids, with few studies focusing on small-bodied fishes such as those found in headwater streams (Gowan et al. 1994, Walker et al. 2013).

Many past studies of movement have indicated that adult fish are sedentary and do not leave a particular reach of the stream. This has been termed the "restricted movement paradigm" by Gowan et al. (1994). Recent studies have shown that individual fish within a population may move due to several factors (Smithson and Johnston 1999, Petty and Grossman 2004). Petty and Grossman (2004) reported that stream flow and the density of conspecifics had effects on the movement patterns of the mottled sculpin (*Cottus bairdi*) in a southern Appalachian stream. It has also been suggested that fishes may move in order to obtain the resources needed for survival including optimal feeding habitats, refugia from extreme temperatures or flow, and optimal spawning habitats (Schlosser 1995, Schlosser and Angermeier 1995).

The movement patterns of small-bodied fishes in headwater streams are poorly understood. There has been limited research regarding the movement patterns of darters and minnows. Most studies involving the movement of small-bodied fishes have utilized a mark and recapture technique employing fin-clipping or visible implant elastomers (Scalet 1973, Mundahl and Ingersoll 1983, Schaefer et al. 2003, Skyfield and Grossman 2008, Roberts et al. 2008). Few movement studies have been conducted using passive integrated transponder tags (PIT tags) in small-bodied fishes (Cucherousset et al. 2005); however, the successful use of PIT tags in larger stream fishes has been well documented (Smithson and Johnston 1999). The use of PIT tags provides several advantages to traditional mark and recapture movement surveys conducted using fin clipping or visible implant elastomers (VIE). PIT tags have been found to have no effect on survival, growth, or swimming ability in small-bodied stream fishes (Ward 2003, Knaepkens et al. 2007, Bolland et al. 2009, Ficke et al. 2012). Additionally, the retention rates of PIT tags placed in fish are higher than those of VIE's (Knaepkens et al. 2007, Bolland et al. 2009). The use of PIT tags allows for a continuous collection of data via flatbed antennas place in the stream (Johnston et al. 2009). The benefits and availability of PIT tags small enough to be used in small-bodied stream fishes provides an ideal device with which to study the movements of these fishes.

The objective of this study was to expand what is known about the movement patterns of these unique, small-bodied, stream fishes by the continuous monitoring of three species of fish, the Kentucky Arrow Darter (*Etheostoma spilotum*), Frecklebelly

Darter (*Percina stictogaster*), and Southern Redbelly Dace, (*Chrosomus erythrogaster*) in two first order Appalachian streams located in southeastern Kentucky.

The Kentucky Arrow Darter (*Etheostoma spilotum*) is a benthic darter species inhabiting tributaries of the upper Kentucky River system of the Ohio River Drainage (Etnier and Starnes 1993a). *Etheostoma spilotum* is the only endemic fish species located in the Kentucky River system (Etnier and Starnes 1993a). It is a relatively large darter species, with adults reaching a maximum total length (TL) of approximately 120 mm. It's distribution is contradictive of it's size, being commonly located in first, second, and third order creeks where it typically inhabits shallow pools and runs with bedrock, boulder, and cobble substrates; and is uncommon in larger streams (Kuehne and Barbour 1983, Etnier and Starnes 1993a, USFWS 2013). An assortment of aquatic invertebrates makes up the diet of *E. spilotum*, with mayfly larvae being the primary food source of adult individuals, particularly members of the families Heptageniidae and Baetidae (Lotrich 1973, USFWS 2013). Lotrich (1973) found that individuals over 70 mm TL feed on small crayfish (<24mm) which is atypical for smaller individuals of *E. spilotum* as well as other species often associated with first and second order streams. Lotrich (1973) proposed that "the utilization of this abundant food source may be the reason for the survival of this large darter in extreme headwaters" due to their removal from direct competition with smaller individuals and other species. Spawning in *E. spilotum* populations typically occurs from April to June when stream temperatures reach approximately 13° C (Lowe 1979). The male makes a nest by fanning out a depression in a sand substratum, defending it from other males, and then putting on a display of rapid dashes, nudging of females, and quivering (Lowe 1979, Kuehne and Barbour 1983). This is responded to by quivering from a female, who then proceeds to a nest site and buries the ventral half of her body into the sand. Once mounted by the male, spawning occurs. The male presumably then defend the nests until the eggs have hatched (Lowe 1979, Kuehne and Barbour 1983, Etnier and Starnes 1993a). *Etheostoma spilotum* is a federally listed species under the Endangered Species Act (USFWS 2016). The Kentucky Department of Fish and Wildlife Resources (KDFWR) consider *E. spilotum* a species of greatest conservation need (KDFWR 2013). The conservation status of *E. spiltoum* is largely contributed to habitat reduction and fragmentation due to anthropogenic activities including surface coal mining, deforestation, and agricultural practices (USFWS 2013, Floyd 2014). *Etheostoma spilotum* has been documented in less than half of its historically recorded sites (USFWS 2013, 2015, Hopkins and Roush 2013, Floyd 2014, Hitt et al. 2016).

The Frecklebelly Darter (*Percina stictogaster*) is a pelagic darter species inhabiting the upper Green River system and portions of the upper Kentucky River system (Kuehne and Barbour 1983, Burr and Page 1993, Etnier and Starnes 1993b). It is the only known fish species to be restricted to these two river systems (Kuehne and Barbour 1983, Burr and Page 1993). It grows to a maximum TL of just over 80 mm and typically inhabits backwater pools with a moderate to low flow and vegetation cover (Kuehne and Barbour 1983, Etnier and Starnes 1993b). *Percina stictogaster* often swims freely through the middle of the water column, uncharacteristic of many darters. This is due to a well-developed swim bladder, making it a much better swimmer than *E. spilotum*, in which a swim bladder is absent (Kuehne and Barbour 1983, Burr and Page 1993, Evans and Page 2003). The primary food sources for *P. stictogaster* are midge larvae, mayfly larvae, stonefly larvae, micro-crustaceans, and amphipods (Etnier and Starnes 1993b). Spawning typically occurs from late February to early April when stream temperatures range from approximately 7-10° C (Eisenhour et al. 2013). Males move in front of the females, prop up on the tips of their pelvic fins, erecting all fins, and occasionally display head bobbing. A male follows a receptive female into an area of gravel substratum and then mounts her. The pair then vibrate together and create a depression in which their caudal regions are buried under the substrate, and spawning occurs. The fertilized eggs are buried in the substrate (Etnier and Starnes 1993b, Eisenhour et al. 2013). The KDFWR considers *P. stictogaster* a species of greatest conservation need (KDFWR 2013). This is largely due to its limited distribution and a lack of knowledge concerning its natural history (Kuehne and Barbour 1983, Eisenhour et al. 2013); though the possibility of habitat fragmentation due to anthropogenic activities such as mining may still apply to this species (Hopkins and Roush 2013).

The Southern Redbelly Dace (*Chrosomus erythrogaster*) is a small cyprinid common in headwater streams throughout upland parts of the Mississippi River Basin, including the Kentucky River system (Etnier and Starnes 1993c, Walker et al. 2013). It grows to a maximum total length of approximately 90 mm and inhabits shallows pools of headwater streams which contain some gravel substrate (Etnier and Starnes 1993c). They are omnivorous species grazing primarily on algae, and feeding on invertebrates when available; a diet similar to the syntopic species *C. cumberlandensis* (Starnes and Starnes 1981, Kohler et al. 2011). Spawning in *C. erythrogaster* populations typically occurs from April to June (Settles and Hoyt 1978). Males gather above a spawning location, usually over the nest of a Stoneroller (*Campostoma sp.*) or Creek Chub (*Semotilus* *atromaculatus*), and females gather in the stream below the spawning location. During this activity females will swim near the group of males; spawning often occurs with a male on each side of a female (Settles and Hoyt 1978, Etnier and Starnes 1993c). *Chrosomus erythrogaster* is a common species in Kentucky's headwaters, though some populations have been negatively impacted by mining activities (Etnier and Starnes 1993c, Hopkins and Roush 2013).

This study was designed and conducted in order to investigate the movement patterns of the Kentucky Arrow Darter, Frecklebelly Darter, and Southern Redbelly Dace in Elisha Creek and Gilbert's Big Creek, Red Bird River, Kentucky. This knowledge will contribute to better explanations of how these species use the habitats available to them in order to meet their needs. It will provide information on when and why the species move in a naturally complex first-order stream in Kentucky. The information obtained from this study will contribute to the conservation of the species.

2. Methods

I. Study Area

The upper Kentucky River system is located along the western edge of the central Appalachian coalfield on the Cumberland Plateau and drains an area of roughly 9,000 km^2 (White et al. 2005). This watershed can be described as a complex network of headwater streams. Four main drainages are contained in the upper Kentucky River system: the North Fork, Middle Fork, South Fork, and the Red River. The study area was located in two streams within Clay and Leslie counties, KY. Elisha Creek and Gilberts Big Creek are tributaries of the Red Bird River (Figure 1, *figures contained in Appendix B*), which flows into the South Fork of the Kentucky River Drainage. The headwaters of Elisha Creek and Gilberts Big Creek are located primarily in the Daniel Boone National Forest; while their confluences with the Red Bird River and lower portions of the streams are located on private lands. Elisha Creek and Gilberts Big Creek were chosen as streams for this study because the *E.spilotum, P. stictogaster, and C. erythrogaster* occur in sympatry in these streams (Baxter 2015).

II. Fish Sampling

Individual of *E. spilotum, P. stictogaster, and C. erythrogaster* were collected from suitable habitats (as described by Etnier and Starnes 1993a, 1993b, 1993c) using a backpack electro-shocker (Smith-Root, Vancouver, WA) between 2013-2015. Upon capture, all specimens were weighed, measured (TL), sexed, and PIT tagged. GPS coordinates of the capture location were recorded for each individual. In cases where sex was unable to be determined, it was recorded as unknown. Data were not collected from juveniles or individuals that did not meet the minimum size and weight requirements to be PIT tagged.

III. PIT Tagging

Upon capture, each individual of *E. spilotum, P. stictogaster, and C. erythrogaster* exceeding 50mm TL was sedated using Tricaine methanesulfonate (MS-222; Finquel, Redmond, WA). A concentration of 60mg/l was used, which is safe and effective for anesthetizing fish (Brandt et al. 1993). A 8 mm HPT8 minichip™ PIT tag (Biomark, Boise, ID) with a unique identification number was implanted into the abdominal cavities of individuals using a MK165 implanter (Biomark, Boise, ID) equipped with a 50mm 16 gauge needle. Once the PIT tag was successfully implanted, a liquid suture was applied to the injection site. The fish were then allowed to recover in an aerated bucket. Upon full recovery, each individual was released at the site of original capture. Procedures related to *E. spilotum, P. stictogaster, and C. erythogaster* capture and handling were reviewed by Eastern Kentucky University's Institutional Animal Care and Use Committee and approved.

IV. Movement Survey

Movements of pit tagged individuals of *E. spilotum, P. stictogaster, and C. erythrogaster* were detected using IS-1001 antennae systems (Biomark, Boise, ID) located along Elisha Creek and at the confluence of Gilberts Big Creek. These systems recorded the unique pit tag number assigned to an individual and the time of passage as the individual swam over the flatbed antenna. Detection efficiency was tested and found to be similar along the entire width of each antenna, 3 m at each confluence and 1.5 m at all other locations. There were 7 antennae systems placed along Elisha Creek (including its confluence), and one antenna system placed at the confluence of the neighboring tributary to the Red Bird River, Gilberts Big Creek (Figure 1). The placement of an antenna system at Gilberts Big Creek's confluence allowed any movement that occurred between the two tributaries (inter-raceway) to be recorded. Each antenna was anchored in the streambed in a location that inhibited movement as much as possible. Data were downloaded from the IS-1001 system twice monthly using a computer containing the software Bioterm (Biomark, Boise, Idaho). After each download, the system's tag memory was erased in order to prevent the memory from becoming full between downloads. Data were downloaded bimonthly in all months with the exception of February through April when weather often prohibited access to the IS-1001 systems. In these months, data were downloaded as often as accessible. Once downloaded, all data were added to a database created using Microsoft Office Access 2010 (Microsoft, Redmond. WA).

V. Spatiotemporal Factors

In addition to the PIT tag number and time that a pit tagged fish swam over an antenna connected to an IS-1001 system; water temperature and light intensity were recorded using a HOBO pendant logger (Onset Computer Corporation, Bourne, MA). To identify high flow events, a barometric pressure HOBO logger was placed at a single location on Elisha Creek to document water depth. The data were downloaded from the HOBO loggers twice monthly using a computer containing the software HOBOware

(Onset Computer Corporation, Bourne, MA). Once downloaded, all data were added to a database created using Microsoft Office Access 2010 (Microsoft, Redmond, WA).

VI. Data Analysis

For the purposes of this study, fishes that exhibited no recorded movement were considered non-mobile. Descriptive statistics were calculated for both mobile and non-mobile individuals using the Data Analysis Tool in Microsoft Excel (Microsoft, Redmond, WA) and SPSS (IBM, Armonk, NY). The distance moved by each individual was calculated using ArcMap 10.1 (ESRI, Redlands, CA) containing shape files of the study area obtained from the 2004 National Hydrography Dataset. Using ArcMap 10.1, containing shapefiles from the 2011 National Land Cover Database, average canopy closure was calculated for the study area. Spearman's rank correlation coefficients were determined for the independent variables water temperature and light intensity, and covariates length (TL) and weight, with distance moved using SPSS. In order to determine the effect of environmental factors (season, temperature, light, depth) on the incidence of movement and total distance moved by pit tagged fishes, General Linear Models (GLM's) were created using SPSS.

3. Results

Over the duration of this study, a total of 182 fishes were PIT tagged and released (Table 1). *Ethostoma spilotum* were tagged in both Elisha Creek and Gilbert's Big Creek; while *P. Stictogaster and C. erythrogaster* were only tagged in Elisha Creek. Each recorded movement and each recapture during sampling was recorded as an individual recapture event. The recapture rate was highest for the *P. stictogaster* (46%), and lowest for *C. erythrogaster* (19%, Table 1, *tables contained in Appendix A*). Based on 2011 National Land Cover data, the average percent canopy closure for Elisha Creek and Gilbert's Big Creek was 91.27±0.68 and 90.81±0.91, respectively. The mean percent canopy closure at antennae locations and locations where individual fish were PIT tagged was 81.88±12.06 and 87.52±1.42, respectively. There was no significant difference between canopy closure of Elisha Creek and Gilbert's Big Creek, or between capture locations. The average length and weight of individuals captured is presented in table 2. There was significant difference in the length and weight of all tagged individuals of *E. spilotum, P. stictogaster, and C. erythrogaster* (r^2=0.23, p<0.001). There was significant difference in the length and weight of all male and female *E. spilotum* (r^2=0.18, p<0.001).

A total of 35 detected intra-raceway movements among 24 individuals were recorded from the summer of 2013 to the spring of 2016 (Table 1). No inter-raceway movement was detected among Elisha Creek and Gilbert's Big Creek. Among all detected movements of mobile individuals (n=35), distances ranged from 41 m to 4,044 m with an average detected movement distance of 795±147 m (Table 1, Figure 2).

The mean depth during the entire study was 0.396 m ± 0.001 m. The mean depth during movement events was 0.403 m ± 0.029 m. The mean temperature during the entire study was 14.74 C ± 0.01 C. The mean temperature during movement events was 13.79 C ± 0.60 C. The mean light intensity during the entire study was 660.29 lux ± 10.27 lux. The mean light intensity during movement events was 2098.45 lux ± 850.21 lux. No significant differences were recorded between the duration of the study and during movement events concerning depth, temperature, and light intensity.

The average length and weight of all mobile individuals of *E. spilotum* were 80±4 mm and 4.9±0.7 g, respectively, with an average distance moved of 973±391 m (Table 3). The average length and weight of all mobile individuals of *P. stictogaster* were 70±1 mm and 2.8±0.1 g, respectively, with an average distance moved of 2450±908 m (Table 3). A single mobile female had a length of 69 mm, weight of 2.7 g, and moved 2098 m (Table 3). The average length and weight of all mobile individuals of *C. erythrogaster* were 71±1 mm and 2.8±0.1 g, respectively, with an average distance moved of 245±134 m (Table 3). A single mobile female had a length of 70 mm, weight of 2.5 g, and moved 643 m (Table 3). No significant difference was found between the length or weight of a mobile and non-mobile individual on any species.

The proportion of movements occurring downstream and upstream for *E. Spilotum, P. stictogaster, and C. erythrogaster* are presented in Table 4. *Etheostoma spilotum* exhibited a greater amount of downstream movement. *Percina stictogaster* exhibited a greater amount of upstream movement. *Chrosomus erythrogaster* exhibited an equal amount of downstream and upstream movement.

The number of PIT tagged fish which exhibited movement was similar among all three species, ranging from 11-14%, with an average rate of approximately 13% (Table 1). There was a positive correlation ($r_8 = 0.415$, N = 36, $p < 0.02$; Figure 3) between distance moved and weight of an individual of *E. spilotum*, but not for *P. Stictogaster* or *C. erythrogaster*.

General linear models showed no significant effects from spatiotemporal factors recorded (temperature, light intensity, water depth) on the distance moved by either species. Species, sex, length, and weight were significant variables and covariates among all combined detected movements ($p < 0.05$). Among the best-fit individual models, the covariates length and weight were significant indicators for movement by *E. spilotum* ($r^2 = 0.28$, $p < 0.04$,); whereas season was the best indicator for movements by *P. stictogaster* ($r^2 = 0.35$, $p < 0.01$). No models indicated a significant relationship between any variables or covariates and movement for *C. erythrogaster*.

4. Discussion

Detected movement rates were regularly low among *E. Spilotum, P. stictogaster, and C. erythrogaster*. This is similar to the findings of other studies investigating the movement of small benthic stream fishes (Roberts and Angermeier 2007, Roberts et al. 2008). Low detected movement may be, in part, influenced by the low recapture rate of individuals. Low recapture rates have been suggested to be evidence that stream fishes are indeed mobile and have escaped the area of interest (Gowan et al. 1994). Possible influences for the low movement rate reported in this study could be the loss of PIT tags by individuals, evasion of sampling efforts within the site, mortality, and limited detection ability of antennae systems. It has been shown that implanted PIT tags have a high retention rate among stream fishes (Knaepkens et al. 2007, Bolland et al. 2009); Tag loss was not thought to have been a factor in this study. Individuals evading capture and recapture during sampling efforts likely occurred among all species in this study. Though escapement rates were unknown, it is assumed escapement had little effect on movement rates due to mobile and non-mobile individuals having similar escapement opportunity. The mortality rates of fishes tagged in this study are unknown. It is likely that many of the individuals which were not recaptured or detected using the flatbed antennae grid can be accounted for by some combination of escapement, mortality, and residency.

There are several factors that may have impacted the detection of PIT tagged fish by the antennae grid used in this study. The size of the PIT tags could have possibly limited detection by the antennae. The Biomark HPT8 minichip™ used in this study was the smallest chip on the market at the time of this study. The HPT8 minichip™ was chosen due to the relatively small size of the fish to be tagged. Tag size restrictions due to the size of individuals to be tagged can impact the detection range of an antennae system; The detection range of PIT tags is decreased as the size of the tag itself is decreased (Johnston et al. 2009). In preliminary testing, it was found that the antennae used, in combination with the HPT8, provided a detection range of approximately 0.5 m. This was unlikely a source of detection issues in the study because antennae were placed at sites where the depth was less than 0.5 m. A second potential source of error in detecting PIT tagged fish relates to the detection efficacy of the antennae across the wetted width of the stream. Since the antennae coverage of the wetted stream width varied from approximately 90% to 100% at typical wetted stream widths. This was also an unlikely source of detection issues in the study. Another potential source of decreased detection efficiency in the PIT tag antennae system used in this study is related to the source of power for the antennae and computers that control them. The remote location and limited access of the study area required the systems used to have their own sustainable power source. A solar panel was used in conjunction with two large, dry cell batteries in order to provide the necessary power required for the computers controlling the antennae. Based on manufacturer stated power consumption, at full capacity the two batteries provided would theoretically have the ability to power one system for approximately 25 hours. During this study, solar radiation was used to recharge the batteries, but this can be a limitation on power (Johnston et al. 2009). Solar-generated electrical power is influenced by weather patterns and canopy closure during certain times of the year. Efforts were made in this study to install solar panels in locations where they could receive the maximum available solar radiation throughout periods of full foliage. However, power fluctuations did occur, i.e., there was a lack of self-test information on the recorded data of the antennae systems during certain periods, and could have resulted in not detecting movements of PIT tagged fish.

Independent environmental variables including temperature, light intensity, water depth, and season were generally insignificant in GLM's developed in this study. Although seasonality was insignificant in the models, there was a noted trend of movements occurring more often during the late winter and early spring. This is to be expected due to breeding seasons and has been noted in other studies of movement patterns in stream fish (Roberts and Angermeier 2007, Walker et al. 2013). Studies of the Leopard Darter and Southern Redbelly Dace reported movements occurring in late summer and early fall; movements thought to be related to resource availability and seeking thermal refuge (Scott 1987, Schaefer et al. 2003, Walker et al. 2013). Few such movements were observed throughout the course of this study. Temperature has been suggested to be an ecological cue to seasonal movements by fish (Mundahl and Ingersoll 1983, Roberts and Angermeier 2007). The role of temperature in influencing the movements of fish species tagged in this study is unclear and needs to be further investigated.

General Linear Model's indicated there was a significant difference in the movements exhibited by *E. Spilotum, P. stictogaster, and C. erythrogaster*. This was expected, given the differences in aspects of the life histories of the species, such as where they often spend their time in the water column (Etnier and Starnes 1993a, 1993b, 1993c). The significant difference in distance moved between the sexes may be indicative of behavior associated with breeding activities given that most detected movement occurred during spawning periods of these fishes. It was expected that this male-female significance might be true among each species, however no statistical significance was discovered considering *E. spilotum*; only one female individual was recorded as mobile for *P. Stictogaster* and *C. erythrogaster*, preventing statistical analysis. Concerning *E. spilotum*, this could possibly be due to bias caused by sample size of the population. It is also possible it could be due to some bias based on detection efficiency of the antennae during certain periods where one sex may be more likely to move, behaviorally speaking. Further investigation concerning the influence of sex on movement exhibited by the PIT tagged species is required

Movements by *E. spilotum* in Elisha Creek and Gilbert's Big Creek were found to be related to body weight, with heavier individuals moving further (Figure 3). A similar relationship has been reported for other small, benthic stream fishes (Schaefer et al. 2003, Petty and Grossman 2004, Walker et al. 2013). The greater movements of heavier fish may be related to the availability of resources and a competitive advantage of larger individuals. Small sample size precluded the determination if *P. stictogaster* and *C. erythrogaster* in the streams examined exhibited a relationship of weight to movements.

This study is a representation of two streams in which *E. Spilotum, P. stictogaster, and C. erythrogaster* are located, the results of which provide information on the movement patterns of these species in a headwater stream. The results provide insight on the factors that influence the movements of these species. This information is especially important in terms of the management of *E. spilotum*, a federally listed species, and *P. stictogaster*, a species of greatest conservation need in Kentucky. It emphasizes the importance of body size and season on the movements of these stream fish. This study also identifies the need for further investigation of the influences of sex, light intensity, temperature, and water depth on the movements of these species. This information may be used to drive management decisions concerning habitat availability and connectivity required for the conservation of the studied species, as well as drive the direction and design of further movement studies of these species.

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APPENDIX A

Tables

Table 1. Movements of PIT tagged Kentucky Arrow Darters (*Etheostoma spilotum*), Frecklebelly Darters (*Percina stictogaster*), and Southern Redbelly Dace (*Chrosomus erythrogaster*) from Elisha Creek and Gilbert's Big Creek, Red Bird River, KY, 2013-2015.

Species	Number marked	Number of detections	Number of detected movements	Number of movers	Mean ± SE distance moved (m)
All species	182	63	35	24	795±(148)
E. spilotum	105 (58%)	36 (34%)	20 (57%)	15 (14%)	730±(221)
P. stictogaster	46 (25%)	21 (46%)	11 (31%)	5 (11%)	1114±(211)
C. erythrogaster	31 (17%)	6 (19%)	4 (11%)	4 (13%)	245±(134)

Table 2. Mean length (mm) and weight (g) of all PIT tagged Kentucky Arrow Darters (*Etheostoma spilotum*), Frecklebelly Darters (*Percina stictogaster*), and Southern Redbelly Dace (*Chrosomus erythrogaster*) from Elisha Creek and Gilbert's Big Creek, Red Bird River, KY, 2013-2015. Individuals for which sex was undetermined were included only in the calculations including both sexes.

Species	N	Mean Length ± (SE)	Mean Weight ± (SE)
E. spilotum	105	80±(1)	4.6±(0.2)
Males	56	84±(2)	5.4±(0.3)
Females	45	76±(1)	3.5±(0.2)
P. stictogaster	46	69±(1)	2.8±(0.1)
Males	28	68±(1)	2.9±(0.1)
Females	4	68±(2)	2.7±(0.3)
C. erythrogaster	31	70±(1)	2.6±(0.1)
Males	18	70±(1)	2.5±(0.1)
Females	8	71±(1)	2.7±(0.1)

Table 3. Mean length (mm), weight (g), and total distance moved (m) by PIT tagged Kentucky Arrow Darters (*Etheostoma spilotum*), Frecklebelly Darters (*Percina stictogaster*), and Southern Redbelly Dace (*Chrosomus erythrogaster*) from Elisha Creek and Gilbert's Big Creek, Red Bird River, KY, 2013-2015. The data presented for female Frecklebelly Darters and female Southern Redbelly Dace are values for the one individual who exhibited movement.

Species	N	Mean (±SE) Length	Mean (±SE) Weight	Mean (±SE) Total Distance Moved
E. spilotum	15	80±(4)	4.9±(0.7)	972.70±(390.98)
Males	7	87±(6)	6.5±(1.3)	1096.02±(672.79)
Females	8	73±(2)	3.5±(0.2)	864.76±(479.79)
P. stictogaster	5	70±(1)	2.8±(0.1)	2450.47±(908.14)
Males	3	71±(2)	2.8±(0.2)	3309.59±(1238.68)
Females	1	69	2.7	2097.93
C. erythrogaster	4	71±(1)	2.8±(0.1)	245.36±(134.30)
Males	3	71±(1)	2.9±(0.1)	112.98±(31.96)
Females	1	70	2.5	642.52

Table 4. Proportion of movements occurring in upstream and downstream directions separated by Kentucky Arrow Darters (*Etheostoma spilotum*), Frecklebelly Darters (*Percina stictogaster*), and Southern Redbelly Dace (*Chrosomus erythrogaster*) from Elisha Creek and Gilbert's Big Creek, Red Bird River, KY, 2013-2015.

Species	Number of movements (%)	Sum of total distances (%)	Mean distance moved (SE)
E. spilotum			
Downstream	2 (50)	819.3 (83)	409.65 (232.87)
Upstream	2 (50)	162.15 (17)	81.08 (3.34)
P. stictogaster			
Downstream	5 (45)	5783.76 (47)	1156.75 (390.45)
Upstream	6 (55)	6468.57 (53)	1078.10 (247.47)
C. erythrogaster			
Downstream	11 (55)	11965.54 (82)	1087.78 (347.43)
Upstream	9 (45)	2624.65 (18)	291.63 (170.25)

APPENDIX B

Figures

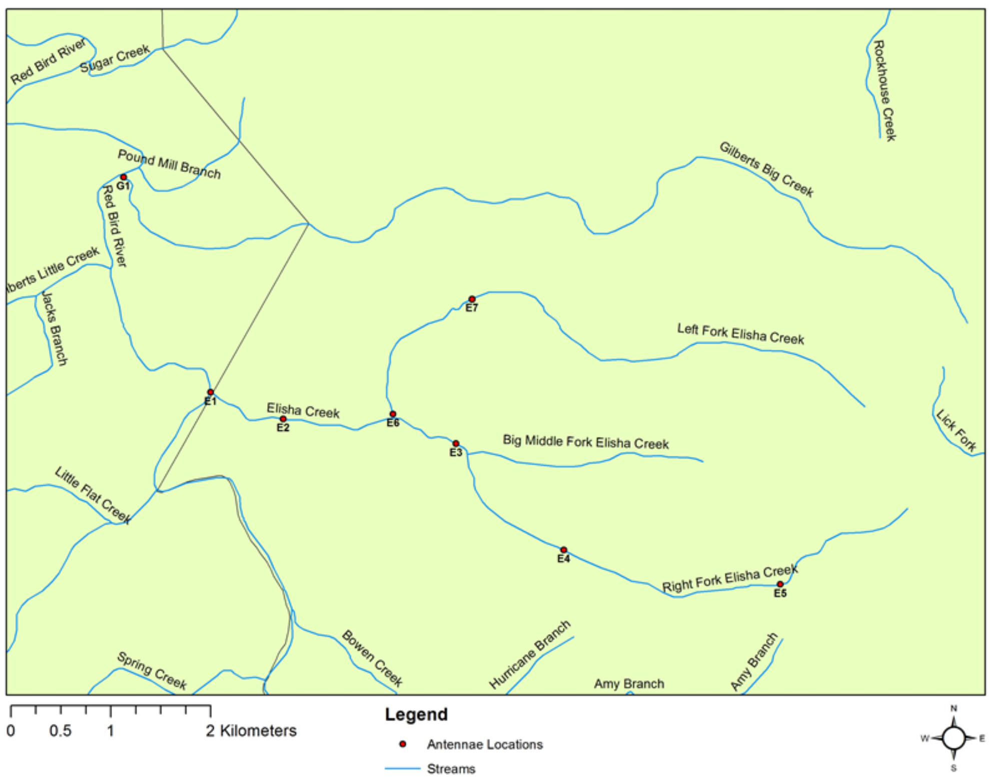

Figure 1. The location of Elisha Creek and Gilbert's Big Creek, tributaries of the Red Bird River, including locations of antennae systems within each stream (G1 and E1-7), in Clay and Leslie counties, KY. (*Source Shapefiles: NHD Plus 100K Streams of Kentucky via kygeonet.ky.gov; Kentucky Counties via kygeonet.ky.gov*)

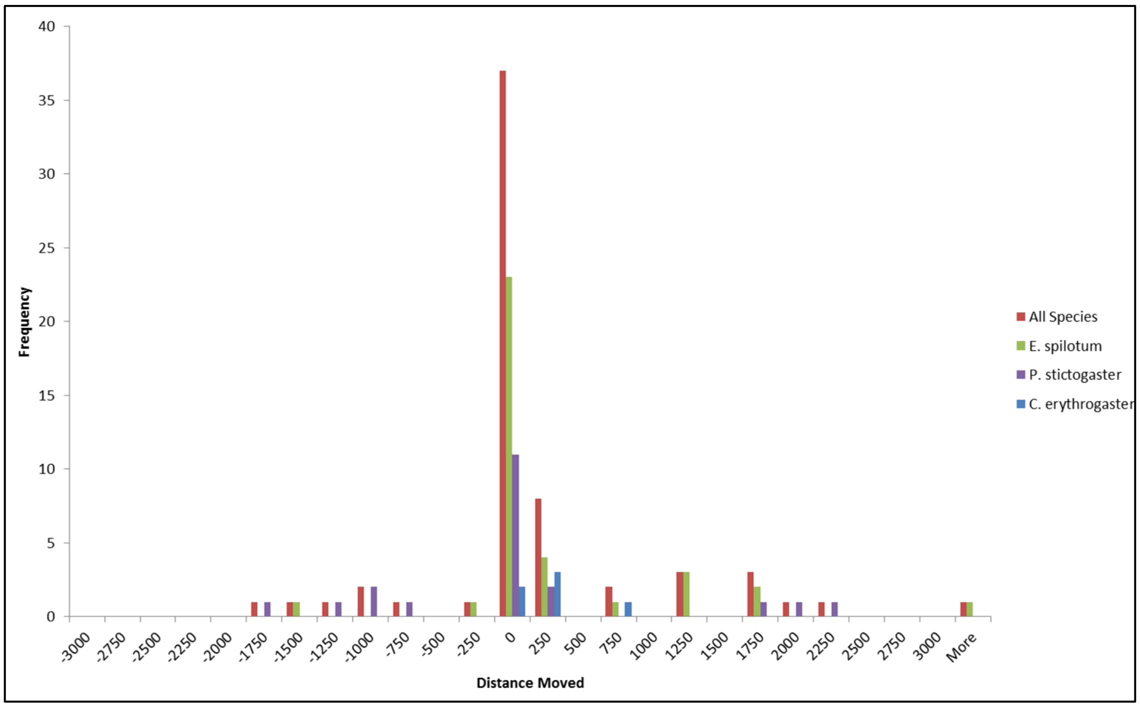

Figure 2. Frequency of distances moved by Kentucky Arrow Darters (*E. spilotum*), Frecklebelly Darters (*P. stictogaster*), and Southern Redbelly Dace (*C. erythrogaster*) from Elisha Creek and Gilbert’s Big Creek, Red Bird River, KY, 2013-2015. Upstream and downstream movement is arbitrarily represented as negative and positive, respectively. Detected movements were classified as any antennae detection event or recapture of a PIT tagged individual.

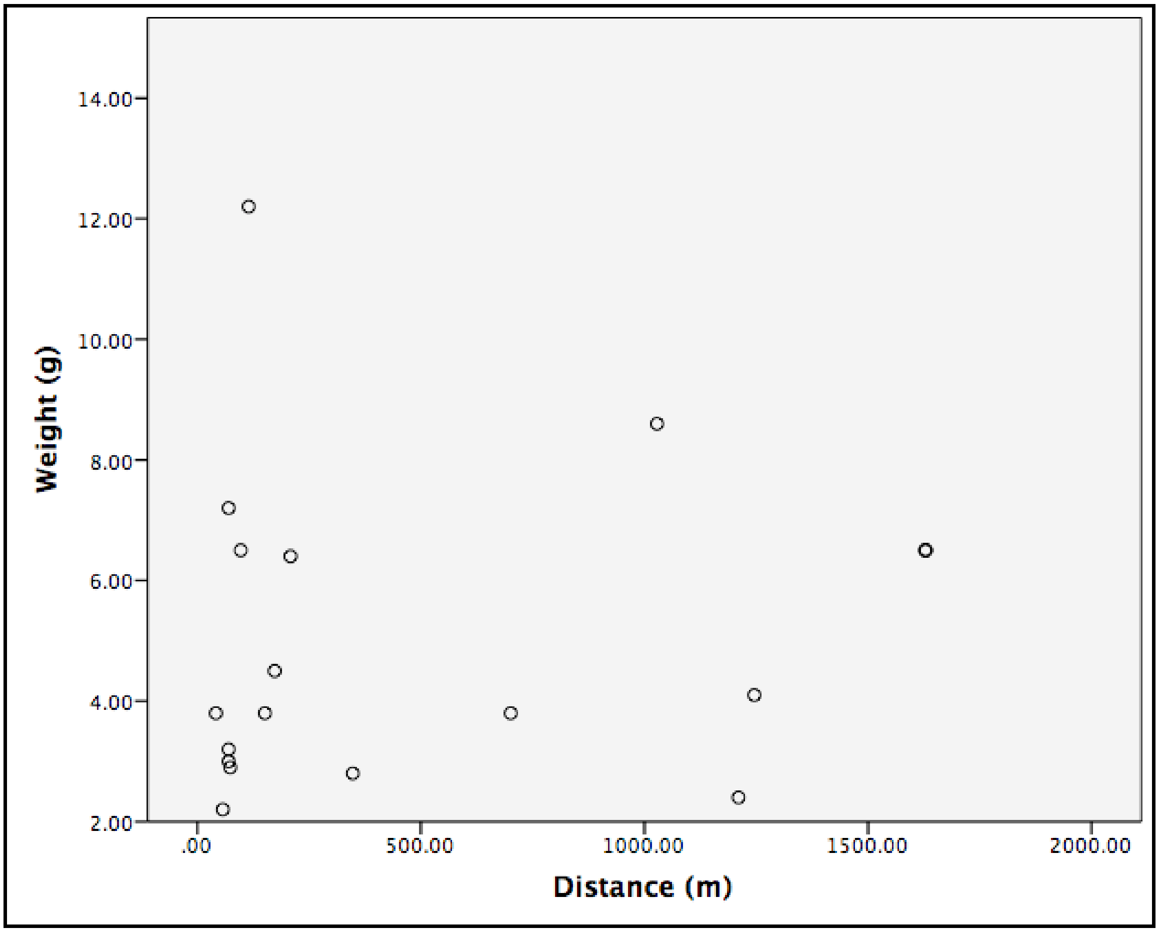

Figure 3. Relationship between an individual's weight and the distance moved for Kentucky Arrow Darters (*E. spilotum*) from Elisha Creek and Gilbert's Big Creek, Red Bird River, KY, 2013-2015. Spearman's correlation coefficient was $r_8 = 0.415$ ($p<0.02$).