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MICROHABITAT PARTITIONING OF AN ASSEMBLAGE OF DARTER SPECIES
WITHIN TWO TRIBUTARIES OF THE TENNESSEE RIVER DRAINAGE IN
NORTHEAST ALABAMA

by

BRIAN THOMPSON

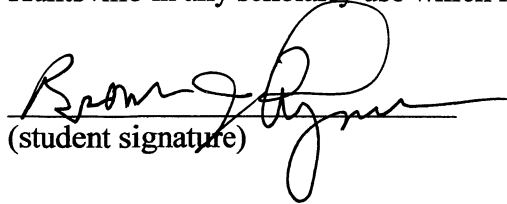
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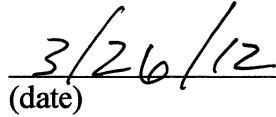
Submitted in partial fulfillment of the requirements
for the degree of Master of Science
in
The Department of Biological Sciences
to
The School of Graduate Studies
of
The University of Alabama in Huntsville

HUNTSVILLE, ALABAMA

2012

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We the undersigned members of the Graduate Faculty of the University of Alabama in Huntsville, certify that we have advised and/or supervised the candidate on the work described in this thesis. We further certify that we have reviewed the thesis manuscript and approve it in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences.

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ABSTRACT
The School of Graduate Studies
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Degree Master of Science College/Dept. Science/Biological Sciences

Name of Candidate Brian Thompson

Title Microhabitat Partitioning of an Assemblage of Darter Species within two tributaries of the Tennessee River Drainage in Northeast Alabama

Habitat partitioning among syntopic darter species (Percidae: Etheostomatini) was examined in the Flint River and Estill Fork of the Paint Rock River in northeast Alabama from September 2010 through September 2011. Substrate composition, depth, and water velocity were important variables by which species segregated. Niche overlap values also indicated a high degree of habitat partitioning among species at both study sites, though a substantial amount of overlap was seen between *Etheostoma simoterum* and *E. caeruleum* at the Estill Fork site. Segregation among species was present throughout the year, but the significance of differences in habitat preferences did vary seasonally, especially during the post-spawn period when habitat heterogeneity was reduced. Intra-specific variation in habitat utilization was present among some species, with males generally preferring higher flow and coarser substrates than females. Habitat segregation appears to be an important mechanism that allows for coexistence of ecologically similar species.

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CHAPTER I

INTRODUCTION

Darters are a diverse group of fishes within the family Percidae (Page 1983). Fishes within this family are characterized by a well-developed spinous section of the dorsal fin that is separated from the second section of the fin by a space or deep notch; usually two anal spines; pelvic fins in the thoracic position, with one spine and five rays; ctenoid scales; and five to eight branchiostegal rays (Boschung & Mayden 2004, Page 1983). With over 250 species in North America, the Percidae is the second richest family of fishes on the continent, following the minnows in the family Cyprinidae (Berra 1981, Boschung & Mayden 2004, Near et al. 2011). The four genera of darters comprise the tribe Etheostomatini, are all endemic to North America east of the continental divide, and can be found in a wide variety of aquatic habitats (Boschung & Mayden 2004, Page 1983). Darters differ from other members of their family by being smaller in size, ranging from 2.5 to a maximum of 16.8 cm, exhibiting some degree of dorso-ventral compression, and by having a dramatically reduced or absent gas bladder (Kuehne & Barbour 1983).

The reduction or loss of a gas bladder is an adaptation in response to darters being predominantly benthic, where there is no need for neutral buoyancy (Page 1983). Though some species are found in other habitats, the main selective force for darters becoming benthic was to occupy shallow riffle systems (Kuehne & Barbour 1983). Riffles are generally devoid of large predators and are high in food availability as

invertebrates use these highly oxygenated riffles for larval growth (Kuehne & Barbour 1983). These characteristics make riffles ideal habitats for fish species adapted to exploit them. Occupation of these shallow areas by darters has led to their reduction in size and body compression in order to increase maneuverability. Living on the bottom of a riffle lessens exposure to current and is accompanied by this streamlining. A flattened venter, seen in many species, reduces exposure to current by shifting the center of gravity closer to the substrate, where the velocity of the water is less than the middle of the water column (Hynes 1970).

Of the four genera of darters, *Etheostoma* is by far the most morphologically and ecologically diverse (Page 1983). With 248 species, it is the most speciose genus of fishes in North America (Near et al. 2011). This genus differs from other genera by lacking the modified scales found along the venter of the genus *Percina* and by lacking the translucent skin characteristic of the genus *Ammocrypta*. *Etheostoma* is represented by 15 subgenera in Alabama (Boschung & Mayden 2004), 5 of which are encompassed by this study.

The subgenus *Etheostoma* is a relatively ancestral group of darters in the genus *Etheostoma* that all inhabit fast riffles systems (Page 1983). Members of this group are characterized by a complete, straight lateral line, complete cephalic canals, connected branchiostegal membranes, and usually four to seven dorsal saddles on the body (Richards 1966). Eyes are set high on a generally large broad head with a short snout that usually has a well-developed frenum (Kuehne & Barbour 1983). Males of this subgenus are usually bright green and are larger than females in several species (Kuehne & Barbour 1983, Richards 1966). Two species of this subgenus are included in this study.

The greenside darter (*Etheostoma blennioides*) is the largest species of the genus *Etheostoma*, reaching over 120 mm in standard length (SL) (Page 1983). It can be distinguished from all other species of darter by a fusion of the skin over the maxilla with the skin of the snout (Kuehne & Barbour 1983, Page 1983). It is further characterized by 5-8 saddles on the dorsum and 6-10 lateral blotches, often shaped like a W (Kuehne & Barbour, Page 1983). Individuals are yellow-green, but breeding males have a dark green head and breast with dark green bars on the sides crossing over the back. The first dorsal fin has a red base and all fins are green (Page 1983). *Etheostoma blennioides* is found in a variety of habitats, but is most commonly seen in medium-sized creeks over rubble-strewn riffles (Kuehne & Barbour 1983). Adult diet consists primarily of Dipteran larvae of the family Chironomidae (midges), but also includes mayfly (Ephemeroptera), black fly (Simuliidae), and caddisfly (Trichoptera) larvae (Hlohowskyj & White 1983).

The banded darter (*E. zonale*) is a moderate-sized darter reaching 62 mm SL (Kuehne & Barbour 1983). It differs from other species of the subgenus *Etheostoma* by having only five branchiostegal rays, as opposed to six in other species (Kuehne & Barbour 1983). It has modally six dark dorsal saddles and 9-13 green vertical bars on the side of a yellow-green body. Dorsal fins are red basally (Page 1983). A distinct pre-orbital bar extends on the lip, while a diffuse suborbital bar extends across the cheek (Kuehne & Barbour 1983). Males are larger (Lachner et al. 1950) and breeding males are dark green with bright green vertical bands encircling the body. Dorsal fins are green over a red basal bar (Page 1983). *Etheostoma zonale* has an extensive range and lives in gravel and rock riffles in moderate-sized streams (Page 1983). In Alabama, the largest populations are associated with mats of aquatic vegetation, particularly *Podostemum* spp.

(Mettee et al. 1996). Adult diet consists mainly of midges and mayfly larvae, with lesser amounts of blackfly and caddisfly larvae (Cordes & Page 1980).

The subgenus *Ulocentra* is considered a slightly more advanced sister-group to the subgenus *Etheostoma* (Richards 1966). The characters which usually distinguish *Ulocentra* from *Etheostoma* are as follows: a blunter snout with frenum absent or slightly developed; lack of breeding tubercles; usually five branchiostegal rays; eight or nine dorsal blotches; and more red on the fins and body (Richards 1966). Additionally, the lateral line is usually slightly arched anteriorly and palatine teeth are always absent (Bouchard 1977). Two species of this subgenus are included in the present study.

The black darter (*E. duryi*) is a moderate-sized darter reaching 60 mm SL (Kuehne & Barbour 1983). The mid-lateral blotches in this species often become fused into a mid-lateral black stripe that differentiates it from other members of the subgenus (Page 1983). No premaxillary frenum is present and the brown or yellow dorsum contains eight saddles. The fourth saddle extends ventrally and connects to the mid-lateral stripe (Page 1983). The first dorsal fin has a bright red ocellus basally and a red marginal or submarginal band. Breeding males are orange ventrally and dorsally and second dorsal fins are mostly red (Kuehne & Barbour 1983, Page 1983). *Etheostoma duryi* is found in Alabama in riffles and pools over gravel, rubble, and slabrock in small and medium-sized streams. Diet consists of midges, and mayfly, caddisfly, and blackfly larvae (Mettee et al. 1996).

The Tennessee snubnose darter (*E. simoterum*) differs from *E. duryi* by having unconnected lateral blotches and the premaxillary frenum is present and moderately developed (Page 1983). A preorbital bar is distinct in small specimens, becoming more

diffuse in larger individuals (Kuehne & Barbour 1983). Pigmentation in the soft dorsal fin produces a series of bands parallel to the fin margin. Anal, pelvic, and pectoral fins are virtually devoid of pigment (Kuehne & Barbour 1983). The breeding male is blue-green overall with orange on the ventral and posterior areas of the body (Page 1983).

Etheostoma simoterum lives in rock and gravel-bottomed pools with moderate current in small to medium-sized streams (Page & Mayden 1981). In Brush Creek, Tennessee, Page and Mayden (1981) most commonly found *E. simoterum* in bedrock pools below a riffle, though some individuals were found in riffles or in slabrock pools along the margins of the stream. Diet was found to consist predominantly of midges along with mayfly and caddisfly larvae.

The subgenus *Nothonotus* is a monophyletic group of primarily deep-bodied riffle-inhabiting species distributed in much of the Ohio, Cumberland, Tennessee, and Arkansas river systems (Wood 1996, Zorach 1972). Species within this group have an elongate, compressed body; deep caudal peduncle; head of moderate length; well-developed frenum; teeth present on the vomer, jaws, and palatine; usually six branchiostegals whose membranes are separate or narrowly conjoined (Zorach 1972). Ten to 15 horizontal lines between scale rows are usually present on the posterior one-third to two-thirds of the body (Zorach 1972). The lateral line is straight and complete and cephalic canals are uninterrupted. Red spots are usually present on the sides of males and females, with breeding males often with shades of deep blue-green (Zorach 1972). One species, the redline darter (*E. rufilineatum*), is included in this study.

Etheostoma rufilineatum can be distinguished from other species of *Nothonotus* by a divided suborbital bar and horizontal dark dashes on the cheek and opercle (Page

1983). The body is yellow to tan with 9-10 dark dorsal saddles. Red spots are present between well-developed horizontal lines on males, and brown to orange spots are present on females (Page 1983). Breeding males are brightly colored with dark blue breast, orange venter, and red-orange bands on the fins. Preferred habitat of *E. rufilineatum* is swift riffles in streams and moderate-sized rivers and it tolerates shallower riffles than do other species of *Nothonotus* (Zorach 1970). Diet consists predominately of midges and blackfly larvae, as well as mayfly, and caddisfly larvae and water mites (Boschung & Mayden, Page 1983).

The polyphyletic subgenus *Oligocephalus* is the largest group in the genus *Etheostoma* (Lang & Mayden 2007). Shared characteristics include: a broad frenum; separate to moderately connected gill membranes; head rather small compared to body; and males usually with a submarginal red band in the spiny dorsal fin (Kuehne & Barbour 1983). The lateral line is usually incomplete and cephalic canals can be interrupted or uninterrupted. Breeding males of this group are usually brightly colored with red, blue, and green on the body and unpaired fins (Boschung & Mayden 2004). The rainbow darter (*E. caeruleum*) is the sole representative of this subgenus in this study.

Etheostoma caeruleum is fairly large and robust, reaching 65 mm SL. The dorsum has 6-10 saddles, with the two most intense ones located just in front of and behind the spiny dorsal fin. Lateral bars number 9-14 (Kuehne & Barbour 1983). The head is conical with a well-defined frenum, and branchiostegals are separate to slightly connected. Breeding males are some of the most colorful darters to be found in Alabama. They have blue and red on the sides of the body and head, while the gill membranes are

typically orange (Mettee et al. 1996). Adult rainbow darters inhabit swift riffles over coarse gravel or rubble (Winn 1958). The diet of *E. caeruleum* consists of midges and mayfly larvae, as well as caddisfly and stonefly (Plecoptera) larvae (Hlohowskyj & White 1983).

The subgenus *Catanotus* is the most derived group of darter species in which all members share the distinct attribute of laying their eggs on the underside of stones (Page 1983). The lateral line is incomplete and the cephalic canals are generally interrupted (Kuehne & Small 1971). Bodies in most species are fairly elongate. The snout is pointed and has a well-defined frenum. The first dorsal fin is much shorter than the second, with thickened fleshy tips in some species (Kuehne & Small 1971). One species of *Catanotus*, the stripetail darter (*E. kennicotti*), is included in this study.

Etheostoma kennicotti reaches a maximum size of about 52 mm SL within the Tennessee River drainage (Page 1983). The second dorsal fin and caudal fin have black bands that give it its common name. *Etheostoma kennicotti* has a tan dorsum with 6-7 brown saddles and a white venter. On the side are 9-11 vertically oblong blotches (Page 1983). It can be distinguished from other *Catanotus* species in Alabama by its deep caudal peduncle, short spiny dorsal fin with a black or brown submarginal bar and yellow fleshy knobs, and moderately connected branchiostegals (Mettee et al. 1996). Adults usually inhabit pools with slabrock substrate in small to moderate streams (Page 1975, Page 1983). Diet consists mainly of midges and mayfly larvae, as well as stonefly larvae (Boschung & Mayden 2004, Page 1975).

Given their specialization for living in shallow riffle areas, most darters have strict habitat requirements (Page 1983). Additionally, the high diversity of darter species

often present within riffle systems would lead one to expect that competition between darter species is high. This high competition leads one to predict a high degree of habitat segregation between species. Studies employing qualitative or univariate techniques to quantify habitat use among darters have, however, found high degrees of overlap (Englert & Segher 1983, Hlohowskyj & Wissing 1986). Hlohowskyj and White (1983) also found a high degree overlap for food resource partitioning among three syntopic species in Ohio.

More recent studies, which have addressed multiple habitat variables, have found a greater degree of habitat partitioning (Henry & Grossman 2008, Kessler & Thorp 1993, Stauffer et al. 1996, Welsh & Perry 1998). Between two closely related species in Kentucky, Kessler and Thorp (1993) found habitat partitioning based on gradients of substrate roughness and size, depth, and flow velocity. Another study demonstrated that habitat partitioning among three darter species in Georgia varied seasonally based on habitat availability (Henry & Grossman 2008). Stauffer et al. (1996) found varying degrees of habitat specialization among a diverse assemblage of darters in Pennsylvania.

To date, no known studies addressing habitat partitioning among darter species within the Tennessee River drainage in Alabama have been conducted. This drainage is ideal for this type of study as it is found within the center of darter diversity (Matthews 1998, Page 1983). A total of 32 species are known to inhabit the Alabama portion of Tennessee River and its tributaries (Boschung & Mayden 2004). Many portions of this drainage are also threatened with impairment from agricultural runoff and urban sprawl, which could adversely impact one of the most diverse fish assemblages in the United States (Tennessee Valley Authority 2008). Achieving a greater understanding of habitat

utilization of darter species within this watershed would aid conservation efforts by providing information on species' habitat requirements.

The purpose of this study is to examine microhabitat utilization and partitioning among a diverse group of darter species within two tributaries of the Tennessee River. Several null hypotheses are proposed about the data collected for this study: 1) Darter species present within each study site are evenly distributed in abundance and diversity across all habitat variables measured and all periods analyzed. 2) Overlap of habitat preference between species is significant across all species interactions. 3) There is no seasonal effect on habitat utilization among species. 4) There is no difference in habitat utilization between sexes of a species sampled.

CHAPTER II

MATERIALS AND METHODS

Data were collected from two study sites. The Estill Fork study site is a continuous 60 meter length of stream located just downstream of its intersection with County Road 140 (34°57'54.78" N, 86°09'12.83" W). Estill Fork is a pristine, second order stream and is one of the four main tributaries that form the Paint Rock River, in Jackson County, Alabama. The Paint Rock River watershed is a subwatershed of the Tennessee River Basin in the Cumberland Plateau region of Tennessee and Alabama. Sampling at Estill Fork was conducted on 11 September and 27 November in 2010 and 22 January, 21 March, 20 May, 15 July, and 24 September in 2011. The Flint River study site is a continuous 60 meter length of stream located just downstream of the confluence of Brier Fork and Mountain Fork, the two main tributaries which form the Flint River, in Madison County, Alabama (34°49'19.74" N, 86°28'57.81" W). The Flint River is similar to Estill Fork in darter species composition but is a fourth order stream with generally higher flow and some impairment due to urban sprawl and agricultural runoff. The Flint River watershed is a subwatershed of the Tennessee River and encompasses portions of the Cumberland Plateau and Highland Rim section of the Interior Low Plateaus. Sampling at the Flint River was conducted on 25 September, 26 November, and 22 December during 2010 and on 26 February, 25 April, 17 June, 29 July, and 28 August during 2011.

Habitat Data Collection

For Estill Fork, a total of fifteen transects were established perpendicular to the flow of the stream at 4-m intervals within the study area. Stream width was generally 10-15m. For the 11 September 2010 sampling event, habitat data were collected for each transect due to a reduction in stream width due to lack of rain. For all other sampling events, data were collected at every other transect for a total of eight transects.

Along each transect, a meter tape was stretched across the stream and depth and flow velocity were recorded at 1m intervals using a Geopacks flowmeter and graduated wading rod (Geopacks 2010). Flow readings were recorded 6 cm from the bottom as this provided the minimum distance from the bottom necessary to allow the flowmeter impeller to rotate freely. Readings were taken at locations free of immediate upstream obstructions, such as large boulders, that would alter flow. Substrate composition was qualitatively classified within each transect sampling for darter species by visually determining percent cover of each substrate class based on a modified version of the Wentworth Scale of classes of rock size. Substrate was separated into five classes: boulder (>256 mm), cobble (64-256 mm), pebble/granule (2-64 mm), sand (0.5-2 mm), and silt (<0.5 mm).

For the Flint River study site, three transects were established at 4-m intervals as at Estill Fork. Stream width at the Flint River was 30-50m, much greater than at Estill Fork, so only three transects were required to attain a comparable data set. A fourth transect was established approximately 40m upstream in a separate riffle, but sampling was only conducted during the November and December 2010 sampling events.

Darter Species Sampling

For each study site, darters were collected using a 3.6 m seine with 3 mm mesh. Sampling was conducted following depth and flow data collection starting with the furthest downstream transect, repeating the process at each subsequent upstream transect. Along each transect, the seine was planted with the brails angled downstream to allow for the current to open the bag of the seine. The person(s) holding the seine also inspected the lead line to ensure it was pinned to the substrate. Starting approximately four meters upstream from the seine, a separate group of two to three samplers would then disturb the substrate by kicking vigorously while moving downstream toward the net. This method, known as the “darter dance,” drives fish downstream and into the seine. The seine was lifted from the water once “dancers” reached the lead line. This process was repeated every three meters along the length of the transect to ensure saturation of habitat gradients. Darter species caught were identified to species, classified as male, female, or juvenile and released.

Data Analysis

For each three-meter section of transect sampled, average depth and average flow velocity were calculated and correlated, along with percent aerial coverage of each substrate class, to darter capture data from the seine sample. Data from each sample event were then grouped into four temporal categories for analysis: 1) full year (data from all sampling events), 2) pre-spawn (October – February), 3) spawn (March – June), and 4) post-spawn (July – September). These categories are based on the spawning period in Alabama for most species analyzed occurring between March and June, with the exception of *E. caeruleum*, which spawns from April to July (Mettie et al. 1996). The

average and standard deviation for depth, flow velocity, and percent coverage for each substrate class were then calculated for each darter species for each temporal category. Only those darter species with five or more records for each time period being analyzed were used in analysis.

Since this study is examining darter species' use of multiple habitat variables, multivariate techniques were used in analysis. All analyses were performed for each temporal category. First, a Canonical Correspondence Analysis (CCA) was performed using CANOCO (V.4) to explore the relationship between species prevalence and habitat variables (ter Braak & Smilauer 1998). A Principal Component Analysis (PCA) was then performed on habitat variables using IBM SPSS 19 (SPSS, Inc. 2010) to reduce the data to a number of principal components that could be used for further analysis.

Prior to PCA, habitat data from both study sites were square root transformed due to moderate positive skewness in most variables. Also, due to the relatively infrequent presence of silt, it was excluded from PCA for both sites. Bartlett's Test of Sphericity was significant for both the Flint River (452.97, $p < 0.001$) and Estill Fork (519.31, $p < 0.001$) and the Kaiser-Meyer-Olkin values (KMO) were above the 0.500 threshold. A Varimax rotation with Kaiser normalization was performed on the resulting principal components. Mean factor scores of each principal component (PC) for each species were then calculated and plotted.

Mean factor scores for each species were analyzed using both univariate and multivariate techniques. First, a one-way Analysis of Variance (ANOVA) and Tukey's HSD post-hoc test (SPSS 19) was performed on each principal component to determine if any significant difference existed among species analyzed. The assumption of

homogeneity of variance was not met for the Flint River analysis and for analysis of the full year and spawn period for Estill Fork, so a Welch's test and Brown-Forsythe test were performed with each of these analyses to ensure robustness of any significance difference in mean factor scores.

To test if any significant difference in habitat utilization existed between sexes of a species was present, a step-wise Discriminant Function Analysis (DFA) (SPSS 19) was performed. The analysis was performed on the mean factor scores of the two sexes of each species for the pre-spawn, spawn, and post-spawn periods to determine if habitat utilization differed based on sex and reproductive activity.

Finally, in order to get an idea of the community structure within each study site, niche overlap between co-occurring species was calculated (NICHE 6.0) using Pianka's (1973) Symmetrical Niche Overlap equation:

$$C_m = 2\sum_i (p_{xi}p_{yi}) / (\sum_i p_{xi}^2 + \sum_i p_{yi}^2)$$

where p_{xi} and p_{yi} represent the proportions of the i^{th} resource used by species x and y.

The resources used in this case would be each three-meter section of transect sampled.

Niche overlap was calculated between species for each sample event and averaged to achieve an average niche overlap for each sample period. Niche overlap values less than 0.500 would indicate significant partitioning between species.

CHAPTER III

RESULTS

Flint River

While eight darter species were caught during sampling, only three species were found in sufficient numbers for analysis. *Etheostoma zonale* was by far the most prevalent species (n = 330) and was found in the central portion of the river, often associated with aquatic vegetation growing on boulders. *Etheostoma duryi* (n = 122) was found predominantly close to the riverbanks in shallower, calmer water. *Etheostoma rufilineatum* (n = 89) was mostly found in a swift, shallow side channel along the eastern bank that had a large cobble and pebble/gravel component in the substrate. The greatest number of individuals for each species was collected during the pre-spawn period and was lowest during spawning (Table 3.1).

Table 3.1. Summary statistics for the seven microhabitat variables at the Flint River study site. Variable means are located on the first line and standard deviations are enclosed in parentheses on the second line.

Sample period	Sample size	Depth (cm)	Flow velocity (m sec-1)	%Boulder	%Cobble	%Pebble/ Gravel	%Sand	%Silt
Full year								
<i>Etheostoma duryi</i>	122	23.66 (7.09)	0.3503 (0.1801)	43.85 (24.52)	24.67 (18.64)	16.93 (14.39)	13.77 (10.59)	1.19 (3.15)
<i>Etheostoma rufilineatum</i>	89	28.29 (12.17)	0.5278 (0.2094)	41.63 (21.57)	34.04 (17.96)	16.91 (7.89)	7.36 (7.03)	0.06 (0.53)
<i>Etheostoma zonale</i>	330	29.77 (10.75)	0.3966 (0.1721)	63.23 (16.11)	13.94 (9.82)	12.94 (10.35)	9.77 (9.45)	0.39 (1.35)
Prespawn								
<i>Etheostoma duryi</i>	58	25.59 (6.08)	0.4141 (0.1741)	40.09 (23.52)	31.21 (21.07)	14.22 (9.40)	13.45 (8.12)	1.03 (3.21)
<i>Etheostoma rufilineatum</i>	47	33.06 (11.91)	0.4922 (0.1525)	41.38 (24.88)	33.83 (20.62)	15.21 (9.15)	9.57 (6.41)	- -
<i>Etheostoma zonale</i>	168	34.57 (9.10)	0.4754 (0.1669)	63.63 (11.49)	14.61 (10.86)	10.12 (5.97)	11.64 (7.90)	- -
Spawn								
<i>Etheostoma duryi</i>	21	27.03 (9.35)	0.31 (0.20)	37.62 (18.35)	28.81 (12.44)	15.95 (7.35)	17.62 (12.21)	- -
<i>Etheostoma rufilineatum</i>	30	24.58 (10.93)	0.5892 (0.2488)	41.50 (18.30)	35.17 (15.62)	17.83 (5.36)	5.50 (7.92)	- -
<i>Etheostoma zonale</i>	33	35.62 (12.48)	0.3552 (0.1503)	63.33 (16.23)	14.39 (12.04)	15.00 (5.45)	7.27 (5.01)	- -
Postspawn								
<i>Etheostoma duryi</i>	43	19.42 (4.88)	0.29 (0.15)	51.98 (26.73)	13.84 (11.69)	21.05 (20.57)	12.33 (12.36)	1.98 (3.64)
<i>Etheostoma rufilineatum</i>	12	18.86 (6.68)	0.5139 (0.2726)	42.92 (15.73)	32.08 (12.33)	21.25 (6.08)	3.33 (3.26)	0.42 (1.44)
<i>Etheostoma zonale</i>	129	22.01 (7.09)	0.3046 (0.1305)	62.67 (20.69)	12.95 (7.49)	16.09 (14.14)	7.98 (11.50)	1.01 (2.01)

Canonical Correspondence Analysis

Canonical Correspondence Analysis was used to compare species prevalence with the habitat variables measured. When the entire data set was examined (full year), a Monte Carlo test exhibited statistical significance for the first axis ($F = 30.94$, $p = 0.002$) as well as all axes combined ($F = 8.19$, $p = 0.002$). The first two canonical axes accounted for 93.3 percent of the variance of the relationship between species and environmental variables. The first canonical axis accounted for 60.5 percent of the total variance and was dominated by the presence of cobble on the positive end and boulder on the negative end. The second axis accounted for 32.8 percent of the total variance and was dominated positively by the presence of sand and negatively by average flow and average depth (Table 3.2).

Table 3.2. Canonical Correspondence Analysis of Flint River darter species and habitat variables for the full year showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.276	0.149
species-environment correlations	0.637	0.467
Cumulative percentage variance of species data	14.2	21.9
Cumulative percentage variance of species-environment relation	60.5	93.3
Environmental Variables	Weighted Eigenvalues	
Average Depth	-0.2902	-0.4457
Average Flow Velocity	0.2568	-0.6855
% Boulder	-0.8794	-0.1845
% Cobble	0.9379	-0.1937
% Pebble/Gravel	0.3183	0.0923
% Sand	0.0425	0.5614
% Silt	0.0761	0.4947

When data were analyzed by reproductive period, a Monte Carlo test showed statistical significance for the first axis ($F = 20.54$, $p = 0.002$) and combined axes ($F = 5.60$, $p = 0.002$) for the pre-spawn period as well as the spawning period (first axis: $F = 10.38$, $p = 0.002$; combined axes: $F = 4.50$, $p = 0.002$). For the pre-spawn period, the first two axes accounted for 94.2 percent of the total variance. The first axis accounted for 68.6 percent of total variance, and was dominated positively by cobble presence and in the negative direction by the presence of boulder. The second axis accounted for 25.6 percent of total variance and was dominated positively by sand and silt presence and negatively by average flow and average depth (Table 3.3).

Table 3.3. Canonical Correspondence Analysis of Flint River darter species and habitat variables for the pre-spawn period showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.346	0.129
species-environment correlations	0.738	0.437
Cumulative percentage variance of species data	19.3	26.4
Cumulative percentage variance of species-environment relation	68.6	94.2
Environmental Variables	Weighted Eigenvalues	
Average Depth	-0.5155	-0.6361
Average Flow Velocity	-0.1988	-0.2985
% Boulder	-0.9521	0.2400
% Cobble	0.8463	-0.4285
% Pebble/Gravel	0.4608	-0.1663
% Sand	0.0734	0.4148
% Silt	0.3757	0.4429

For the spawning period, the first two axes accounted for 96.5 percent of total variance. The first axis accounted for 57.6 percent of the variance and was dominated in the positive direction by boulder presence and negatively by cobble presence. The

second axis accounted for 38.9 percent of the variance and was dominated positively by sand presence and negatively by average flow (Table 3.4).

Table 3.4. Canonical Correspondence Analysis of Flint River darter species and habitat variables for the spawn period showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.469	0.317
species-environment correlations	0.752	0.617
Cumulative percentage variance of species data	21.5	35.9
Cumulative percentage variance of species-environment relation	57.6	96.5
Environmental Variables	Weighted Eigenvalues	
Average Depth	0.5980	0.0394
Average Flow Velocity	-0.5994	-0.6937
% Boulder	0.8328	-0.1759
% Cobble	-0.8815	-0.1472
% Pebble/Gravel	-0.3423	-0.1747
% Sand	-0.0630	0.8334
% Silt	-	-

A Monte Carlo test of the canonical axes for the post-spawn period showed no significance for the first axis ($F = 6.93$, $p = 0.106$) or combined axes ($F = 1.83$, $p = 0.066$). The first two axes accounted for 94.5 percent of the total variance. The first axis accounted for 59.8 percent of the variance and was dominated positively by cobble presence and average flow velocity (Table 3.5).

Table 3.5. Canonical Correspondence Analysis of Flint River darter species and habitat variables for the post-spawn period showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.202	0.117
species-environment correlations	0.501	0.495
Cumulative percentage variance of species data	12.2	19.3
Cumulative percentage variance of species-environment relation	59.8	94.5
Environmental Variables	Weighted Eigenvalues	
Average Depth	-0.1130	-0.4967
Average Flow Velocity	0.5937	-0.0592
% Boulder	-0.2918	-0.6633
% Cobble	0.8382	0.3102
% Pebble/Gravel	0.0876	0.4070
% Sand	-0.2330	0.4481
% Silt	-0.1169	0.4722

Figures 3.1 through 3.4 show the results of the CCA depicted graphically showing the first and second canonical axes. The difference in the sexes for *E. duryi* during the spawn period and *E. rufilineatum* during the post-spawn period were not included in the analysis because less than five individuals of a particular sex were caught for those sample periods.

For the full year as well as for each reproductive period, *E. duryi* was closely associated with smaller substrate classes (sand, silt) and negatively correlated with average depth and flow velocity. There appears to be some separation for habitat preference between the sexes for the prespawn period.

Etheostoma rufilineatum is strongly associated with average flow and cobble. For the pre-spawn period, females appeared to prefer slightly deeper water than males. During the spawn period, males appear to prefer areas of greater flow than do females.

Etheostoma zonale is strongly associated with boulder presence and, to a lesser degree, average depth, although this relationship is somewhat diminished during the post-spawn period. The greatest habitat partitioning between sexes occurred during the spawn period, when females appear to be found in deeper water.

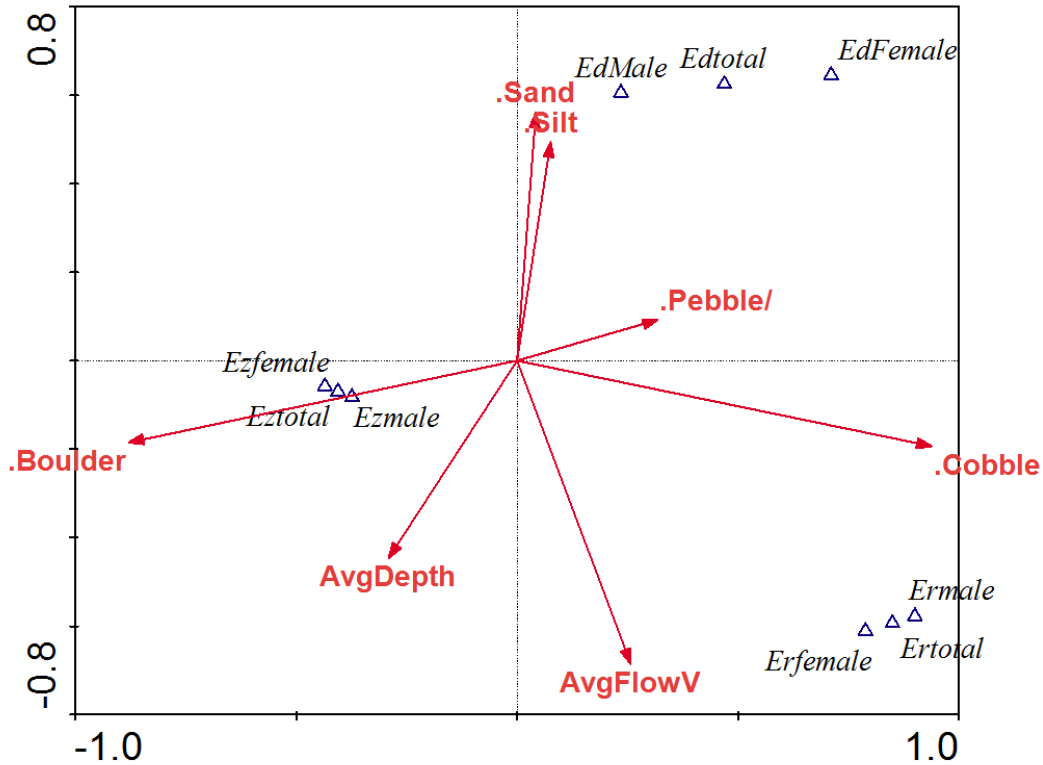


Figure 3.1. CCA-ordination plot of Flint River data for the full year depicting weighted loadings (\rightarrow) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Ed = *E. duryi*; Er = *E. rufileineatum*; Ez = *E. zonale*.

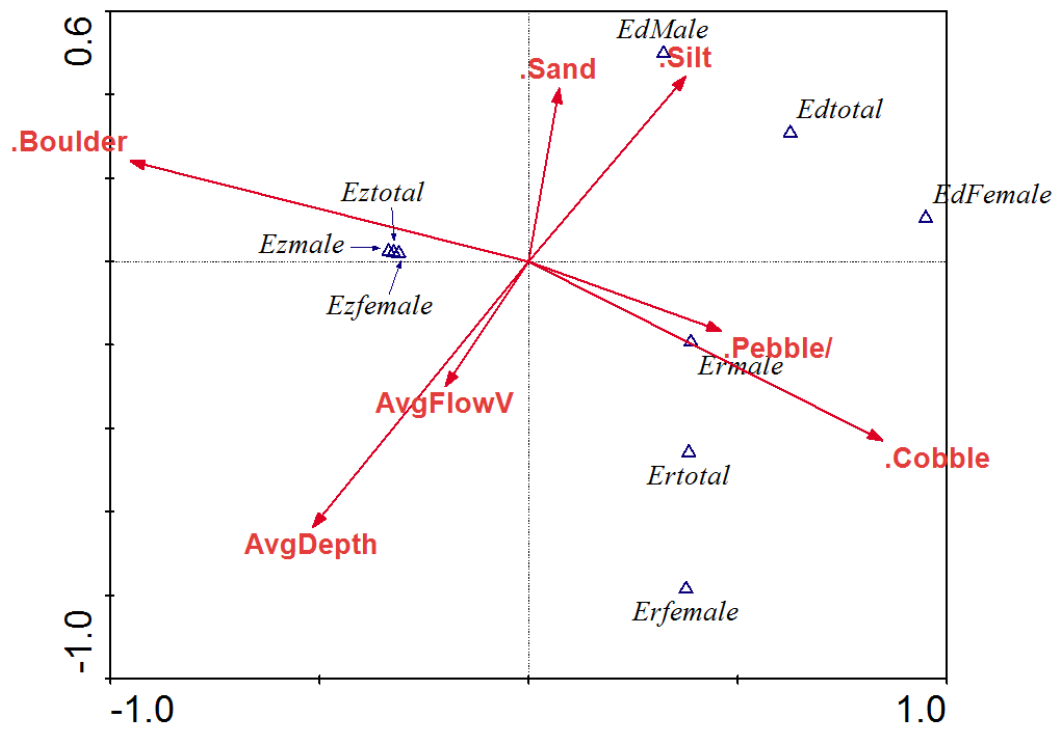


Figure 3.2. CCA-ordination plot of Flint River data for the pre-spawn period depicting weighted loadings (\rightarrow) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Ed = *E. duryi*; Er = *E. rufilineatum*; Ez = *E. zonale*.

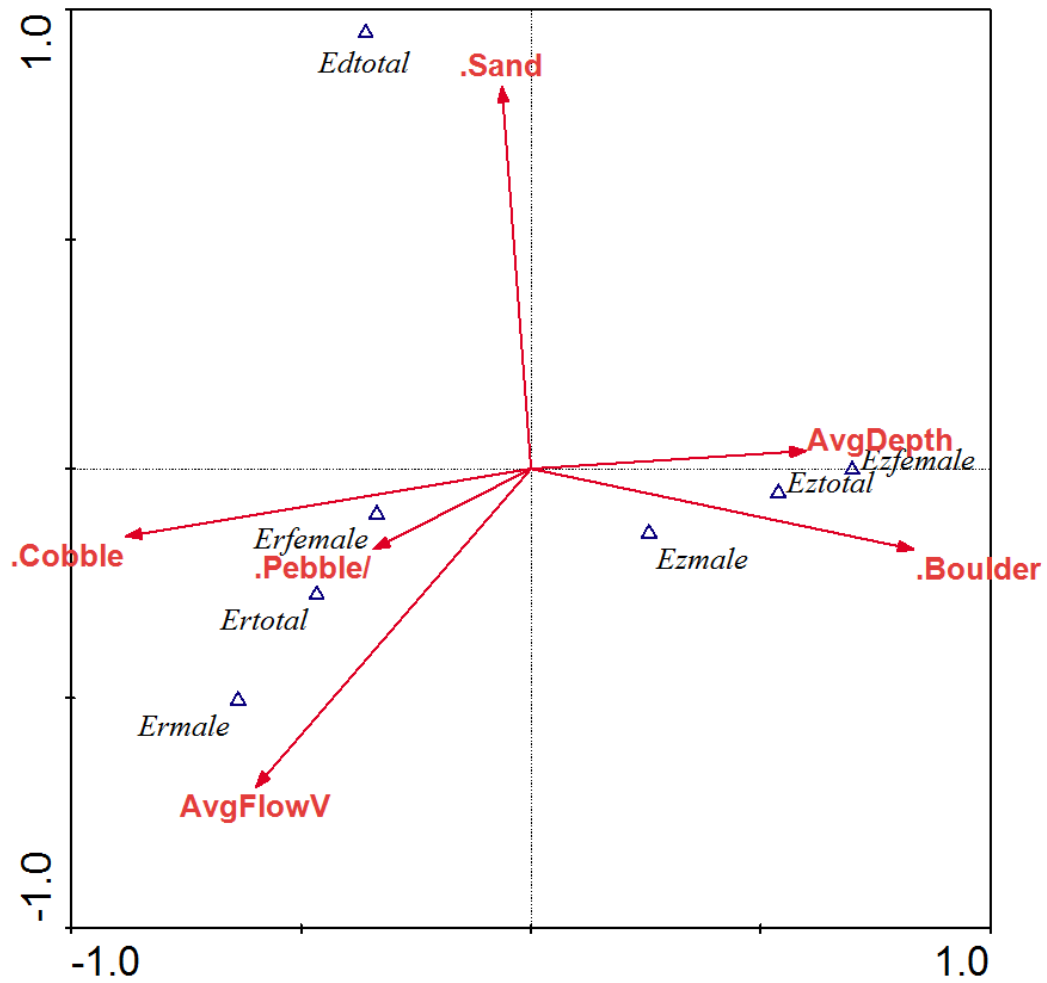


Figure 3.3. CCA-ordination plot of Flint River data for the spawn period depicting weighted loadings (\rightarrow) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Ed = *E. duryi*; Er = *E. rufilineatum*; Ez = *E. zonale*.

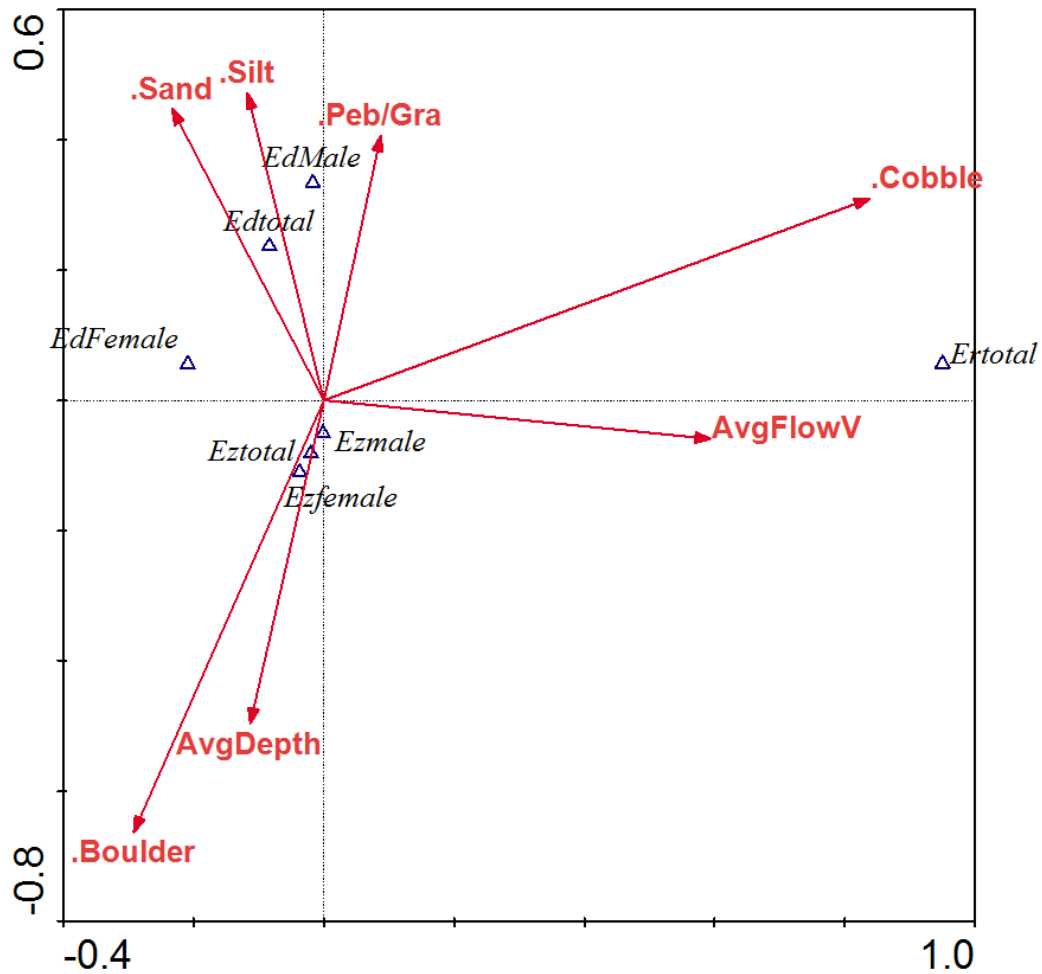


Figure 3.4. CCA-ordination plot of Flint River data for the post-spawn period depicting weighted loadings (\rightarrow) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Ed = *E. duryi*; Er = *E. rufilineatum*; Ez = *E. zonale*.

Principal Component Analysis

A Pearson Correlation analysis of the six habitat variables showed that 12 of the 15 possible correlations were found to be significant at the $p = 0.01$ level, indicating a high degree of collinearity. The PCA of the habitat variables resulted in three principal components. Communalities for the habitat variables were all above 0.800, indicating the extracted components accounted for a large part of the variation within the original

variables. The eigenvalue of the third principal component (PC) was below 1.00, but was included for further analysis since it accounted for 17.96 percent of the total variance. The three PCs extracted accounted for 93 percent of the total variance within the measured habitat variables (Table 3.6).

Table 3.6. Total variance explained by the three extracted Principal Components of habitat variables for the Flint River study site.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	1.822	45.538	45.538	1.822	45.538	45.538	1.703	42.583	42.583
2	1.181	29.533	75.071	1.181	29.533	75.071	1.011	25.281	67.864
3	.719	17.964	93.035	.719	17.964	93.035	1.007	25.171	93.035
4	.279	6.965	100.000						

Extraction Method: Principal Component Analysis.

The highest loadings in PC 1 were for cobble (0.919) and boulder (-0.915), so this component was renamed “substrate” for further analysis. The highest and only significant loading for PC 2 was average depth (0.990) and average flow (0.989) for PC 3 so these two components were renamed “depth” and “flow” accordingly.

Table 3.7. Factor loadings of the original habitat variables onto Principal Components for the Flint River study site.

	Component		
	1	2	3
sqrtCobble	.919	.001	.138
sqrtboulder	-.915	.154	-.031
sqrtAvgFlow	.110	.090	.989
sqrtAvgDepth	-.097	.990	.090

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

Analysis of the factor scores by species for the three principal components indicated an outlier for depth for *E. duryi*. This component score was determined to represent an extreme case, so it was deleted from further analysis.

For substrate (PC1), *E. zonale* exhibited a negative mean factor score for all periods analyzed, indicating an association with boulder. *Etheostoma duryi* and *E. rufilineatum* both exhibited positive mean factor scores, indicating a stronger association with cobble, although *E. duryi* did exhibit a slightly negative mean factor score during the post-spawn period.

Etheostoma zonale was consistently associated with greater depths (PC2) than the other two species when mean factor scores were examined. *Etheostoma rufilineatum* was

found in deeper water than *E. duryi* when examining the full year and pre-spawn period, but mean factor scores showed *E. duryi* in slightly deeper water for the spawn and post-spawn periods.

Examination of flow (PC3) factor scores showed that *E. rufilineatum* was consistently found in areas of relatively high flow. *Etheostoma duryi* had the lowest mean factor scores for all periods analyzed, indicating it was consistently found in slower currents than the other species. *Etheostoma zonale* was generally found in areas of flow between that of *E. duryi* and *E. rufilineatum*, but was found in areas of greater flow during the pre-spawn period (Table 3.8).

Table 3.8. Mean factor scores for the three Principal Components from the Flint River study site. Standard deviations are enclosed in parentheses on the second line.

Sample period	Sample size	Substrate (PC1)	Depth (PC2)	Flow (PC3)
Full Year				
<i>Etheostoma duryi</i>	121	0.4366 (1.1496)	-0.3717 (0.7187)	-0.3490 (1.0529)
<i>Etheostoma rufilineatum</i>	89	0.7690 (1.0840)	0.0296 (1.1160)	0.5182 (1.0156)
<i>Etheostoma zonale</i>	329	-0.3727 (0.6855)	0.1103 (1.0020)	-0.0071 (0.9178)
Prespawn				
<i>Etheostoma duryi</i>	58	0.7193 (1.2650)	-0.1269 (0.6445)	-0.0185 (1.0132)
<i>Etheostoma rufilineatum</i>	47	0.8571 (1.3074)	0.5112 (0.9731)	0.3410 (0.6687)
<i>Etheostoma zonale</i>	168	-0.3843 (0.6884)	0.5555 (0.8563)	0.3827 (0.8352)
Spawn				
<i>Etheostoma duryi</i>	20	0.7844 (0.7802)	-0.0567 (0.7159)	-0.7411 (1.2559)
<i>Etheostoma rufilineatum</i>	30	0.7115 (0.8013)	-0.3446 (1.0722)	0.8133 (1.2461)
<i>Etheostoma zonale</i>	33	-0.2993 (0.7828)	0.5977 (0.9291)	-0.2781 (0.8347)
Postspawn				
<i>Etheostoma duryi</i>	43	-0.1065 (0.9267)	-0.8474 (0.5644)	-0.6123 (0.8751)
<i>Etheostoma rufilineatum</i>	12	0.5678 (0.6879)	-0.9215 (0.7824)	0.4749 (1.3838)
<i>Etheostoma zonale</i>	129	-0.3758 (0.6603)	-0.5902 (0.7699)	-0.4475 (0.8164)

ANOVA

A one-way ANOVA of the three principal components for the entire year resulted in significant difference in mean factor scores for substrate ($F = 79.004$, $df = 540$, $p < 0.001$), depth ($F = 11.078$, $df = 538$, $p < 0.001$), and flow ($F = 21.005$, $df = 540$, $p < 0.001$). Post-hoc testing revealed a significant difference among all three species for both substrate and flow. For depth, the mean factor score for *E. duryi* was significantly less

than that of *E. rufilineatum* and *E. zonale*, indicating it was found in significantly shallower water (Tables 3.9-3.11).

Table 3.9. Homogeneous subsets of darter species based on post-hoc analysis for PC 1 (Substrate) for the full year at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05		
		1	2	3
<i>E. zonale</i>	330	-.3721972	.4457441	.7690369
<i>E. duryi</i>	122			
<i>E. rufilineatum</i>	89			
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Table 3.10. Homogeneous subsets of darter species based on post-hoc analysis for PC 2 (Depth) for the full year at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. duryi</i>	121	-.3713656	.0295785
<i>E. rufilineatum</i>	89		
<i>E. zonale</i>	329		
Sig.		1.000	.774

Means for groups in homogeneous subsets are displayed.

Table 3.11. Homogeneous subsets of darter species based on post-hoc analysis for PC 3 (Flow) for the full year at the Flint River site.

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05		
		1	2	3
<i>E. duryi</i>	122	-.3527347	-.0093633	.5182420
<i>E. zonale</i>	330			
<i>E. rufilineatum</i>	89			
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

A one-way ANOVA for the pre-spawn period again showed a significant difference in mean factor scores for substrate ($F = 47.844$, $df = 272$, $p < 0.001$), depth ($F = 14.764$, $df = 272$, $p < 0.001$), and flow ($F = 4.892$, $df = 272$, $p = 0.008$). For substrate, post-hoc testing indicated the mean factor score for *E. zonale* was significantly different than that of the other two species as individuals had a stronger association with boulder. The factor scores for depth and flow for *E. duryi* were both significantly different than those for *E. zonale* and *E. rufilineatum*, indicating it was found in calmer, shallower water (Tables 3.12-3.14).

Table 3.12. Homogeneous subsets of darter species based on post-hoc analysis for PC 1 (Substrate) for the pre-spawn period at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. zonale</i>	168	-.3843435	.7193037
<i>E. duryi</i>	58		
<i>E. rufilineatum</i>	47		
Sig.		1.000	.682

Means for groups in homogeneous subsets are displayed.

Table 3.13. Homogeneous subsets of darter species based on post-hoc analysis for PC 2 (Depth) for the pre-spawn period at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. duryi</i>	58	-.1269488	
<i>E. rufilineatum</i>	47		.5112323
<i>E. zonale</i>	168		.5554596
Sig.		1.000	.950

Means for groups in homogeneous subsets are displayed.

Table 3.14. Homogeneous subsets of darter species based on post-hoc analysis for PC 3 (Flow) for the pre-spawn period at the Flint River site.

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05	
		1	2
<i>E. duryi</i>	58	-.0185295	
<i>E. rufilineatum</i>	47		.3410033
<i>E. zonale</i>	168		.3827093
Sig.		1.000	.956

Means for groups in homogeneous subsets are displayed.

Results of the one-way ANOVA for the spawning period were found to be similar to that of the pre-spawn period. Mean factor scores for substrate ($F = 18.196$, $df = 83$, $p < 0.001$), depth ($F = 8.112$, $df = 81$, $p = 0.001$), and flow ($F = 14.345$, $df = 83$, $p < 0.001$) all exhibited a significant difference. As in the pre-spawn period, post-hoc analysis revealed a significant difference in mean substrate factor score for *E. zonale* compared to the other two species. For depth and flow, however, results differed from pre-spawn analysis. The mean factor score for depth for *E. zonale* was significantly different, indicating it was found in significantly deeper water. For flow, *E. rufilineatum* exhibited

a significant difference from the other two species as it was found in areas with greater flow velocity (Tables 3.15-3.17).

Table 3.15. Homogeneous subsets of darter species based on post-hoc analysis for PC 1 (Substrate) for the spawn period at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. zonale</i>	33	-.2961959	
<i>E. rufilineatum</i>	30		.7115058
<i>E. duryi</i>	21		.8210128
Sig.		1.000	.865

Means for groups in homogeneous subsets are displayed.

Table 3.16. Homogeneous subsets of darter species based on post-hoc analysis for PC 2 (Depth) for the spawn period at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. rufilineatum</i>	30	-.3445917	
<i>E. duryi</i>	20	-.0567365	
<i>E. zonale</i>	32		.5976553
Sig.		.512	1.000

Means for groups in homogeneous subsets are displayed.

Table 3.17. Homogeneous subsets of darter species based on post-hoc analysis for PC 3 (Flow) for the spawn period at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. duryi</i>	21	-.7443455	.8132568
<i>E. zonale</i>	33	-.2927135	
<i>E. rufilineatum</i>	30		
Sig.		.288	1.000

Means for groups in homogeneous subsets are displayed.

For the post-spawn period, mean factor scores were significantly different for substrate ($F = 10.210$, $df = 183$, $p < 0.001$) and flow ($F = 7.375$, $df = 183$, $p = 0.001$) but were not significantly different for depth ($F = 2.765$, $df = 183$, $p = 0.066$). Post-hoc analysis showed that the mean factor scores for substrate and flow for *E. rufilineatum* differed significantly from those of *E. duryi* and *E. zonale*, indicating it was found in swifter water over substrate with a higher cobble component (Tables 3.18 & 3.19).

Table 3.18. Homogeneous subsets of darter species based on post-hoc analysis for PC 1 (Substrate) for the post-spawn period at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. zonale</i>	129	-.3758209	.5677832
<i>E. duryi</i>	43	-.1065140	
<i>E. rufilineatum</i>	12		
Sig.		.379	1.000

Means for groups in homogeneous subsets are displayed.

Table 3.19. Homogeneous subsets of darter species based on post-hoc analysis for PC 3 (Flow) for the post-spawn period at the Flint River site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. duryi</i>	43	-.6122713	
<i>E. zonale</i>	129	-.4474846	
<i>E. rufilineatum</i>	12		.4748897
Sig.		.774	1.000

Means for groups in homogeneous subsets are displayed.

Discriminant Function Analysis

For *E. duryi*, a step-wise DFA showed that there was a significant difference in habitat utilization between the sexes for the pre-spawn period, with substrate and depth being the two most important principal components for predicting separation ($X^2 = 18.453$, $df = 2$, $p < 0.001$). The resulting discriminant function accounted for 53.4 percent of the variation between sexes. Females were found among substrates with a decreased boulder content and in areas with decreased water flow compared to males.

A step-wise DFA performed for *E. rufilineatum* showed significant differences in habitat utilization for both the pre-spawn and spawn period. During the pre-spawn period, depth was the principal component that predicted separation ($X^2 = 5.930$, $df = 1$, $p = 0.015$), accounting for 35.3 of the variation between sexes. Females were found in significantly deeper water than males. For the spawn period, flow velocity best predicted separation, accounting for 35.8 percent of the variation between sexes ($X^2 = 3.772$, $df = 1$, $p = 0.05$), with males found in areas of greater flow than females.

For *E. zonale*, difference in habitat utilization between sexes was only present for the spawn period. Flow was the predictor of separation, with males being found in areas of higher flow than females ($X^2 = 7.783$, $df = 1$, $p = 0.005$) (Table 3.20).

Table 3.20. Significant differences between sexes for habitat Principal Components for darter species at the Flint River site based on Discriminant Function Analysis.

Species	Variable (PC)	Sample Period	mean factor score (male)	mean factor score (female)	Discriminant Function
<i>E. duryi</i>	Substrate	pre-spawn	0.1458 (1.1120)	1.3338 (1.1372)	0.399
	Depth	pre-spawn	-0.3068 (0.6673)	0.0657 (0.5692)	
<i>E. rufilineatum</i>	Depth	pre-spawn	0.2056 (0.8637)	0.8896 (0.9868)	0.143
<i>E. rufilineatum</i>	Flow	spawn	1.3149 (0.9158)	0.4297 (1.3509)	0.147
<i>E. zonale</i>	Flow	spawn	0.2326 (0.9611)	-0.5846 (0.7977)	0.302

Mean factor scores of variables included in the discriminant function are included with standard deviations in parentheses.

Niche Overlap

Niche overlap values for species at the Flint River site were all well below 0.500, indicating a low degree of niche overlap (Table 3.21). With the exception of the spawn period, *E. duryi* and *E. zonale* exhibited the greatest amount of overlap, especially during the post-spawn period. *Etheostoma duryi* and *E. rufilineatum* were rarely found together, with no overlap occurring during post-spawn sampling. Co-occurrence of *E. zonale* and *E. rufilineatum* was only slightly higher.

Table 3.21. Pianka's Symmetrical Niche Overlap by sample period for the three species sampled at the Flint River site.

Sample period	<i>E. duryi</i> x <i>E. zonale</i>	<i>E. duryi</i> x <i>E. rufilineatum</i>	<i>E. zonale</i> x <i>E. rufilineatum</i>
Full year	0.251	0.043	0.129
Pre-spawn	0.280	0.081	0.197
Spawn	0.084	0.029	0.120
Post-spawn	0.334	0.000	0.036

Estill Fork

At the Estill Fork study site, a total of five species were found in sufficient numbers for analysis. *Etheostoma simoterum* was the most prevalent species collected (n = 326) and was found throughout the study site. *Etheostoma caeruleum* (n = 143) was also widespread through much of the study site. *Etheostoma blennioides* (n = 23) was found in deeper portions of the study site mostly along the eastern bank. *Etheostoma rufilineatum* (n = 53), like the Flint River site, was found in the swiftest portions of the riffle system. *Etheostoma kennicotti* (n = 65), which was mostly represented by smaller, first-year adults, was found predominantly over sand and pebble substrates in areas of low flow (Table 3.22).

Table 3.22. Summary statistics for the seven microhabitat variables at Estill Fork. Variable means are located on the first line and standard deviations are enclosed in parentheses on the second line.

Sample Period	Sample size	Depth (cm)	Flow velocity (m sec- 1)	% Boulder	% Cobble	% Pebble/ Gravel	% Sand	% Silt
<i>Etheostoma blennioides</i>	23	29.44 (12.44)	0.2242 (0.1863)	25.43 (17.51)	32.83 (21.84)	18.26 (7.17)	19.78 (15.92)	3.48 (9.35)
<i>Etheostoma caeruleum</i>	143	26.13 (16.28)	0.1564 (0.1060)	16.26 (16.49)	25.56 (16.23)	26.75 (12.94)	28.57 (22.37)	2.59 (5.13)
<i>Etheostoma kennicotti</i>	65	21.52 (10.42)	0.1604 (0.1490)	9.38 (17.51)	17.23 (11.53)	30.85 (15.32)	37.77 (19.30)	4.62 (7.46)
<i>Etheostoma rufilineatum</i>	53	25.80 (11.22)	0.3201 (0.2033)	23.30 (15.63)	29.53 (11.65)	22.36 (9.84)	24.53 (17.02)	0.28 (1.52)
<i>Etheostoma simoterum</i>	326	28.30 (14.76)	0.1773 (0.1300)	17.48 (19.42)	25.20 (17.13)	24.36 (13.72)	30.12 (22.96)	2.73 (6.07)
Prespawn								
<i>Etheostoma blennioides</i>	5	39.82 (12.29)	0.4414 (0.1285)	30.00 (17.32)	40.00 (14.14)	20.00 (10.00)	10.00 (7.07)	- -
<i>Etheostoma caeruleum</i>	56	35.19 (16.65)	0.1722 (0.1108)	12.77 (12.54)	24.20 (13.20)	27.32 (13.04)	32.68 (20.87)	2.68 (5.80)
<i>Etheostoma kennicotti</i>	12	32.62 (12.80)	0.1758 (0.1316)	5.42 (9.40)	14.58 (9.64)	28.75 (11.70)	42.92 (18.02)	7.50 (9.65)
<i>Etheostoma rufilineatum</i>	24	32.58 (9.96)	0.3567 (0.1241)	26.04 (17.94)	30.00 (7.80)	18.96 (10.21)	25.00 (19.28)	- -
<i>Etheostoma simoterum</i>	98	37.37 (15.09)	0.2068 (0.1209)	11.89 (14.79)	21.38 (11.73)	26.73 (11.86)	36.73 (20.41)	3.37 (7.45)
Spawn								
<i>Etheostoma blennioides</i>	5	33.70 (7.51)	0.2946 (0.1932)	44.00 (13.56)	18.00 (6.78)	14.00 (4.90)	24.00 (17.72)	- -
<i>Etheostoma caeruleum</i>	42	23.84 (13.06)	0.1779 (0.1095)	22.26 (22.26)	20.95 (12.36)	27.98 (14.27)	25.95 (19.33)	2.86 (4.70)
<i>Etheostoma kennicotti</i>	43	19.83 (7.48)	0.1772 (0.1632)	10.23 (19.91)	16.86 (10.18)	33.60 (16.01)	36.28 (18.33)	3.02 (5.89)
<i>Etheostoma rufilineatum</i>	15	22.96 (8.78)	0.3888 (0.3098)	22.00 (16.45)	27.33 (16.02)	26.00 (10.39)	23.67 (16.31)	1.00 (2.80)
<i>Etheostoma simoterum</i>	144	27.60 (12.60)	0.2052 (0.1424)	22.01 (23.75)	20.80 (14.49)	22.85 (15.46)	32.01 (25.27)	2.33 (4.73)
Postspawn								
<i>Etheostoma blennioides</i>	13	23.82 (11.11)	0.1136 (0.0870)	16.54 (12.14)	35.77 (25.81)	19.23 (6.41)	21.92 (16.27)	6.15 (11.93)
<i>Etheostoma caeruleum</i>	45	17.01 (12.48)	0.1168 (0.0860)	15.00 (12.97)	31.56 (20.75)	24.89 (11.51)	25.89 (26.20)	2.22 (4.71)
<i>Etheostoma kennicotti</i>	10	15.49 (9.61)	0.0696 (0.0391)	10.50 (14.03)	22.00 (17.67)	21.50 (12.92)	38.00 (25.30)	8.00 (9.19)
<i>Etheostoma rufilineatum</i>	14	17.21 (8.47)	0.1836 (0.0818)	20.00 (9.41)	31.07 (12.28)	24.29 (6.75)	24.64 (14.61)	- -
<i>Etheostoma simoterum</i>	84	18.94 (11.29)	0.0952 (0.0706)	16.25 (13.24)	37.20 (20.72)	24.17 (12.24)	19.17 (17.25)	2.68 (6.33)

Canonical Correspondence Analysis

When data were examined for the year, a Monte Carlo test exhibited statistical significance for the first canonical axis ($F = 8.753$, $p = 0.002$) as well as all axes combined ($F = 2.443$, $p = 0.002$). Similar results were seen for both the pre-spawn (first axis: $F = 7.794$, $p = 0.002$; all axes: $F = 1.982$, $p = 0.006$) and spawning (first axis: $F = 4.990$, $p = 0.016$; all axes: $F = 2.025$, $p = 0.002$) periods. The post-spawn period, as with the Flint River site, did not show significance at the 0.05 level for the first axis ($F = 2.76$, $p = 0.490$) or combined axes ($F = 1.172$, $p = 0.232$).

For the year as a whole, the first two canonical axes accounted for 82.4 percent of the total variance. The first canonical axis accounted for 53.7 percent of the total variance and was dominated by average flow velocity and the presence of boulder and cobble on the positive end and the presence of smaller substrate categories on the negative end. The second axis accounted for 28.7 percent of the total variance and was dominated positively by average flow velocity and the presence of pebble/gravel and sand and negatively by average depth and the presence of cobble (Table 3.23).

Table 3.23. Canonical Correspondence Analysis of Estill Fork darter species and habitat variables for the full year showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.127	0.068
species-environment correlations	0.506	0.410
Cumulative percentage variance of species data	5.1	7.8
Cumulative percentage variance of species-environment relation	53.7	82.4
Environmental Variables	Weighted Eigenvalues	
Average Depth	0.1767	-0.4548
Average Flow Velocity	0.8279	0.5001
% Boulder	0.5147	-0.2899
% Cobble	0.4868	-0.3995
% Pebble/Gravel	-0.4724	0.3713
% Sand	-0.3925	0.3445
% Silt	-0.3994	-0.0853

The first two canonical axes for the pre-spawn period accounted for 85.9 percent of the total variance. The first axis accounted for 62.4 percent of the variance and, as with the year as a whole, was dominated by average flow velocity and the presence of boulder and cobble on the positive end and the presence of smaller substrate categories on the negative end. The second canonical axis accounted for 23.5 percent of the total variance and was dominated by cobble on the positive end and the presence of sand and silt on the negative end (Table 3.24).

Table 3.24. Canonical Correspondence Analysis of Estill Fork darter species and habitat variables for the pre-spawn period showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.283	0.106
species-environment correlations	0.717	0.518
Cumulative percentage variance of species data	14.2	19.6
Cumulative percentage variance of species-environment relation	62.4	85.9
Environmental Variables	Weighted Eigenvalues	
Average Depth	-0.1018	-0.2206
Average Flow Velocity	0.8794	-0.1683
% Boulder	0.6204	0.2253
% Cobble	0.4920	0.5046
% Pebble/Gravel	-0.4331	-0.1017
% Sand	-0.3810	-0.3293
% Silt	-0.3383	-0.3429

During the spawning period, the first two canonical axes accounted for 79.2 percent of the total variance. The first axis, dominated by the presence of pebble/gravel in the positive direction and average depth in the negative direction, accounted for 47.7 percent of the total variance. The second axis accounted for 31.5 percent of the total variance and was dominated in the positive direction by average flow velocity and the presence of silt in the negative direction (Table 3.25).

Table 3.25. Canonical Correspondence Analysis of Estill Fork darter species and habitat variables for the spawn period showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.146	0.107
species-environment correlations	0.606	0.518
Cumulative percentage variance of species data	7.9	13.7
Cumulative percentage variance of species-environment relation	45.7	79.2
Environmental Variables	Weighted Eigenvalues	
Average Depth	-0.7502	-0.0758
Average Flow Velocity	-0.2713	0.9180
% Boulder	-0.6238	0.0169
% Cobble	-0.2834	0.3517
% Pebble/Gravel	-0.7711	-0.0464
% Sand	0.2072	-0.1242
% Silt	0.2893	-0.3348

For the post-spawn period, the first two canonical axes accounted for 70.3 percent of the total variance. The first axis accounted for 37.7 percent of the variance and was dominated by average flow velocity in the positive direction and the presence of silt in the negative direction. The second axis accounted for 32.6 percent of the variance and was dominated in the positive direction by average depth the presence of silt and by the presence of pebble/gravel in the negative direction (Table 3.26).

Table 3.26. Canonical Correspondence Analysis of Estill Fork darter species and habitat variables for the post-spawn period showing total variance explained by the first two canonical axes and weighted eigenvalues of habitat variables.

Axes	1	2
Eigenvalues	0.158	0.137
species-environment correlations	0.541	0.522
Cumulative percentage variance of species data	6.0	11.3
Cumulative percentage variance of species-environment relation	37.7	70.3
Environmental Variables	Weighted Eigenvalues	
Average Depth	-0.1184	0.4113
Average Flow Velocity	0.9522	0.1088
% Boulder	0.1545	0.0932
% Cobble	-0.2225	0.1191
% Pebble/Gravel	0.1065	-0.3367
% Sand	0.2171	-0.0981
% Silt	-0.4001	0.3558

Figures 3.5 through 3.8 show the results of the CCA depicted graphically showing the first and second canonical axes. For all periods, *E. simoterum* is located near the origin of the axes, indicating it is a generalist, though it is somewhat associated with an increase in depth, particularly among males. As was seen at the Flint River site, *E. rufilineatum* is strongly associated with higher flow velocity, with the presence of larger substrate classes also influencing distribution during the pre-spawn period. *Etheostoma blennioides* is most strongly associated with increased average depth and the presence of cobble and boulder. However, there is a strong association with increased flow during the pre-spawn period. For *E. kennicotti*, the strongest association is with smaller substrate classes, though a slight shift is seen during the post-spawn period where there is a stronger association with the presence of cobble. The distribution of *E. caeruleum* is very much like that of *E. simoterum*, as it is located near the origin. Substrate class,

particularly pebble/gravel and sand does, however, appear to be of greater influence for *E. caeruleum*.

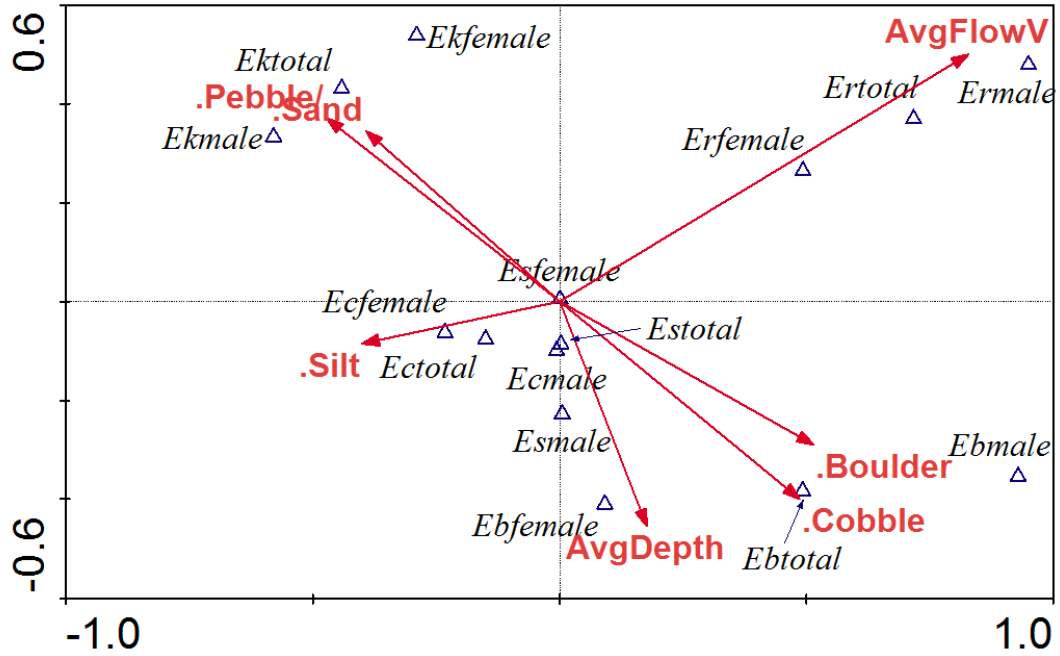


Figure 3.5. . CCA-ordination plot of Estill Fork data for the full year depicting weighted loadings (\rightarrow) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Eb = *E. blennioides*; Ec = *E. caeruleum*; Ek = *E. kennicotti*; Er = *E. rufilineatum*; Es = *E. simoterum*.

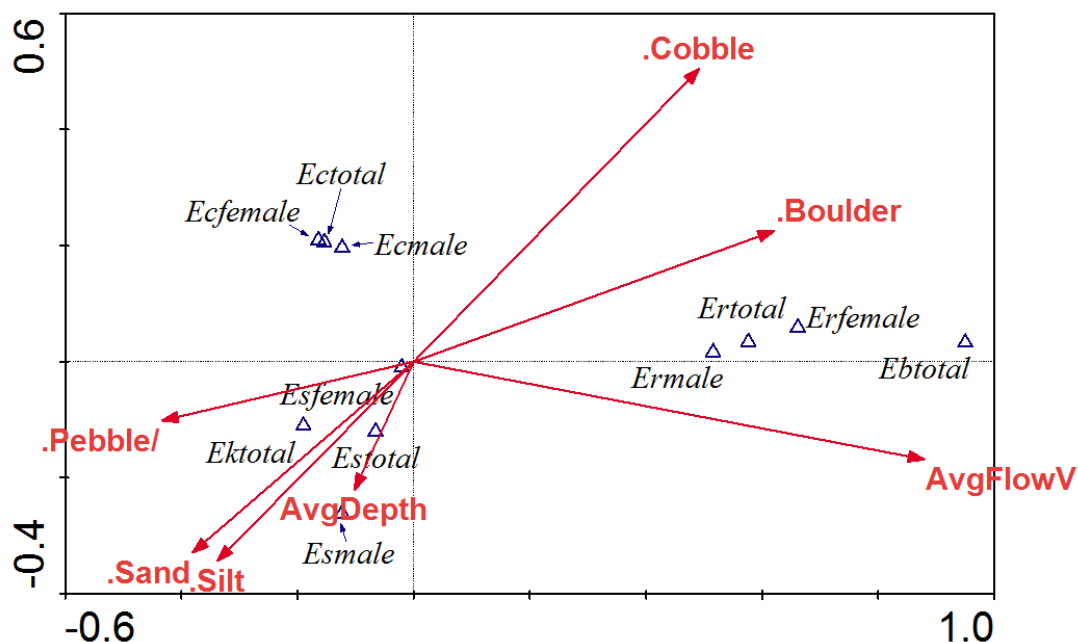


Figure 3.6. CCA-ordination plot of Estill Fork data for the pre-spawn period depicting weighted loadings (\rightarrow) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Eb = *E. blennioides*; Ec = *E. caeruleum*; Ek = *E. kennicotti*; Er = *E. rufilineatum*; Es = *E. simoterum*.

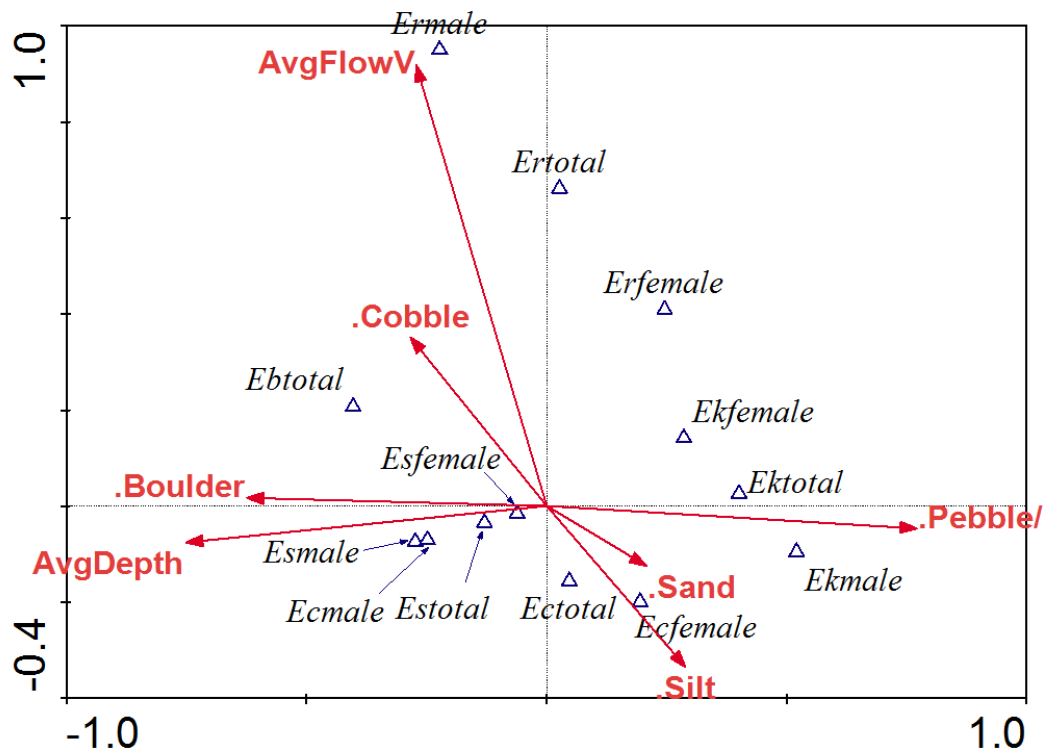


Figure 3.7. CCA-ordination plot of Estill Fork data for the spawn period depicting weighted loadings (\rightarrow) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Eb = *E. blennioides*; Ec = *E. caeruleum*; Ek = *E. kennicotti*; Er = *E. rufilineatum*; Es = *E. simoterum*.

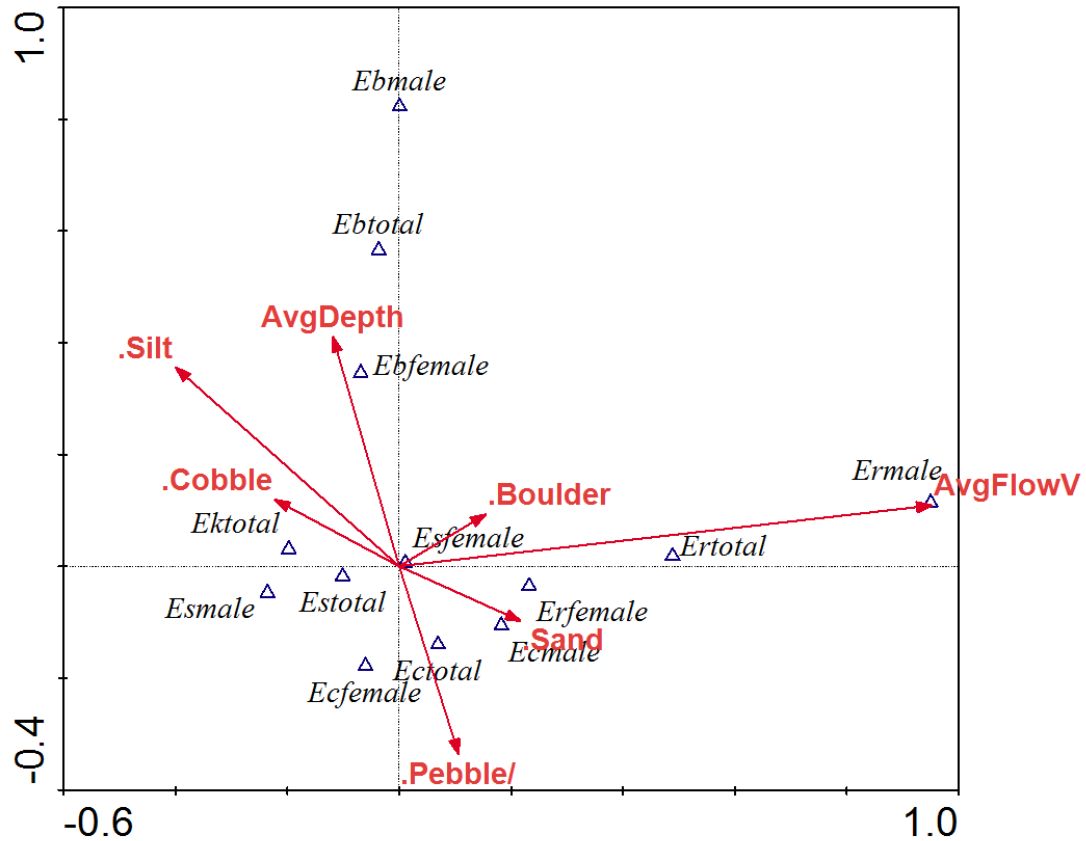


Figure 3.8. CCA-ordination plot of Estill Fork data for the post-spawn depicting weighted loadings (→) of habitat variables on the first two canonical axes and darter species (Δ) association with habitat variables. Species abbreviations are: Eb = *E. blennioides*; Ec = *E. caeruleum*; Ek = *E. kennicotti*; Er = *E. rufilineatum*; Es = *E. simoterum*.

Principal Component Analysis

A Pearson Correlation analysis of the six habitat variables revealed a total of 11 of 15 possible correlations were significant at the $p = 0.01$ level, indicating a high degree of collinearity. Analysis of variables showed that cobble, sand, and average flow had an adequate MSA for analysis. The resultant PCA yielded two principal components. Communalities for the three variables were above 0.850, indicating the extracted components accounted for a large part of the variation within the original variables.

Though the eigenvalue of the second PC was slightly below 1.00, it accounted for 29.44 percent of the total variance and was included for further analysis (Table 3.27).

Table 3.27. Total variance explained by the two extracted Principal Components of habitat variables for the Estill Fork study site.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	1.858	61.925	61.925	1.858	61.925	61.925	1.730	57.667	57.667
2	.883	29.436	91.362	.883	29.436	91.362	1.011	33.695	91.362
3	.259	8.638	100.000						

Extraction Method: Principal Component Analysis.

The significant loadings in PC 1 were for cobble (0.932) and sand (-0.927), so this component was renamed “substrate” for further analysis. The significant loading for PC 2 was average flow velocity (0.993) and was renamed “flow”. The PCs extracted accounted for 91.4 percent of the total variance among habitat variables (Table 3.28).

Table 3.28. Factor loadings of the original habitat variables onto Principal Components for the Estill Fork study site.

	Component	
	1	2
sqrtCobble	.926	.116
sqrtSand	-.927	-.109
sqrtAvgFlow	.121	.993

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser

Normalization.

Examination of substrate factor scores indicated that *E. kennicotti* was consistently associated with areas with a higher sand content, while *E. blennioides*, with the exception of the spawn period, was found over substrates with a greater cobble component. *Etheostoma rufilineatum* was also consistently found over substrates with a higher cobble content, though not as pronounced as *E. blennioides*. Mean factor scores for *E. simoterum* indicated association with slightly sandier substrates, though not as pronounced as *E. kennicotti*. Mean factor scores for *E. caeruleum* were close to zero, indicating it was found over a mixture of substrate types. It is of note that, with the exception of *E. kennicotti*, all species were associated with substrates with a greater cobble component during the postspawn period.

As at the Flint River site, *E. rufilineatum* was consistently found in areas of increased flow velocity, though it is somewhat diminished for the post-spawn period.

With the exception of the post-spawn period, *E. blennioides* was also positively correlated with flow velocity. Mean factor scores for *E. caeruleum* and *E. kennicotti* were negative or near zero for all periods analyzed, indicating they were more likely to be found in calmer waters. Mean factor scores for *E. simoterum* showed it was somewhat positively correlated with flow velocity for the pre-spawn and spawning periods, but was found in calmer waters during the post-spawn period (Table 3.29).

Table 3.29. Mean factor scores for the two Principal Components from the Estill Fork study site. Standard deviations are enclosed in parentheses on the second line.

Sample period	Sample size	Substrate (PC1)	Flow (PC2)
Full Year			
<i>Etheostoma blennioides</i>	23	0.4798 (1.0269)	0.1505 (1.2622)
<i>Etheostoma caeruleum</i>	143	0.0772 (1.0143)	-0.1786 (0.8041)
<i>Etheostoma kennicotti</i>	65	-0.4498 (0.7429)	-0.1501 (1.0177)
<i>Etheostoma rufilineatum</i>	53	0.1939 (0.7380)	0.8664 (1.1171)
<i>Etheostoma simoterum</i>	326	-0.0095 (1.0447)	-0.0432 (0.9672)
Prespawn			
<i>Etheostoma blennioides</i>	5	0.7967 (0.6392)	1.5613 (0.6208)
<i>Etheostoma caeruleum</i>	56	-0.0740 (0.8641)	-0.0281 (0.7887)
<i>Etheostoma kennicotti</i>	12	-0.7149 (0.7342)	0.0635 (0.8830)
<i>Etheostoma rufilineatum</i>	24	0.1852 (0.6770)	1.1801 (0.7140)
<i>Etheostoma simoterum</i>	98	-0.3303 (0.8289)	0.2575 (0.8372)
Spawn			
<i>Etheostoma blennioides</i>	5	-0.1423 (0.6308)	0.6846 (1.3570)
<i>Etheostoma caeruleum</i>	42	-0.0572 (0.7929)	0.0083 (0.7984)
<i>Etheostoma kennicotti</i>	43	-0.4459 (0.6265)	-0.0354 (1.0818)
<i>Etheostoma rufilineatum</i>	15	0.0622 (0.7730)	1.1035 (1.5831)
<i>Etheostoma simoterum</i>	144	-0.2307 (0.9405)	0.1819 (0.9633)
Postspawn			
<i>Etheostoma blennioides</i>	13	0.5972 (1.2028)	-0.5975 (0.7901)
<i>Etheostoma caeruleum</i>	45	0.3907 (1.2863)	-0.5402 (0.7207)
<i>Etheostoma kennicotti</i>	10	-0.1487 (1.1134)	-0.8997 (0.4493)
<i>Etheostoma rufilineatum</i>	14	0.3497 (0.8235)	0.0747 (0.6848)
<i>Etheostoma simoterum</i>	84	0.7439 (1.0797)	-0.7799 (0.7078)

ANOVA

For the full year, a one-way ANOVA of the two principal components resulted in a significant result for substrate ($F = 5.488$, $df = 609$, $p < 0.001$) and flow ($F = 12.632$, $df = 609$, $p < 0.001$). Post-hoc testing resulted in two homogeneous subsets for both principal components. For substrate, the first subset was composed of *E. kennicotti* and *E. simoterum*. The second was again composed of *E. simoterum* and the remaining three species. These results indicate that *E. kennicotti* is associated with significantly finer substrate than *E. caeruleum*, *E. rufilineatum*, and *E. blennioides*. For flow, post-hoc analysis resulted in two homogeneous subsets with *E. rufilineatum* in one subset and the remaining four species in the other. This result indicates that *E. rufilineatum* was found in areas with significantly higher flow than other species sampled (Tables 3.30 & 3.31).

Table 3.30. Homogeneous subsets of darter species based on post-hoc analysis for PC 1 (Substrate) for the full year at the Estill Fork site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. kennicotti</i>	65	-.4498496	
<i>E. simoterum</i>	326	-.0095271	-.0095271
<i>E. caeruleum</i>	143		.0771659
<i>E. rufilineatum</i>	53		.1938721
<i>E. blennioides</i>	23		.4798318
Sig.		.121	.063

Means for groups in homogeneous subsets are displayed.

Table 3.31. Homogeneous subsets of darter species based on post-hoc analysis for PC 2 (Flow) for the full year at the Estill Fork site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. caeruleum</i>	143	-.1785665	
<i>E. kennicotti</i>	65	-.1501033	
<i>E. simoterum</i>	326	-.0432260	
<i>E. blennioides</i>	23	.1505416	
<i>E. rufilineatum</i>	53		.8664330
Sig.		.362	1.000

Means for groups in homogeneous subsets are displayed.

For the pre-spawn period, mean factor scores for substrate ($F = 5.375$, $df = 194$, $p < 0.001$) and flow ($F = 12.792$, $df = 194$, $p < 0.001$) were found to be significantly different among species. For substrate, post-hoc testing resulted in three homogeneous subsets. The first consisted of *E. kennicotti*, *E. simoterum*, and *E. caeruleum*. The second again contained *E. simoterum* and *E. caeruleum* as well as *E. rufilineatum*. The third subset consisted of *E. rufilineatum* and *E. blennioides*. These results indicate that *E. kennicotti* was found on substrates with a significantly higher sand component than *E. rufilineatum* and *E. blennioides*. Additionally, *E. blennioides* was found on substrates with a higher cobble component than *E. caeruleum* and *E. simoterum* (Table 3.32).

Table 3.32. Homogeneous subsets of darter species based on post-hoc analysis for PC 1 (Substrate) for the pre-spawn period at the Estill Fork site.

Tukey HSD

species	N	Subset for alpha = 0.05		
		1	2	3
<i>E. kennicotti</i>	12	-.7149417		
<i>E. simoterum</i>	98	-.3303411	-.3303411	
<i>E. caeruleum</i>	56	-.0740049	-.0740049	
<i>E. rufilineatum</i>	24		.1852337	.1852337
<i>E. blennioides</i>	5			.7966907
Sig.		.226	.445	.270

Means for groups in homogeneous subsets are displayed.

Post-hoc analysis of flow for the pre-spawn period resulted in two homogeneous subsets, with *E. caeruleum*, *E. kennicotti*, and *E. simoterum* in the first, and *E. rufilineatum* and *E. blennioides* in the second. This result indicated *E. rufilineatum* and *E. blennioides* were found in areas with significantly greater flow velocity than the other three species (Table 3.33).

Table 3.33. Homogeneous subsets of darter species based on post-hoc analysis for PC 2 (Flow) for the pre-spawn period at the Estill Fork site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. caeruleum</i>	56	-.0281380	
<i>E. kennicotti</i>	12	.0635244	
<i>E. simoterum</i>	98	.2574690	
<i>E. rufilineatum</i>	24		1.1801310
<i>E. blennioides</i>	5		1.5613479
Sig.		.881	.719

Means for groups in homogeneous subsets are displayed.

Analysis of the spawn period showed no significant difference in substrate mean factor scores among species ($F = 1.553$, $df = 248$, $p = 0.188$). Analysis of mean factor scores for flow did yield a significant result ($F = 4.213$, $df = 248$, $p = 0.003$). A Welch's test was not significant ($t_{4, 23.1} = 2.048$, $p = 0.121$), but a Brown-Forsythe test was significant ($F_{4, 29.5} = 2.860$, $p = 0.041$), so there is some question about the robustness of the significant difference in mean factor scores among species.

Post-hoc analysis for flow resulted in two homogeneous subsets. The first contained *E. kennicotti*, *E. caeruleum*, *E. simoterum*, and *E. blennioides*. The second subset contained *E. simoterum*, *E. blennioides*, and *E. rufilineatum*. This result indicated *E. rufilineatum* was found in areas with significantly greater flow velocity than *E. caeruleum* or *E. kennicotti* (Table 3.34).

Table 3.34. Homogeneous subsets of darter species based on post-hoc analysis for PC 2 (Flow) for the spawn period at the Estill Fork site.

Tukey HSD

species	N	Subset for alpha = 0.05	
		1	2
<i>E. kennicotti</i>	43	-.0353842	
<i>E. caeruleum</i>	42	.0082834	
<i>E. simoterum</i>	144	.1818677	.1818677
<i>E. blennioides</i>	5	.6845837	.6845837
<i>E. rufilineatum</i>	15		1.1034863
Sig.		.277	.085

Means for groups in homogeneous subsets are displayed.

For the post-spawn period, a one-way ANOVA exhibited results similar to the spawn period, with a significant difference between mean factor scores for flow ($F = 5.01$, $df = 165$, $p = 0.001$) but no significant difference present among mean factor scores for depth ($F = 1.901$, $df = 165$, $p = 0.113$). Two homogeneous subsets were created as a

result of post-hoc analysis. Members of the first subset were *E. kennicotti*, *E. simoterum*, *E. blennioides*, and *E. caeruleum*. The second subset included *E. caeruleum* and *E. rufilineatum*. This result indicated that *E. rufilineatum* was found in areas of significantly higher flow than all species but *E. caeruleum* (Table 3.35).

Table 3.35. Homogeneous subsets of darter species based on post-hoc analysis for PC 2 (Flow) for the post-spawn period at the Estill Fork site.

Tukey HSD^{a,b}

species	N	Subset for alpha = 0.05	
		1	2
<i>E. kennicotti</i>	10	-.8997486	
<i>E. simoterum</i>	84	-.7799120	
<i>E. blennioides</i>	13	-.5974770	
<i>E. caeruleum</i>	45	-.5401595	-.5401595
<i>E. rufilineatum</i>	14		.0746792
Sig.		.552	.076

Means for groups in homogeneous subsets are displayed.

Discriminant Function Analysis

When a step-wise discriminant function analysis was performed, only *E. simoterum* and *E. rufilineatum* showed significant separation of the sexes. This separation was only present during the post-spawn period. For *E. simoterum*, separation was based on flow, which accounted for 28.1 percent of the variation between sexes ($X^2 = 6.703$, $df = 1$, $p = 0.01$). Females were found in areas of greater flow than were males. A similar result was seen for *E. rufilineatum*, with flow being the important predictor for separation ($X^2 = 5.862$, $df = 1$, $p = 0.015$). The resultant discriminant function accounted for 63.2 percent of the variation between sexes. Males were found in areas of greater flow compared to females (Table 3.36).

Table 3.36. Significant differences between sexes for habitat principal components for darter species at the Estill Fork site based on Discriminant Function Analysis.

Species	Variable (PC)	Sample Period	mean factor score (male)	mean factor score (female)	Discriminant Function
E. rufilineatum	Flow	post-spawn	0.6341 (0.6641)	-0.2361 (0.4870)	0.665
E. simoterum	Flow	post-spawn	-0.9974 (0.5353)	-0.6002 (0.7846)	0.086

Mean factor scores of variables included in the discriminant function are included with standard deviations in parentheses.

Niche Overlap

Niche overlap values among species at the Estill Fork study site can be seen in Table 3.37. Due to a small sample size per sample event, *E. blennioides* was not included in this analysis. Overlap values were not as small as the Flint River site, but a significant amount of species partitioning was present.

Etheostoma simoterum exhibited the greatest amount of niche overlap among co-occurring species, particularly with *E. caeruleum*, with which it exhibited significant overlap except during the post-spawn period. There was also a significant amount of niche overlap for *E. simoterum* and *E. kennicotti* during the pre-spawn period. All other niche values between species exhibited significant partitioning. *Etheostoma rufilineatum* exhibited the greatest amount of niche partitioning across all species, particularly with *E. caeruleum* and *E. kennicotti*.

Table 3.37. Pianka's Symmetrical Niche Overlap by sample period for four of the five species sampled at the Estill Fork site.

Sample Period	<i>E. simoterum</i> x <i>E. caeruleum</i>	<i>E. simoterum</i> x <i>E. rufilineatum</i>	<i>E. simoterum</i> x <i>E. kennicotti</i>	<i>E. caeruleum</i> x <i>E. rufilineatum</i>	<i>E. caeruleum</i> x <i>E. kennicotti</i>	<i>E. rufilineatum</i> x <i>E. kennicotti</i>
Full year	0.524	0.277	0.385	0.084	0.307	0.092
Pre-spawn	0.539	0.271	0.503	0.150	0.220	0.147
Spawn	0.661	0.257	0.377	0.066	0.408	0.111
Post-spawn	0.422	0.325	0.169	0.053	0.281	0.000

CHAPTER IV

CONCLUSION

Analysis of species and habitat data suggest a difference in habitat utilization among species along depth, flow, and substrate gradients for both study sites, which is similar to results for other studies examining habitat partitioning among darters (Henry & Grossman 2008, Kessler & Thorp 1993, Stauffer et al. 1996, Welsh & Perry 1998). At the Flint River site, the most important factor for interspecific partitioning between *E. duryi* and *E. rufilineatum* was flow velocity as *E. rufilineatum* consistently preferred areas of higher flow than did *E. duryi*. This is not surprising as *E. rufilineatum* has been noted to be found in fast-flowing riffles (Stiles 1972), while *E. duryi* prefers slow to moderate flow (Kuehne & Barbour 1983).

Interspecific partitioning between *E. duryi* and *E. zonale* was most strongly influenced by depth and substrate composition based on data analysis. As stated previously, *E. zonale* was most often found in the central portion of the riffle system during sampling. This area had the highest boulder content and the greatest depths. In contrast, *E. duryi* was found in areas along the shallower periphery of the stream where the boulder component was not as high.

Habitat partitioning between *E. zonale* and *E. rufilineatum* was driven most strongly by substrate composition. Mean factor scores for the substrate principal component were significantly different between species for all four periods analyzed. Based on analyses and in-field observations, *E. rufilineatum* preferred areas with a

substantial cobble component, while *E. zonale* was strongly associated with boulders as previously mentioned.

Niche overlap values served as a tertiary indicator that a high degree of partitioning was occurring among these three species. The highest value occurred between *E. duryi* and *E. zonale* during the post-spawn period (0.334), but still did not represent a significant amount of overlap. Species exhibited significant partitioning throughout the year.

Partitioning among species at Estill Fork was not as clear as the Flint River site. Depth was not determined to be a significant source of variation in the data based on PCA. This is not surprising as Estill Fork is a smaller order stream and there was less variation in depth within the study site compared to the Flint River site, so fluctuations in depth were generally not as great. This fact equated to a reduction in variables available for analysis coupled with an increase in species to be analyzed. Habitat preferences among species and subsequent partitioning, however, were discernible.

In general, *E. kennicotti* preferred the calmest areas over substrate with a larger sand component when compared to other species. Of note is the fact that this is not the substrate composition where *E. kennicotti* is typically found elsewhere. Adults usually prefer shallow pools with slab-rock or boulder substrate, which can be used for cover or spawning, but juveniles are known to frequent deeper pools with different substrate (Page 1975). This atypical substrate preference, coupled with the fact that most individuals captured were smaller adults (~30mm), indicates this could represent a transitional habitat from juvenile to adult.

Etheostoma simoterum appears to be the generalist of this assemblage as it was fairly ubiquitous during sampling except for the swiftest parts of the riffle. Statistical analyses support this conclusion as ordination graphs show *E. simoterum* close the origin of axes and mean factor scores for the two principal components were usually in the middle portion of the range when compared to other species. It was generally found over coarser substrates than *E. kennicotti* and could be found in slightly swifter flow.

Etheostoma caeruleum preferred habitat conditions similar to that of *E. simoterum* for this species assemblage. The two species showed significant niche overlap for three of the four periods sampled and exhibited similar mean factor scores for principal components analyzed. Ordination plots also showed these species in close proximity to one another. As with *E. kennicotti*, this is not typically where *E. caeruleum* is found. Adult usually prefer areas of moderate to swift flow over cobble and pebble/gravel substrates (Kuehne and Barbour 1983, Mettee et al. 1996, Page 1983). This shift from preferred habitat may be due to the presence of *E. rufilineatum*, which has a very similar habitat preference (Page 1983).

As at the Flint River site, *E. rufilineatum* was consistently associated with the areas of the riffle system with the greatest flow velocity. Individuals were also generally associated with coarser substrates than *E. kennicotti*, *E. caeruleum* or *E. simoterum* with the exception of the post-spawn period.

Etheostoma blennioides preferred areas of flow greater than those of *E. kennicotti*, *E. caeruleum*, and *E. simoterum*, but generally less than those of *E. rufilineatum*. Data also indicate a preference for larger substrate classes than other species sampled. Though depth was not included in the PCA, ordination plots indicate that *E. blennioides* preferred

areas of deeper water compared to the other species, which is what was observed in the field.

Niche overlap, though higher than the Flint River site, still shows a significant amount of habitat partitioning except in the case of the overlap between *E. simoterum* and *E. caeruleum*, which showed significant overlap for three of the four periods sampled. It is worth noting that, in both the Estill Fork and the Flint River study site, niche overlap appears to be independent of habitat heterogeneity. The post-spawn period, as previously mentioned, had the least amount of habitat heterogeneity as lack of rainfall had decreased flow and stream width at both sites, yet two of the three niche overlap values for the Flint River and four of the six values for Estill Fork were lowest during this period.

Competition and subsequent habitat partitioning among similar species is most often driven by food availability (Page 1983). Given that the species sampled for this study are known to have very similar diet preferences, it would seem that competition for that food source would be based on space, i.e., that food-niche differentiations are a result of where they feed, rather than what they feed on (Page 1983). At the Flint River study site this partitioning can clearly be seen.

Wehnes (1973) concluded that subterminal mouths were more adapted to feeding on insect larvae inhabiting the tops of rocks and that terminal mouths are more adaptive for feeding on the sides of rocks. Page (1983) also showed that riffle-inhabiting species are generally more robust with a well-developed frenum. *Etheostoma rufilineatum*, with its terminal mouth, robust body form and well defined frenum is, therefore, more adapted to feeding on aquatic invertebrates on the sides of rocks in swift-flowing riffles than is *E. duryi*, which is more adapted to feeding on the tops of rocks and loose substrates with its

subterminal mouth with no frenum. Since *E. duryi* must feed on top of the substrate, fighting swifter flow to attain food would also prove too energetically costly, so it is found in calmer waters.

The central section of the study site is characterized by aquatic vegetation growing on boulder, habitat which *E. zonale* is uniquely adapted to utilize (Kuehne and Barbour 1983, Page 1983). It would therefore stand to reason that it would outcompete other syntopic species for this habitat niche, and is indeed often found in the greatest numbers when mats of aquatic vegetation are present (Boschung & Mayden 2004, Mettee et al. 2006). Due to its morphology and feeding strategy, *E. duryi* would have to feed on the tops of boulders where currents are much stronger and energy expenditure would be much greater, giving it a distinct disadvantage. Individuals of *E. zonale* likely used downstream sides of boulders where flow was drastically decreased as refugia from the current when not foraging among the aquatic vegetation.

Individuals of *E. rufilineatum* were collected from the central portion of the stream, but never in high numbers. This is likely due to competitive pressures from *E. zonale*. *Etheostoma rufilineatum* was found in much greater density in shallower areas that lacked boulders to provide refuge from the fast current. No individuals of *E. zonale* were captured in these areas.

A similar but more complex scenario appears to be present at the Estill Fork site. *Etheostoma rufilineatum* was again found to dominate the high-flow riffle areas to which it is uniquely suited. *Etheostoma caeruleum*, another species with a robust body form and terminal mouth with a well-developed frenum, was not associated with areas of higher flow volume. This shift in habitat preference could be due to the presence of

another riffle species competing for the same habitat. Competitive interactions between these two species have not been specifically addressed and are a possible area for future study.

As seen in niche overlap values, *E. caeruleum* was commonly found with *E. simoterum*, a species in the subgenus *Ulocentra* similar to *E. duryi*. Like other species in the subgenus *Ulocentra*, *E. simoterum* has a blunt head and subterminal mouth adapted for feeding on the top of rocks (Wehnes 1973), whereas *E. caeruleum* is suited to feed mostly on the sides of rocks. This may lead to a sort of vertical habitat partitioning, allowing this relatively high amount of niche overlap to be tolerated by each species since feeding patterns may lessen competitive interactions. There may also be some difference in diet as Hlohowskyj and White (1983) found that *E. caeruleum* fed more heavily on larvae of Ephemeroptera, Plecoptera, and Trichoptera species than did syntopic species in two rivers in northeastern Ohio, while the diet of *E. simoterum* in Brush Creek, Tennessee was predominantly Dipteran larvae of the family Chironomidae (Page & Mayden 1981).

The preference for coarser substrates appears to be the main partitioning mechanism for *E. blennioides*. Previous studies have shown that the most important habitat characteristic for *E. blennioides* is the rocky substrate where it is most commonly found (Miller 1968, Page 1983). Indeed, a study of substrate showed *E. blennioides* preferred the largest substrates when tested alone and in the presence of *E. caeruleum* and *E. flabellare*, two species with which it co-occurs in Estill Fork (Hlohowskyj & Wissing 1986). This preference for larger substrates is likely due to its ability to better exploit this habitat due to its larger size, which also likely decreases the energetic cost of occupying

areas of moderate to fast current. This habitat preference may also be a partial reason as to why *E. blennioides* was only found in limited numbers at the Estill Fork study site, as suitable habitat was only present within two or three 4-meter long sections along the eastern bank of the stream.

As previously mentioned, *E. kennicotti* was not found over the substrate composition typically preferred by large adults. These smaller adults may still be utilizing the habitat more commonly occupied by juveniles to feed on copepods and ostracods (Page 1975). Indeed, a large juvenile population of *E. kennicotti* was found in a slow-flowing pool with sandy substrate adjacent to the main channel.

An examination of the seasonality of the partitioning between species showed a consistent pattern, though the significance of differences among species did fluctuate. At the Flint River site, *E. duryi* was found in the calmest waters throughout all periods. *Etheostoma zonale* was consistently found in the deepest water, with currents generally stronger than those preferred by *E. duryi*, and over substrate with the greatest boulder content. *Etheostoma rufilineatum* was consistently found in water with the highest flow, of a depth between that of *E. zonale* and *E. duryi*, over substrates with the highest cobble content. Niche overlap values did show an increase in partitioning from the pre-spawn period to the spawn period as territoriality likely increased during spawning.

Trends between pre-spawn and spawn periods for the Estill Fork site were similar to those at the Flint River site. The biggest shift between habitat types for these two periods appears to have occurred with *E. blennioides*. During the pre-spawn and post-spawn periods, *E. blennioides* was found over the coarsest substrates of all species sampled. The ordination plot and mean factor score of the substrate PC indicate a shift

by *E. blennioides* to finer substrates during the spawn period. *Etheostoma blennioides* spawns in aquatic algae or over sand (Miller 1968), so this shift in substrate preference is likely due to spawn behavior.

Also of interest is the reduction in significance of the difference in mean factor scores of habitat PCs between species for the spawning period. One would expect the opposite effect as territoriality increases during spawning, but this may represent a shift from preferring certain habitat types during other parts of the year to guarding discrete territories during spawning. This hypothesis is supported by the fact that niche overlap values decreased compared to the pre-spawn period for four of the six species interactions analyzed. The increase in niche overlap between *E. simoterum* and *E. caeruleum* could be explained by a difference in spawning behavior. *Etheostoma simoterum* females attach their eggs to the sides of rocks (Page & Mayden 1981) while those of *E. caeruleum* bury themselves in the substrate (Page 1983), so as with foraging habitat, this could be an example of vertical partitioning taking place. The Estill Fork system is also not as dynamic as the Flint River riffle system, so differences in habitat utilization are more subtle.

The dissimilar results of the post-spawn period compared to the rest of the sampling periods for both study sites could be due in large part to a reduction in habitat availability. A lack of rainfall during the post-spawn period reduced the size and flow of the stream and confined it to the main channel along the eastern shoreline. Substrate was far less heterogeneous with a reduced sand/silt component as finer substrates were located along the periphery of both streams.

When examining habitat utilization between the sexes of a species, only a few significant differences were found. This is partially due in some cases to an insufficient number of a particular sex being sampled to allow for statistical analysis. Of particular interest is the lack of males of *E. duryi* during the spawning period. Mostly gravid females were captured during sampling, raising the question of whether a shift in habitat may have occurred for this species that was not encompassed by the sample site. Another explanation is that high water and extremely fast currents made sampling difficult, allowing for capture rate to drop. Indeed, the number of individuals for both *E. duryi* and *E. zonale* were lowest during the spawn period.

The differences in habitat utilization among the sexes of *E. duryi* for the pre-spawn period can likely be explained by secondary sexual dimorphism. In general male darters are somewhat larger in size, with larger fins, than females, especially during spawning. This can aid in sexual recognition during spawning, as male fins are often brightly colored, or increase swimming power (Page 1983). The latter consequence allows for males to utilize areas of faster flow, and subsequently coarser substrates, than females.

A similar situation was present for *E. rufilineatum* and *E. zonale* during the spawn period. Larger males, with enlarged fins for display and spawning, are able to utilize habitats with faster flow than are the smaller females. The difference between sexes of *E. rufilineatum* during the pre-spawn period is likely similar to the spawn results. Males were found in shallower water, which generally correlates to higher flow.

Results for the differences in habitat utilization among the sexes of *E. simoterum* and *E. rufilineatum* at Estill Fork are somewhat peculiar because they only occur during

the post-spawn period when there was the least amount of habitat heterogeneity due to a lack of rainfall. For *E. simoterum*, the discriminant function explains a relatively small amount of the total variation between the sexes (28.1 percent), so biological significance is minimal. This difference in flow preference could be an indicator of a sort of resting phase for males that have moved into calm waters following high energy expenditures associated with defending females from other males and spawning.

The pattern of males of *E. rufilineatum* occupying areas of higher flow does mimic results found at the Flint River site for the spawn period. It is somewhat curious that this trend was not seen during pre-spawn or spawn period when flow regimes were more heterogeneous. This could be an artifact of small sample size as the number of males was just large enough for analysis to be performed.

When comparing the two study sites, several differences are evident. First, the Flint River site has generally greater depth, higher flow, and coarser substrate than the Estill Fork study site. These differences likely have a direct impact on the diversity and composition of species found at each site. All species sampled for this study can be found in both river systems (Boschung & Mayden 2004, Mettee et al. 1996). In fact, all species present at the Estill Fork site, with the exception of *E. simoterum*, were captured at the Flint River site, just not in numbers great enough for analysis. The reason for this decrease in diversity at the Flint River site is likely due to the aforementioned differences in habitat. The higher flow velocity would preclude *E. kennicotti* from being found in high numbers at the Flint River site as it was found in areas with the least flow at Estill Fork. *Etheostoma blennioides* was present in deeper pools at the Flint, but was likely not caught in greater numbers due to a limited amount of this habitat, as was the case at Estill

Fork. *Etheostoma caeruleum* may not have been found in high numbers at the Flint River due to the presence of both *E. rufilineatum* and *E. zonale* occupying habitat where it was most likely to be found.

The presence of *E. zonale* in itself may also be a factor in the reduction of species diversity in the Flint. It was by far the most dominant species and occupied the central portion of the study site. Other species that could occupy this habitat may be competitively excluded by *E. zonale*. Gray et al. (2005) found a significant shift in habitat utilization by *E. olmstedii* in the presence of *E. zonale*. A similar pattern could exist in the Flint. *Etheostoma zonale* is found in Estill Fork south of the study site, but not in great numbers (B. Stallsmith pers. comm.). This is likely due to a difference in habitat availability compared to the Flint River.

The main goal of this study was to determine what habitat parameters darter species are using for intra-specific and inter-specific partitioning. While this study accomplished the goal of attaining knowledge of the mechanisms species are using, much is yet to be done to attain a full understanding of this complex system. One shortcoming of this study was that only one riffle in each stream could be sampled and analyzed. Due to this research design, only certain habitat types could be sampled. Species that could be present but occupy different habitat within the river systems could have therefore been missed. Also, our sampling technique could have led to some pseudoreplication as darters caught at a particular transect/station could move to another sampling transect/station and be caught again. This study was also constrained by accessibility to the two streams. Habitats that could not be easily accessed were not able to be sampled. Future studies encompassing a greater portion of each stream would be beneficial by

encompassing a wider array of habitats and increasing the size of the data set for analysis. In particular, the lack of data on males of *E. duryi* during the spawn period leaves a gap in the knowledge of this species in the Flint River.

Further study of *E. kennicotti* in Estill Fork would confirm if individuals sampled for this study truly were occupying a transitional habitat. Also, several species, including *E. jessiae* and *E. nigrum* were only captured during the spawn period, suggesting they are moving into the riffle system to spawn and occupy separate habitats during other portions of the year. Many species are known to undergo an annual cycle of inter-habitat movements (Page 1983). Knowing where these species are during other portions of the year will aid in fully understanding their ecology.

Studies on habitat utilization of different life-stages of a particular species would be helpful. This study focused specifically on adults, but many juveniles were caught during sampling. Further knowledge is needed on juvenile habitats for many species within these two systems to get a better picture of an entire life cycle for a species.

APPENDIX

Principal Component Analysis Plots

Flint River

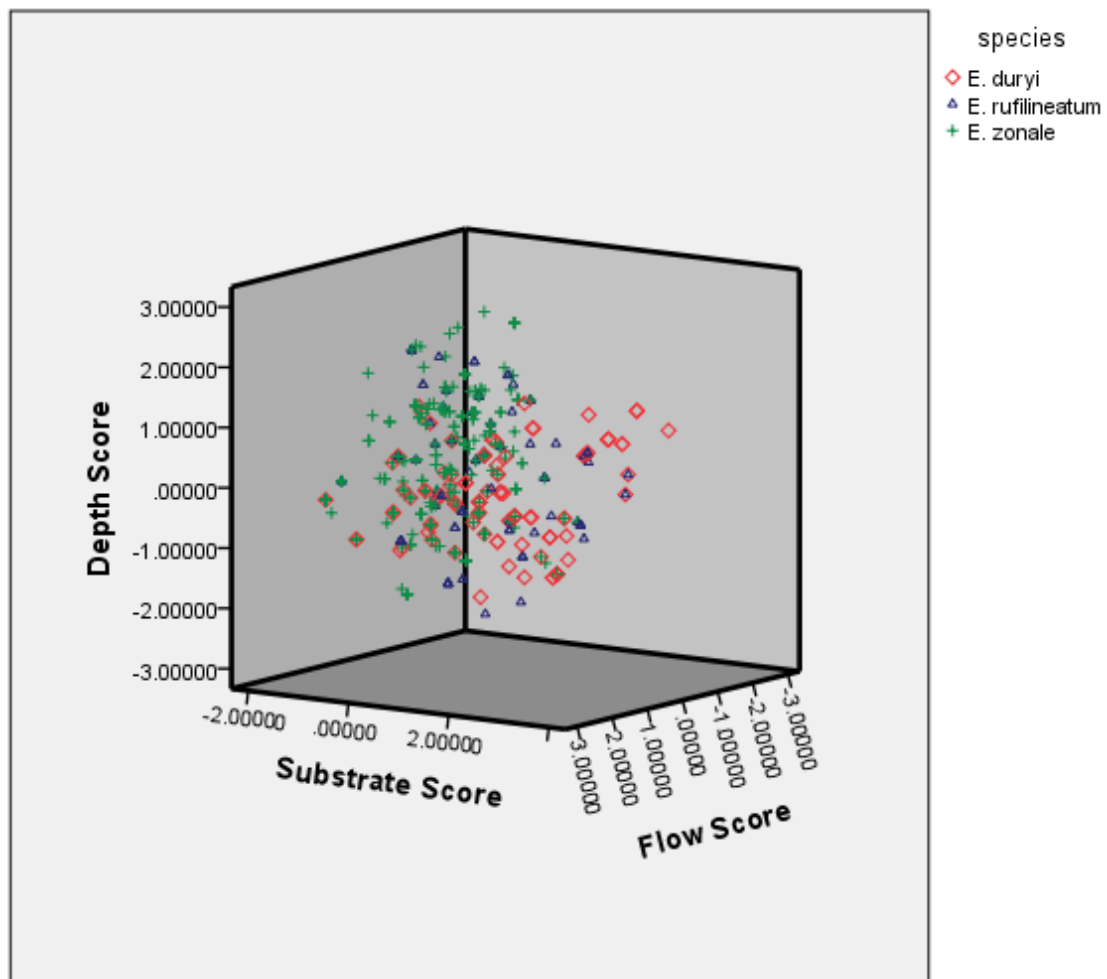


Figure A.1. Projections of individuals of darter species from the Flint River study site for the full year on rotated principal components I (Substrate), II (Depth), and III (Flow).

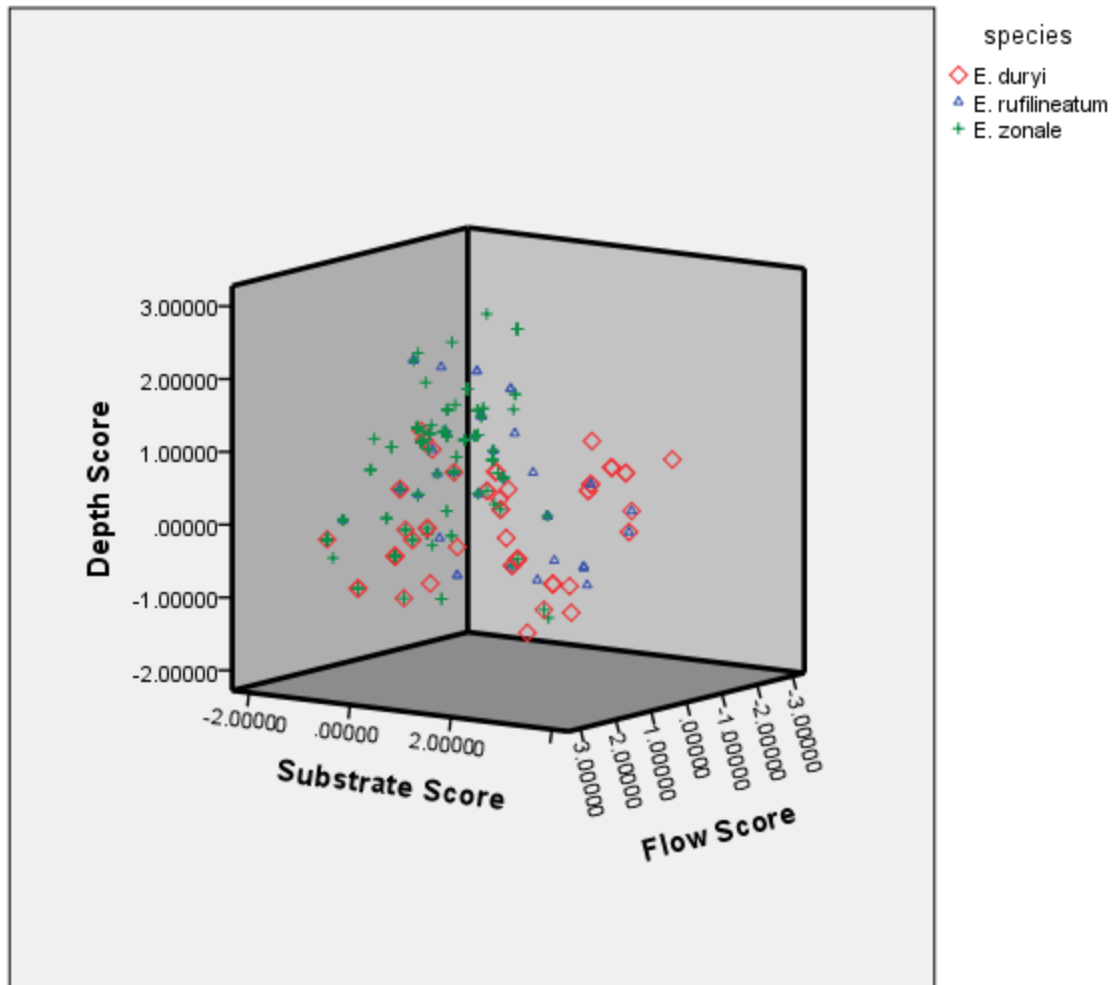


Figure A.2. Projections of individuals of darter species from the Flint River study site for the pre-spawn period on rotated principal components I (Substrate), II (Depth), and III (Flow).

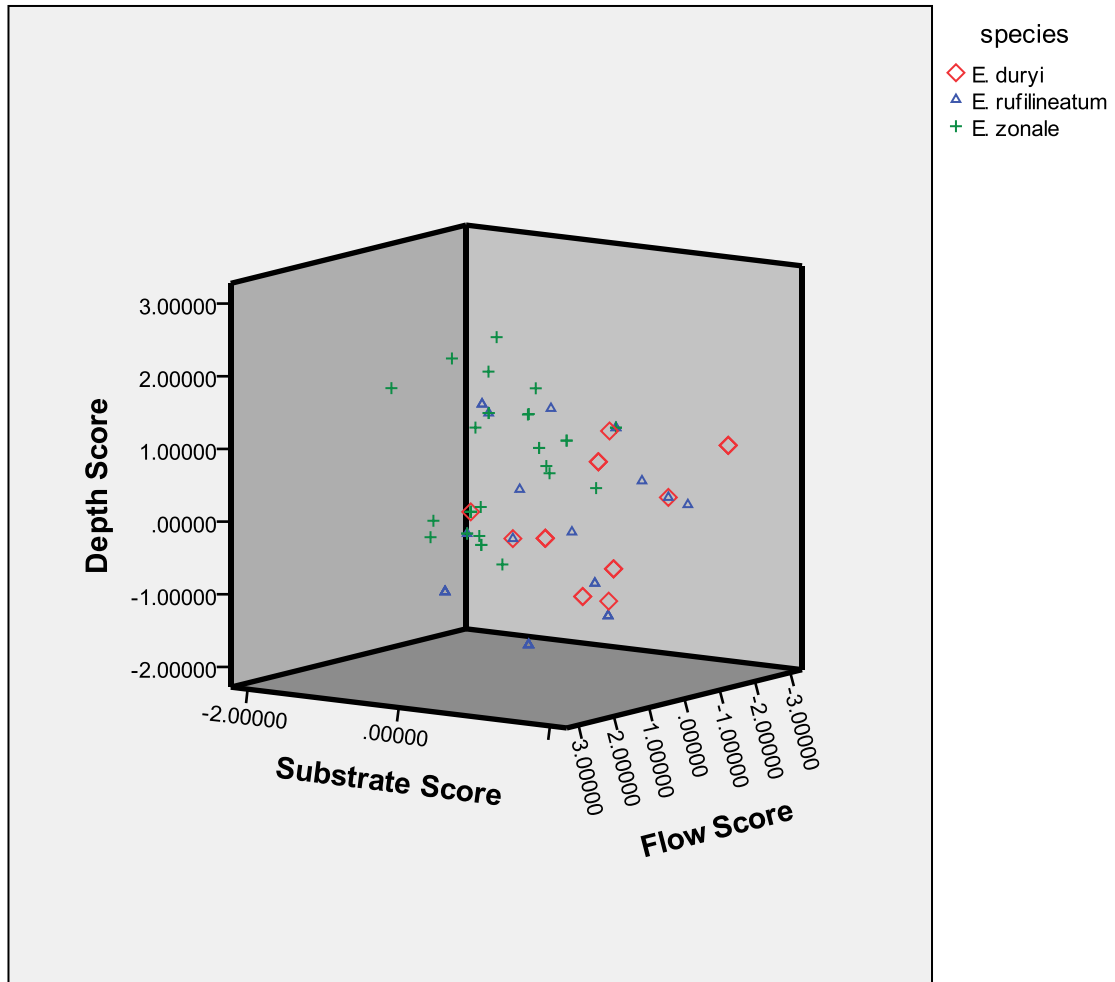


Figure A.3. Projections of individuals of darter species from the Flint River study site for the spawn period on rotated principal components I (Substrate), II (Depth), and III (Flow).

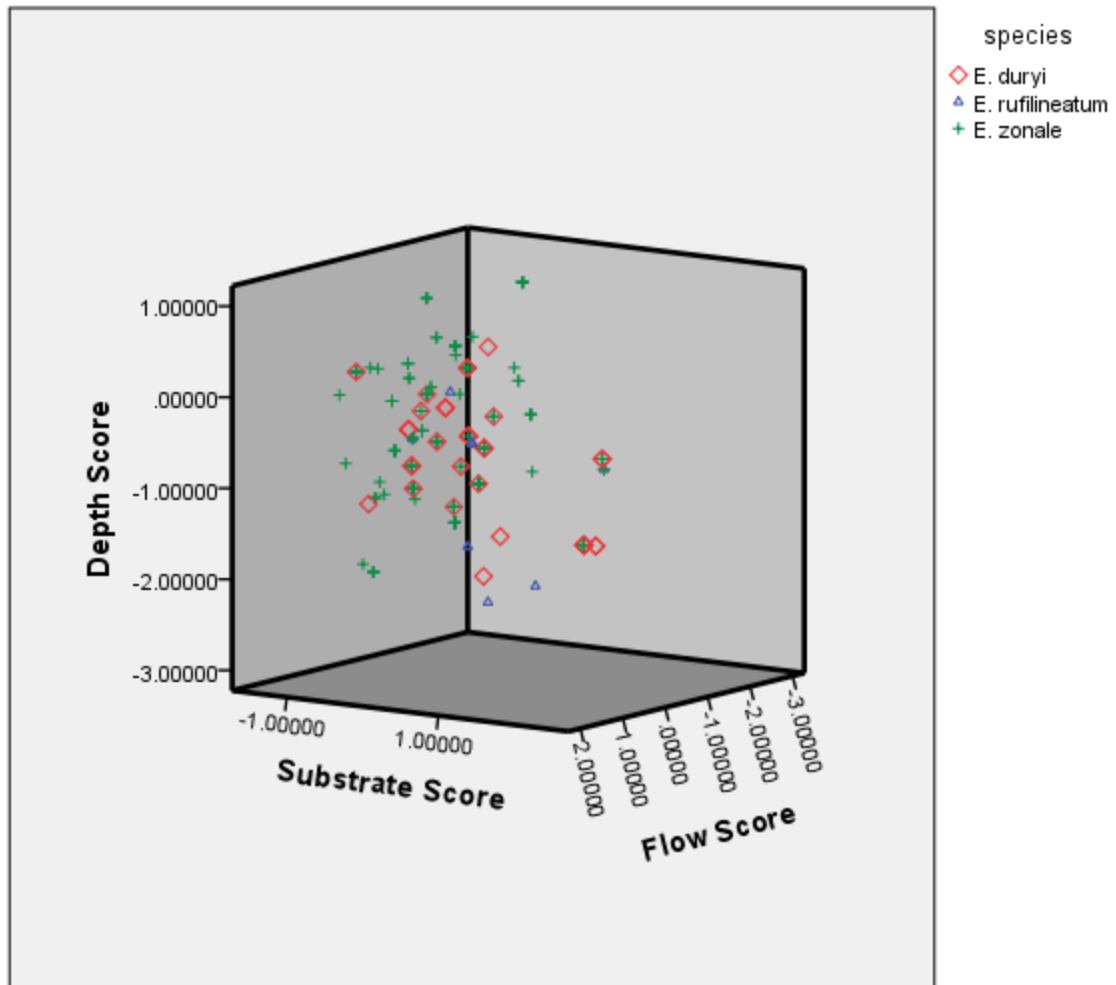


Figure A.4. Projections of individuals of darter species from the Flint River study site for the post-spawn period on rotated principal components I (Substrate), II (Depth), and III (Flow).

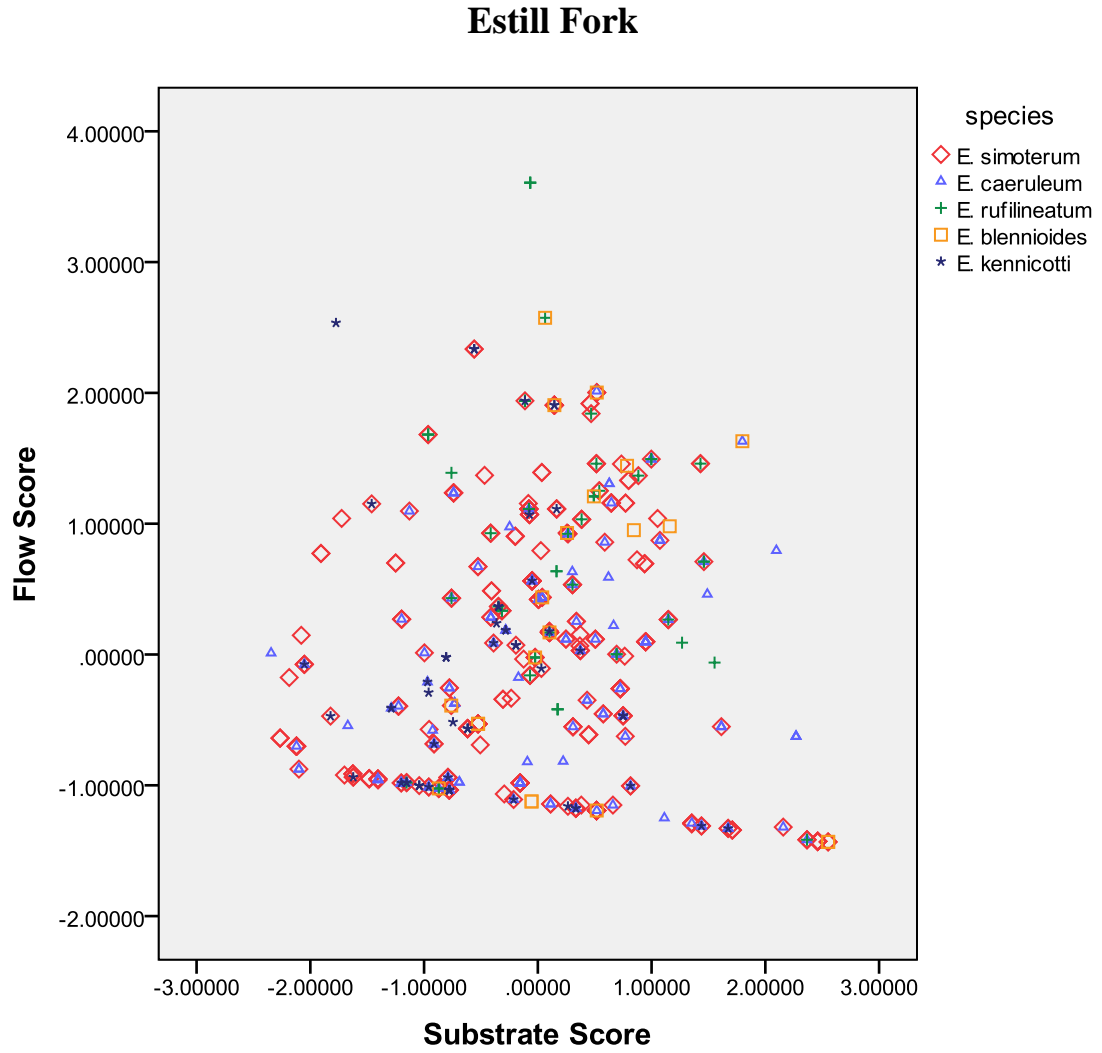


Figure A.5. Projections of individuals of darter species from the Estill Fork study site for the full year on rotated principal components I (Substrate) and II (Flow).

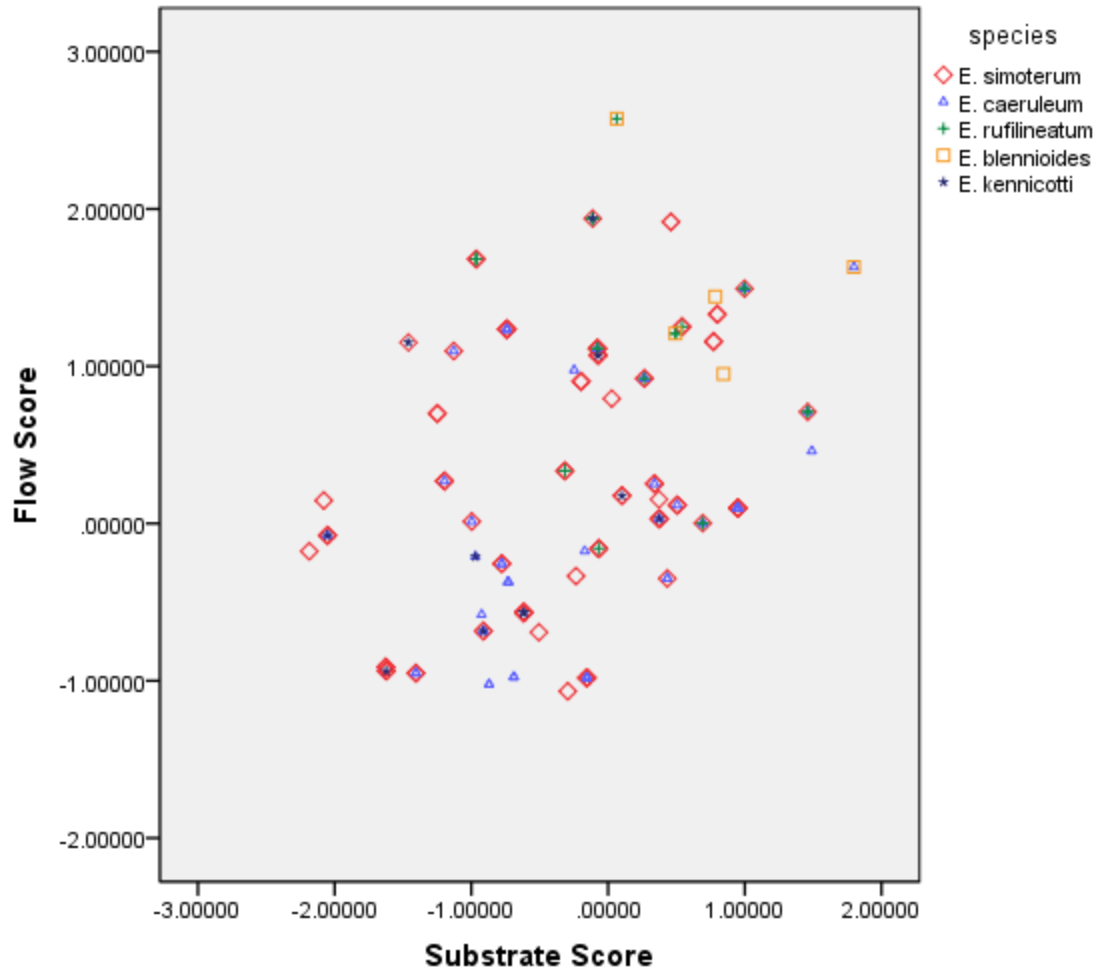


Figure A.6. Projections of individuals of darter species from the Estill Fork study site for the pre-spawn period on rotated principal components I (Substrate) and II (Flow).

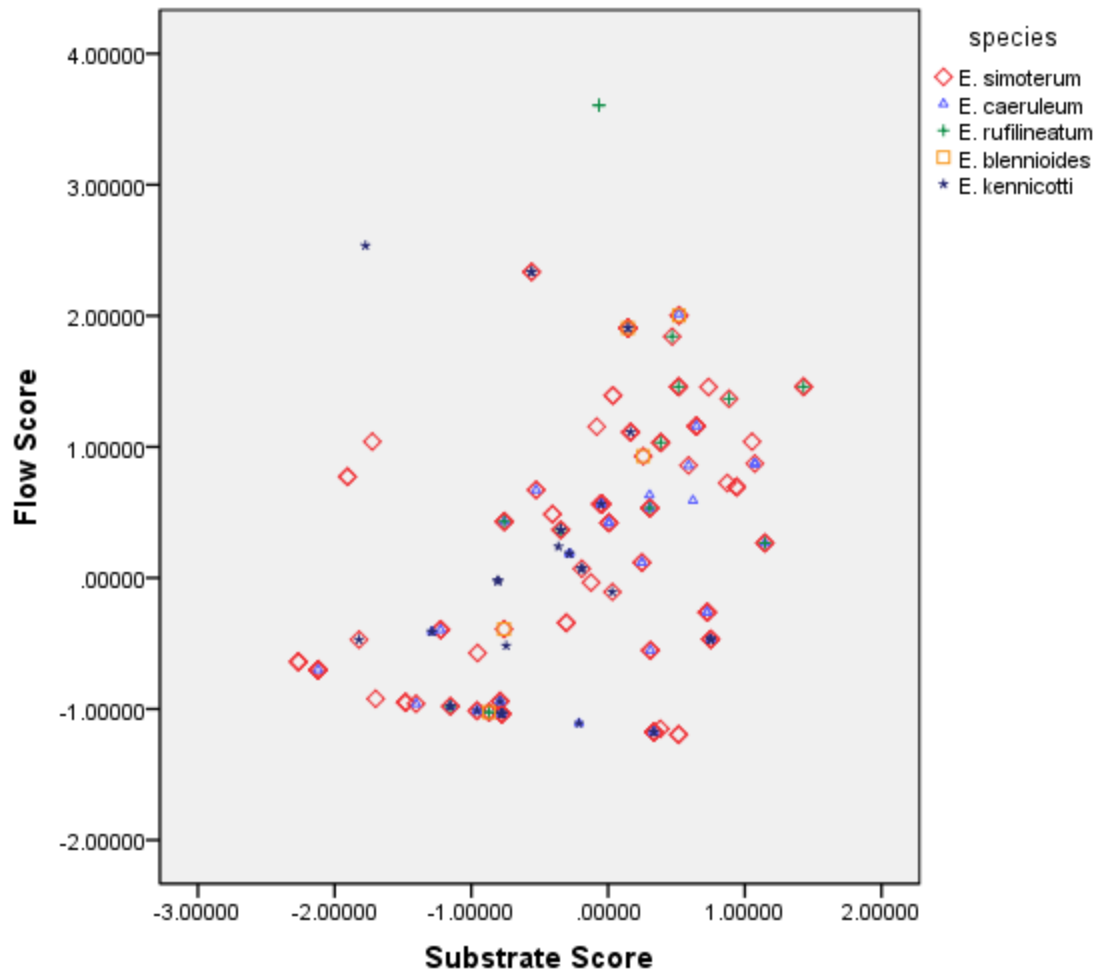


Figure A.7. Projections of individuals of darter species from the Estill Fork study site for the spawn period on rotated principal components I (Substrate) and II (Flow).

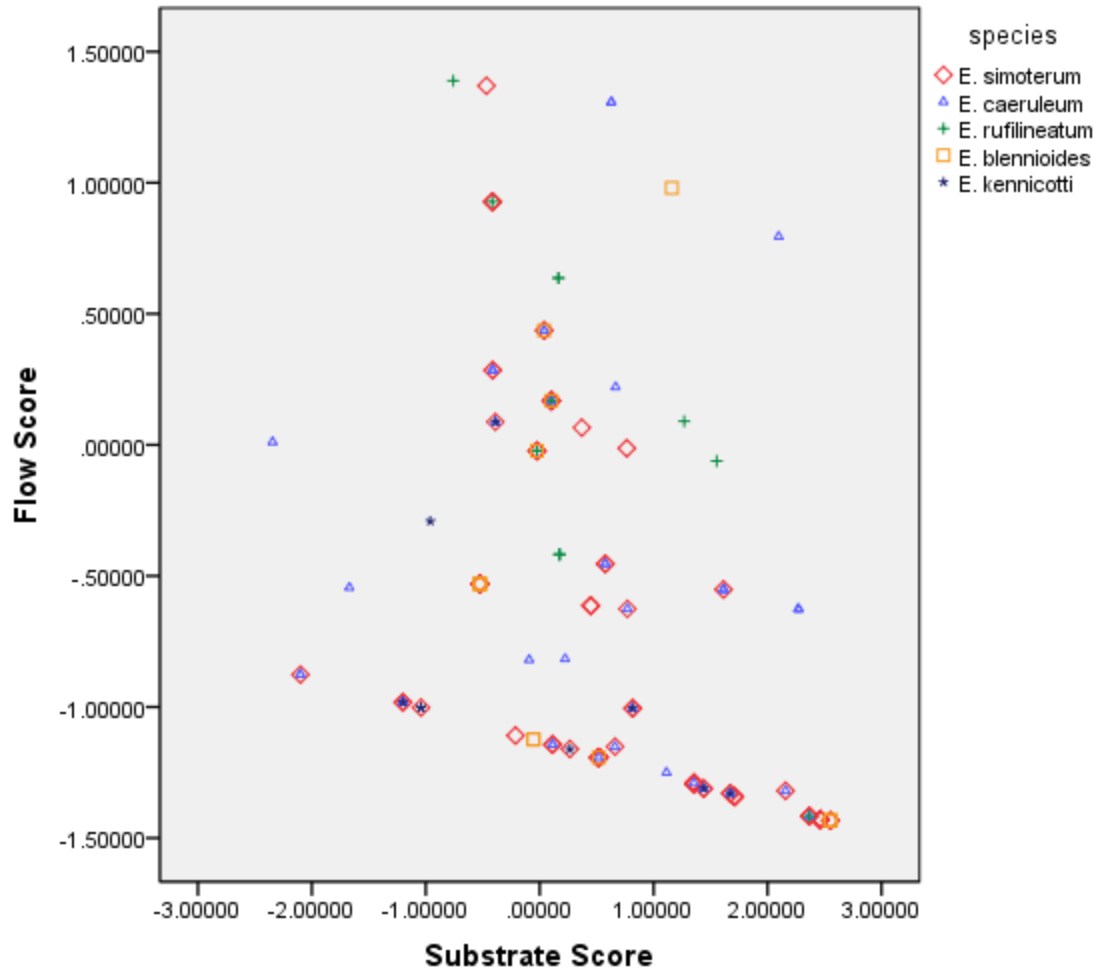


Figure A.8. Projections of individuals of darter species from the Estill Fork study site for the post-spawn period on rotated principal components I (Substrate) and II (Flow).

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