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DISTRIBUTION AND LIFE HISTORY ASPECTS OF *ETHEOSTOMA CROSSOPTERUM* (PERCIDAE), THE FRINGED DARTER, IN THE CACHE RIVER DRAINAGE, ILLINOIS

by

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ABSTRACT

Etheostoma (*Catonotus*) *crossopterum*, the fringed darter, first discovered in Illinois in 1997, occupies only two third order streams (Big and Mill Creeks) of the middle Cache River drainage. I sampled 28 streams for *E. crossopterum* over a two-year sampling period (2000-2001). I found the species to be restricted to these two streams, but in 13 of their second order tributaries in the western Shawnee Hills. The highest densities were in small, headwater creeks with pebble/cobble substrates, clear water, stable banks, and shaded pools. Judging from the few streams occupied and the potential and historical threats (i.e., stream channelization, bank destabilization, sedimentation) to habitat destruction in the middle Cache River drainage, I recommend this species be considered for listing by the State of Illinois as a threatened species.

Nesting and spawning occurred in 9 streams and commenced when water temperatures reached 21.5° C; in 2001, nests were found from 23 March to 12 June. Analysis of a gonadosomatic index for males and females supported a March to June nesting season. In 2001, I found 117 nests, with most under flat stones (mean diameter = 3.6 cm) and guarded by single males. Nests were located in quiet (mean velocity = 0.05 m/s), shallow (mean depth =12.5 cm), pools or runs. Males were polygamous and nests often contained two or more egg clutches laid by two or more females. As a consequence, some males guarded as many as 500 eggs averaging 2.18 mm in diameter.

An in-stream cage with 10 chambers, stocked with 10 pairs of adults, one pair for each chamber of the cage, was examined daily to determine the number of eggs laid per day, length of spawning, and the total number of eggs laid by a female in one season.

Daily fecundity ranged from 1-30 eggs (mean = 8), all deposited on the underside of ceramic tiles. Maximum (110) and mean clutch sizes (52) (range = 1-110) were smaller than those in the wild, probably because males had access to only one female in each chamber. Counts of ripe ovarian eggs in 17 females prior to and during spawning revealed an average clutch size of 150 (SD = 88.4).

Of the 18 streams sampled in Illinois in which *E. crossopterum* was found, Cooper Creek has the highest densities (0.907 darters/m^2), while Big Creek and Little Creek had the highest number of nests, and the largest number of young and juveniles. If these three creeks remain relatively undisturbed, *E. crossopterum* should continue to thrive well into the future.

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DEDICATION

I dedicate this thesis to my parents, Robert and Pamela Elkin, my parents, and to my grandfather, Virgil Ball. My parents encouraged me to never lose hope on finishing my degree. My grandfather always believed in me no matter what I chose to do with my life. He would be very happy to know I have chosen a career in Ichthyology.

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INTRODUCTION

The fringed darter, *Etheostoma crossopterum*, was first discovered in 1997 in southern Illinois in six streams located in the middle Cache River drainage (Poly and Wilson 1998). Prior to its recognition in Illinois, *E. crossopterum* was referred to as *Etheostoma squamiceps*, the spottail darter. The Illinois records represent a small isolated population of *E. crossopterum* on the very northern edge of the species range. *Etheostoma crossopterum* is also found in the lower Cumberland River drainage, Kentucky and Tennessee, in the lower Tennessee River drainage, Tennessee and Alabama, in the Middle Duck River System, Tennessee, and in Reelfoot Lake tributaries, Tennessee (Page et al. 1992). The reported habitat of adults is rocky pools of headwaters, creeks, and small rivers (Page and Burr 1991).

Etheostoma crossopterum is one of 23 members of the subgenus *Catonotus*, all of whom share a specialized reproductive behavior referred to as egg-clustering (Page 1985). A male *Catonotus* sets up a territory under a suitable nesting substrate (e.g., flat rock) in an upland headwater stream. Males are strikingly colored with dark and light vertical bars on the body and a blackened head. When a female enters the male's territory, he courts her. After the female is enticed by the male, she inverts and lays her eggs in a single layer on the underside of the nesting substrate while the inverted male fertilizes the eggs. The female leaves after spawning, and the male guards the eggs until hatching.

In the subgenus *Catonotus*, characteristics that separate *E. crossopterum* from other species are: 1) fairly large body size; 2) three dark spots at the base of the caudal fin; 3) scales on the nape, prepectoral area, and breast, and usually on the cheeks and

opercles; 4) body and fin colorations not chromatic; 5) infraorbital canal complete or incomplete with 7-8 pores; and 6) dorsal fin spines with fleshy knobs in both males and females (Page et al. 1992)

The fringed darter is distinguished from similar species by having a white edge on the second dorsal fin of the breeding male, and small black tips on the rays may be visible. In addition, the second dorsal fin has three branches per ray, and the third branch is much longer than the second branch (Page and Burr 1991).

Other than the recent discovery of this fish in southern Illinois, and notes on its distribution and nesting sites (Poly and Wilson 1998; Poly 2000), little else has been reported on the biology of *E. crossopterum*. With funding from the United States Fish and Wildlife Service and the SIUC Graduate School, I undertook a two-year study of the distribution, habitat, population size, and reproductive biology of *E. crossopterum* in southern Illinois streams. My ultimate goal was to generate quantitative, biological data (i.e., densities, clutch size estimates) that might be useful to future management and protection of the species. Ecological information collected on *E. crossopterum* is critical to management practices that are being implemented currently in the Cache River drainage. It is important to research this species in Illinois because it represents a small, isolated population that could become threatened if continued human induced degradation occurs in the Cache River drainage.

My hypotheses for this study, useful to the management of *E. crossopterum* in Illinois, include the following:

Ho: Geographic distribution of *E. crossopterum* will be in the middle Cache River watershed in southern Illinois.

Ho: Habitat of *E. crossopterum* will be in headwater streams with cobble/pebble substrates.

Ho: Clutch sizes will differ between nests found in the wild versus artificial nest tiles used in an in-stream cage.

Ho: Gonadosomatic index (reproductive condition) values will be higher for females when compared to males during the reproductive period.

STUDY AREA

The Cache River lies at the "biological midpoint of North America"- one of only six areas in the U.S. where four or more physiographic regions overlap (Illinois Department of Natural Resources 1998). The Cache River drainage encompasses 19 of 21 watersheds and drains 248,482 hectares in Alexander, Johnson, Massac, Pope, Pulaski, and Union counties in extreme southern Illinois. The headwaters of the Cache River originate near Cobden in Union County, and the river meanders 2,604 km throughout the southernmost part of the state before emptying into the Mississippi River through a diversion ditch near the city of Mounds, Pulaski County, (Illinois Department of Natural Resources 1998). Post Creek cutoff, built in 1915, divided the Cache into two separate drainages- the upper and lower- and diverted all flow from the upper Cache River through a dredged channel in eastern Pulaski County directly to the Ohio River 35 km above Cairo. The upper Cache River is 314 hectares in area, while the lower Cache River is 305 hectares in area. At the extreme southern tip of Illinois, the land flattens and is poorly drained (Illinois Department of Natural Resources 1996). A southern flora is present in this area on the northernmost extent of the Coastal Plain that stretches to the Gulf of Mexico (Illinois Department of Natural Resources 1996). Swamps, dominated by bald cypress (some of the oldest and largest trees in Illinois) and tupelo, are reminiscent of Louisiana bayous.

Channel entrenchment and lateral gully formation are some major problems in the upper Cache River (Camacho et. al 1990). The upper reaches flow through the hills of the Ozark plateau, and the lower reach flow through flatter Coastal Plain where drainage is slow and wetlands become more abundant (Illinois Department of Natural Resources

1996). As the river leaves the uplands it drops as much as 4.6 m per 1.6 km (Illinois Department of Natural Resources 1998). The steep fall gives even small streams the power to cut out gorges and canyons as much as 61 km deep, exposing bedrock (Illinois Department of Natural Resources 1998). The lower Cache flows through a flood plain 2.4 to 3.2 km wide and falls only 0.30 m per 2.6 km on average (Illinois Department of Natural Resources 1998). Flooding and sedimentation are the major problems in the lower Cache River (Camacho et. al 1990). Tributary streams contribute to the sedimentation problem because of an increase in silt load, and the river is not able to transport sediment out of the water (Camacho et. al 1990). The Cache River floodplain functions much like a flood-control reservoir because of the relatively steep slopes of upland streams in the drainage combined with very flat slopes in the valley itself (Illinois Department of Natural Resources 1997). Stream flow in the lower Cache River moves in opposite directions: either east towards two 1.2-meter-diameter culverts in the Cache River Levee and then into the Post Creek cutoff, or west through the original river channel to the Cache River diversion channel, and then to the Mississippi River (Illinois Department of Natural Resources 1997).

The geology of the upper Cache River consists of unglaciated sandstone bedrock, and the lower Cache River has limestone and sandstone bedrock. Although the lower and middle stretches of the Cache River consist of cypress swamps and mud-bottomed sloughs, many of the headwater streams are clear with gravel, and some are spring-fed (Smith 1971). The upland soils are formed of loess and are primarily silt loams susceptible to severe erosion on slopes (Cache River Watershed Resource Planning Committee 1995).

There are many negative, human induced impacts on the region. The main impact is excessive or poorly treated sewage from a few small municipal sewage treatment plants (Illinois Department of Natural Resources 1998). Physical changes to the landscape such as land clearing, erosion, fragmentation of habitat, introduction of non-native plants, and drainage and other changes to hydrology have had more profound ecological effects in the drainage than pollution (Illinois Department of Natural Resources 1998). Many of the tributaries of the Cache River drain agricultural fields, and erosion of stream banks is an annual problem.

Approximately 360 km of the Cache River and its tributaries have been channelized. Channelized stream segments contribute large amounts of sediment from bank and channel erosion, and herbicides used on the banks are easily washed into the streams (Cache River Watershed Resource Planning Committee 1995). Channelized streams and ditches have homogenous aquatic habitats with little structure or complexity, resulting in degraded fish communities (Cache River Watershed Resource Planning Committee 1995).

MATERIALS AND METHODS

DISTRIBUTION

I visited all 19 localities reported in Poly and Wilson (1998) in an effort to confirm the presence of the species at all of their sites. I searched the SIUC Fluid Vertebrate Collection database to generate geographic location data for stream sites reported in Poly and Wilson (1998). I chose potential new sampling sites after studying 7.5' quadrangle maps that showed some elevation around small headwater streams in the middle Cache River. During the summer (June-September), fall (September-December), winter (December-March), and spring (March-June) of 2000-2001, I sampled 28 sites (includes Poly and Wilson's sites) in the middle Cache River drainage to obtain a better understanding of the fish community and the relative population numbers of *E. crossopterum*.

Two to three people assisted me during each sampling trip. If *E. crossopterum* was found, a voucher specimen was taken, and the site was searched for possible nesting habitat in the spring. I did not search drainages outside the Cache River in southern Illinois because no records of a species of *Catonotus* (other than *E. flabellare*) have been reported outside the Cache River drainage (Smith 1979) or extreme southeastern Illinois (Poly and Wilson 1998). In 2001, I narrowed my observation and sampling to only those eight streams that supported the highest population numbers of the species.

Vouchered records were plotted on a base stream map of southern Illinois after obtaining specimen records from the SIUC Fluid Vertebrate Collection and the Illinois Natural History Survey. I plotted all known records of *Etheostoma squamiceps* in Illinois

streams from the same two state collections so that comparisons of geographic range would be clear.

Quadrangle maps (7.5') were studied to record township, range, and section data for each site from which voucher specimens were taken. Latitude and longitude coordinates were taken using a commercially produced CD Rom (Street Atlas USA 1997 ©).

A conservation status index by Burr and Stewart (sensu 1998) was calculated to develop a concept of relative rarity for both *E. crossopterum* and *E. squamiceps*. This index: [$D = (Si + 2S + 4A)^n$], where D = distributional score, Si = number of sites, S = number of streams, A = number of areas, and n = variable used to approximate actual endangerment results in scores of 1-32 of a given species being assigned the status of endangered, and scores of 33-69 considered threatened.

HABITAT

I made habitat measurements at 100-meter transects in each stream sampled. I took 10 to 15 depths with a meter stick at the beginning, middle, and end of each transect. I made 10 to 15 random substrate evaluations. The sizes of substrate were then classified according to the modified Wentworth Scale (Hynes 1970) (Table 1). I measured three random stream (wetted) widths from one bank to the other within each transect using a 70 meter tape measure. I recorded other measures of physical habitat, including pH, dissolved oxygen, air temperature, and water temperature. I measured pH with either a Hach meter or a Hach tester kit (Hach Chemcal Co.).

Table 1. Suggested terminology and categories for particle-sizes used for substrate analysis after Hynes (1970).

Name of Particle	Range of size in mm
Boulder	>256
Cobble	64-256
Pebble	32-64; 16-32
Gravel	8-16; 4-8; 2-4
Very Coarse Sand	1-2
Coarse Sand	0.5-1
Medium Sand	0.25-0.5
Fine Sand	0.125-0.25
Very Fine Sand	0.0625-0.125
Silt	0.0039-0.0625
Clay	<0.0039

Dissolved oxygen was measured with a Hach tester kit (Hach Chemical Co.). Air and water temperature were taken with a handheld Celsius thermometer at 0.6 m water depth for every season. I recorded water velocity with a Swoffer velocity meter, or by floating an object over a measured distance (10 times).

I also made regular field notes of surrounding land use and other potential factors that might detrimentally influence streams. Making a percentage estimate based on my judgement of the percent cover in trees/grasses or residential and agriculture acreage in and around each stream determined surrounding land usage. I recorded common invertebrates by observation and their relative abundance by estimating percentages within a 100-meter transect.

DENSITIES

I used a minnow seine (3-meter long by 1.5 meter wide; 0.32 cm. mesh) in pools and the set-and-kick method in riffles and runs (Jenkins and Burkhead 1994) to collect darters (15-meter transects). A seine haul was used to collect darters in pools (Jenkins and Burkhead 1994). Each of two people grabbed hold of a brail and walked downstream with the lead line dragging on or above the substrate. A bag develops in the seine from holding onto the brails near the float lines. The middle of the net is lifted and the remaining net is thrown upstream. The set-kick involved setting a seine at the downstream end of a riffle or run; one person stayed with the seine net at the downstream end, and the other person kicked rootwads and turned rocks from upstream to

downstream within that macrohabitat. The seine was brought out of the water and fish were identified to species, enumerated, and returned to the stream above the beginning of that transect. My unit of effort was the 10 minutes a seine was in the water being used.

Root wads and debris in the stream transect were kicked out by placing a seine net around that root wood or debris in the water. One person held the seine net of both brails, and the other person kicked the root wad or debris out to obtain any fish in these areas. The seine net was picked up by both people to inspect fish in the net.

I used d-ringed dipnetting (1.2 m x 1.2 m) in shallow areas where the seine net would not have done an effective job in obtaining fish. The dipnet was also used towards the end of the spawning season to capture juveniles.

I used the set-and-kick method in riffles and runs to estimate population densities of the fringed darter during the fall season. I placed block nets (0.32 cm mesh) at each end of a 15-meter transect during every season. Within that transect, one person kicked into the downstream block net by disturbing all parts of the habitat. A 3-meter seine was picked up at the downstream end. Fish were placed in a live bucket, identified to species, enumerated, and released back into the stream above the transect. I recorded total lengths of fish (to nearest 0.1 mm) using Mitutoyo dial calipers.

Samples of each fish species were taken from each stream at least once to verify identification of local fish assemblages. If after subsequent visits I obtained fish species different from previous visits, I would take specimens as additional vouchers. I preserved fish samples in ten percent formalin. After one week of preservation, I washed

the sample in water and transferred the fish to 70 percent ethanol. I cataloged the fish into the SIUC Fluid Vertebrate Collection.

REPRODUCTION

Nesting

During the spring of 2001, I searched for nests in nine streams over a 6-hour period in 100-meter transects by turning over any suitable substrate (e.g., flat rock, metal lid, log) and looking for embryos or nest guarding males. I recorded 13 different parameters at each nest site including: 1) length and width of clutch and egg stage; 2) length, width, and depth of nest substrate; 3) depth of water in front of nest substrate and five random depths around that substrate; 4) velocity of water at front, back, and each side of the nest; 5) five randomly selected egg diameters using dial calipers (nearest 0.1 mm); 6) distance to nearest bank using a 60-meter tape measure; 7) distance to nearest neighbor; 8) substrate under nesting rock; 9) nest cavity depth; 10) total length and standard length of each male or female captured under nesting rock; 11) habitat type (i.e., riffle, pool, or run); 12) surrounding land use and other impacts to the stream; and 13) air and water temperature.

Cage Study

I used a plexiglass cage (from Weddle and Burr 1991) with dimensions of 100 cm wide by 76 cm long and 20 cm high to record daily egg counts (Figure 1). The cage consisted of ten separate chambers (Figure 2) with a hinged lid on top of each chamber. A metal bar over the top of the cage locked the chambers into place so that enclosed

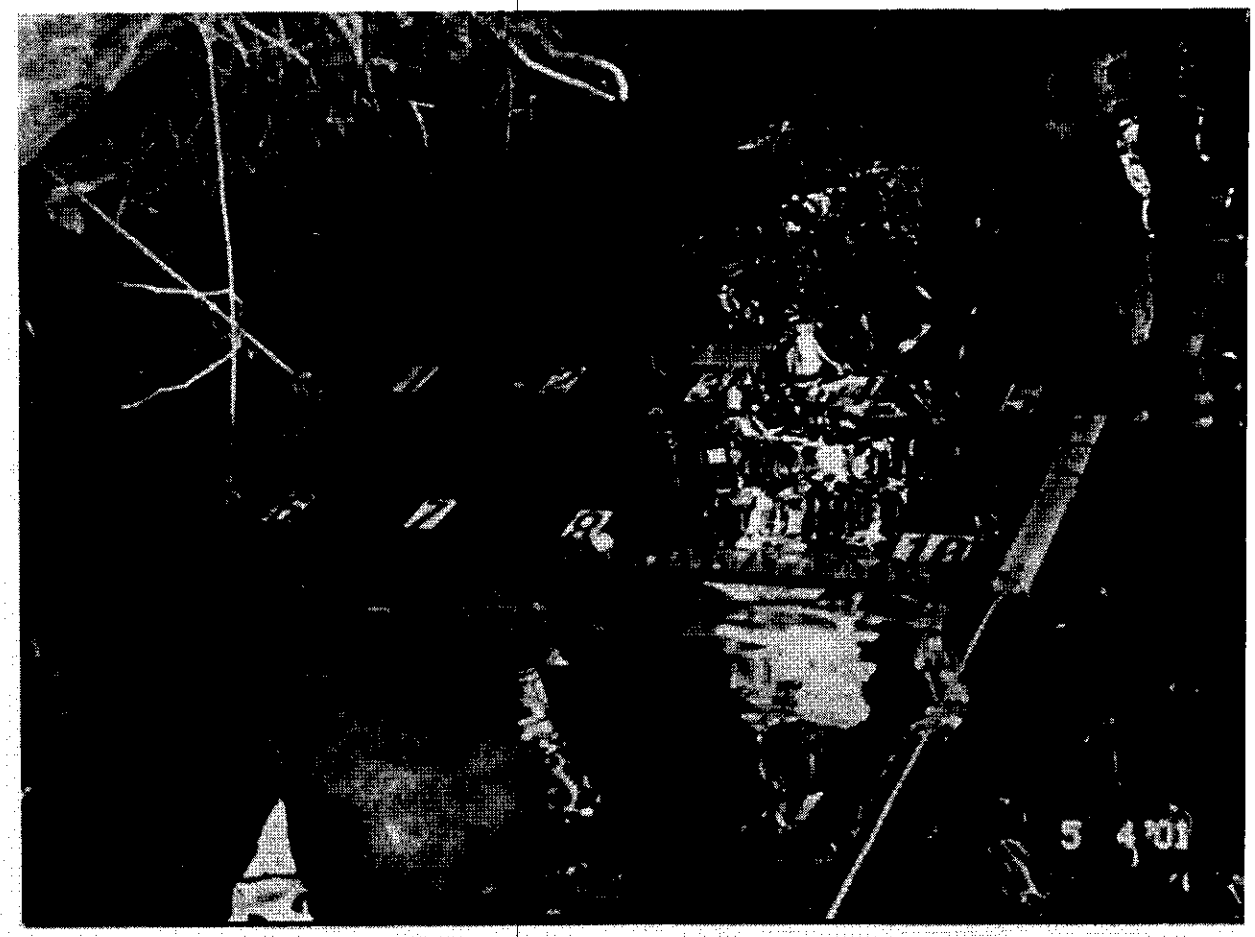

Figure 1. In-stream cage- Little Creek- Body Barn Road; 200 meters upstream of bridge crossing Union Co., Illinois, 2001.

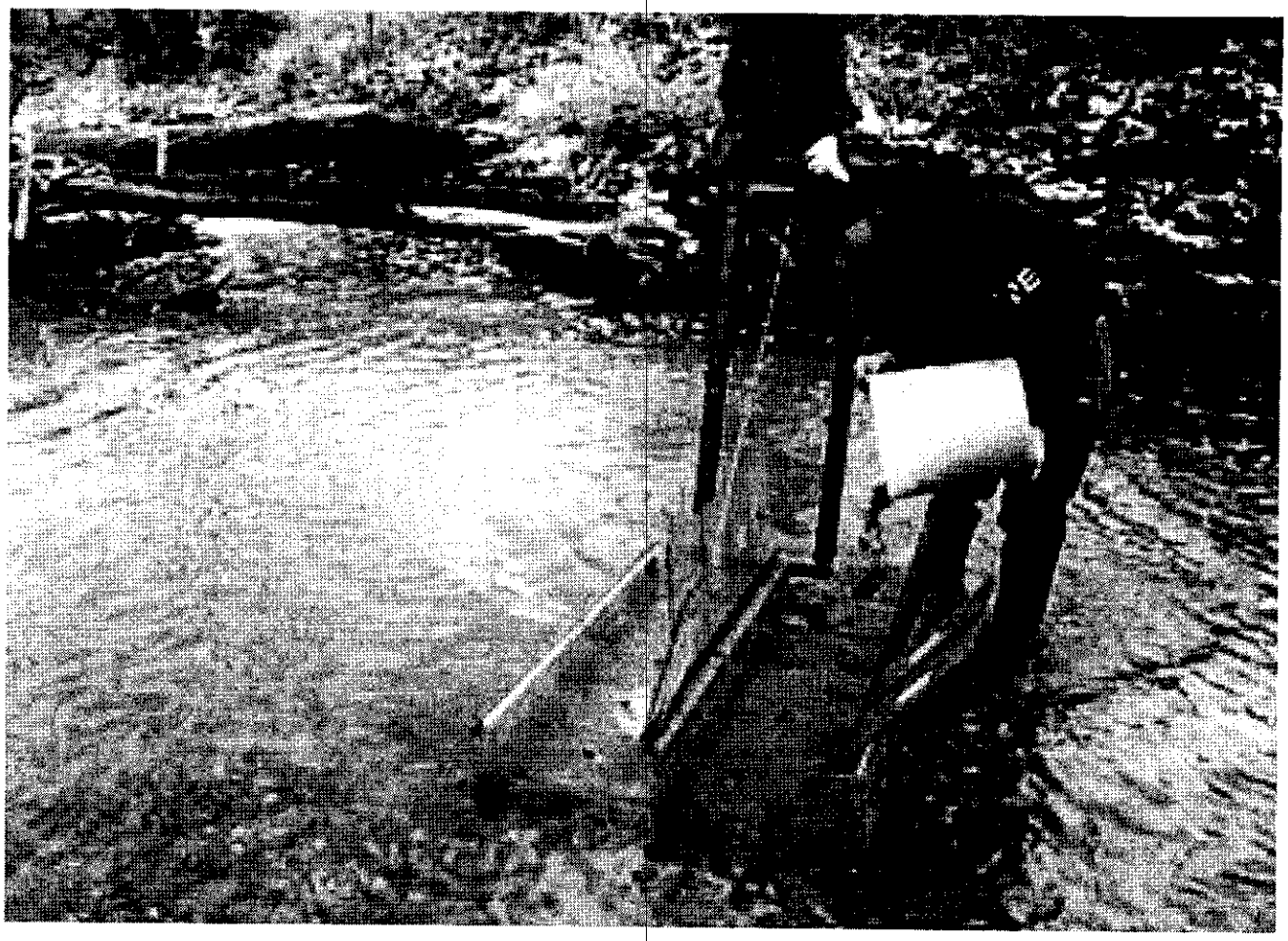

Figure 2. In-stream cage showing ten chambers in Little Creek on Body Barn Road; Union Co., Illinois, 2001.

darters could not escape. The metal bar had a key lock to discourage vandalism. Mesh wire (0.32 cm) covered the back of the cage to allow invertebrates and water into the cage. Slits (6 cm long by 2 cm wide) in front of the cage allowed for water to pass through and for hatchlings to disperse. The cage was placed in a 0.5-meter deep pool and tied off at the front end to a downed tree and to another tree by the back end of the cage.

I placed an Optic Stowaway temperature logger in the stream next to the cage, and the logger recorded hourly water temperatures from 23 February to 12 June 2001.

Before placement of adults into the chambers, I left the cage alone for three weeks to allow invertebrates to colonize. Two centimeters of stream gravel, and a half-cylindrical ceramic spawning tile (Piller and Burr 1998) was placed in each chamber. I stocked the cage with adults from the headwaters of Mill Creek. I randomly chose adults, sexed them, measured their total and standard lengths, and placed a large male and ripe female in each chamber. I waited three days before checking each chamber to allow the fish to adjust to the cage.

Every day at 730 hours, I measured water and air temperatures, water velocity, and depth readings around the cage. During each visit, I checked each spawning tile for the number of eggs. I marked with a grease pencil the placement of eggs on the underside of the tiles so that I would not count the same eggs twice. I also noted the location of the male and female in each chamber. I re-stocked the cage three times during the entire cage study, which was conducted from 23 February to12 June 2001.

Gonadosomatic index (GSI)

I measured each fish with dial calipers to obtain a total and standard length. I obtained total weights of each fish from an Explorer Ohaus micro-fine scale that measured to the nearest 0.0000 ug. I dissected each darter with micro-fine scissors and a scalpel. I exposed internal organs to remove the stomach, intestine, and gonads (Erickson et al. 1985). The body, stomach, and intestine were removed and placed into a vial with 70% ethanol. The testes or ovaries were then removed and placed into a separate vial with 70% ethanol. The body of the fish was preserved in a separate vial in 70% ethanol. I weighed the gonads and stomach/intestine on an Explorer Ohaus micro-fine scale. Ovaries with eggs were teased apart, and a total count of the eggs was taken. Egg diameters were measured using Mitutoyo dial calipers. I made a total count of eggs and determined the dry weight (drying oven for 48 hours) of a subsample. A subsample of eggs was obtained by counting half of the total count of eggs.

Statistical Analyses

I used Principal Components Analysis (PCA) (Johnson and Berk 2000) to statistically compare five habitat variables (average width, average substrate size, average depth, average water temperature, and average velocity) to identify which variable(s) were responsible for most of the variation in streams sampled. PCA was used to identify a series of vectors that are at right angles to one another so that normalized data have maximum variance along the first principal component axis (Quicke 1993).

PCA was used because seven of my habitat variables were highly correlated using multiple correlations. PCA placed habitat components together that were statistically independent. Eigenvalues are given for each component, which represents an overall

variance. PCA was used to analyze densities in relation to five habitat variables measured in 16 streams.

I used multiple stepwise regression in the forward direction to predict which (if any) variables measured at a nest site were predicting clutch size. The variables used in the model were rock length, substrate area, nest cavity depth, and water temperature. Multiple stepwise regression established structural relationships among these variables. I entered the model with a high alpha level (0.250), and the alpha was 0.100 for leaving the model. I used JMP IN 4 (Johnson and Berk 2000) to analyze my data and Excel (2000) to create figures of nesting variables.

RESULTS

DISTRIBUTION

I obtained *E. crossopterum* at 18 of 28 sites sampled (Table 2-3) (Figure 3). Big Creek at Interstate 57 contained seven adults, but no juveniles or nests were found in 2001. Little Creek at Body Barn Road contained adults, nests, and juveniles. Cooper Creek contained adults only. Eighteen sites contained adults and of those, nine sites had nests (Table 2).

My samples and the records I compiled indicated the species is restricted to two major stream systems, Big and Mill Creeks and six of their tributaries in the Cache River drainage (Figure 3). Big and Mill Creeks drain 23,569 hectares of a possible 271,342 hectares drained by the entire Cache River. Of the 2604 km length of the Cache River (Brigham 1978), the entire Illinois range of *E. crossopterum* occupies only 50.6 km. The Illinois range of *E. crossopterum* thus occupies less than 9% of the Cache River drainage.

All sites where I obtained the species were streams of upland character (mean elevation = 70 m)(i.e., moderate gradient, rocky substrates, usually shaded, and often spring-fed). Sites that apparently lacked the species were more lowland (mean elevation = 67 m) in character or had been modified (e.g., channelized, banks denuded) to such an extent that I judged suitable habitat (e.g., cobble/pebble substrate) for the species to be lacking. Appendix A contains latitude and longitude coordinates for each stream.

All streams occupied were perennial and structurally unmodified by humans (i.e., not channelized). Seventeen streams had 2-3 meter wide buffer zones of mature tree growth.

Table 2. Stream sites that yielded samples of *E. crossopterum* in the middle Cache River drainage, 2000-2001.

Stream	Location	Stream order	Dominant substrate	Stream type	# of nests or juveniles	Potential impacts
Little Cr.	Balcom, Body Barn Rd.;US 51; T13S R1W Sec.18	Second	Pebble/gravel	Intermittent	42 Nests	Road runoff; creosote from railroad ties
Big Cr.	Nash Rd.; US 51 T13S R1E Sec.100	Second	Cobble	Perennial	10 nests	Road and agricultural runoff
Crooked Cr.	Dongola Lake Rd. T12S R1W Sec.16	Second	Cobble	Perennial	15 nests	Agricultural runoff; drainage pipe
Crooked Cr.	Pisgah Rd. T12S R2W Sec.6	Second	Clay/silt	Perennial	4 nests	Road and agricultural runoff; bank erosion
Lingle Cr.	Springville Hill Rd. T13S R2W Sec.7	Second	Cobble/gravel	Perennial	8 nests	Road and agricultural runoff; ATV use
Mill Cr.	Redbud Lane T13S R1W Sec.13	Second	Cobble/silt	Perennial	0	Agricultural runoff
Mill Cr.	Quarry Rd. T13S R1W Sec.7	Second	Cobble/boulder	Intermittent	4	Road and agricultural runoff; bank erosion
Big Cr.	Church Rd. T12S R2W Sec. 34	Second	Cobble/gravel	Perennial	10 nests	Road and agricultural runoff; bank erosion
Big Cr.	Interstate 57 T12S R1W Sec.16	Second	Cobble/silt	Perennial	0	Bank erosion; agricultural and road runoff
Cooper Cr.	Alexander/Union County line T13S R2W Sec.31	Second	Gravel/cobble	Intermittent	0	Road and agricultural runoff; ATV use
You Be Hollow	Shawnee Forest T13S R2W Sec31	Second	Cobble	Intermittent	2 nests	Road runoff; culvert

Table 2. continued

Stream	Location	Stream order	Dominant substrate	Stream type	# of nests or juveniles	Impacts
Big Cr.	Dongola Ballpark T13S R1W Sec.18	Second	Cobble/Pebble	Perennial	15 nests	Road runoff
Lingle Cr.	Rt. 127 N. Kinder Lane T13 S R2W Sec.8	Second	Cobble	Intermittent	0	Road and agricultural runoff
* Tributary to Hartline Cr.	Elco, IL; .5 miles West of Rt. 127 T14S R2W Sec.18	Second	Cobble/Boulder	Perennial Intermittent	0	Road runoff; industrial plant
Little Cr.	Little Creek Ln.; Rt.51 T13S R1W Sec.12	Second	Cobble	Perennial	0	Road and agricutural runoff
Little Cr.	East of Wetaug; Old 51 T14S R1W Sec.1	Second	Cobble	Perennial	0	Road and agricultural runoff
Tributary to Mill Creek	Wetaug and Mill Creek Rd. T14S R1W Sec.23	Second	Cobble	Intermittent	0	Road and agricultural runoff
*Little Cr.	Perks and Old 51 T14S R1W Sec.12	Second	Cobble	Perennial	0	Road and agricultural runoff

- Cr. = creek
- T = township
- R = range
- S = section
- TRS derived from quadrangle maps

* vouchered specimens taken only; no habitat measurements

Table 3. Stream sites that did not yield *E. crossopterum* in the middle Cache River drainage, 2000-2001.

Stream	Location	Stream order	Dominant substrate	Stream type	Potential impacts
Jackson Cr.	7.4 km West of Ullin T14S R2W Sec.13	Second	Gravel	Ephemeral	Road runoff; bank erosion
Sandy Cr.	6.4 km NW of Tamms T14S R4W Sec.15	Second	Gravel/silt	Ephemeral	Road runoff; bank erosion
Jim Cr.	4.8 km WNW of Tamms T14S R4W Sec.3	Second	Gravel	Ephemeral	Road runoff; bank erosion
Cypress Cr.	Christian Chapel Rd T12S R1E Sec.4.	Second	Boulder/cobble	Ephemeral	Agricultural runoff; bank erosion
Cypress Cr.	9.7 km SE of Dongola T13S R1E Sec.26	Second	Silt/clay	Ephemeral	Agricultural runoff; bank erosion
Ambeer Cr.	8.1 km South of Tamms T14S R4W Sec.5	Second	Silt/gravel	Ephemeral	Road runoff; bank erosion
Dutch Cr.	Airport Road; 1.6 km West of Rt.127 T13S R1W Sec.12	Second	Silt/gravel/cobble	Perennial	Road runoff; bank erosion
Tributary to Limekiln Slough	Mallard Ln.; off Shawnee College Rd. T14S R2E Sec.26	Second	Silt/clay	Perennial	Agricultural runoff

- Cr = creek
- T = township
- R = range
- S = section

Table 3 continued

Stream	Location	Stream order	Dominant substrate	Stream type	Potential impacts
Little Cache Cr.	Vienna at Tunnel Hill park T12S R2W Sec.30	Second	Boulder/cobble /silt	Perennial	Bank erosion
Wolf Cr.	5 miles south of Tamms T15S R3W Sec.17	Second	Cobble/silt	Perennial	Bank erosion; road runoff

- Cr = creek
- T = township
- R = range
- S = section

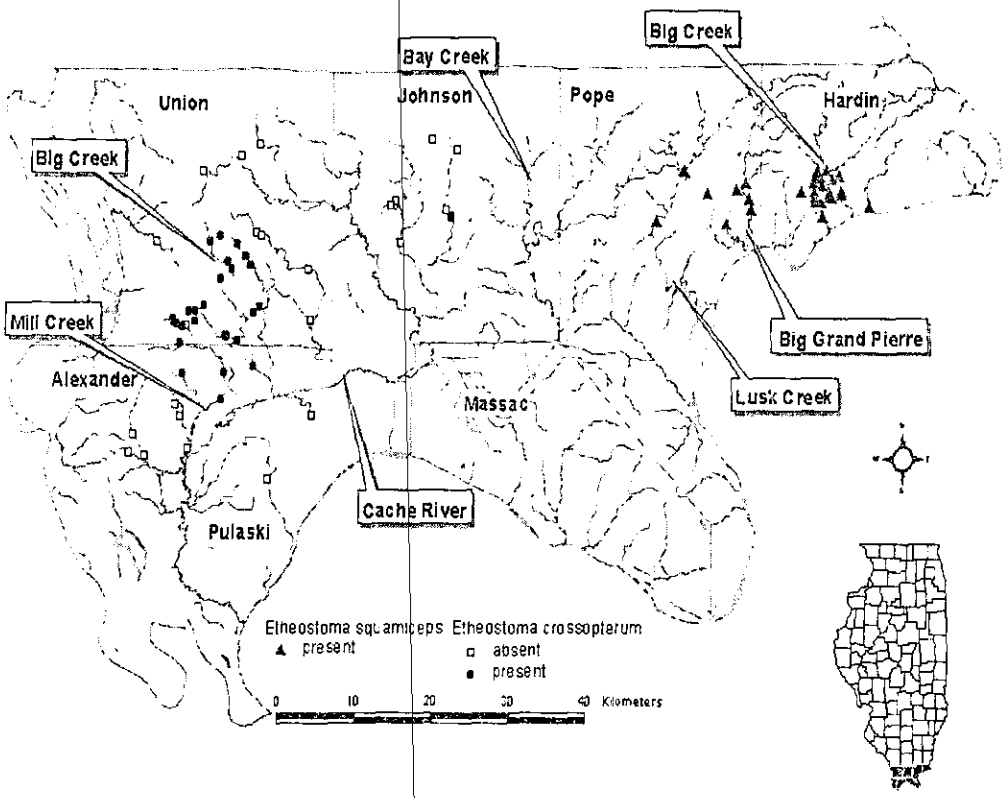

Figure 3. Geographic distribution of *E. crossopterum* and *E. squamiceps* in southern Illinois. Records of *E. crossopterum* are from my field work, Poly and Wilson (1998), and Poly (2000). Records of *E. squamiceps* are from vouchered specimens at Illinois Natural History Survey and Southern Illinois University. Base map from U.S. Dept. of Commerce. Bureau of Census. TIGER files. 1998. Graphic Source: ArcView.

HABITAT

I found adults of *Etheostoma crossopterum* primarily over pebble or cobble-sized substrates in second order headwater streams in pool habitats with zero to minimal flow. I collected juveniles in adjacent run habitats (N = 100; mean velocity = 5-10 m/s; S.D. = 3.5). Spawning and nesting occurred in nine streams where I measured habitat variables. In the 18 streams sampled with *E. crossopterum*, average width was 14.7 ft., average substrate size was 58.4 mm., average depth was 8.28 in., average water temperature was 18.7° C, and average velocity was 4.69 m/s

A Principal Components Analysis (PCA) of five habitat variables showed that each was independent of the other (Table 4). As width, depth and velocity increased in principal component 1, substrate size and average water temperature decreased. The first two components explained 62% of the variation in habitat data. Figure 4 shows an ordination plot of PC1 versus PC 2, and this shows streams grouped together by related habitat variables. Appendix B shows average habitat variables measured for 17 streams.

DENSITIES

Densities varied from 0.002 to 0.907 (mean = 0.090) (Table 5). Densities of darters were significantly related when compared to principal component 1 ($r^2 = 0.18$; $p = 0.10$) (alpha = 0.10) (Figure 5). Low densities were associated with increasing width, increasing depth, and increasing velocity. Densities were highest in Cooper Creek (0.91 darters/m^2), but this data point was a major outlier compared to other density estimates, and it was removed from the analysis. You Be Hollow averaged 0.14 darters/m^2 with decreasing width, depth, and velocity. Principal component 2 was not significantly related to fish density in a linear regression ($r^2 = 0.12$; $p = 0.20$;) (Figure 6).

Table 4. Principal Components Analysis in relation to *E. crossopterum* densities for five habitat variables measured in 17 streams in the middle Cache River drainage, 2000-2001.

Parameter Estimates Term	Estimate	Std Error	t ratio	Prob > t
log (avg. water temperature)	0.9345	0.4197	2.23	0.11
log (avg. substrate size)	-0.4888	0.1460	-3.35	0.04
PC 1	-0.4634	0.1006	-4.61	0.02
log (avg. depth)	0.4711	0.2542	1.85	0.16
PC 2	0.6824	0.3087	2.21	0.11
Whole Model	$r^2 = 0.97$	$p = 0.02$		
Eigen Values	PC1 = 1.6574	PC2 = 1.4213		

- log = log transformation
- PC = principal component

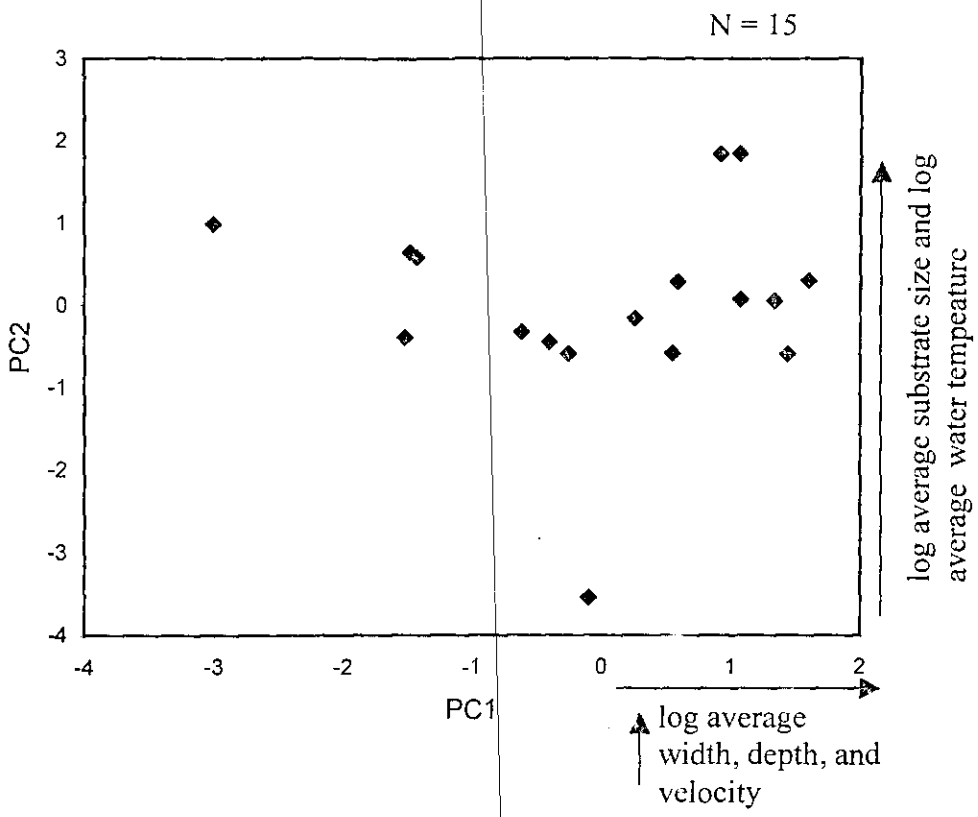

Figure 4. Ordination plot showing principal component one versus principal component two for five habitat variables taken in 17 streams.

Table 5. Densities per m² of *E. crossopterum* for 17 stream sites in the middle Cache River drainage, 2000-2001.

Stream	# of darters	Unit length (m)	Mean width (m)	Density (darters/m²)
Little Cr.-BBarn	11	100	5.12	0.0215
Big Cr.-Nash	93	100	6.89	0.1350
Crooked Cr.-Lake	10	100	3.84	0.0260
Crooked Cr.-Pisgah	26	100	4.05	0.0642
Lingle Cr.-Springville	10	100	2.65	0.0377
Mill Cr.-Redbud	26	155	6.43	0.0261
Mill Cr.-Quarry	10	100	4.82	0.0207
Big Cr.-Church	4	100	4.88	0.0082
Big Cr.-I57	7	300	5.61	0.0042
Cooper Cr.	278	100	2.62	0.9070
You Be Hollow	83	150	4.05	0.1370
Little Cr.-Old 51	3	100	7.28	0.0038
Trib. Big Cr.-Dongola	5	300	10.8	0.0015
Lingle Cr.-Kinder	2	100	4.42	0.0045
Little Cr.-Little Cr. Ln	25	200	6.13	0.0204
Little Cr.-Wetaug	31	25	4.57	0.2170
Trib. Mill Cr.-Wetaug	20	34	5.30	0.1110

- Cr. = Creek
- Trib. = Tributary

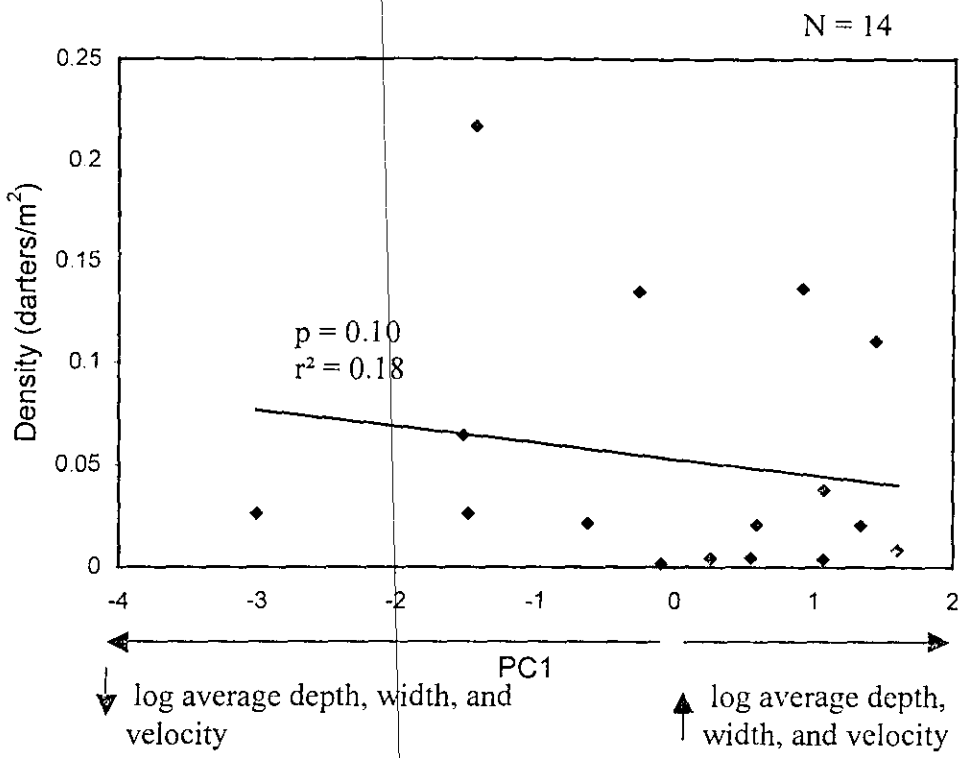

Figure 5. Principal component 1 (average width, depth, and velocity) in relation to fish densities for 16 streams located in the middle Cache River drainage, southwestern Illinois, 2001.

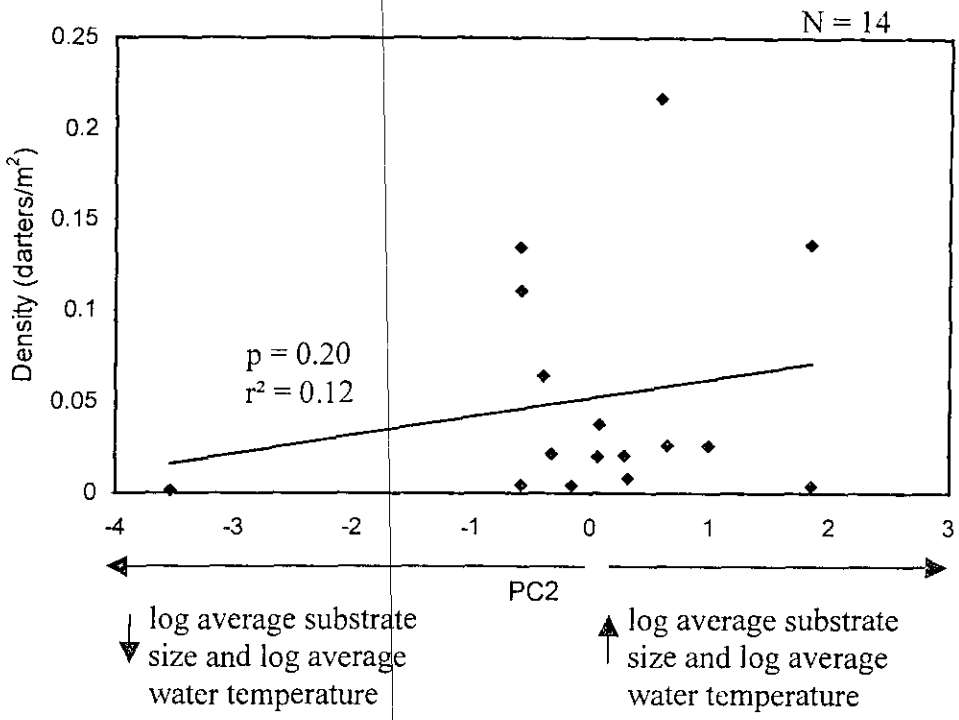

Figure 6. Principal component 2 (substrate size and water temperature) in relation to fish densities for 16 streams located in the middle Cache River drainage, southwestern Illinois, 2001.

Highest densities (Table 5 from previously) found over cobble/pebble substrates in headwater streams with little surrounding land use impacts (Table 6).

Cypress Creek, a major tributary just east of Big Creek, contained zero *E. crossopterum*, and the stream was heavily impacted by agricultural runoff. Average water temperature was the only variable that was not significantly related to densities. As all five variables (width, depth, velocity, substrate size, and water temperature) increased, densities of *E. crossopterum* declined.

Seasonal changes in the density of *E. crossopterum* were found during 2000-2001 (Figure 7). Four streams were examined over 3 or 4 seasons. The seasons were: fall (September-December), winter (December-March), spring (March-June), and summer (June-September). In three of the four streams sampled, *E. crossopterum* total numbers were higher in the summer. Juvenile *E. crossopterum* were found in pools with vegetative cover (i.e; algae). A series of juvenile *E. crossopterum* (10.7- 20.9 mm SL) were collected during June 2001 (Table 7) to get an idea of length measurements after the spawning season. Juveniles were found in groups of 100 or more at the end of the spawning season in June. Table 8 shows the number of darters associated with each macrohabitat. Pools accounted for the highest numbers of *E. crossopterum* (Table 8) adults.

Relative abundances of five fish species occurring in 17 streams in southwestern Illinois were recorded (Table 9). Species abundances are for every stream reach combined for each site. *Etheostoma crossopterum* dominated in total numbers over the

Table 6. Estimated percentages of land usage for 17 streams in the middle Cache River drainage, 2000-2001.

Stream	% Trees/Grasses	% Residential	% Agriculture
Little Cr.- Body Barn	60	30	10
Big Cr.- Nash	40	20	40
Crooked Cr.- Lake	40	10	50
Crooked Cr.- Pisgah	40	10	50
Lingle Cr.- Springville	20	10	70
Mill Cr.- Redbud	5	10	85
Mill Cr.- Quarry	20	15	65
Big Cr.- Church	30	20	50
Big Cr.- I57	20	30	50
Cooper Cr.	50	15	35
You Be Hollow	75	0	25
Little Cr.- Old 51	30	40	30
Trib Big Cr.- Dongola.	20	80	0
Lingle Cr.- Kinder	35	10	55
Little Cr.- Little Cr. Ln.	55	10	35
Little Cr.- Wetaug	20	40	40
Trib. Mill Cr.- Wetaug	15	10	75

- Cr. = creek
- Ln. = lane
- Trib. = tributary

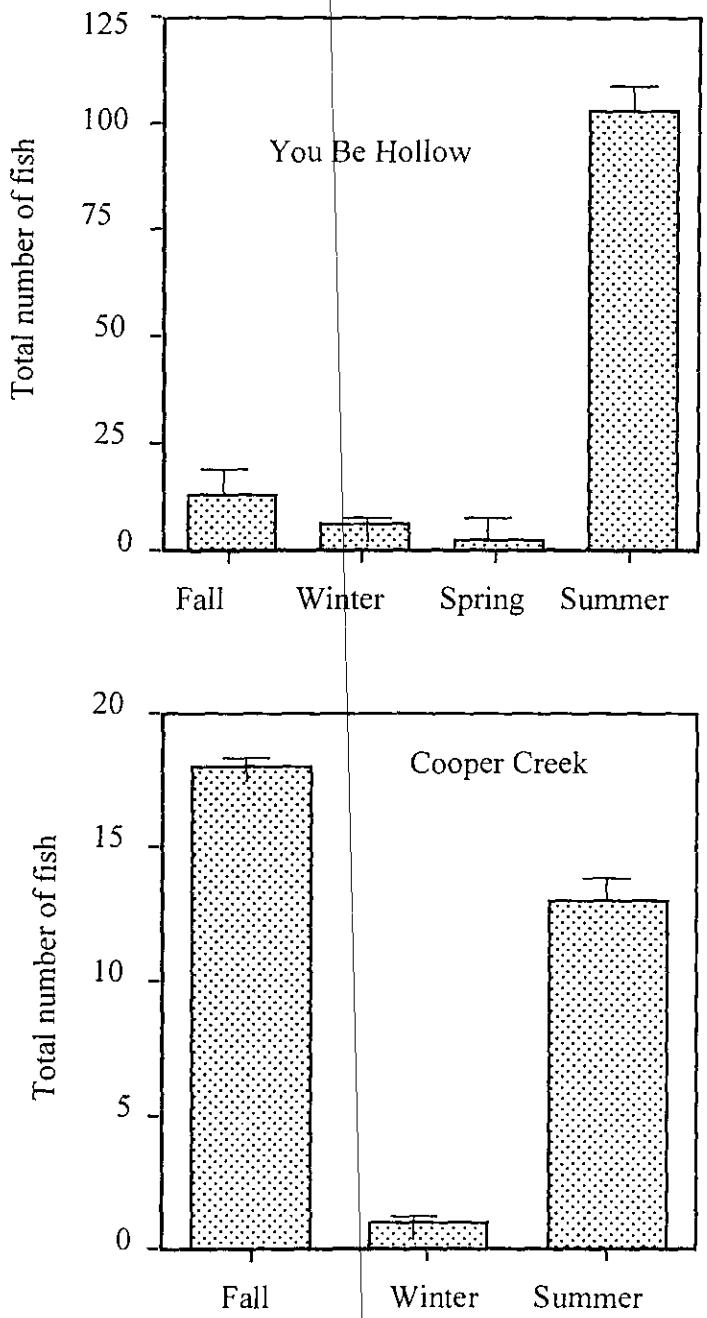

Figure 7. Seasonal changes in total number of *E. crossopterum* for four streams in the middle Cache River drainage, southwestern Illinois, 2001.

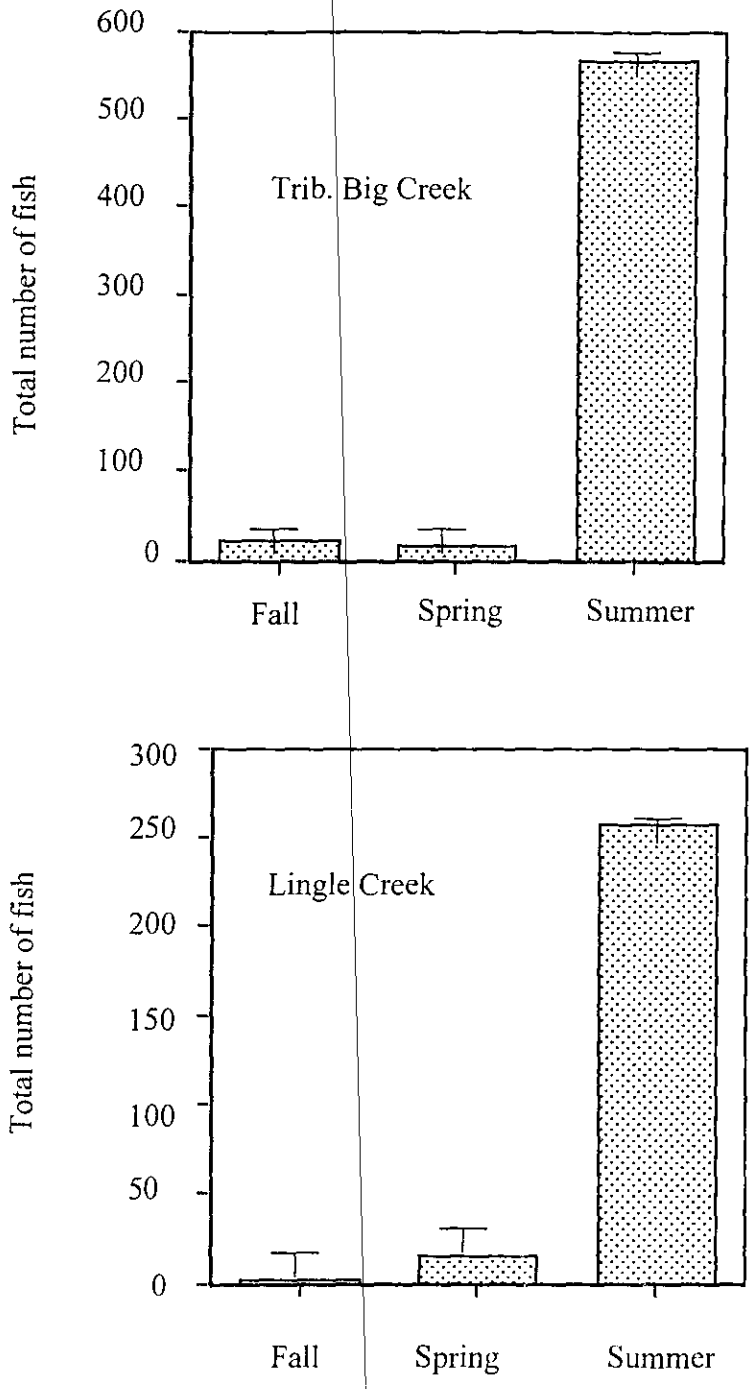

Figure 7 continued. Seasonal changes in total number of *E. crossopterum* for four streams in the middle Cache River drainage, 2001.

Table 7. Juvenile length measurements for *E. crossopterum* collected from Little Creek, the middle Cache River drainage, Illinois, 2000-2001.

Date	Range	Mean	S.D.
29 May 01	15.8-20.9	18.2	1.87
04 June 01	10.7-18.7	13.9	2.45
12 June 01	11.1-19.8	14.9	4.90

• S.D. = standard deviation

Table 8. Total number of darters per unit length using the set-and-kick method for three macrohabitat types in 16 stream sites in the middle Cache River drainage, 2000-2001.

Stream	Macrohabitat	Unit length(m)	Total # of darters
Little Cr.-Body Barn	Riffle	20	0
	Pool	30	1
Big Cr.-Nash	Riffle	30	1
	Pool	70	0
Crooked Cr.-Lake	Pool	100	1
Crooked Cr.-Pisgah	Pool	15	9
Lingle Cr.-Springville	Riffle	100	0
	Pool	200	1
Mill Cr.-Redbud	Pool	12	1
Mill Cr.-Quarry	Riffle	200	9
	Run	200	15
	Pool	200	30
Big Cr.-Church	Pool	50	4
Big Cr.-I57	Pool	50	1
	Run	50	0
Cooper Cr.	Pool	100	300
Little Cr.-Old51	Pool	4	5
Trib.Big Cr.-Dongola	Riffle	30	0
	Pool	70	13
Lingle Cr.-Kinder	Pool	100	1
Little Cr.-Little Cr. Ln.	Pool	6	500
Little Cr.-Wetaug	Pool	50	7
Mill Cr.-Wetaug	Pool	3	25

•Cr. = Creek

Table 9. Percent relative abundance of five most dominant fish species in 17 sites in the middle Cache River drainage, 2000-2001

Stream	*Etheostoma crossopterum*	*Semotilus atromaculatus*	*Pimephales notatus*	*Lythrurus umbratilis*	*Campostoma anomalum*
Little Cr.-Body Barn	36.2	52.1	1.37	1.76	21.3
Big Cr.-Nash	5.34	4.80	0.457	3.52	1.88
Crooked Cr.-Lake	1.30	14.0	17.4	89.2	47.3
Crooked Cr.-Pisgah	9.17	0.087	2.74		
Lingle Cr.-Springville	2.58	15.0	2.74	0.661	23.5
Mill Creek-Redbud Ln.	0.384		49.3		
Mill Cr.-Quarry	1.40	1.31			
Big Cr.-Church	0.105	7.07	3.65	3.74	3.14
Big Cr.- I57	0.244	1.75	18.3		2.09
Cooper Cr.	6.98		0.913	0.441	0.419
You Be Hollow	2.62	0.437			
Little Cr.- Old 51	1.08		3.20		0.209
Trib. Big Cr.-Dongola	1.60	3.41			0.209
Lingle Cr.-Kinder Ln.	0.070				
Little Cr.-Little Cr. Ln.	17.4			0.661	
Little Cr.-Wetaug	1.08				
Trib. Mill Cr.-Wetaug	0.698				
Total numbers	2867	1145	219	454	955

- Cr. = Creek
- Trib. = Tributary
- Ln. = Lane

four other syntopic species of fish. An estimated population size of *E. crossopterum* is 27,337 based on Table 8. Population size was figured by totaling the number of darters found in the total number of meters of stream with *E. crossopterum* and extrapolating out based on the drainage area of the middle Cache River.

REPRODUCTION

Nesting

Nesting occurred from 23 March to 12 June 2001. A total of 127 nests were found: 10 nests in 2000 and 117 in 2001. Of nine sites where nests were found, an average of 10 nests was found in a 100-meter transect. During the 2000 nesting season, only 10 nests were found, with two under cobble, and 8 nests under pebble substrates (32-64 mm diameter). The dominant (74%) substrate used for nesting in 2001 was pebble (89 of 120 nests) (Table 10). Cobble (18%) was used for 21 of the nests, logs (5.0%) for 6 nests, and rusted metal (1.7%) for 2 nests. Logs (bark) contained nests in Big Creek at Nash Road, and the two ceramic tiles I placed into Lingle Creek on Springville Hill Road. Multiple stepwise regression in the forward direction showed nest cavity depth and water temperature to be the most significant variables affecting clutch size (Table 11). Appendix B shows the habitat variables taken at each nest site. Nest cavity depth ($p = 0.057$) and water temperature ($p = 0.090$) were significant based on the alpha level (0.100) I set to leave the model. The overall model ($r^2 = 0.06$; $p = 0.071$) had a significant probability with a low r^2 value.

Two darter nest sites were re-used by *Pimephales notatus* at the end of the *E. crossopterum* spawning season. One marked nest had a *Cottus carolinae* hiding under it.

Table 10. Type of substrate utilized for nesting by *E. crossopterum* in 9 stream sites in the middle Cache River drainage, 2000-2001.

Nesting substrate	# of nests	Mean substrate area (length x width) (cm^2)
Cobble	21	25.0
Pebble	89	6.0
Ceramic Tile	2	75.0
Rusted Metal	2	124
Logs (Bark)	6	50.0
Total	120	Mean = 56.0; S.D. = 46.0

Table 11. Multiple stepwise regression results for four measurements taken at 91 nest sites in nine streams in the middle Cache River drainage, Illinois, 2001.

Variable	F ratio	probability
Nest cavity depth	3.72	0.06*
Rock length	2.94	0.09*
Substrate area	0.227	0.64
Water temperature	0.469	0.50

Whole Model $r^2 = 0.06$ $p = 0.07$

Crayfish were occasionally found under or near nests of *E. crossopterum*.

Nest substrate sizes ranged in greatest length from 1.5 to 12.3 cm (mean = 2.5 cm). Substrate directly under a nest rock was silt/sand/gravel (100%). Nests were in water that ranged from 5.03 to 38.1 cm (mean = 22.3 cm) in depth for nine streams. Average velocity around a nest was 0.1 m/s. Eighty-one nests (75.7%) were found in pools, and 25 nests (23.4%) were found in runs.

Estimated nest clutch sizes ranged from 18 to 500 eggs (mean = 100). Embryos were uniformly distributed in a single layer on the underside of a substrate. I observed five nests with clumping of eggs (i.e., one or more eggs on top of one another). Dimensions of clutch masses ranged from 2.5 to 30.4 mm in length and 2 to 12.5 mm in width. Average egg diameter under a nesting substrate was 2.18 mm. Several eggs were in different developmental stages under a nesting substrate. Some eggs had yolk sacs while other eggs had larval fish inside the embryo about to hatch.

Multiple correlations showed nest cavity depth and egg size correlated at 0.677 ($p = 0.024$). Pairwise correlations (Spearman's Rho) showed water depth and egg size with a significant, positive probability at 0.001.

There was no significant relationship between male total length ($N = 12$) and nest cavity depth ($r^2 = 0.0001$; $p = 0.973$). Males captured guarding nests ranged from 41 to 80 mm total length (mean = 62.1 mm). The density of males observed in a nesting reach ranged from 1 to 10 (mean = 5). I observed only one male under each nest. After lifting up a nesting substrate, a male would remain in place where the substrate had covered him. I observed males under nest rocks that had 10 eggs being guarded after other eggs had hatched (presence of egg scars). If a male left after lifting up a nest rock, he would

return after about two minutes. Males never ventured more than one meter from their nest. I also observed up to three interloping *E. crossopterum* around a nest site. I never observed males eating while guarding a nest. There was no significant relationship between male total length and clutch size (N = 12) ($r^2 = 0.167$; $p = 0.212$).

Embryos were golden-orange when first laid. Egg sizes ranged from 1.95 to 2.4 mm (mean = 2.18 mm). In 100 of 120 nests, I observed embryos in different developmental stages on a nest substrate. Embryos were always touching one another.

In-stream cage study

Two of seven nests had one egg each. Chamber one had one egg at two different times, with the female replaced once. Water temperature ranged from 3.3 to 21.5° C from 23 February to 12 June. Average water temperature was 14.9° C, with an average air temperature of 16.5° C. Spawning commenced on 18 March at 12° C. In seven of ten in-stream cage chambers, females laid from 1 to 110 embryos; the pair in chamber seven had the most numerous clutch size. The range in number of embryos laid per day was from 1 to 30 (mean = 7). Depths around the cage ranged from 29.2 to 40.2 cm (mean = 33.2 cm). The velocity ranged from 0 to 0.1 m/s around the cage. Daily egg counts for three females were found to count the number of eggs laid per day in an in-stream cage (Figure 8). I chose these three females because two females laid one egg each, and five females stopped spawning after a three days in the cage. Three males inside the three different chambers of the cage killed each of the females in their chambers.

Gonadosomatic Index- (GSI)

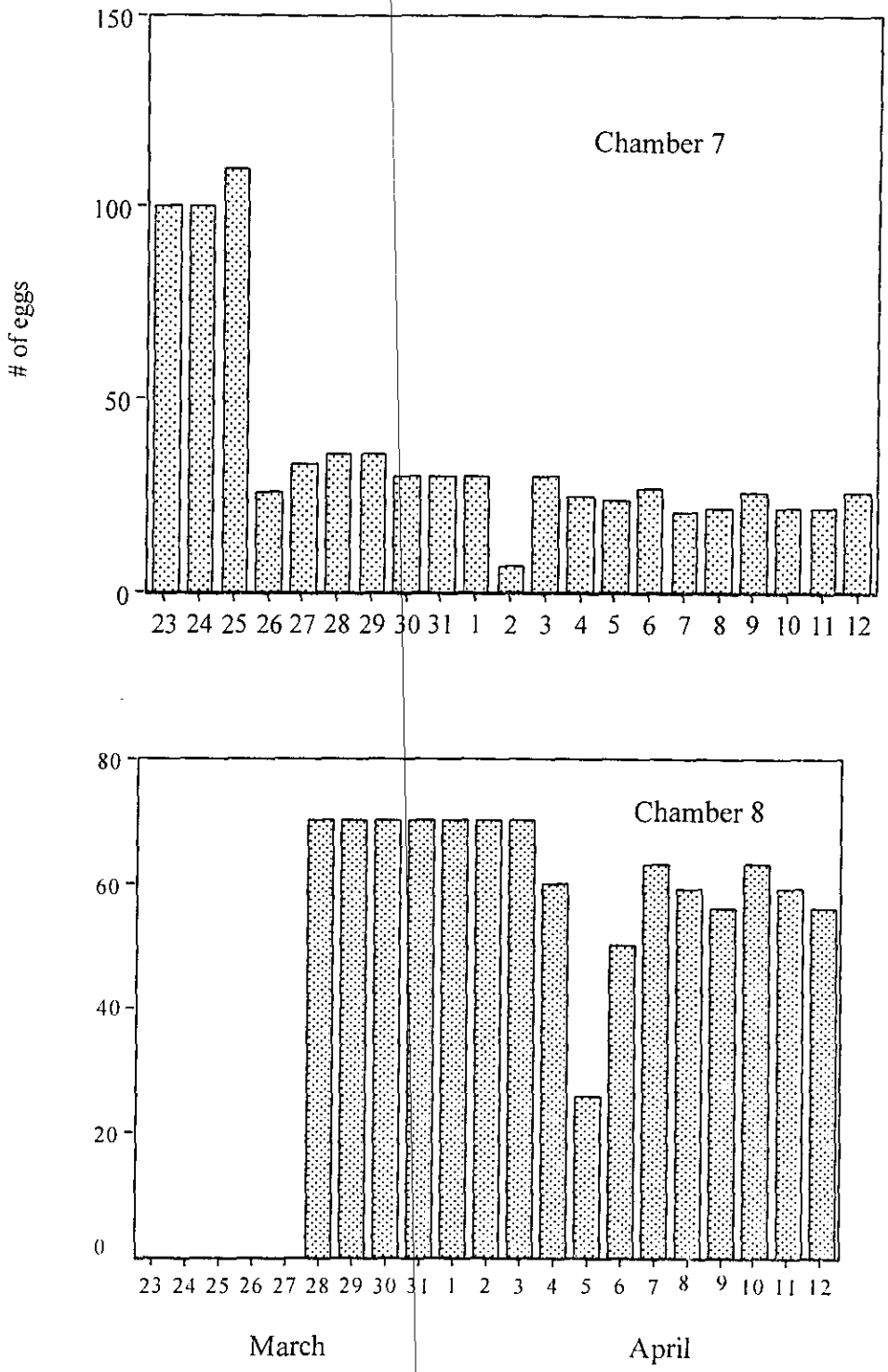

Figure 8. Daily egg counts for *E. crossopterum* in an in-stream cage in Little Creek, Union Co., Illinois, 2001.

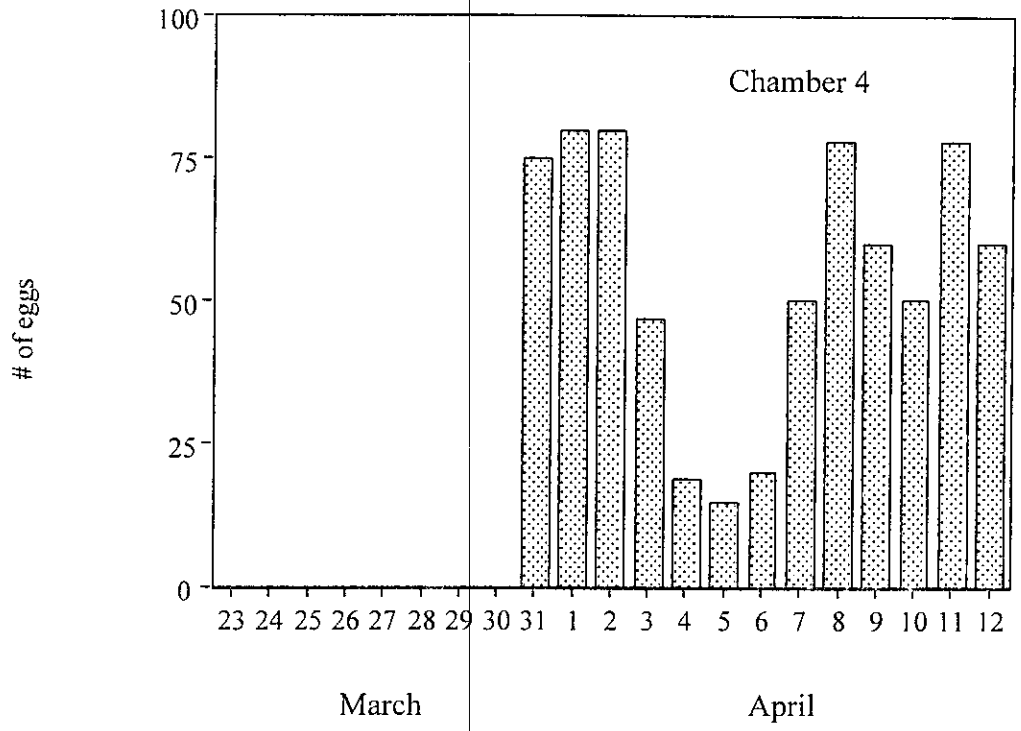

Figure 8 continued. Daily eggs counts for *E. crossopterum* in an in-stream cage in Little Creek, Union Co., Illinois, 2001.

Female (N = 21) GSIs ranged from 0.08 to 0.40 (mean = 0.18) for females (Appendix C). Male (N = 44) GSIs ranged from 0.02 to 0.15 (mean = 0.06) (Appendix D). An increase in total or standard length for *E. crossopterum* shows an increase with either total weight or dry weight. There was a significant positive relationship between the log transformation of female standard length and total body weight (N = 20) ($p = 0.0002$; $r^2 = 0.53$) (Figure 9) and between the log transformation of male standard length and total body weight (N = 44) ($p < 0.001$; $r^2 = 0.80$) (Figure 10). Male *E. crossopterum* from the SIUC Fluid Vertebrate Collection (average standard length = 55.0 mm) were greater in standard length than females (average standard length = 48.0 mm) from Alabama, Kentucky and Tennessee.

Figure 11 (N = 21) shows a significant relationship of the log transformation of female standard length and ovary weights ($p < 0.0015$; $r^2 = 0.42$). Appendix D illustrates the results of ovary and testes weights. Egg diameters ranged from 0.243 mm to 0.271 mm inside the ovaries of females. No difference in egg diameters was measured from left to right ovary. Stomach/intestine weight is much lower in females versus the ovary weight (average stomach/intestine weight = 0.23 g; average ovary weight = 0.39 g) (Appendix C). In males, stomach/intestine weight is higher than testes weight (average stomach/intestine weight = 0.21 g; average testes weight = 0.06 g) (Appendix D).

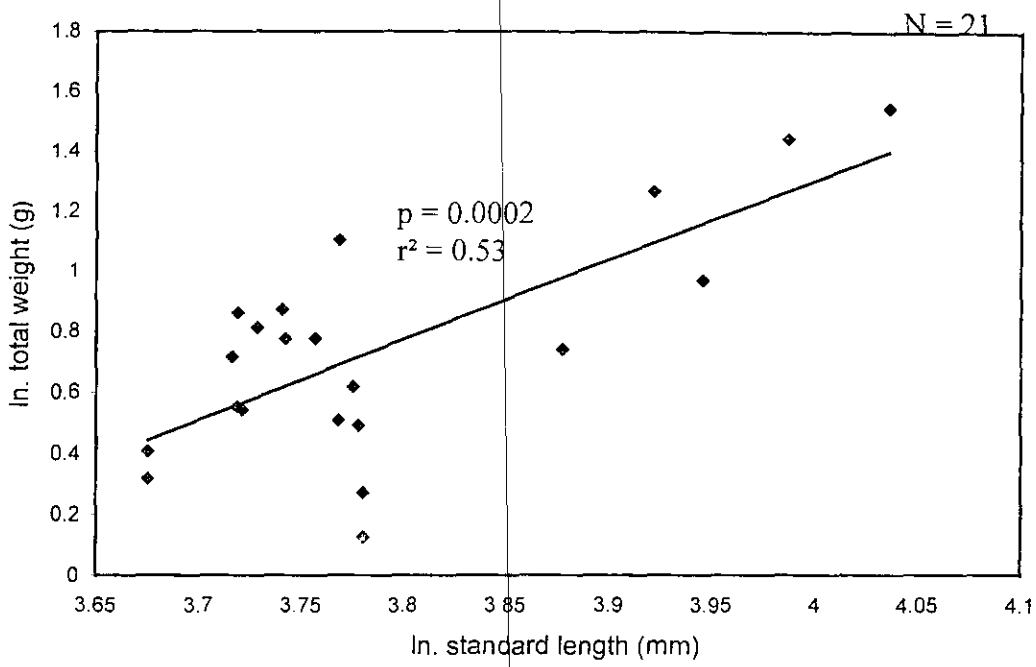

Figure 9. Female standard length and associated total body weights for 21 females collected in the middle Cache River drainage, Illinois, 2000-2001

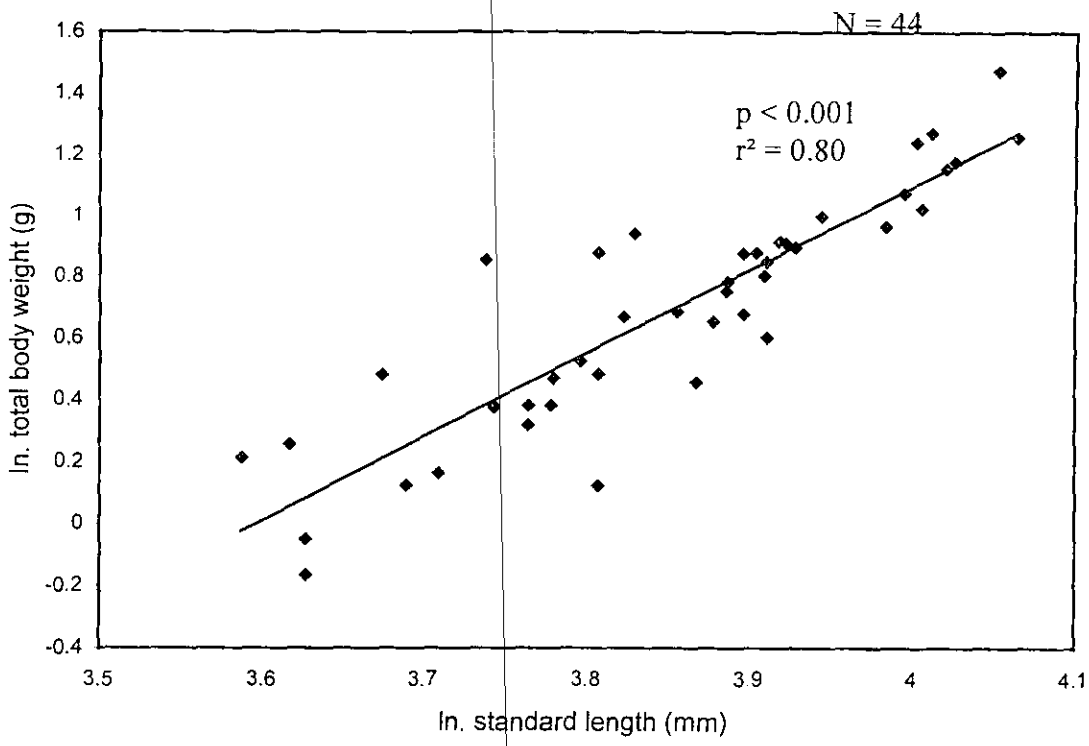

Figure 10. Male standard length and associated total body weights from 44 males collected in the middle Cache River drainage, Illinois, 2000-2001.

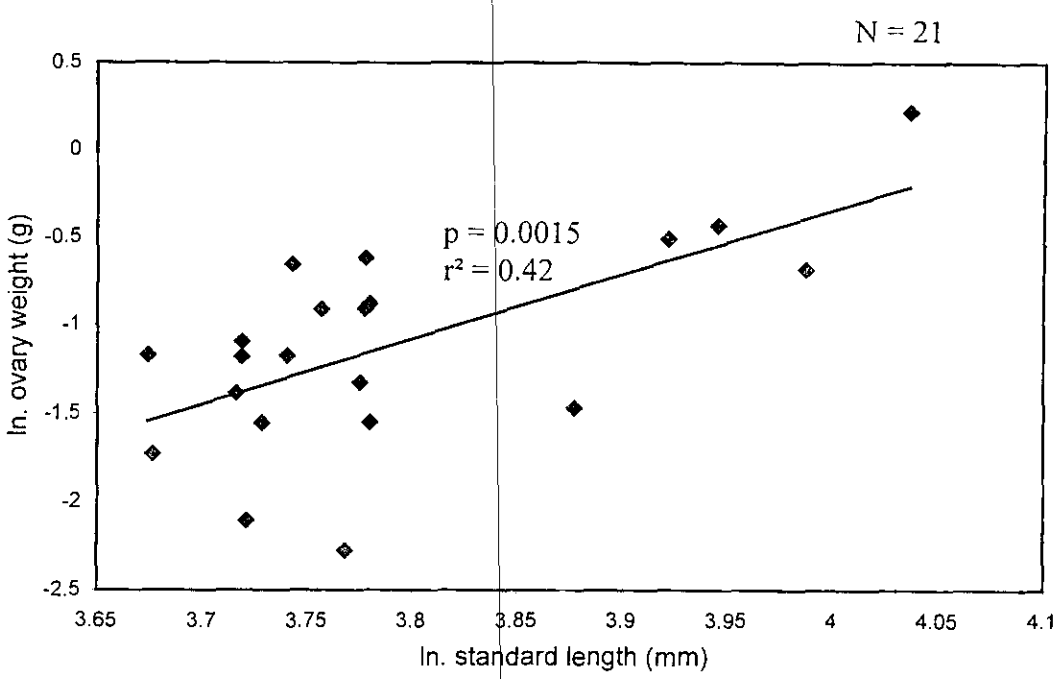

Figure 11. Female standard length and associated ovary weights for 21 females collected in the middle Cache River drainage, Illinois, 2000-2001.

DISCUSSION

DISTRIBUTION

The distribution of *E. crossopterum* is restricted to the middle Cache River drainage in southwestern Illinois, where it occupies only two third order tributaries, Big and Mill Creeks. Its limited distribution is partly the result of somewhat narrow habitat requirements- small, spring-fed headwater streams with cobble/pebble substrates- and historical factors. Outside of Big and Mill Creeks, much of the lower Cache River watershed consists of heavily degraded (i.e., bank erosion, channelized) streams with slow to moderate water velocities. The Illinois populations are isolated from those in the central range of this species in eastern Kentucky, western Tennessee, and the border of Tennessee and Alabama (Page and Burr 1991). *Etheostoma crossopterum* does not occur in streams south and west of Tamms, Illinois because these streams are ephemeral and cobble/pebble substrates are lacking.

I searched 17of 19 streams in which Poly and Wilson (1998) found *E. crossopterum.* I sampled 11 additional streams that had not been collected prior to 2000. There is one historical record (1951) for *E. crossopterum* in Little Cache Creek, but a male in breeding condition is needed to confirm it was indeed *E. crossopterum.* Other than the Little Cache Creek record, no reliable records of the species have been found outside Big and Mill Creeks. Historic sampling outside Big and Mill Creeks has not revealed any species of *the E. squamiceps* group (Smith 1979, Phillippi et al.1986, Muir et al. 1995, and Bennett et al. 2001). No vouchered but unpublished records are known from major museum depositories of Cache River fishes (i.e., INHS, SIUC, UMMZ).

There are no records of *E. crossopterum* from drainages (e.g., Big Muddy River, Saline River) surrounding the Cache River (Smith 1979, Burr and Warren 1994, and Cook 1994).

Only a small percentage (1.9%) of the Cache River is inhabited by *E. crossopterum*, which places this species in danger of extirpation. There is no formal conservation status for *E. crossopterum*, even though Poly and Wilson (1998) suggested the species for listing. A distributional score of 34 for *E. crossopterum* placed this species in the threatened category according to the scores given by Burr and Stewart (1998) for 30 listed fishes in Illinois. The Cache River has been altered hydrologically for years since the 1915 building of the Post Creek cutoff. This changed the flow of the river and separated the Cache River into upper and lower portions. It is important to consider the conservation status of *E. crossopterum* because of its limited Illinois range, and the environmental array of changes that have occurred in the Cache River.

The range of *E. squamiceps* is restricted to three direct tributaries of the Ohio River in extreme southeastern Illinois. A conservation score of 34 was determined for *E. squamiceps*, which also places this species in the threatened category (sensu Burr and Stewart 1998). The two closely related species are separated by the Bay Creek drainage that falls geographically in between the Cache River and Lusk, Big Grand Pierre, and Big Creeks.

Etheostoma crossopterum has six geographically isolated populations. *E. squamiceps* is more evenly distributed with no isolated populations. The center of distribution of both these species is in the Kentucky-Tennessee area. The isolation of populations could be the result of geological or glacial displacements that occurred in the

region, or human alterations of the Cumberland and Tennessee drainages over the past 150 years. The Illinois range of *E. crossopterum* lies between two major physiographic features, the Coastal Plain and the Shawnee Hills. Both features may have acted as barriers for *E. crossopterum* that could have led to the isolated population in Illinois. Braasch and Mayden (1985) provided the only explanation for the unusual distribution of *E. crossopterum* and invoked an historical phylogenetic argument. Ohio drainage access was gained when the Ohio and Cumberland rivers formed a single system (Braasch and Mayden 1985). Another theory is the ancestral "*E. squamiceps*" species gained access to the Ohio River drainage through stream capture between the Ohio and Cumberland drainages (Braasch and Mayden 1985). The diversion of the Ohio River into the Cumberland and diversion of the Mississippi River to join the new Ohio River near Cairo, Illinois, would have affected populations of *E. crossopterum* (Braasch and Mayden 1985). Several range modifications have occurred for the species: 1) invasion of the upper Caney Fork through stream capture from the Duck River; 2) Cypress Creek invasion (middle Tennessee drainage) thru stream capture from the Duck River; 3) populations dispersed west onto the western Highland Rim; and 4) Mississippi Embayment populations were mostly eliminated, except those in western Tennessee and southern Illinois (Braasch and Mayden 1985).

HABITAT

I found the largest populations of *E. crossopterum* in pools (mean depth = 29 cm), and occasionally runs (5-10 m/s), of second and third order streams of moderate gradient over pebble/cobble substrates. Page (1975) and Braasch and Mayden (1985) stated that the preferred habitat of *E. crossopterum* is small quiet streams with large flat rocks or

bedrock substrates. Smaller populations may be found in first order gravelly streams as well as large streams around quiet margins and undercut banks (Page 1975). Page and Burr (1991) stated that rocky pools and adjacent riffles of headwaters, creeks, and small rivers is the habitat for *E. crossopterum*. Etnier and Starnes (1993) found that in Tennessee, preferred habitats are gently flowing pool areas or riffles with slab-rock rubble substrates in small streams. Mettee et al. (1996) stated that boulders in pool areas with reduced flow, slab-rock riffles, and flowing pools with eroding depressions is the habitat for *E. crossopterum* in northern Alabama. In Kentucky, Burr and Warren (1986) found *E. crossopterum* to inhabit upland, headwater streams and creeks. Except for the bedrock, eroding depressions, and riffle habitation, my habitat assessment verifies what has been reported previously for this species outside of the Illinois range.

Seasonal habitat occupation varies between the nesting and non-reproductive seasons. During the nesting season, *E. crossopterum* was found in pools with cobble/pebble substrates suitable for nesting. Pools underlain by gravel strewn with slab cobble comprise the habitat during the peak spawning periods (Burr and Warren 1986). During the nonspawning season, adults occurred in moderate current of riffles underlain by slab, cobble, and pebble substrates (Burr and Warren 1986). I found *E. crossopterum* adults in non-nesting cobble/pebble pools with 0-5 m/s velocity. Young individuals tend to occupy a variety of stream habitats but show a strong affinity for slab riffles (Burr and Warren 1986). Juvenile *E. crossopterum* were usually found along the side of the stream channel and under algae covered rocks in the middle of the channel.

Etheostoma crossopterum is associated with headwater streams, and the widths in these streams are narrow enough that the species can dominate because other fish

species may require wider streams. The important components for habitat were increasing average width, increasing average depth, and increasing average velocity with decreasing average substrate size and decreasing average water temperature. Page and Swofford (1984) found specializations in darters that live in several habitats using canonical vectors with various morphometric measurements taken along with habitat variables. Page and Swofford (1984) found strong correlations between combinations of morphological characteristics and habitat variables for *Etheostoma* species suggesting that the habitat has been a prime influence in the evolution of darter morphology. There are no comparable data from other studies that might verify or falsify my findings.

Surrounding land use and in-stream impacts affected several stream habitats that are occupied by *E. crossopterum*. Agriculture is the primary use of land in southwestern Illinois. The EPA water quality report from 1998 identified impacts on streams throughout Illinois. The middle Cache River watershed had several impacts. In Mill Creek, impacts were: 1) nonirrigated crop production; 2) channelization; and 3) flow regulation/modification. Hartline Creek impacts were: 1) nonirrigated crop production; and 2) channelization. One impact on Lingle Creek was animal holding/management areas at a slight risk. Big Creek was impacted from nonirrigated crop production, pasture land, and animal holding/management areas. Little Creek impacts were nonirrigated crop production and channelization.

Mean water temperature fluctuated at different sites, and this depended, in part, on the presence of springs and the degree of canopy cover. The spring-fed stream at Big Creek Nash road had the coldest average water temperature. I found the latest date for nests (12 June) at this site, and this probably relates to the cooler (15° C) temperature

here when compared to other streams sampled in June. As the seasons changed and day length increased, water temperatures increased. Photoperiod is believed to be the external initiation factor for spawning to commence in darters, which makes this factor more critical than water temperature (Hubbs 1985). With increasing daylight and the coldest temperature (12 ° C), darters peak in spawning activities (Hubbs et al. 1968).

DENSITIES

The range in mean densities (mean = 0.09) for all streams with *E. crossopterum* was 0.002 to 0.91 darters/m². In a Kentucky stream, Page (1974) found a mean density of adults in slab pools of 0.07 darters/m²; in slab riffles, densities were 0.37 darters/m². Cooper Creek supported the highest densities, and it is a second order stream with nesting rocks suitable for *E. crossopterum*. Cooper Creek also provides protection from predators because of the small size of the stream. Agriculture impacts the stream on one side, but there is a buffer strip of trees covering the entire stream. Buffer strips of tree growth provide suitable canopy cover and roots that could filter out chemicals used on the agricultural fields. *E. crossopterum* is able to dominate because many other fish are not specialized enough to inhabit these shallow, narrow, and slow flowing waters.

Etheostoma crossopterum was the dominant darter in each case where it occurred with another darter species, or it was the only darter in a given reach of stream. Of the five dominant fish species in streams where *E. crossopterum* occurred, it was always the most abundant species. A rough calculation based on the site map (Figure 3) showed *E. crossopterum* to occupy 50 km of streams. Densities can be used to assess the quality of streams in southern Illinois. There are no comparable data on densities for other species of *Catontous*.

REPRODUCTION

Nesting

Bark, ceramic tiles, and rusted metal were used for spawning substrates in three streams with *E. crossopterum*. Page (1974) found *E. squamiceps* to spawn on slab rocks. Poly (2000) found *E. crossopterum* to spawn on slab rocks, tires, and any suitable nesting substrate that was available. Piller and Burr (1998) found *E. chienense* to spawn on any substrate that was available, including tires and glass bottles. Poffinbarger (2000) found *E. flabellare* laying eggs on substrates with a mean of 20.3 cm. Ceramic tiles were placed out into streams with *E. chienense* to provide additional spawning substrates (Piller and Burr 1998). Tiles and slates were used in Lake Waccamaw, North Carolina for *E. longmanum* egg production (Lindquist et al. 1984).

Nest clutch sizes of *E. crossopterum* ranged from 18-500 eggs, and in *E. squamiceps* from 8 to 1500 eggs (Page 1974). Males in both species set up territories before spawning commenced, and large males successfully defended their nest rocks against smaller males, probably because of their size dominance. Size dominance and nest guarding is needed to defend against other potential predators such as crayfish.

I found the bluntnose minnow, *Pimephales notatus* re-using two nesting substrates of *E. crossopterum* for its nest sites. Nest sites of *E. crossopterum* were commonly re-used by other individuals of the species near the end of the spawning season. Poffinbarger (2000) found re-used *P. notatus* nests that *E. flabellare* occupied earlier in the spring.

I found one male guarding three nests at the same time. There is no literature to date on this behavior among species of *Catonotus*. One male could be guarding three

nests that might not be fertilized by that guarding male. The reason for one male guarding three nests could be the competition for prime sites because of nest-reuse. Weddle and Burr (1991) found *E. rafenisquei* to have multiple clutches. Page (1974) found that at least three female *E. squamiceps* spawned with one male in aquarium studies. Assuming females lay multiple clutches (sensu Heins 1986) in one season, a given female contributes about 10 eggs to a nest. Multiple clutching may be a strategy developed to ensure that a portion of eggs will hatch. By laying eggs in several different nests, this allows darters to save energy instead of using all their energy laying eggs in one nest. The reason for laying eggs in several different nests (multiple clutching) could be the need to allocate energy into laying eggs at various times throughout the nesting season. Multiple females are reported to lay eggs in one nest (Page 1974). By laying the eggs in intervals, this might be a way for females to save energy over the spawning season. Heins and Rabito (1986) found evidence for multiple clutches in the genus *Notropis*. Size distributions of ova in ovaries indicated multiple clutching in *Notropis*. After dissecting female *E. crossopterum*, large eggs were found with small and medium sized eggs attached. Multiple clutching is evident when looking at the size distribution of eggs in *E. crossopterum*.

A minimum nest cavity depth of 2.0 cm is critical for male and female inversion during spawning. Males and females invert in a head to head spawning position (Page 1974) under a nesting substrate. Larger females have a greater number of mature ova (Heins and Baker 1993) and need a deep enough nest cavity to invert for egg laying and to lay an entire clutch. Increase in egg size and parental care decrease fecundity, while an increase in body size will increase fecundity (Helfman et al. 1997). Males need space

under the nesting substrate to guard eggs, pick off fungus or dead eggs, and to rub or clean eggs with their thickened napes.

Egg diameters of *E. crossopterum* averaged 2.18 mm on the underneath sides of substrates. Page (1974) found an average egg diameter of 1.8 mm in *E. squamiceps*. Poffinbarger (2000) found *E. flabellare* egg diameters to average 1.73 mm. In a life-history study of *E. percenurum* , Layman (1991) found that eggs [or embryos] averaged 2.3 mm in diameter, were translucent gold, had one or more amber oil globules, and were indented on one side.

Pebble/cobble substrate (mean = 20 cm) was the dominant nesting substrate when clutch sizes exceeded 350 eggs. Silt/sand, gravel, and silt/gravel were the dominant substrates under nests of *E. crossopterum*, and this could be due to the ease of moving this material out of the way. Male were observed pushing the substrate to each side to situate their bodies under the nest.

I found no significant effect of nearest nesting neighbor on clutch sizes. Poffinbarger (2000) found no significant differences among nearest nesting neighbor and clutch sizes of *E. flabellare*. Constanz (1979) found subordinate male tessellated darters to clean attached eggs of dominant males nests when they were away from their nests.

Lengths and weights are more highly correlated in males than in females. Males apparently place energy into attaining a large body size, greater fin lengths, and a variety of color displays. Large bodies with expanded fins helps males attract females and aids in defending nesting territories against potential predators. Females place more energy into egg production rather than somatic growth, and this explains why for ovary weights always exceed testis weights.

Environmental variation could be the cue for darters to spawn because of the change in seasons. Coldest temperatures are found prior to spring, which is the start of the darter spawning season. An increase in spring water temperature is crucial to the onset of spawning in *E. crossopterum*. Water temperature of 15° C (range = 12-21 ° C) was found for *E. crossopterum* clutch sizes of 100 to 300 eggs. Page (1974) found spawning to occur among *E. squamiceps* at water temperatures between 14-19° C. When water temperatures exceeded 20° C, 18 eggs were found. Lower clutch numbers could be due to eggs having hatched previously or that increasing water temperatures cause females to lay fewer eggs or eggs may not develop because of increased (> 21° C) water temperatures. Increasing photoperiod along with the coldest water temperatures (12° C) appears to be the cue for spawning to take place and for the largest clutch sizes to be found.

I only looked at only four variables in my stepwise regression model, and there could be other variables that are important in determining clutch size. An interaction of variables may be important in clutch size variation, and it is possible that other measurements taken or not taken could be influencing clutch sizes.

In-Stream Cage Study

Clutch sizes for the in-stream cage were small (range = 1- 110 eggs) compared to clutches in the wild (range = 18-500 eggs). Weddle and Burr (1991) found *E. rafinesquei* females to spawn 500-1000 ova during an in-stream cage study in Kentucky. Gale and Deutsch (1985) found tessellated darters (*Etheostoma olmstedi*) to be multiple clutchers in an in-stream cage. Only one female and one male were present at a time in each of the ten chambers. Polygamy is common among several species of darters, including *E.*

crossopterum. Page (1974) found one male *E. squamiceps* spawning with three females at different time intervals in aquarium studies. Eggs were laid in intervals over the course of a two-month period. Smaller clutch sizes could be due to the confinement of males and females to a chamber. Males in the chambers often killed the females. In the wild, the female leaves after the egg-laying interval, while the male stays to guard the nest. In each of the chambers, neither males nor females had any way of escaping, and males may have felt threatened by the females being so close to the nest, and this ended in the death of some females.

Photoperiod and water temperature are the cues for darters to start spawning. Average water temperature of the cage was around 14.9° C. Weddle and Burr (1991) found *E. rafinesquei* to spawn at temperatures below 21.5° C in an in-stream cage. Weddle and Burr (1991) found *E. rafinesquei* batch fecundity to be negatively influenced by water temperatures in excess of 21.5° C.

A major flood disrupted the spawning activities of *E. crossopterum* inside the cage. A flood of this magnitude causes nest substrates to turn over and be dispersed far from the guarding male. Substrates with eggs can be completely displaced after a flood, and the hatching success is unknown but presumed to be zero percent.

Confinement to individual chambers could be stressful to either or both sexes. In one chamber, eggs appeared to have been eaten because 100 eggs were originally present, but the following day there were only 70 eggs. Eggs had not hatched because they had just been laid two days earlier, and yolk was still present. Depth, flow, siltation, food supply, and confinement inside a chamber could have been several factors leading to smaller clutch sizes on artificial tiles.

Egg-clustering is a unique behavior among species of *Catonotus*, and this makes for an interesting study for *E. crossopterum*. "Egg-clustering breeding behavior (Page 1985) and several forms of egg mimics (Page and Swofford, 1984; Knapp and Sargent 1989; Page and Bart 1989) make species of *Catonotus* one of the most morphologically and behaviorally interesting assemblages of North American fishes and a prime target for studying character evolution" (Porterfield et al. 1999).

Gonadosomatic index- (GSI)

GSIs were higher in females than males. GSI tends to be higher in females around the start of the spawning season. Male GSIs are higher in February and remain steady throughout the spawning season. Larimore (1957) found warmouth females to have higher GSI values than male warmouths. The difference in body weight and total lengths leading to variations in GSIs of males and females may have to do with males guarding nests. Poly (2000) found male *E. crossopterum* to be larger in length than females. Museum specimens of *E. crossopterum* from the Southern Illinois University Fluid Vertebrate Collection showed male standard and total lengths to be greater than females. Ovary weights may be greater than testes weights because females need to ensure the success of eggs hatching or recruitment of *E. crossopterum* by producing several eggs.

Future research on *E. crossopterum* should focus on the current population size in the middle Cache River drainage. Artificial ceramic tiles could be used to increase nest productivity and presumably recruitment. Habitat for young of the year could be increased with the implementation of stream restoration practices. This would involve researching newberry weirs to see if habitat increases for *E. crossopterum*. Newberry

weirs are rock riffle structures designed to stop downgrading of the channel and to slow or halt bank erosion. Newberry weirs create pools above the rock riffle structures, which could provide increased habitat for *E. crossopterum*. Monitoring population numbers is important for the future to detect any positive or negative changes in population size. Buffer zones of two to three meters of tree growth is important to maintain so that bank erosion is kept at a minimum. First and second order streams are important to protect because these streams provide habitat for *E. crossopterum*. *Etheostoma crossopterum* population numbers were higher in these streams than higher order streams (> third order) because cobble/pebble is dominant and depths are shallower. Population numbers over a number of years could remain steady or increase as a result of stream restoration practices in the Cache River drainage.

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APPENDIX A.

Latitude and longitude coordinates for 28 streams sampled in the middle Cache River drainage, 2000-2001.

1) Big Creek- Nash Rd.- N37° 27.21' W89° 12.98'
2) Big Creek- Big Creek Church Rd.- N37° 24.76' W89° 09.94'
3) Big Creek- Interstate 57- N37° 23.93' W89° 09.94'
4) Tributary to Big Creek- Dongola- N37° 21.86' W89° 09.52'
5) Little Creek- Balcom – N37° 24.90' W89° 13.12'
6) Little Creek- Body Barn Rd.- N37° 24.12' W89° 11.29'
7) Little Creek- Little Creek Ln.- N37° 24.20' W89° 10.75'
8) Little Creek- Perks and Old 51- N37° 19.20' W89° 09.74'
9) Crooked Creek- Pisgah Rd.- N37° 20.10' W89° 11.21'
10) Crooked Creek- Dongola Lake Rd.- N37° 20.61' W89° 12.63'
11) Lingle Creek- Springville Hill Rd.- N37° 21.67' W89°17.30'
12) Lingle Creek- Kinder Ln.- N37° 21.20' W89° 15.70'
13) Mill Creek- Redbud Ln.- N37° 19.84' W89° 13.18'
14) Mill Creek- Quarry Rd.- N37° 22.35' W89° 14.39'
15) Cooper Creek- N37° 19.94' W89° 16.53'
16) You Be Hollow- N37° 20.74' W89° 18.88'
17) Tributary to Hartline Creek- N37° 18.38' W89° 16.43'
18) Jackson Creek- N37° 16.82' W89° 16.37'
19) Sandy Creek- N37° 14.94' W89° 20.13'
20) Little Creek- Wetaug- N37° 20.93' W89° 10.78'
21) Cypress Creek- Christian Chapel Rd.- N37° 25.11' W89° 06.80'
22) Cypress Creek- Hickory Bottoms- N37° 21.54' W89° 04.17'
23) Jim Creek- N37° 13.60' W89° 18.95'
24) Ambeer Creek- N37° 10.50' W89° 18.28'
25) Dutch Creek- N37° 25.73' W89° 17.31'
26) Tributary to Limekiln Slough- N37° 19.82' W89° 04.50'
27) Little Cache Creek- N37° 25.39' W88° 52.86'
28) Wolf Creek- N37° 11.29' W89° 19.20'

APPENDIX B.

Five habitat variables measured for 17 streams in the middle Cache River drainage, 2000-2001.

Stream	Average width (cm)	Average substrate size (mm)	Average depth (cm)	Average water temperature (° C)	Average velocity (m/s)
Lingle Cr. Springville	41	40.8	5.00	19.4	4.38
Crooked Cr. Lake Rd.	60	19.1	3.74	23.7	4.32
You Be Hollow	63	35	1.38	14.4	2.5
Crooked Cr.- Pisgah	63	10	1.57	22	2.5
Little Cr.- Old 51	113	15.1	1.82	26	7.5
Cooper	41	395	0.800	24.1	2.5
Little Cr.- Body Barn Rd.	60	36.5	3.37	19.4	8.13
Big Cr.- Nash Rd.	107	58.1	5.12	15.9	5.83
Big- Dongola	53	49.6	5.83	18.5	5.36
Big Cr.- Church Rd.	72	30.5	4.53	17.7	2.5
Mill Cr.- Quarry	75	89.6	2.36	8.85	7.5
Big- I57	87	80	2.50	8	6.39
Little Cr.- Wetaug	71	0.0039	5.51	21.5	2.5
Lingle Cr.- Kinder	69	34	5.91	23	5
Mill Cr.- Redbud	100	28	3.24	22.4	7.5
Mill Cr.- Wetaug	82	97	1.04	22.3	2.5
Little Cr.- Little Cr. Ln.	95	32.5	4.92	30.3	7.5

- Cr. = creek
- Ln. = lane

APPENDIX C.

Length, weight, ovarian clutch size, and GSI of 21 female *E. crossopterum* from Mill and Little Creeks, Union Co., Illinois, 2001.

Stream	Date	S.L.(mm)	Total Weight (g)	S/I weight(g)	Ovary weight (g)	No. of eggs	GSI
Mill Cr.	23 March 01	43.8	1.13	0.09	0.42	112	0.4
Mill Cr.	23 March 01	43.8	1.31	0.18	0.21	67/47(L/R)	0.2
Mill Cr.	23 March 01	43.3	3.01	0.49	0.54	98/128(L/R)	0.2
Mill Cr.	23 March 01	43.7	1.64	0.29	0.40	73/65(L/R)	0.3
Mill Cr.	23 March 01	51.7	2.64	0.36	0.65	95/71(L/R)	0.3
Mill Cr.	13 April 01	48.3	2.10	0.17	0.23	69/83(L/R)	0.1
Mill Cr.	13 April 01	43.6	1.86	0.13	0.27	61/57(L/R)	0.2
Mill Cr.	13 April 01	39.5	1.37	0.13	0.18	43/40(L/R)	0.1
Mill Cr.	13 April 01	39.4	1.50	0.12	0.31		0.2
Mill Cr.	13 April 01	41.3	1.72	0.11	0.12		0.08
Mill Cr.	13 April 01	43.3	1.66	0.36	0.10	29/51(L/R)	0.08
Mill Cr.	13 April 01	41.2	1.74	0.14	0.33	64/77	0.2
Mill Cr.	13 April 01	42.8	2.18	0.18	0.40	69/133	0.2
Mill Cr.	13 April 01	42.1	2.40	0.20	0.31	87/71	0.1
Mill Cr.	13 April 01	41.1	2.05	0.24	0.25		0.1
Mill Cr.	13 April 01	41.2	2.37	0.27	0.31		0.1
Mill Cr.	13 April 01	41.6	2.26	0.10	0.21		0.1
Little Cr.	23 March 01	56.6	3.55	0.20	0.60	212	0.3
Little Cr.	19 April 01	50.5	2.17	0.16	0.52	81	0.2
Little Cr.	19 April 01	42.2	4.70	0.50	1.25	136	0.3
Little Cr.	20 April 01	53.9	4.24	0.41	0.51	114/142	0.1

- Cr.= creek
- L/R = left/right ovary
- S.L. = standard length
- S/I = stomach/ intestine
- GSI = gonadosomatic index

APPENDIX D.

Length, weight, testes weight, and GSI of 44 male *E. crossopterum* from Mill, Little, Crooked, Cooper, and Big Creeks in southwestern Illinois, 2001.

Stream	Date	Standard length(mm)	Total weight(g)	Stomach/intestine weight(g)	Testes weight(g)	GSI
Mill Cr.	23 March 01	49.9	1.83	0.26	0.07	0.04
Mill Cr.	23 March 01	48.7	2.12	0.33	0.16	0.09
Little Cr.	23 Feb 01	43.8	1.60	0.24	0.05	0.04
Little Cr.	07 June 01	56.0	3.25	0.18	0.06	0.02
Little Cr.	07 June 01	49.2	2.00	0.07	0.03	0.02
Little Cr.	18 April 01	51.6	2.72	0.17	0.02	0.01
Big Cr.	30 March 01	49.8	2.24	0.19	0.04	0.02
Little Cr.	07 June 01	47.2	2.00	0.09	0.03	0.02
Mill Cr.	13 April 01	39.4	1.61	0.29	0.04	0.03
Mill Cr.	13 April 01	54.7	3.47	0.24	0.09	0.03
Little Cr.	07 June 01	55.2	3.57	0.16	0.10	0.03
Crooked Cr.	11 Sept 00	47.8	1.58	0.12	0.07	0.05
Cooper Cr.	02 Oct 00	45	1.13	0.22	0.15	0.17
Crooked Cr.	18 June 01	37.2	1.29	0.13	0.14	0.12
Crooked Cr.	18 June 01	36.1	1.23	0.13	0.12	0.11
Little Cr.	19 June 01	58.2	3.52	0.23	0.10	0.03
Big Cr.	02 Mar 01	45	2.41	0.09	0.13	0.06
Little Cr.	07 June 01	57.5	4.38	0.22	0.18	0.04
Little Cr.	07 June 01	46	2.56	0.19	0.17	0.07
Little Cr.	07 June 01	42	2.35	0.20	0.19	0.09
Little Cr.	19 June 01	48.7	2.13	0.27	0.18	0.10
Little Cr.	19 June 01	50.3	2.50	0.47	0.17	0.08
Little Cr.	19 June 01	55.7	3.18	0.52	0.17	0.06
Little Cr.	19 June 01	54.3	2.94	0.32	0.19	0.07
Little Cr.	19 June 01	49.2	2.41	0.36	0.22	0.12
Little Cr.	19 June 01	45	1.62	0.27	0.20	0.15
Little Cr.	19 June 01	49.9	2.35	0.31	0.21	0.10
Little Cr.	19 June 01	45.7	1.95	0.31	0.22	0.13
Mill Cr.	15 March 01	53.7	2.63	0.18	0.04	0.02
Mill Cr.	15 March 01	48.3	1.93	0.12	0.07	0.04
Mill Cr.	15 March 01	54.9	2.80	0.23	0.06	0.02
Mill Cr.	15 March 01	50.8	2.46	0.07	0.03	0.01
Mill Cr.	15 March 01	43.7	1.46	0.11	0.02	0.01
Mill Cr.	15 March 01	49.6	2.42	0.19	0.09	0.04
Mill Cr.	15 March 01	50.5	2.49	0.21	0.08	0.04
Mill Cr.	15 March 01	42.2	1.46	0.20	0.08	0.06
Mill Cr.	15 March 01	43.1	1.37	0.18	0.06	0.05
Mill Cr.	15 March 01	44.5	1.69	0.24	0.07	0.05
Mill Cr.	15 March 01	40.8	1.17	0.12	0.08	0.07
Mill Cr.	15 March 01	42.2	1.45	0.15	0.06	0.05
Mill Cr.	15 March 01	40.0	1.13	0.13	0.05	0.05
Mill Cr.	15 March 01	43.1	1.46	0.17	0.09	0.07
Little Cr.	15 March 01	37.6	0.950	0.08	0.11	0.13
Little Cr.	15 March 01	37.6	0.846	0.08	0.11	0.15

•Cr.= Creek

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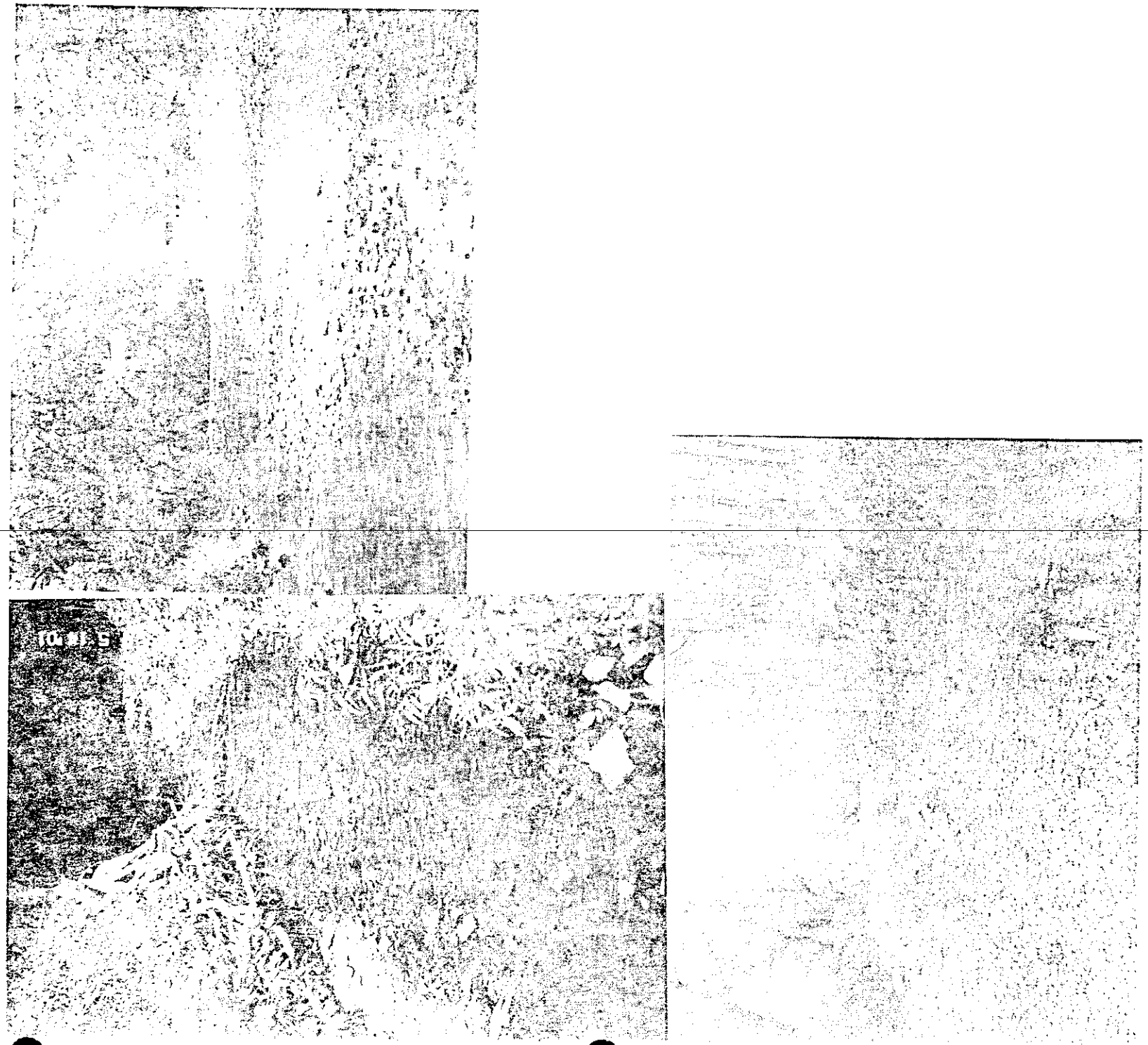

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