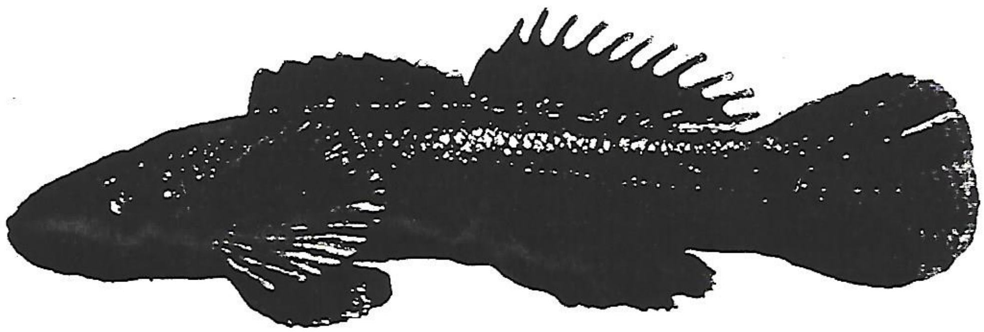
Final Report

Distribution, nesting biology, population estimates, and spawning habitat improvement of the relict darter, *Etheostoma chienense* (Pisces: Percidae), Bayou du Chien, Kentucky



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JOB PERFORMANCE REPORT

State: Kentucky

Project No. E-2-9 APR - 8 2002

Section: Research and Survey

Program: Nongame

Project Title: Statewide Endangered Species

Study Title: Relict Darter Threat Analysis and Habitat Improvement

Study Objectives:

1. Recovery of the Relict Darter

Job 1. Recovery of the Relict Darter

Job Objectives:

1. Review of pertinent literature.
2. Field research and final report.

Activities: Kyle R. Piller and Brooks M. Burr have completed their research on the relict darter and submitted the final report. See the attached final report.

Location: Western Kentucky.

Target Date of Accomplishment: June 30, 1996.

Significant Deviations: None

Comments: None

Recommendations: See the attached report.

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Don Walker	Roy Grimes	C. Tom Bennett
P-R Coordinator	Director Wildlife Division	Commissioner

Date: 9-23-96

ABSTRACT

The relict darter, Etheostoma chienense, is a federally endangered species endemic to the upper reaches of the Bayou du Chien drainage of western Kentucky. Between 1994 and 1996, multiple sites within the drainage were sampled in an effort to determine present distribution and population status. Prior to this study, E. chienense was known from only nine sites. Six new localities of occurrence were discovered during this study.

Etheostoma chienense inhabits undercut banks in gently flowing (0.2-0.6 m/sec) water over sand or gravel, and is often associated with instream cover in the form of leafy detritus, human refuse, and partially submerged logs and sticks. The distribution and abundance of E. chienense seems to be affected by the availability of suitable spawning substrate and cover. Anthropogenic modifications, including channelization and the removal of riparian vegetation, in the early part of the century have significantly reduced the amount of spawning substrate and suitable habitat. Etheostoma chienense inhabits approximately 47,100 linear meters of stream. Population estimates taken in spring of 1996 based on the known range revealed that the E. chienense population ranged from 9,533 and 31,293 individuals. Over the two year study, sampling with seines indicated that sustainable relict darter populations were found in unchannelized sections of stream with adequate riparian zones. In contrast, heavily modified stream reaches lacked large numbers of relict darters.

The relict darter is an egg-clustering species of the subgenus Catonotus that spawns from mid-March to early June at water temperatures ranging from 11 to 22° C. Nests were found at a mean depth of 16.96 cm (4.5-38 cm) and the cavity between the stream bottom and the spawning substrate averaged 2.92 cm (2-5 cm). Etheostoma chienense seems to be opportunistic in its choice of egg-deposition substrates as nests were discovered on the underside of a natural and anthropogenic materials. The majority

majority of nests were found on small logs and sticks, live tree roots, and rocky substrates, but 37% of the nests were found on materials of anthropogenic origin including plastic milk jugs, metal materials, concrete slabs, roofing shingles, and glass. Clearly, the availability of suitable spawning substrata is one of the main factors affecting the success of the population.

As a result of the paucity of suitable spawning substrates in the drainage, several reaches of stream were seeded with ceramic tiles to increase reproductive success. Between 25 and 88% of the tiles were utilized for spawning at least once during the study, while several were used multiple times. The results indicate that the number of eggs (based on the maximum) deposited on the ceramic tiles was significantly larger than the number deposited on the naturally occurring substrates in both 1995 ($t=4.111$, $p<0.0005$) and 1996 ($t=3.8111$, $p<0.0003$).

In addition, two laboratory experiments were performed to attempt to determine nest preferences of the species. In the first experiment, females were given a choice between two spawning substrata of different sizes. Six of eight laboratory spawnings occurred on the larger substrates, but no significant differences were found at the .05 level ($p=.1445$). In the second experiment, females were added to aquaria with a large (68-72 mm SL) and a small (60-64 mm SL) male. Seven spawnings occurred with the larger male, while only one occurred with the smaller male and a significant result was obtained ($p=0.0351$).

During laboratory experimentation, several spawnings were video taped. Spawning occurred between 20 and 21° C. Males and females spawned in a head-to-head pattern and remained inverted for 1.52 to 3.00 ($\bar{x}=2.218$) seconds, during which time ova were released. The spawning behavior and position of <u>E. chienense</u> is identical to that observed for three species in the E. squamiceps and may represent an additional synapomorphy uniting the species group.

ACKNOWLEDGEMENTS

We would like to thank many individuals for assistance in the field, most notably, Kenneth M. Cook, Andy B. Mowery (Murray State University), and David J. Eisenhour, all of whom accompanied us on many of our trips to Kentucky. Zachary U. Campbell, Marcos J. DeJesus, Tonya R. Dempsey, Joseph M. Maushard, William J. Poly, Rex M. Strange, and Allan K. Wilson also aided in the field on various occasions. Special gratitude goes to Kenneth M. Cook who helped cut the ceramic tiles and to William J. Poly who provided several publications that were important in the completion of this project. The state of Kentucky and the U. S. Fish and Wildlife Service granted appropriate permits for research with endangered species.

This research was funded primarily through a contract with the Kentucky Department of Fish and Wildlife Resources and the Southern Illinois University at Carbondale (SIUC) Board of Trustees. Sonjie Schwartz, SIUC Office of Research and Development, has assisted with numerous logistical problems. We are indebted to Tim Slone for his assistance with a variety of fiscal matters, and to his predecessor Gary Sherman for supporting this project from the beginning. Richard G. Biggins, U. S. Fish and Wildlife Service, Asheville, North Carolina, has been a constant source of advice regarding the myriad of issues involving endangered species, private property rights, proper permits, and other pertinent matters. We are especially appreciative of our department and institution, Southern Illinois University at Carbondale, for providing the intellectual freedom to conduct research on the most fascinating group of vertebrates, the fishes.

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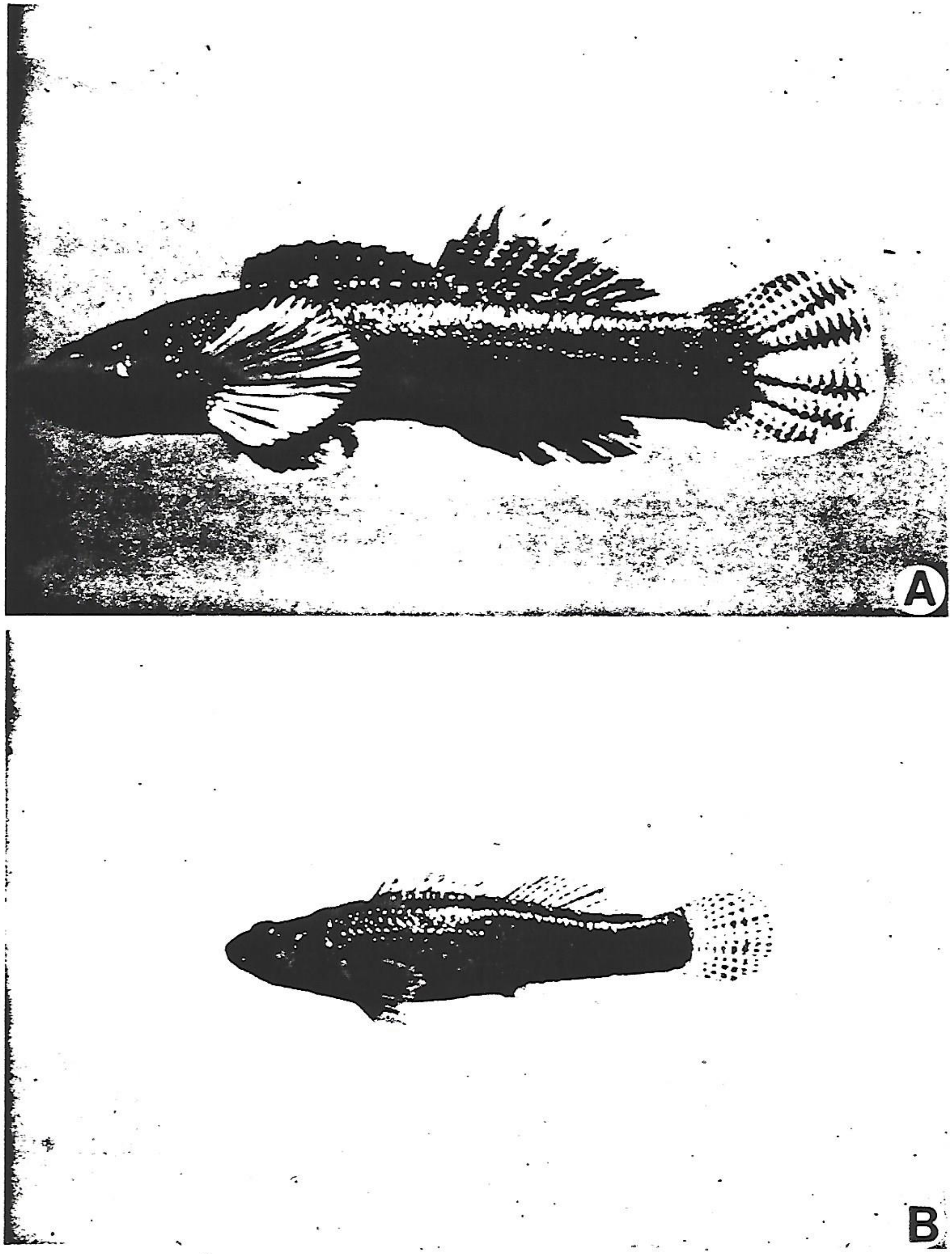
INTRODUCTION

Kentucky has one of the most speciose fish faunas in North America, only Tennessee and Alabama have more taxa. This is due, in part, to Kentucky's physiographic and geological diversity and rich icthyological history that has documented the taxonomy, distribution, and ecology of many of the species (Burr 1980, Page and Burr 1991, Burr and Warren 1986). Although most of the sport-fishes are well-known, we lack detailed biological and distributional information for many non-game taxa.

One such species, the relict darter, Etheostoma chienense Page and Ceas, is a rare and poorly known fish endemic to the Bayou du Chien drainage of western Kentucky (Fig. 1). Surveys of the Bayou du Chien drainage by Webb and Sisk (1975) and Warren et al. (1994) revealed that the relict darter occurred at only nine sites and was known to spawn in only one tributary in the upper reaches of the drainage. Historically, the relict darter was probably more widespread throughout the drainage, but extensive channelization and removal of woody riparian vegetation in the early part of the century have significantly reduced instream flows and the availability of instream cover and suitable spawning habitat. The relict darter is presently listed as federally endangered and receives protection under the Endangered Species Act of 1973 (Biggins 1993). Factors responsible for its listing include: restriction to a small watershed, a lack of suitable spawning substrate, and at the time of listing, only one reported spawning reach.

The relict darter is one of eighteen recognized species in the subgenus Catonotus (Page et al. 1992, Jenkins and Burkhead 1993), one of the largest and most thoroughly studied darter groups. Catonotus is unique among percids, because all members engage in a complex form of reproduction referred to as egg-clustering behavior (Page 1983). Outside Catonotus, egg-clustering within Percidae is known only in species of the darter subgenus Boleosoma of Etheostoma (Page 1983).

Fig. 1 Etheostoma chienense from Jackson Creek, Lawrence Rd., Graves Co., KY. A) A 70 mm SL male collected 18 April 1991; B) a 46 mm SL female collected 18 March 1990. Photos by P. A. Ceas.


A
B

The Etheostoma squamiceps complex is one of three morphologically and behaviorally distinct groups within Catonotus. The complex contains ten species and most taxa can only be distinguished morphologically as nuptial males. The unique second dorsal fin patterns of nuptial males are the primary characters useful in identification. All females and nonbreeding males are drab and mottled brown in coloration and fail to develop distinguishing attributes like those of nuptial males (Page et al. 1992).

Although the subgenus Catonotus is one of the most thoroughly studied subgenera of darters, detailed information regarding the nesting biology of any of the species is lacking. This study was undertaken to provide information on the ecology, nesting biology, and distribution of the relict darter, and to attempt to enhance recruitment by improving darter spawning habitat. In addition, we conducted laboratory experiments to determine nest rock size and mate choice preferences in an effort to provide management recommendations on the dynamics of spawning of this jeopardized species.

STUDY AREAS

The entire Bayou du Chien drainage served as the study area. The Bayou du Chien is a sand, gravel, and mud-bottomed stream system that is located on the Coastal Plain of extreme western Kentucky (Fig. 2 and 3). It originates in southwestern Graves County and flows in a northward arc through Hickman and Fulton counties to its confluence with the Mississippi River near Hickman, Kentucky. It drains approximately 554 km^2, most of which is fertile farmland (Burr and Warren 1986), and all but the terminal 8-10 km of the Bayou du Chien has been subject to channelization (Webb and Sisk 1975).

Webb and Sisk (1975) provided an annotated list of fifty-three species that are known to occur or to have occurred in the Bayou du Chien drainage. Semotilus atromaculatus, Fundulus olivaceus, and Erimyzon oblongus are three species commonly collected in smaller tributaries of the Bayou du Chien. Cyprinella venusta, Lythrurus fumeus, Lythrurus umbratilis, Noturus nocturnus, Percina vigil, and Etheostoma histrio, are frequently collected in the main channel of Bayou du Chien. In addition, other species collected in the Bayou du Chien drainage include Esox americanus, Opsopoeodus emiliae, Cyprinella lutrensis, Pimephales promelas, Phenacobius mirabilis, Catostomus commersoni, Gambusia affinis, Aphredoderus sayanus, Centrarchus macropterus, Lepomis cyanellus, L. gulosus, L. macrochirus, Micropterus salmoides, Pomoxis annularis, P. nigromaculatus, Etheostoma chlorosoma, and E. gracile.

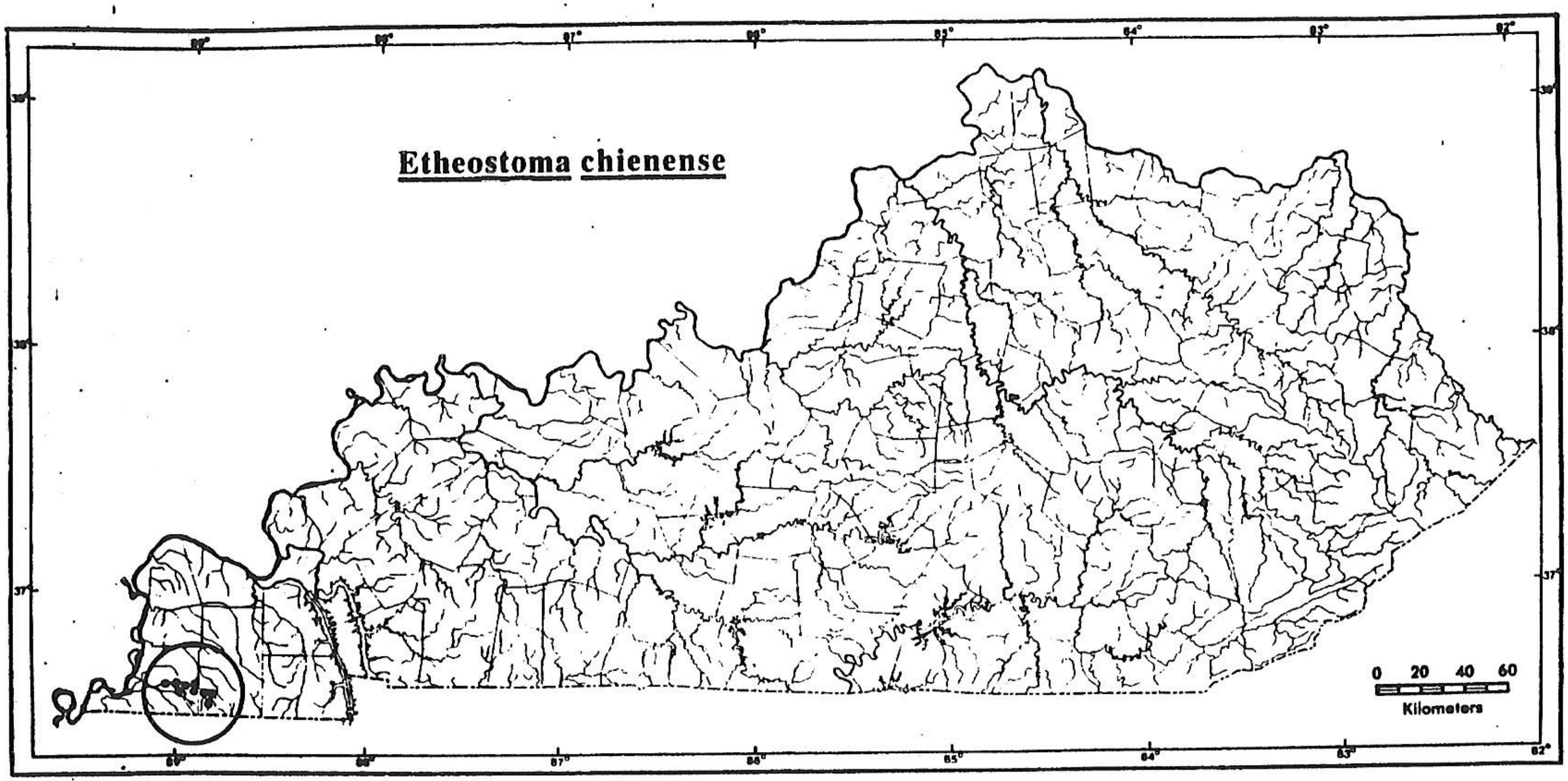


Fig. 2. Record stations for <u>Etheostoma</u> <u>chienense</u> in the Bayou du Chien drainage, Kentucky.

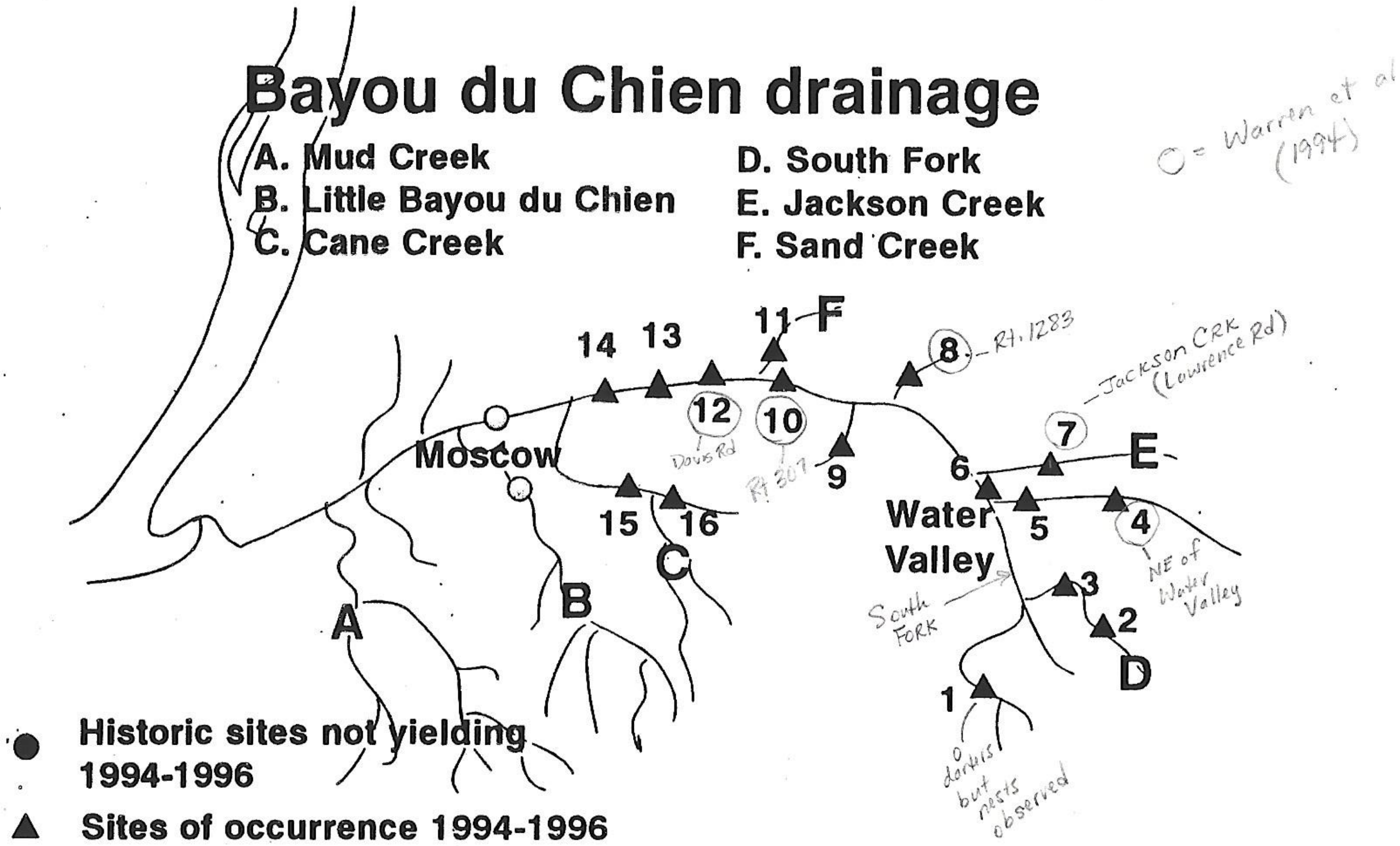


Fig. 3. Distribution of the relict darter, Etheostoma chienense, Bayou du Chien drainage, Graves, Hickman, and Fulton counties, western Kentucky, 1994-1996.

METHODS

Monthly visits were made to Bayou du Chien and many of its tributaries during late summer and early fall of 1994 and 1995 to assess the distribution and population status of the relict darter. Collection localities were primarily sites sampled by Webb and Sisk (1975) and Warren et al. (1994), but several others within the drainage were sampled to attempt to discover new localities of occurrence. Because of the endangered status of the species, no specimens were vouchered from any new sites of occurrence. Sampling was accomplished using standard minnow seines (Jenkins and Burkhead 1994) and dip nets, and all captured darters were measured to the nearest millimeter (SL) and then released. Length data was utilized to analyze population structure by way of length-frequency estimation.

In the spring of 1996, we obtained a population estimate for each site where E. chienense was captured. All estimates are based on 200 linear meter sections (100 m for each stream bank). Areas in the middle section of a stream were excluded because of the affinity of E. chienense for undercut banks and other near-bank cover. Several 10-20 meter sections were measured and sampled at each site. We attempted to capture darters within each section by "kicking" around instream objects and beneath undercut banks. A block net was used in small tributaries to help increase catch rate and avoid darter escape. After sampling several sections, We obtained a mean darter density for each site by averaging the results obtained from each 10-20 meter section sampled. In addition, the amount of suitable habitat (i.e., undercut banks or instream cover) was also estimated for each 200 meter study area. Population estimates were obtained by multiplying the mean darter density by the amount of suitable habitat at each site.

In addition to a population estimate for each site, we obtained a drainage wide population estimate by averaging the results from the site by site analysis. The total linear stream meters inhabited was multiplied by the average amount of suitable habitat obtained in the areas where E. chienense was captured to arrive at the total amount of suitable habitat. The lower range of the population estimate was determined by multiplying the total amount of suitable habitat by the average darter density within the drainage. The resultant population estimate should be less than the actual number because not all darters were captured and because the relict darter probably inhabits additional stream kilometers. To determine an upper range, we used the greatest darter density and multiplied it by the total amount of suitable habitat.

The site by site population estimates were obtained from a one-time sampling effort, which may not accurately describe the population status of E. chienense. To account for the single census bias, the following categories were utilized to classify the abundance of relict darters at each site over the two year sampling period. 1). Rare- species captured or vouchered only once or very infrequently; 2) Uncommon- captured semi-regularly, but usually only in small numbers; 3) Common- collected regularly, and usually found in moderate to large numbers; 4) Abundant- commonly collected in large numbers, one of the dominant species.

During the spring of 1995 and 1996, we attempted to locate nests by searching 200 linear meters of habitat at each known site of occurrence. Egg clutches (number of eggs laid on a given substrate at the time of inspection) were located by overturning instream objects or by feeling the undersides of immovable structures, including live tree roots, bridge supports, and large logs. Movable structures that harbored nests were removed from the stream and eggs were either counted on site or a photograph was taken for later enumeration. After counting/photographing, the nest was returned to the stream. Field notes were routinely taken to record the size of nest guarding males, depth of the nest in the water column, distance of the nest off the bottom, nest substrate dimensions, water velocity (floating and timing an object over a known distance; Orth

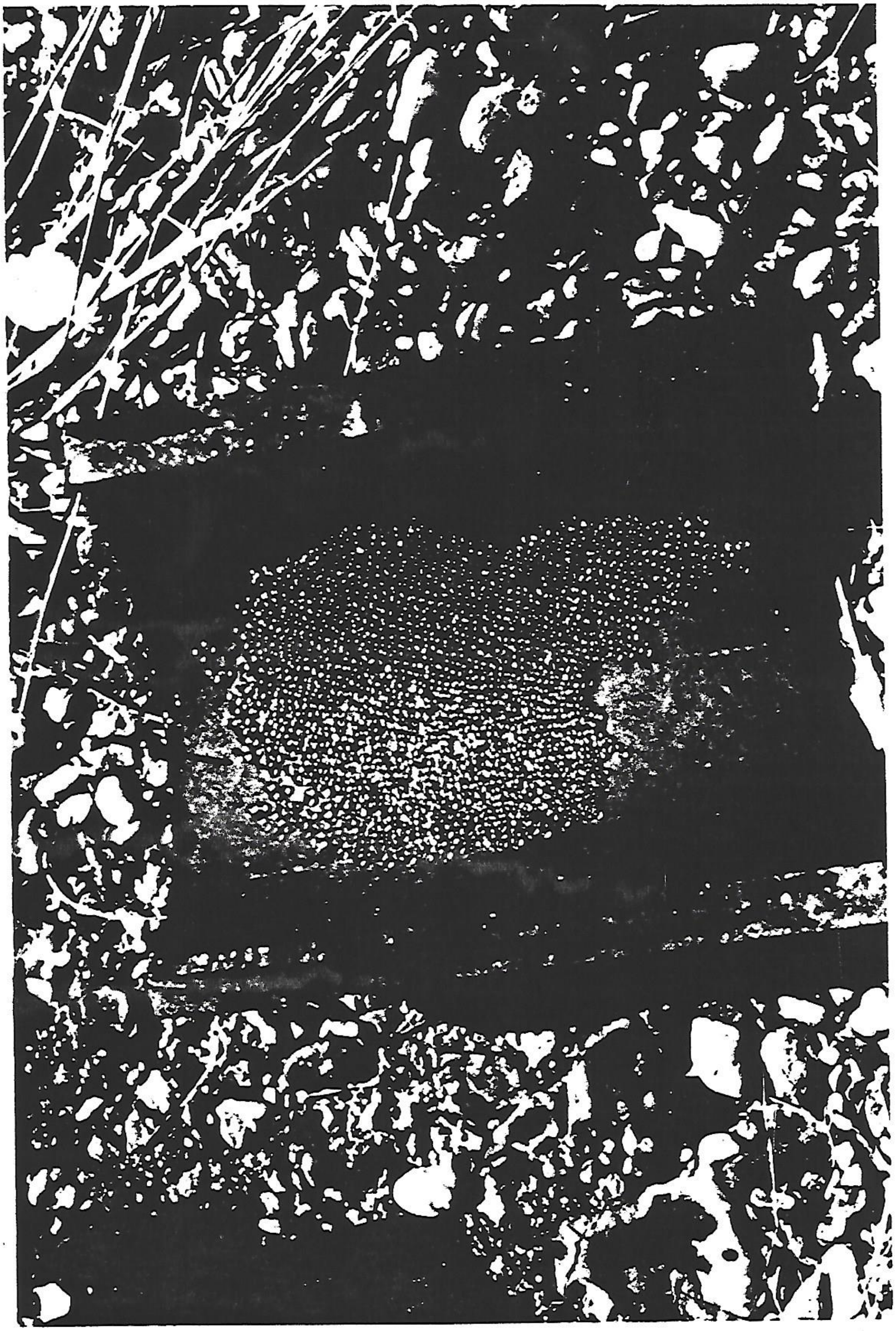
1983), and water temperature. Substrate classifications follow Cummings (1962). After cessation of the breeding season, field work was reduced to once per month so as to not disturb the darters any further and to allow recruitment into the population.

The lack of suitable nesting substrate in Bayou du Chien has been implicated as one factor preventing population increase. To potentially increase nest productivity, artificial spawning substrate was added to selected stretches of stream. It consisted of half-cylindrical ceramic field tiles with a length of 14-16 cm and an inside diameter of 10 cm (Fig. 4). Tiles were placed approximately 2-3 meters apart. Each tile was pressed one to two centimeters into the substrate and had an undersurface area of 131.9 - 150.4 cm^2. Sites were visited biweekly to inspect each tile for egg masses. A photograph was taken of each tile with eggs and an attempt was made to capture any darters beneath the tiles by placing an aquarium net at one end of the tile and flushing the darters beneath the tile into the net. Each captured darter was sexed and measured to the nearest millimeter (standard length).

On 11 March 1995, twenty tiles were added to Jackson Creek, Lawrence Rd., Graves Co., Kentucky. Ten tiles were placed approximately 125 meters downstream from the bridge and another ten tiles were added 30 meters upstream from the bridge. An additional twenty tiles were placed at Bayou du Chien, Rt. 1283, Graves/Hickman Co., Kentucky, on 16 March 1995. The first group of ten tiles was set approximately 40 meters downstream from the bridge while the remaining ten tiles were set 15 meters upstream from the bridge.

During the second year of the study, an additional twenty tiles were again placed at Jackson Creek, Lawrence Rd., Graves Co., Kentucky, on 30 March 1996. All tiles were placed upstream from the bridge in the same area utilized in 1995. An additional 16 tiles were set at Bayou du Chien, 2422 Rd., Graves Co., Kentucky, on 30 March 1996, approximately 30 meters upstream from the bridge. During both years of the study,

Fig. 4. Ceramic tile used as an artificial spawning substrate by Etheostoma chienense.



several tiles were lost, buried or washed downstream. As a result, new tiles were added to replace tiles that were lost during the study. A more detailed description of each site is given in the results section.

Egg clutches attached to the artificial spawning substrata were photographed on successive visits, which allowed both a maximum and average clutch size per tile to be obtained. Because naturally occurring nests were only photographed once, the numbers presented in the results section are based on a one-time observation, rather than a maximum or average number. we assessed relict darter nest productivity (maximum [or average] number of eggs observed per tile) by photographing and/or counting each nest that was discovered.

To determine the actual success of the tiles, the data was analyzed two ways. First, the maximum nest productivity (represented by a subscript "m") of the tiles was compared to the number of eggs observed on natural substrata. Next, with the same data we compared the average nest productivity (represented by a subscript "a") per tile over the interval in which the tile had eggs versus the number of eggs found on the natural materials. The use of both data sets allowed us to determine if the tiles increased both the maximum numbers of eggs and if the average nest size over the incubation period was also increased.

In addition to field studies, two laboratory experiments were performed. The U. S. Fish and Wildlife Service granted a permit (PRT-797421) to collect and transport live relict darters from Bayou du Chien to the SIUC Vivarium. After transport to the laboratory, darters were immediately added to 1 of 10 twenty-gallon aquaria. Each aquarium had a bottom substrate that consisted of natural pea-sized aquarium gravel. Water temperature in each aquarium was maintained at (18-22 °C) with a standard aquarium heater and photoperiod was controlled to match ambient conditions. Darters were fed a diet of frozen bloodworms and when possible live "California blackworms."

Detailed color and spawning descriptions came from direct observation or video tape in the laboratory.

Experiment 1 examined nest rock size and female choice. The aim of the experiment was to determine if substrate size is an important parameter to females. One male (range 57-71 mm SL) and one female (range 50-60 mm SL) were placed into each of five aquaria with two flat rocks of unequal size (mean difference = 47.69%). The amount of surface area available for egg-deposition was determined by tracing the outline of each rock onto graph paper and the outline was measured with a polar planimeter to obtain the area of each rock. Large rocks had surface areas ranging from 206.32-230.20 cm^2 while small rocks ranged from 96.52-114.97 cm^2 (Table 1).

Experiment 2 investigated female mate choice. A large (68-72 mm SL) and a small (60-64 mm SL) male were added to each aquarium along with one female (48-62 mm SL) and two ceramic tiles identical to the ones previously described (Table 2). The goal was to determine if females had a preference in mate size. A choice was considered to be made when eggs were deposited under a tile and guarded by a male. After completion of each replication, pairs (or trios) of darters were returned to their respective streams of origin.

Statistical Analysis

During 1994, E. chienense nests were photographed, but only a rough estimate of the number of eggs in each nest was made, rather than each egg being counted individually as in 1995 and 1996. To account for the discrepancy and the possible error in estimation, only data obtained in 1996 and 1996 are including in the nesting biology analysis. Length-frequency, distributional, and population status information are based on the data obtained 1994, 1995, and 1996.

Table 1. Values for experiment 1 testing the nest rock size preference of Etheostoma chienense.

Large rock	Small rock	Substrate Used	M (mm)	F (mm)	Tank #	Spawned	Hatched
230.1	109.3	small	58	58	7	03/22/95	03/29/95
230.2	114.9	large	70	61	10	03/23/95	eaten
230.2	114.9	large	72	62	10	03/27/95	04/07/96
206.3	96.5	large	58	58	2	03/30/95	eaten
230.2	111.5	large	62	56	1	03/30/95	eaten
217	102.5	large	69	59	8	03/30/95	eaten
224.3	110.6	small	66	56	5	03/31/95	04/08/95
217	102.5	large	66	50	8	04/02/95	04/11/05

Table 2. Combinations, dates, number of eggs, and hatching temperatures of aquarium experiment 2 investigating female mate choice in Etheostoma chienense.

Tank #	Large Male (mm)	Small Male (mm)	Female choice	Female (mm)	Spawned	Hatched	# of Eggs	Temperature
3	68	62	large male	52	03/27/96	eaten by male	41	21
2	70	62	small male	55	03/27/96	04/08/96	108	21
1	71	64	large male	58	03/29/96	eaten by male	54	20
9	72	61	large male	48	04/10/96	eaten by male	11	21
3	68	62	large male	62	04/10/96	eaten by male	92	21
7	70	60	large male	55	04/12/96	eaten by male	43	20
8	68	62	large male	52	04/12/96	04/22/96	110	20
2	68	62	large male	60	04/16/96	eaten by male	54	20

All statistical analyses were performed using the SIUC mainframe version of the Statistical Analysis Systems package (Statistical Analysis Systems Institute, Inc., Cary, NC, 1985) following the statistical procedures outlined in Zar (1996). In all analyses, statistical significance was considered to be $p < 0.05$. Means and standard deviations were determined for the number of eggs on both the artificial and natural substrata, height of nest substrata above the stream bottom, and nest depth. A simple t-test was used to determine if there were significant differences between the nest productivity of the artificial and natural substrata. Correlation coefficients (r) were all Pearson product moments.

Similar materials (i.e., logs, sticks, bark) were combined into a single category and an average was obtained. An ANOVA was performed to assess nest size similarity (or differences) among the different types of substrata. After a significant F-ratio was determined, we used a *post hoc* Tukey pair-wise comparison to determine which groups were significantly different.

To test whether the observed frequencies in the laboratory experiments deviated significantly from the hypothesized value of a 1:1 ratio ($p=0.50$), a one-tailed binomial exact test was implemented.

RESULTS

General Habitat

The relict darter was most commonly collected in the upper reaches of the drainage in both the mainstem of Bayou du Chien and many of its smaller tributaries. It was most frequently captured in deep undercut banks with rooted vegetation in less that 0.5 meter of water, but was also collected within 1-2 meters of the stream bank beneath instream objects such as leaf-litter, partially submerged logs and sticks, and human refuse. Occasionally individuals were collected in mid-stream areas with gently flowing water, but rarely was a relict darter collected in riffle habitats. Apparently, the species limits its activities to undercut banks or other near bank areas. In the spring, some adult males leave the undercut banks to guard territories beneath instream objects, but seining indicated that many nuptial males and gravid females remained beneath undercut banks and may attach eggs to the ceilings of these habitats. Males were darkened and had dorsal fins knobbed, while females would release eggs with the slightest pressure on their abdomens. On one occasion, we observed a large nuptial male dart out from beneath an undercut bank at a smaller male passing by. The larger male was in full spawning coloration and was either guarding a nest or attempting to entice a female into his territory. The males butted heads a few times and eventually the smaller male left the territory of the larger male.

Population Estimates

Sixteen of the twenty-eight sites sampled or reconnoitered within the Bayou du Chien drainage produced E. chienense adults, juveniles and/or nests (Table 3) (Appendix A). Etheostoma chienense presently inhabits a total of 94,200 linear meters of stream (47,100 m for each stream bank) and population estimates suggest that the total drainage

Table 3. Sites surveyed in the Bayou du Chien drainage that produced *Etheostoma chienense*, with habitat data, population estimates, and population status for each site of occurrence. Site numbers refer to numbered sites in Fig. 3.

Locality	Suitable habitat (m/200 m)	Density (darters/m)	Darters (N)	Population status	Riparian zone (m)
Site 1. Trib. of Bayou du Chien, Rt. 94/45	120	0	0	rare	1-2
Site 2. South Fork Bayou du Chien, Kingston Rd.	20	0.100	2	rare	1-2
Site 3. South Fork Bayou du Chien, Pea Ridge Rd.	80	0.100	8	uncommon	0-1
Site 4. Bayou du Chien, 2422 Rd	60	0.256	15	common	1-2
Site 5. Bayou du Chien, Bard Rd.	10	0.100	1	rare	0
Site 6. Bayou du Chien/Jackson Cr./South Fork BDC	88	0.200	2	uncommon	1-2
Site 7. Jackson Creek	140	0.755	106	abundant	4-6
Site 8. Bayou du Chien, Rt. 1283	140	0.430	60	common	3-4
Site 9. Unnamed trib. Bayou du Chien, Rose Rd.	50	0.200	10	uncommon	3-4
Site 10. Bayou du Chien, Rt. 307	130	0.500	65	common	2-3
Site 11. Sand Creek, Rt. 307	100	0	0	rare	2-3
Site 12. Bayou du Chien, Davis Rd.	130	0.375	49	common	2-3
Site 13. Bayou du Chien, Howell Rd.	100	0.066	7	uncommon	0-1
Site 14. Bayou du Chien, Rt. 51	100	0.230	23	uncommon	2-3
Site 15. Cane Creek, Howell Rd. KY 1070	40	0.500	20	uncommon	0-1
Site 16. Cane Creek, Coolie Rd.	100	0.066	7	rare	2-3

population is between 9,533 and 31,293 individuals. Following is a summary of all known sites of occurrence of E. chienense. Each section gives the population estimates taken in spring 1996, population abundance status at each site as observed over the two year sampling period, and a detailed habitat description of each locality of occurrence.

Site 1. Tributary to Bayou du Chien, near intersection of Rt. 45 and Rt. 94, 0.5 km S. Water Valley, Graves Co., rare. Two unguarded nests were found on 9 April 1994 and a single nest was discovered on the underside of a broken piece of concrete on 15 April 1995. Although it is evident that reproduction occurs at this site, it is likely that few individuals inhabit this locality. Habitat modification and degredation at the sites are acute. Cultivated fields extend to the edge of the stream and only a few scattered trees remain along the bank. The tributary has been channelized and the stream flow velocity is low. The bottom substrate consists entirely of mud and silt. Return trips to collect additional specimens proved unsuccessful. It is possible that the spawning adults may have been waifs that were washed downstream during a flooding event. No darters were captured at this site during the 1996 survey, therefore we have a population estimate of zero individuals for this site.

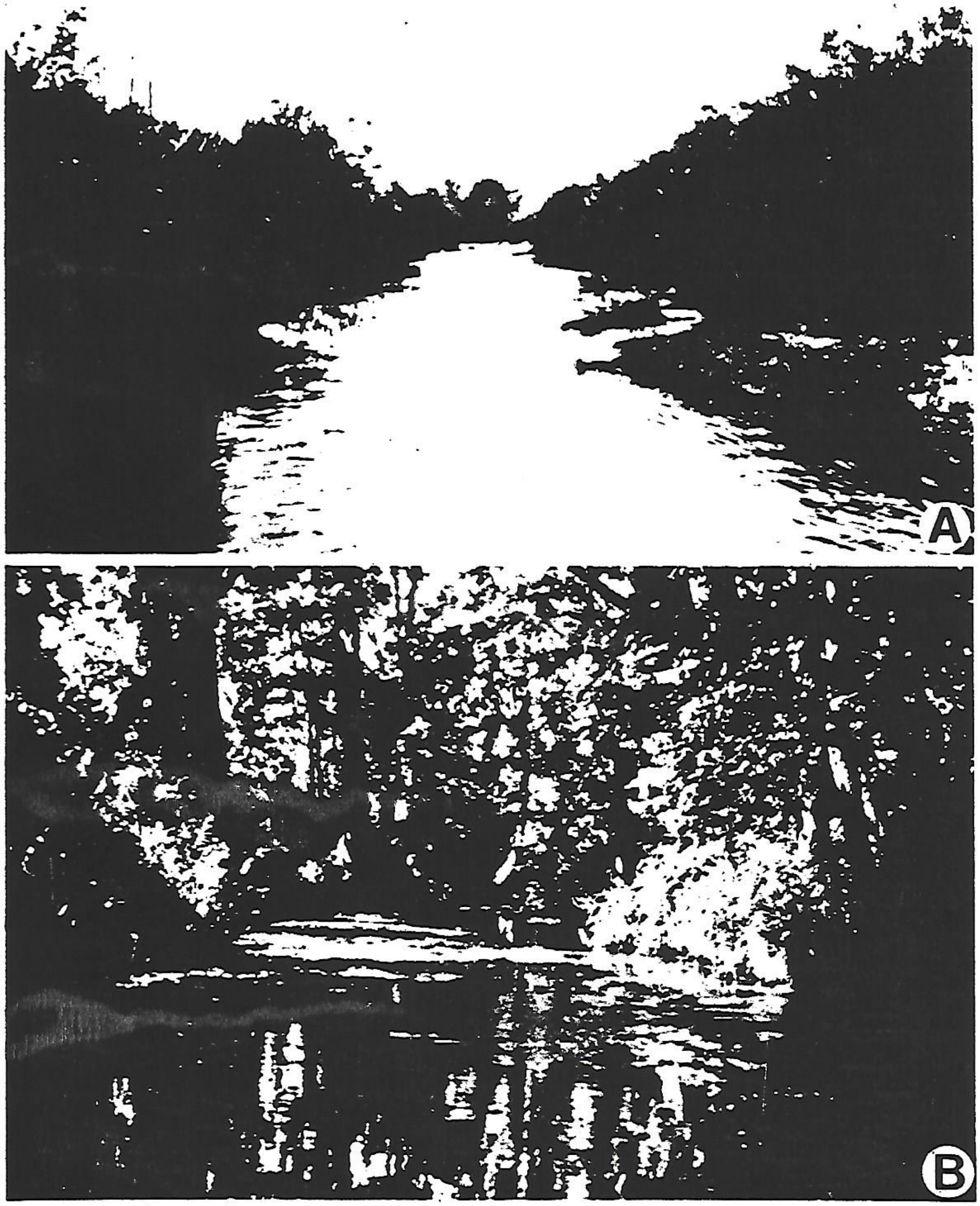
Site 2. South Fork, Kingston Rd., Graves Co., rare. This site was sampled on 27 August 1994 and 11 March 1995 and failed to produce any E. chienense, but on 2 April 1996, a 66-mm male and a 46-mm female were captured beneath a small log that was anchored to the bank. Both were in breeding condition and were apparently ready to spawn. Additional sampling failed to produce other E. chienense. Suitable habitat and instream cover is limited. The substrate consists entirely of sand and virtually no undercut bank habitat is available. We conservatively estimate 1 meter of suitable habitat per ten linear meters of stream. A narrow 1-2 meter riparian zone exists, but trees are sporadic along the stream bank. As judged from the low darter densities and the lack of suitable habitat, we estimate that 2 E. chienense inhabit the sample area.

Site 3. South Fork Bayou du Chien, Pea Ridge Rd., 2.4 km E Water Valley, Graves Co., uncommon. This site was sampled by Webb and Sisk (1975) and Warren et al. (1994) and both failed to capture any E. chienense. During this study, the site was sampled 3 times and initially produced two adults (71 and 62 mm SL) on 11 March 1996 that were collected approximately 40 m downstream from the bridge under a partially submerged railroad tie. Two other individuals (74 and 58 mm) were collected in a deep undercut bank in water 0.75-1 m deep. The riparian zone upstream from the bridge has been removed and the stream bed has been channleized. Subsequent sampling trips have produced only small numbers of individuals, and no more nests. Downstream from the bridge are long stretches of sand raceways and numerous deep pools. Few undercut banks or spawning habitat is available and although a narrow riparian zone remains it consists largely of herbaceous vegetation. We estimate that 8 E. chienense may inhabit the 200 linear meters sampled.

Site 4. Bayou du Chien, 2422 Rd., 3.2 km NE Water Valley, Graves Co., common. This locality is one of the upper most sites surveyed in the main channel of Bayou du Chien. The stream averages 2 to 3 m in width and the substrate consists entirely of sand and gravel. The majority of suitable habitat lies upstream from the bridge. A 1-2 m wide woody riparian zone is present on both sides. During 1996, artificial spawning substrate was added approximately 30 m upstream from the bridge. The downstream reach has been significantly modified. Cultivated fields extend to the stream's edge and woody bankside vegetation has been almost completely removed. We conservatively estimate that 15 E. chienense occupy the sample area.

Site 5. Bayou du Chien, Bard Rd., Graves Co., rare. This site averages approximately 1-2 m in width and no woody riparian zone is present (Fig 5A). The stream has been dredged and straightened and little or no undercut bank habitat is available. The only instream cover is a few scattered pieces of limestone rip-rap which

Fig. 5. Contrast in localities where Etheostoma chienense was collected during the 1994-1996 survey. A) A highly modified reach, Bayou du Chien, Bard Rd., Graves Co., KY; B) ideal habitat, Jackson Creek, Lawrence Rd., Graves Co., KY.


A
B

was used for bank stabilization. This is the most anthropogenically modified site sampled in the drainage. The amount of suitable habitat and darter density is extremely low. We estimate that 1 darter occupies the 200 linear m reach sampled.

Site 6. Bayou du Chien, South Fork Bayou du Chien, and Jackson Creek, Rt. 45, Graves Co., uncommon. All three of these localities were lumped into a single site because of their geographic propinquity. All three sites merge within a 100 m stretch. This site maintains water year round due to several cold-water springs that feed South Fork Bayou du Chien. The site was eutrophic with much filamentous green algae present along the stream bottom, even during mid-April. Eutrophication is likely a consequebce of the extremely small riparian zone and the agricultural field that borders the south side of the stream. The north side has an adequate riparian zone that is approximately 3-4 m wide. This site yielded a single male guarding a nest attached to the underside of a rubber tire on 16 April 1995. No other darters were captured during the study. We estimate that 2 darters occupy the sample site.

Site 7. Jackson Creek, NE Water Valley on Lawrence Rd. (formerly Roy Lawrence Dr.), Graves Co., abundant. Prior to 1994, Jackson Creek was the only stream in the Bayou du Chien drainage known to harbor a spawning population of E. chienense. (Page et al. 1992, Warren et al. 1994). It is one of the upper-most sites surveyed in the drainage and is the type locality of E. chienense (Page et al. 1992). Jackson Creek is a first order tributary that averages 1-3 m in width. Substrate consists of sand and gravel, and although no slabrocks are present, a few areas contain small concentrations of cobble (64-256 mm). Downstream from the bridge, there is an extensive tree canopy covering the stream. The riparian zone, consisting of both deciduous trees and herbaceous vegetation, extends 4-6 m in width on both sides (Fig 5B). A substantial amount of undercut bank habitat is present downstream from the bridge. Above the bridge, a cattle pasture borders both sides of the stream and although a few large trees are still present along the surrounding fenceline, the riparian zone has been significantly reduced. A substantial amount of woody debris that exists in the riparian zone (above and below the bridge) is deposited into the stream. This in turn provides a surplus of spawning substrate and cover for darters and other aquatic species. Jackson Creek has been relatively unaffected by anthropogenic modifications and the pristine nature of the stream undoubtedly contributes to the healthy population at this site. Extrapolating, we estimate that 106 darters occupy the site. The population in Jackson Creek is the largest population in the drainage and is one of the primary areas of recruitment.

Site 8. Bayou du Chien, Rt. 1283, 4.5 km N. Water Valley, Hickman/Graves Co., common. This site has been subject to a variety of human modifications. Above the bridge, the stream is approximately 4-6 m wide and a narrow woody riparian zone exists on both sides of the stream. There is approximately 20 m of suitable habitat immediately upstream from the bridge, while the remaining reach has been channelized and little suitable habitat is present. Silt covered substrates, sluggish flow, and lack of heterogeneity undoubtedly contribute to low densities. Ceramic tiles were placed in the area immediately upstream during 1995. Although the area below the bridge has been partially channelized, the stream flows more freely, has a higher velocity, and harbors a greater density of E. chienense. Sand is the dominant substrate below the bridge, but there is a small area (100 m^2) near the bridge that has many cobble-sized rocks available for spawning. The woody riparian zone has been significantly reduced, but the remaining vegetation extends about 1-2 m on either side of the stream. We estimate that 60 individuals occupy the sample area that stretches from approximately 25 m above the bridge to 75 m below.

Site 9. Unnamed tributary, Bayou du Chien, Rose Rd., 6.7 km SE Fulgham, Hickman Co., uncommon. This site failed to yield specimens during both of the previous surveys, but several adults and nests were discovered on 31 March 1996. There is an abundance of woody material available for nesting, but little undercut bank habitat exists. Flow is extremely low and it is likely that this small tributary dries completely during the late summer and early fall months. Several females also were collected on 9 April 1996, but no other nests were discovered. The population estimate for this site is 10 individuals.

Site 10. Bayou du Chien, Rt. 307, 4.5 km S. Fulgham, Hickman Co., common. The Rt. 307 site yielded darters during both of the previous surveys and was relatively common in this study. A multitude of spawning habitat and cover is available including tree roots extending into the water, logs and sticks deposited from the overhanging tree canopy (2-3 m riparian zone), and deep undercut banks. This reach of Bayou du Chien has been partially straightened and widened. We estimate that 65 darters occupy the sample area that stretches from 50 m above the bridge to 50 m below.

Site 11. Sand Creek, Rt. 307, 4.0 km S. Fulgham, Hickman Co., rare. This small stream can be classified as ephemeral or intermittent. The stream commonly lacks water or dries to isolated pools during summer and early fall. Sand Creek was sampled nine times during the study. Several adults were captured in 1994, but few individuals since. A total of four nests were found during the three year study. It failed to yield any specimens during the Warren et al. (1994) study, but produced specimens for Webb and Sisk (1975), although the vouchers remain unavailable. The riparian zone averages approximately 1-2 meters wide and an ample amount of undercut bank habitat is available. Although it was shown that spawning does occur, it is likely that many E. chienense larvae become trapped in isolated pools during times of low water. We estimate that zero darters occupy this site. None were captured during 1996, and few others were captured during previous visits. Low water levels and the intermittent nature of the stream seem to be the main reasons that few individuals or nests have been discovered at this locality.

Site 12. Bayou du Chien, Davis Rd., 4.8 km SW Fulgham, Hickman Co., common-uncommon. The Davis Rd. site yielded three total specimens for Webb and Sisk (1975) and Warren et al. (1994). Various collection at this site resulted in unequal numbers. On 8 August 1995, 15 individuals were collected in less than 30 minutes, while on 23 March 1996 only 4 individuals were collected in the same time frame. Nesting does occur and several nests were photographed during 1994 and 1996. Reaches above and below the bridge are similar. An ample woody riparian zone remains along the stream edge, but channelization is evident. A substantial amount of spawning habitat is present and many undercut banks exist. For the sample area which extends from immediately below the bridge to 100 meters downstream, we estimate that 60 darters inhabit the site.

Site 13. Bayou du Chien, Howell Rd., Hickman Co., uncommon. This site has a mainstem character averaging 7-8 m in width with a strong flow. There are no alternating stretches of riffle-pool habitats and undercut bank microhabitat are limited. This site failed to yield any specimens via seining, but two unguarded nests attached to small sticks were found on 2 May 1996. Below the bridge, the south stream bank almost completely lacks woody vegetation. Only a few scattered trees are present along the bank. On the north side a riparian zone greater than 5-6 m extends along the bank. Above the bridge, a buffer zone that is 3-4 m wide borders both sides of the stream. Although a suitable riparian zone is present at this site, the channel has been straightened and ditched. No adults were ever captured, but two nests were discovered in the area sampled. We estimate that 2 darters occupy the area.

Site 14. Bayou du Chien, Rt. 51, 0.8 km SE Clinton, Hickman Co., uncommon. Prior to this study, the farthest downstream localities of occurrence were Bayou du Chien, 6.4 km N of Cayce, Hwy. 239, Fulton Co., and Bayou du Chien, 0.8 km N. Moscow, Hickman Co. Additional collection efforts at these localities proved unsuccessful. Bayou du Chien, Rt. 51, is presently the farthest downstream site of occurrence. This new locality was first discovered on 27 August 1995. Five individuals (28-45 mm) were captured near the bridge. Subsequent collections also have yielded specimens, but always in low numbers. Little spawning habitat is available. Undercut banks are scattered and some woody material has been deposited into the stream from the remaining woody vegetation present. Upstream from the bridge, the riparian zone remains intact, but the stream channel has been ditched and straightened. The sampled area stretches 50 m above and below the bridge. We estimate that 23 darters occupy the area.

Site 15. Cane Creek, Howell Rd., uncommon. Two nests were found in 1994, a single nest was discovered on 31 March 1995, and several others were found during 1996. The area beneath the bridge and immediately upstream contained the only suitable habitat or cover within 200 m of the bridge. The spawning habitat consisted of several pieces of limestone rip-rap and small rocks. The area downstream has an extremely low flow rate and little undercut bank habitat. Riparian buffer zones extend 1-2 m to either side, and siltation is extremely high. Several silt tolerant species were collected downstream including Ameiurus natalis and Lepomis cyanellus. The area upstream lacks a riparian zone and tilled fields extend up to the streams edge. The population estimate for this site may be a misleading. Virtually all of the suitable habitat present occurs immediately below the bridge within a 5-6 m area. We estimate that 20 darters occupy the sample area, most occurring in the vicinity of the limestone rip-rap.

Site 16. Cane Creek, Coolie Rd. rare. This site was sampled three times and failed to yield any adult E. chienense via seining. Two nests attached to the underside of a large wooden board were discovered on 2 May 1996, but no males were guarding the eggs at the time of discovery. This site has been moderately developed. A small 2-3 m buffer zone borders both sides of the creek.. The creek is averages 2-3 m in width and is fairly shallow, but probably maintains flow year round. Much woody debris is scattered throughout the stream, but little undercut bank habitat is present. Because, no darters were ever captured and only two unguarded nests were discovered, We estimate that 2 darters occupy the site.

Reproductive Habits

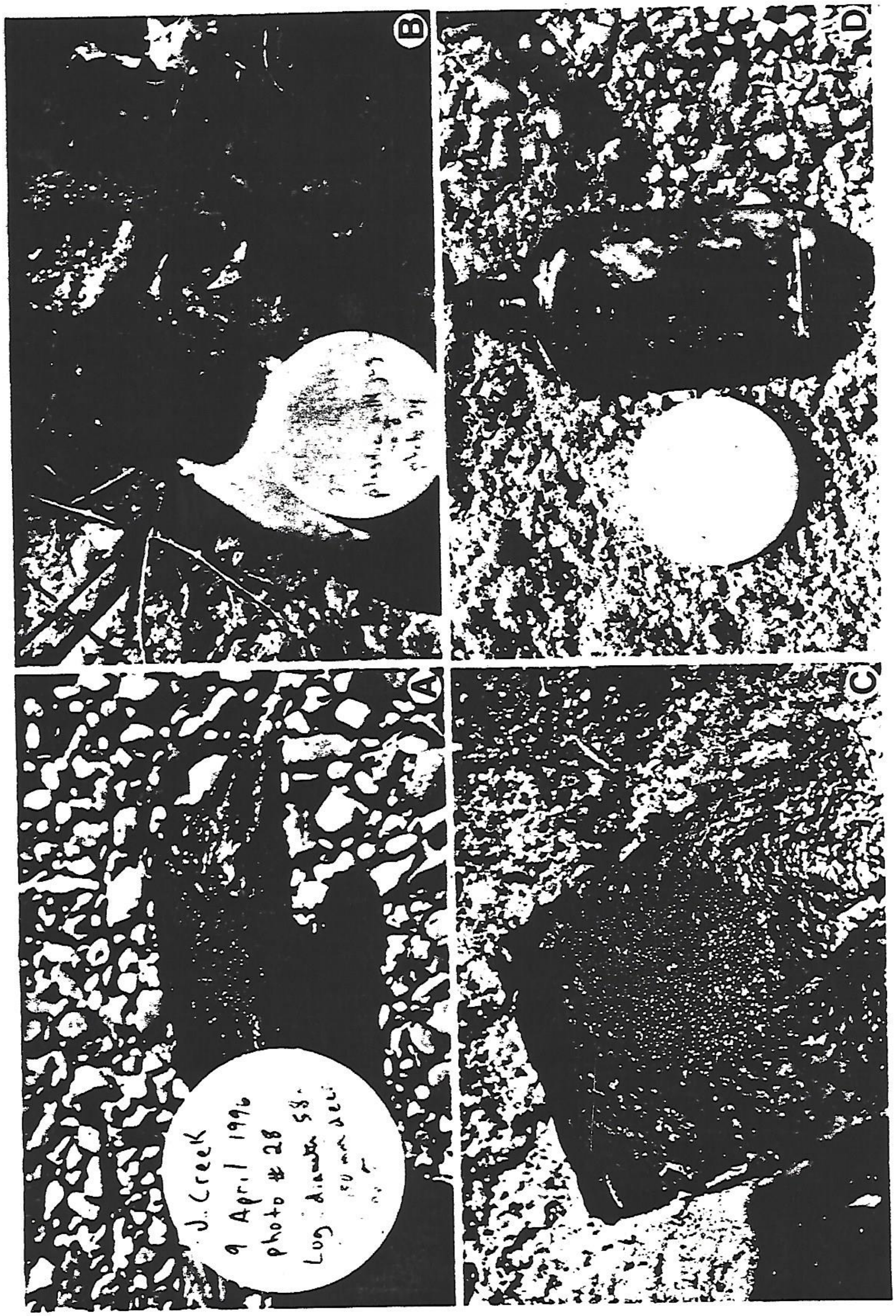
Nests of Etheostoma chienense were found from 16 March to 8 June 1995 and 21 March to 24 May 1996 at water temperatures ranging from 11 to 22 °C. The length of the sampling season was shortened in 1996 due to time constraints. Nests were found at depths of 4.5-38 cm ($\bar{x}$=16.96,SD=0.7992) in slow to moderate flow ($\bar{x}$=0.40, 0.25-0.60 m/sec) and the cavity between the stream bed and the spawning substrate ranged from 2-5 cm (x=2.92 cm, SD=8.604). Breeding males and females were significantly different in length (t=18.74, p<0.0001). Nest guarding males were larger and ranged from 55 -76 mm SL (x=62.5 mm) while gravid females were 36-68 mm SL ($\bar{x}$=48.7 mm). Nuptial males guarded eggs for 10-13 days at 20 °C and abandoned the nest soon after the eggs hatched. Freshly deposited eggs were a translucent golden-orange color and averaged 1.86 mm in diameter (1.7-2.0 mm, n=35).

One-hundred sixty-six nests were discovered on 16 different types of substrata (Table 4). Small rocks, woody debris, and live tree roots were the dominant materials utilized. Nests were found on sticks with diameters of 2-14 cm ($\bar{x}$=7.88 cm) (Fig 6A) and rocks with surface areas of 32-540 cm^2 ($\bar{x}$= 166cm^2). In addition to naturally occurring materials, 37% of the E. chienense nests were found on materials of anthropogenic origin. When present, rubber tires were utilized most often, but other materials including; plastic (Fig. 6B), roofing shingles (Fig. 6C), glass (Fig. 6D), concrete/cinder blocks, bricks, metal road signs, an aerosol can, concrete slabs, and even a discarded air conditioner were used as egg-deposition substrates.

Table 4. Number of nest found and type of nest substrate of <u>Etheostoma</u> <u>chienense</u>, Bayou du Chien drainage, western Kentucky, 1994-1996.

Substrate	1994	1995	1996
Natural rock	10	25	9
Concrete/cinder block	4	2	16
Brick	0	1	2
Limestone rip-rap	0	0	5
Live roots	17	10	0
Logs/sticks	23	14	29
Bark	1	3	5
Boards	2	0	5
Rubber tires	3	3	16
Rubber mat	0	0	5
Roofing paper/shingle	1	2	0
Plastic	0	1	2
Blacktop aggregate	1	1	0
Metal	1	5	4
Glass	0	0	1
Clay	3	0	0
Totals	66	67	99

Fig. 6. Spawning substrata used by Etheostoma chienense. A) small stick; B) plastic milk container; C) roofing shingle; D) glass bottle.


B
D
A
C
J. Creek
9 April 1996
photo # 28

Nests with clutches of eggs attached to naturally occurring materials contained a range of 12-789 eggs ($\bar{x}$=254.6) in 1995 and from 12-1275 ($\bar{x}$=342.9) in 1996 (Table 5). In 1995, roofing shingles had the highest mean nest clutch size among substrates ($\bar{x}$=555.5), while in 1996 the highest mean number of eggs was found on rubber materials ($\bar{x}$=498.3) including rubber tires and floor mats.The mean clutch size was determined for each substrate type and ANOVA was used to ascertain whether there were significant differences between the size of egg masses attached to the different types of materials utilized as spawning substrata. No significant differences were found between mean clutch sizes in 1995 (F=1.89, p=0.11), but a significant ANOVA was detected in 1996 (F=2.98, p=0.01). A Tukey test revealed that rocks ($\bar{x}$=188.9) and rubber materials ($\bar{x}$=498.3) were the only two substrates that differed significantly in size.

Several substrata harbored multiple nests and guarding males. Many of the E. chienense nests lacked physical barriers between clutches of eggs. It was not unusual to find egg masses less than 10 cm apart and guarded by separate males. Logs and sticks were the most common substrata containing multiple egg masses. Several of the larger-sized logs harbored three or four nests at a time with each being guarded by a single male. A small (304 cm^2) cinder block held two nests less than 4 cm apart and because no guarding males were captured, it was difficult to assess whether a single male was guarding both nests or whether there were two males each guarding a single nest while tolerating the presence of another male. Rubber tires routinely contained multiple nests. One particular tire contained five fresh nests, all greater than 10 cm apart, while holding the remnants of four other recently hatched egg masses. Again, no guarding males were captured so it was not possible to asses how many males were guarding the eggs. On 31 March 1995, at Bayou du Chien, Rt. 1283, a metal air conditioner cover was found that harbored four nests all 20-25 cm apart from all other nests. On corner was firmly anchored to the stream bank while the remaining area floated on the water surface. As

Table 5. Mean clutch size of each type of spawning substrate utilized by Etheostoma chienense, Bayou du Chien drainage, Kentucky 1995-1996.

Substrate	1995	1996
Natural rock/limestone rip-rap	329.8	188.9
Concrete/cinder block/brick	213	351.2
Roofing shingle	555.5	ND
Plastic	52	457
Woody materials	216.3	316.4
Rubber materials	239	498.3
Metal	444.5	288.9
Blacktop	94	ND
Glass	ND	80

*ND=no data

mentioned earlier, egg-clustering is a behavior in which eggs are laid in a single layer on the underside of a substrate, but occasionally, nests were found that contained eggs that were double layered. Double layered nests were found on both logs and sticks, a small piece of particle board and occasionally on artificial tiles. Usually, only a few eggs within the nest were found in this condition.

Artificial Spawning Substrata

During 1995, 100% of the tiles at Jackson Creek were guarded at least once during the study (Table 6) and 70% of the tiles contained eggs, including several that were used multiple times. The size of egg masses deposited on the tiles ranged from 197-1266 ($\bar{x}_m$=683.3 and $\bar{x}_a$=617.3) and guarding males ranged from 58-70 mm SL. Fifty percent of the tiles at Bayou du Chien, Rt. 1283 were guarded in 1995. Only 25% of these tiles contained egg masses and all where in the upstream locality. The remaining ten tiles downstream were in swift deep water and never contained darters or eggs. The mean clutch size based on the maximum number of eggs observed ranged from 93-658 ($\bar{x}_m$=369.0) while the number of eggs based on the average ranged from 93-361 ($\bar{x}_a$=263.0).

The relict darter guarded and spawned beneath 88% of the tiles at Jackson Creek in 1996. Clutches based on both the maximum and average number of eggs observed ranged from 11-1182 ($\bar{x}_m$=518.5 and $\bar{x}_a$=473.0). Guarding males ranged from 55-74 mm SL. Bayou du Chien, 2422 Rd., was visited fourteen times during 1996. Sixty-five percent of the tiles were guarded and contained eggs at least once during the survey. Clutch sizes ranged from 19-1132 ($\bar{x}_m$=600.7 and $\bar{x}_a$=506.7) and guarding males ranged from 56-75 mm SL. No significant differences were detected between the size of nest guarding males in the first half ($\bar{x}$=64.94) or second half ($\bar{x}$=65.40) of the 1995 and 1996 spawning seasons (t=-0.3188, p<0.7511). There were no significant differences between

Table 6. Summary of artificial spawning substrate usage by *Etheostoma chienense* in the Bayou du Chien drainage, Kentucky, 1995-1996.

	1995		1996	
Characteristic	Jackson Creek	Bayou du Chien, Rt. 1283	Jackson Creek	Bayou du Chien, Pea Ridge Rd.
Average number of sites available	20	20	17	20
Number of visits to each site to check tiles	15	11	14	14
% of tiles guarded at least once	100	50	88	65
% of tiles with at least one nest	70	25	88	65
Guarding males (mm SL)	(58-70)	(58-70)	(55-74)	(56-75)
Mean tile clutch size (based on maximum)	683.3	369.0	518.5	600.7
Mean tile clutch size (based on average)	617.3	263.0	473.0	506.7
Range of clutch sizes	(197-1266)	(93-658)	(11-1182)	(19-1132)

clutch sizes between 1995 and 1996 based on both the maximum and average clutchs observed (F_m=1.23, p=0.3072 and F_a=1.44, p=0.2407).

To determine the success of the ceramic tiles for increasing nest productivity, we obtained a grand mean for each year by combining the results obtained for each site (Table 7). During 1995, the mean clutch size for artificial substrata of Jackson Creek and Bayou du Chien, Rt. 1283, based on the maximum number of eggs observed, averaged 613.40 (range= 93-1116, SD=346.08) while the mean nest size based on the average number observed on successive visits was 538.55 (range= 93-1266, SD=337.41). The natural substrata had clutches ranging from 12 to 789 ($\bar{x}$=254.60, SD=183.77) in 1995. The clutch size based on both the maximum and average number of eggs observed per tile was significantly greater than the clutch sizes found on the naturally occurring materials (t_m=4.111, p<0.0005 and t_a=3.32, p<0.0003). Significant differences were also found during 1996. The combined clutch size from Jackson Creek and Bayou du Chien, 2422 Rd., ranged from 11 to 1132 ($\bar{x}_m$=560.60 , SD=336.72 and $\bar{x}_a$=486.21, SD=305.73). In 1996, the mean clutch size on the naturally occurring substrates was 342.90 eggs (SD=247.29, range= 25-1275). In 1996, a t-test revealed that nest sizes based on both the maximum and average size observed on artificial tiles were significantly greater than naturally occurring egg masses (t_m=3.811, p<0.0003 and t_a=2.709 p<0.008). Simple linear regression revealed that there were moderate positive correlations between male size and the maximum number of eggs observed per tile (r_m=0.4716, p<0.0056) (Fig. 7A) and between male size and the number of eggs per tile based on the average number observed over the interval (r_a=0.5032, p<0.0028) (Fig. 7B).

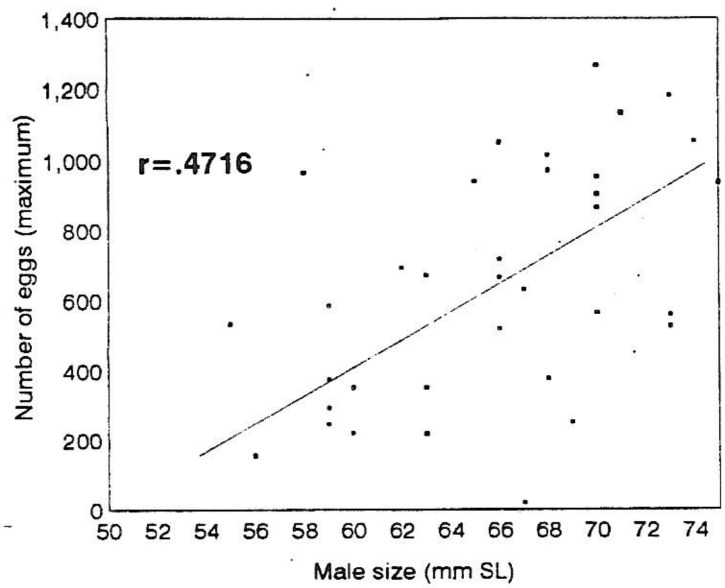
It was a common occurrence to find large nests with clutches of eggs in 4 or 5 different stages of development, indicating that male E. chienense were polygynous. In addition, on 25 April 1996, we captured a single male along with three females (40, 41, 42 mm SL) beneath a single tile at Bayou du Chien, 2422 Rd.

Table 7. Comparison of Etheostoma chienense nests attached to artificial and natural substrata based on A) maximum and B) average clutches observed. Significant differences denoted by a (***).

	Artificial	Natural	t-value	p
1995	$\bar{x}$=613.4 (93-1266)	$\bar{x}$=254.6 (12-789)	4.111	$p<0.0005$***
1996	$\bar{x}$=560.6 (11-1182)	$\bar{x}$=342.9 (25-1275)	3.811	$p<0.0003$***

	Artificial	Natural	t-value	p
1995	$\bar{x}$=538.5 (93-1266)	$\bar{x}$=254.6 (12-789)	3.320	$p<0.0003$***
1996	$\bar{x}$=490.48 (11-1182)	$\bar{x}$=342.9 (25-1275)	2.709	$p<0.008$***

A



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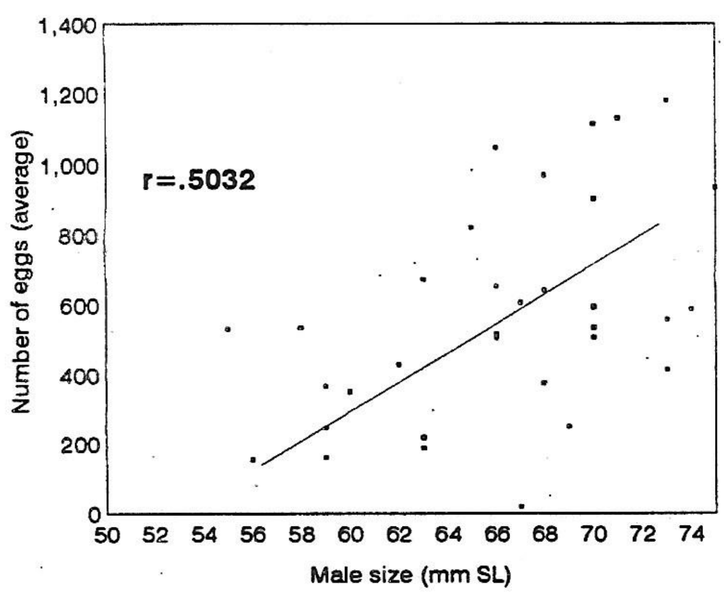

Fig. 7. Scatterplots of male size (mm SL) versus clutch size on artificial tiles based on; A) maximum number of eggs; B) average number of eggs.

In addition to E. chienense, several other aquatic organisms utilized the ceramic tiles for shelter. Occasionally, creek chubs, Semotilus atromaculatus, were captured beneath unguarded tiles without eggs. In addition, the midland water snake, Nerodia sipedon, and several crayfish species, Cambarus spp. and Orconectes spp., were sometimes captured beneath the tiles. No aquatic organisms other than E. chienense were ever captured beneath tiles that harbored eggs.

Ceramic tiles were fairly stable at the Jackson Creek and Bayou du Chien, 2422 Rd., sites. Although tiles were occasionally flipped or buried during natural flooding events which routinely occurred during the spring, most remained in the study area. Several times, E. chienense deposited eggs on the top of tiles that were flipped over from increased water velocity. Although flooding occurs in the headwaters of Bayou du Chien and many of the tributaries in the upper reaches of the drainage, the intensity of these events is much less than that which occurs in the downstream localities. Several tiles were placed in the main channel of Bayou du Chien at Rt. 1283 in 1995. Even during normal flow, tiles were buried, washed downstream, or flipped over.

Sexual Dimorphism

Although nuptial males fail to develop bright chromatic colors characteristic of many other species of Etheostoma, several distinct changes occur during the spawning season. The head and nape of territorial males became extremely swollen and blackened. Males developed 7-8 alternating black vertical bars on the side of the body. The bars were located on the posterior half of the body beginning just below the origin of the second dorsal fin and proceeded posteriorly to the origin of the caudal fin. In addition, a single intense vertical bar was usually found directly behind the opercular flap at the origin of the first dorsal fin. All fins with the exception of the pectorals became blackened while both the caudal and dorsal fins retained their transparent banding patterns. In early spring, males acquired swollen knobs at the tips of the second dorsal rays. Females and non-breeding males are mottled brown and remain relatively unchanged throughout the year.

Length-frequency estimates of both males and females from spring 1994 to spring 1995 revealed four age classes (Fig. 8A). Separate length-frequency estimates were then determined for both males (Fig. 8B) and females (Fig. 8C) from the spring of 1994, 1995, and 1996. Males were approximately 40 mm SL by age 1 and between 52 and 62 mm SL at age 2. Age 3 males ranged from approximately 63-76 mm SL. On the other hand, females were slightly smaller than males at each age class. Females were almost 35 mm SL by age 1, and ranged from 47-54 mm SL for age 2. Age three females were 55-68 mm SL.

Laboratory Experimentation

In addition to field studies, two laboratory experiments were performed in aquaria maintained in the SIUC Vivarium. Spawning occurred as early as three hours after introduction of adults into an aquarium, while most individuals spawned within a few days. Eggs were commonly eaten by the guarding males within 1 to 2 days. Eggs that were not eaten hatched within 10-13 days at 20-21 °C.

The goal of experiment 1 was to determine if nest substrate size was an important parameter to females.. Eight total spawning events occurred with six pairs of darters spawning on the larger rocks and two pairs spawning on the smaller rocks. The expectation for spawning sites was (4:4) for large and small rocks. Although 75% of the spawning occurred on the underside of the larger rocks, the choice of nest substrate size by females were not significnatly different than random ($p=0.1445$).

Laboratory experiment 2 was designed to determine if females had a preference in the size of a mate. Again, eight total spawning events occurred, seven with the larger

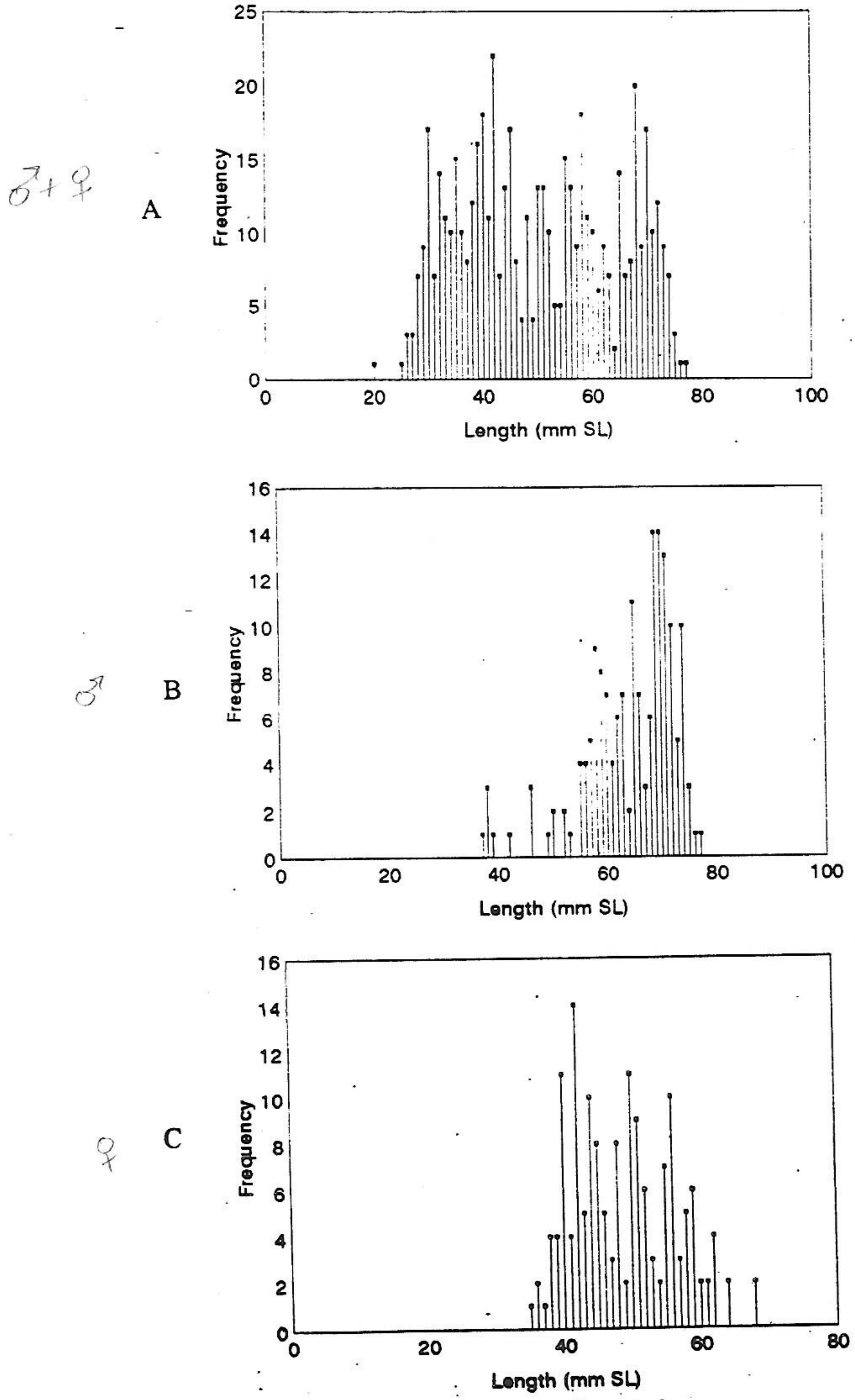

Fig. 8. Length-frequency graphs of <u>Etheostoma chienense</u> based on; A) both sexes combined; B) males spring 1994, 1995, and 1996; C) females spring 1994, 1995, and 1996.

males and 1 with the smaller male. A significant devation from randomness was obtained (p=0.0351).

Several aquarium spawnings were observed and taped with a VHS video camera. After attaining a nest-rock territory, a male would periodically leave the nest to chase and court a female. A female ready to spawn followed the male beneath the rock or tile substrate. Prior to each spawning act, the female raised her head to view the ceiling of the spawning substrate, apparently determining where to attach the eggs. The spawning act was initiated by the female. She inverted herself by rolling in either a clockwise or counterclockwise direction. During the inversion, the female pressed her genital papilla flat against the spawning substrata. As soon as the female began to invert, the male immediately positioned himself alongside the female in a head-to-head relationship and quickly inverted. The tumultuous vibrating act of egg-fertilization and deposition only lasted for 1.52-3.00 sec. ($\bar{x}$=2.218 sec., n=68). Both the male and female returned to the upright position following each spawning act. Approximately 15 to 623 seconds ($\bar{x}$=84.3 sec., n=58) elapsed between inversions. It was not possible to determine the number of eggs laid by E. chienense during each inversion. Following each spawning event, the female would remain stationary until she was ready to spawn again. The male remained active and continually swam in a circular motion near the head region of the female. Apparently the male was trying to entice the female to spawn again by laterally displaying his barred pattern and erect median fins. Occasionally a male would press his second dorsal fin against the spawning substrate and display the swollen tips of the second dorsal fin rays.

After completion of the spawning act, male E. chienense aggressively guarded the egg masses. Additional males within the territory of a guarding male were not tolerated. Smaller males were driven off by guarding males. Guarding males maintained their characteristic vertical barred patterns and swollen blackened head and nape. Males routinely tended the eggs by fanning them with the second dorsal fin and occasionally they removed dead or fungused eggs to avoid contamination of the entire nest.

Predation

The creek chub, Semotilus atromaculatus, is the most common species of fish present in Jackson Creek and in the upper reaches of Bayou du Chien. Several studies including Barber and Minckley (1971), Newsome and Gee (1978), and Keast (1985) have investigated the diet of the creek chub. All three studies indicate that by age IV (>81 mm SL) S. atromaculatus is predominately a piscivore, with as much as 70% of the diet consisting of fish. During this study, no creek chubs were preserved or examined internally for E. chienense larvae or juveniles. The abundance of S. atromaculatus in principal areas of recruitment could severely impact the relict darter population.

A single 96-mm SL Micropterus salmoides (SIUC 26073) was captured at Bayou du Chien, Rt. 1283, on 4 May 1996 with the caudal region of an E. chienense adult protruding from its mouth. After further examination, it was determined that the specimen was a partially digested E. chienense female that was gravid. In addition to M. salmoides and S. atromaculatus, several other piscine species, Lepomis cyanellus, L. macrochirus, L. gulosus, and Esox americanus, could potentially prey upon adult and larval E. chienense. Besides piscine predators, several species of water snakes, Nerodia spp., were also observed throughout the drainage. Although no Nerodia were preserved or examined for E. chienense, they were quite abundant and may prey upon juveniles and adults.

DISCUSSION

Our results demonstrate that the distribution and abundance of *E. chienense* is both a function of the availability of quality habitat and the amount of suitable spawning substrata. The most viable populations were found in areas having gently flowing water, good undercut bank habitat, low silt load, and a suitable quanitity spawning substrata or instream cover. Adults occurred almost exclusively in reaches with appropriate cover and spawning substrata and were absent or in low abundance at sites that lacked these features.

It is difficult to ascribe aquatic habitat degradation to one or even two sources, but channelization and the removal of riparian vegetation are two factors that have reduced heterogeneity in streams of the midwestern and southeastern United States (Karr and Schlosser 1977, Williams et al. 1989, Schlosser 1991, Warren and Burr 1994). Channelization is a common and biologically controversial practice that is primarily aimed at controlling flooding and increasing the drainage rate of agricultural land. Stream channelization has severely impacted Bayou du Chien by changing stream flow patterns, reducing in-stream flows, and decreasing aquatic habitat complexity. Historically, Bayou du Chien was presumably a free-flowing stream with alternating areas of riffles, runs, and pools, and although a few of these reaches remain, much of the stream has been converted to a deep ditch with uniform depth, velocity, and substrate. During times of high water or flooding, velocity rates are at their most extreme through channelized reaches of the stream. Straightening of the stream is aimed at increasing the drainage, but it also destroys and removes much of the instream cover. Many aquatic organisms cannot withstand increased flow rate and often seek alternative shelter during flooding events (Hynes 1970). Furthermore, channelized sections of stream often have little or no flow when water levels are below normal. Many aquatic organisms, including darters, prosper in flowing waters and cannot withstand stagnant or low oxygenated waters. In addition, the removal of streamside vegetation is a common practice associated with stream channelization and is executed to increase the amount of tillable agricultural land. Streamside vegetation is critical maintaining water quality. First, the tree canopy normally shades the stream, thus helping to maintain normal water temperatures (Hansen 1971, Karr and Gorman 1975). Second, rooted bankside vegetation assists in reducing bank erosion and stream turbidity by decreasing soil loss (Emerson 1971, Hansen 1971). Finally, woody vegetation provides allochthonous input into the stream and most importantly provides spawning substrate and cover for darters and other aquatic organisms.

The major impacts of anthropogenic modifications on the North American ichthyofauna have been well documented. Burr and Warren (1986) noted that in the state of Kentucky stream channelization and the concomitant removal of woody vegetation have most negatively affected streams in the lowland regions of the lower Green River and Coastal Plain, including Bayou du Chien. Several other studies including Etnier (1972), Trautman and Gartman (1974), and Congdon (1971) have documented the decrease of fish and invertebrate biomass following a stream channelization event.

Although no pre-modification data are available for comparison, several reaches of stream within Bayou du Chien have been channelized and deforested to different degrees, thus allowing comparisons with the drainage. Etheostoma chienense is abundant at only one site in the drainage, Jackson Creek, an unchannelized stream with a wide (4-6 m) riparian zone. A plethora of woody debris at this site provides an abundance of spawning substrata, and consequently Jackson Creek harbors the most viable population of E. chienense. The species is considered common at 4 additional localities. These sites have been moderately modified, but still have adequate quantities of spawning materials and instream cover. At 11 of the remaining sample sites the species is rare or uncommon. Stream reaches such as those at Bayou du Chien, Bard Rd. and South Fork Bayou du Chien, 2422 Rd., have been radically modified, and lack suitable riparian zones, and therefore contain low numbers of E. chienense.

Welsch (1992) provided minimum requirements for the reforestation of open lands and for the management of existing streamside buffer zones for the purpose of sediment removal, input of organic material into the stream, and for maintenance of suitable water temperatures. Ideally, streamside buffer zones should consist of three distinct zones. Zone 1 nearest the streambank should be approximately 5 m in width and left unmanaged. It should consist of a mix of native riparian trees and shrubs that can provide organic input into the stream in the form of leaf fall, large woody debris, and detritus. Zone 2 also should consist of native tree and shrubs, but should have an average width of 20 m. The purpose of zone 2 is for filtration, denitrification, and sediment and nutrient removal from runoff. Zone 2, known as the managed forest zone, should have trees and shrubs periodically harvested to help maintain consistent vegetation growth. The runoff control zone (Zone 3) should be at the outward edge of zone 2 and average 6 m in width. The vegetation should consist of grasses and shrubs that need to be periodically mowed or grazed to help maintain growth. As mentioned before, the Bayou du Chien watershed is used presently for tillable agriculture, and because of this, most of the streamside vegetation has been removed. Most sample sites on Bayou du Chien only have a buffer zone of 2-3 meters, rather than the 30 proposed by Welsch (1992). An increase in the riparian zone of only 10 additional meters could positively benefit the E. chienense population.

The introduction of ceramic tiles significantly increased nest productivity as judged from both the maximum and average clutch sizes observed. Egg-clusters on the artificial tiles were almost double the size of the clutches attached to naturally occurring materials. The tiles appear to be beneficial in two ways. First, when compared to most other naturally occurring materials, the tiles offer more flat surface area for egg-deposition. The narrow surface area of small logs and sticks limits the number of eggs deposited and thereby decreases nest productivity. During late spring 1995, a flooding event in Jackson Creek washed several small rocks (cobble size) into the study area. Within the next several weeks, numerous egg masses were found on these cobbles. Clearly, attaching eggs to objects of this size only limits the number of eggs laid and guarded. Possible explanations for the use of small nest materials may be that other suitable nesting sites were already occupied or that females prefer to spawn on rocky materials rather than on alternative substrates. Further experimentation is needed to determine spawning substrate preferences. Secondly, although the artificial tiles are vulnerable to natural flooding, they are more stable than many of the substrates such as bark or small sticks that were only loosely attached to the stream bank. On successive visits to a site, we routinely observed the absence or destruction of nests attached to unstable materials. These materials were vulnerable to periodic spring floods, whose effects have already been enhanced due to prior straightening of the stream channel.

The use of the artificial spawning tiles varied between sites and years. The majority of the tiles were utilized in 1995 and 1996, with the exception of the tiles that were placed at Bayou du Chien, Rt. 1283, in 1995. Only five clutches were attached to the tiles placed in the upstream locality. The remaining ten tiles downstream never contained darters or eggs. Although darters were captured in the downstream area, the water is to swift and deep for spawning and guarding to occur.

Winn (1958) stated that the fantail darter, Etheostoma (Catonotus) flabellere, only needed a space of 1.5-2.5 cm for spawning to occur, just enough room so that the female could remain inverted while resting on her dorsal fins with her genital papillae pressed flat against the substrate. Members of the E. squamiceps complex are generally larger than other members of the other two Catonotus species groups and therefore need a larger space for inversion. Therefore, substrates that lack a cavity of 2.0-5.0 cm are useless to E. chienense

Several clutches found on both naturally occurring materials and the artificial spawning substrates contained eggs that were double layered. Page and Mayden (1979) found a single E. oophylax (reported as E. neopterum) nest that was double layered. They suggested that the small surface area of the nest substrate necessitated an additional layer of eggs. We failed to find evidence to support their hypothesis. Eggs were double-layered presumably because of miscues or misplacement of eggs during spawning events. Several of the double layered clutches had large amounts of surface area available for attachment of additional eggs and did not necessitate an additional layer of eggs. Sometimes the cavity beneath the spawning substrates is dark. The simple inability to see may explain the occasional double-layering of eggs.

Although no nests were discovered beneath undercut banks, it is possible E. chienense utilizes undercut banks for spawning. Both gravid males and females were commonly collected in these habitats. Undercut banks may be the primary spawning habitat for E. chienense, with other near bank materials used secondarily.

Nesting on the undersides of rocks has been observed for five species in the E. squamiceps complex, including E. squamiceps (Page 1974), E. crossopterum (Page 1974), E. oophylax (Page and Mayden 1979), E. nigripinne (Braasch and Mayden 1985), and E. olivaceum (Page 1980). All of these studies indicate that slabrocks are the most common spawning material utilized by Catonotus. Etheostoma olivaceum, which has been found guarding nests attached to tiles and tin cans (Page 1980), and E. chienense, which uses a wide variety of materials (Burr and Cook 1994, Piller and Burr 1995) represent the only two published records of a member of Catonotus utilizing substrates other than slabrocks.

The use of a wide array of spawning substrata by E. chienense presents an interesting evolutionary question within the subgenus Catonotus. Is the use of alternative substrata a derived condition in the subgenus, and by inference the ancestral condition was greater spawning substrate specificity, or is it that members of the group originally used a wide-variety of materials for egg-deposition and a few have become more limited in their choices (i.e. slabrocks)? The most tenible position is that the use of alternative nest substrata is a derived trait and the use of slabrocks is ancestral. Intuitively, this makes the most sense. Most members of Catonotus inhabit slabrock streams with abundant rocky materials for egg-deposition. Braasch and Mayden (1985) provided various hypotheses to explain the present distribution of species of Catonotus. They suggested that ancestral Catontous of the E. squamiceps complex originally inhabited slabrock pools in throughout the Cumberland River drainage and by inference utilized stony materials for egg-deposition. Access was gained to the Ohio River either by stream capture or when the Ohio and Cumberland Rivers formed a single river system. From the Ohio River, individuals could have entered several of the tributaries on the Coastal Plain. With the exception of E. chienense and E. oophylax, all other ancestral E. squamiceps-neopterum populations in Kentucky have been eliminated.

Howell and Dingerkus (1978) and Etnier and Starnes (1993) stated that other members of the E. squamiceps complex inhabiting Coastal Plain streams lacking abundant slabrocks may be logically assumed to utilize nest substrata other than slabrocks for egg-deposition. Recent observations of two additional members within the E. squamiceps complex confirm their assumptions. The crown darter, Etheostoma corona, endemic to the Cypress Creek system of northern Alabama and south-central Tennessee, and the guardian darters, E. oophylax, found in tributaries of the Tennessee River in Kentucky and Tennessee, have been found to utilize woody materials, road signs, cherty-cobble, and cinder blocks for egg-deposition (P. Ceas pers. comm. and A.

Mowery pers. comm.). Experimentation involving spawning substrate preferences in these species and other mambers of the E. squamiceps complex would be of considerable evolutionary interest.

The occurrence of multiple clutches on a single spawning substrate reflects the paucity of suitable nesting substrates in stream reaches where E. chienense occurs. As mentioned previously, it was a common occurrence to find substrata with multiple clutches and males with no physical obstruction between guarding males A single slabrock with two nests attached was found during life-history studies involving E. squamiceps (Page 1974) and E. olivaceum (Page 1980). Although two distinct egg masses were discovered, observations indicated that there were physical obstruction between clutches of eggs and guarding males. The life-history studies were performed in Big Creek, Hardin Co., Illinois and Brush Creek, Smith Co., Tennessee. Both streams have an abundance of slabrocks available for spawning and apparently when there is a plethora of spawning materials available there is no need for darters to "share" substrates.

The inclusion of behavioral characters along with morphological, meristic, and biochemical data sets allow us to better understand the evolutionary relationships within a group of organisms. Mayr (1963) stated that behavioral characters are often superior to morphological characters in studies involving sister species (or groups). At present, we have detailed morphological and meristic data on all members of Catonotus (Braasch and Mayden 1985, Page et al. 1992). The inclusion of reproductive and behavioral information can offer additional synapomorphies useful in corroboratting the relationships among sister groups. Although the sister relationships of the species groups within Catonotus are relatively clear (Braasch and Mayden 1985, Page et al. 1992), the verification of a head-to-head spawning pattern, and brief inversion by both the male and female E. chienense, substantiate the existing topology. Aquarium spawning has been observed for four species within the E. squamiceps complex, including E. squamiceps

(Page 1974), E. olivaceum (Page 1980), E. crossopterum (Page 1974), and E. chienense (This study). Although we lack detailed spawning information on the other six species in the complex, all of which are morphologically and ecologically similar, it is likely that the other members of the complex spawn in a similar manner and that this behavior will prove to be an additional synapomorphy that unites all members of the E. squamiceps complex.

The results of the length-frequency estimation indicate that the age groupings of E. chienense are similar to those which has been reported for E. squamiceps (Page 1974) and E. olivaceum (Page 1980). Although age groupings here are comparable to what has been previously reported, age determination by length-frequency estimation is often unreliable (Jearld 1983). Without large sample sizes (500+ individuals) it is difficult to delineate age-classes because of overlapping groups among year classes. The low sample sizes in this study weaken the reliability of the age-classes.

The aim of experiment 1 was to determine if nest rock size (i.e., small vs. large) conveyed the quality of a male to a female. When given a choice, a female presumably will spawn on the larger of the two substrates. A female relinquishes several benefits by spawning on materials of smaller sizes because larger spawning substrates can provide benefits that smaller, less stable substrates cannot. Larger substrates are usually heavier and have a lower probability of turning over and destroying the eggs (Brown and Downhower 1982). Females may also be choosing larger nest substrates because they allow more room for egg-deposition. The final benefit is that aggressive males obtain the larger and better nest sites. The female benefits because her eggs will be protected by aggressive males which generally are the better defenders of nests (Downhower and Brown 1980). By choosing a smaller nest, a female may be decreasing her own reproductive success, which may inevitably decrease population numbers as a whole

Laboratory experiment 2 explored whether females preferred to mate with larger males. Seven of the eight spawnings occurred with larger males. It has been shown in many fish species including the mottled sculpin, Cottus bairdi, river bullhead, Cottus gobio, and the closely related spottail darter, Etheostoma squamiceps, that females prefer to spawn with larger males (Brown and Downhower 1982, Bisazza and Marcanto 1988, Bandoli in press). In most species, females spawn with larger males presumably because the large body size of the male is an indicator of advantageous characteristics that may be passed on to her offspring. By choosing a larger male, a female's own fitness may be increased (via increased egg survival) by the better defense rates of larger males (Turner 1986). In addition, it is generally assumed that larger males are older, more experienced, better defenders of nests, and they often tend to control the larger and superior nesting sites (Downhower and Brown 1980). Generally, the selection of a larger male results in a better nest site, with more area for egg-deposition, resulting in an increase in the reproductive success of the female.

Laboratory experiments designed to examine mate choice and spawning substrate preferences were based on small samples, a constraint in working with federally endangered species. Nonetheless, the raw numbers provide evidence that female choice is a prevailing factor in nest success and population dynamics

STATUS, THREATS, AND RECOMMENDATIONS

In this two-year study, 6 additional sites of occurrence of E. chienense were discovered and it is likely that other intervening areas not sampled also harbor viable populations. Historic localities, including two sites near Moscow, Kentucky still remain void of relict darters. Collections by Webb and Sisk in the 1970s are the only vouchered specimens this far downstream and the species seems to be extirpated from these localities. Occurrences of E. chienense downstream of the Bayou du Chien, Rt. 239 bridge, which is sluggish and swamp-like is extremely unlikely.

At the time of the Warren et al. (1994) survey, conducted during the fall, many of the small tributaries including Sand Creek, Rt. 307 and Cane Creek, Coolie Rd were totally dry or comprised of only isolated pools. Although several of these localities produced individuals during this study, it is likely that these intermittent streams contribute little to recruitment. During times of low rainfall, nests may become desiccated because of low water levels or young-of-the-year may become trapped in isolated pools and become subject to predation by birds or other organisms.

Presently, E. chienense is maintaining an effective population size and the potential for downlisting from federally endangered to federally threatened is conceivable in the future. Several factors relevant to retention of the species endangered status include the following: 1) two sites that yielded specimens for Webb and Sisk (1975), Bayou du Chien, Hwy. 239 and Little Bayou du Chien, Hwy. 239, failed to produce any specimens during this study; 2) range restriction of the species to only the upper reaches of the Bayou du Chien drainage; 3) the continuing effects of natural flooding and drought at several points along the stream; including drying of small tributaries during times of low flow; 4) evidence of pesticide and sediment runoff; 5) potential for heavy predation of larval E. chienense in the primary nesting areas; 6) potential for further habitat alteration (i.e., dredging, snag removal, channelization, reduction of buffer zones); and most importantly, 7) the lack of knowledge regarding the degree of recruitment of individuals into the population. No larval E. chienense were discovered during the study and only a few juveniles were captured in late spring and early summer. After determining the habitat of larval E. chienense and the degree of recruitment into the existing population, an informed descision regarding endangered or threatened status will be made.

Etheostoma chienense has persisted in spite of various threats, but it cannot be assumed that its viability will remain indefinitely. A single toxic chemical spill in to Jacskon Creek or an extremely dry spring and summer could have devastating effects on population numbers. We recommend that the following steps be implemented and that E. chienense be monitored for several years.

1. Known sites of occurrence should be sampled periodically to determine trends in distribution and population abundance.

2. Habitat preferences of juvenile and larval E. chienense need to be determined. Although we have documentation that nesting occurs at several localities in the upper reaches of the drainage, the biology of larvae is unknown, and consequently recruitment estimates are lacking.

3. Habitat quality be maintained throughout the drainage. The proper authorities (U. S. Army Corps of Engineers, U. S. Fish and Wildlife Service, Kentucky Department of Fish and WIldlife Resources) should attempt to closely monitor the entire drainage and limit the number of permits granted to snag, channelize, or modify the existing watershed.

4. In the future, voluntary planting of woody riparian vegetation along stream banks to help decrease sedimentation, provide suitable habitat for terrestrial organisms, and most importantly, provide spawning substrata for relict darters should be strongly suggested to private landowners. The specification proposed by Welsch (1992) are perhaps unrealistic for the Bayou du Chien. The entire watershed is in private ownership with agriculture being the primary use of the watershed. The creation of large buffer zones, even an increase of 10 m of woody streamside vegetation would almost certainly be beneficial to the species.

5. Annual addition of spawning substrate (i.e., ceramic tiles). As shown previously, the artificial spawning substrate provides an effective management tool that increases nest productivity and presumably enhances survivorship and recruitment. Seeding stream reaches throughout the upper portion of the drainage for several consecutive years may significantly increase or at least maintain current population numbers.

Life-history information gathered in this study indicates that E. chienense is similar to other members of the E. squamiceps complex. Etheostoma chienense deviates by attaching eggs to a wide variety of both anthropogenic and naturally occurring materials, but the size of nest guarding males, length of spawning season, spawning behavior, size of nests, resource defense polygyny, and egg incubation periods are all analogous to what has been reported for other members in the complex (Page 1974, Page and Mayden 1979, Page 1980, Braasch and Mayden 1985). The spawning behavior of Etheostoma chienense, including the use of the fleshy masses (egg-mimics) on the tips of the second dorsal fin, head-to-head spawning pattern, brief inversion by the male and femlae, and although it was not observed, the depostion of 2-5 eggs per inversion (Page, 1974, Page 1980, Page and Swofford 1984, Page and Bart 1989, Knapp and Sargent 1989). A summary table of information on the life-history and nesting biology of E. chienense is provided (Table 8).

Table 8. Summary of information on the life-history characteristics of Etheostoma chienense in the Bayou du Chien drainage, Kentucky.

Charactersitics	Life-history data
Distribution	upper half of the Bayou du Chien drainage
Habitat of adults	Undercut banks with rooted vegetation, half meter deep
Habitat of juveniles and larvae	Unknown
Sexual dimorphism	Males larger, darkened, and knobbed second dorsal fin
Maximum size	Males 76 mm SL; females 68 mm SL
Longevity	3 + years
Spawning season	Mid-March to early June
Spawning temperatures	11-22°C
Nesting site	Sticks, logs, rocks, and any firm anthropogenic material present
Size of natural nests	12-1275 eggs
Size of artifical nests	11-1266 eggs
Incubation period	10-13 days at 20° C
Spawning position	Head-to head, both male and female only briefly invert

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Appendix A

Sites surveyed and reconnoitered for relict darters and/or nests, 1994-1996. All historic localities and sites previously sampled can be found in Webb and Sisk (1975) and Warren et al. (1994). A. Sites yielding E. chienense and or nests. BAYOU DU CHIEN DRAINAGE: Jackson Creek, 2.6 km NE Water Valley on Lawrence Boyd Rd., Graves Co., 5 March 1994, as above, 19 March 1994, as above, 16 April 1994, as above, 7 May 1994, as above, 16 May 1994, as above, 26 may 1994, as above, 27 Aug 1994, as above, 11 March 1995, as above, 16 March 1995, as above, 22 March 1995, as above, 25 March 1995, as above, 31 March 1995, as above, 5 April 1995, as above, 9 April 1995, as above, 13 April 1995, as above, 15 April 1995, as above, 25 April 1995, as above, 28 April 1995, as above, 3 May 1995, as above, 5 May 1995, as above, 11 May 1995, as above, 24 May 1995, as above, 8 June 1995, as above, 18 July 1995, as above, 2 Sept. 1995, as above, 23 March 1996, as above, 30 March 1996, as above, 2 April 1996, as above, 9 April 1996, as above, 12 April 1996, as above, 16 April 1996, as above, 18 April 1996, as above, 25 April 1996, as above, 27 April 1996, as above, 2 May 1996, as above, 4 May 1994, as above, 9 May 1996, as above 13 May 1996, as above, 17 May 1996, as above 21 May 1996, as above, 24 May 1996. Bayou du Chien, 2422 Rd., Graves Co., 9 March 1994, as above, 7 May 1994, as above, 16 May 1994, as above, 27 Aug 1994, as above, 23 March 1996, as above, 30 March 1996, as above, 2 April 1996, as above, 9 April 1996, as above, 13 April 1996, as above, 16 April 1996, as above, 18 April 1996, as above, 25 April 1996 as above, 27 April 1996, as above, 2 May 1996, as above, 4 May 1996, as above, 9 May 1996, as above, 13 May 1996, as above, 17 May 1996, as above, 21 May 1996, as above, 24 May 1996. Bayou du Chien, Rt. 307, Hickman Co, 5 March 1994, as above, 26 as above, March 1994, as above 7 May 1994, as above, 16 May 1994, as above, 26 May 1994, as above, 27 Aug 1994, as above, 25 March 1996, as above, 21

March 1996, as above, 30 March, 1995, as above, 27 April 1996, as above, 2 May 1996. Bayou du Chien, Rt. 1283, Graves/Hickman Co., 5 March 1994, as above, 26 March 1994, as above, 7 May 1994, as above, 26 May 1994, as above, 27 Aug 1994, as above, 16 March 1995, as above, 22 March 1995, as above, 25 March 1995, as above, 31 March 1995, as above, 5 April 1995, as above, 9 April 1995, as above, 13 April 1995, as above, 15 April 1995, as above, 25 April 1996, as above, 2 May 1996, as above, 9 May 1996, as above, 17 May 1996. Bayou du Chien, Davis Rd., 4.8 km SW Fulgham, Hickman Co., 19 March 1994, as above, 7 May 1994, as above, 16 May 1994, as above, 26 May 1994, as above, 27 Aug 1994, as above, 23 March 1996, as above, 16 April 1996, as above, 2 May 1996. South Fork Bayou du Chien, Pea Ridge Rd., Graves Co., 11 March 1995, as above, 31 March 1995, as above, 2 April 1996. Bayou du Chien, Rt. 51, Hickman Co., 27 Aug 1994, as above, 25 March 1995, as above, 4 May 1996, as above, 17 May 1996. Cane Cr., Howell Rd., Hickman Co., 9 April 1994, as above, 16 April 1994, as above, 31 March 1995, as above, 9 April 1996, as above, 4 May 1996. Sand Cr., Rt. 307, Hickman Co., 5 March 1994, as above, 26 March 1994, as above, 7 May 1994, 31 March 1995. Bayou du Chien, unnamed tributary, Rose Rd., 2.25 km NNE Water Valley, Hickman Co., 31 March 1995, as above 9 April 1996. Cane Cr., Coolie Rd., Hickman Co., 2 May 1996. Trib. Bayou du Chien, Rt. 94/45, Graves Co., 9 April 1994, as above, 31 March 1996. Bayou du Chien, Bard Rd., 13 May 1996. South Fork Bayou du Chien, Kingston Rd., Graves Co., 2 April 1996. Bayou du Chien/Jackson Cr./South Fork Bayou du Chien, Rt. 45, Graves Co., 16 April 1996. Bayou du Chien, Howell Rd, Hickman Co., 2 May 1996.

B. Sites surveyed or reconnoitered that did not yield relict darters and/or nests.
BAYOU DU CHIEN DRAINAGE: Cane Cr., 1529 Rd, Hickman Co., 27 Aug 1995. (lowland fauna, low flow). Bayou du Chien, Rt. 239, 0.8 km E. Moscow, Hickman Co.,

27 Aug 1995 (good flow, undercut banks present, sand-bottomed). Little Bayou du Chien, Rt. 1125, 5.2 km SSW Buda, Fulton Co., 27 August 1995 (low flow, little habitat). Little Bayou du Chien, Rt 781/94, Fulton Co., 27 August 1995 (low flow). Bayou du Chien, Howell Rd, Hickman Co., 21 March 1996 (high flow, deep pools). Bayou du Chien, Rt. 94 E of Water Valley, 0.5 mi. W. of Bethel Church Rd., Graves Co., 2 April 1996, (very silty with low flow). Cane Cr., 1529 Rd., Hickman Co., 13 April 1996 (Extrememly low flow, silty). Bayou du Chien, Hwy. 239, Hickman Co., 13 April 1996 (high water, strong flow, some suitable habitat). Bayou du Chien, Pea Ridge Rd., Graves Co., 16 April 1996 (much garbage present, stagnant). Cane Cr., Rt. 307, Hickman Co., 16 April 1996 (low flow, high silt). Pond Br., Rt. 307, Hickman Co., 16 April 1996 (low flow, little habitat). Cane Cr., Rt. 51, Hickman Co., 16 April 1996 (no habitat). Little Bayou du Chien, Rt. 1125, 5.2 km SSW Buda, Fulton Co., 18 April 1996 (mud banks, silty). Trib. of Little Bayou du Chien, Jefferies Rd/Rt. 94, 4.9 km W. Cayce, Fulton Co., 18 April 1996 (sluggish and silty). Little Bayou du Chien, Rt. 781/94, 3.2 km W. Cayce, 1.6 km E. Cayce, Fulton Co., 18 April 1996 (swamp-like, turbid). Verhine Cr., Rt. 94, Fulton Co., 18 April 1996 (cypress trees present, no flow, deep). Rush Cr., Rt. 94, Fulton Co., 18 April 1996 (silty, low water levels, no habitat). OBION RIVER DRAINAGE: Brush/Barn Cr, Smith Boyd Rd., Graves Co., 18 July 1996, as above, 21 March 1996. Brush Cr., Cuba Rd/Pilot -Oak Rd., Graves Co., 2 May 1996.