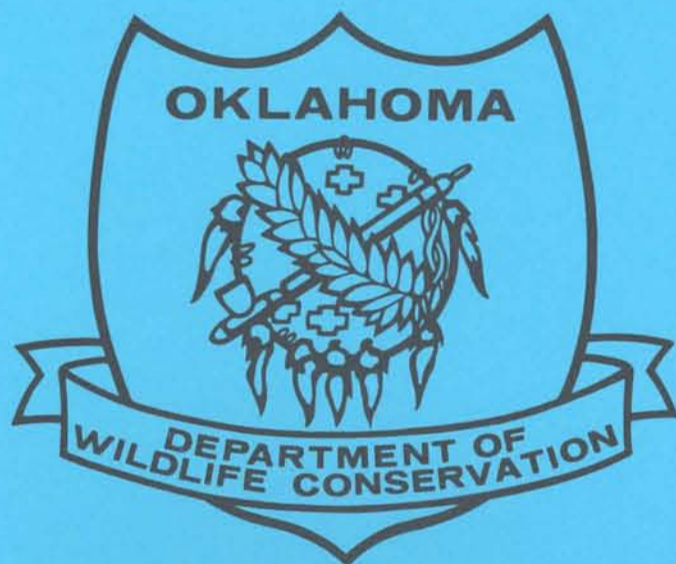


FINAL REPORT

ENDANGERED SPECIES ACT (SECTION 6)



FEDERAL AID PROJECT NO. E-2-R-1

REPRODUCTIVE ECOLOGY OF THE LEOPARD DARTER, Percina pantherina

JOB NO. 1

OCTOBER 1, 1986 through SEPTEMBER 30, 1988

FINAL REPORT

FINAL REPORT

ENDANGERED SPECIES ACT (SECTION 6)

ABSTRACT

FEDERAL AID PROJECT NO. E-2-R-1
REPRODUCTIVE ECOLOGY OF THE LEOPARD DARTER, Percina pantherina

JOB NO. 1

OCTOBER 1, 1986 through SEPTEMBER 30, 1988

FINAL REPORT

STATE: OKLAHOMA

PROJECT NUMBER: E-2-R-1

PROJECT TITLE: Reproductive Ecology of the Leopard Darter, *Percina pantherina*

STUDY TITLE: Reproductive Ecology of the Leopard Darter, *Percina pantherina*

PERIOD COVERED: October 1, 1986 to September 30, 1988

OBJECTIVE NUMBER: 1

JOB NUMBER: 1

ABSTRACT

Leopard darters were sampled monthly from August 1985 to September 1988 at six study sites in Glover River, McCurtain County, Oklahoma. Habitat measurements were made at capture locations to determine microhabitat preferences, and along transects to determine habitat availability at each site. Leopard darters inhabited pools during most of the year except March and April. Within the pools they were found at water depths of 20-100 cm over rubble/boulder substrates with no detectable current velocity. Leopard darters migrated from pools to riffle tailwaters in late February and early March. Spawning occurred from mid-March through mid-April and only certain riffles were used for spawning. Eggs were buried in deposits of fine gravel at water depths of 30-90 cm with a current velocity of 10-30 cm/s.

Juvenile leopard darters as small as 18 mm SL were captured in mid-May and inhabited the same areas of pools as adults. Growth was rapid with an adult size attained in about five months. Maximum longevity appeared to be about 18 months. Leopard darters had very specific habitat preferences and models based on the amount

of preferred habitat at a site resulted in an accuracy of about 80% in predicting leopard darter occurrence. Population abundance at each study site appeared to fluctuate appreciably from year to year. A significant relationship was found between leopard darter population abundance at a site and the amount of preferred habitat available. Leopard darter populations appear dependent on annual recruitment for maintenance and both pool habitats and spawning riffles may be limiting.

REPORT CONTENT

I. Objective:

To define the spawning season and the spawning habitat utilized by the leopard darter.

II. Introduction:

The leopard darter, *Percina pantherina*, is a small percid fish endemic to streams in the Little River drainage of Oklahoma and Arkansas (Fig. 1). O. P. Hay made the first collection of *P. pantherina* in 1884, but these specimens were incorrectly identified and were not recognized as being *P. pantherina* until about 1970 (Jim Williams, U. S. Fish and Wildlife Service; personal communication). In 1927, Hubbs and Ortenburger (1929) collected a single specimen of *P. pantherina* from the Mountain Fork River, Arkansas, and provisionally identified it as *Hadropterus macrocephalus*. The authors commented on the specimen's anomalous cheek scalation and body coloration, and mentioned that it might be a new species. As more collections were made in the Little River drainage of Oklahoma and Arkansas, it became apparent that this darter was indeed a new species. It was formally described as *Hadropterus pantherinus* by

Moore and Reeves (1955). Bailey et al. (1954) synonymized *Hadropterus* with *Percina*, and assigned the name *Percina pantherina* to the leopard darter.

Only 109 specimens of *P. pantherina* had been collected prior to 1975 (Eley et al. 1975). Because of its rarity in collections, several researchers and collectors recommended that *P. pantherina* be given special protection (Miller and Robison 1973; Buchanan 1974; Cloutman and Olmsted 1974; Robison et al. 1974; Hubbs and Pigg 1976). The U.S. Fish and Wildlife Service listed *P. pantherina* as threatened and designated critical habitat in the upper Little River, Glover River, and the upper Mountain Fork River (U. S. Fish and Wildlife Service 1978) (Fig. 2).

Percina pantherina has been assigned to the subgenus *Alvordius* by Collette (1965) and Page (1974). Other species placed in this subgenus include *P. maculata*, *P. macrocephala*, *P. peltata*, *P. crassa*, *P. roanoka*, *P. nottoagramma*, and *P. gymnocephala*. According to phylogenies constructed by Page (1974, 1981), *P. maculata*, the blackside darter, appears to be the species most closely related to *P. pantherina*. Hubbs and Raney (1939) reported that *P. maculata* was probably a complex of subspecies, and Moore and Reeves (1955) hypothesized that a population of *P. maculata* became isolated in the upper Little River tributaries and evolved into *P. pantherina*. Mayden (1985) proposed that the Kiamichi, Little, and Ouachita rivers once shared a common Ouachita Highland drainage. Presumably, *P. pantherina* evolved in this drainage as a result of vicariant events that left it isolated from its sister species, *P. maculata*, which is generally confined to lowland streams (Mayden 1985).

Populations of leopard darters are known to occur in the following areas (Fig. 3):

- 1) Little River upstream from Pine Creek Reservoir
- 2) Glover River upstream from Hwy. 3-7 bridge

- 3) Mountain Fork River upstream from Broken Bow Reservoir
- 4) Robinson Fork upstream from its confluence with Rolling Fork River
- 5) Cossatot River upstream from Gillham Reservoir.

Populations have also been found in some of the larger tributaries of the above rivers (Leon et al. 1987; Lechner et al. 1987). The downstream limits of the distributions of leopard darters can be clearly defined in all of the rivers except the Glover as the free-flowing area immediately upstream from reservoir headwaters. Historically, populations of leopard darters were known to inhabit the lower Mountain Fork and Cossatot rivers (Eley et al. 1975), but these populations have apparently been extirpated since the construction of Broken Bow and Gillham reservoirs, respectively. Population abundances in Cossatot River and Robinson Fork River are small and confined to small sections (Leon et al. 1987). Population abundances in Mountain Fork, Glover, and Little rivers are larger than those in Arkansas streams, but the most abundant populations are found in the section of Glover River upstream from Carter Creek to the town of Battiest, Oklahoma (personal observations) (Fig. 4). Glover River was chosen as the study area for this project because it supports the most abundant populations of leopard darters and is the only natural, free-flowing river in the Little River drainage.

Robison (1978) outlined the suspected life history and habitat of the leopard darter based on collection records and museum specimens. He concluded that leopard darters were most common in clear, moderately-swift water flowing over gravel substrates. Based on examination of museum specimens, he found that females contained from 450 to 2500 total ova, both sexes had enlarged genital papillae in the spring, and black fly larvae (*Simuliidae*) were the most common food items. Jones et al. (1984) found leopard darters to be predominantly pool-dwellers rather than riffle-

dwellers as suggested by Robison (1978). Leopard darters generally occurred at depths of 20 to 80 cm in areas with little or no detectable current over rubble and boulder substrates. Densities of leopard darters in riffle areas increased in the spring but no observations of spawning activity were made (Jones et al. 1984).

III. Study Area:

Glover River is a major tributary in the Little River drainage of southeastern Oklahoma and southwestern Arkansas. The river originates in the Beaver's Bend Hills subsection of the Ouachita Mountains in northern McCurtain County, Oklahoma, and flows south toward the Little River (Fig. 1). The Glover drainage basin is 56.3 km long, 32.2 km wide, and drains about 876 km². The mainstem is 53 km long and the East and West forks are 35 and 33 km long, respectively. The mean gradient of the Glover River is 2.3 m/km, and ranges from 19 m/km near the source to 1 m/km at the mouth (U. S. Army Corps of Engineers 1975). The Glover basin is composed largely of sandstone and shale sedimentary rocks of Cambrian or Ordovician to Pennsylvanian origin (Thornbury 1965). The Glover River bed is composed predominantly of Pennsylvanian and Mississippian Stanley Shale (Flawn et al. 1961).

The upper reaches of the Glover drainage are characterized by heavily forested (oaks and pines) mountainous ridges with steep slopes. Commercial timber harvesting and poultry farming are the principal economic activities in this area. The lower reaches flow through fertile lowlands and the floodplain of the Gulf Coastal Plain. These areas are devoted principally to livestock grazing.

Stream habitat of Glover River upstream from one of its major tributaries, Carter Creek (Fig. 4), consists of shallow, wide pools with bedrock, boulder, and rubble substrates separated by riffles, chutes, and low falls over bedrock and boulders.

Stream habitat below Carter Creek consists of long, deep pools, separated by shallow riffles of rubble and gravel substrates. Periodic flooding in all areas keeps the stream well scoured and results in substrates dominated by bedrock, boulders, and rubble. During summer months, extensive growths of water willow (*Justicia americana*) develop in shallow, slow-current areas, and cattails (*Typha* sp.) grow along the shorelines of pools. Six study sites in Glover River (Table 1) were selected based on the relatively high densities of leopard darters at these sites. The study sites were distributed as follows: sites 1 and 2 on mainstem Glover, sites 3, 4 and 5 on the West Fork, and site 6 on the East Fork (Fig. 5). Sites 1, 2, and 3 were pool habitats and sites 4, 5, and 6 each contained riffle and pool habitats (Table 1).

IV. Methods:

Habitat Preference

Leopard darters were sampled monthly by snorkeling within an area delineated by three to five habitat transects established at each site. The transects were perpendicular to stream flow and spaced 15-m apart. Masks and snorkels were used for underwater sampling during summer, but drysuits, hoods, and gloves were required during fall, winter, and spring. Observations of leopard darter swimming and feeding behaviors and interactions with other species were periodically recorded in the field. Hand-held dipnets (16x26-cm aquarium nets) and an underwater electrofisher (James et al. 1987) were used to capture leopard darters encountered while snorkeling. The exact location where a diver first sighted a leopard darter and initiated capture was marked with a small weighted float. The floats were made of a 10x10x1-cm styrofoam block attached to a 40-g lead weight by a 2-m long section of monofilament fishing line. The microhabitat at each capture location was characterized by measuring water

depth, substrate type, and current velocity at the point where the lead weight was placed. Eight additional measurements of depth and substrate were made at 25-cm intervals along imaginary X-Y axes to quantify the microhabitat in a 1-m² area (Fig. 6). Water depth was measured to the nearest cm with a meter stick, substrate was coded according to a modified Wentworth Particle Size scale (Table 2), and current velocity was measured to the nearest 2 cm/sec with a pygmy-gurley current meter. The mean depth, modal substrate value, and current velocity were used to characterize the microhabitat at each capture location and were used to construct frequency distributions estimating habitat preference throughout the year. Habitat availability was determined at each site by measuring water depth, substrate, and current velocity at 1-m intervals along the habitat transects. Depth, substrate, and current velocity values from capture locations and transect points at each site were compared by analysis of variance (Sokal and Rohlf 1981) to determine if differences between preferred habitat of leopard darters and habitat availability exist among sites. In addition, depth, substrate, and current velocity values from capture locations at each site were compared by analysis of variance to determine if preferred habitat of leopard darters differed between the sites. The point measurements made along transects at each site were also used in an analysis of variance to determine if habitat availability differed between sites.

Handling stress on captured specimens was reduced by holding specimens in a water-filled graduated cylinder while measurements of total length (TL, mm) and standard length (SL, mm) were made with a small, flexible metric ruler (Litvak 1983). Length-frequency distributions were constructed for each month by pooling 1986-1988 data. Sex of adults was determined by examination of mid-ventral scalation (Page 1976) with a 10x hand lens. Any anomalies or ectoparasites on specimens were noted.

Habitat Suitability

Quantitative comparisons of habitats where leopard darters were present and absent were made to identify characteristics of suitable and non-suitable habitats. Summer habitat data from the six Glover River study sites were combined with summer habitat data collected by Leon et al. (1987) at 34 sites in reaches of Arkansas streams considered to be potential leopard darter habitat by Eley et al. (1975). Habitat characteristics at each site were determined by measuring water depth, substrate type, and current velocity at 1-m intervals along three transects spaced 15-m apart. The point measurements were intended to represent average values of depth, substrate, and current velocity for a segment 1-m wide extending 7.5 m upstream and downstream from the transect for a total segment area of 15 m^2 (Fig. 7). Five transects were originally established at Glover sites 1 and 4, but data from the lowermost and uppermost transects were deleted for this analysis. The following variables were used to characterize the habitat at each site:

MD = mean depth (cm)

SD = standard deviation of depth

MS = mean substrate value

SS = standard deviation of substrate value

MC = mean current velocity (cm/s)

SC = standard deviation of current velocity

EL = elevation above mean sea level (m)

GR = stream gradient (m/km)

SW = maximum stream width (m)

An additional variable, the amount of preferred habitat (PH, m^2) at each site, was included in the analysis. Values of PH were calculated by summing all 15 m^2 segments

that had depth, substrate, and current velocity values within the preferred range as determined from the frequency distributions described in the previous section (Figs. 8-10). Sites were grouped by presence or absence of leopard darters. A stepwise discriminant function analysis was used to determine which variables were most important in distinguishing between the two groups. This analysis was used because it results in an equation that includes the combination of variables that best separate the two groups and may be used to classify future observations (Pielou 1984). Data from 29 sites in Mountain Fork, Glover, and Little rivers collected by Lechner et al. (1987) were used as an independent data set to test predictions of presence or absence resulting from the discriminant function analysis.

Population Abundance

Minimum population abundance estimates were made at sites 1-5 during the summers of 1987 and 1988. The population at site 6 was sampled during the summer of 1986 and 1988. Estimates were made at each site by capturing all leopard darters encountered while snorkeling within the area delineated by habitat transects. Divers made repeated passes through the sample area until no leopard darters were found. The captured darters were measured and enumerated, then released as close to the original capture location as possible.

The amount of preferred habitat (PH) and population abundance at each study site were used in a linear regression analysis (Sokal and Rohlf 1981) to determine the relationship between preferred habitat area and the abundance of leopard darters. Only the population abundance estimates made in August 1986, July 1987, July 1988, and September 1988 were used in the analysis because these estimates were made at each site under similar stream conditions over a two or three day period.

Spawning Habitat

Underwater observations of spawning of leopard darters were made in Glover River during March and April of 1986-87; high water and turbid conditions during spring months in 1988 precluded any observations of spawning. Detailed descriptions of male and female behaviors during spawning acts were recorded immediately following each observation. When spawning acts were observed, the spawning sites were marked with a fluorescent-yellow, metal washer (8-cm in diameter) placed at the exact site of egg deposition. Habitat characteristics of spawning sites were quantified using the same procedure described above for determining habitat preference.

Habitat characteristics of riffles used for spawning were quantified using procedures described above for the presence/absence analysis except that the three transects across riffles were only 5-m apart. Fifteen riffles in the Mountain Fork, Glover, and Little rivers were sampled during the spawning season for the presence or absence of spawning individuals. All riffles were within areas that supported leopard darter populations. The variables MD, SD, MS, SS, MC, and SC were used to characterize each riffle. Riffles were segregated by the presence or absence of spawning individuals and a discriminant function analysis was used to determine which variables were most important in distinguishing riffles that were used for spawning from those that were not.

In January, 1988 two artificial stream units were constructed in the laboratory to conduct experiments on spawning site preference. Each unit was configured so that a variety of water depths, substrates, and current velocities were available for spawning. Specific substrates (sand, fine gravel, coarse gravel, or rubble) were placed in removable trays to allow the stream configuration to be changed easily and also to provide an accurate count of the number of eggs laid per clutch by removing

the tray on which a spawning act was observed. Unfortunately, water levels in Glover River during March and April, 1988 precluded the collection any leopard darters for use in the artificial stream experiments.

Fecundity

An estimate of fecundity was made by collecting and counting eggs spawned by pairs of leopard darters held in aquaria. In March, 1986 and April, 1987 a pair of leopard darters were collected from Glover River and transported to the laboratory. In both years the individuals were held in a 150 l fiberglass aquarium at a water temperature of 18-20 °C and a photoperiod of 13L:11D. The substrate in the aquarium (coarse and fine gravel mixture) was siphoned every two days to remove eggs. The eggs collected from the aquarium were counted, measured, and held in glass bowls at a water temperature of 18-20 °C.

Another estimate of fecundity was made by counting the ova in five museum specimens collected during spring months. The diameter of each ovum in the preserved specimens was measured to the nearest 0.05 mm using an ocular micrometer mounted in a dissecting microscope.

V. Results:

Habitat Preference

Leopard darters inhabited pools exclusively except during the spawning season in March and April. Individuals were captured most often at depths ranging from 30 to 100 cm over rubble and boulder substrates with little or no detectable current velocity (Figs. 8-10). In these areas, leopard darters were typically observed cruising 5-10 cm above the substrate, stopping often to pick prey items from the periphyton. Leopard darters were rarely seen resting on the substrate and appeared to

be capable of maintaining position in the water column with minimal effort. They fled in a burst-swimming behavior when large piscivorous fishes (i.e., smallmouth bass and green sunfish) approached, although no predation was ever observed. Individuals occasionally swam into crevices or under slabs to escape our nets. The few leopard darters captured during periods of extremely low water temperatures (2-6 °C) were found under large rocks. Benthic fishes that were commonly observed with leopard darters were (in decreasing order of abundance) orangebelly darters (*Etheostoma radiosum*), channel darters (*Percina copelandi*), logperch (*P. caprodes*), and johnny darters (*E. nigrum*). At a supplemental study site in lower Glover River (R23E T5S Sec. 9) leopard darters were captured with blackside darters (*P. maculata*) and dusky darters (*P. sciera*).

Significant differences existed in the seasonal average depths at which leopard darters were captured (ANOVA $F=16.6$, $P<0.001$). Deepest and shallowest habitats were used in winter and fall, respectively (Fig. 8). Substrate types at capture locations differed significantly among seasons (ANOVA $F=6.47$, $P<0.001$), with rubble/boulder preferred during summer and gravel/rubble preferred during spring (Fig. 9). There was a significant difference in seasonal current velocities used (ANOVA $F=16.5$, $P<0.001$), with some use of areas with current during winter and spring (Fig. 10).

Young-of-the-year leopard darters as small as 18 mm SL inhabited the same pool areas as adults; no significant differences existed between depths ($t=0.45$, $P>0.66$), substrates ($t=0.151$, $P>0.88$) or current velocities ($t=0.191$, $P>0.85$) inhabited by juveniles and adults. No significant differences existed between depths ($t=0.92$, $P>0.36$), substrates ($t=0.69$, $P>0.49$), or current velocities ($t=0.61$, $P>0.54$) occupied by males and females.

No significant differences were found between substrate preference and substrate

availability (ANOVA $F=0.39$, $P>0.85$) or between current velocity preference and current velocity availability (ANOVA $F=0.90$, $P>0.45$) among the study sites. However, a significant difference existed between water depth preference and water depth availability at the sites (ANOVA $F=6.42$, $P<0.001$). No significant differences existed in water depth, substrate, or current velocity preference between the six study sites (Table 3). However, significant differences existed among depths, substrates, and current velocities available at the six sites (Table 3).

Habitat Suitability

The stepwise discriminant function analysis selected the variable PH (m^2 of preferred habitat), as the most important variable distinguishing sites with leopard darters present from sites with leopard darters absent. From the habitat preference analysis (Figs. 8-10) we concluded that the preferred water depths, substrate types, and current velocity were 24-76 cm, rubble/boulder, and no current, respectively. The analysis resulted in a canonical correlation of 0.746 and calculated canonical variable values for the two groups (leopard darters present vs. absent) from the formula:

$$\text{Canonical Variable} = 1.2035 - (0.0049 \times \text{PH})$$

The mean canonical variable value for sites with leopard darters was -1.89, and was 0.63 for sites without leopard darters. The above formula correctly assigned 36 of the 40 sites (90%) to their original group (Fig. 11). A test of the predictive accuracy of the above formula was performed with data from 23 sites in Mountain Fork River, 5 sites in Little River, and 1 site in lower Glover River. These sites were not randomly selected by the investigators (Lechner et al. 1987), but rather, only those sites that were determined to be potentially suitable for leopard darters based on visual examination were selected. At each of these sites, a value for PH was

calculated and used to produce a canonical value. The value -0.00000125 was used as the midpoint between the present versus absent groups. Sites with a calculated canonical value less than the midpoint ($PH > 240 \text{ m}^2$) were predicted to have leopard darters and those with values greater than the midpoint ($PH < 240 \text{ m}^2$) were predicted to have no leopard darters. The discriminant function correctly predicted the presence or absence of leopard darters at 23 of 29 sites (79%) (Table 4). Two of the incorrect predictions were at sites where only one leopard darter was found; these sites may not represent permanent populations. One of the incorrect predictions was at a site in lower Mountain Fork River where leopard darters have been extirpated.

Age and Growth

Adults in winter and spring months ranged from 55 to 80 mm SL (Fig. 12). Young-of-the-year leopard darters were first captured in May and averaged 26 mm SL. By late July, adults were 75-85 mm SL and juveniles were 35-55 mm SL (Fig. 12). No large adults (>80 mm SL) were found after the end of September and young-of-the-year attained adult size (55-70 mm SL) by September (Fig. 12). Populations were dominated by young-of-the-year from September through the next spawning season in March. The sex ratio did not deviate significantly from a 1:1 ratio (276 males, 286 females, $X^2=0.178, P>0.25$).

Monthly growth rates of juvenile leopard darters approximated 10-15 mm SL from May through August (Fig. 13). Growth rates decreased to about 10 mm SL for the period September through April (Fig. 13).

Parasitism

Leopard darters with parasitic copepods (*Lernaea* sp.) attached to the base of either the dorsal fins or the pectoral fins were occasionally found. Small leeches were also occasionally found attached to either the pectoral fins or the caudal fin.

The leeches did not appear to cause any noticeable damage to their hosts; however, the copepods caused large wounds at attachment sites. Parasites were found on 30 individuals from 1986-1988. Except for two darters captured in November, all parasitized individuals were captured during the summer. The frequency of individuals having parasites averaged 28.55% (3-100%) at sites where at least one individual had parasites.

Population Abundance

Minimum population abundance estimates ranged from 1 leopard darter at site 5 in September 1987 and 1988 to 90 individuals at site 1 in June 1987. Mortality rates at the study sites from July through September averaged 60.5% (23.4-85.7%) in 1987 and 58.3% (35.7-77.7%) in 1988. The highest mortality rates were found at site 6 (85.7% in 1987 and 77.7% in 1988), a headwater site on the East Fork. Population abundances at all sites throughout summer months were lower in 1988 than in 1987 (Fig. 14).

Reduced recruitment in 1988 may have been caused by adverse stream conditions during the spawning season of 1988. Stream discharges from mid-March through early April in 1987 were relatively stable, whereas 1988 was marked by three periods of high flow during this period (Fig. 15). High flows may have interrupted spawning and/or destroyed eggs and larvae in 1988.

A significant relationship existed between amounts of preferred habitat and leopard darter population abundances at the study sites in August 1986 ($F=13.16$, $P<0.05$), July 1987 ($F=11.78$, $P<0.05$), July 1988 ($F=12.12$, $P<0.05$), and September 1988 ($F=10.14$, $P<0.05$) (Fig. 16). The linear regression formulas each explained about 75% of the variation in abundance (Fig. 16) and could be used to make relatively accurate predictions of leopard darter abundances at a site based on preferred habitat area.

Spawning Season

Spawning occurred from mid-March through mid-April in 1986-1988. Spawning began on March 9, 1986, at a water temperature of 17 °C, and on March 12, 1987, at a water temperature of 12 °C. Initiation of spawning at different temperatures on about the same date in the two years may indicate that day length was more important than water temperature in inducing spawning (Hubbs 1985). No spawning acts were observed in 1988 but gravid females were found on riffles on 7 March at a water temperature of 13 °C. Leopard darters were found in riffles as late as 16 April, 1988, at a water temperature of 15 °C. The spawning season in 1988 was probably interrupted because of three heavy rainfall events that caused high flows in Glover River (Fig. 15).

Spawning Migration

Leopard darters occurred exclusively in the tailwaters of riffles in late February or early March of 1986-1988 when water temperatures were about 10 °C. The average number of leopard darters collected at sites 5 and 6 during the late summer and fall months was 2 and 4, respectively. During the spawning season, as many as 10 darters occurred in the riffle area at site 5 and as many as 18 at site 6. Conversely, no leopard darters inhabited the riffle area immediately downstream from the pool at site 3 during the spawning season where about 15 leopard darters were found during the summer and fall months. Leopard darters did not necessarily utilize the nearest riffle for spawning, but appeared to select specific spawning riffles on which as many as 20-25 individuals were found. The relatively high densities found on some but not all riffles during the spring suggested that leopard darters underwent a migration from pools to specific spawning areas.

Spawning Behavior

In a typical spawning event, a gravid female, followed by one or more males,

moved from the riffle tailwaters upstream into the riffle. The female moved slowly over the gravel and rubble and occasionally settled on the substrate. Males appeared to establish and defend "moving territories" around a gravid female and attempted to chase other males away from the female. One of the males, usually the largest, attempted to position himself directly on top of the female. Unreceptive females immediately swam away but the male or males continued to follow. If a female was receptive, a male positioned himself with his pelvic fins on her spinous dorsal fin. With both fish oriented in the same direction, the male curved his body into an S-shape and the pair began to vibrate rapidly, presumably releasing gametes. During the vibrations, the female's genital papilla became buried in the gravel. The male appeared to begin vibrating before the female. Contact with the enlarged midventral scales of males in the genus *Percina* may stimulate females to release eggs (New 1966; Page 1976). The vibrating movements of the pair buried the fertilized eggs in fine gravel. The water-hardened eggs were non-adhesive and demersal. No eggs remained on the surface of the substrate following a spawning act. The vibrations lasted 3-5 sec and were followed by an inactive period of 3-10 min. During the resting phase, both fish remained stationary on the substrate. The female and attendant males then selected another spawning site and repeated the spawning act. Females engaged in as many as six spawning acts during a 30 min period. When multiple spawning acts occurred, the eggs were deposited within a 0.5 m² area. Occasionally, one or two smaller, supernumerary males joined a pair already spawning. These males, facing in the same direction as the original pair, vibrated while making contact along the side of the female. Analogous behaviors have been observed in only two other darter species (Reeves 1907; New 1966). Parental care of eggs or larvae has never been observed in any species of *Percina* (Page 1983) and none was observed in leopard

darters. Logperch, channel darters, and orangebelly darters were observed on the riffles while leopard darters were spawning, and on two occasions, predation on leopard darter eggs by channel darters was observed.

Spawning Habitat

Spawning sites were located at depths of 30-90 cm over predominantly gravel substrates where current velocities were 0-50 cm/s (Fig. 17). Eggs were buried in deposits of fine gravel (3-10 mm in diameter) in the interstices of coarse gravel and rubble. Underwater observations made at several riffles in Glover River revealed that some riffles were not used for spawning, despite habitation of adjacent pools by leopard darters. In general, the riffles where spawning activities were observed had deposits of fine gravel at water depths of 50-100 cm in the less turbulent tailwater areas having current velocities of 5-30 cm/sec.

The discriminant function analysis selected MD (mean depth) and MS (mean substrate) as the most important variables in separating the two groups of riffles.

The formula:

$$\text{Canonical Variable} = -9.6 - (0.13 \times \text{MD}) + (2.17 \times \text{MS})$$

was used to classify the riffles into two groups. The mean canonical variable value for riffles with spawning activity was -2.4, and was 1.2 for riffles with no spawning activity. The analysis correctly classified all 15 riffles to their original group (Fig. 18). Spawning would be predicted to occur on riffles having a canonical variable value of ≤ 0.0 .

Fecundity

Total numbers of distinguishable ova in preserved specimens ranged from 294 to 757 with a mean of 465 ova per female. Diameter-frequency distributions showed a decrease in the frequency of ova >0.5 mm in diameter after the spawning season (Fig.

19) suggesting ova <0.5 mm in diameter were not released during spawning. A relationship between standard length and fecundity (Fig. 20) suggests that larger fish produce more eggs ($F=4.89$, $P<0.15$).

The pair held in captivity in 1986 spawned 26 clutches over about 120 days with an average time between spawns of about 4.6 days. In 1987, the pair collected in April spawned only four clutches in captivity; the female had probably already spawned several clutches in the stream before being captured. Egg clutches from the 1986 pair contained 15-146 eggs with an average of 58.5 eggs per clutch. The eggs had a mean diameter of 1.37 mm (range 1.25-1.5) and hatched in about seven days at 20 °C. The larvae exhibited a distinctive swimming-up behavior in the glass bowls. If newly-hatched larvae exhibit the swimming-up behavior in the stream, they could easily drift downstream into pools where they may complete their early life history.

If female leopard darters spawn 58.5 eggs every 4.6 days in natural systems, an individual female could potentially spawn 6-7 times and produce about 350-410 eggs over a 30-day spawning season. This prediction is relatively close to the actual ova counts made from museum specimens (Fig. 19).

Food Habits

Mayfly nymphs (Ephemeroptera: Baetidae and Heptageniidae), blackfly larvae (Diptera: Simuliidae), and midge larvae (Diptera: Chironomidae) were the only food items found in stomachs of 19 leopard darters examined (Table 5). Blackfly larvae and mayfly nymphs were the major food items in thirteen leopard darter stomachs examined by Robison (1978).

VI. Discussion:

Habitat preferences revealed by our study are in general agreement with the description of leopard darter habitat by Jones et al. (1984). However, our data further revealed that leopard darters exhibited a seasonal shift in water-depth preference. Darters chose deepest habitats during winter months, perhaps in an effort to avoid freezing conditions that occasionally occur in the shallow areas of pools. Leopard darters preferred smaller substrates and moving water during spring. These areas, characteristic of riffle tailwaters and apparently necessary for successful spawning, were selected even when pools with rubble and boulder substrates and no detectable flow were available. No significant difference in habitat preferences of young-of-the-year versus adult leopard darters was apparent. Although no darters smaller than 18 mm SL were collected, it is doubtful that larval leopard darters inhabit areas that are very different from those used by juveniles or adults. Leopard darter preference for substrate and current velocity based on capture locations was not significantly different from the average substrate type and current velocity available in the pools. However, leopard darter preference for water depth was significantly different from the average depth found in the pools. Leopard darters appear to be very specific in their microhabitat preferences; they inhabited similar microhabitats at all study sites even though the study sites showed differences in the habitats available (Table 3).

Kuehne and Barbour (1983) stated that the distributions of blackside darters and leopard darters are probably allopatric; however, the supplemental study site in lower Glover River (R23E T5S Sec. 9) represents an area of sympatry. Further studies are needed to determine if hybridization occurs between these closely related species in this area.

The predictive formula derived by discriminant function analysis was about 80% correct in predicting the occurrence of leopard darters at any site in the Little River drainage. Due to the difficulty in collecting and identifying leopard darters, this formula may prove to be a practical tool for habitat assessment because a relatively accurate prediction of leopard darter occurrence can be made by simply measuring the amount of preferred habitat in 45-m long stream section. Leopard darters can be expected to occur at sites where the amount of preferred habitat is greater than 240 m². Absence of leopard darters at such sites would suggest that the areas are on the periphery of leopard darter distribution and may be used only periodically. Alternatively, a factor other than depth, substrate, or current velocity may be responsible for rendering the area unsuitable. For example, a site (M48, Table 4) in Mountain Fork River at Beaver's Bend State Park where leopard darters occurred prior to the construction of Broken Bow Reservoir (Frank Cross, University of Kansas; personal communication) was judged suitable based on the amount of preferred habitat. No leopard darters were found during several collecting efforts and we concluded that a factor other than depth, substrate, or current velocity was responsible for the absence the species. Fluctuations in water depth, current velocity, and water temperature associated with hydropower generation probably render the reach unsuitable for leopard darters. In addition, this predictive model may also be useful in identifying habitat modifications that would enhance a site's suitability for leopard darters.

Our length-frequency distributions (Fig. 11) suggest that leopard darters have a maximum longevity of about 18 months. Although we did not capture any Age-I darters after September, it is possible that a few individuals may survive to reproduce a second time at Age-II. However, all spawning individuals appeared to be Age-I

darters. Only the smallest darter species have a longevity of less than two years (Page 1983) and thus leopard darters appear to be unique; they are relatively large darters with a short longevity. Mortality rates of Age-I darters following spawning appear to be high, but rapid growth of Age-0 darters allows achievement of adult size in about 5-6 months. The growth rates for leopard darters (Fig. 12) are higher than those that have been reported for any other darter species (Page 1983). Leopard darter populations are apparently dependent on successful annual recruitment for maintenance.

Leopard darter mortality from July to September was about 59% in both 1987 and 1988. Mortality of each life history stage was not estimated, but at least some egg mortality was due to predation by channel darters. Predation on juvenile and adult darters was not observed and although predation on other darter species has been documented (Page 1983) leopard darters are probably not abundant enough to be a common food item. However, because of their low abundances any predation could play a major role in juvenile and adult leopard darter mortality. Parasitism by copepods caused relatively large wounds on leopard darters and although they are common parasites of darters (Page 1983), the mortality directly or indirectly due to parasites could not be determined.

Leopard darter populations appear to fluctuate from year to year (Fig. 13) and stream conditions during early life history stages may be important in determining population abundances. The relationship between leopard darter abundances and preferred habitat area (Fig. 15) suggests that populations at a specific site are limited by the available habitat. This relationship may be useful in predicting the impact of habitat changes on leopard darter populations.

Leopard darters select specific spawning habitat and restrict spawning to certain

riffles for spawning. The observation of higher numbers of darters on a riffle than were believed to inhabit adjacent pools suggests that leopard darters migrate to specific riffles for spawning. The discriminant function analysis of riffles during the spawning season resulted in a complete separation between riffles that were used for spawning and those that were not. The predictive model developed by this analysis is likely to be useful in identifying important spawning areas and may provide a means to identify management options that could improve unused riffles to make them more preferable.

VII. Conclusions and Recommendations:

Juvenile and adult leopard darters inhabit pools exclusively during summer, fall, and winter. They specifically inhabit areas within the pools that have water depths of 25-75 cm, substrates of rubble and boulder, and no detectable current velocity. Leopard darter occurrence can be predicted based on the presence or absence of this habitat and population abundance is proportional to the amount of preferred habitat available. These specific areas of preferred habitat must be managed and protected in order to ensure the survival of leopard darters. Any major stream alteration such as channelization, impoundment, or diversion that would cause a change in the habitat preferred by leopard darters would result in a major decrease in leopard darter population abundance. The habitat at specific sites should be monitored frequently to detect any habitat degradation.

Leopard darters spawn from mid-March through mid-April on deposits of fine gravel at water depths of 25-90 cm and current velocities of 10-35 cm/s. Spawning does not occur on all riffles but only on those with the specific habitat characteristics described above. Proper management of leopard darters must include the

identification, protection, and maintenance of access (by darters) of suitable spawning areas.

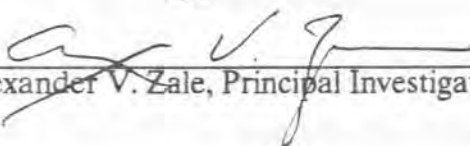
The leopard darter's life history is characterized by rapid growth to maturity, short longevity, and high mortality of post-spawning and young-of-the-year individuals. These characteristics, as well as stochastic environmental effects, cause leopard darter population abundances to fluctuate drastically from year to year. Leopard darter management plans should include annual monitoring of population abundance at specific sites to identify general trends in population dynamics.

The relationship between leopard darter population abundance and preferred habitat area indicates that this habitat may be a limiting factor. Spawning occurred only on riffles with specific characteristics which suggests that spawning habitat may also be limiting. Future research projects should be designed to evaluate the possibility and overall effect of pool and riffle habitat modifications on leopard darter populations.


VIII. Prepared by: Paul W. James, Student Investigator

O. Eugene Maughan, Principal Investigator

and


Alexander V. Zale, Principal Investigator

IX. Date: November 18, 1988

X. Approved by: 
Dr. Harold Namminga
Federal Aid/Research Coordinator

Literature Cited

- Bailey, R. M., H. E. Winn, and C. L. Smith. 1954. Fishes from the Escambia River, Alabama and Florida, with ecologic taxonomic notes. Proceedings of the Academy of Natural Sciences of Philadelphia 106:109-164.
- Buchanan, T. M. 1974. Threatened native fishes of Arkansas. Pages 67-92 in Arkansas natural area plan. Arkansas Department of Planning, Little Rock.
- Cloutman, D. G., and L. L. Olmsted. 1974. A survey of the fishes of the Cossatot River in southwestern Arkansas. Southwestern Naturalist 19:257-266.
- Eley, R. L., J. C. Randolph, and R. J. Miller. 1975. Current status of the leopard darter, *Percina pantherina*. Southwestern Naturalist 20:343-354.
- Flawn, P. T., A. Goldstein, Jr., P. B. King, and C. E. Weaver. 1961. The Ouachita system. University of Texas, Austin. 401 pp.
- Hubbs, C. 1985. Darter reproductive seasons. Copeia 1985:56-68.
- Hubbs, C., and J. Pigg. 1976. The effects of impoundments on threatened fishes of Oklahoma. Annals of the Oklahoma Academy of Science 5:113-117.
- Hubbs, C. L., and A. I. Ortenburger. 1929. Fishes collected in Oklahoma and Arkansas in 1927. Publications of the University of Oklahoma Biological Survey 1:47-105.
- Hubbs, C. L., and E. C. Raney. 1939. *Hadropterus oxyrhynchus*, a new percid fish from Virginia and West Virginia. Occasional Papers of the University of Michigan Museum of Zoology No. 396. 9 pp.
- James, P. W., S. C. Leon, A. V. Zale, and O. E. Maughan. 1987. Diver-operated electrofishing device. North American Journal of Fisheries Management 7:597-598.

- Jones, R. N., D. J. Orth, and O. E. Maughan. 1984. Abundance and preferred habitat of the leopard darter, *Percina pantherina*, in Glover Creek, Oklahoma. *Copeia* 1984: 378-384.
- Kuehne, R. A., and R. W. Barbour. 1983. The American darters. University Press of Kentucky, Lexington. 177 pp.
- Lechner, M., A. V. Zale, S. O'Donnell, and B. Ben. 1987. Distribution and abundances of the leopard darter, *Percina pantherina* (Moore and Reeves), in the upper Mountain Fork and upper Little River drainages. Final Report, U. S. Army Corps of Engineers, Tulsa, Oklahoma. 27 pp.
- Leon, S. C., M. T. Ferguson, O. E. Maughan, and A. V. Zale. 1987. Distribution and abundances of the leopard darter, *Percina pantherina* (Moore and Reeves). Final Report, U. S. Army Corps of Engineers, Tulsa, Oklahoma. 34 pp.
- Litvak, M. K. 1983. A method for measuring small fish. *Progressive Fish-Culturist* 45:61.
- Mayden, R. L. 1985. Biogeography of Ouachita Highland fishes. *Southwestern Naturalist* 30:195-211.
- Miller, R. J., and H. W. Robison. 1973. The fishes of Oklahoma. Oklahoma State University Press, Stillwater. 246 pp.
- Moore, G. A., and J. D. Reeves. 1955. *Hadropterus pantherinus*, a new percid fish from Oklahoma and Arkansas. *Copeia* 1955:89-92.
- Page, L. M. 1974. The subgenera of *Percina* (Percidae: Etheostomatini). *Copeia* 1974:66-86.
- Page, L. M. 1976. The modified mid-ventral scales of *Percina* (Osteichthyes:Percidae). *Journal of Morphology* 148:255-264.
- Page, L. M. 1981. The genera and subgenera of darters (Etheostomatini). *Occasional*

- Papers of the University of Kansas Museum of Natural History No. 90. 69 pp.
- Page, L. M. 1983. Handbook of darters. TFH Publications, Neptune City, New Jersey. 271 pp.
- Pielou, E. C. 1984. The interpretation of ecological data. John Wiley and Sons, New York. 263 pp.
- Robison, H. W. 1978. The leopard darter (a status report). Endangered Species Report No. 3, U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 28 pp.
- Robison, H. W., G. A. Moore, and R. J. Miller. 1974. Threatened fishes of Oklahoma. Proceedings of the Oklahoma Academy of Science 54:139-146.
- Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman, New York. 859 pp.
- Thornbury, W. D. 1965. Regional geomorphology of the United States. John Wiley and Sons, New York. 609 pp.
- U.S. Army Corps of Engineers. 1975. Environmental statement, Lukfata Lake, Glover Creek, Oklahoma. U.S. Army Corps of Engineers, Tulsa District, Tulsa, Oklahoma.
- U.S. Fish and Wildlife Service. 1978. Final threatened status and critical habitat for the leopard darter. Federal Register 43:3711-3716.

Table 1. Locations and descriptions of study sites in Glover River, McCurtain County, Oklahoma.

Site	Location	Description
1	mainstem Glover (R23E T3S Sec. 32)	pool about 75 m upstream from bridge on Weyerhauser Road No. 52000
2	mainstem Glover (R23E T3S Sec. 7)	East Fork-West Fork confluence pool on upstream side of bridge on Weyerhauser Road No. 53000
3	West Fork (R23E T3S Sec. 7)	pool about 100 m upstream from bridge on Weyerhauser Road No. 53100
4	West Fork (R23E T2S Sec. 20)	riffle and pool on downstream side of bridge on Weyerhauser Road No. 74260
5	West Fork (R23E T2S Sec. 6)	riffle and pool on downstream side of bridge on Weyerhauser Road No. 61000
6	East Fork (R23E T2S Sec. 27)	riffle and pool on downstream side of bridge on Weyerhauser Road No. 53100

Table 2. Modified Wentworth particle-size scale for coding substrate.

Value	Particle Size (mm in diameter)	Description
1	-----	detritus, muck
2	<0.004	mud, clay
3	0.004-0.05	silt
4	0.06-2.00	sand
5	2.00-64.0	gravel
6	65-255	rubble
7	256-1000	boulder
8	>1000	bedrock

Table 3. Results of analysis of variance tests of habitat preference among the six study sites and habitat availability among the six study sites.

Variable	F	P
Habitat Preference		
Depth	0.80	>0.5
Substrate	1.76	>0.1
Current Velocity	2.07	>0.05
Habitat Availability		
Depth	23.81	<0.001
Substrate	2.52	<0.05
Current Velocity	7.43	<0.001

Table 4. Results of discriminant function predictions of leopard darter presence/absence at 29 sites (M = Mountain Fork River sites, L = Little River sites, G = Glover River sites).

Site	PH (m ²)	Canonical Value	Prediction
M6	360	-0.575	Correct
M7	270	-0.130	Correct
M11	195	0.240	Incorrect
M12	255	-0.056	Correct
M17	135	0.537	Incorrect
M18	1095	-4.206	Correct
M19	240	0.018	Incorrect
M20	370	-0.624	Correct
M23	315	-0.353	Correct
M24	555	-1.538	Correct
M28	420	-0.871	Correct
M29	465	-1.094	Correct
M31	270	-0.130	Correct
M32	120	0.611	Incorrect
M36	345	-0.501	Correct
M38	270	-0.130	Correct
M39	285	-0.204	Correct
M40	1680	-7.096	Correct
M43	75	0.833	Incorrect
M45	360	-0.575	Correct
M46	560	-1.563	Correct
M47	510	-1.316	Correct
M48	285	-0.204	Incorrect
L53	300	-0.278	Correct
L56	420	-0.871	Correct
L57	390	-0.723	Correct
L58	855	-3.020	Correct
L59	300	-0.278	Correct
G7	150	0.463	Correct

Table 5. Frequency (%) and mean number of food items found in stomachs of 19 leopard darter museum specimens.

Taxon	Frequency	Mean	Range
Ephemeroptera			
Heptageniidae	47.4%	2.0	1-6
Baetidae	52.6%	15.5	1-51
Diptera			
Simuliidae	5.3%	17.0	17
Chironomidae	26.3%	1.6	1-3

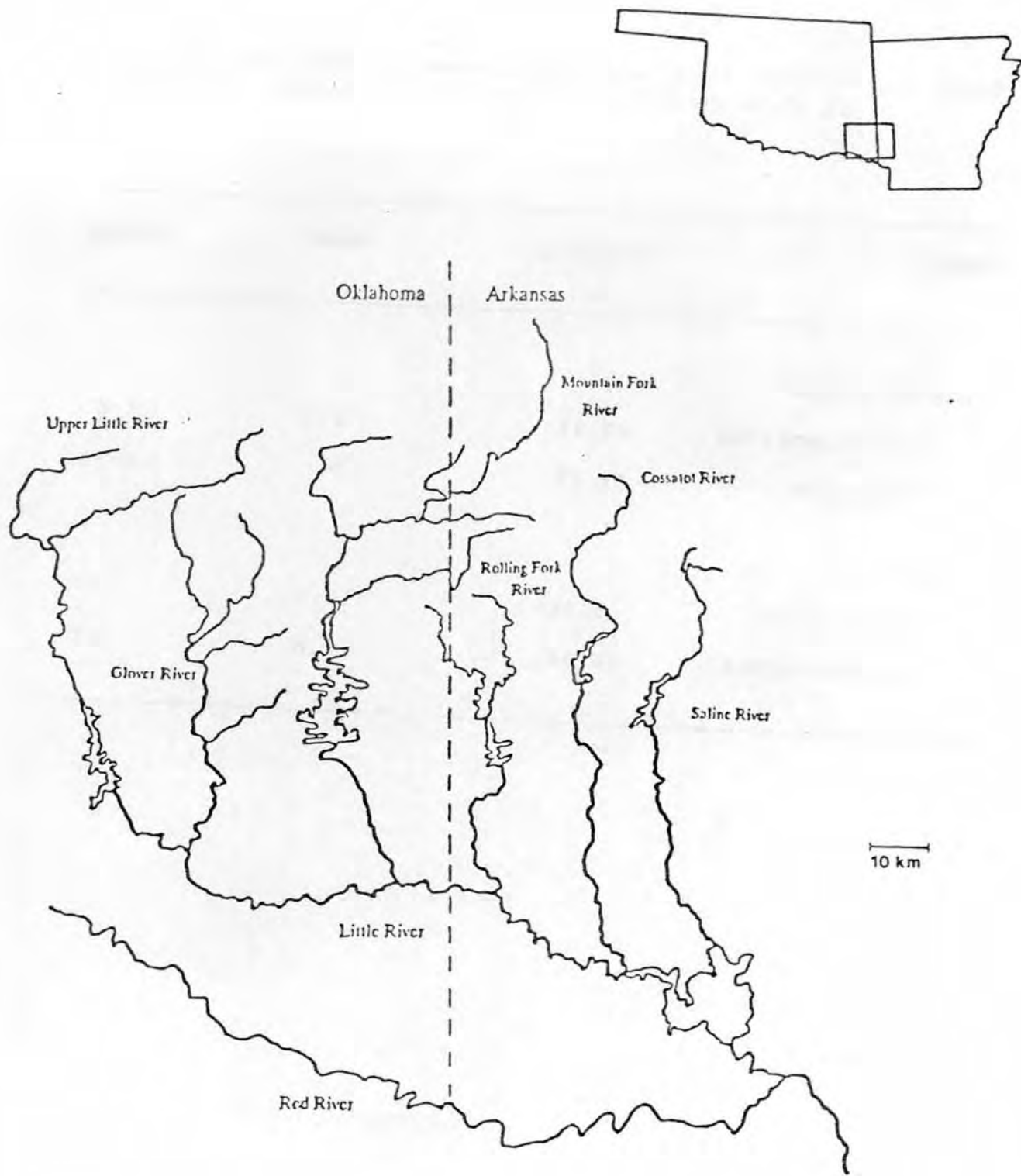


Figure 1. Little River drainage in Oklahoma and Arkansas.



Figure 2. Little River drainage showing official critical habitat for the leopard darter.

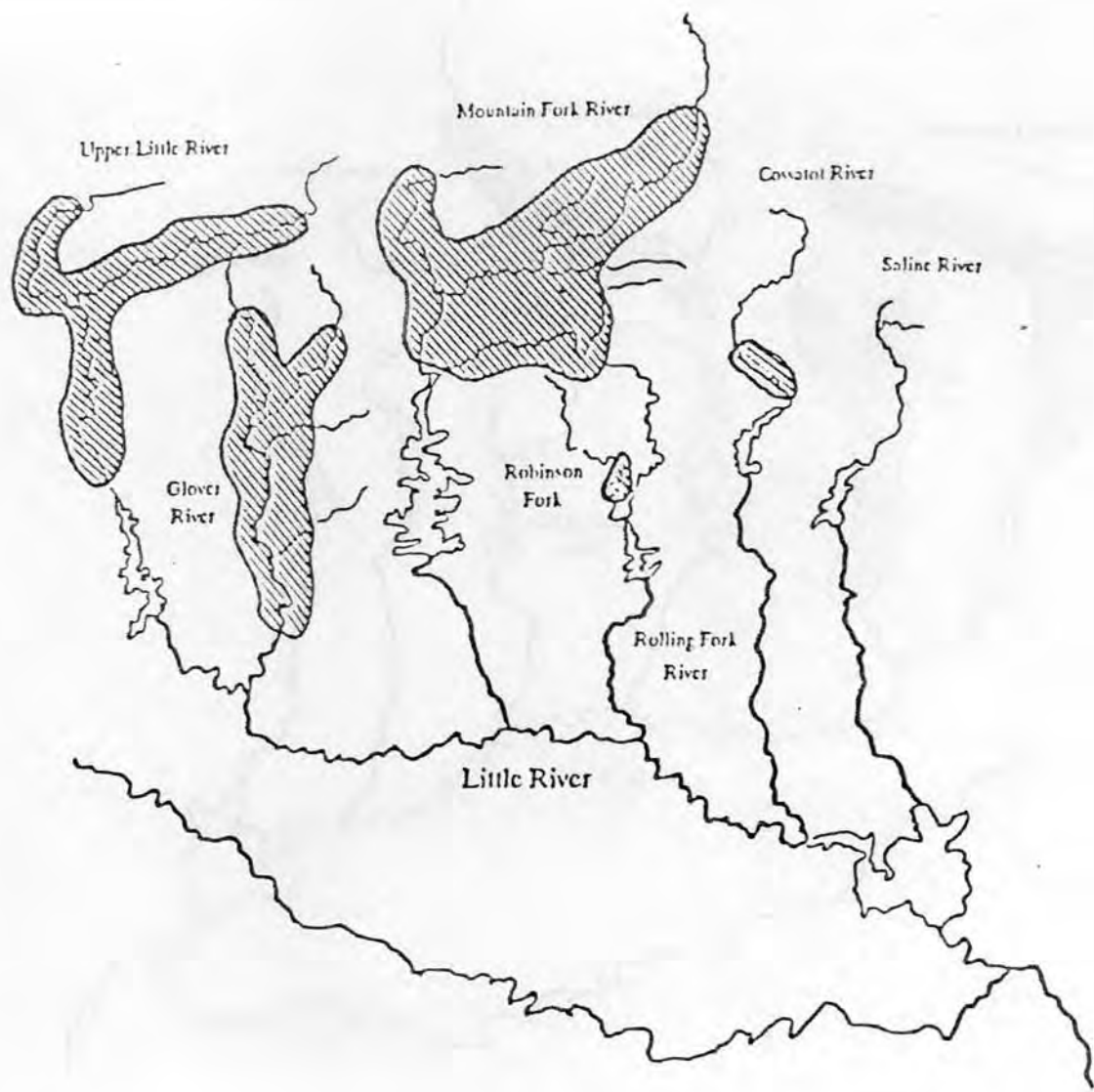


Figure 3. Little River drainage showing distribution of the leopard darter.



Figure 4. Glover River in McCurtain County, Oklahoma.



Figure 5. Study sites in Glover River, McCurtain County, Oklahoma.

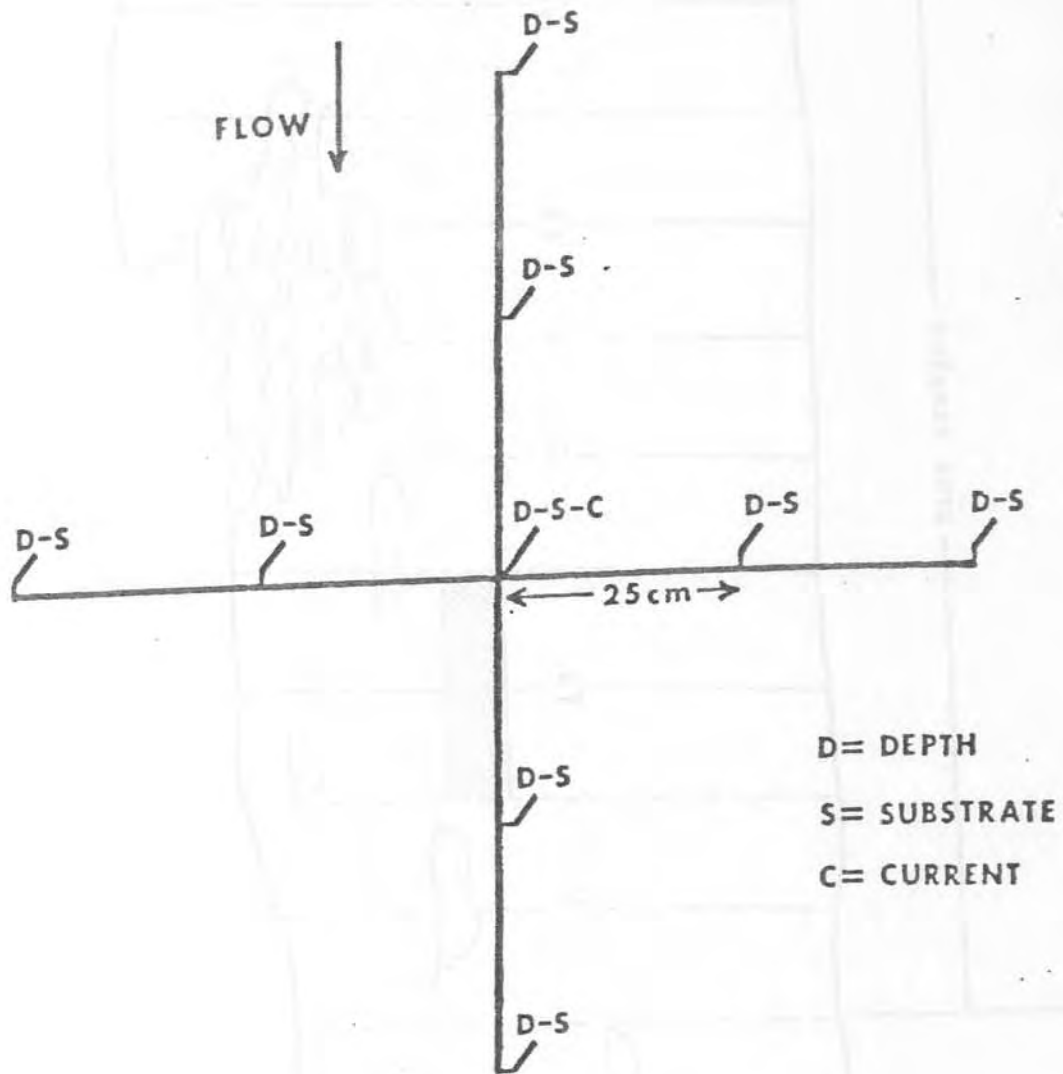


Figure 6. Diagram of measurements taken at leopard darter capture locations.

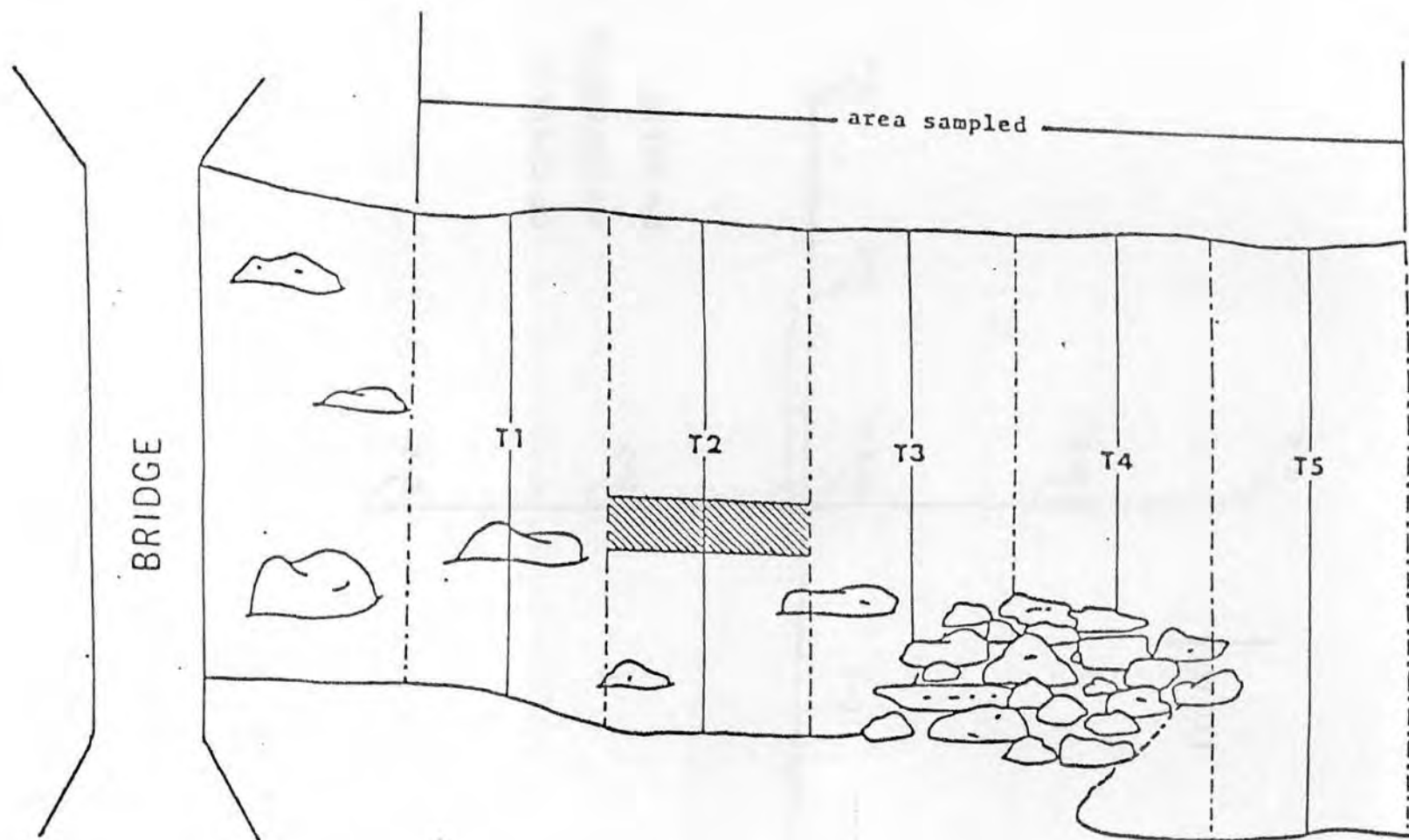


Figure 7. Diagram of a typical study site showing habitat transects (T1-T5), example of an area represented by a point measurement (shaded), and total area sampled.

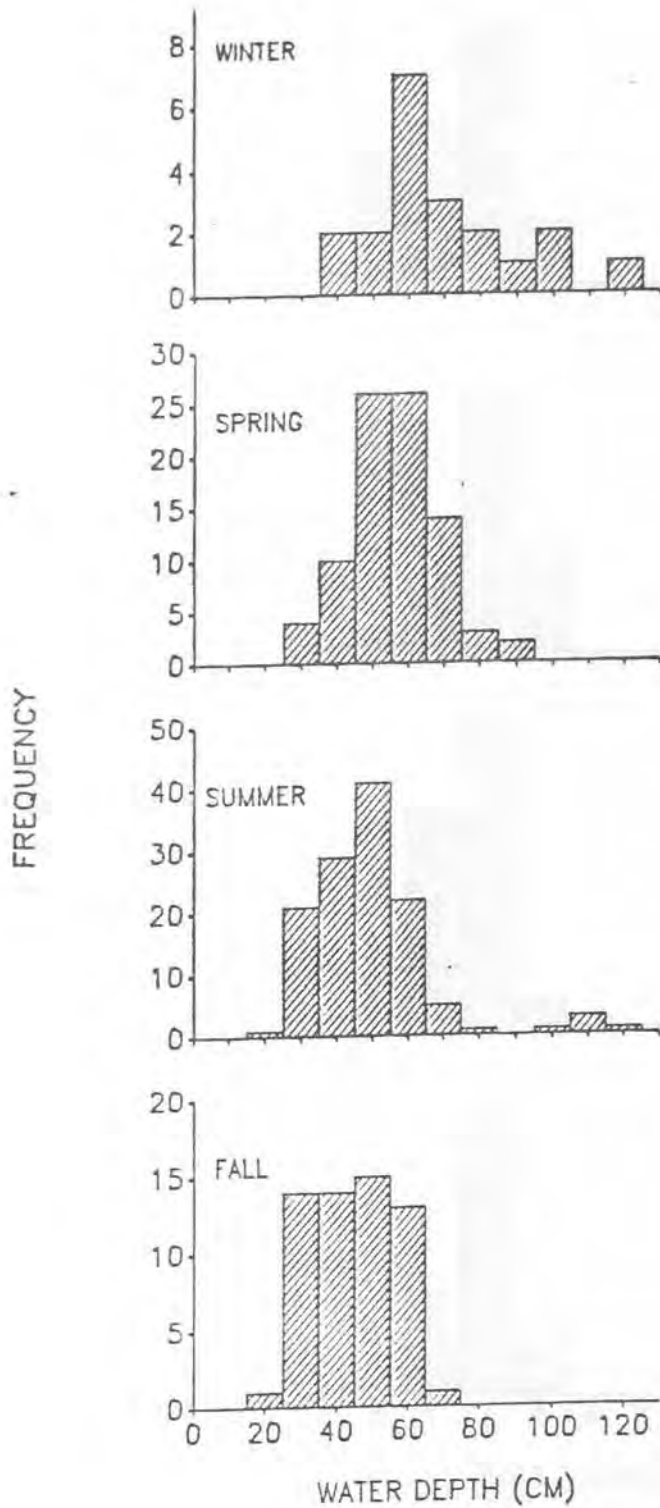


Figure 8. Seasonal frequency distributions of water depth at leopard darter capture locations in Glover River 1986-1988.

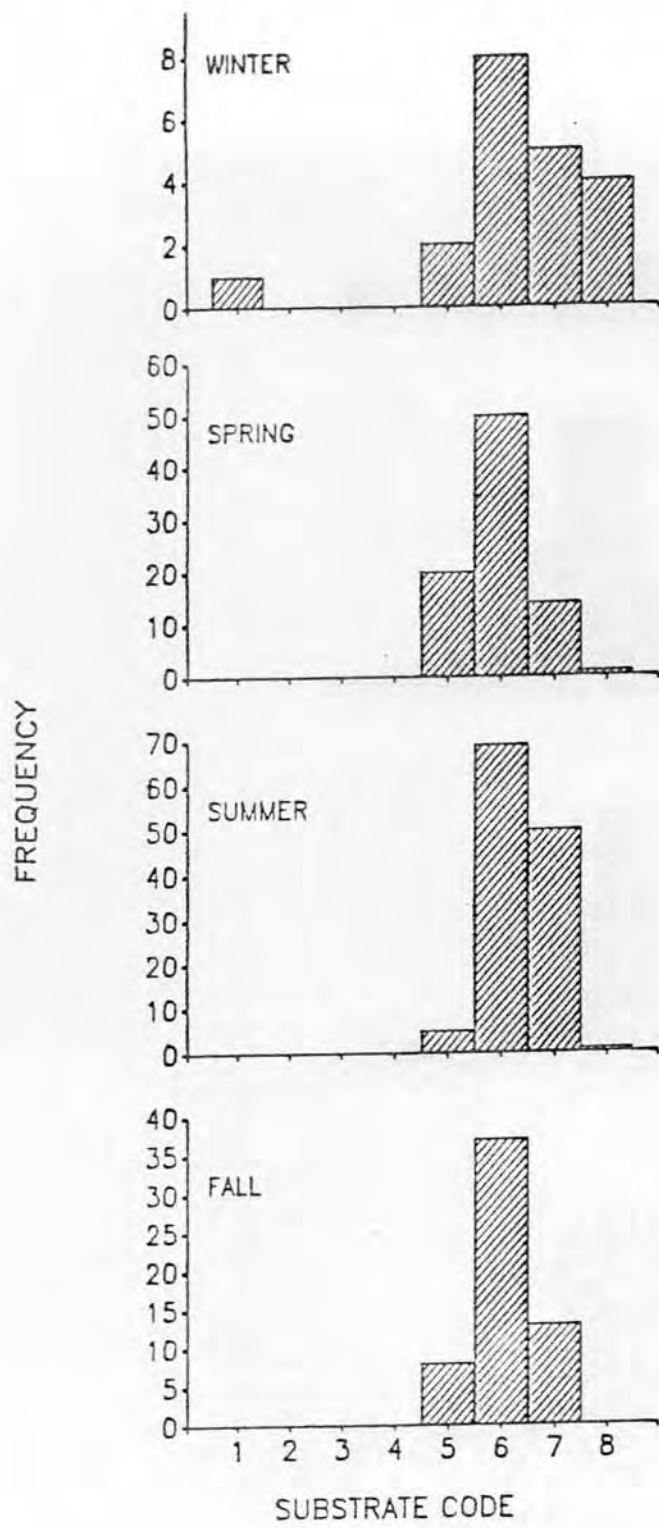


Figure 9. Seasonal frequency distributions of substrate type at leopard darter capture locations in Glover River 1986-1988.

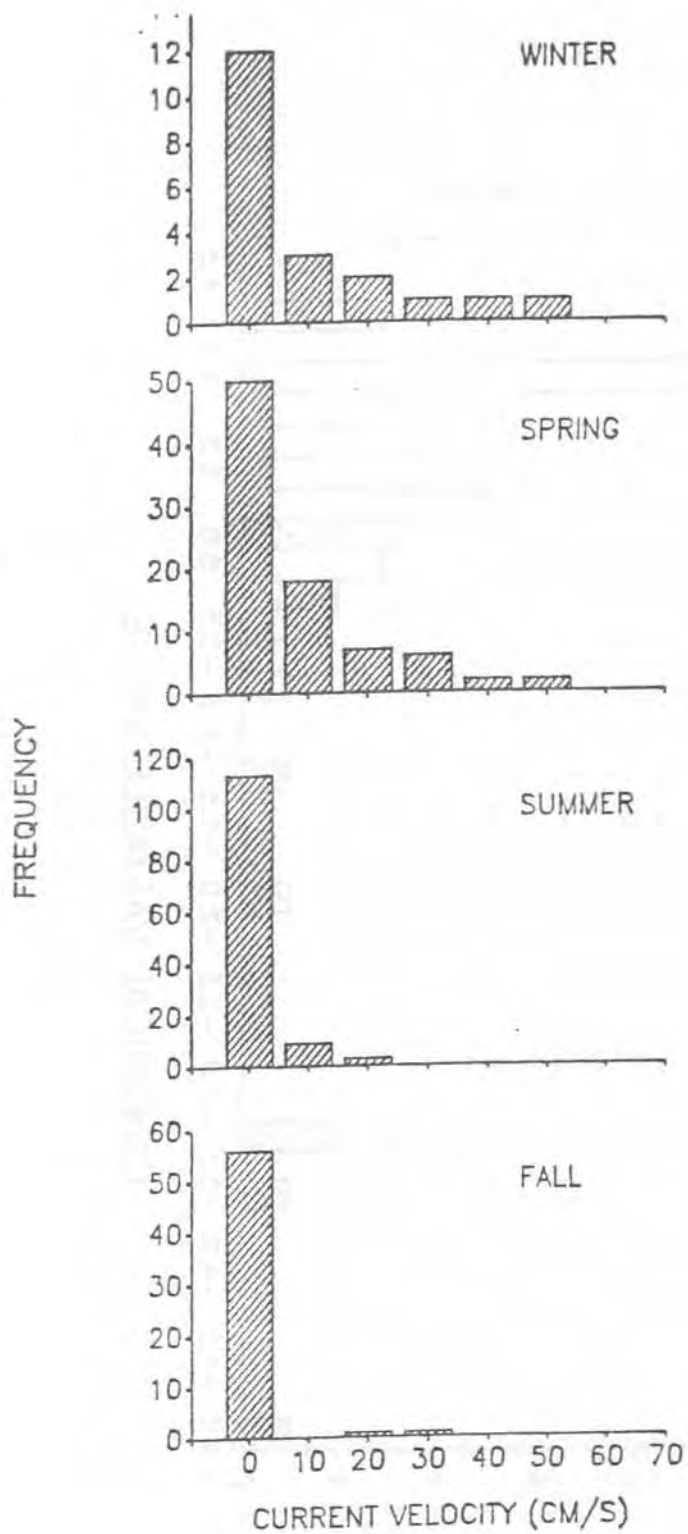


Figure 10. Seasonal frequency distributions of current velocity at leopard darter capture locations in Glover River 1986-1988.

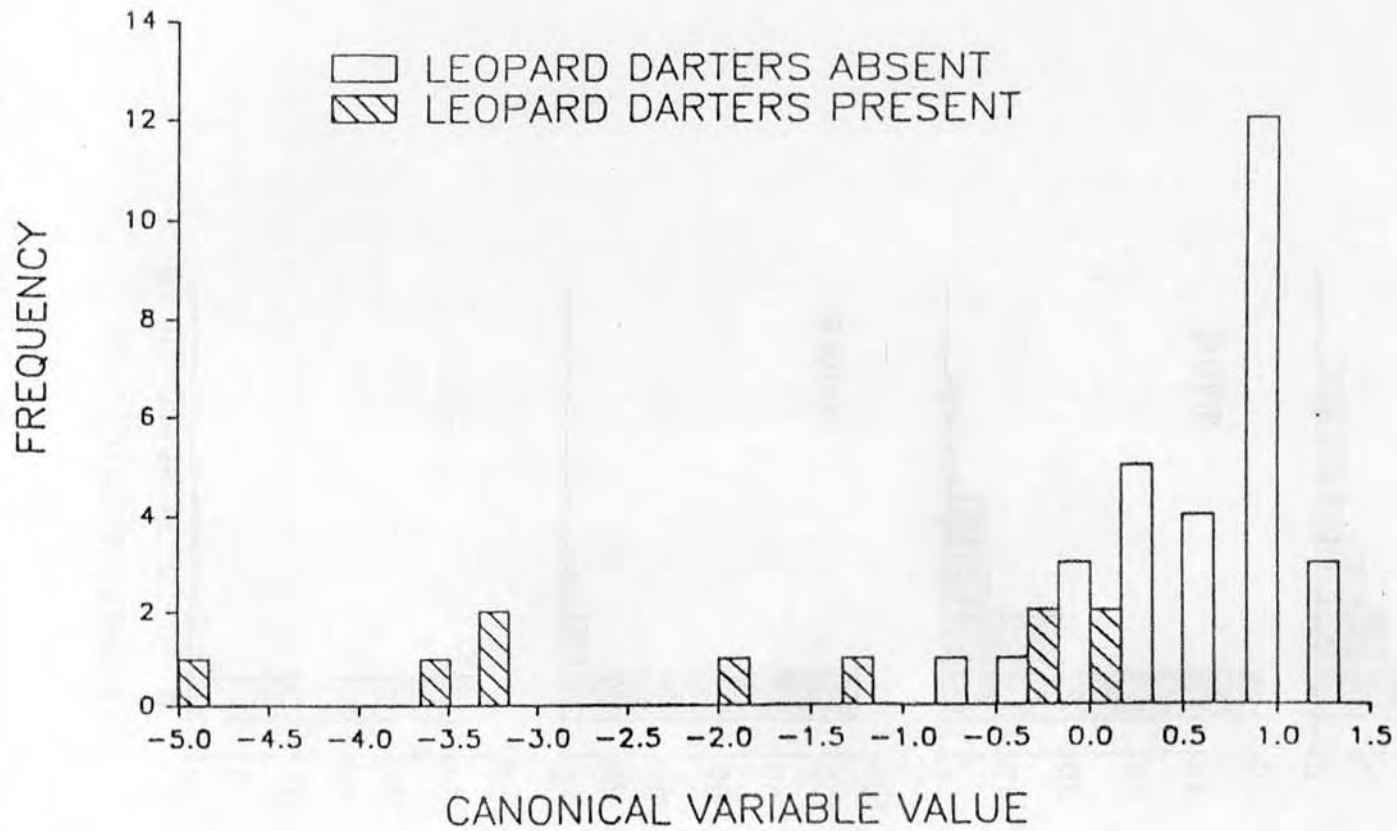


Figure 11. Frequency distribution of canonical variable values from discriminant function analysis of 40 sites in the Little River drainage.

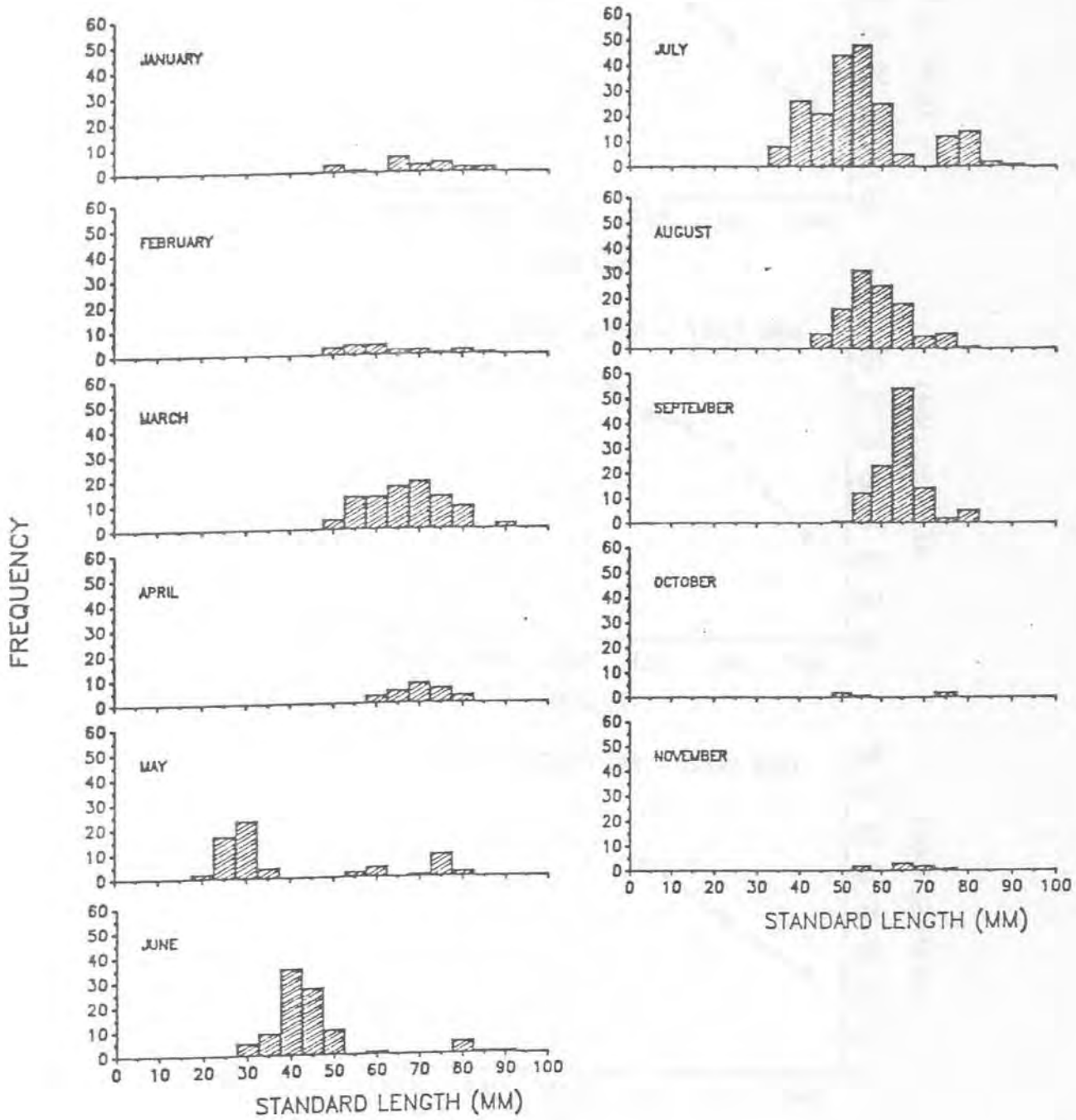


Figure 12. Monthly length-frequency distributions for leopard darters captured in Glover River 1936-1988.

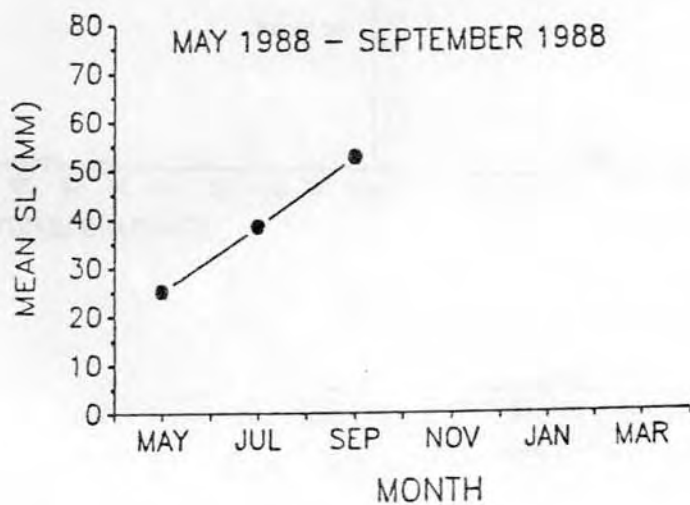
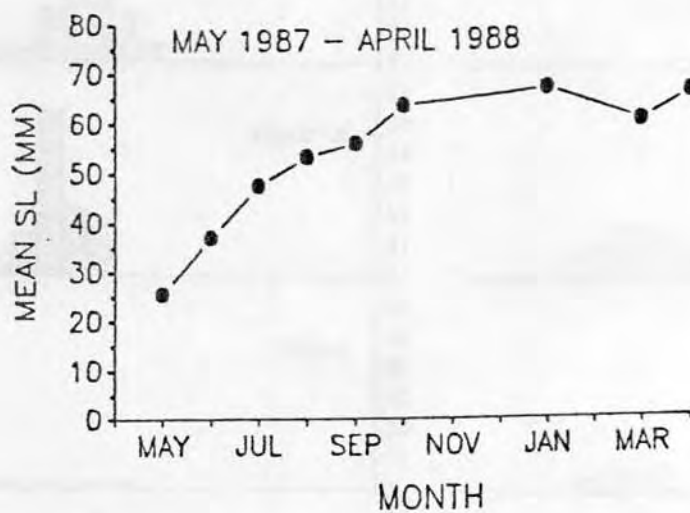
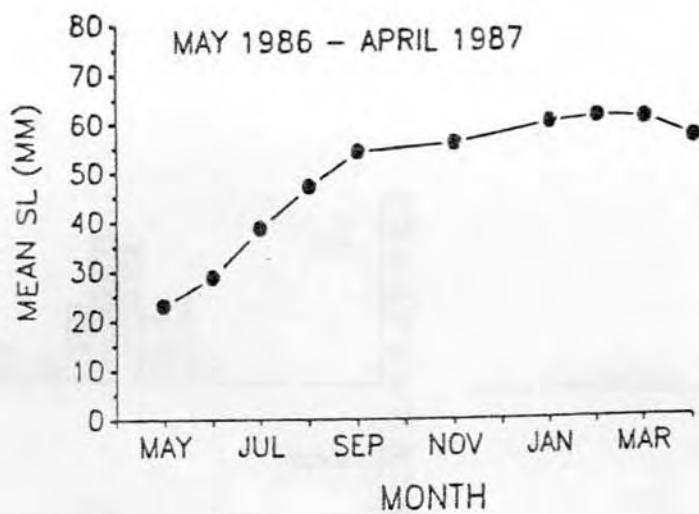


Figure 13. Growth curves of leopard darters captured in Glover River 1986-1988.

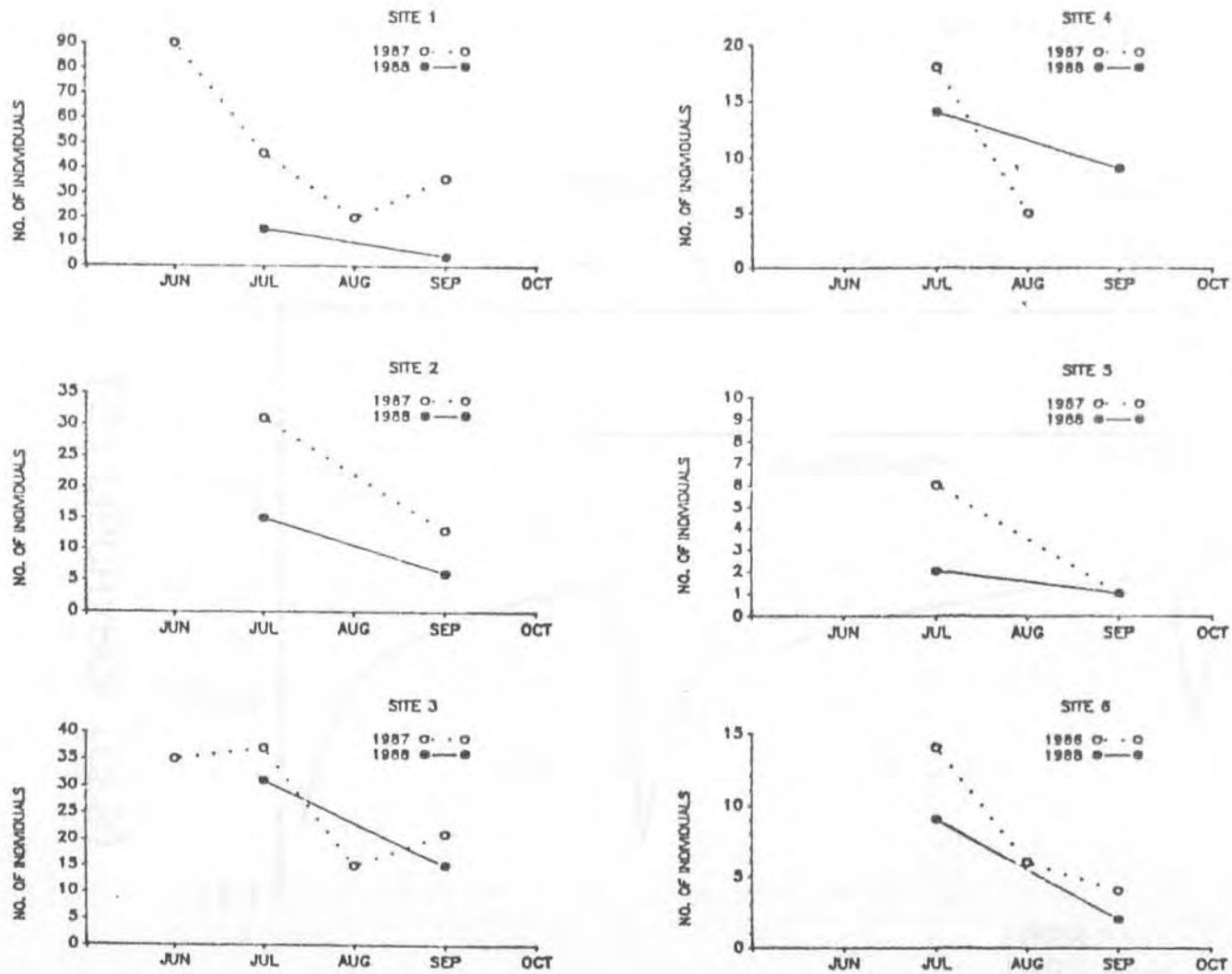


Figure 14. Leopard darter population abundances at six sites in Glover River.

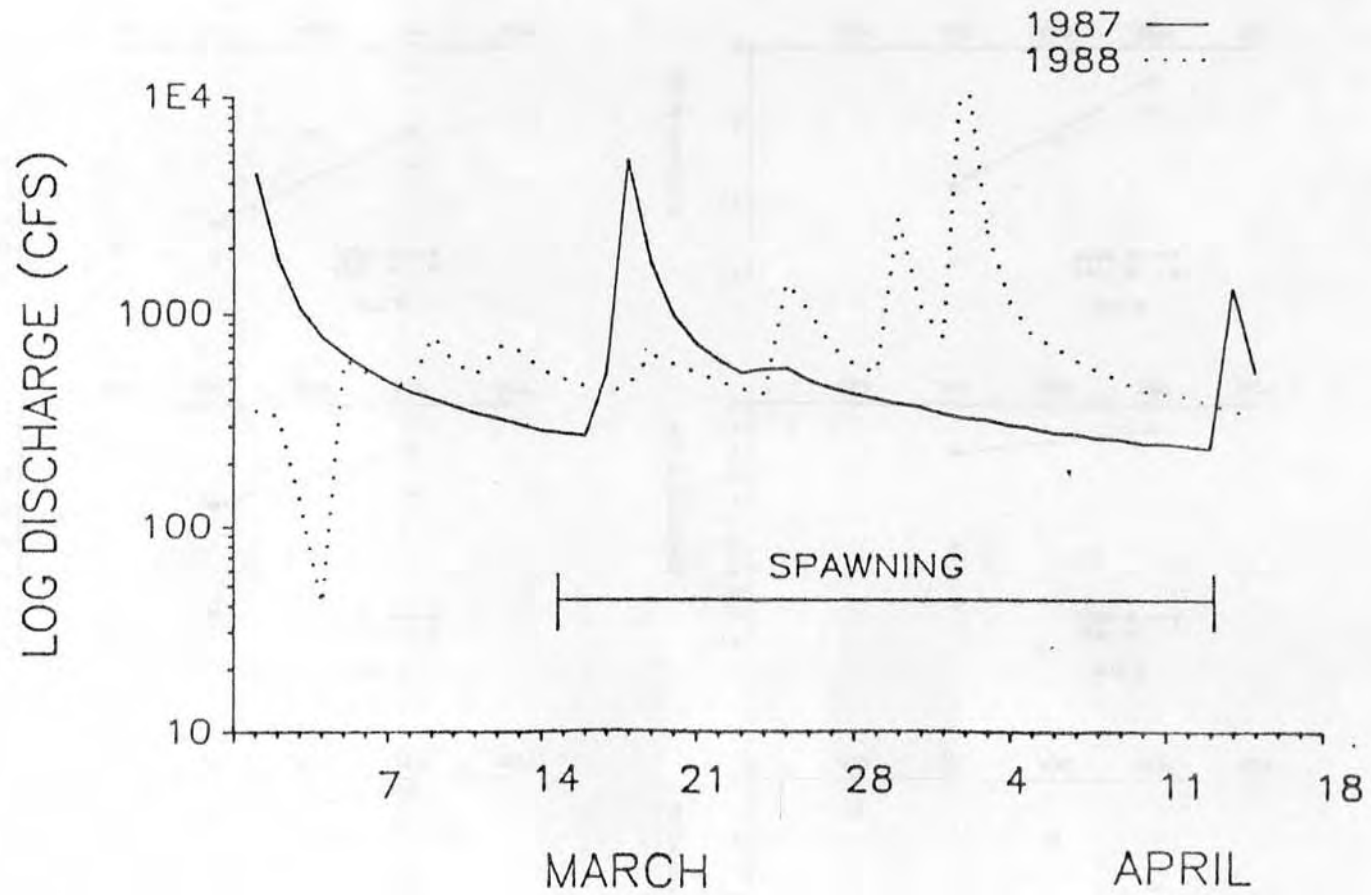


Figure 15. Mean daily discharges from Glover River during March and April 1987-1988.

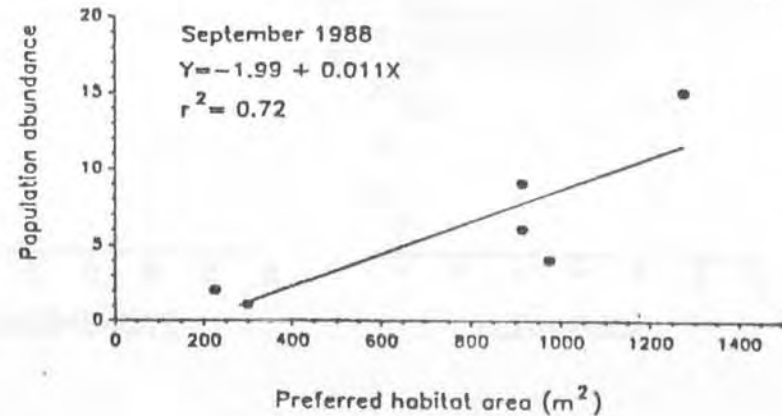
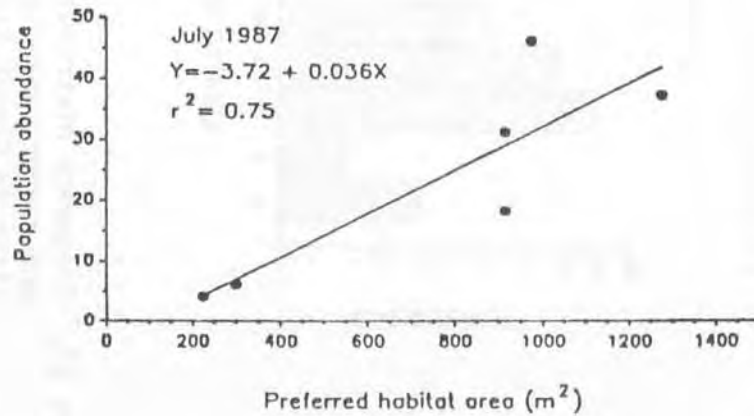
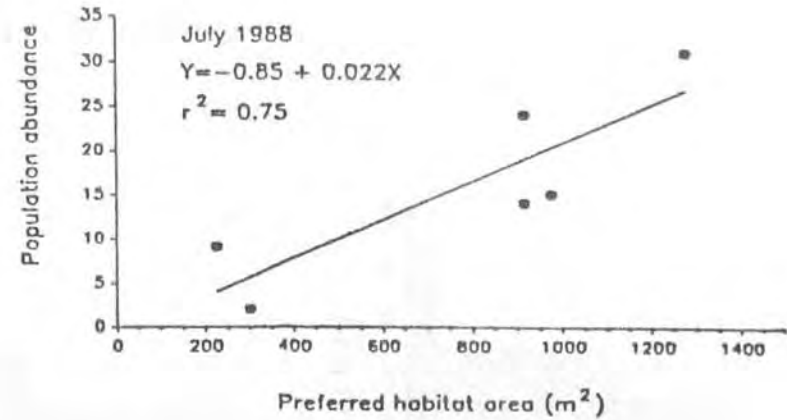
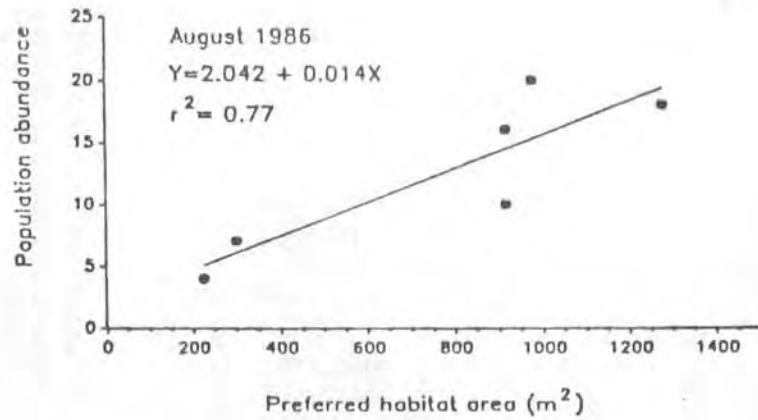


Figure 16. Relationships between leopard darter population abundances and preferred habitat area at six study sites in Glover River 1986-1988.

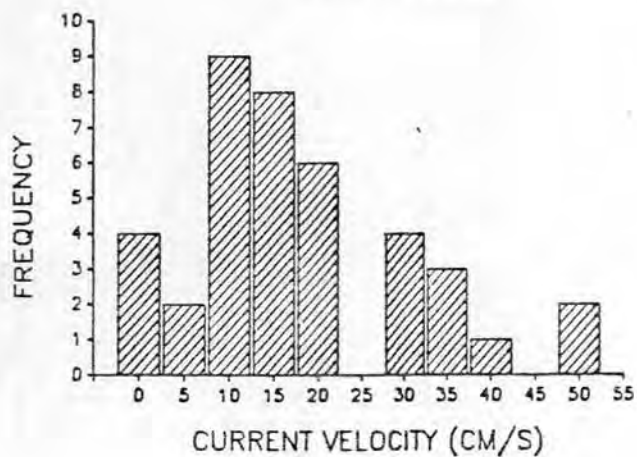
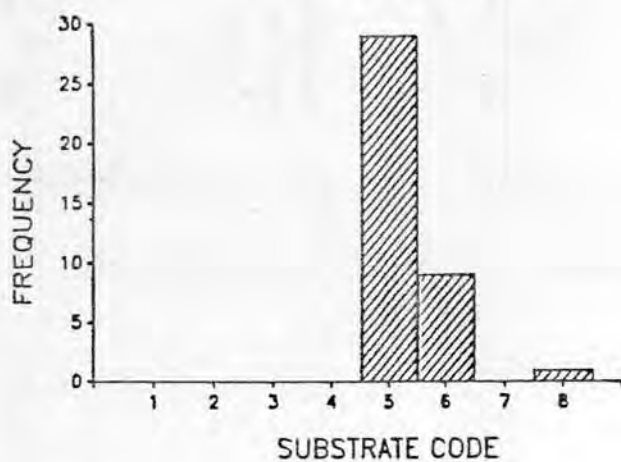
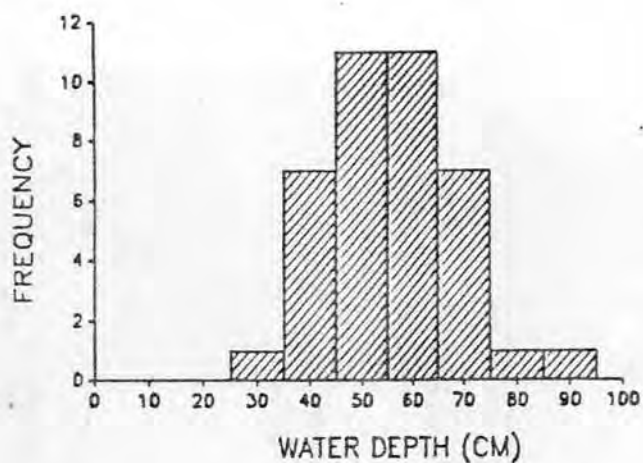


Figure 17. Frequency distributions of water depth, substrate type, and current velocity at leopard darter egg deposition sites in Glover River 1986-1988.

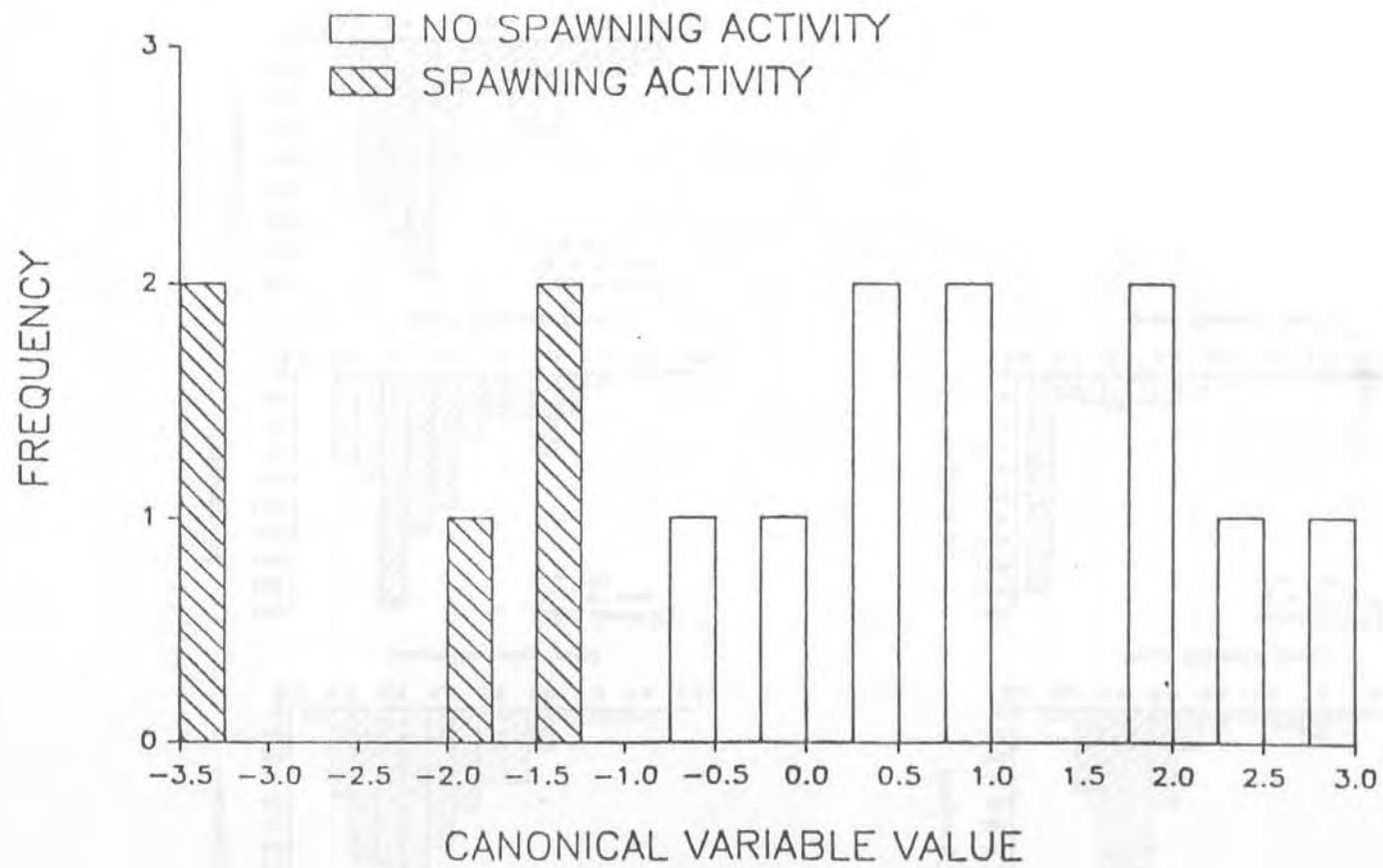


Figure 18. Frequency distribution of canonical variable values from discriminant function analysis of 15 riffles in the Little River drainage.

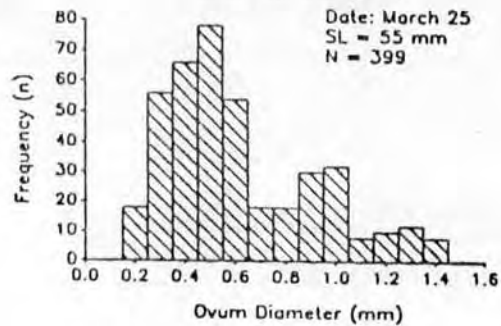
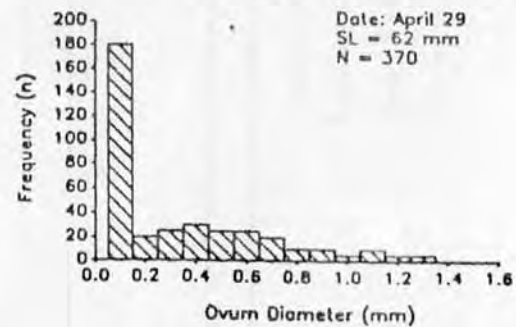
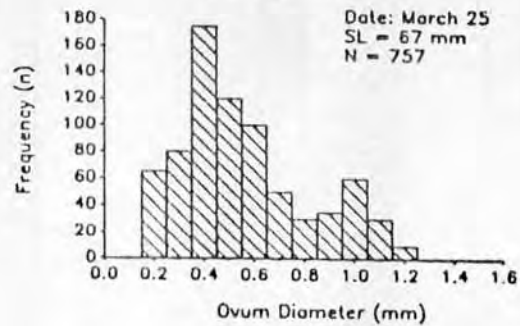
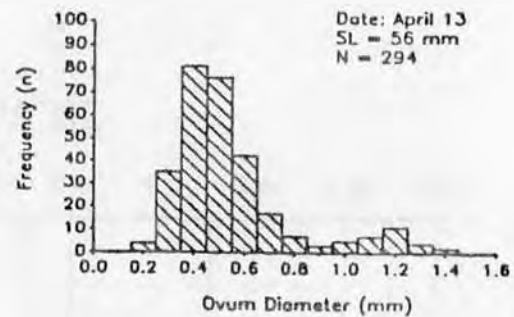
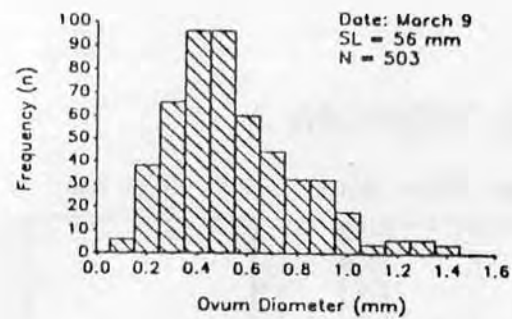


Figure 19. Frequency distributions of ovum diameter in five leopard darter museum specimens.

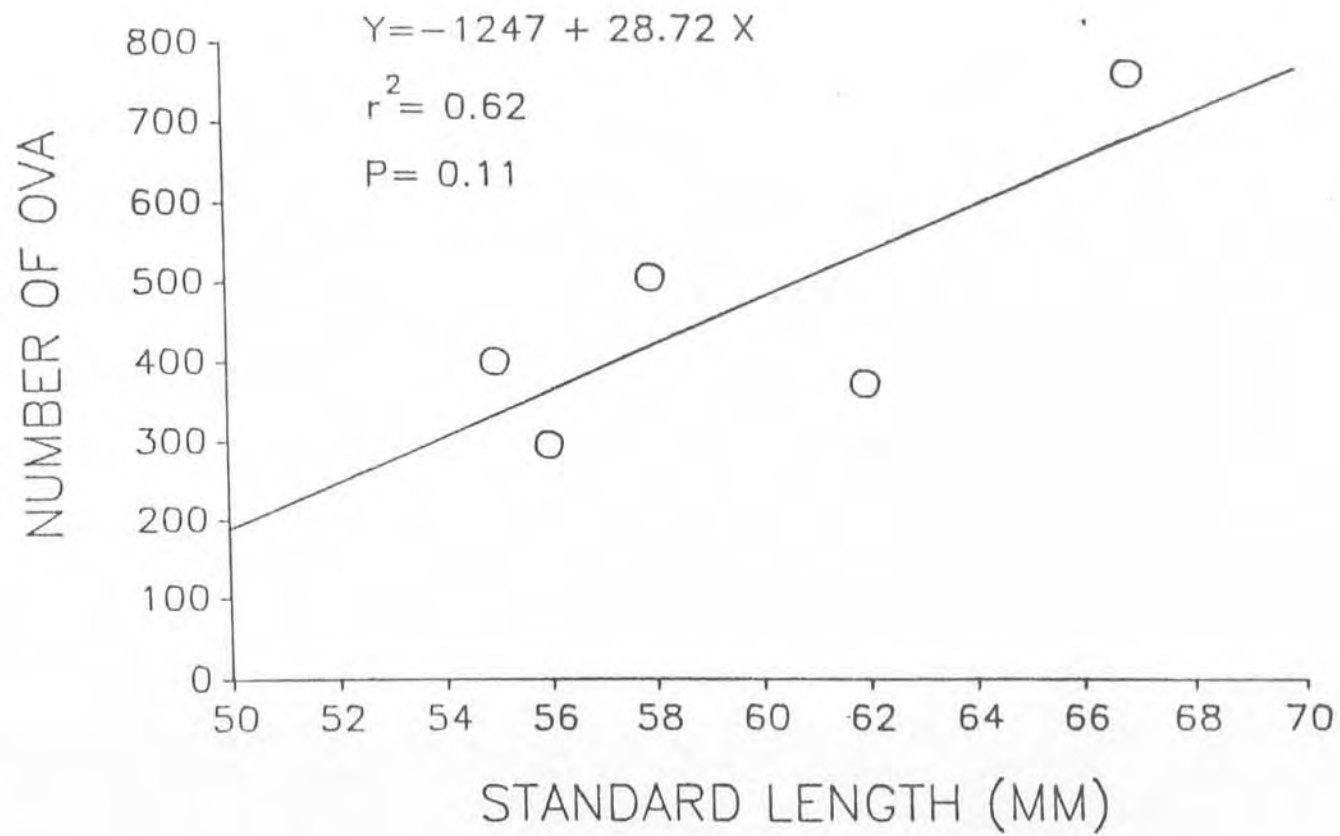


Figure 20. Relationship between fish length and number of ova in five leopard darter museum specimens.

