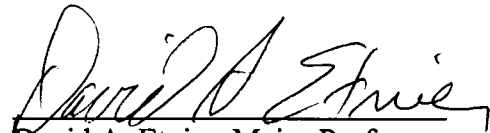


To the Graduate Council:

I am submitting herewith a thesis written by Christopher James Paxton entitled "The Life History and Ecology of the Redband Darter, *Etheostoma (Oligocephalus) luteovinctum* Gilbert and Swain (Osteichthyes: Percidae)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.


David A. Etnier, Major Professor

We have read this thesis
and recommend its acceptance:




Accepted for the Council:


Associate Vice Chancellor
and Dean of the Graduate School

THE LIFE HISTORY AND ECOLOGY OF THE REDBAND DARTER,

Etheostoma (Oligocephalus) luteovinctum GILBERT AND SWAIN

(OSTEICHTHYES: PERCIDAE)

A Thesis

Presented for

The Master of Science Degree

The University of Tennessee, Knoxville

by

Christopher James Paxton

December 1998

DEDICATION

For my wife Margaret Angela

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ABSTRACT

An investigation of the ecology and life history of the redband darter, *Etheostoma luteovinctum*, was conducted in Titan Creek of the Duck River system in Maury Co., Tennessee, from March 1996 through June 1997. In Titan Creek, a second order spring influenced tributary, adult and juvenile *E. luteovinctum* commonly inhabited pools and runs of silt, fine gravel, and sand. At one year of age, both sexes reached maturity and spawned. Sexual dimorphism in coloration persists after one year of age and intensified in nuptial males. Spawning occurred from February to May at water temperatures of 15 to 18° C. Spawning *E. luteovinctum* observed in the wild and held in aquaria buried eggs in sand or fine gravel. Female *E. luteovinctum* produced group-synchronous ova with a mean clutch size for mature and maturing ova of 85. Mean diameter for ripe ova was 1.27 mm. Aquarium held fertilized eggs hatched in six to nine days at a water temperature of 21° C. Larvae had concentrations of round melanophores on top of the head and along the dorsum. Modal myomere counts were 15 preanal, 19 postanal, and 34 total. Early growth was rapid, with males growing faster than females, and both sexes attained a maximum age of 2+ years. A three pass population estimate with 95 percent confidence intervals indicated that redband darters were the fourth most abundant species at the study site; the observed sex ratio was 1:1. Redband darters were found to be opportunistic invertivores with dipteran larvae of the family Chironomidae dominating the diet numerically, seasonally, and by volume for all size classes of redband darters in Titan Creek.

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CHAPTER I

INTRODUCTION

The redband darter, *Etheostoma luteovinctum* Gilbert and Swain, was described from five specimens collected in the Stones River (Gilbert, 1887). *Etheostoma luteovinctum* (Figure 1) is restricted to the Tennessee and Cumberland River drainages (Page, 1983) in the Central or Nashville Basin portion of Tennessee and the adjacent Barrens Plateau of the Highland Rim (Figure 2). It is widespread in the Duck River system of the Tennessee River drainage. In the Cumberland River drainage it is a common species in the headwaters of the Caney Fork system and is localized in the Stones River system (Etnier and Starnes, 1993). Due to its restricted range and recent habitat alteration, the Tennessee Wildlife Resource Agency (TWRA) declared the redband darter as Wildlife in Need of Management (TWRA Proclamation 94-16, 1994), although it appears to be under no immediate threat (Etnier and Starnes, 1993) and continues to be a common species in many areas within its range.

Systematics

Etheostoma luteovinctum has been placed in the subgenus *Oligocephalus* (Percidae, tribe Etheostomatini) of which eight species, *E. asprigene*, *E. bison*, *E. caeruleum*, *E. ditrema*, *E. kantuckeense*, *E. luteovinctum*, *E. spectabile*, and *E. swaini*, are found in Tennessee (Page, 1981; Ceas and Page, 1997). Species of this subgenus are small, laterally compressed fishes with incomplete lateral lines, narrowly to moderately



Figure 1. *Etheostoma luteovinctum*, female (left) and male (right).



Figure 2. Tennessee range map for *Etheostoma luteovinctum*. Closed circles represent known localities for *E. luteovinctum*. Adapted from Etnier and Starnes, 1993.

joined branchiostegal rays, moderate scale counts, and dorsal saddles that can be inconspicuous or number eight or more (Etnier and Starnes, 1993). Females of this subgenus are known to bury their eggs in the substrate or attach them to plants (Page, 1981), and males have brilliant colors on their bodies and dorsal fins in reds, blues, and greens (Etnier and Starnes, 1993).

The redband darter was redescribed from the examination of 505 museum specimens to determine the extent of morphological variation in populations (Rogner, 1981). Rogner's study suggested that *E. luteovinctum* is most closely related to the orangethroat darter, *E. spectabile*, based on electrophoretic evidence, and that *E. luteovinctum* likely originated through differentiation from a primitive *E. spectabile* ancestor. A combination of two characters, infraorbital canal interrupted and supratemporal canal usually interrupted, is shared in *Oligocephalus* only by *E. spectabile* (in the broad sense) and *E. luteovinctum* (Page, 1981). Both species occur in the upper Caney Fork and upper Stones River systems of the Cumberland river drainage, where they are potentially sympatric but have yet to be collected together (Etnier and Starnes, 1993). Subsequently, *E. spectabile* was considered a complex of similar species, with two new species, *E. bison* and *E. kantuckeense*, described from the Tennessee river drainage (Ceas and Page, 1997). Three yet to be described species in the complex occur in the Cumberland river drainage (pers. com., Dr. Pat Ceas. Eastern Kentucky University, Richmond Kentucky).

Biology

Only limited biological and life history information on the redband darter is available. It is known that they inhabit pools and runs of small streams and springs with a moderate gradient. The substrate of these streams may consist of limestone bedrock, rubble, gravel, or silt (Etnier and Starnes, 1993). Breeding males of *E. luteovinctum* show distinct sexual dimorphism in color and are tuberculate from at least March 26 until April 28 in the Duck River, Tennessee, based on nuptial specimens with tubercles developing first and remaining longest on the anal fin (Collette, 1965). Size distribution has been obtained from several large collections of redband darters. *Etheostoma luteovinctum* reaches sexual maturity at one year of age and a total length (TL) of 50-55 mm (Etnier and Starnes, 1993). Adults attain a maximum size ranging from 60 to 70 mm TL by the end of their normal life span, believed to be two years of age (Etnier and Starnes, 1993). The digestive tracts of previously collected redband darters contained midge larvae, but other taxa associated with spring influenced habitats are possible (Etnier and Starnes, 1993). Additional morphological and meristic descriptions are provided in Page, 1983; Kuehne and Barbour, 1983; and Etnier and Starnes, 1993.

Locality and Objectives of Study

The objective of this study was to collect life history information on the redband darter, a species of native fish that is deemed "wildlife in need of management" by the State of Tennessee. It was also the objective of this study to determine if a habitat

specific species such as the redband darter can maintain a stable population with continued recruitment in proximity to a large industrial facility.

The primary study site for this project is in Maury County, Tennessee (Figure 3) on property owned by an automobile manufacturer, the SATURN Corporation. Redband darters were collected at the SATURN site in 1985, 1986, 1987, and 1993 by biologists from Roy F. Weston, Inc. (WESTON) as part of pre-, mid-, and post-construction biological and water quality surveys (WESTON, 1994). Of particular interest to WESTON were streams of the Duck River system that bordered or were in proximity to the SATURN site, including Carters Creek, McCutcheon Creek, West Branch McCutcheon Creek, and an unnamed tributary to Carters Creek. The unnamed tributary was later named Titan Creek by SATURN and WESTON biologists and hereafter will be referred to as Titan Creek.

Apparent populations of redband darters on their property led SATURN to fund research on the life history of this species in March of 1996. Due to its accessibility from SATURN property and workable size by one person, Titan Creek was selected as the primary study site.

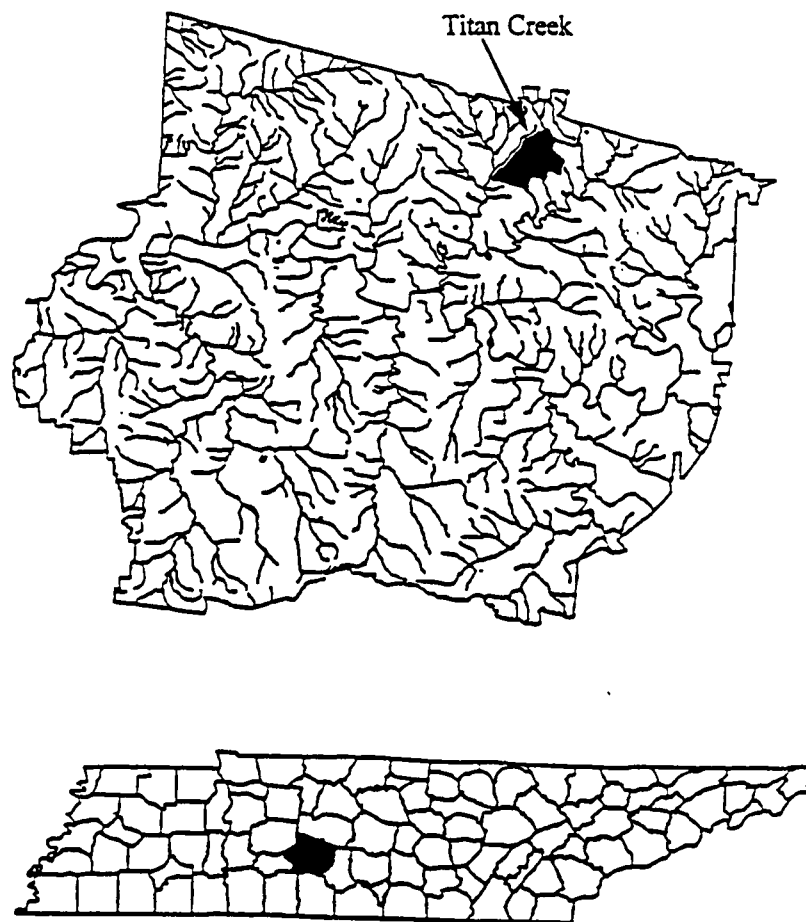


Figure 3. Titan Creek study site, Maury Co., Tennessee. SATURN Corp. property boundary indicated by shaded area.

CHAPTER II

STUDY AREA

Boundaries of Study Area

Titan Creek (Figure 4) is a spring influenced second order tributary to Carters Creek originating approximately 2.1 km north of the SATURN site, flowing southwest for 4.9 km, and forming the site's western border (WESTON, 1994). The drainage area of Titan Creek covered approximately 4.2 square km, which included all developed and undeveloped areas on the SATURN site (WESTON, 1994). Of this, approximately 3.2 square km of developed land drains to Titan Creek before its confluence with Carters Creek at creek km 7.4 (WESTON, 1994).

Description of Study Site

The study area on Titan Creek began on SATURN property at Little Brown Jug Trail overpass and continued upstream approximately 500 m. All collections, observations, and population estimates being used for the study were conducted within this reach. A lower reach of 80 m, a middle and upper reach of approximately 100 m, all containing representative habitat types found in Titan Creek, were selected within the 500 m reach for population estimates. The mean widths of these three reaches were 3.66, 1.98, and 3.66 m respectively.



Figure 4. Titan Creek study site, lower reach pool, Maury Co., TN.

Habitat and Parameters

The habitat types found in Titan Creek consist of pools, sluggish runs, and small riffles. Pools ranged in depth from 0.2 to 1.4 m and made up the widest expanse of the creek at a maximum of 5.5 m. Runs ranged from 10 to 30 mm in depth and were 0.5 m maximum width. The predominant substrates in Titan Creek were sand, fine to coarse gravel, and silt. Siltation in Titan Creek is due to an increased sediment load from the influence of livestock farming upstream from SATURN property. An increase in siltation can cause the spawning habitat to become degraded, increase egg mortality, and decrease larval growth and development (Kohler and Hubert, 1993). Minimal current flows over gravel areas found at the end of runs, in small riffles, and shoal areas. A dense canopy and riparian vegetation provide some pockets of fallen leaf litter, logs, and sticks. These areas of habitat were associated with undercut banks and pool areas. Aquatic vegetation was predominantly green algae due to the sediment load and influence of cow manure present in Titan Creek. Occasional patches of water cress, *Nasturtium*, were found along small sand bar areas. Water temperature and pH were recorded monthly from March 1996 through March 1997 for Titan Creek. The pH ranged from 7.0 to 7.5 throughout the year, while temperature ranged from 16 to 23°C. These field parameters did not differ from those recorded by WESTON biologists during spring and fall surveys of Titan Creek (WESTON, 1994).

The lower 1,490 meters of Titan Creek's banks and bed were lined with rip-rap during the construction of the SATURN facility. Although collections of fishes were taken from this reach during the initial investigation of the study site and periodically

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throughout the study, data collected here were not included in the final analysis. It is of interest that redband darters were collected in this area during the winter months but not found there during the summer. It was speculated by biologists from WESTON that redband darters moved downstream to deeper waters in Carters Creek during the summer months, although sufficient evidence to support this hypothesis was not found during this study. It is possible that during summer months the rip-rapped portion of Titan Creek becomes uninhabitable. Elevated water temperatures caused by a lack of riparian vegetation and tree canopy may create a thermal barrier hindering the migration of redband darters.

CHAPTER III

MATERIALS AND METHODS

Introduction

The life history of the redband darter described herein required visual monitoring of general and spawning behaviors of this species in the stream both with the naked eye and with binoculars. Photographs were taken on site to document various behaviors as well as habitat, spawning areas, and intensity of color in breeding males. Further observations of reproductive behavior and spawning between aquarium held breeding pairs have been video taped and photographed in the laboratory. Preserved specimens were examined in the laboratory to determine age and growth, diet, and fecundity.

Field Methods

Collections and observations were made in Titan Creek at monthly intervals beginning 3 March 1996 through 27 June 1997. These intervals were interrupted only by high water due to excessive rain, or snow during winter months. Observations and collections were made more frequently once the spawning season began.

Collection and Preserved Specimens

Field collections were made with a 1.8 m by 1.2 m seine of 4.8 mm mesh, by dipnets, or by electrofishing with a backpack shocker. A total of 369 adult and juvenile redband darters was collected during the study. Of these collections, 156 specimens were

retained and preserved in a 10% formalin solution in the field and later transferred to 50% isopropanol for laboratory examination and permanent storage at the University of Tennessee, Knoxville. All other redband darters were measured to the nearest mm standard length (SL), sexed if possible, and released. A maximum of 10 to 20 redband darters was collected per sampling interval. This was considered necessary to avoid depletion at the study site while obtaining an adequate number of specimens for study. Monthly collections were made during daylight hours only so that observations of darter behavior and collected sympatric species could be noted throughout the study. Breeding pairs of redband darters collected at the field site for reproductive observation in aquaria were placed into aerated foam coolers for transportation back to Knoxville.

Population Estimation

Using a removal-depletion method (Van Deventer and Platts, 1983), fishes were collected from two of the three site reaches on 26 and 27 June 1997 by electrofishing. A block net was placed at the upstream and downstream boundary of the reach being sampled before the electrofishing began. This was to insure that no fish entered or left the study site during sampling. This criterion, as well as keeping current and voltage settings constant and maintaining a constant sampling effort, met all assumptions associated with this sampling technique (Armour et al., 1983; Van Deventer and Platts, 1983). Three removal passes in an upstream direction were then performed to deplete all fish populations. A maximum-likelihood population estimate was calculated from this data using the statistical software MicroFish 3.0 (Van Deventer and Platts 1989). The lower reach of the study area was not included in the maximum-likelihood population

estimate. Due to a lack of flow in this reach and extreme turbidity caused by collection, depletion by electrofishing was found to be impossible. Instead, three passes were made upstream that consisted of ten seine hauls per pass. It was not possible to combine the data from all three reaches due to differences in sampling techniques. However, total numbers of redband darters collected in the lower reach are given. Juvenile or young of the year (YOY) redband darters were not included in the population estimate due to the lowered catchability associated with YOY fishes (Armour et al., 1983).

Laboratory Methods

One hundred fifty-six redband darters from Titan Creek were collected and preserved for laboratory examination during this study. Individual specimens were measured in mm SL and TL, sexed, and aged. Sex was determined by examination of the gonads when not apparent by breeding color. Age was determined to the nearest month by examining the length-frequency distribution of 202 redband darters (156 preserved specimens and 46 released specimens). Age was also confirmed by counting annuli on scales (Page, 1974; Shute et al., 1982) removed from the dorsum near the junction of the first and second dorsal fins (Carney and Burr, 1989). March, the month assumed to be near the middle of the reproductive season based on gonadal-somatic index (GSI), was used as month zero. Growth was examined by studying the development of aquarium reared specimens as well as preserved YOY specimens.

Dietary Analysis

The stomachs of 156 redband darters were examined in order to identify food items in the diet and their relative importance. Identification and analysis of contents were restricted to the stomach because the digestion of food is more complete in the intestine (Layman, 1984). Stomachs were extracted from the body and dissected. The contents within were teased apart and examined under a binocular dissecting microscope. All food organisms were then sorted, counted, and identified to the lowest practicable taxonomic unit. The number of total food items found in the stomachs and the number of stomachs in which each food item occurred were recorded. A visual estimate was made and recorded to determine which taxon contributed the greatest volume to each stomach (Etnier, 1971). Stomach contents were also analyzed to determine seasonal and size specific variation found in the diet.

Gonadal Analysis and Reproductive Condition

Gross examinations of ovaries were performed to determine the reproductive condition of female redband darters. Ovaries were removed from the body and placed into a petri dish for examination under a dissecting microscope. Females were categorized into one of five stages based on their ovarian development: latent (LA), early maturing (EM), late maturing (LM), mature (MA), and ripe (RE) (Heins and Rabito, 1986). A mature-ripening (MR) category was added between the MA and RE stages to describe females with unovulated ripening oocytes (Parrish et al., 1991). Reproductive females were classified as MA, MR, or RE, and non-reproductive females were classified as LA, EM, and LM (Heins and Rabito, 1986).

A gonadal-somatic index (GSI) was calculated for 62 preserved females to determine when the redband darter spawned. A gonadal-somatic index is a commonly used method for determining the development and maturity of the gonads (Nielsen and Johnson, 1985). GSI was determined by expressing the mass of the ovary as a percentage of the adjusted body mass (Schenck and Whiteside, 1977; Lindquist et al., 1981; Layman, 1993). Ovaries and the eviscerated body (specimen minus the ovaries, liver, and gut) were blotted dry with tissue paper and weighed to the nearest 0.01 mg. Ova were excised from the ovary and sorted into different categories based on color, shape, and size. Clutch size was determined by counting the mature and ripening ova of 19 MA and MR females following criteria on the developmental stages of eggs (Heins and Baker, 1988) in group synchronous ovaries (Wallace and Selman, 1981). Due to the difficulty involved with determining if RE females had already spawned, clutch size was based on counting ova from MA and MR females (Heins and Baker, 1989; Parrish et al., 1991). Fecundity was expressed as the number of MA and MR ova counted in a sexually mature female.

The diameter of all ripe ova from RE females was measured to determine the size of ova. RE females were chosen because ripe ova had completed the yolk loading process of egg development and were assumed to be at a maximum size (Heins, et al., 1992). The diameters of all ripe ova were averaged to determine a mean size. Averaging the smallest and largest dimensions was necessary due to the irregular shape of preserved ova (Heins and Machado, 1993). Measurements of ova were made using an ocular micrometer (calibrated with a stage micrometer) in a dissecting microscope.

The reproductive condition of male redband darters was determined by examining the size and appearance of the testes, intensity of body coloration, and the presence or absence of breeding tubercles (Layman, 1993). Mature males (MA) had greatly enlarged, spongy, opaque white testes. They were also tuberculate on their anal fin, and showed extremely vivid coloration of the body that consisted of red vertical banding and blue-green branchiostegal rays, venter, and anal fin. Latent males (LA) had small, flattened, translucent yellow testes. There were no tubercles present on their anal fin, and they showed only faint red markings or banding on the body.

A GSI for 60 male redband darters was determined by expressing the width of the left testis as a proportion of the square-root of the standard length (Schenck and Whiteside, 1977; Layman, 1984; Lindquist et al., 1981). The testes were removed from the body and then separated. The greatest width of the left testis was measured to the nearest 0.01 mm using digital calipers

Selection of Breeding Pairs and Documentation of Reproduction

Breeding pairs of redband darters were collected on site for aquarium observations beginning 29 February and continuing through 20 March, 1997. A total of 14 redband darters was collected for these observations. Two breeding pairs were initially placed in the observation aquarium on 29 February 1997. On 20 March 1997, an additional four males and six females were added to the same aquarium. Male specimens were chosen based on their size and intensity of breeding colors. Individuals that appeared most vivid and were 45-50 mm (TL) were assumed to be sexually mature. Female specimens were selected based on the same criteria and visible signs of sexual

maturity. Females appearing to have the most distended abdomens were presumed to be gravid and sexually mature. Male and female specimens were placed in a foam cooler equipped with a battery powered aerator for transportation from Titan Creek back to Knoxville. Breeding pairs of redband darters were placed into a 113.5 l aquarium for reproductive observation in the laboratory. Specimens were transferred to plastic bags from the foam cooler and floated in the aquarium for 30 minutes while adding one cup of water from the aquarium to the bag every ten minutes for acclimation of temperature and adjustment of pH. There was no attempt to maintain or simulate water temperatures in the aquarium with those of Titan Creek. The aquarium was placed beside glass doors to maintain the seasonal photo-period during observations. All water and substrates used in the aquarium were transported from Titan Creek. Substrates consisted of fine and coarse gravel, sand, silt, and cobble stones associated with riffle areas believed to be the spawning habitat. The aquarium was equipped with an under-gravel filter and power-head pump to provide current in order to simulate spawning habitat. A video camera equipped with a macro lens was mounted on a tripod and connected to a VCR and television. The television was used as a monitor that enabled behaviors to be observed away from the aquarium and on a larger than life scale. Breeding behaviors such as courtship, aggression, and spawning were video taped in order to allow analysis of behaviors at slower speeds. Still photographs were also taken of the breeding pairs during spawning. Aquarium held redband darters were fed a diet of frozen brine shrimp, live California blackworms, and live isopods and amphipods collected from nearby springs.

Collection, Rearing, and Hatching of Eggs

Once spawning was observed, the location was marked in the aquarium. Eggs buried in the substrate could then be removed from this location for rearing and further study. An attempt was made to siphon the eggs from the substrate as a method of collection. This method was abandoned due to the adhesive nature of the eggs and the damage caused to them when separated from the substrate. The most successful method for retrieving eggs was to bury a plastic container into the substrate and fill it with sand, which appeared to be the preferred spawning substrate. Heavier stones were placed around the container, making only the area within the container available for spawning. Once spawning activity ceased, the container was removed from the aquarium, fitted with its lid, and sealed. This enabled entire clutches of eggs to be removed from the aquarium for rearing without damage.

All eggs collected were transported to Conservation Fisheries Inc. (CFI) for rearing. The plastic container was placed into a slow drip flow through tank that allowed continuous circulation of water over the eggs. Eggs and larval redband darters were preserved in a five percent buffered formalin solution at different days of development. This was necessary to attain and document a series of larval stages for growth and developmental studies. Larvae were illustrated using a camera lucida and binocular dissecting microscope. Terminology used to describe larval stages of development follows Snyder et al., (1977) and Snyder (1983).

CHAPTER IV

RESULTS AND DISCUSSION

Food Habits

Food Items Consumed Overall

A total of 14 taxa was identified from the stomachs of 156 male and female redband darters of all size classes examined from Titan Creek (Table 1). Of the 156 stomachs examined, 148 contained the remains of various food items, and eight were empty. Redband darters were found to be opportunistic feeders that consumed various aquatic invertebrates. The only restricting factor influencing the choice of food item was apparently gape size of the individual. For all size classes of redband darters, the predominant food item, both by volume and by consumed numbers, was aquatic dipteran larvae of the family Chironomidae (Figure 5). The aquatic dipteran families Ceratopogonidae, Psychodidae, and Tipulidae were also present in the diet. Other aquatic insects identified were mayfly larvae of the family Heptageniidae and alderfly larvae of the family Sialidae. All aquatic insect families other than Chironomidae were found to be negligible in occurrence and volume in the diet.

Crustaceans consisting of amphipods, cladocera, copepods, isopods, and ostracods were second in occurrence as a food item collectively (Table 1). Isopods were found to be the most heavily preyed upon crustacean and were the second most dominant taxon by

Table 1. Dietary analysis of all *Etheostoma luteovinctum* stomachs examined.

Taxon	%PO	%PFO	%DV
Annelida			
Piscicolidae	0.11	2.03	
Crustacea			
Amphipoda	0.86	10.14	1.35
Cladocera	2.66	2.7	
Copepoda	2.06	4.05	
Isopoda	7.84	47.3	33.11
Ostracoda	0.64	7.43	
Ephemeroptera			
Heptageniidae	0.11	2.03	
Megaloptera			
Sialidae	0.34	5.41	3.38
Diptera			
Ceratopogonidae	1.16	14.86	
Chironomidae	82.23	95.95	47.97
Psychodidae	0.45	4.05	
Tipulidae	0.19	2.7	
Gastropoda			
Pleuroceridae	0.56	8.11	6.76
Pelecypoda			
Sphaeriidae	0.79	10.81	7.43
Number of fish examined			156
Number of empty stomachs			8

%PO=Percent composition of the total number of food items found in all stomachs

%PFO=Percent frequency of occurrence of food item found in all food containing stomachs

%DV=Percent of food containing stomachs in which food category was dominant by volume

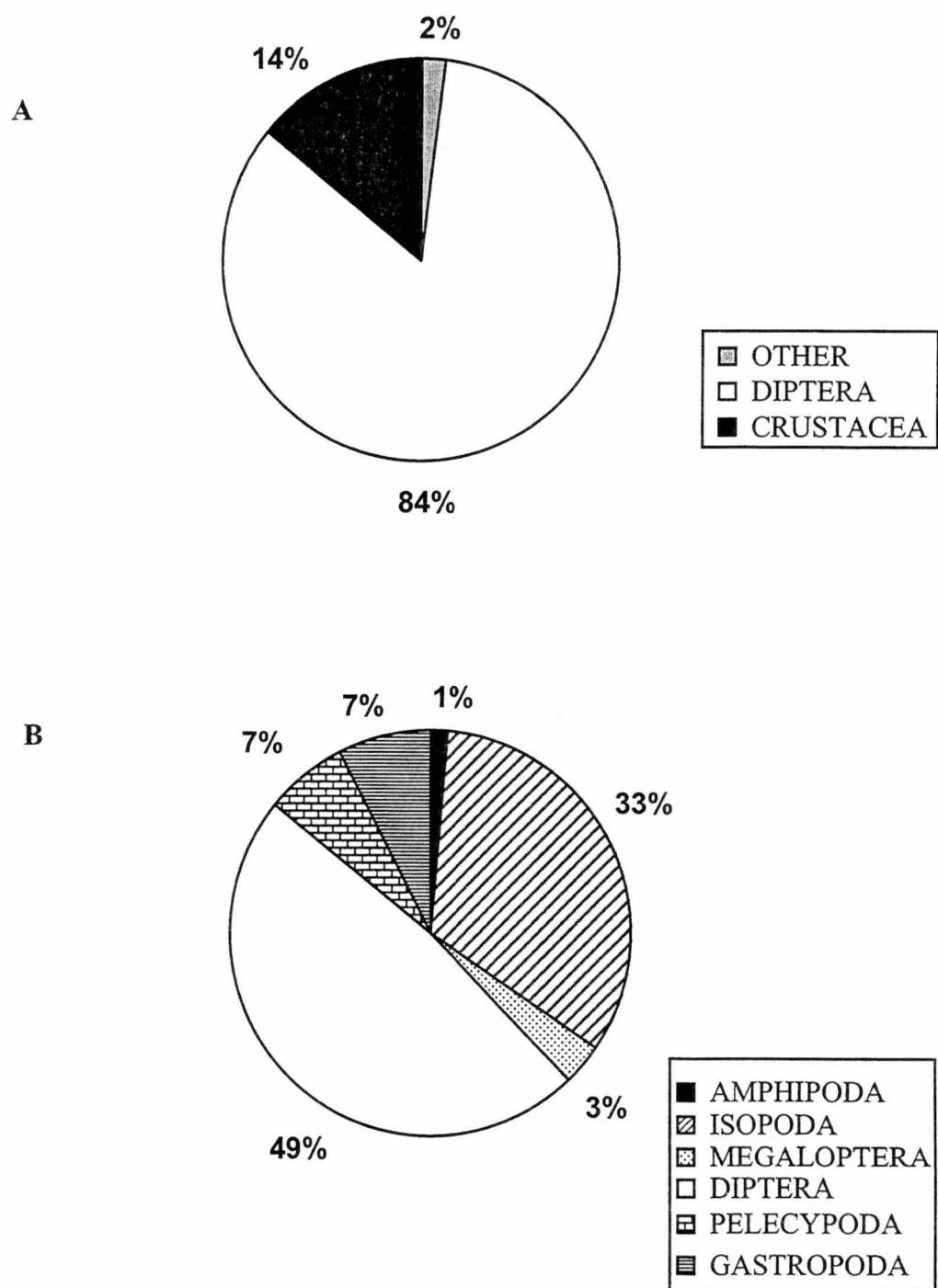


Figure 5. Percent composition of food items (A) found in all stomachs and (B) dominant by volume. All seasons of the year and size classes of *E. luteovinctum* represented.

volume overall (Figure 5). Other non-insect food items identified were fingernail clams of the family Sphaeriidae and snails of the family Pleuroceridae. These two families comprised collectively 14 % of the diet by volume overall. Leeches of the family Piscicolidae were present as a non-aquatic insect food item but were also negligible in occurrence and volume.

Food Items Consumed by Size Class

Size specific variation in food items (Table 2) indicated that the predominant food item by composition for all size ranges was dipteran larvae. Redband darters ≤ 25 mm SL were presumably restricted by their small gape size to smaller prey items (Figure 6). The diet of redband darters within this size range, all juveniles, was dominated in occurrence and volume by chironomids. The diet of juveniles shifted to include a variety of crustaceans when they attained a length of 26-35 mm SL (Figure 6). Larger redband darters, 36-45 mm SL up to the 1 year age class at 46-55 mm SL, were found to consume the widest variety of taxa. It is within this size range that predation on snails and fingernail clams increased, presumably due to the increase in gape size (Figure 7). The two year age class, 56-61 mm SL, still fed predominantly on chironomids, which were again dominant by volume and occurrence. The two year age class consisted of four individuals whose overall contribution to the dietary analysis did not differ significantly from that of the one year age class and therefore did not warrant a separate graphic figure.

Table 2. Stomach content analysis of size-specific variation in diet for
Etheostoma luteovinctum (n=156).

Taxon	< 25 mm SL			26-35 mm SL			36-45 mm SL			46-55 mm SL			56-61 mm SL		
	%PO	%PFO	%DV	%PO	%PFO	%DV	%PO	%PFO	%DV	%PO	%PFO	%DV	%PO	%PFO	%DV
Annelida															
Pisicolidae															
Crustacea															
Amphipoda				2.9	30		0.16	2.67		0.1	2.44				
Cladocera	2.21	16.67		33.82	30		1.07	9.33		0.42	12.2				
Copepoda				23.67	30		0.08	1.33							
Isopoda	1.66	5.56	5.56	16.91	60	50	0.49	2.67		6.18	63.41	21.95	3.39	50	25
Ostracoda				0.48	10		1.31	13.33		0.1	2.44				
Ephemeroptera															
Heptageniidae							0.16	2.67		0.1	2.44				
Megaloptera															
Sialidae							0.41	6.67	4	0.21	4.88	2.44			
Diptera															
Ceratopogonidae							1.88	21.33		0.63	17.07		0.85	25	
Chironomidae	96.13	100	94.44	19.81	90	20	81.8	100	53.33	91.31	100	58.54	94.92	100	50
Psychodidae							1.31	2.67							
Tipulidae							0.41	5.33							
Gastropoda															
Pleuroceridae				1.93	30	20	0.33	5.33	1.33	0.52	12.2	9.76			
Pelecypoda															
Sphaeriidae				0.48	10	10	1.15	13.33	10.67	0.42	14.63	5.56	0.85	25	25
Number of fish examined			18			10			79			45			4
Number of empty stomachs			0			0			4			4			0
Number of full stomachs			18			10			75			41			4
%PO=Percent composition of the total number of food items found in all stomachs															
%PFO=Percent frequency of occurrence of food item found in all food containing stomachs															
%DV=Percent of food containing stomachs in which food category was dominant by volume															

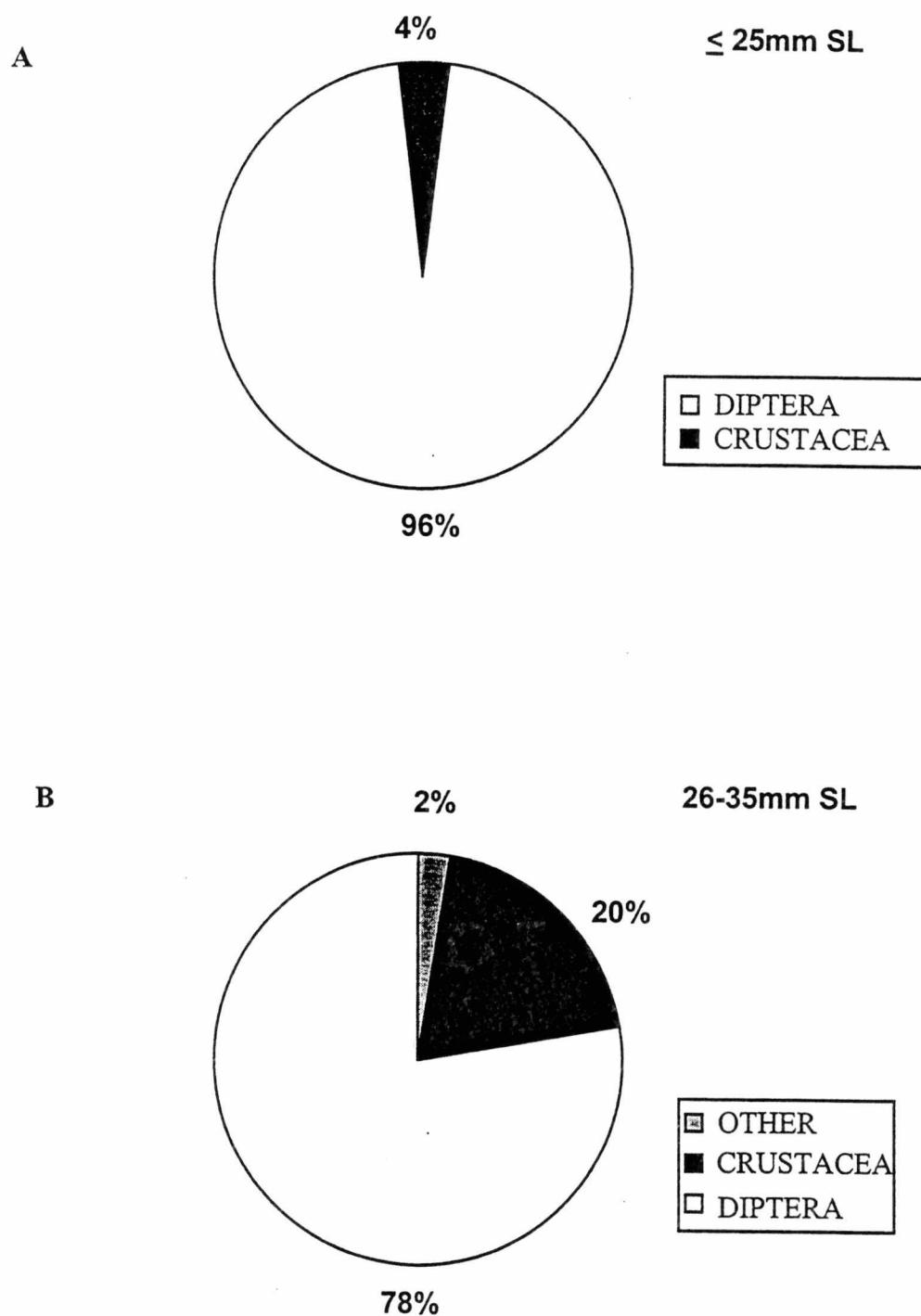


Figure 6. Size specific variation in percent composition of the diet for (A) $<25\text{mm SL}$ (B) 26-35mm SL *E. luteovinctum*.

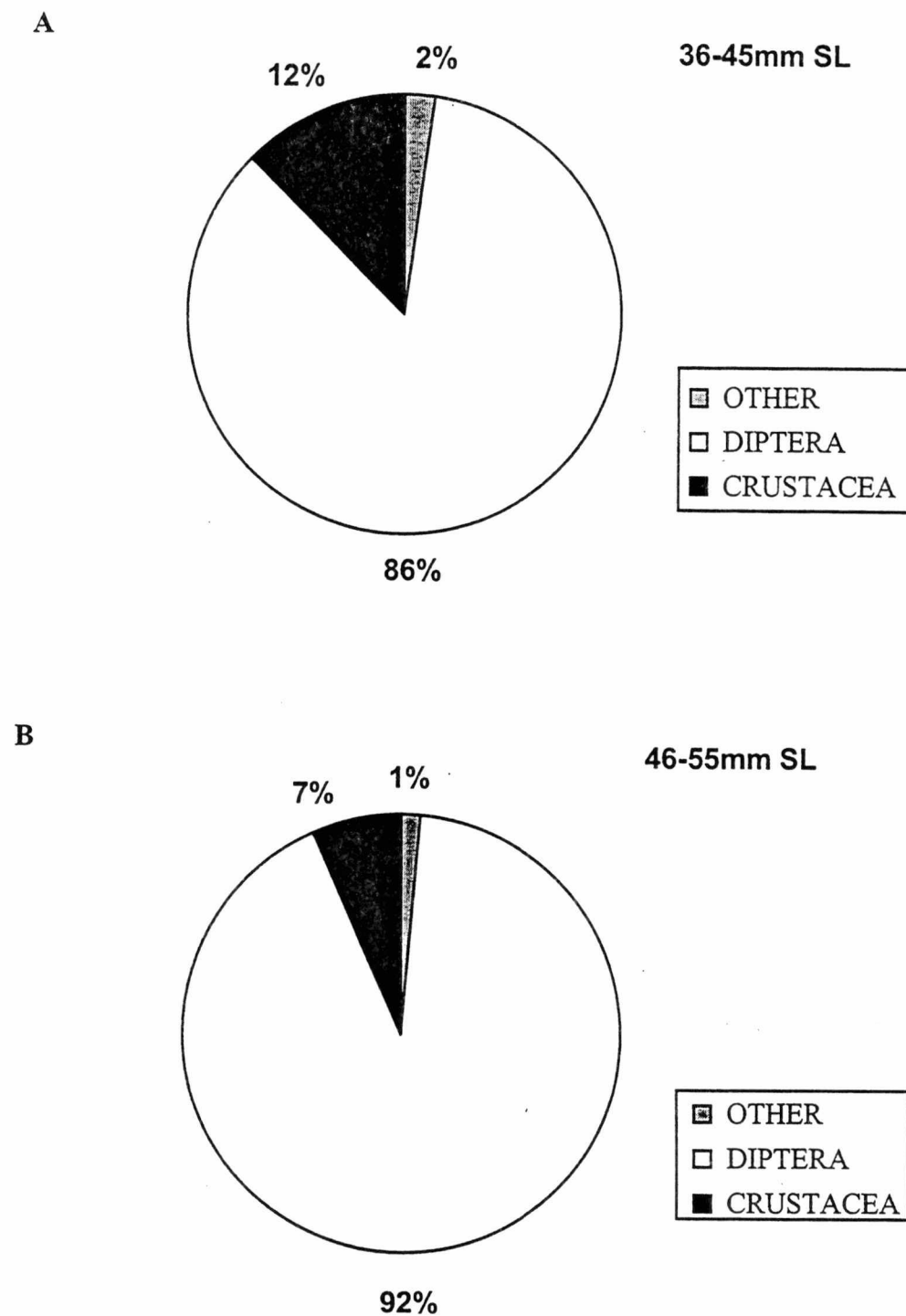


Figure 7. Size specific variation in percent composition of the diet for (A) 36-45mm SL (B) 46-55mm SL *E. luteovinctum*.

Food Items Consumed by Season

Seasonal dietary trends (Table 3) were determined by percent occurrence and percent by volume data. Chironomids were consumed throughout the year, representing from 60 to 97 % by occurrence and 50 to 86 % by volume the dominant food item found in all food containing stomachs. Crustaceans were also consumed in all months. Representation of a distinct dietary shift by volume to isopods during winter and to fingernail clams during fall were the most noticeable seasonal trends found. Fingernail clams and snails were not found in the stomachs examined during spring. The volume of food in stomachs was found to be the highest in the winter and spring (December-May) (Figure 8) and lowest in summer and fall (June-November) (Figure 9).

Larval Feeding Behavior

Aquarium held larval redband darters began feeding on live brine shrimp nauplii upon absorption of the yolk sac. Larvae exhibited a pelagic stage that varied in length from four to seven days before becoming completely benthic. When fed during this developmental period, aquarium held larvae were observed searching for food items throughout the water column. Although rearing tanks were equipped with adjustable flow valves to produce continuous current and oxygenation, larvae did not appear to be rheotactic or exhibit schooling behavior. These behaviors have been observed in other larval benthic fishes such as madtoms (Dinkins and Shute, 1996). It is likely that larvae are carried downstream by the current associated with the riffle and pool spawning habitat. The early pelagic foraging stage and absence of rheotaxis may suggest a means of dispersal for larval redband darters in the wild.

Table 3. Stomach content analysis of seasonal variation in diet for *Etheostoma luteovinctum* (n=156).

Taxon	WINTER		SPRING		SUMMER		FALL	
	%PO	%PFO	%PO	%PFO	%PO	%PFO	%PO	%PFO
Annelida								
Piscicolidae	0.36	3.85	0.1	2.7				
Crustacea								
Amphipoda	2.49	15.38	0.1	2.7	0.28	3.23		
Copepoda			0.1	2.7				
Isopoda	23.84	80.77	1.96	21.62	2.84	19.35	9.26	18.75
Ostracoda	3.56	23.08			0.57	3.23		
Ephemeroptera								
Heptageniidae	0.36	3.85						
Megaloptera								
Sialidae					1.14	12.9		
Diptera								
Ceratopogonidae	3.91	30.77			0.85	9.68	1.85	6.25
Chironomidae	62.99	96.15	97.73	100	93.47	96.77	81.48	100
Psychodidae	0.71	7.69						
Tipulidae	0.36	3.85						
Gastropoda								
Pleuroceridae	0.36	3.85						
Pelecypoda								
Sphaeriidae	1.07	11.54			0.85	9.68	7.41	31.25
Number of fish examined		26		42		34		16
Number of empty stomachs		0		5		3		0
Number of full stomachs		26		37		31		16
%PO=Percent composition of the total number of food items found in all stomachs								
%PFO=Percent frequency of occurrence of food item found in all food containing stomachs								
%DV=Percent of food containing stomachs in which food category was dominant by volume								

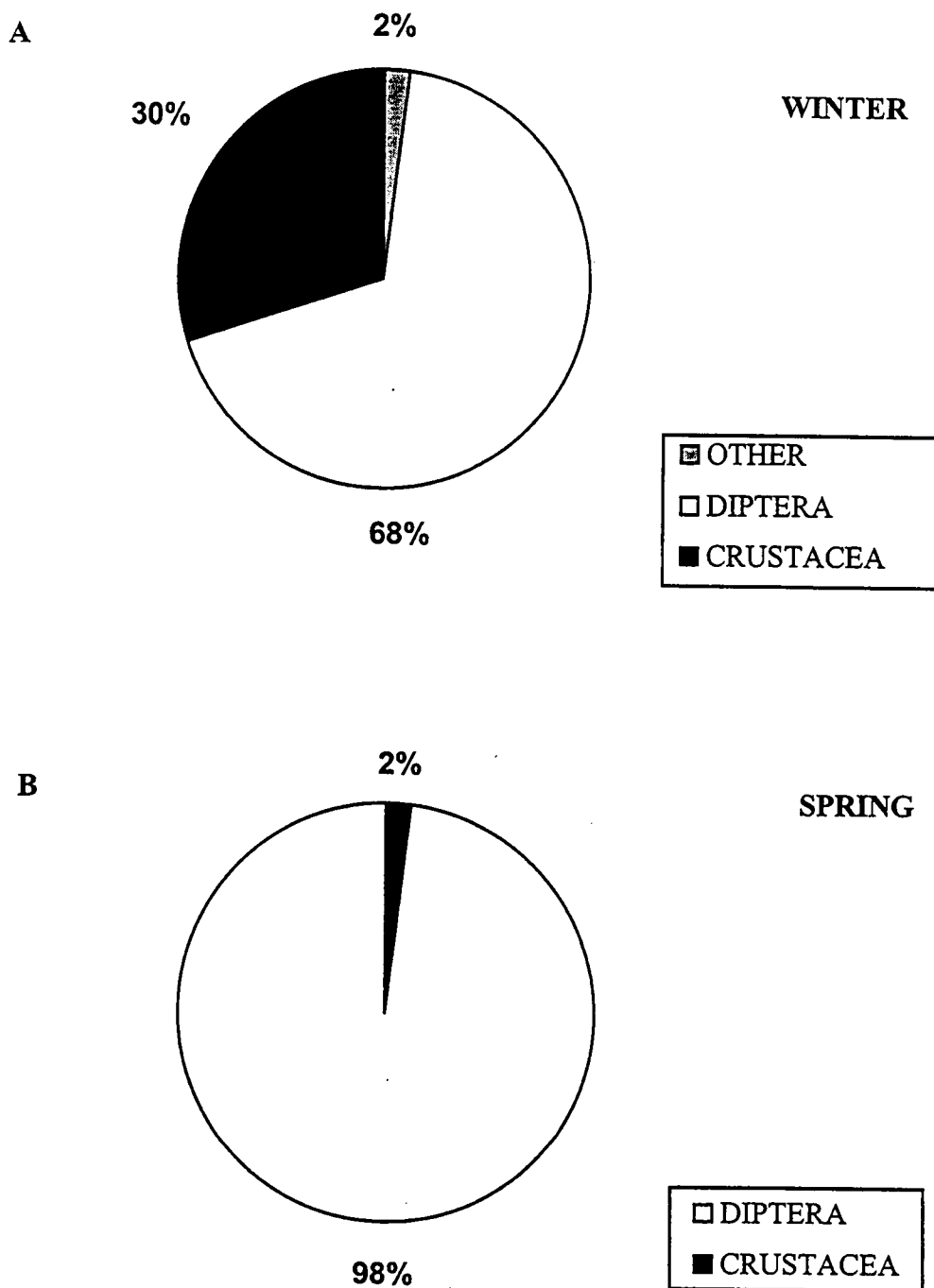


Figure 8. Stomach content analysis of seasonal variation in diet (A) winter and (B) spring.

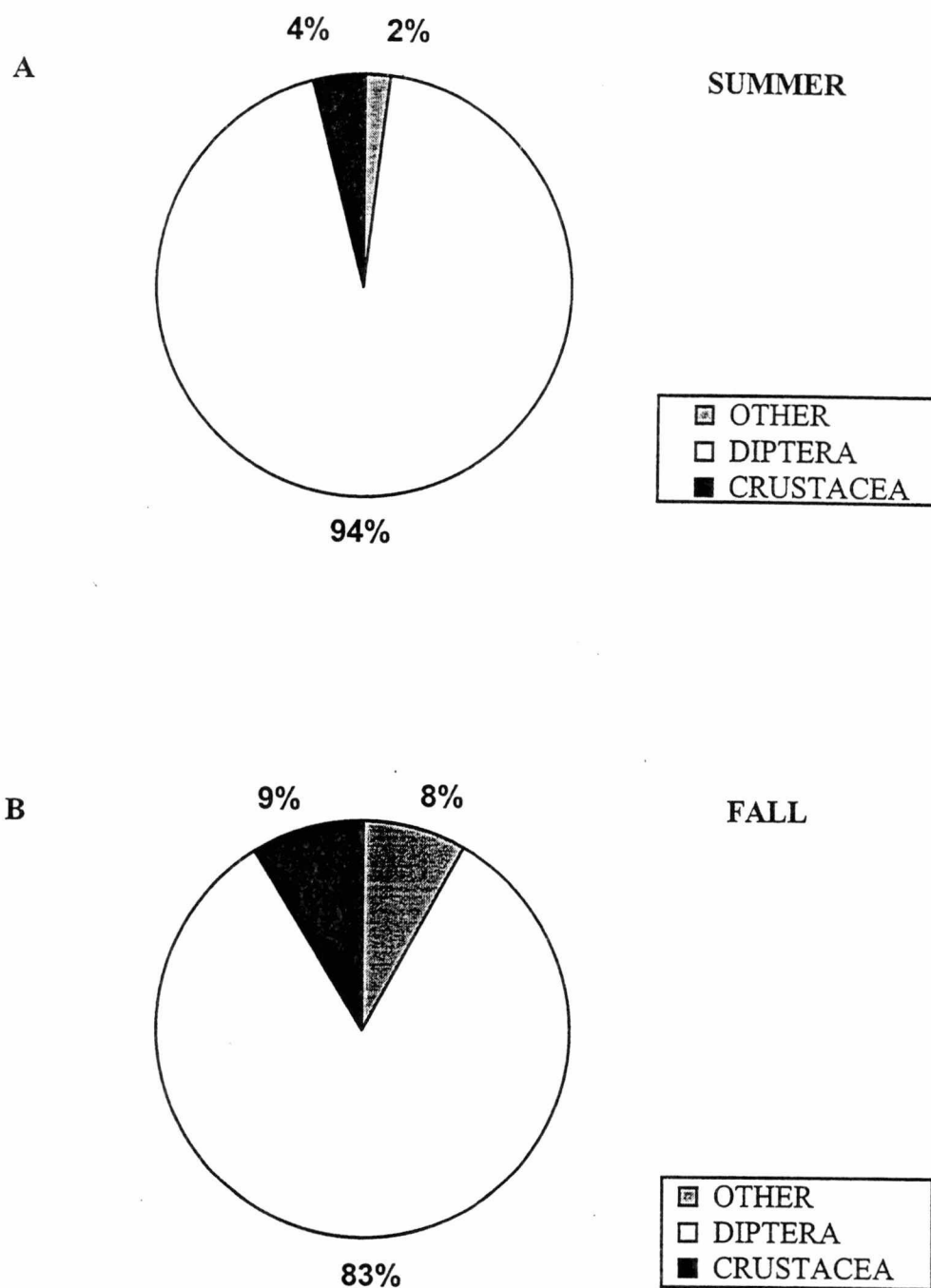


Figure 9. Stomach content analysis of seasonal variation in diet (A) summer (B) fall.

Location of Feeding

Based on the composition of the redband darter's diet and personal observation, feeding occurred predominantly in pools and areas of little current in Titan Creek. There was a noticeable lack of black fly larvae (Simuliidae) found in the 156 stomachs that were examined. Black fly larvae, which are found in flowing water (Brigham, Brigham, and Gnafka, 1982) and in the diets of other species of *Oligocephalus* (Grady and Bart, 1984; Ruple, McMichael, and Baker, 1984), occurred in the riffle areas of Titan Creek (WESTON, 1994 and pers. obs.). The absence of this dipteran family from the diet suggests that foraging occurred in the non-riffle areas of Titan Creek.

Age and Growth

Age Determination

The examination of scale annuli and length frequency distributions indicates that three age classes of redband darters were present in Titan Creek. A catchable size class of juveniles (0+ age group) was not evident in monthly collections until May following the spawning season. Scale analysis separated older age groups into 1+ and 2+ cohorts. Based on the GSI of males and females, the majority of redband darters presumably spawned during March and April. With this assumption, and using both length frequencies and scale annuli, it was possible to determine the age of an individual to within approximately one month (Ruple, McMichael, and Baker, 1984). Annuli apparently formed by the end of winter, which closely corresponds to the beginning of the

spawning season (late February). This was indicated by the presence of a new annulus located near the scale margin for specimens that were one year of age. Generally, the location of an annulus was found at the outer edge of closely spaced ridges on the scale. Zones of widely spaced ridges correspond to periods of faster growth, while zones of closely spaced ridges correspond to periods of slower growth.

During the May 1996 to June 1997 study period, 202 male and female redband darters (156 specimens preserved, 46 captured and released) were measured to develop a length frequency distribution (Figure 10). This distribution indicates that three year classes were present in Titan Creek at any given time. These classes represent individuals from the 1995, 1996, and 1997 spawning seasons.

Etheostoma luteovinctum collected during the study ranged in size from 15 to 61 mm SL. Individuals less than 15 mm were probably not vulnerable to seining and therefore were not present in the samples. Members of the 1997 year class were not collected while sampling until May of that year. Early growth was rapid, with YOY reaching 61.3 % of their eventual total length by the end of their first year (1997 year class, mean SL at 12 months of age = 37.4 mm). At 12 months, males averaged 39 mm SL and were slightly larger than the females average 35.8 mm SL. At two years (24 months), males averaged 50.6 mm SL and again were larger than females, which averaged 47.4 mm SL. Although male redband darters were larger on average than females, the largest individual, collected in October 1996, was a female 61 mm SL and determined to be 31 months of age. This female measured 76 mm total length (TL) and

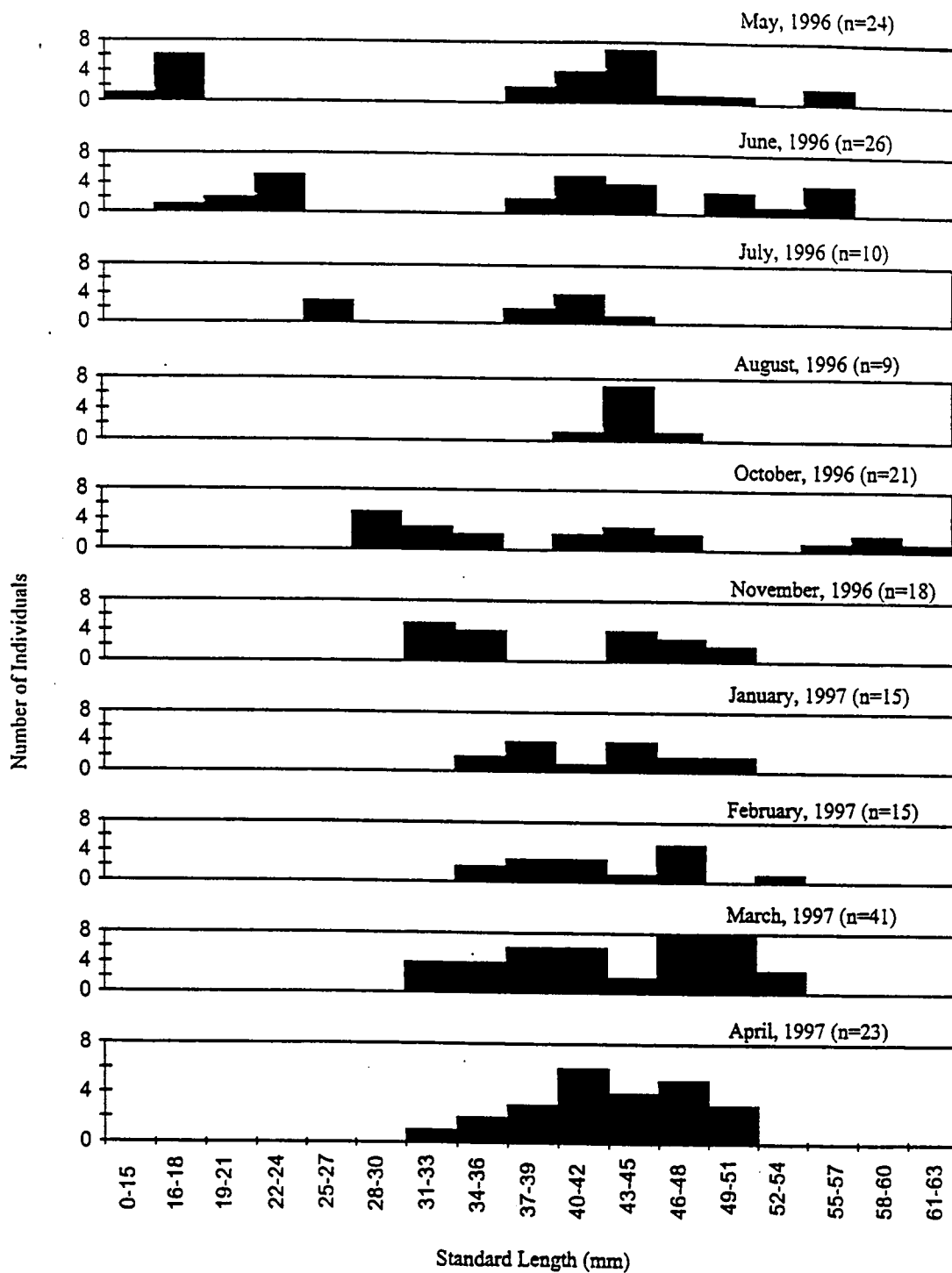


Figure 10. Length frequency histogram for *Etheostoma luteovinctum* (n=202).

surpassed the previous known maximum length record for this species. The largest male, 60 mm SL (73 mm TL), was collected in October 1996 and determined to be 31 months of age. The rate of growth decreased during the first winter following hatching and continued to decrease during the second and third year of life. The relationship between standard length (Y) and age in months (X) for males is described by the linear equation $Y = 13.837 \text{ Log } X + 6.3851$, $R^2 = 0.9417$; for females this relation is described by the linear equation $Y = 13.760 \text{ Log } X + 4.0136$, $R^2 = 0.9463$ (Figure 11).

Percent Composition of Age Classes

The age composition for 202 redband darters collected from Titan Creek was compiled both numerically (Table 4) and by percentage. Assuming that field collections

Table. 4. Age composition of 202 redband darters collected from Titan Creek.

SEX	0+	1+	2+	Total
Male	37	68	3	108
Female	50	39	5	94
Total	87	107	8	202

sufficiently represent all age classes in the population in proportion to their relative number, 43 % were less than 12 months old (0+), 53 % were 12 -23 months old (1+), and 4 % were 24-35 months old (2+). No individuals examined were older than 31 months.

Description of Fertilized Egg

Fertilized eggs were adhesive over the entire surface, often resulting in a coating of silt particles. Chorionic fibrils attached to the egg were short and varied in length,

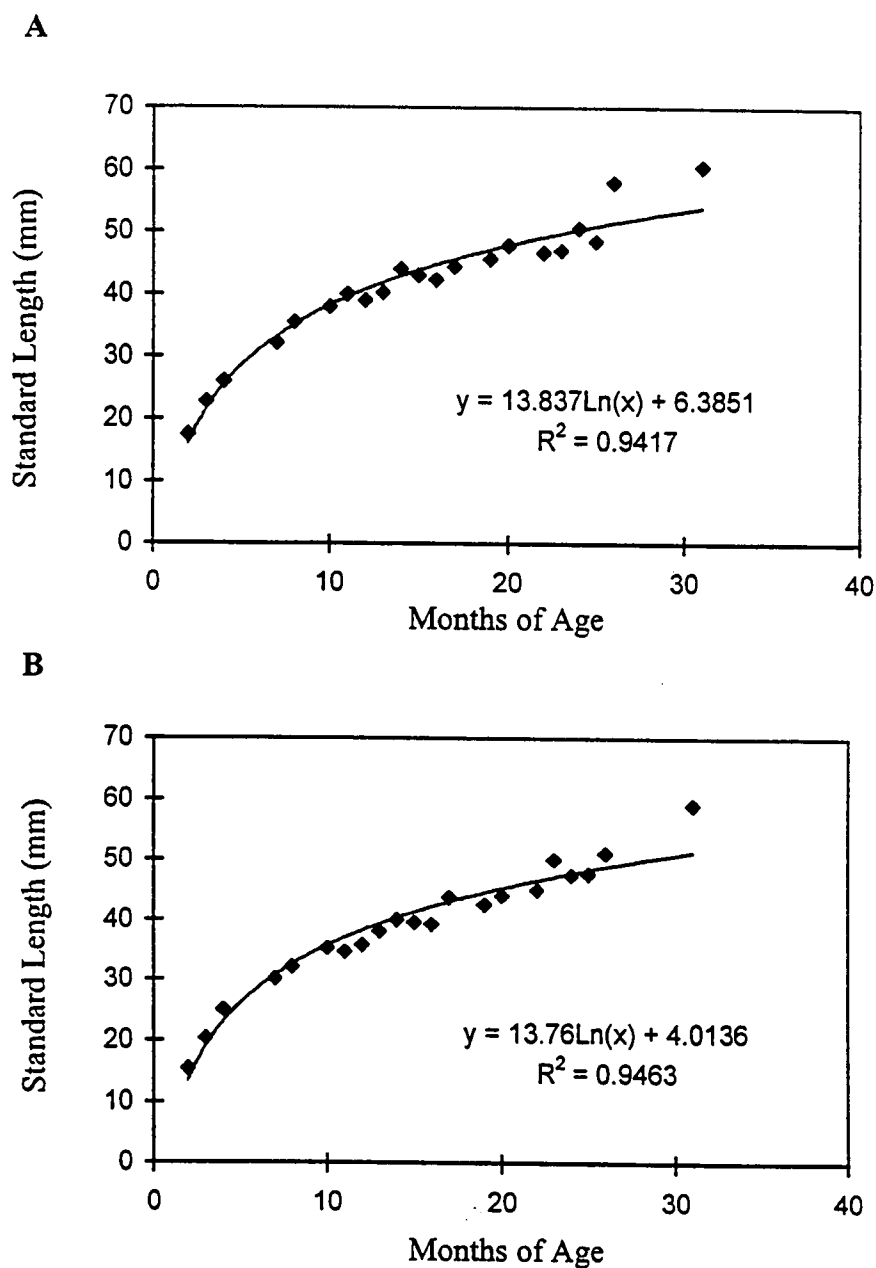


Figure 11. Growth curves for (A) male (dots represent sample means for 108 specimens) and (B) female (dots represent sample means for 94 specimens) *Etheostoma luteovinctum*.

forming clusters of filaments 0.1 to 0.6 mm. These filaments were also adhesive and covered with silt particles. Once placed into a watch glass and gently cleansed using an eye dropper and fresh water, fertilized eggs were observed to be spherical in shape and translucent to amber in color, and contained a single large oil droplet (0.5 to 0.8 mm). Small concentrations of pigment were also visible inside the egg prior to hatching (pers. com. P. L. Rakes, Conservation Fisheries Inc., Knoxville TN). Hatching occurred within six to nine days at a water temperature of 21° C.

Larval Development

Protolarvae (Figure 12) averaged 5.0 mm TL based on preserved specimens (n=3; range 4.8 to 5.2 mm TL) and had fully absorbed their yolk sac within 24 to 48 hours from the time of hatching. Protolarvae were translucent to opaque in color with well developed pectoral fins, eyes, and jaws. The yolk sac was large and speckled with melanophores. Melanophores were also located mid-laterally along the myosepta, along the dorsum in two distinct rows, and on top of the head where they were most concentrated and prominent. Thirteen to 15 preanal myomeres and 18 to 20 postanal myomeres were visible. Myomere counts remained fairly consistent throughout all three stages of larval development. Modal myomere counts for all three stages of larval development were 15 preanal and 19 post anal.

Mesolarvae (Figure 13) averaged 7.5 mm TL (n=4; range 7.2 to 8.0 mm TL). Pelvic fin formation was not yet evident. Dorsal spine formation had begun and was visible at the base of the dorsal fin fold. Ray formation had begun in the caudal, anal, and

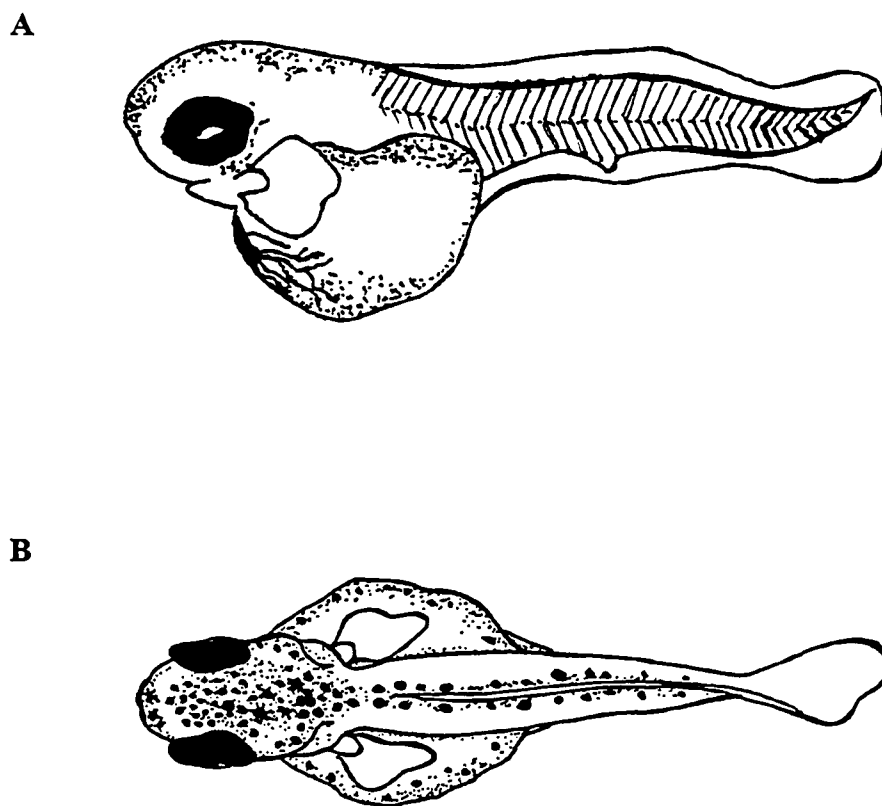
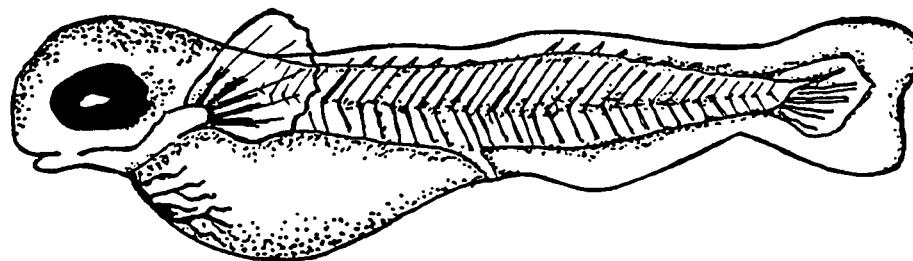


Figure 12. *Etheostoma luteovinctum* protolarva (5.0 mm TL).
(A) lateral view, (B) dorsal view

A



B

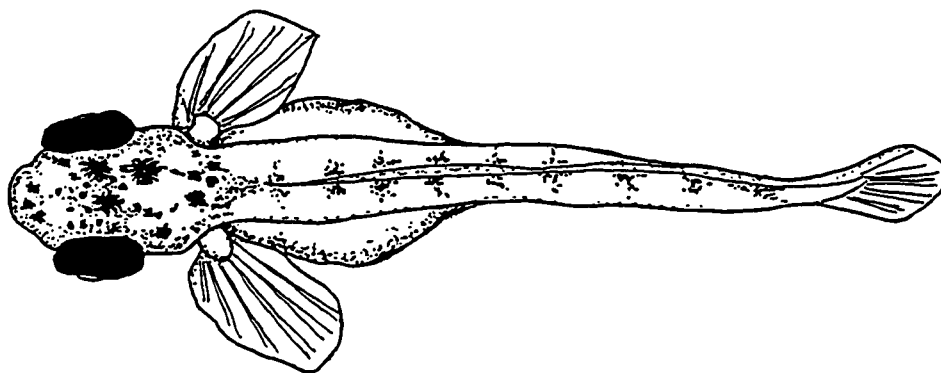
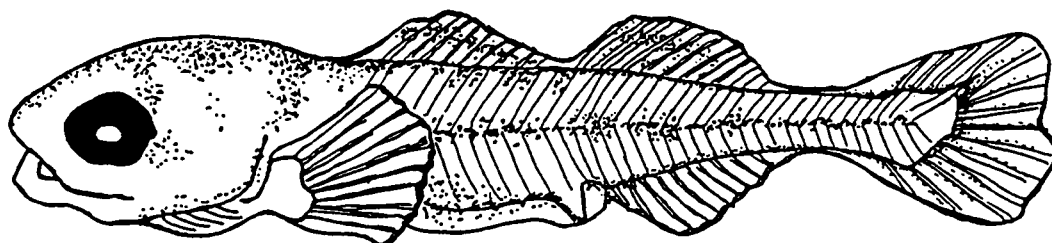


Figure 13. *Etheostoma luteovinctum* mesolarva (7.5 mm TL).
(A) lateral view, (B) dorsal view

soft dorsal fin, but was most prominent in the pectoral fins. The urostyle was upturned at the end of the vertebral column. Larger stellate melanophores were prominent and concentrated on top of the head. The two rows of melanophores along the dorsum were now increasing in concentration and expanding across the dorsum. Metalarvae (Figure 14) averaged 11.8 mm TL ($n=3$; range 11.5 to 12.5 mm TL). Pelvic fin formation began in the metalarval stage with rudimentary fins having noticeable rays. Ray formation in all other fins was complete by this stage. Pectoral and all median fins had begun to increase in length. Branchiostegal rays were visible, the yolk sac was greatly reduced, and the overall coloration of the body was a dull yellow. The melanophores along the dorsum were developing into distinct dorsal saddles. Lateral pigmentation now consisted of an interrupted series of distinct blotches along the myosepta. Concentrations of melanophores were visible in the interradiial membranes of both dorsal fins. The pectoral and caudal fin rays were also speckled with melanophores as were the abdomen and opercles. The snout, interorbital, and occipital regions of the head were now heavily pigmented. The metalarval stage of development lasted six to eight days and was concluded by the complete formation of the pelvic fins.

A 22 mm TL juvenile (Figure 15, 18 mm SL) collected from Titan Creek was compared to an aquarium reared 30-day-old juvenile 26 mm TL (22 mm SL). Both individuals possessed the full complement of spines and rays with segmentation in the principle rays of all fins evident. Seven distinct saddles were visible along the dorsum. Lateral pigmentation consisted of seven to eight distinct blotches along the lateral line.

A



B

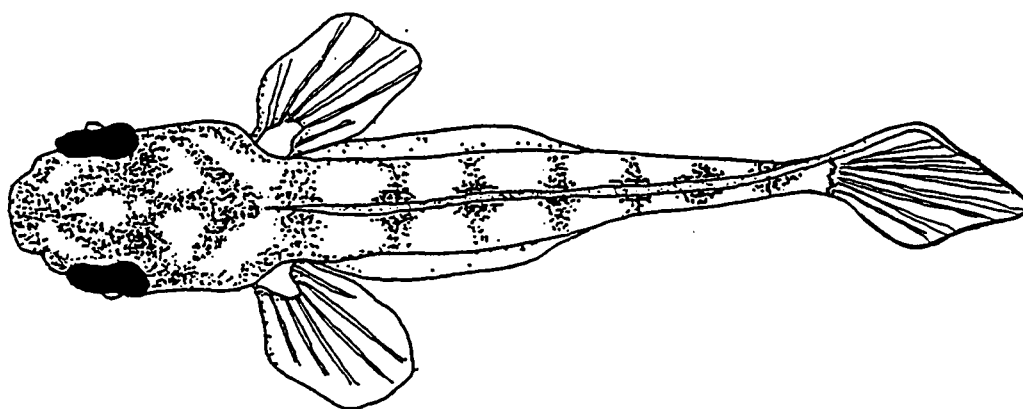
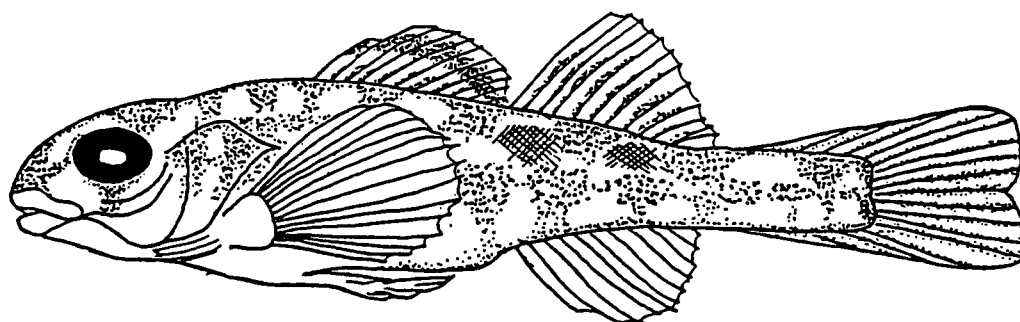


Figure 14. *Etheostoma luteovinctum* metalarva (11.5 mmTL)
(A) lateral view, (B) dorsal view

A



B

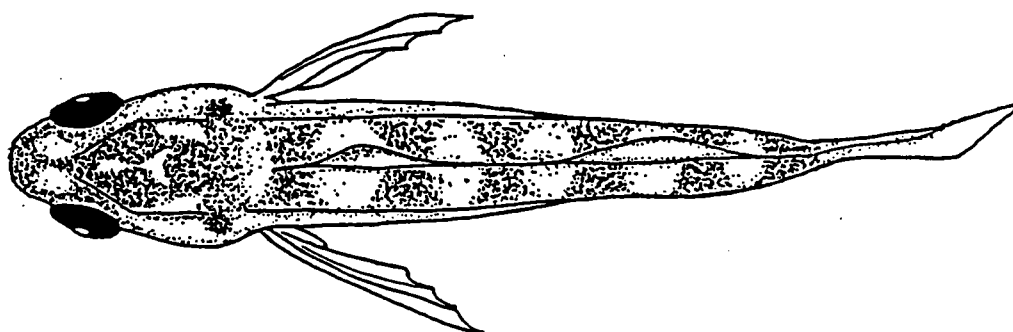


Figure 15. *Etheostoma luteovinctum* juvenile (22 mm TL).
(A) lateral view, (B) dorsal view

A suborbital bar was evident as well as a distinct band in the spinous dorsal fin. Extent of squamation of both juvenile individuals approximated that of an adult.

Reproductive Cycle

Sexual Maturity

All males (n=60) and all but one female (n=62) collected from Titan Creek during the 1997 spawning season were sexually mature adults based on gonadal development. The smallest adult female collected was 38 mm SL, and the smallest adult male was 37 mm SL. The sexually immature female, 31 mm SL, was collected on March 20, 1997, and was EM. It is possible that this female would have reached the MA (reproductive) stage, but probably would not have spawned before the season ended. Based on this data, nearly all redband darters reach sexual maturity and spawn during the first reproductive season following hatching. There was no evidence found to support the hypothesis that an EM (immature) female continued to develop and spawn later than all other mature females, which spawned and were LA by the end of May.

Gonadal-Somatic Indices

A GSI was calculated for 60 male and 62 female redband darters collected from Titan Creek during the months of January through June 1997. Monthly changes in mean GSI for males and females (Figure 16) indicated that gonadal development reached its peak during March.

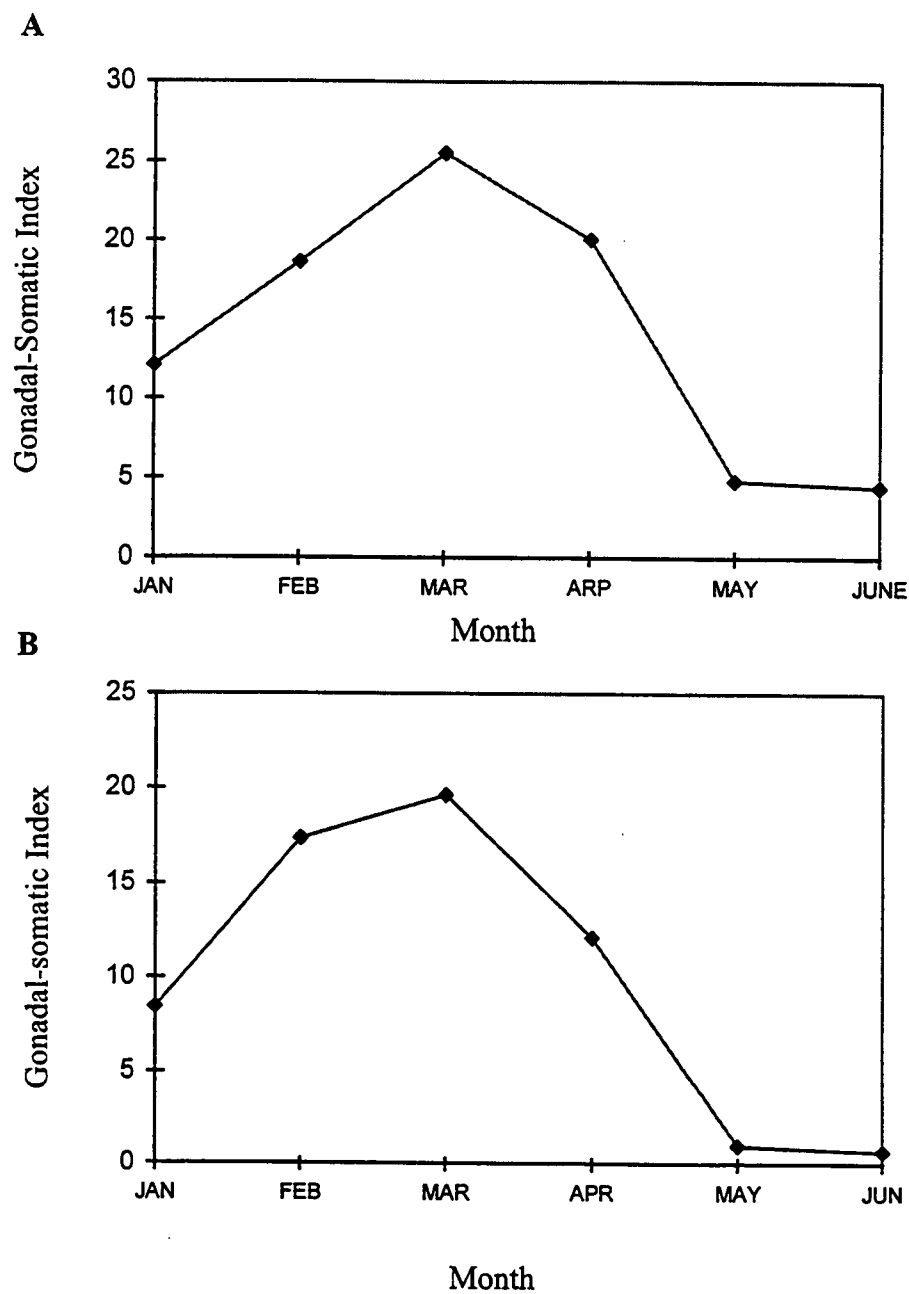


Figure 16. Monthly changes in mean gonadal-somatic indices for (A) male (n=60) and (B) female (n=62) *Etheostoma luteovinctum*.

Male Gonadal Development and Reproductive Cycle

Mean monthly GSI for 60 male redband darters was from 12 % in January and 4.4 % in June, peaking at 25.5 % of the adjusted body weight during March. The enlargement of the testes increased most rapidly during the month of February. Although breeding tubercles were not present on the anal fin of all males collected in February, four males were found already tuberculate at this time. The smallest of these males was 40 mm SL. All males (n=39) collected in March and April were tuberculate on their anal fin and MA. Breeding tubercles were no longer present on males of all sizes collected 29 May and 26 June. The testes of all males examined from the May and June collections were found to be LA. The genital papilla of larger preserved male specimens collected during the spawning season appeared to be slightly enlarged, but this was not apparent in all breeding males collected.

Males of one year of age and older displayed distinct sexual dimorphism in breeding color prior to and during the spawning season. Noticeable increases in coloration intensity were observed, beginning in December, for male redband darters in the wild. Breeding males were observed to have the most vivid colors during the months of March and April (Figure 17).

Female Gonadal Development and Reproductive Cycle

The changes in monthly mean GSI for 62 female redband darters ranged from 8.4 % in January to a peak of 19.6% of the adjusted body weight during March to less than 1 % in June. Overall, female redband darters did not show dramatic changes in coloration prior to or during the spawning season (other than some females appearing

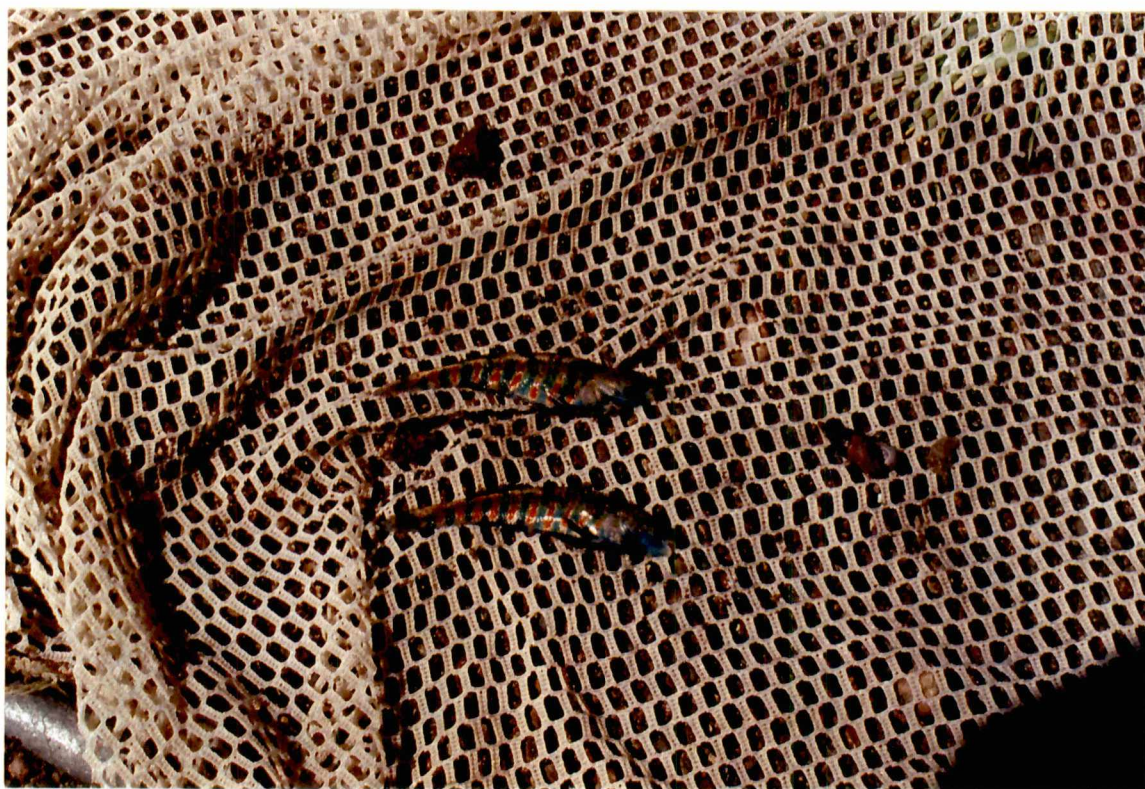


Figure 17. Male *Etheostoma luteovinctum* in breeding color.

to have a darkening of their normal pigment shade). The largest females examined (> 55 mm SL) were occasionally observed to have vivid red spots or blotches on their lower sides, which were otherwise faint or absent during the rest of the year. The distended abdomen of breeding females was the only observable distinct morphological change in appearance. The distention of the abdomen during the spawning season resulted from the continued maturation of ova. The genital papilla of preserved female specimens was examined and appeared to be slightly enlarged in some females during the spawning season, but this character was not consistent for all females or evident in the field.

All female redband darters collected and examined in January 1997 were found to be in the EM stage of ovarian development, with ovaries small, thin, and transparent to slightly translucent. Ovaries increased in size in February and continued enlarging until March. Five of eight females collected in February were LM and three were EM. In March, 11 of 16 females were MA, two females were MR, two females were RE, and one female was EM. All females collected in April were reproductive. Five of 11 females examined were MA, one was MR, and five were RE. Ovaries were found to have decreased greatly in size by the third week in May. All females collected on 29 May and 26 June (n=18) were LA. Therefore, 98 % of all females collected in March and April (n=27) were in one of three stages of reproductive development MA, MR, or RE (Table 5). Percent frequency of occurrence histograms were generated to illustrate the continued ovarian development of all females (n=62) collected from January to June 1997 (Figure 18).

Table 5. Monthly summaries of reproductive condition of *E. luteovinctum* females. The total number of females examined from each month (n) is shown as are the percent frequencies of occurrence for each stage of reproductive condition: LA, latent; EM, early maturing; LM, late maturing; MA, mature; MR, ripening; RE, ripe.

Reproductive condition (%)							
Month	n	Latent	Early Maturing	Late Maturing	Mature	Ripening	Ripe
January	9	0	100	0	0	0	0
February	8	0	37	63	0	0	0
March	16	0	6	0	68	13	13
April	11	0	0	0	45	10	45
May	9	100	0	0	0	0	0
June	9	100	0	0	0	0	0

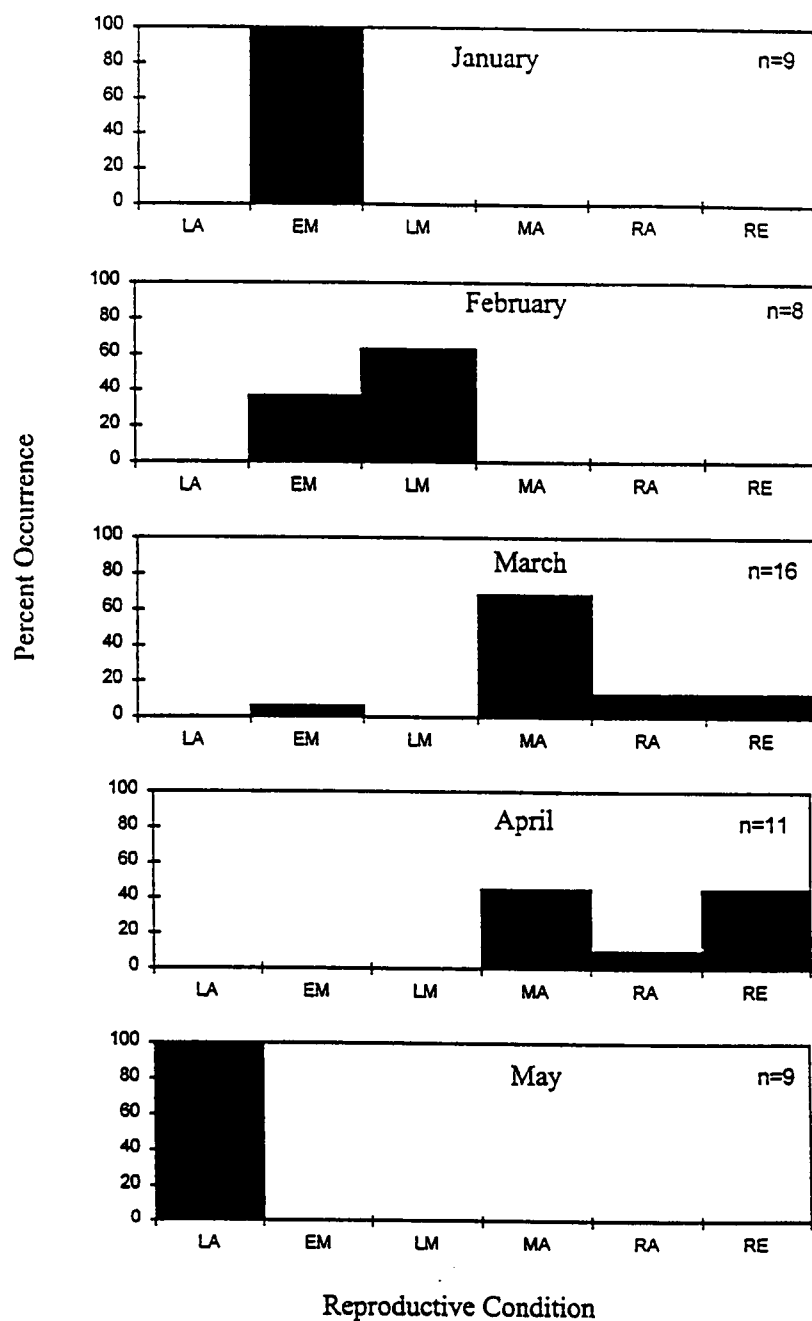


Figure 18. Percent frequency histogram of ovarian development for female *Etheostoma luteovinctum*.

Egg Dimension and Clutch Size

The mean egg diameter (not "water hardened") of all individual RE females (n=7) varied in size from 1.18 to 1.30 mm. The overall mean diameter for all ripe ova (n=523) was 1.27 mm. Clutch size (number of mature ova) was defined as a group of larger developing oocytes from MA and MR females. This group of oocytes was a relatively synchronous population of mature ova destined to be spawned within a short time as fully formed eggs (Wallace and Selman, 1981, Heins and Rabito, 1986, Heins and Machado, 1993). Numbers of mature ova ranged from 33 to 146, with a mean of 85.1 among 19 females 31 to 56 mm SL. The mean number of mature ova was 66.7 for 9 females of the 0+ age class (31 to 37 mm SL). The mean number of mature ova for 9 females of the 1+ age class (40 to 50 mm SL) was 98.4. Of the 2+ age class (56 mm SL) only one female in reproductive condition was collected during the 1997 spawning season. This female contained 127 mature ova. Regression analysis of *E. luteovinctum* revealed a linear relationship between the number of ova produced and the size of the female. The relationship between number of mature (MA) and maturing (MR) ova (Y) and standard length (X) is described by the linear equation : $Y = 3.1817X - 43.722$, $R^2 = 0.6027$. A significant positive correlation ($r = 0.7884$, $p < 0.05$) existed between the number of MA and MR ova produced and standard length (Figure 19). The protocols used to describe ovarian condition and developing ova and oocytes were chosen in an attempt to aid in standardizing methodologies used in life history studies of stream fishes (Heins and Baker, 1989).

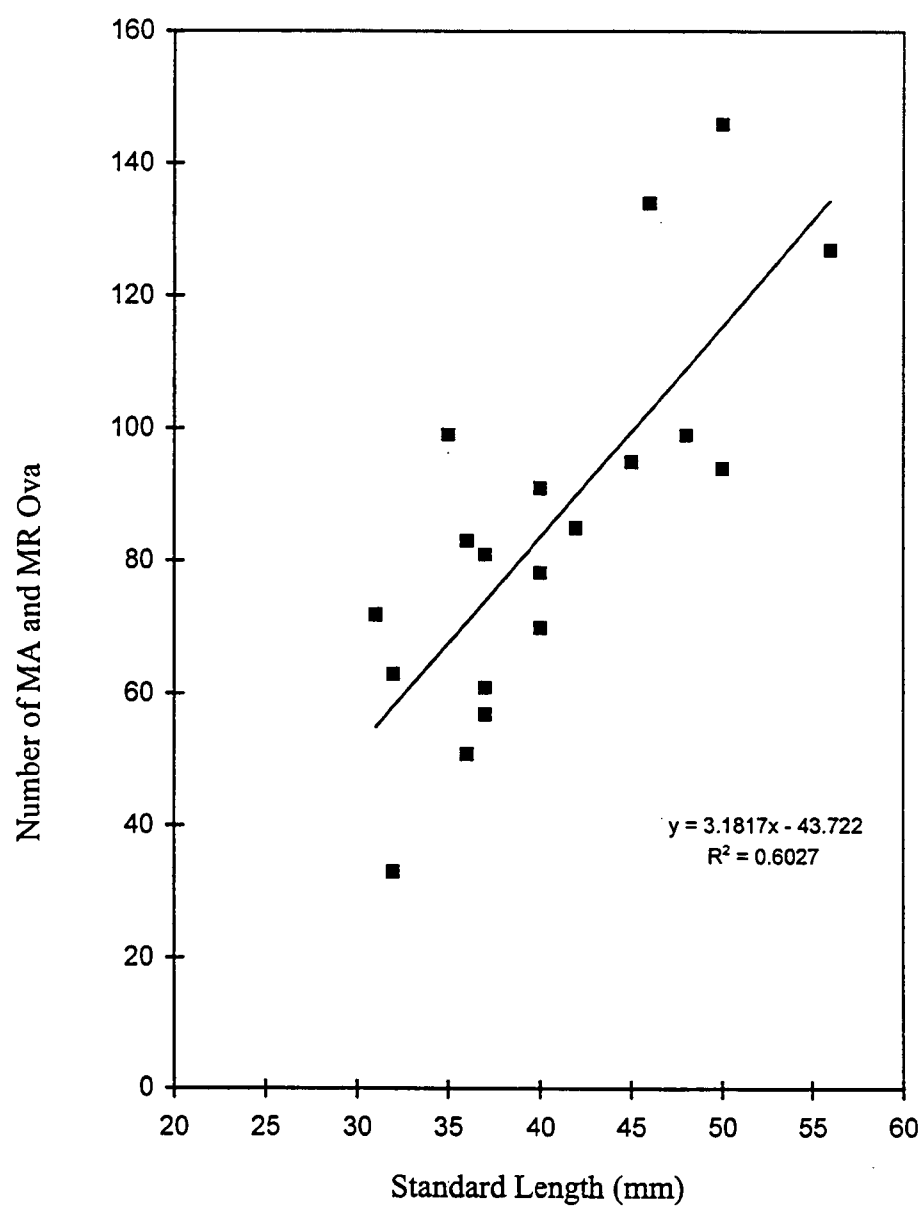


Figure 19. Standard length (mm) to number of mature (MA) and maturing (MR) ova regression for female (n=19) *Etheostoma luteovinctum*.

Spawning Behavior

On 29 February 1997 (1400 h), two male and two female *E. luteovinctum* collected from Titan Creek were placed in a 113.5 l aquarium. Both males and females were assumed to be in breeding condition based on their physical appearance. Males were brilliantly colored, and the abdomens of both females were visibly swollen (Figure 20). The aquarium substrate consisted of fine sand and gravel. There were a few larger cobbles placed in one end of the aquarium to provide cover.

Spawning was first observed within 30 minutes of the same day at 1430 h. The larger (60 mm SL) and apparently dominant of the two males remained close to both females, while the smaller male remained at one end of the aquarium hidden beneath the large cobbles. Both females vibrated their heads side to side in the presence of the attending male. This behavior also occurred when the attending male would brush against the soft dorsal fin of the female with the ventral side of his head. The male then settled behind the female, with the ventral side of his head resting on the female's caudal peduncle. Females were observed pushing their snouts into the substrate and burrowing themselves, from a vertical position, completely into the substrate with forcible thrusts of their caudal fin. Their heads would quickly reappear, leaving their abdomen and often the remainder of their entire body buried. No burrowing behavior was exhibited by either male. The dominant male arched his back and pressed himself against the top of the female (Figure 21). Both male and female then quivered, rapidly fanned their pectoral fins, and pushed downward and forward through the substrate, apparently spawning (Figure 22).



Figure 20. Male and female *Etheostoma luteovinctum* in breeding condition.



Figure 21. Male and female *Etheostoma luteovinctum* in spawning position.



Figure 22. Spawning pair, male and female *Etheostoma luteovinctum*.

Eggs were presumably fertilized and buried in the substrate at this time. The dominant male spawned with both females between 1430 and 1500h. These spawnings were photographed with a 35 mm camera. A single spawning involving the same male and one of the same females was videotaped the following day, 1 March 1997 (1710 h). No further spawning events were observed between these four individuals. These courtship and spawning behaviors are similar to those reported for other egg burying species of *Oligocephalus* (Ruple, McMichael, and Baker, 1984; Scalet, 1973; Winn, 1958).

On 20 March 1997 (1902 h), an additional four male and six female redband darters collected from Titan Creek were placed in the aquarium. These individuals were assumed to be in breeding condition based on the same physical criteria as the initial breeding pairs. The initial two breeding pairs were not removed from the aquarium. The largest male (60 mm SL) of the initial two remained the dominant male of the five total males that were now in the aquarium. However, aggressive behavior toward other males, not seen between the initial pair of males, was now observed. The dominant male repeatedly left the females he was attending to chase away challenging males and then quickly returned to the females. Both the dominant and challenging male positioned their bodies parallel to one another and fully erected their dorsal fins while tilting their bodies to the side, presumably to exaggerate their size. This display resulted in the dominant male remaining with the females while the submissive male retreated. Also observed during display behavior between males was a noticeable change in body coloration. On one occasion, a smaller male was observed to darken in body color until turning black. This behavior has also been reported for the rainbow darter, *Etheostoma caeruleum*,

during spawning (pers. com., Becky Fuller. Florida State University, Tallahassee Florida.).

The displaying of dorsal fins was also observed during courtship behavior with females. Males displayed their dorsal fins to approaching females or as they approached a female. Males would then pursue females and nip at their caudal peduncle. Upon coming to rest behind the female, a male would relax his dorsal fins. Once the additional six females were placed into the aquarium, spawning began within two minutes. A total of ten individual spawning events and all associated behaviors of courtship and aggression was videotaped. The dominant and largest male spawned eight times within a thirty minute period. Females were observed to spawn more than once during this time period. No further spawnings were observed this day. Two spawnings were observed the following day, 21 March 1997 (1240 h). No further spawning was observed in the aquarium throughout the remainder of March. All redband darters used for aquarium observations were preserved for gonadal examination.

After spawning was completed, neither the male nor the female remained in the spawning area or appeared to protect the spawning site. The dominant male was no longer aggressive toward other males in the aquarium once all spawning activity had ceased. Aggression was displayed by the dominant male only during courtship of a female or while attending females that were buried in the substrate.

Observations of spawning behavior and individual spawnings were observed in Titan Creek on 29 February, 20 March, 17 April, and 27 April 1997. Aggregations of 10+ to 30+ redband darters, containing one to four large males (50-55 mm SL) per group,

were observed in Titan Creek throughout the spawning season. These groups were found in pool areas 45 to 300 mm in depth, ranging in temperature from 15 to 18° C, and located above and below small riffles (Figure 23). Spawning was observed to occur only in these pooled areas associated with a riffle. No spawning was observed in the riffles. The largest males appeared to have territorial boundaries and were continually surrounded by females of all sizes. When females would burrow into the substrate in the presence of an attending male, or when spawning would occur, smaller males would rapidly move to the spawning site and be quickly chased away by the dominant male. The larger and apparently dominant males did not leave the group of females they were attending to chase each other. Aggression was displayed only toward smaller males. These behaviors were directly similar to spawning behaviors observed in the aquarium. Aggregations of spawning redband darters were no longer present in any habitat type in Titan Creek by 29 May 1997.

Based on compiled data from all reproductive male and female specimens collected, observations of spawning behavior in Titan Creek and in aquaria, spawning occurred from 29 February to 27 April 1997. This time frame would be comparable to the speculated spawning season for this species (Etnier and Starnes, 1993; Page, 1983) based on tuberculate males (Collette, 1965). However, it is probable that spawning begins in early to mid February and continues into early May.



Figure 23. Spawning pool and riffle site for *Etheostoma luteovinctum* in Titan Creek, Maury Co., TN.

Even though males were found to outnumber females overall (Table 4), a chi-square test indicated that the observed sex ratio did not differ significantly from the expected 1:1 sex ratio found among most darter populations (Page, 1983). The overall ratio for 108 males and 94 females was 1.15:1. A chi square (χ^2) value of 0.96 ($P > 0.05$) indicated there was no significant difference between the observed and expected (1:1) overall sex ratio.

Population Estimate

A maximum likelihood population estimate, with upper and lower 95 % confidence intervals, was calculated for *Etheostoma luteovinctum* and all other sympatric species collected from the middle and upper reach of the study site (Table 6). The population estimate indicated there were 35 and 79 *E. luteovinctum* per 100m in the middle and upper reach, respectively. This estimation also indicated a density of approximately 4 individuals per square meter in the middle reach and approximately 8 individuals per square meter in the upper reach. It was not possible to use the same population estimate method at the lower reach. However, seining yielded a total of 53 *E. luteovinctum* collected for the lower 80 m (Table 6). Comparing the overall population estimates for all six species collected in the middle and upper reach of the study site, redband darters ranked fourth in abundance in this 200 m section of the study site.

Table 6. Population estimate with 95 % confidence intervals for *Etheostoma luteovinctum* and all sympatric species collected in Titan Creek, Maury Co., TN.

SITE 1		Total Catch	Length	Mean Width	Surface Area	
Lower Reach	Species					
	<i>Campostoma oligolepis</i>	1	80 m	3.66 m	292.8 m	
	<i>Luxilus chrysocephalus</i>	9				
	<i>Pimephales notatus</i>	110				
	<i>Semotilus atromaculatus</i>	26				
	<i>Fundulus notatus</i>	3				
	<i>Fundulus olivaceus</i>	1				
	<i>Gambusia affinis</i>	21				
	<i>Lepomis cyanellus</i>	6				
	<i>Etheostoma crossopetrum</i>	25				
	<i>Etheostoma luteovinctum</i>	53				
SITE 2		Population Estimate	95% Confidence Interval	Length	Mean Width	Surface Area
Middle Reach	Species					
	<i>Pimephales notatus</i>	89	75-107	100 m	1.98 m	198 m
	<i>Semotilus atromaculatus</i>	63	60-69			
	<i>Fundulus notatus</i>	9	36-49			
	<i>Lepomis cyanellus</i>	11	9-11			
	<i>Etheostoma crossopetrum</i>	67	62-75			
	<i>Etheostoma luteovinctum</i>	35	33-41			
SITE 3		Population Estimate	95% Confidence Interval	Length	Mean Width	Surface Area
Upper Reach	Species					
	<i>Pimephales notatus</i>	209	179-239	100 m	1.79	179
	<i>Semotilus atromaculatus</i>	61	56-70			
	<i>Fundulus notatus</i>	30	29-34			
	<i>Lepomis cyanellus</i>	8	8-10			
	<i>Etheostoma crossopetrum</i>	128	108-149			
	<i>Etheostoma luteovinctum</i>	79	72-89			

Interspecific Interaction

The only other species of Etheostominae that was abundant and occurred in Titan Creek with *E. luteovinctum* was the fringed darter, *Etheostoma crossopterum*. The fringed darter was the most abundant species of darter in Titan Creek based on a three pass population estimate (Table 6). The stomach contents of six preserved *E. crossopterum*, collected during the population estimate, contained chironomid larvae. It is likely that competition for food does occur between these two species. However, interspecific competition for spawning habitat is unlikely between these two species due to the fact that *E. crossopterum* spawns and deposits its eggs in a single layer on the underside of stones (Etnier and Starnes, 1993). Other sympatric species that were abundant in Titan Creek included *Pimephales notatus*, *Semotilus atromaculatus*, *Gambusia affinis*, and *Fundulus notatus*.

Parasitism

A total of eight leeches of the family Piscicolidae (Maloney and Chandler, 1976) was found on eight of the 156 redband darters collected and preserved from Titan Creek during the March 1996 to June 1997 study period. Two leeches were found attached to the anal fin, two were attached to the caudal fin, and four were attached at the base of the pectoral fin.

Predation

There was no direct evidence of predation on redband darters in Titan Creek. However, potential predators of the redband darter were the green sunfish, *Lepomis*

cyanellus and the banded sculpin, *Cottus carolinae*. The stomach contents of four *L.*

cyanellus collected from Titan Creek were examined but did not contain redband darters.

Although banded sculpins have been occasionally collected in Titan Creek (WESTON, 1994) and are known to feed on benthic fish species such as darters (Starnes, 1977), none were collected or found during this study.

CHAPTER V

OUTLOOK AND DISCUSSION

The redband darter population in Titan Creek has apparently increased and is increasing since the 1985, 1986, 1987, and 1993 biological and water quality surveys were conducted on the streams bordering or in proximity to the SATURN Corporation site (WESTON, 1994). The drainage area and run off from all developed areas on the SATURN property continues to drain into Titan Creek. No evidence was found during this study to indicate that this practice is adversely impacting the Titan Creek ichthyofauna. It should be noted that the lands acquired for the construction of the SATURN facility were predominantly livestock and row crop agricultural lands. These lands bordering Titan Creek have remained fallow since 1986 (pers. com., Gerald Dinkins, 3D International, Knoxville Tennessee), throughout this study, and have continued to remain fallow through June 1998 (pers. obs.). It is likely that the decreased silt load in Titan Creek, historically produced by run off from row crop agriculture and bank and riparian devastation from livestock, has enabled not only redband darters to increase in number but also all other fish populations.

It is of interest that two generations of redband darters, propagated from wild caught parents collected from Titan Creek in February and March 1996, were successfully reared in captivity from 1997 and 1998 spawnings. Although redband darters are a common species within their range (Etnier and Starnes, 1993), they are a species that is restricted geographically. In the future, if there is a need to change the status of the

redband darter from "wildlife in need of management" due to habitat encroachment, techniques for propagation of this species are available.

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