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Life History of the Tricolor Shiner, *Cyprinella trichroistia* (Teleostei: Cyprinidae), in Alabama

Jeremy H. Scott[1,*] and Richard L. Mayden[2]

Abstract - Relatively little is known about the life history of *Cyprinella trichroistia* (Tricolor Shiner), a species endemic to the Coosa and Cahaba rivers in Alabama, Georgia, and Tennessee above the Fall Line. The life history of this species was investigated using a series of collections obtained through the University of Alabama Ichthyological Collection spanning 27 months and collected from Hubbard Creek in the Coosa River System. A total of 6366 specimens were examined for age and growth, reproductive cycle, food habits, parasites, habitat, and associated species. Males and females have similar length-weight relationships, but males attain a larger size. Data analysis and field observations indicate that the species has a single reproductive season beginning in April and ending in August with peak activity in June. Numbers of mature ova of females ranged from 32 to 148, and was highly correlated to female size and weight. At any given time in the population, there were three (possibly four) age classes noted. The habitat for the Tricolor Shiner was primarily clear, flowing streams with a rocky substrate suitable for crevice spawning to occur. The diet consisted mainly of aquatic insects; the only parasites observed were internal nematodes and external trematodes.

Introduction

Cyprinella trichroistia (Jordan and Gilbert in Jordan and Brayton 1878) (Tricolor Shiner) is a typical representative of the genus *Cyprinella*, the second-largest genus of North American cyprinids, encompassing 27 to 30 species, depending on classification (Mayden 1989, Nelson et al. 2004). It is identified as an elongate, slightly compressed fish with a large, triangular head and a long, pointed snout with a terminal oblique mouth (Mettee et al. 1996). The species derives its name from the breeding coloration of adult males, in which the dorsal fin is orange basally, black posteriorly, and white medially. The Tricolor Shiner is an upland endemic of the Cahaba and Coosa river systems in Alabama, Georgia, and Tennessee, where it is commonly associated with small-to-moderate size streams with rocky substrates and high habitat diversity (Boschung and Mayden 2004, Ferguson 1989).

Except for reproductive behavior (Ferguson 1989, Stephens and Mayden 1998), little is known about the life history of this shiner. The Tricolor Shiner is the sister species of *C. gibbsi* (Howell and Williams) (Tallapoosa Shiner), which is a sister species to the imperiled *C. caerulea* (Jordan) (Blue Shiner) (Broughton and Gold 2000, Mayden 1989). The Blue Shiner is on the federal list of threatened and endangered species (threatened) and is regarded

[1]CH2M HILL, 1766 Sea Lark Lane, Navarre, FL 32566. [2]Department of Biology, Saint Louis University, 3507 Laclede Avenue, St. Louis, MO. *Corresponding author - jeremy.scott@ch2m.com.

to be endangered by the American Fisheries Society (Warren et al. 2000). Because of phylogenetic relatedness and apparent ecological similarities to the Blue Shiner, it has been suggested that the Tricolor Shiner may serve as a surrogate model to develop proactive conservation strategies for the Blue Shiner (Stephens and Mayden 1998). The Tricolor Shiner was used to experimentally examine the effects of suspended sediment on reproductive success in the *Cyprinella* group (Burkhead and Jelks 2001).

This study combines methods used in two of the most detailed life-history studies of North American minnows: that of *C. callistia* (Jordan) (Alabama Shiner) by Ferguson (1990) and that of *Pteronotropis hubbsi* (Bailey and Robison) (Bluehead Shiner) by Fletcher and Burr (1992). In addition, our analyses of life-history attributes are based on thousands of specimens sampled over a two-year interval. The large number of specimens analyzed enables greater precision in the delineation of age structure, growth rates, reproductive cycles, and diet.

Methods

Specimens of the Tricolor Shiner used in this study were taken from Hubbard Creek, a tributary of Cheaha Creek, in the Coosa River system, Clay County, AL. Monthly samples were taken from November 1976 to January 1979. In hopes of conducting life-histories studies, H.T. Boschung supervised collection and deposition of 6366 Tricolor Shiners in the University of Alabama Ichthyological Collection (UAIC). Specimens were examined to determine length-weight relationships, growth, reproductive cycles, food habits, and parasites. Habitat and species associates were based on field observations.

Because species were preserved in 10% formalin, aging by otoliths was not possible; the mild Alabama winters also preclude accurate age estimation from scale annuli. Standard length (SL) of 6366 specimens was measured using electronic dial calipers to determine growth rates and estimate age. Annual cohort and ages were estimated by plotting length frequencies in 3-mm size classes, determining the modal size-class for each month, and plotting the mean lengths of all specimens in monthly modal size-classes over the sampling interval. This simple arithmetic weighting technique (Ricker 1975) allowed detection of annual cohorts across the sampling interval and was used to estimate age classes present in the total sample.

To determine reproductive timing and fecundity, 25 representative adult specimens of each sex were eviscerated, and the bodies dry blotted and weighed to the nearest 0.01 g on a top-loading electronic balance. Gonads of both sexes were dissected free, dry blotted, and weighed to the nearest 0.01 g. The ratio of eviscerated body weight/gonad weight were plotted to determine the gonadosomatic index (GSI). Ovaries and ova were assessed for relative developmental states using the methods promulgated by Heins and Dorsett (1986); ovaries were dissected, and the mature and ripe ova counted and measured with an ocular micrometer in a dissecting microscope.

Diet was determined by examination of the anterior alimentary canal of 25 adult specimens from each month over the sampling interval. Highly macerated contents were determined to be immature stages of aquatic insects and identified to the family level using Merritt and Cummins (1996). Additionally, internal and external parasites were noted during anatomical study of specimens.

Fieldwork was conducted in April 2000 in Hubbard Creek to observe characteristics of the species in their habitat, as well as associated species. Spawning behavior was observed while snorkeling at eight different sites over five days. Visibility was good; vertical water clarity was about 1.5 m and horizontal about 3 m. Five total hours of spawning observations were made in 30-minute intervals spanning five days. Spawning habitat and behaviors were documented in field notes after each interval. Nine different spawning assemblages were observed. Species associates were observed as well; these were later collected by seining and returned to the laboratory for identification.

Results

Reproductive season and age and growth

The GSI indicated seasonal variation in both sexes, but only females showed differences sufficient to delimit the spawning season (Fig. 1). In 1977, spawning began in late April and ended in late July or August; in 1978, it may have begun by mid-April and lasted until late August. Specimens

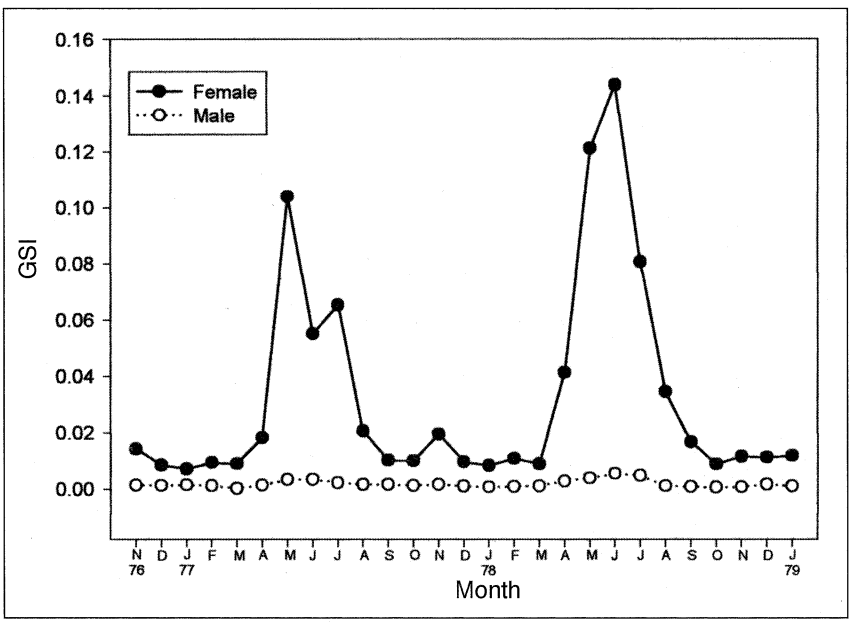

Figure 1. Average male and female gonadosomatic index (GSI) by month for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January 1979. Each point represents the average for that sex for that particular month.

from 1977 showed bimodal peaks, the largest in May and the secondary peak in July. No bimodality was evident in 1978, but relative ovarian mass was larger (Fig.1).

There is concordance between the GSI and the length-frequency distribution (Fig. 2). The young-of-year (YOY) appeared in monthly samples in August in 1977 and 1978, when 9.3 to 14.5-mm SL. The distribution of annual cohorts indicates that four, possibly five, age classes are present: 0, 1, 2, 3, and possibly 4. Plotting of the modal monthly size-classes enables visualization of the natural annual cohorts, especially in 1975 and 1976 (data interpolated). The oldest age-3 fish appear to senesce as early as July (1975) to as late as December (1973), and both putative age-4 fish disappear from the population in July (1973 and 1974; Fig. 2). The hiatus between age-3 and putative age-4 in the 1974 annual cohort suggests the age-4 determination might be valid. These specimens ranged 57.4 to 66.9-mm SL. The length-frequency distribution also suggests that six annual cohorts, 1973 to 1978, are represented in the specimens collected over 27 months from 1976 to 1979.

Growth in both sexes fits a logistic model, with males growing faster and larger than females after about 35-mm SL (Fig. 3). The data fit for both models was exceptional: the relationship between the standard length and the body weight for males ($r = 0.969$, $r^2 = 0.939$) was curvilinear, and was not significantly different from that of females ($r = 0.962$, $r^2 = 0.925$).

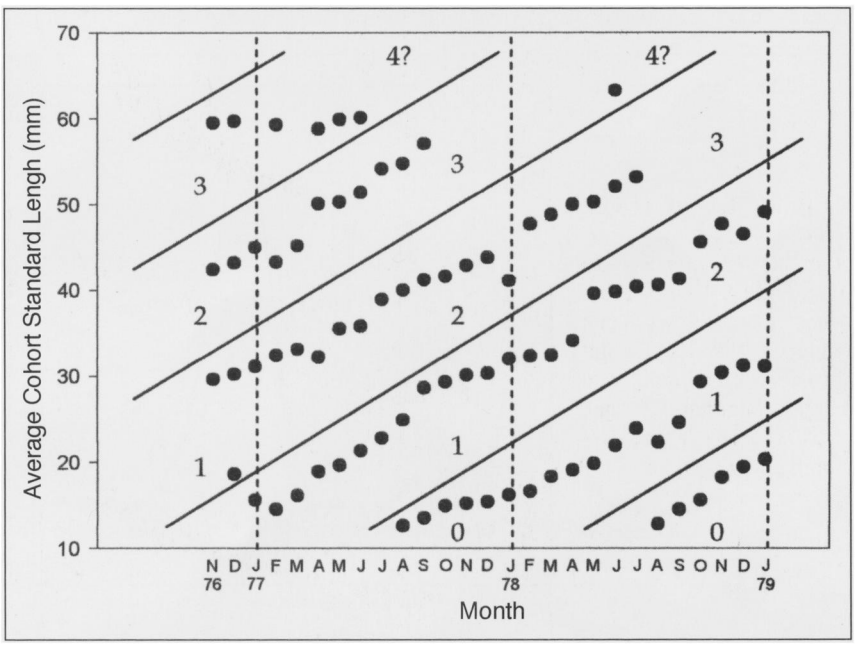

Figure 2. Age cohorts of *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January 1979. Each point represents the average functional age group classes of individuals, and each line represents an age cohort developing over time.

Reproductive cycles

Male GSI began to increase in April of both years, indicating the beginning of the reproductive period. Testes development peaked in May or June and then began to return to normal weight in July or August, depending upon the year. Monthly variations in GSI developmental patterns of males are consistent with the length-frequency analysis indicating recruitment primarily in the months of April through July. Female GSI also began to increase in April of both years, and peaked in May, June, and July. Like males, ovary development decreased precipitously in July and August, indicating the completion of the reproductive season. These data support the observation that breeding begins in April and declines between July and August for both years.

The average number of mature ova per month increased significantly ($p < 0.05$) in April and conversely declined in August (Fig. 4). Fecundity estimates of mature ova of reproductively active individuals ranged from 32.1 to 147.6 (mean = 94.4, SD = 47.2), and size of ova was greater than 1 mm in diameter. Beyond the breeding season, number of ova ranged from 9.5 to 33.6 (mean = 15.2, SD = 5.8), and size generally ranged from 0.20 to 0.25

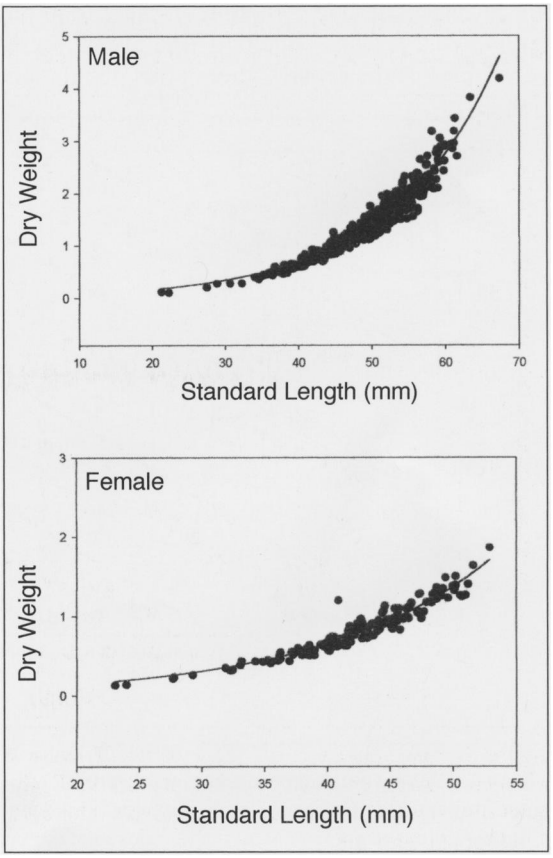

Figure 3. Length-weight relationships for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January 1979. Each point represents the length and weight recorded for each individual over the 27-month study.

mm in diameter. For mature, spawning females only mature and ripe ova sensu Heins and Dorsett (1986) were counted. Some of the ova were classified as ripe and were invariably located at the posterior end of the ovary. Ripe ova were observed in June and July of both years, and size ranged from 1.10 to 1.25 mm (mean= 1.19 mm, SD = 0.05 mm).

The cyclic variation in monthly mean ova diameters paralleled the annual GSI pattern (Fig. 5). Throughout most of the year, ova diameter remained generally constant at about 0.25 mm. During the reproductive season, ova diameter ranged from 0.35 to 1.25 mm and averaged 0.54 mm. The largest ova were observed in June, diameter 0.70 to 1.25 mm (mean = 0.94 mm, SD = 0.32 mm).

Only those females found to be over two years old contained mature ova, suggesting maturity at this age. Males also demonstrated much larger testes size in their second year, indicating they too must become sexually mature at age 2+. Mature males ranged 45 to 55 mm and weighed 1.5 to 2.5 g, while females ranged 40 to 50 mm and weighed between 1.0 and 2.0 g.

Dietary analyses

The diet of the Tricolor Shiner consisted mainly of aquatic insects (Table 1). Typically 0 to 3 food items were found in the stomachs of examined individuals. By far, Dipterans (mainly chironomids) made up the greatest percentage of the species' diet in this population, accounting for 36% of the

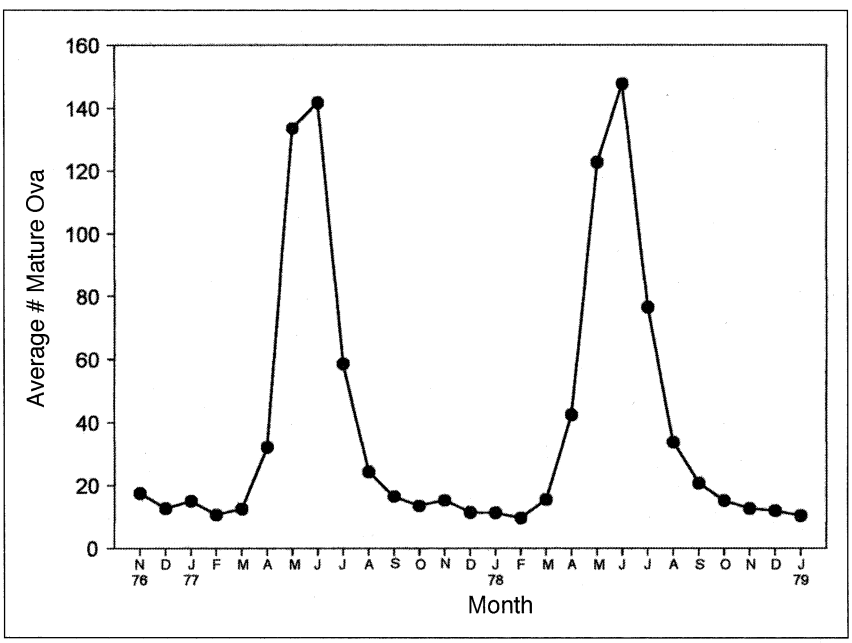

Figure 4. Average number of ova per female produced by month for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January 1979. Each point indicates the average of all the females total eggs produced in that month.

total. Aquatic beetles were also a significant portion of their diet, composing almost a quarter (23%) of total food items consumed. Many other orders of

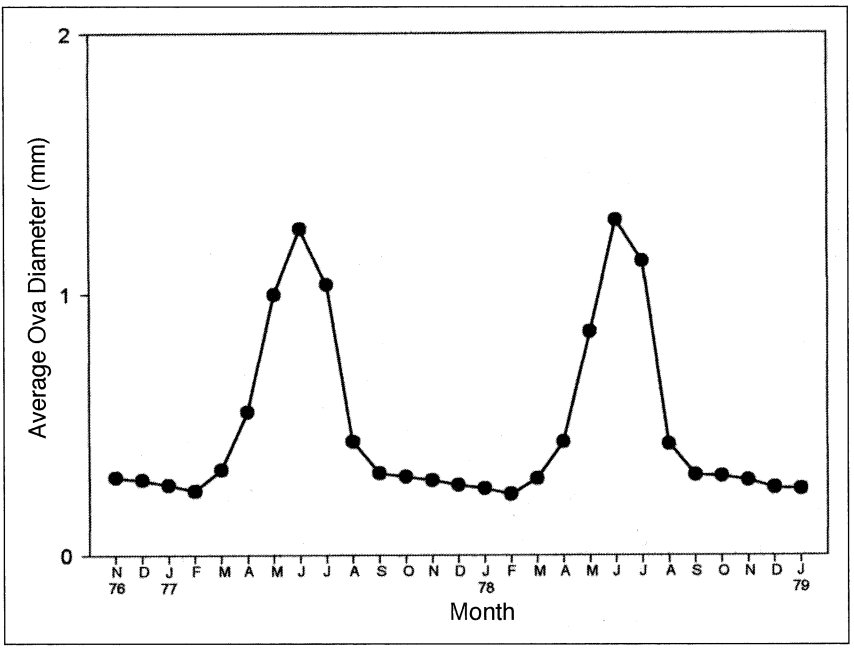

Figure 5. Average ova diameter by month for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January. Each point represents the average size of all the females total eggs produced in that month.

Table 1. Diet composition for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January 1979.

Annelida	Plecoptera	Coleoptera	Plantae
Oligochaeta	Chloroperlidae	Carabidae	Hydrophytes
Arachnoidea	Peltoperlidae	Chrysomelidae	Seeds
Araneae	Perlidae	Curclionidae	Fungi
Hydracarina	Perlodidae	Dytiscidae	Hyphomycetes
Crustacea		Elmidae	Teleostei
Amphipoda	Hemiptera	Gyrinidae	Catostomidae
Insecta	Corixidae	Hydrophilidae	Cyprinidae
Collembola	Gerridae	Psephenidae	
Ephemeroptera	Homoptera	Scirtidae	
Baetidae	Hydrometridae	Hymenoptera	
Baetiscidae	Veliidae	Formicidae	
Ephemeridae	Megaloptera	Vespidae	
Ephemerillidae	Corydalidae	Diptera	
Heptageniidae	Trichoptera	Ceratopogonidae	
Isonychiidae	Hydropsychidae	Chironomidae	
Odonata	Leptoceridae	Culicidae	
Coenagrionidae	Philopotamidae	Simuliidae	
Libellulidae	Lepidoptera	Tabanidae	
Orthoptera	Noctuidae	Tipulidae	

invertebrates and representatives of two families of fishes were eaten, but infrequently (Fig. 6). The diet of Tricolor Shiners differed markedly between seasons. In the spring (March–May), shiners consumed Dipterans much more so than any other type of insect; however, in the summer (June–August), the shiners' diet shifted predominantly to Coleopterans (Fig. 7). In the winter (December–February), Plecopterans played a large role in the fishes' diet, and in the fall (September–November), Dipterans returned as the dominant food source.

Parasitism

All associated parasites on the Tricolor Shiner occurred in one of two groups. Internal parasites consisted exclusively of nematodes found in the intestines. It remains unclear as to whether these worms were actually endoparasites, or perhaps food items. Given their relative size and abundance when found, however, it is believed they were not part of the diet, but rather parasitic on the fish in which they occurred. The other class of parasites observed were all ectoparasitic trematodes found typically on fins, *Posthodiplostomum minimum*, which typically results in "black spot disease" (Post 1987).

Behavioral and ecological observations

Stephens and Mayden (1998) documented 22 discrete reproductive behaviors for the Tricolor Shiner occurring in two classes: aggression and courtship. Field observations of both courting and spawning pairs in April 2000 revealed 6 of these described behaviors (Table 2). The behaviors generally occurred along rocky crevices near the bottom of pools with relatively slow current. The crevices typically overhung a shelf and were usually no more than a few centimeters wide. Male shiners generally aggregated around these shelves and "chased" off any other fish species entering the spawning area. No sneaker males were noted as described by Ferguson (1989) for those in captivity, and no mass-spawning behaviors were observed. Males and females congregated together, and courtship displays were conducted by isolated individuals closer to the crevice shelves than the general assemblage. No feeding was observed by the courting pairs, but other fish not participating in spawning were observed feeding normally. Feeding in the

Table 2. Reproductive behaviors observed for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, during April 2000.

Behaviors	Description
Aggressive	
Butting	Nest defender butts intruder
Nipping	Nest defender bites intruder's anal fin
Chasing	Nest defender chases intruder away from nest site
Parallel swim	Nest defender and intruder swim parallel for a short span
Courtship	
Solo spawn	Male turns vent toward crevice and swims alongside
Spawning	Female swims with vent toward crevice with male behind fertilizing eggs

water column was widespread; however the majority of shiners were concentrated between the middle and top and tended to rely on visual foraging.

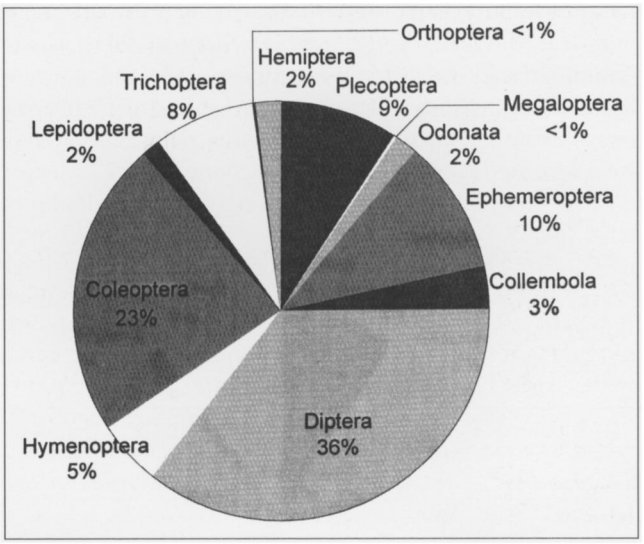

Figure 6. Total insect diet composition for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January 1979.

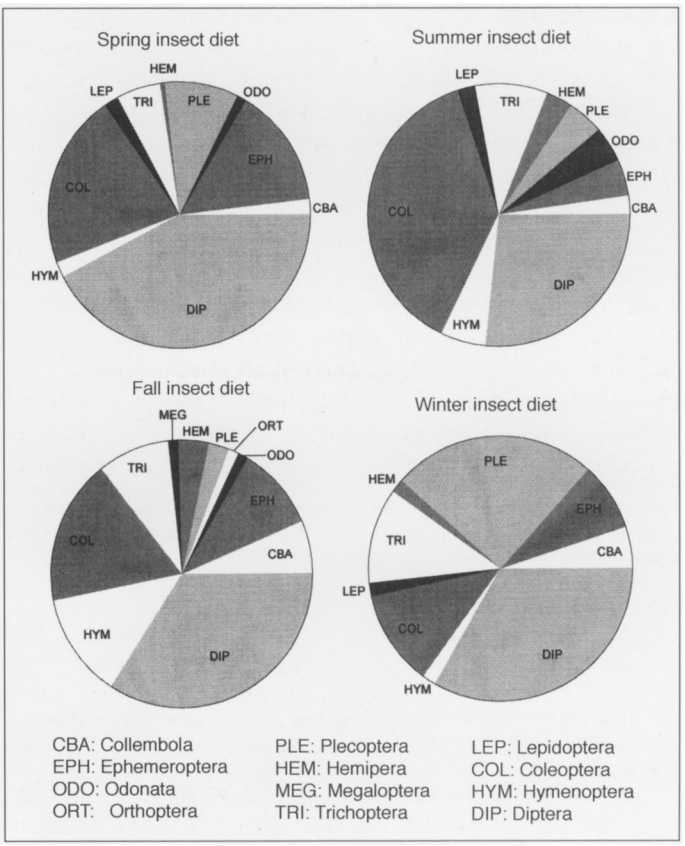

Figure 7. Seasonal insect diet composition for *C. trichroistia* (Tricolor Shiner) from Hubbard Creek, between November 1976 and January 1979.

Habitat and associated species

The typical habitat of the Tricolor Shiner is deep pools in small- to medium-sized clear, cool streams having slow to moderate current (Boschung and Mayden 2004, Etnier and Starnes 1993). These habitats generally include a rocky substrate with crevices, shelves, and outcroppings suitable for breeding activity. The observed habitat of Hubbard Creek consisted of approximately 0.6- to 1-m deep pools with cobble and bedrock substrate. Investigating recorded collections from UAIC, 27 species of fish were found to be located in Hubbard Creek along with the Tricolor Shiner. However, of these species, only a few were ever found in close proximity to the spawning sites of Tricolor Shiners (Table 3). The species collected were typically darters observed near the bottom of the pools, with a few individuals of cyprinids (*Notropis asperifrons*) found in the dense populations of non-courting Tricolor Shiners or nearer to the water surface.

Table 3 Associated species noted for the tricolor shiner, *C. trichroistia*, from Hubbard Creek.

Scientific name	Common name
Cyprinidae	
Campostoma oligolepis Hubbs & Greene	Largescale Stoneroller
Cyprinella callistia Jordan	Alabama Shiner
Cyprinella venusta Girard	Blacktail Shiner
Hybopsis lineapunctata Clemmer & Suttkus	Lined Chub
Lythrurus lirus Jordan	Mountain Shiner
Notropis asperifrons Suttkus & Raney	Burrhead Shiner
N. stilbius Jordan	Silverstripe Shiner
N. xaenocephalus Jordan	Coosa Shiner
Phenacobius catostomus Jordan	Riffle Minnow
Semotilus atromaculatus Mitchill	CreekChub
Catostomidae	
Hypentelium etowanum Jordan	Alabama Hog Sucker
Moxostoma duquesnei Lesueur	Black Redhorse
Ictaluridae	
Ameiurus natalis Lesueur	YellowBullhead
Fundulidae	
Fundulus stellifer Jordan	Southern Studfish
Cottidae	
Cottus carolinae Gill	Banded Sculpin
Centrarchidae	
Ambloplites ariommus Viosca	Shadow Bass
Lepomis cyanellus Rafinesque	Green Sunfish
L. gulosus Cuvier	Warmouth
L. macrochirus Rafinesque	Bluegill
L. megalotis Rafinesque	Longear Sunfish
Micropterus coosae Hubbs & Bailey	Redeye Bass
M. punctulatus Rafinesque	Spotted Bass
Percidae	
Etheostoma coosae Fowler	Coosa Darter
E. jordani Gilbert	Greenbreast Darter
E. stigmaeum Jordan	Speckled Darter
Percina kathae Thompson	Mobile Logperch
P. nigrofasciata Agassiz	Blackbanded Darter

Discussion

Life-history studies are essential in understanding a species' habits, environment, life strategies, and conservation. Using information from length-frequency, cohort analysis, and gonadal development, it is now understood that the Tricolor Shiner reproduces beginning in April–May and ending in July–August, and at any given time there are three (possibly four) separate age classes present in a population. The number and size of ova also follow this temporal pattern of development.

Previous studies have indicated that the Tricolor Shiner diet is comprised of about half terrestrial and half aquatic insects dominated by mayfly nymphs (Etnier and Starnes 1993). The results presented in this study indicate that the diet of the Tricolor Shiner is primarily aquatic macroinvertebrates and directly affected by the season as to what insects were taken and in what numbers. A temporal shift from summer and winter suggest that the shiner is an opportunistic feeder based on the availability of insect larva; however, dipteran midges remain the primary food source for the species throughout the year.

Field observations showed that courtship behavior during spawning followed a ritualistic pattern of antagonistic as well as courtship actions to prevent other males from intruding on nesting sites and attracting females for spawning. These results are congruent with existing literature on the behaviors of crevice-spawning cyprinids.

By interpolating the findings of in this study, the Tricolor Shiner could be used as a surrogate species for conservation models aimed at its endangered relative the Blue Shiner. The degradation of habitat, particularly blue-green algal growth and siltation, along with late maturity and low fecundity have been implicated in the decline in Blue Shiners in much of Alabama (Boschung and Mayden 2004). Burkhead and Jelks (2001) proposed that turbidity disrupted visual cues critical to successful spawning of the Tricolor Shiner, resulting in up to 20% fewer eggs being spawned. This trend would likely be reciprocal in the imperiled Blue Shiner given the similarity of the two species.

Developing life-history studies of species like the Tricolor Shiner is essential to establishing long-term strategies for the preservation of this fish and its habitat, as well as providing a basis of knowledge for the understanding of other similar sympatric species.

Acknowledgments

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