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Life History and Ecology of the Barrens Topminnow, *Fundulus julisia* Williams and Etnier (Pisces, Fundulidae)

Patrick L. Rakes
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Patrick L. Rakes entitled "Life History and Ecology of the Barrens Topminnow, *Fundulus julisia* Williams and Etnier (Pisces, Fundulidae)." I have examined the final electronic 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 Animal Science.

David A. Etnier, Major Professor

We have read this thesis and recommend its acceptance:

Larry Wilson, Gary McCracken, Neil Greenberg

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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LIFE HISTORY AND ECOLOGY OF THE BARRENS TOPMINNOW,

FUNDULUS JULISIA WILLIAMS AND ETNIER

(PISCES, FUNDULIDAE)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Patrick L. Rakes

May 1989

For Kelly, Gretchen and Sean--

may they all appreciate it someday--

and, with my appreciation,

for my parents.

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ABSTRACT

The life history of the Barrens topminnow, Fundulus julisia, was investigated in a two-year study from 1983 to 1985. The species is restricted to a few isolated springs and groundwater-influenced upland streams on the Barrens Plateau in the vicinity of Manchester in middle Tennessee. In most aspects of its behavior, including feeding and reproduction, this topminnow differs little from other members of the same genus. The fish is an opportunistic carnivore, feeding upon aquatic insects, crustaceans, and gastropods, as well as terrestrial insects that fall in the water. Like other killifish, F. julisia has an extended breeding season that is bimodal with peaks in late spring and late summer. The requirement of filamentous algae and/or other aquatic plants for a spawning site and the paucity of permanent upland waters supporting their growth is apparently the primary factor limiting the species to its currently restricted distribution. F. julisia has a maximum lifespan of about three years, but mortality is high among adults following reproduction and relatively few survive to spawn more than once. Mortality is higher in males than in females older than one year, presumably due to selective predation upon the more brightly colored males. Growth in the first year is very rapid when compared to other Fundulus. Possible predators of young Barrens topminnows are numerous, but piscivorous wading birds are probably the primary predators of adults. A potentially serious threat to all Barrens topminnow populations is Gambusia affinis which bears live young continuously throughout the warmer months and tends to grossly outnumber topminnows where the two are syntopic. The mosquitofish has

been known to replace native species elsewhere. Droughts of the past few years as well as habitat alterations by man, especially those affecting groundwater levels, also may endanger the species' survival.

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CHAPTER I

INTRODUCTION

The Barrens topminnow, Fundulus julisia (Figure 1), is a small killifish known only from the Barrens Plateau region of middle Tennessee (Figures 2-3). Specimens were first collected during preimpoundment surveys in the late 1930s at three localities from the Duck River system near Manchester by the Tennessee Valley Authority . These were sent to the University of Michigan Museum of Zoology where Carl L. Hubbs recognized them as an undescribed species. They were catalogued, however, as F. albolineatus, the whiteline topminnow, a closely related species (now presumed extinct) known previously from only a few collections taken from Big Spring, Huntsville, Alabama in 1889. Subsequent collections of Barrens topminnows (BTMs) were made in the 1960s and 70s from headwaters of both the Duck and Cumberland river systems on the Barrens Plateau. No collections of the species have been made from Duck River tributaries since 1964, however, and the species may have been extirpated from that drainage prior to recent reintroductions. All these specimens were considered to be whiteline topminnows until work by J. S. Ramsey, R. W. Bouchard, J. D. Williams, and D. A. Etnier culminated in the description of Fundulus julisia (Williams and Etnier, 1982). The same paper also redescribes F. albolineatus and includes a diagnosis of the subgenus Xenisma which includes these two species as well as F. rathbuni, F. catenatus, F. stellifer, and the recently described F. bifax (Cashner et al., 1988). Further taxonomic treatments of the subgenus include Wiley (1986) and Rogers and Cashner (1987).

Figure 1. Adult Fundulus julisia. Top: 66 mm SL male; middle: 68 mm SL male; bottom: 64 mm SL female. (Top male is an undescribed color morph that was photographed alive by the author to illustrate the green lateral pigmentation which turns blue in preservative; bottom two photos by B. H. Bauer.)(Color prints are provided in ten original copies of this thesis.)

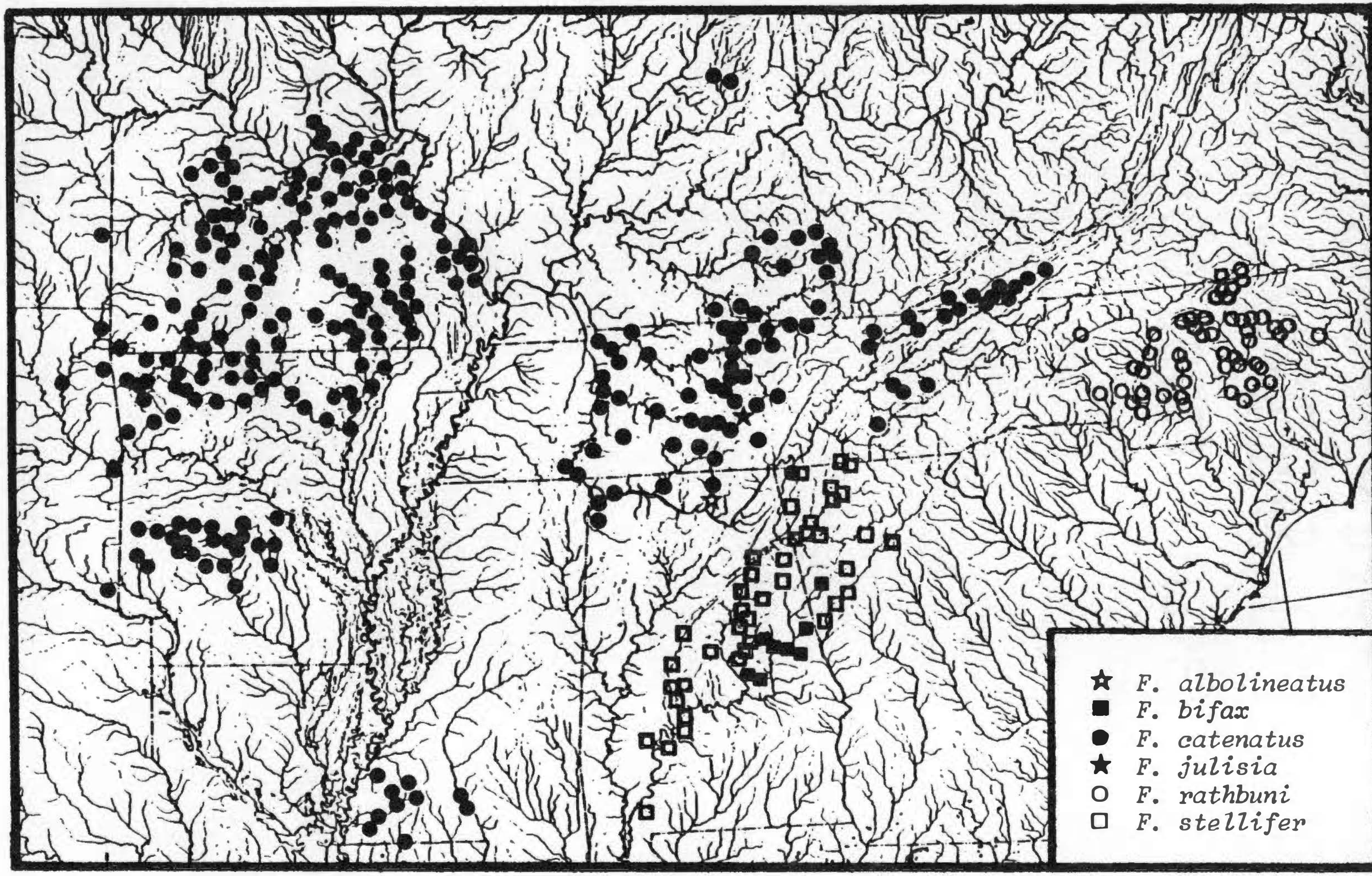

Figure 2. Collection localities for the subgenus Xenisma (modified from Lee, et al., 1980; Bart and Cashner, 1980; and Cashner, et al., 1988).

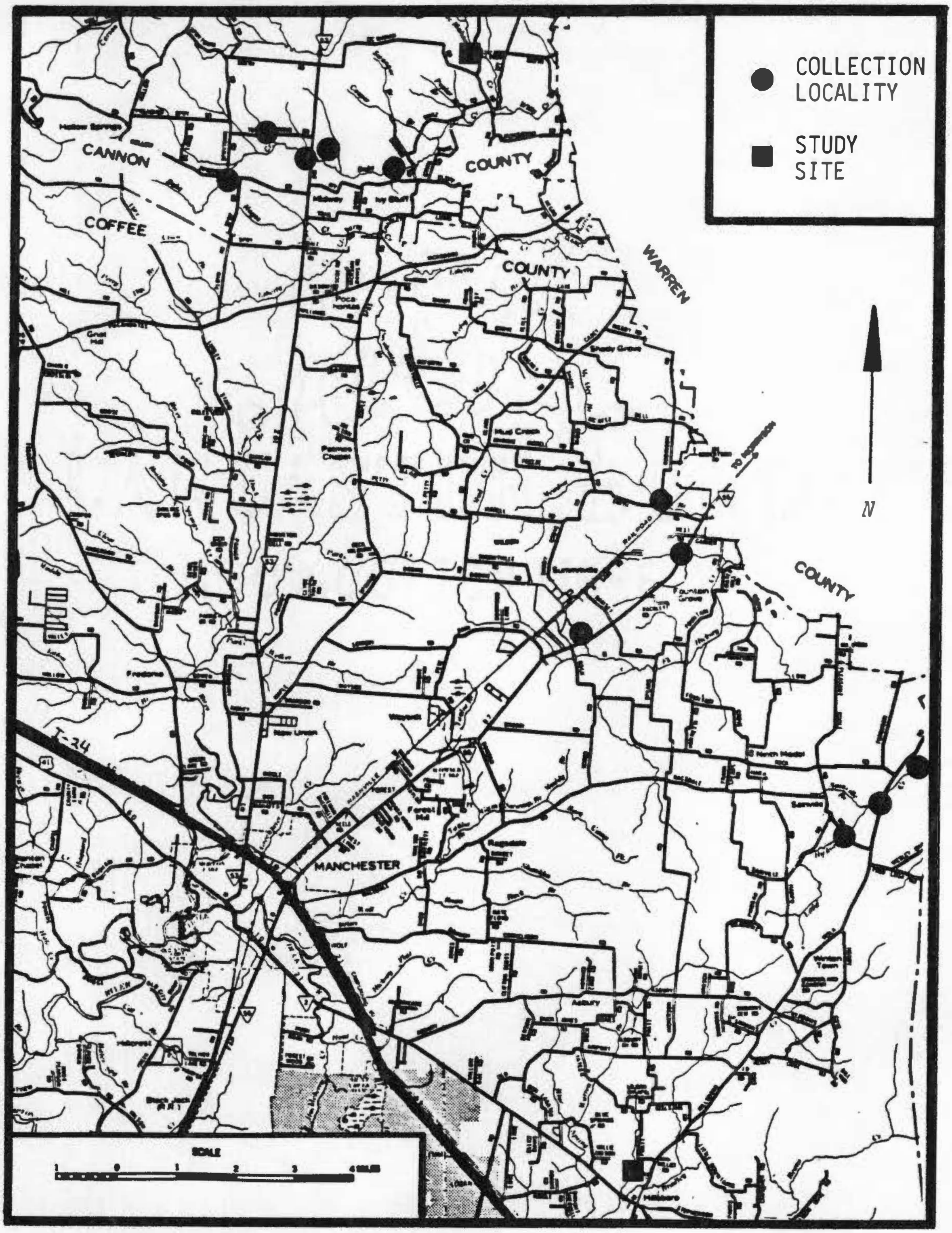

Figure 3. Collection localities for Fundulus julisia on the Barrens Plateau near Manchester, Tennessee (modified from Etnier, 1983).

In 1980 and 1981, late summer droughts threatened to extirpate the (then) only known populations of BTMs in tributaries of West Fork Hickory Creek near Summitville, and 60 individuals were maintained in aquaria by Jeff Prestwich, a Tennessee Wildlife Resources Agency (TWRA) officer, until conditions improved. Since then, several more populations have been discovered in Cumberland drainages and at one location in the Elk River system (Figure 3) during state- and federally-funded surveys conducted by D. A. Etnier. These finds lead Etnier (1983) to consider the species marginally threatened rather than critically endangered. TWRA has entered into cooperative agreements with the landowners of the type locality and an additional locality utilized in this study, and has been pursuing plans for agreements at other localities and to establish new breeding populations in suitable habitats in the Duck and Cumberland drainages near Manchester, as recommended by Etnier (1983). Ten adults were placed in Short Spring (Duck River drainage) in December 1981, followed by 70 adults and 30 juveniles in 1983. One hundred juveniles were also placed that year in nearby Wiley Springs.

As implied above, Barrens topminnows are often associated with springs. Although at first the species appeared entirely limited to pristine, heavily vegetated spring-type habitats, subsequent surveys indicated that the essential habitat characteristics appeared to be permanent waters (or nearby access to them during drought conditions) with slackwater areas and aquatic vegetation or algae (Etnier, 1983). The fish was observed to tolerate fairly high water temperatures and turbidity, predators such as bass and sunfish, and competitors such as *Gambusia affinis* and *Fundulus catenatus* as long as the above habitat conditions were present. The primary limiting factor appeared to be aquatic vegetation which might be required for a spawning substrate. The present study confirms those early suspicions for the most part.

As with many small, economically unimportant freshwater fish, knowledge concerning the biology and life history of topminnow species in the genus Fundulus is fragmentary. Early studies of a few species were mostly anecdotal descriptions of reproductive and other behaviors (Newman, 1907; Mast, 1915; Chidester, 1916; Richardson 1939; Koster, 1948; Carranza and Winn, 1954). Data for many species are still limited to habitat descriptions of collection localities in systematic treatments and general surveys and notes in regional texts (i.e., Pflieger, 1975). More complete data are available for some species in several graduate student theses (Young, 1950; Schmelz, 1964; Byrne, 1976; Kneib, 1976) as well as miscellaneous journal articles (Rice, 1942; Hunt, 1953; Clemmer and Schwartz, 1964; Simpson and Gunter, 1956; Brummett, 1966; Goodyear, 1970; Lotrich, 1975; Kneib, 1978). Only two studies deal with any aspect of the biology of members of the subgenus Xenisma-- an analysis of the food of F. catenatus (McKaskill et al., 1972) and a comparison of meristic and morphometric variation in F. catenatus and F. stellifer (Thomerson, 1969).

By far the most work on the genus has concentrated on a single species, F. heteroclitus, the mummichog, which has been a "white rat" for more diverse studies in physiology, embryology, endocrinology, genetics, geographic variation, and behavior and motor patterns than any other teleost (for recent reviews see Able and Hata, 1984; Atz, 1986; Able and Felley, 1986; Kneib, 1986a; Powers et al., 1986; Taylor, 1986; Weisberg, 1986). As a result, *F. heteroclitus* and other distantly related euryhaline species are frequently the standard against which findings for *F. julisia* are compared since less is known about more closely related interior upland *Fundulus* species. This study adds to that information in general and, more specifically, provides knowledge useful for the preservation of a species whose continued survival appears uncertain.

CHAPTER II

METHODS AND MATERIALS

1. INTRODUCTION

Observations of Barrens topminnows began in October, 1983 when 12 fish were collected from the Elk River population and maintained in aquaria. These fish and two generations of their offspring were observed through October, 1985. Field work began in March, 1984 and continued through October followed by additional field work in March, 1985. Studies were conducted at a number of topminnow localities on the Barrens Plateau, but most work was done at two sites: an unnamed tributary to McMahan Creek on the J.D. Lewis farm in Cannon County (tributary to Witty Creek and the Barren Fork River) and Pond Spring, a 1/4 hectare spring-fed pond on Mrs. Florence Fults' farm near Hillsboro in Coffee County (discharges into Bradley Creek and the Elk River). The sites were visited 12 and 6 times, respectively, in 1984 and once each in 1985.

2. FIELD METHODS

When a helper was available most fish captured for study were taken with a seine (1.8 m x 3 m, 4.8 mm mesh). A large dipnet (4.8 mm mesh) and cylindrical funnel-ended minnow traps constructed from 7 mm square galvanized steel mesh also were employed, primarily when working alone. The traps were `fished' for approximately 24 hours with varying success.

Captured fish were held in 20-liter buckets until they were released or fixed in 10% formalin (and later preserved in 50% propanol). All topminnows were anaesthetized with quinaldine sufficient to render them unconscious within 10-20 seconds (determined by trial additions). The fish were then placed in a shallow dish of water and measured (SL, TL) to the nearest millimeter and inspected to determine sex, presence of abnormalities or parasites, and reproductive condition. Finally, a pelvic fin was clipped with dissecting scissors or its absence noted during a mark-recapture, multiple census at each study locality (5 census samples March 31-June 10 at the Cannon County site, and 6 samples, June 10-October 14 at Pond Spring). More than 700 fish were captured and released during the study. Only 33 were preserved due to the threatened status of the species.

Additional field work consisted of measuring water temperature and pH and noting relative flow rates, water levels, and water clarity, as well as any significant changes in aquatic macrophytes. Aquatic invertebrates were qualitatively sampled by kicking into screens in addition to those taken when collecting fish. Topminnow eggs were sampled by manually searching through clumps of filamentous algae and other macrophytes. Feeding, reproductive, and other behaviors were observed both with the naked eye and the aid of 10 x 50 mm binoculars.

3. LABORATORY METHODS

Data collected in the field during the multiple mark-recapture censuses were used to calculate a modified Schnabel population estimate and 95% confidence intervals (Ricker, 1975) for each study population.

Length and sex data were used to construct length-frequency histograms, calculate sex ratios, and plot average growth. Growth curves and equations were fitted and derived, respectively, by the Slidewrite Plus program (Advanced Graphics Software, Inc., Sunnyvale, CA). Histograms were used to delineate age-classes and calculate survival rates for the Cannon County population (Heinke's combined survival rate) (Ricker, 1975). Scales removed from the right side of the body just anterior to the dorsal fin origin of preserved specimens were used to determine time of annulus formation and to verify ages.

The gut contents of 33 BTMs were examined under a binocular dissecting microscope to determine prey items, which were identified to the lowest practicable taxon. Both the number of prey items in each food category and the category comprising the largest volume (Etnier, 1971) were recorded for each stomach. This procedure was also performed on a few individuals of other syntopic species (Lepomis cyanellus, Gambusia affinis, and Hemitremia flammea). Mature and immature ova were counted and recorded from the ovaries of four gravid female BTMs.

Fish were held in the laboratory in a number of different aquaria with varying substrates and macrophytes to observe feeding behavior and inter- and intraspecific interactions (concurrently held species were F. catenatus and Ambloplites rupestris). Most breeding behavior was observed in 38-liter aquaria that were bare except for spawning mops made from yarn. Photoperiod was controlled at varying ratios and water temperature maintained at at least 21 C. Food consisted of Tetramin flakes, frozen brine shrimp, live freshly-hatched brine shrimp, mosquito larvae, and chopped worms.

Eggs recovered from mops were incubated in Petri dishes in either a solution of methylene blue or the presence of filamentous algae (*Pithophora* or *Cladophora*) to retard fungal infections. Development of eggs and fry was observed and recorded using a binocular dissecting microscope and photographed with an attached 35 mm camera. Older fry were anaesthetized with quinaldine as described above to immobilize for measurements and observations. Terminology used to describe early development followed Hardy (1978).

CHAPTER III

STUDY AREA AND HABITATS

As noted above, Fundulus julisia is limited to the Barrens Plateau region of middle Tennessee, a portion of the Highland Rim or Interior Low Plateau geomorphic province as defined by Fenneman (1917) and more specifically the Highland Rim section of this province (Theis, 1936). The Highland Rim Plateau in this area is characterized by low relief--elevations mostly around 300 to 330 m above sea level-- and is sharply defined to the east and west by the escarpments ascending to the Cumberland Plateau and descending to the Nashville Basin, respectively.

Theis (1936) describes the strata underlying the area which produces its plateau character as all calcareous and/or siliceous for the most part and Mississippian in age. The uppermost layers, the St. Louis limestone and Warsaw formations, consist mostly of limestone, and weather to produce fertile soils. The Fort Payne chert formation underlying these, however, produces very unproductive soil. This stratum is exposed on much of the Barrens and thus seems to be responsible for the name of the area.

As might be guessed from the low relief and calcareous nature of the underlying rock, streams draining the plateau generally flow in broadly rounded, shallow valleys and Karst topography is common. Streams which are tributaries to three separate major drainage systems are weakly divided from one another on the Barrens Plateau. The Duck River receives those flowing generally southward and westward, the Elk River those which flow mostly south from the southeast edge of the plateau, and the Barren Fork River those flowing east and north. As noted earlier, all extant populations of BTMs are known only from streams in the Barren Fork system except for the Pond Spring population. Field work was initiated at several of these sites and then concentrated at the tributary to McMahan Creek on the J.D. Lewis farm due to the size and easy accessibility of the population there. The Pond Spring population was also studied intensively for the same reasons as well as to ascertain a precise estimate of its size and whether it differed in any respects from the other populations.

The habitats at the two principal study sites are different in nearly every respect except for their strong groundwater influence and open aspect. The McMahan Creek tributary is a small brook about 150 m in length that arises from a small spring-fed pool. The upper 100 m of the brook consists of alternating pools and riffles with a variety of substrates-- bedrock, chert gravel, and mud and silt. The brook empties into a small mud-bottomed stock pond (about 10 x 15 m). Water leaving the pond is diverted into and travels about 50 m down a narrow, overgrown roadside ditch to McMahan Creek. On the opposite side of the road, however, what appears to be the old lower section of the brook continues in a straight line to McMahan Creek. Although this section held water and several fish species, including BTMs, during the first part of the study period, it later dried up completely while the upper section of the brook continued to have a weak flow. Water levels at this time contrasted markedly with an early spring flash flood that I witnessed in which water rose more than a meter at the mouth of the brook and the pond overflowed across the road into the lower section of the brook. Several alterations in brook morphometry were noted following this flood and on two other visits as well, probably suggestive of the frequency of such events.

Under normal conditions, water depth in most sections of the brook seldom exceeds a few centimeters (15-20 cm in some pools) and the stock pond is mostly shallow with a maximum depth of around 1.5 m. The temperature range observed during the study period was 12 C in March and 33 C in August. Aquatic plants include rushes (Juncus) lining the brook and pond and false loosestrife (Ludwigia palustris) in the upper sections of the brook. Filamentous algae (Cladophora and Pithophora) flourishes in all sunlit waters of the tributary and nearly completely clogged the stock pond and many pools in the brook at times. Two somewhat unusual characteristics of this habitat also are notable. Water at the source of the brook was quite acidic (pH 6 or less) during the study period but pH gradually increased to 7.0-7.5 or more by the time it reached the stock pond and McMahan Creek. No fish were ever found in the upper sections of the brook with pH less than about 6.2. Finally, both the brook and stock pond were heavily used (and polluted) by a herd of dairy cattle, especially during the hotter months.

Pond Spring is a large (about 1/4 hectare) inundated sinkhole with a mostly calcareous sand and silt substrate perforated in a number of areas by strongly upwelling groundwater. No permanent streams enter the pond, but the outflowing stream produced by its springs is quite impressive (several cubic m/sec). Depths are mostly 20-50 cm, but range as deep as 2 m in one area. Due to the large volume of groundwater constantly entering the pond, water temperatures may vary enormously between areas at any given time-- once from 19 C near upwellings to 30 C or more in the shallows. The pH in most of the pond was usually quite basic (pH 8 or more), but sometimes approached 7.5 in an entering streambed.

The pond is partially surrounded by a stand of trees, but the watershed above it consists mainly of row crop fields. During periods of substantial rainfall most of the pond becomes turbid with suspended silt washed in from the fields. The pond banks are lined with grasses and rushes (Juncus) with pondweed (Potamogeton) abundant in the warm, shallow margins around the perimeter of the pond. Eelgrass (Valisneria americana) occurs in the outflow which is surrounded by dense stands of bur reed (Sparganium). Hornwort (Ceratophyllum) is common in the pond and outflowing stream and mats of stonewort (Chara) are typically found around the upwellings. Filamentous algae (Cladophora and Pithophora) flourishes everywhere, sometimes so extensively that it piles up in a thick, rotting mat near the outflow.

CHAPTER IV

RESULTS AND DISCUSSION

1. GENERAL CHARACTERISTICS AND BEHAVIOR

Fundulus julisia is physically described in detail in Williams and Etnier (1982). A notable addition to that description, discovered in both populations in this study, is a different color morph seen in nuptial males. Rather than a pale yellow submarginal and black marginal band on the caudal and posterior dorsal fins (Figure 1, page 2), the fins of these males are translucent blue basally with white and orange submarginal bands and a black margin. In all other respects pigmentation is the same. These morphs were actually more numerous than the yellow-finned ones in the two principal study areas. Like most species of *Fundulus*, female BTMs are much less colorful (Figure 1) than males, appearing mostly dull olive though with yellow-tinged ventral fins. Males are much brighter during the breeding season, but even juveniles can be distinguished from females by the iridescent green coloration on their sides.

In many aspects of their general behavior (feeding and reproductive behaviors are discussed in later sections), Barrens topminnows are similar to other members of the genus *Fundulus*, preferring shallow, slackwater habitats rather than flowing or deep waters most of the time. The only times BTMs were observed in riffles or other areas with current or in deep waters was when they were taking flight to escape a predator (or observer) or attempting to maintain position during a flood. They do, however, seem to possess a strong urge to migrate upstream, at least in the spring, as evidenced by their occupancy of primary streams and springs, appearance in temporary waters above permanent ones, and tendency to leap from aquaria and holding buckets where water flows in (traits observed to be shared by a frequently syntopic species, *Hemitremia flammea*). Unless fleeing or chasing prey, BTMs move about leisurely, often pausing to inspect the surface or substrate, suspended motionless except for their pectoral and caudal fins which constantly wave at the margins.

Social behavior varies both with ontogeny and season. Larvae and juveniles aggregate in large, loose schools in very shallow waters (less than 5 cm) when feeding and move together in dense schools when frightened. Agonistic behavior was observed among even the youngest larvae, but was short-lived and infrequent, even "playful" in appearance. With the advent of the breeding season in March, schooling behavior ceases for the males, which aggressively maintain some distance between one another. Females and small males continue to aggregate, but generally in much smaller groups (2-10). All fish are relatively less wary during the warmer months, but by the end of the summer (September) they again become what can only be described as furtive, with all fish maintaining tight schools that effectively telegraph any disturbance such as an observer's movement. When behaving this way the fish were difficult to observe and were collected very effectively with minnow traps.

Individual fish fleeing from collectors were frequently observed burying into detritus or algae as well as leaping from the water, sometimes onto emergent plants or land before flipping back into the water. Much of the time they would also dart a short distance and then freeze and were very difficult to see, camouflaged by their olive coloration against the usually algae-coated substrate. Schooling, burying, and leaping out of the water are all predator escape behaviors observed in numerous other cyprinodontids (Minckley and Klaasen, 1969; Goodyear, 1970; Colgan, 1974).

2. DIET AND FEEDING BEHAVIOR

Like most topminnows in the genus Fundulus, Barrens topminnows were found to be carnivorous. As Atmar and Stewart (1972) have reported for F. notatus and McCaskill et al. (1972) for the closely related F. catenatus, BTMs were found to be opportunistic, unspecialized predators, taking whatever aquatic invertebrates were available (Table 1). Crustaceans and immature aquatic insects dominated the diet approximately equally by volume for the sum of all fish examined (Figure 4).

The taxa in Table 1 generally reflect the most precise practicable identifications. Gastropods examined were tentatively identified as physids and planorbids due to partial digestion and disintegration of the shells. Physella spp. and Planorbella sp. were collected at the Lewis population locality. Among the crustaceans, isopods and amphipods were also generally too well digested or too young to identify (Lirceus fontinalis and gammarid amphipods were collected from the Lewis locality). No attempt was made to precisely identify smaller crustaceans, but the most common copepods were harpacticoids along with a few

Table 1. Gut contents of juvenile and adult *Fundulus julisia* from two localities.

	Lewis Population						Pond Spring Population						All Fish					
Age Group:	J[1]			A[2]			J			A			J			A		
Food Category	%C[3]	%F[4]	%D[5]	%C	%F	%D	%C	%F	%D	%C	%F	%D	%C	%F	%D	%C	%F	%D
Gastropoda	<1	25	--	2	10	10	--	--	--	1	14	--	<1	14	--	2	18	6
Crustacea																		
Isopoda	--	--	--	--	--	--	--	--	--	11	43	43	--	--	--	3	18	18
Amphipoda	<1	12.5	--	<1	10	--	--	--	--	18	29	29	<1	7	--	5	18	12
Ostracoda	2	62.5	--	36	40	10	8	50	--	54	71	14	3	36	--	42	53	12
Copepoda	29	75	22	6	50	--	17	83	17	3	29	--	28	79	20	5	41	--
Cladocera	31	50	11	12	40	10	43	83	33	2	29	--	33	71	20	9	35	6
Aquatic Insects																		
Odonata	--	--	--	--	--	--	2	17	17	--	--	--	<1	7	7	--	--	--
Trichoptera	--	--	--	<1	10	10	--	--	--	--	--	--	--	--	--	<1	6	6
Diptera																		
larvae	16	87.5	56	30	90	30	27	50	33	8	56	--	18	71	47	22	76	18
pupae	<1	25	11	5	60	--	--	--	--	3	43	14	<1	14	7	4	53	6
Others	--	--	--	2	30	--	--	--	--	2	29	--	--	--	--	2	29	--
Terrestrial Insects	<1	12.5	--	5	60	30	1	17	--	--	--	--	<1	14	--	4	35	18
Miscellaneous	20	25	--	2	20	--	2	17	--	--	--	--	17	21	--	1	12	--
Number of guts examined which contained food	8			10			6			7			14			17		

[1] J = Juveniles; 9-25 mm. SL.
[2] A = Adults; 26-75 mm. SL.
[3] %C = Percent composition of the total number of food items found in all guts examined.
[4] %F = Percent of guts that contained food category (percent frequency of occurrence).
[5] %D = Percent of guts in which food category was dominant by volume.

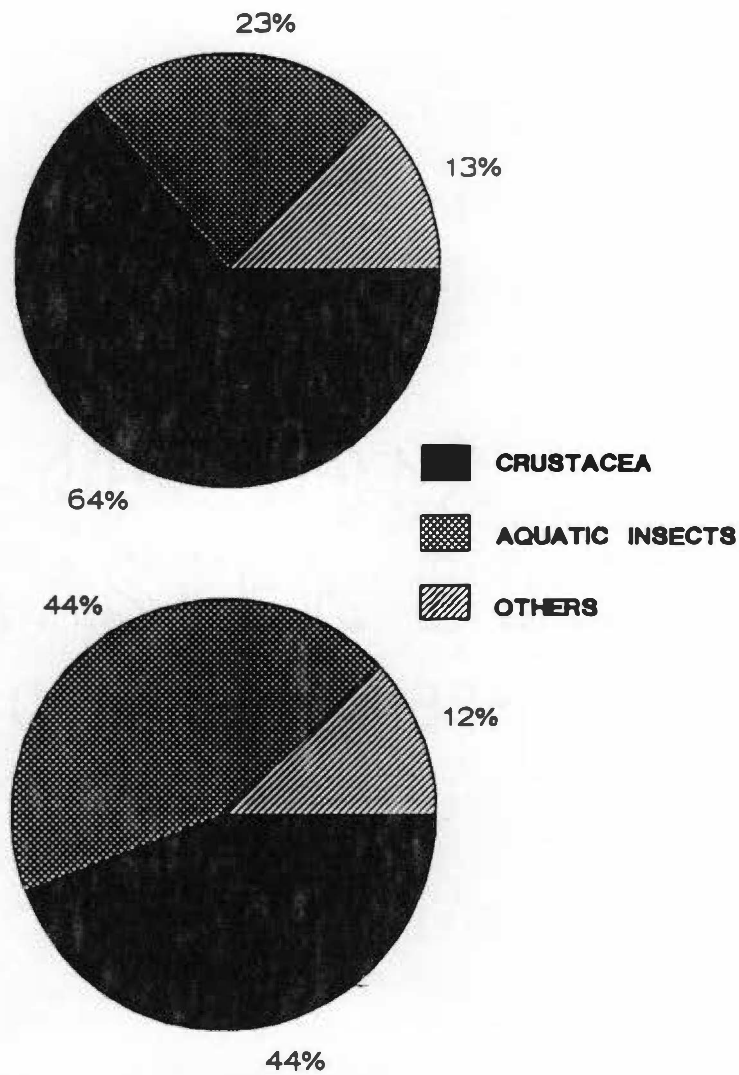

Figure 4. Summary of gut contents of all <u>Fundulus julisia</u> which were examined. Top: percent composition of the total number of food items found in all guts; bottom: percent of guts in which the food category was dominant by volume.

cyclopoids, and nearly all cladocerans were chydorids. Most immature aquatic insects were not identified more precisely than order, except for dipteran larvae which consisted entirely of chironomids (Chironomus, Polypedilum, Tanytarsus, and Procladius spp.) and ceratopogonids (Palpomyia spp. and Atrichopogon sp.). Other aquatic insects included caenid and baetid ephemeropteran larvae, a corixid nymph, an adult elmid beetle, a dytiscid beetle larva (Laccophilus sp.), and an arctiid lepidopteran larva (possibly terrestrial). Terrestrial insects consumed included carabid beetles, one zygopteran, numerous ants, and various adult dipterans. Miscellaneous prey included rotifers, nematodes, flatworms, and what appeared to be more than 90 extremely small flatworms in one gut.

Although none were found in any guts examined, it seems likely that topminnows would also prey upon eggs and fry of their own and other species if given the opportunity. Cannibalism was observed in aquaria-held BTMs and studfish and has been noted for other species of Fundulus (Newman, 1907; Carranza and Winn, 1954; Able and Hata, 1984), although such behavior is probably less frequent under natural conditions. Studfish have been found to consume small fish (McCaskill, et al. 1972) and in Pond Spring massive numbers of Hemitremia and Gambusia fry were observed from early spring to early summer (when only one BTM was killed).

A few guts contained varying quantities of filamentous algae, probably ingested with prey items taken from masses of algae. Only two guts were found to be empty, one of which was from a female whose tail had been cut off just anterior to the peduncle presumably by some predator many hours earlier judging from the fungal growth on the wound.

Since relatively few guts were examined, any generalizations based on the data collected are tenuous, but a few conclusions based upon the diet of those Barrens topminnows which were examined can be offered. The juveniles examined (<25 mm SL) relied primarily upon midge larvae, which most frequently constituted the greatest volume in guts, and small crustacea, which were the most numerous item consumed (Figure 5). Those in the Lewis population contained mostly dipteran larvae and pupae by volume, whereas the Pond Spring population contained roughly equivalent volumes of immature aquatic insects and crustaceans (Figure 6). The smallest juveniles examined (9.5 - 13.5 mm SL) contained almost entirely small crustaceans. This could be due to gape size limitations, but possibly of equal or greater importance is the tendency of BTMs this size to remain very near or at the surface and any available cover (observed both in aquaria and the field). Larger juveniles ranged widely in depth, frequently spending the majority of their time near the bottom. This correlates well with the predominance of midge immatures in most guts of fish this size, though some individuals still consumed mostly crustaceans, an indication of opportunistic feeding habits and/or the possibility of differing hunting strategies or search images among different individuals.

Adult and larger juvenile Barrens topminnows (>25 mm SL) consumed a wider variety of larger prey items and exhibited more marked differences between the two populations (Figure 6). Guts of individual

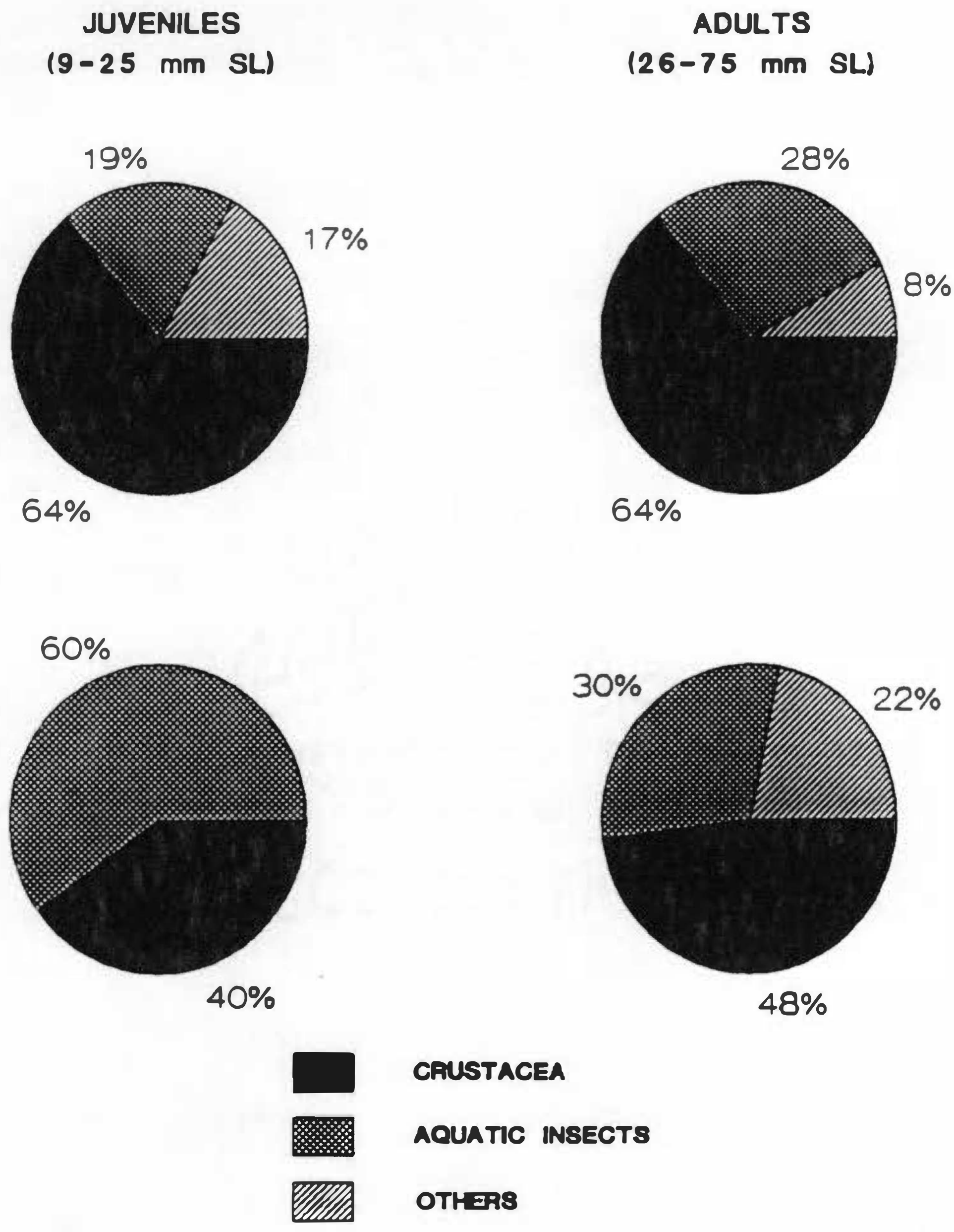

Figure 5. Variation in gut contents between juvenile and adult <u>Fundulus julisia</u>. Top: percent composition of total number of food items found in all guts; bottom: percent of guts in which food category was dominant by volume.

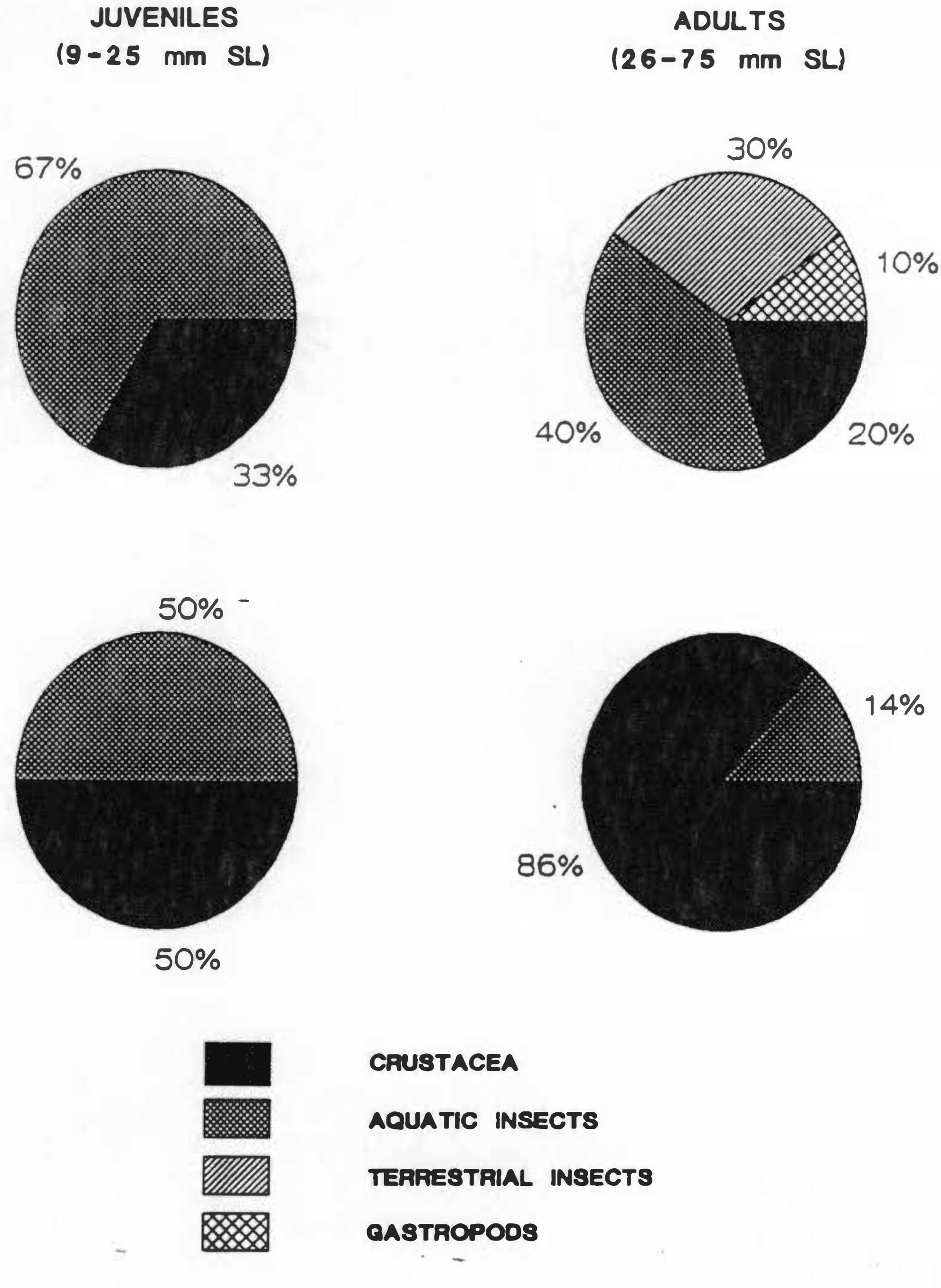

Figure 6. Variation in gut contents between juvenile and adult *Fundulus julisia* from two populations. Top: Lewis population; bottom: Pond Spring population (percent of guts in which food category was dominant by volume).

adults contained a wide variety of food organisms, often both benthic and terrestrial (presumably taken from the surface). As with smaller fish, differences between individuals were also often great-- one 68 mm SL female consumed five of the six gastropods found in adult guts, for example. The same female also contained the 20 mm hind limb of a grasshopper which would have been far too large for her to consume whole. Topminnows were observed several times in the field attacking insects on the surface that were too large to consume. Most frequently, however, large topminnows were observed feeding on the bottom or on submerged vegetation.

As can be seen in Figure 6, there was a substantial difference in the prey items consumed in greatest volumes between the Pond Spring and Lewis populations. The Lewis fish consumed mostly insects, while the Pond Spring fish consumed mostly large crustaceans (isopods and amphipods). This is entirely reasonable given the differences between the two habitats and an opportunistic feeding style. The Lewis population's brook habitat probably presents a far greater relative availability of aquatic and terrestrial insects compared to Pond Spring which supports large populations of isopods, amphipods, and ostracods. However, individual preferences among the fish examined may confound such broad generalizations based on data derived from such a small sample.

A final preliminary finding of possible significance and worthy of further study was discovered while examining the gut contents of a few *Gambusia* and *Hemitremia* juveniles (< 20 mm SL) from Spring Pond. *Gambusia* were found to rely heavily upon copepods which were the dominant food item by volume in all guts examined (n=4). *Hemitremia* guts were nearly equally dominated by ostracods (n=4). These were surprisingly different from the same size class topminnow guts which contained a wide variety of prey items (Table 1, page 19). Food resource partitioning or specialization by these syntopic species in Pond Spring could be indicated by these findings.

Barrens topminnows held in aquaria are quick to adapt to feeding upon frozen foods such as brine shrimp and dry flake food and may be quickly reinforced to anticipate feeding by visual and auditory cues. In the lab, larvae were fed freshly-hatched live brine shrimp for the most part, but they would feed on fine dry food as early as three days after hatching (<8 mm TL). In aquaria and in the field, topminnows of all sizes will sample any small item that might be edible, whether stationary, moving on the surface or bottom, or drifting in the water. Although fast-moving prey are captured in a rapid dash, their typical method of prey capture involves slowly approaching a potential prey item, followed by a stationary pause to inspect it before biting or inhaling it.

3. REPRODUCTION

Breeding Season

The breeding season of Barrens topminnows was found to be comparable to other *Fundulus*. At the Lewis locality males were observed in breeding colors as early as mid-March at water temperatures of 14-15 C. Eggs were stripped from females and collected from clumps of algae in mid-April in water that was 18 C. Judging from the intensity of male coloration and the number of gravid females collected, spawning activity peaked in late May and early June in water as warm as 30 C, but a few individuals spawned as late as early August at the Lewis locality as evidenced by newly hatched fry collected in mid-August. At the Pond Spring locality, length-frequency data (discussed below-- see Figure 8) suggests that spawning may not terminate until an even later date (late August or September). Findings for many other temperate *Fundulus* indicate similarly long breeding seasons (Koster, 1948; Carranza and Winn, 1954; Greeley and MacGregor, 1983; Kneib, 1986b).

Considerable disagreement has existed as to whether photoperiod or water temperature is the primary factor initiating reproductive activity in *Fundulus* spp. Work in the last decade or so (reviewed by Taylor, 1986) indicates that both stimuli are critical in *F. heteroclitus*. Long days are essential for sexual maturation, but warm enough temperatures are also required. Prior exposure to both short days and low temperatures enhances the response to increases in both, and low temperatures may be required for early oocyte development. Furthermore, members of this species, as has been reported for *F. grandis* by Greeley and MacGregor (1978, 1983), usually undergo at least a temporary gonadal regression at some point in the breeding season before favorable photoperiods and water temperatures decline, although lower water temperatures and perhaps enhanced nutrition can delay or prevent this "refractory period".

Although this study did not determine the minimum temperature at which *Fundulus julisia* will spawn, water temperatures in BTM habitats exceeded 12 C a month or more before spawning began and were generally

15-20 C when evidence of spawning was first observed. In the laboratory, fish maintained at room temperature (19-24 C) on a 12:12 hour light:dark photoperiod would not spawn while, simultaneously, fish of the same age and condition and at the same water temperature would do so on a 13:11 or 15:9 hour cycle. Field observations of breeding coloration suggest that breeding activities may be initiated as soon as daylength exceeds 12 hours.

Termination of spawning activities may be due ultimately to declining photoperiod, but a more direct cause for cessation in individual fish (and eventually most of a population) seemed to be loss of condition. As noted above, this may be due to a "refractory period" that is endogenously controlled or induced by high water temperatures, or it may simply be due to inadequate nutrition to maintain reproductive condition in warmer water. Both field and laboratory observations of F. julisia strongly suggest that loss of condition and even mortality may frequently follow a mature individual's reproductive efforts well before the end of the breeding season or similar lab-induced conditions. Length-frequency analyses discussed below revealed that relatively few individuals survived through more than one breeding season and that mortality was greatest following a major reproductive peak. Also notable was the much lower mid-summer mortality and greater late-season reproduction at Pond Spring, perhaps a consequence of lower water temperatures and/or greater food availability than at the Lewis locality.

When fish were induced to spawn in aquaria, individual females produced eggs for a period of about a month, following which they rapidly declined in condition, becoming emaciated and frequently dying despite a constant and abundant supply of food. The constant stress of courting males seemed to be largely responsible, since females isolated at the first signs of decline were able to recover their former condition. Though males remained in good condition for much longer during breeding conditions, dominant males tended to decline and be replaced by subordinates, after which they also died if not isolated.

Again, these observations suggest that the overall stress and energy expenditure of reproduction is the ultimate cause of the termination of breeding activities and often the mortality of individual topminnows. It also would explain the observed bimodal pattern of reproductive activity seen in each population, a pattern noted and similarly explained by Kneib (1986b) in southern but not in northern populations of _F. heteroclitus_. If this hypothesis is correct, Barrens topminnows are largely annuals in their reproductive life history, with only a small percentage of the population reproducing more than once. It might also be one reason why populations are clustered around areas of strong groundwater influence where water temperatures are more moderate during the summer months, possibly decreasing this tendency.

Spawning Sites

Although Barrens topminnows appear relatively opportunistic in the choice of a spawning site, egg morphology, habitat, and field observations suggest that submergent vegetation-- especially filamentous algae-- is the most preferred and ultimately successful site. In aquaria, attempts at spawning were observed virtually everywhere, but eggs were only recovered (and actually observed being deposited) in mops set at various depths. Most eggs recovered in the field were located in clumps of filamentous algae at or near the surface ($n<20$). Exceptions included a number of dried-out eggs exposed above the water line on emergent sedges and attached algae and a few eggs deposited in a mass of grass roots on the edge of a bedrock-bottomed pool with no other vegetative cover.

These observations coupled with the egg morphology of BTMs strongly suggest that the availability of successful spawning sites may be the primary ecological factor limiting the distribution of the species. As described in more detail below, eggs are initially colorless and translucent with a few long chorionic fibrils that act as anchors to the spawning substrate. When exposed or attached to a uniform substrate, the eggs are highly visible (and therefore vulnerable to predation). Entangled in vegetation, they can be fairly difficult to see, but they are almost perfectly camouflaged in clumps of filamentous algae which generally trap thousands of air bubbles generated in photosynthesis. The eggs are so similar to air bubbles that they are indistinguishable visually and finding them requires handling the algae until all the air bubbles have burst. It thus appears strongly possible that _F. julisia_ eggs have evolved to resemble trapped air bubbles. This alone may explain why the species seems tied to open sunlit waters with abundant algal growth. The concentrations of all BTM populations in upland waters with a reliable groundwater supply is also likely a result of another factor affecting successful spawning site selection.

Eggs deposited near the surface in vegetation require a steady water level to survive. Although deposition of eggs in free-floating mats of algae alleviates this requirement, these are largely limited to sunny areas-- springs, spring runs, and associated upland bogs.

Another observation relevant to spawning site selection concerns the susceptibility of the eggs to fungi. In the laboratory, eggs that were not incubated in a fungal inhibitor such as methylene blue or acriflavin frequently were killed by fungi. If, however, they were incubated in the presence of some of the filamentous algae (Cladophora or Pithophora) from the field, fungal infections were never observed on fertilized eggs. That this alga may somehow inhibit fungal growth seems further supported by the observation that it also tended to prevent the establishment and growth of any other algae in aquaria. This has also been reported under certain conditions both in aquaria and in the field by Fitzgerald (1969).

Finally, it is interesting to note the extreme divergence in spawning sites between F. julisia and another member of the subgenus Xenisma, F. catenatus, which spawns in fine gravel and sand and has associated differences in egg morphology (many short, adhesive chorionic fibrils that trap and completely coat the egg with sand grains).

Fecundity

Due to the extremely few individuals killed and dissected and the unavailability of other preserved specimens, seasonal gonadal indices for Barrens topminnows could not be obtained. Four mature females (39-68 mm SL) killed during the spawning season had an average of 83 (65-

106) mature and 201 (120-250) immature ova, but as Kneib (1978) noted, ova counts do not yield actual fecundity estimates without data on spawning frequency and duration for fish which spawn repeatedly over a long breeding season. Kneib and Stiven (1978) calculated actual fecundity for *Fundulus heteroclitus* and noted that it was about double the total number of oocytes found in females in spawning condition. If that conclusion is valid for *F. julisia*, then the four females mentioned above could potentially have produced an average of more than 500 ripe ova during the breeding season.

Most individuals in aquaria (40-45 mm SL) only produced a dozen or so eggs that were collected from the mops before the fish lost condition, presumably due to the stress of close confinement with aggressive males. However, one female produced at least 60 eggs over about a two week interval and another produced 17 eggs in a single episode (1-6 was the normal number). Based on these observations and the ova counts noted above, it seems likely that under less stressful natural conditions a conservative estimate of actual fecundity exceeds 300 eggs over the breeding season.

Other species of *Fundulus* vary enormously in fecundity. Taylor (1986) reported that *F. heteroclitus* females may produce 100-300 eggs per day for 3-5 days in just one of the early semilunar cycles during the spawning season. *Fundulus majalis* is similarly fecund, producing as many as 800 eggs (Newman, 1909). At the opposite end of the continuum are species such as *F. luciae* which rarely contain more than 20 mature ova (Kneib, 1978). *Fundulus julisia* seems similar in many respects to

F. diaphanus (200-250 mature ova)(Hardy, 1978). Both deposit a few eggs at a time in vegetation and their egg morphology is very similar.

Reproductive Behavior

General observations. Observations of reproductive behavior both in aquaria and in the field revealed that Barrens topminnows exhibit motor patterns that are similar in most respects to those observed in other *Fundulus* (Newman, 1907; Richardson, 1939; Koster, 1948; Carranza and Winn, 1954; Able and Hata, 1984) and even in more distantly related cyprinodontids such as *Cyprinodon* (Barlow, 1961; Echelle, 1973) and the aplocheilid cyprinodontiform, *Nothobranchius* (Haas, 1976). A recent definitive description of reproductive behavior of members of the *F. heteroclitus*-*F. grandis* complex (Able and Hata, 1984) is strikingly similar to much of the behavior observed in this study and Able and Hata's ethological terms (capitalized) will be employed here. Most behavior was observed in aquaria and, as will be noted, probably differs in some respects from that in natural conditions. Observations in the field were very difficult to obtain due to the extreme sensitivity of the topminnows to any movement or even motionless observers anywhere nearby, causing them to hide or flee. The observations that were obtained required field glasses and a great deal of patience.

Territoriality. The defense of fixed territories was observed on one occasion in the field during the breeding season and numerous times in aquaria with a breeding season photoperiod. In aquaria, males were observed defending a portion or the entirety of the tank, initially with numerous aggressive interactions between males, but generally evolving into a single male's dominance of the entire tank, and all other fish except receptive females being driven into hiding most of the time. This situation often became so extreme and the dominant male so aggressive that he would incessantly pursue any other fish (of either sex), and in a small tank without sufficient cover, would eventually kill them. Females were also observed on several occasions actively defending localized feeding areas in aquaria.

In the field such strong territoriality was rarely observed. Females were never observed exhibiting aggressive behavior and males much less frequently than in the lab. On 8 July 1984 at about 4 p.m., some males were observed in Pond Spring defending territories around clumps of filamentous algae. The water temperature was 24 C and the clarity very low due to recent rains. Larger males defended fixed "prime" territories (large clumps of algae) against all other males and similar-sized adults of other species (fish less than about 20 mm TL were ignored). Smaller males moved about individually or in small, loose schools, frequently courting females which generally ignored them. These males sometimes interacted aggressively, but in every encounter with territory-holding males they were quickly driven away. Females were observed both alone and in the small schools, mostly feeding or presumably seeking food. Whenever a female came near a large male's territory she was actively courted (see descriptions of motor patterns below) by the male. Most females immediately fled from the male's advances, but occasionally they would remain in the territory for a while or inspect the algae, which greatly increased the intensity of the male's courtship and aggressive behavior towards any trespassers.

The territorial behavior observed at Pond Spring could be of limited occurence in Barrens topminnow populations, but due to the paucity of successful field observations, it is possible that such behavior is temporally limited on a daily cycle. Koster (1948) concluded that spawning activity in _Plancterus_ (_Fundulus_) _kansae_, the plains killifish, was controlled by daily fluctuations in water temperature, but water temperature fluctuates very little in Spring Pond during the day except in extremely shallow areas. The time of day of the above observations and the observed tendency of aquaria-held fish to initiate courtship and territorial behavior early and late in the day when sunlight from windows penetrated the sides of tanks strongly suggests that the angle of incidence of sunlight may be an important factor in initiating such activities. This possibility would appear to be supported further by the intensified brilliance of male coloration when light is reflected laterally off the sides of the fish. Under vertically incident light males are nearly as dull as females.

Koster (1948) also found that plains killifish males only loosely defended transient, moving territories-- a behavior that was observed on several occasions among BTMs at the Lewis locality. Thus, it appears that the territorial behavior of Barrens topminnows varies among, and probably even within, populations. Kodric-Brown (1981) has reported that _Cyprinodon_ exhibits even more varied breeding systems which are dependent on such factors as population density, food availability, and the physical dimensions of the habitat. Each system is a facultative response by the fish to such environmental conditions and they switch systems if conditions change. Such behavioral shifts may also occur in other cyprinodontids, such as *Fundulus julisia*.

Motor patterns. As noted above, motor patterns observed in *F. julisia* frequently were identical to those described by Able and Hata (1984) for *F. heteroclitus* and many of their terms are used here.

Aggressive interactions by BTMs began with an Aggressive Approach by one or both fish with median fins and often opercula expanded. If this did not result in the Flight of one individual, a Lateral Display by one or both followed in which the aggressor fish(es) oriented itself(themselves) laterally to the opponent with fins and opercula flared and the body rigidly S-shaped. During these behaviors, pigmentation often intensified noticeably in males, with the head and opercular regions sometimes becoming unusually dark. In most cases a Lateral Display was immediately followed by the Flight of one fish with the other Chasing and sometimes Biting. Rarely, two adult males would continue to escalate aggressive behavior to higher levels. Tail Wags began as a slow wagging of the entire rigid body of the fish while stationary in the Lateral Display and increased in intensity until the fish were taking turns "shoving" and "butting" each other with their tails and opercula. This would often turn into "whirling", with both fish chasing each other at high speed in a tight circle. Rarely, Jaw Locking occurred: the fish grasped jaws and shook each other violently.

The full extent of these aggressive motor patterns was observed only in aquaria, with Tail Wags being the greatest extreme observed in the field between adults. In larvae, however, (some less than 25 mm TL) pairs of fish were observed both in the field and aquaria exhibiting everything except Jaw Locking (play?). Another remarkable observation was an extended aquarium battle displaying all these motor patterns (except Jaw Locking) between an adult male F. julisia and a rock bass (Ambloplites rupestris) of the same size that ended only when the rock bass was removed from the tank.

In courtship behavior, males typically swam toward females in a Courting Approach (median fins folded). Females responded either by Fleeing or by seemingly ignoring the male at first, swimming a short distance and pausing, then repeating this sequence. This behavior caused the male to Loop and Circle around the female and Follow her sporadic movements so as to place her between him and any substrate she approached. It also increased his aggression towards any other fish that approached or were nearby. At this point courtship would sometimes break off as the female Fled, but if she were ready to spawn she always approached and Inspected a substrate (usually mops in aquaria, algae in the field). This further intensified the actions of the male and he would sometimes briefly make lateral contact with the female. If the female then Nipped at the substrate, spawning immediately occurred with the female assuming an S-shape that oriented her anal sheath and head towards the substrate and the male simultaneously assuming a parallel posture (the Clasp) with his dorsal and anal fins wrapped over the female's. Both fish vibrated rapidly while in the Clasp, releasing any eggs and sperm, then literally "burst" away from the spawning site, entangling and hiding the eggs in the eddies created in the spawning medium.

These observations closely parallel those made by Able and Hata (1984) of F. heteroclitus in that the female, not the male, selects the actual spawning site and that her Nipping the substrate is the cue for the spawning clasp. Remarkably, in the closer subgeneric relative, F. catenatus, which is a benthic spawner over fine gravel and sand, it appears that the male selects the spawning site, remains stationary there, and attracts females to Nip at the site with a head dipping display (pers. obs.).

4. EGGS AND EARLY DEVELOPMENT

Eggs

All eggs spawned in aquaria were 2.0-2.3 (mostly 2.2) mm in diameter and completely colorless and translucent. A few eggs stripped from females in the field were found to be orange-amber in color (as are mature ovarian oocytes), but all eggs recovered from vegetation were colorless. Chorionic fibrils were variable in quantity and appearance, but were generally long (up to 100 or more mm) and few in number and formed a tangled filament or filaments, frequently incorporating fibrils from more than one egg. The filaments were extremely adhesive and elastic in freshly spawned eggs, much less so in older eggs. Although eggs removed from spawning sites were often tangled in the fibrils, they were easily removed except at a small point of primary attachment, leaving an entirely naked, translucent chorion through which all internal structures and development could be observed. All eggs contained a mass of 20-30 oil droplets just inside the top of the yolk that varied in size from minute to about 0.1 mm in diameter.

Development

Embryos. See Appendix A.

Yolk-sac larvae. All larvae observed at hatching were yolk-sac larvae 7.0-7.2 mm total length (TL). Yolk-sac size was variable, but generally equal to or smaller than the belly (distinguishable by lack of chromatophores and vitelline vascularization). The air bladder was visible, spherical, and less than one-half the width of the body at its site. Larvae were negatively buoyant for 8-36 hours as the air bladder enlarged. The yolk-sac was generally absorbed within 24-72 hours, but some fry fed in less than 24 hours while still negatively buoyant. Oil globules were present in the belly for 12-24 hours after yolk-sac absorption.

Myomeres numbered 8-9 + 23-24. The dorsal fin fold originated at myomere 13 or 14. The only fin rays evident were caudal (10-13). The mouth was terminal and oblique (upturned 45 degrees).

Pigmentation was much as described in Appendix A for advanced embryos except xanthophores were confined to remnants of the yolk-sac, myomeres and head, forming a cluster on the snout and a strip ventrolaterally along the jaws and under the eyes. Fine melanophores formed a mid-dorsal streak and postanal midventral streak and were scattered thinly over the dorsum; the brain was covered with closely spaced large melanophores as was the remnant of the yolk-sac. Eyes were opaque and checkered with xanthophores, melanophores and metallic silver pigment (also present laterally behind the pectoral fins). Larvae were otherwise translucent with the dorsum infused with light green.

Larvae. See Appendix B.

5. POPULATION CHARACTERISTICS

Population Size

Analysis of the mark-and-recapture data using the modified Schnabel method yielded a final estimate of 534 adult topminnows in the Lewis population and 1493 in the Pond Spring population with respective 95% confidence intervals of 476-599 and 965-2424. These estimates conservatively support those of Etnier (1983). Most of the assumptions of such a study were presumed to have been met or closely approximated. The studies were conducted mostly during the spawning season, but young-of-the-year (Y-O-Y) were ignored, eliminating recruitment error (and the Y-O-Ys contribution to the population size). Some error due to mortality may have been attached to the estimates due to the length of time of the studies (76 days for the Lewis and 120 days for the Pond Spring population). If so in either case, the estimates represent population size at some time during the study rather than on the final date. Due to the localized nature of both populations migration was probably insignificant.

These two populations (or subpopulations) represent a significant portion of all known Barrens topminnows. Assuming the accuracy of the above estimates and those of Etnier (1983), the sum of all known BTM populations was probably around 5000 individuals in 1984.

Sex Ratio

The number of individuals of known sex collected at the two primary study sites are listed in Table 2. A sex ratio of 1:1.27 (males to females) was found for the sum of all fish examined from both

Table 2. Sex ratios of Barrens topminnows collected from two populations from March 1984 to March 1985.

	Lewis Population			Spring Pond Population		
Age Group	Male		Female	Male		Female
0+	157	:	171	133	:	164
>0+	18	:	43 **	1	:	15 **
all	175	:	214 *	134	:	179 *
	(all ages of both populations = 309 : 393 *)					

* $p \leq .05$, ** $p \leq .01$ (X^2 test)

populations (n=702) and was significantly different from a 1:1 ratio ($p \leq .01$). The ratios for each population were 1:1.22 (Lewis, n=389, $p \leq .05$) and 1:1.33 (Pond Spring, n=313, $p \leq .05$). Chi square analyses by year classes revealed that while females were always more numerous than males, the ratio in fish of the youngest year class in each population was not significantly different from 1:1. Among older fish, however, females were significantly more numerous than males: 1:1.82 in the Lewis population (n=82, $p \leq .01$) and 1:15.0 in Pond Spring (n=16, $p \leq .01$). The latter ratio is likely biased by the small number of fish examined and the relatively greater ease of capture of large gravid females as compared to large males when seining the pond, but the numerous predators in the pond habitat may also have been a factor. Sampling bias should not have been significant with the Lewis population. These findings suggest that mortality is greater among males than females in fish older than one year-- a reasonable hypothesis given the brighter coloration of the males and their aggressive, territorial behavior during the reproductive season. Similar findings have been reported for *F. heteroclitus* by Kneib (1976). Haas (1976) even found that male annual killifish, *Nothobranchius*, are preferentially selected by avian predators in experimental situations.

Age Composition and Mortality

Examination of scales and length-frequency distributions of fish from the Lewis population (Figure 7) revealed that a maximum of four distinct age classes was present only during the breeding season. The frequency distributions from both study sites (Figures 7-8) also suggest that growth and age composition of topminnow populations may be fairly variable among and within populations from year to year.

At the Lewis locality nearly all 2+ age group and many age 1+ fish died by the end of the summer, leaving mostly the 0+ and a much reduced 1+ age group. The maximum age attained appeared to be rarely more than two years and one-third or less of the 1+ age group survived to spawn the following year. Mortality in this age group was high following a reproductive peak in July and it appears that it may vary significantly from year to year judging from the comparison of the March length-frequency distributions of successive years. The estimated combined survival rate of 1+ and older fish in March 1984 was 29.9% (n=277),

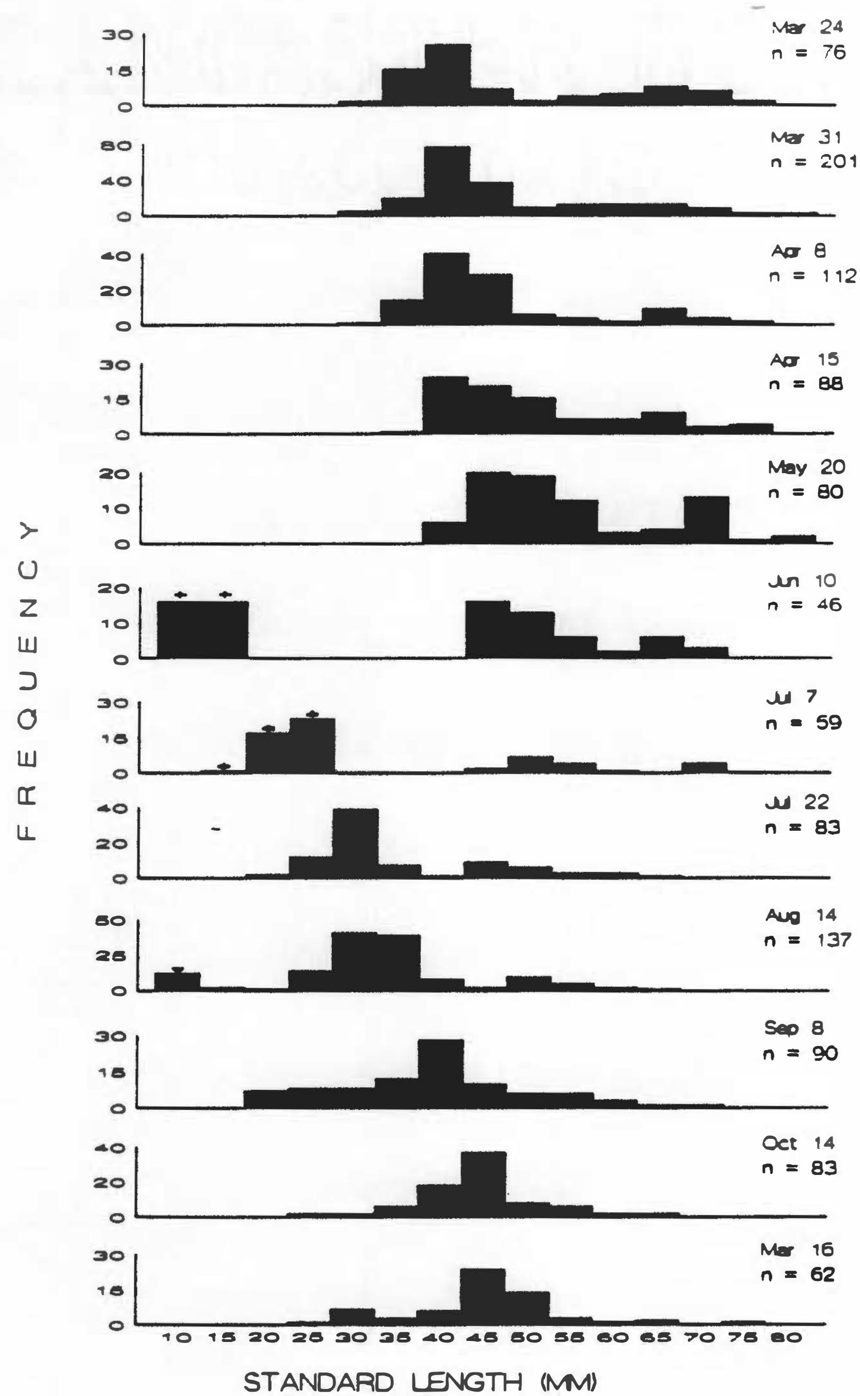

Figure 7. Length-frequency distributions of Fundulus julisia collected from the Lewis population March 1984 to March 1985.

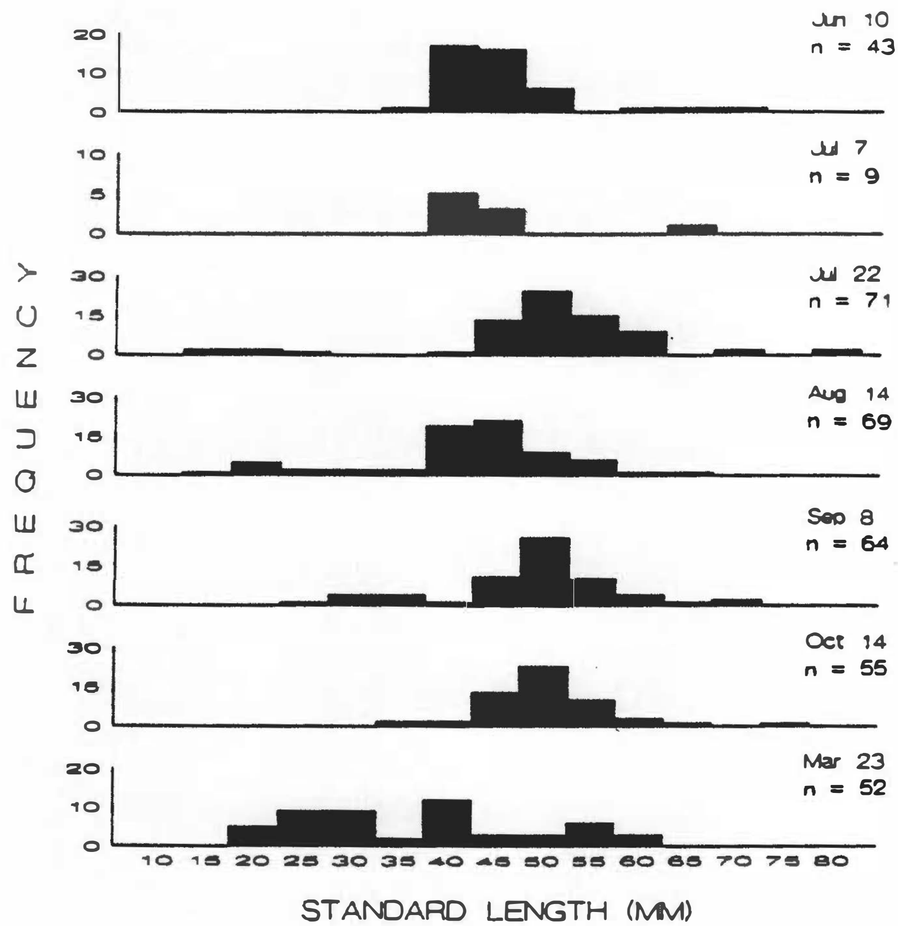

Figure 8. Length-frequency distributions of <u>Fundulus julisia</u> collected from the Pond Spring population June 1984 to March 1985.

while the following year was 22.6% (n=62). The accuracy of either of these figures is questionable, however, since they assume equal recruitment for the three age classes involved (highly doubtful), not to mention the small sample size of the latter figure.

The size (length) compositions of the 0+ age groups in the successive March distributions in Figure 8 are also markedly different (average 40.3 vs. 45.5). If growth rates were relatively constant for the two years it would appear that the March 1984 0+ age group were nearly all spawned in mid-June since the two subclasses in the 1985 0+ age group were observed to have been spawned in late May and early August. Variabilities of weather probably strongly shape the size and composition of age classes each year by affecting both reproductive timing and success as well as growth. One example of how this may occur was the observation of a number of dead eggs in emergent vegetation just above the water level which had recently fallen at the Lewis locality on 19 May, 1984.

Variability in age composition between populations also was evident when comparing the length-frequency distributions from the two study sites (Figures 7-8). The first young-of-the-year appear about two weeks later at Pond Spring than at the Lewis locality and the March 1985 distribution indicates that many young were produced there as late as late August and/or September. Coincidentally, there is not a July crash in the 1+ age group (thereby allowing more late season reproduction) which appears to suffer greatest mortality in the winter months. Finally, large fish (in excess of 70 mm SL) were present in the late summer and fall months whereas they disappeared in July in the Lewis population. These differences are probably attributable to the cooler more constant summer water temperatures at Pond Spring (for further discussion see "Breeding Season" above).

Examination of scales revealed that annuli are probably laid down following the spawning season since they were only present in 1+ or older individuals and were absent in fish approaching one year of age in the Spring. The oldest and largest fish whose scales were examined was a 66 mm SL female who possessed two annuli plus 5-6 circuli in October. It thus appears that the rare fish nearing or exceeding 70 mm SL during the breeding season are approximately three years of age and that this is the maximum age attained. The largest fish collected during the study period were 79-80 mm SL (96-98 mm TL).

Growth

Figure 9 illustrates the growth of Barrens topminnows in the lab and at the Lewis locality from March 1984 to March 1985 (early development and growth is described in detail above in "Eggs and Early Development"). Equations of curves fitted to the data can be found in Appendix C.

The average growth of the 0+ age class at the Lewis locality from 1984 to 1985 roughly parallels the growth of the largest individuals reared in aquaria, although the wild fish grew somewhat faster, attaining an average length of nearly 40 mm SL in about three months. By mid-October the wild juveniles averaged 42.5 mm SL at an age of about four months. Growth was then very slow through February with the age class averaging 45.5 mm SL in mid-March. During the same year the older age

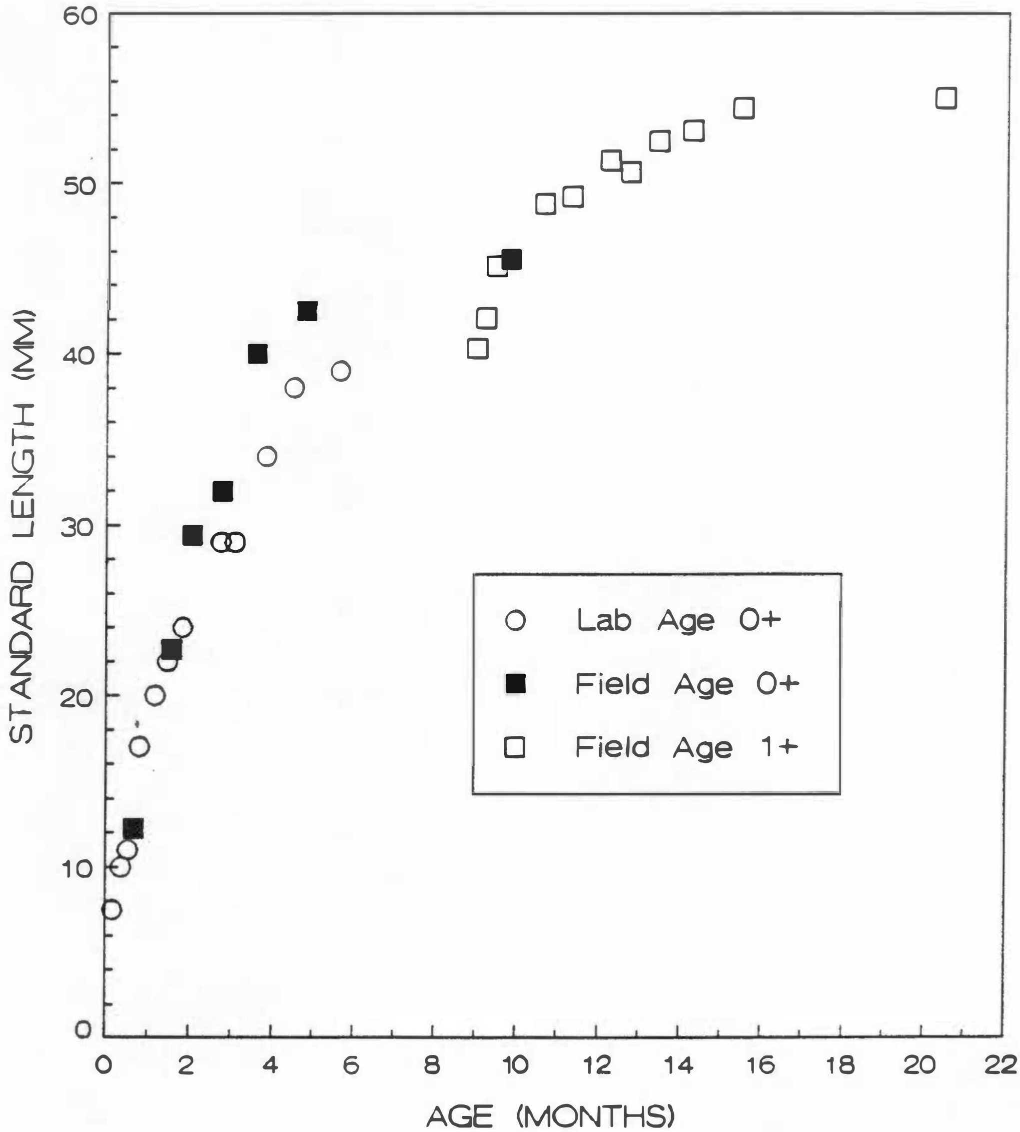

Figure 9. Growth of Fundulus julisia in lab aquaria and in the field in 1984 and 1985 (lengths are from individual fish in the lab and averages of year classes in the field).

class fish underwent rapid early growth which leveled off in late May as the breeding season reached full swing. By mid-October the average length of the 1+ age class was about 54.5 mm SL, an increase of 15 mm. These fish also grew very little over the winter, averaging 55 mm SL in March. Figures for this age class may be slightly inaccurate due to overlap of the older age classes beginning in September requiring somewhat arbitrary divisions between them (see Figure 7). Because of this and small sample sizes, no attempt was made to describe detailed growth of the oldest age classes.

In summary, the growth of Barrens topminnows may vary considerably from year to year, but they generally attain lengths of around 40 to 45 mm SL by the end of their first growing season, 55-60 mm at the end of two, 65-70 after three, and rarely, 75-80 mm sometime during a fourth and final season. This is very fast growth in the first few months, considerably faster than for *F. heteroclitus*, for instance, which has an identical lifespan and averages only 30-35 mm SL at the end of the first season although it catches up in the second and eventually reaches greater lengths than *F. julisia* (Kneib and Stiven, 1978). *Fundulus majalis* has been reported to grow even faster, (Clemmer and Schwartz, 1964).

Interspecific Interactions

Potential competitors with Barrens topminnows vary depending on the population locality. *Lepomis cyanellus*, *L. macrochirus*, and *Hemitremia flammea* were ubiquitous and probably compete for food with topminnows to some extent at all ages. A possibly more serious competitor found at Pond Spring and most other topminnow localities is Gambusia affinis. At all these sites, Gambusia outnumber topminnows by a wide margin and, unlike the topminnows, the livebearers reproduced continuously throughout the warmer months at Pond Spring. Presumably similar diets and feeding habits of both adults and fry of the two species as well as piscivory strongly suggest that the poeciliid is limiting the size of the topminnow population at the very least. Many instances of replacement of native species (e.g., Poeciliopsis occidentalis, Cyprinodon spp., and other Gambusia spp.) by Gambusia affinis have been documented (Schoenherr, 1981). In no instance has actual competition for a limited resource been demonstrated as the means of replacement. Instead, predation by G. affinis upon young of other species and dominance in aggressive interactions appears responsible. Interestingly, a number of the native species apparently continue to survive only in clear, spring-fed waters that are high in carbonates; habitat which is generally avoided by mosquitofish according to Schoenherr (1981). Only circumstantial evidence exists at this time, but prime topminnow habitats at other localities on the Barrens Plateau containing only Gambusia might be the results of recent competitive exclusion since the mosquitofish was not present on the Barrens Plateau only a few decades ago (D. A. Etnier, personal communication). The Spring Pond and type locality populations, in particular, merit close monitoring to see if topminnow numbers are declining. At several localities outside the study sites (i.e., McMahan Creek and nearby Duke Creek) Fundulus catenatus may be a significant competitor also.

Potential predators of Barrens topminnows include Cottus carolinae (Spring Pond), Micropterus salmoides, Lepomis spp., Nerodia spp., coleopteran (Cybister), hemipteran (Belostoma), and arachnid species, kingfishers (Megaceryle alcyon), and predatory wading birds (especially great blue herons, Ardea herodias, and green herons, Butorides striatus) which are probably the primary predators of adult BTMs. No direct evidence of predation was noted, but all these predators were noted in habitats occupied by topminnows, and a L. cyanellus was observed attempting to catch BTM larvae.

The extent of parasitism on F. julisia is difficult to assess due to the few fish killed and dissected. No external parasites were noted and the only internal parasites found were large nematodes found in the body cavities of two unusual individuals that were visibly swollen externally and killed for that reason. Adult fish held in aquaria were susceptible to bacterial and fungal diseases if stressed or wounded by other fish and frequently succumbed to the former or unknown causes following breeding.

CHAPTER V

CONCLUSIONS AND OUTLOOK

The limited distribution of the Barrens topminnow seems to be almost entirely a consequence of reproductive adaptations requiring aquatic vegetation, particularly filamentous algae (particularly *Cladophora* or *Pithophora*?), as a spawning substrate. This study suggests that the ideal habitat for the species would be extensive, permanent upland marshes. These were probably once widespread on the Barrens Plateau and much of the rest of the Interior Low Plateau during the cooler, moister climates of the Pleistocene and early Holocene, as indicated by the palynological studies of Delcourt (1979) in this area. Presently, however, such habitat is largely seasonal, and much has been drained and converted to farmland or other uses, limiting the topminnow to a few widely disjunct localities where springs and strong groundwater influence provide suitably stable habitat.

A severe drought this past summer (1988) prompted a visit to several of the localities. Those with the strongest groundwater supplies such as Pond Spring and the type locality were much reduced in flow, but still supported adequate habitat and topminnows for those populations to survive the dry conditions. Other sites, however, were completely dried up or reduced to isolated, stagnant pools that supported few if any fish (about 40 BTMs were rescued from a small pool that was all that remained of the upper brook at the Lewis locality). Such populations have suffered severe losses and warrant close monitoring the next few years to see if they recover. At present, the total number of

BTMs is probably less than half what it was in 1984-85. All populations could be threatened if another equally dry year follows this one or drier climatic conditions or reductions in the water table reduce groundwater flow on the Barrens. Other threats to individual populations potentially include replacement by *Gambusia affinis* and pollution from agricultural run-off, especially at Pond Spring.

On a more positive note, efforts are currently underway to establish breeding populations by concerned aquarists and killifish breeders. Since the species adapts well to aquaria and is easy to breed, there will hopefully soon be back-up populations for each of those on the Barrens Plateau should any ever be eliminated and a source for additional introductions to suitable habitats that lack *Fundulus julisia*.

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APPENDIXES

APPENDIX A

EMBRYONIC DEVELOPMENT

Development at 24-26 C (times are increasingly approximate with age and highly temperature-dependent):

<u>Hour</u>

1:00 - blastoderm aggregating, early blastocap formation.
1:40 - blastodisc formed, one-cell stage.
1:50 - first cleavage, 2-cell stage.
2:25 - second cleavage, 4-cell stage.
2:50 - third cleavage, 8-cell stage.
3:10 - blastomeres arranged in symmetric, parallel rows.
3:30 - 16-cell stage
5:00 - approximately 64-cell cylindrical early morula.
5:35 - morula with serrate border, beginning to spread out and flatten onto yolk.
8:10 - morula flat-bottomed with perimeter of periderm one-fourth morula's diameter.
8:45 - morula indenting yolk, round-bottomed.
9:15 - morula trapezoidal in cross-section, blastocoel formation?
10:30 - corona of periblast expanded, increasing radius of blastula by about one-half.
11:30 - blastula cone-shaped, indenting yolk with outside surface concentric with yolk surface.
12:30 - blastula flattened, expanding, top and bottom surfaces concentric with yolk surface.
13:15 - blastocoel barely visible.
16:00 - blastula covering nearly one-fourth of yolk; slight swelling at site of embryonic axis.
18:00 - germ ring visible around margin of blastoderm with thickness slightly increased at site of embryonic axis.
21:00 - gastrula covering about one-half of yolk; embryonic shield well-developed.
23:00 - embryonic axis visible as faint streak; mass of oil droplets at site of closure of blastopore.
27:00 - gastrula covering about five-sixths yolk; embryonic axis well-formed with optic vesicles and brain divisions rudimentary; first two somites forming.
31:00 - blastopore closed with many small oil droplets in vicinity; Kuppffer's vesicle present; rudimentary pericardial cavity forming; otic vesicles forming; optic vesicles and brain divisions advanced; blood islands forming on yolk; 5 somites; no chromatophores.
37:00 - 9-12 somites; future lenses appear as slight dimples in optic vesicles; first xanthophores appearing on yolk and somites; caudal bud expanded, tapered.

44:00 - heart contracting, no circulation yet; lenses complete; no otoliths; xanthophores numerous with melanophores now on yolk and first appearing on embryo lateral to hindbrain and otic vesicles; caudal bud clubbed; 19-20 somites.

48:30 - xanthophores and melanophores numerous on yolk, expanding in size; still only about five melanophores on embryo near otic vesicles and incipient belly wall; future otoliths appear as amorphous aggregation of fine granules; circulation confined to a single vessel running the length of the embryo and continuing around the yolk from the tail to the heart.

55:30 - otoliths now a larger, less diffuse cluster of granules; lateral vitelline circulation established with colorless blood; all chromatophores much enlarged with a double row of xanthophores now bordering incipient belly wall and forming parallel rows on somites (4 per somite, dorso- and ventrolateral positions); tail attached to yolk-sac at tip, but free posterior to anus.

62:30 - otoliths now condensed collections of granules; incipient pectoral buds visible.

69:00 - incipient dorsal fin fold forming low mound.

73:30 - dense dusting of minute metallic and dark chromatophores on eyes and sparsely over brain; blood red; no erythrophores; large stellate melanophore on ventricle; pectoral buds established; belly wall (with xanthophore border) expanded to one-third width of somites; tail free with caudal fin and dorsal fin fold present as low, thin ridges; otoliths complete; embryo rights self with tail if chorion turned.

84:00 - eyes well-pigmented, but translucent; very faint green pigment over brain; melanophores and xanthophores superstellate; belly walls two-thirds somite width; pectoral buds are small flattened cones; low anal and dorsal fin folds forming.

92:00 - many fine melanophores appearing lateral to brain and forming mid-dorsal streak; first metallic pigment visible on belly walls which now equal somites in width; still no erythrophores.

95:00 - brain greatly enlarged; much green pigment present on dorsum; xanthophores huge, stellate on optic lobes, snout, and one on posterior of each eye; eye pigment dense but not opaque; tail curls to reach pectorals; pericardial cavity and heart enlarged so that atrium is anterior to jaw; fin folds established, forming continuous strip around caudal bud which has two conspicuous basal xanthophores; erythrophores now visible under eyes; incipient gut visible.

103:00 - erythrophores expanded between and around opaque eyes; yolk melanophores now aligned with blood vessels; xanthophores extremely stellate; on somites two midlateral xanthophores per somite form midlateral bands while those in dorsolateral and ventrolateral positions alternate in height to form zigzag lines; rows of xanthophores also form border along somite-belly and belly-yolk junctures and form circle around the pectoral fins; also one xanthophore on each opercle and on snout anterior to each eye.

119:00 - pectoral fin slightly smaller than eye; tail reaches eye; caudal fin with three xanthophores in center; liver and gut established; all pigment more extensive with many new small melanophores on sides of embryo.

130:00 - pectoral fins now frequently moving, equivalent in size to eye, have several proximal melanophores; caudal fin same size as pectoral with three vertical xanthophores; lateral circulation now present on pericardial cavity; tail reaches snout.

144:00 - first caudal rays appearing.

166:00 - caudal fin with 6 rays.

185:00 - yolk contracting in size, about six times as large as belly; tail curled so that caudal fin is on top of head, has 6 rays outlined with melanophores; melanophores on yolk stellate, those on embryo responsive to light; all other chromatophores contracted.

195:00 - yolk now about three times belly; air bladder visible.

200:00-250:00 - hatching follows rapid absorption of yolk (becomes smaller than belly) and greatly increased activity of embryo (somersaults in chorion with rapid eye, mouth, and opercular respiratory movement); caudal fin rays 10-13; temperature increases precipitate process; larvae break chorion with lash of tail and 'somersault' free.

APPENDIX B

LARVAL DEVELOPMENT

Development at 20-26 C (lengths are total followed by standard length in parentheses):

Day
5 : 8-9 (6 3/4-7) mm; 3-6 fold-like incipient pectoral rays; mid-ventral yolk-sac xanthophores absent, melanophores disappearing anteriad; incipient dorsal and anal fins appearing as increases in height of fin folds in addition to melanophores at the anal fin site.
6 : caudal rays 16-17 with 3 vertical bands of fine xanthophores at future sites of first joints; 8-10 pectoral rays; anal fin rays beginning to form at postanal myomeres 5-9, fin 1 1/4-1 1/2 times fin fold in height; incipient dorsal fin appearing as increasing fin fold thickness and melanophores at postanal myomeres 6-11; air bladder now conical, nearly reaches anus posteriad.
7 : 9-9 1/2 (7-7 1/4) mm; 13-14 segmented caudal rays; 9-10 pectoral rays with increased melanophores present; remnants of mid-ventral yolk-sac melanophores absent to nearly so; 5 anal rays and 1-2 incipient dorsal rays.
8 : 9-10 (7-7 1/2) mm; 14-15 segmented caudal rays; 8-9 anal rays, nearly reach margin of fin on postanal myomeres 6-11; 3-5 extremely short incipient dorsal rays starting at postanal myomere 8, dorsal fin fold disappearing anteriad; all xanthophores have disappeared (now silver?)-- iridescent silver pigment now present on belly and lower opercles, iridescent green pigment appearing dorsally on melanophores; body papillose--scales forming.
10 : 10-10 1/2 (8-8 1/2) mm; 14-15 segmented caudal rays; 8-11 pectoral rays; 10 anal rays, anal fin now 2-3 times decreasing height of ventral fin fold; 5-6 dorsal rays, dorsal fin fold reduced to body surface between dorsal and caudal fins; 5 branchiostegal rays; tiny pelvic fin buds visible; pigment: overall pale green with fine melanophores scattered over body laterally, lateral belly walls with silver-white coating forming vertical stripes on posterior half, melanophores clustered on air bladder and vertebrae. 13 days: 11 (8 3/4) mm; caudal fin with about 26 rays, 15 segmented; 8-12 pectoral rays; 10 anal rays; 7-8 dorsal rays; fin folds much reduced--dorsal absent except near caudal fin, anal still complete anterior to anal fin but reduced to body surface posteriad.
15 : 12-12 1/2 (9-10) mm; 16 segmented caudal rays; 8-12 pectoral rays; 11-12 anal; 8-9 dorsal; pelvic fin buds enlarging; mouth strongly upturned now.
19 : 14 1/2 (12 1/2) mm; 12 pectoral rays; 12 anal; 9 dorsal; about

5 incipient pelvic rays; only remnants of anal fin fold still present; pigment: body evenly speckled with small melanophores except for mid-dorsal and mid-ventral streaks, iridescent green chromatophores present laterally, two pairs of melanophores conspicuous on venter of maxillae.

23 : 16 1/2 (13 1/4) mm; 5 branched caudal rays; all other fin rays as above with melanophores outlining rays except in pelvic and and lower two-thirds of pectoral fins; pectorals now with a few segmented rays; lateral melanophores now outlining scales, those dorsal are larger and still scattered; lower half of opercles and belly now iridescent silver as are eyes except for melanophores closely surrounding pupil and speckled dorsally.

27 : 17 (13 1/2) mm; 7 branched caudal rays; 10 dorsal rays; fine melanophores now outline dorsal scales and sprinkle maxillae; mid-ventral strip of melanophores now forms 'V' where splits around anal fin and anus; mid-dorsal gold spot forming anterior to dorsal fin.

31 : 19 1/2 (15 1/4) mm; 11 branched caudal rays; 12 pectoral rays, dorsal six outlined with melanophores; 12 anal rays; 11 dorsal; body becoming opaque, evenly covered with scattered fine melanophores on head, maxillae, and outlining scales except on lower half of opercles and sides; vertical white stripes laterally between pectoral and pelvic fins.

36 : 22 (18) mm; 14 branched caudal rays; 15 pectoral rays; 6 pelvic rays; body olive-tan and opaque now.

43 : 24 1/2 (19 1/2) mm; anal fin rays starting to branch; 5 branched pectoral rays; iridescent green chromatophores on brain, nasal openings, eyes, and centers of lateral scales; gold spot at anterior base of dorsal fin still small; anterior dorsolateral scales with 5 annuli.

52 : 27 1/2 (22) mm; mid-dorsal gold spot larger, now conspicuous.

75 : 32-35 (25-28) mm (3 fish); 15-16 branched caudal rays; 6-8 branched anal rays; 7-8 branched dorsal rays; 6 branched pectoral rays; eyes, brain, and nasal openings iridescent green with large melanophores on brain and clustered mid-dorsally anterior to gold spot; larval stage nearly complete.

85 : by lengths of around 36-37 mm TL (29-30 mm SL) larval stage is complete and males distinguishable by lateral iridescent green pigmentation; this size observed as early as 60-70 days in the field.

APPENDIX C

EQUATIONS OF CURVES FITTED TO FIGURE 9 -- GROWTH OF *FUNDULUS JULISIA*

Lab Age 0+ : $Y = 5.5 + 14.1X - 2.4X^2 + 0.2X^3$
Rval = 0.994

Field Age 0+ : $Y = 1.9 + 16.8X - 2.2X^2 + 0.1X^3$
Rval = 0.996

Field Age 1+ : $Y = -67.6 + 21.6X - 1.3X^2 + 0.03X^3$
Rval = 0.985

VITA

Patrick L. Rakes was born January 1, 1959 in Aldershot, Surrey, Great Britain, but grew up in Rogers, Arkansas beginning that same year. There, he attended elementary and junior high school and in June, 1977 he graduated Valedictorian from Rogers Senior High School. In September he entered the University of the South in Sewanee, Tennessee on a National Merit Finalist Scholarship. While studying there he was a member of the Order of Gownsmen, an officer of the Chi Psi fraternity, a Wilkin's Scholar, and a member of Phi Beta Kappa. He received a Bachelor of Science degree (Magna cum Laude) with a major in Natural Resources and a minor in Biology in May, 1981. In September, 1982 he entered the University of Tennessee in Knoxville, serving as a graduate teaching assistant, and received a Master's degree in Zoology in May, 1989.

The author is a member of the Southeastern Fishes Council, the Association of Southeastern Biologists, and the American Society of Ichthyologists and Herpetologists. He is married to the former Kelly Frye, and has two children, Gretchen, 9, and Sean, 3. Although planning to continue graduate studies towards a doctoral degree at UT, he first plans to continue working with Aquatic Specialists of Knoxville establishing a captive breeding program for threatened and endangered southeastern fishes.