Larval fish collections using lighted traps in spring pools in the Barrens Plateau of middle Tennessee

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Abstract - We used lighted larval traps to assess reproduction by fish species inhabiting nine spring pools in the Barrens Plateau region of middle Tennessee in the spring, summer, and fall of 2004. The traps (n = 162 deployments) captured the larval or juvenile forms of *Gambusia affinis* (Western Mosquitofish) (n = 168), *Etheostoma crossopterum* (Fringed Darters) (n = 128), *Hemitremia flammea* (Flame Chubs) (n = 43), the imperiled *Fundulus julisia* (Barrens Topminnows) (n = 10), and *Forbesichthys agassizii* (Spring Cavefish) (n = 1). The larval forms of four other species (Families Centrarchidae, Cyprinidae, and Cottidae) were not collected, despite the presence of adults. Larval Barrens Topminnow hatched over a protracted period (early June through late September); in contrast, hatching intervals were much shorter for Fringed Darters (mid-May through early June) and Flame Chubs (early-to-late May). Juvenile Western Mosquitofish were collected between early June and late August. Our sampling revealed that at least two species (Flame Chubs and Fringed Darters) were able to reproduce and thrive in habitats harboring the invasive Western Mosquitofish; whereas Barrens Topminnows could not.

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Introduction

Numerous springs dot the landscape of the Barrens Plateau region in middle Tennessee and provide habitat for rare spring-associated fishes including *Forbesichthys agassizii* (Putnam) (Spring Cavefish), *Fundulus julisia* Williams and Etnier (Barrens Topminnow), and *Hemitremia flammea* (Jordan and Gilbert) (Flame Chub) (Etnier and Starnes 2001). Spring habitats throughout the Barrens Plateau region have been altered during development of farmlands and nurseries, which has limited already-rare spring dwelling fishes to a few locales (Rakes 1989). Another anthropogenic manipulation of the ecosystems in the Barrens Plateau region includes the liberal transplanting of *Gambusia affinis* (Baird and Girard) (Western Mosquitofish). The negative effects of introduced Western Mosquitofish on native fish assemblages (especially larvae) have been well documented (Belk and Lydeard 1994, Courtenay and Meffe 1989, Lydeard and Belk 1993, Myers 1967, Rincon et al. 2002). Mills et al. (2004) reported that no larval *Iotichthys phlegethontis* (Cope) (Least Chubs) survived in enclosures with high mosquitofish densities and Laha and Mattingly (2007) confirmed that Western Mosquitofish predation can eliminate larvae and fry of native species, including Barrens Topminnows. Reproduction (i.e., producing larval offspring) by Barrens Topminnows in the presence of Western Mosquitofish has been documented, but subsequent recruitment of juveniles to the spawning population is rarely observed (Goldsworthy and Bettoli 2006; Watts 2009). During the course of evaluating the efficacy of a re-introduction program using hatchery-reared Barrens Topminnows, larval fishes were sampled in a variety of habitats stocked with topminnows in the Barrens Plateau region in middle Tennessee. Goldsworthy and Bettoli (2006) reported where they caught larval Barrens

Topminnows using light traps and noted the presence (or absence) of Western Mosquitofish. However, a depauperate literature exists on the biology of spring-associated fish species in Tennessee and few larval fish studies exist for these fishes. In this paper, we report on the catches of larval forms of five species we encountered in nine spring pools in the Barrens Plateau region and comment on the efficacy of lighted larval traps in detecting reproduction by those fishes. Lighted traps have been developed and used for decades to sample larval fishes in freshwater and marine habitats (e.g., Floyd et al. 1984a; Bryan and Scarnecchia 1992; Sponaugle and Cowen 1996); however, we are unaware of any larval fish studies of spring-associated fishes, especially troglophilic species, using any approach or gear. We also describe patterns of emergence for four species captured in enough numbers to draw inferences regarding when and how often they spawned.

Study Area

Larval sampling was conducted at nine sites; four of the nine sites were part of a small complex of seven natural and man-made spring pools located on private property (the Clayborne Complex; 35^{o} 30.351'N, 85^{o} 54.757'W) in the headwaters of the West Fork of Hickory Creek watershed in Coffee County, Tennessee. The four pools ranged in size from 16 to 59 m^{2} and three of the four pools were stocked with *F. julisia*. One of the pools (number 4) was isolated from all other pools by virtue of its elevation. The resident fishes in this complex of pools (except pool 4) were *Etheostoma crossopterum* Braasch and Mayden (Fringed Darter), *Semotilus atromaculatus* (Mitchill) (Creek Chub), *F.*

agassizii, *G. affinis,* and *H. flammea* (Johnson 2004). Water temperatures discharged from the springhead as measured by a recording data logger averaged 14.9 $^{\circ}$C annually.

Adjacent to and downstream of the Clayborne Complex were two small (~ 50 m^2 each) pools on the Sain Property (35° 30.359'N, 85° 54.714' W) that had been stocked with *F. julisia*. The resident fishes in these two pools were *E. crossopterum, G. affinis,* and *H. flammea* (Johnson 2004*).*

We also sampled two sites that drained into Little Hickory Creek in Coffee County, Tennessee. The most upstream site (Cunningham Barn; 35° 29.569'N, 85° 55.309'W) was a spring-influenced, excavated pool with a surface area of 191 m^2 that averaged 18.8 $^{\circ}$C. Resident fish fauna included *Lepomis cyanellus* Rafinesque (Green Sunfish), *L. macrochirus* Rafinesque (Bluegill), *G. affinis*, and *E. crossopterum.* The downstream site (Cunningham Lower; 35° 29.992'N, 85° 54.426'W) consisted of two small (56-68 m^2), excavated pools that were connected by a spring run. Both sites (and all pools) on the Cunningham Property were stocked with *F. julisia* in 2003 and 2004.

The type locale (35° 32.968'N, 85° 59.015'W; Williams and Etnier 1982) for the Barrens topminnow is a spring pond emanating from a cave located in the headwaters of the West Fork of Hickory Creek in the Caney Fork River watershed. A previous landowner constructed a small dam in the late 1970s or early 1980s to create a pool (approximately 150 m^2) for *Oncorhynchus mykiss* Walbaum (Rainbow Trout). The trout dispersed downstream, but the pool acted as a refuge for Barrens Topminnows and the dam (and subsequent modifications to it) prevented Western Mosquitofish invasion (Williams and Etnier 1982). A seine survey in April 2004 collected only *F. julisia* and *Cottus carolinae* (Gill) (Banded Sculpin). From 18 May 2003 to 21 April 2004, water temperatures at the type locale averaged 14.0^{o}C with a minimum temperature of 8.8^{o}C and a maximum temperature of 16.8^{o}C.

Methods

The light traps we used were a modified design of the light traps used by Hartman (1994) which were based on a Tennessee Valley Authority design, as reported in Goldsworthy and Bettoli (2006). The main body of the trap consisted of a 10-cm long section of 28-cm (inside diameter) PVC pipe with about 1/3 of the circumference removed for an entrance. The entrance consisted of two pieces of clear acrylic plastic angled inward to create a 3-mm wide opening. The main body was glued to an 18-inch square piece of black plastic with holes drilled in each corner through which 10-inch bolts were passed to affix a similar piece of plastic, which acted as the removable top. A matching piece of 25-mm thick foam was attached to the removable top to make the traps float. With the exception of the Cunningham Barn site and type locale, the traps floated in shallow water, with the bottom of the traps less than 0.5 m off the bottom. At the two larger sites, the traps floating in water ~ 1 m deep. The light source was a 12-hour photochemical stick that produced green light. In a laboratory trial, six of nine captive-reared Barrens Topminnows (20-25 mm TL) placed into a 720-l tank entered a lighted trap suspended in the tank and in a second trial none entered an unlit trap. Thus, we assumed that the trap would be sufficient for determining the presence/absence of larval topminnows and we also assumed that the traps would capture other phototropic larval fishes. Floyd et al. (1984b) considered light traps semiquantitative samplers at best because the frequencies (and intensity) of light that larvae respond to are unknown and turbidity in their study stream affected efficacy. Although we did not measure water transparency or turbidity, water clarity at each site was high and characteristic of small, spring-influenced pools.

Traps were deployed overnight at nine sites (one trap per site) at approximately weekly intervals between May and August 2004 and every two weeks in August and September 2004. Traps were retrieved the following morning and the contents of each trap was washed through a 100-micron mesh net and fixed in 10% formalin solution. In the laboratory, samples were transferred to ethanol before processing. All larvae were subsequently counted and measured for total length; the total lengths of captured fishes were plotted over time for those sites where sufficient numbers of fish were collected in order to discern the duration of larval emergence. We obtained hatchery-propagated larval Barrens Topminnows to serve as a reference and used larval fish keys (Heufelder and Fuiman 1982, Hogue et al. 1976) to identify other species. Sampling at these sites using seines and backpack DC electrofishing gear to meet other project objectives during (and in the months and years preceding) our sampling yielded species inventories of adult fish species known to inhabit each site, which aided in the identifications of larval forms.

Results and Discussion

The lighted traps (n = 162 deployments) captured a total of 350 larval or juvenile fishes, representing five species, in spring pools in the Barrens Plateau region. Those species were Barrens Topminnows, Flame Chubs, Fringed Darters, Western Mosquitofish, and Spring Cavefish (Table 1).

Fundulus julisia

Larval Barrens Topminnows (n = 10) were collected at only three of the nine sites sampled with light traps, despite the fact that all nine sites harbored adult Barrens Topminnows through either stocking or via natural reproduction (i.e., the type locale). The first three larval Barren Topminnows were collected between 9 June 2004 and 8 July 2004 at two reintroduction sites stocked with propagated adults, one of which also contained Western Mosquitofish (Fig. 1). The low catches (or absence) of larval Barrens Topminnows was probably due to the presence of Western Mosquitofish at most of the sites sampled with light traps (Goldsworthy and Bettoli 2006), given that they will readily prey upon larval Barrens Topminnows *ex situ* (Laha and Mattingly 2007). Etnier and Starnes (2001) noted that Barrens Topminnows can continue spawning into August and September and the capture of the fourth larval topminnow (10.0 mm TL) at the type locale on 24 September 2004 would support their observation that the spawning period is protracted. The six juvenile Barrens Topminnows (17 - 24 mm TL) were all collected at the type locale between 5 July and 24 September 2004.

Hemitremia flammea

Forty-three Flame Chubs were collected in the light traps. The first larval Flame Chubs were collected on 7 May 2004 at the Clayborne 7 spring pool (Fig. 2). Most measured between 6.2 mm TL and 8.2 mm TL with one fish that measured 15 mm TL;

similar-sized Flame Chubs were collected throughout May. June samples collected progressively larger fish. Flame Chubs in the 9 June 2004 sample averaged 17.6 mm TL and in the 19 June sample the average size was 22.9 mm TL. No fish larger than 29.7 mm TL was collected in the traps after the 19 June sample. Flame chub larvae were collected at five of the six sites where adults were known to inhabit (Table 2).

Etheostoma crossopterum

Fringed Darter larvae (n = 128) were collected at four of the seven sites inhabited by adults (Table 2), but only at the upper Sain site were they collected in sufficient numbers to discern emergence times. Prelarvae (i.e., yolk-sac present) appeared in light trap samples from 13 May to 9 June 2004 (Figure 3). Post-larval Fringed Darters were collected on 9 June (11.8 mm TL) and 17 July (13.2 mm TL). The smallest Fringed Darter was 5.8 mm TL; the largest was 21.5 mm TL (17 June) and the last date of capture of any fringed darters was 8 July 2004. Poly (2000) observed Fringed Darter nests from mid-April to late May in a variety of degraded habitats in southern Illinois streams. Given the changes in lengths of Fringed Darter larvae collected over time the spawning season (and subsequent hatching period) spanned about 27 days (Figure 3). Gregory and Powles (1985) noted that larval Iowa Darters (Girard) *E. exile* collected by light traps emerged over 22 days in an Ontario lake. Other *Etheostoma* species (e.g., *E. simoterum* [Cope] [Snubnose Darter]; *E. blennoides* Rafinsesque [Greenside Darter]) were among the most common larvae captured in the light traps deployed by Hartman (1994) in a small Tennessee stream. Floyd et al. (1984b) also reported large catches of four *Etheostoma* species in light traps deployed in a small Kentucky stream. Thus, members of this genus are phototaxic and readily susceptible to capture using lighted larval traps, as Gregory and Powles (1985) also noted. The absence of larval Fringed Darters in traps deployed at three sites where adults were collected (Table 2) suggests those particular sites provided suitable adult habitat, but not good spawning or juvenile nursery habitat.

Gambusia affinis

Western Mosquitofish (n = 168) were the most common species captured in the light traps. Western Mosquitofish are livebearers; hence, no true larval Western mosquitofish were collected. Young specimens (41% were less than 20 mm) were collected from the beginning of May to the end of September (Fig. 4). The smallest Western Mosquitofish collected was 9.5 mm TL and the largest was 32 mm TL. No noticeable growth trend was evident, which is typical for a species that spawns multiple times between May and September (Vargas and de Sostoa 1996); those authors and others have noted that viviparity, multiple broods per female, and large larvae provide Western Mosquitofish with a competitive advantage over oviparous species. Adult Western Mosquitofish were present at seven of the nine sites we sampled and juveniles were captured in light traps at six of those seven sites (Table 2).

Forbesichthys agassizii

Adult Spring Cavefish are a troglophilic species associated with, but not restricted to, caves (Etnier 1983). In a Missouri study, adults appeared to occupy spring pools at night and retreat to subterranean cover near sunrise (Adams et al. 2002). We collected adult Spring Cavefish at three pools in the Clayborne Complex (Table 2). However, only one larval Spring Cavefish (8.0 mm TL) was collected and it was caught at Clayborne 1, the springhead pool on 9 June 2004. Adams et al. (2002) caught no larval Spring Cavefish in light traps deployed at a Missouri spring site. Adult Spring Cavefish are negatively phototactic (Poulson 1963) and low catches of larvae in light traps might be expected. However, Adams et al. (2001) caught more than 100 larval and juvenile Spring Cavefish using lighted traps at an Illinois spring. In two previous studies, Spring Cavefish fry (which hatch underground) appeared in the epigean portions of their spring habitats in early March (Poulson 1963) and between January and April (Adams et al. 2001) and we did not begin deploying traps until May. However, our catch of a larval individual in early June indicates that spawning and hatching can occur much later than previously reported. The absence of Spring Cavefish larvae in the catches at the two pools downstream of the springhead is additional, albeit weak, evidence that spawning and hatching do not occur in surface waters and are restricted to subterranean habitats, as noted by Poulson (1963), Smith and Welch (1978), and Adams et al. (2002).

Species that were not collected in the larval traps

Adult Creek Chubs were observed at two of the nine sites but their larvae were not collected by the light traps (Table 2). Floyd et al. (1984b) collected larval Creek Chubs in light traps set in a Kentucky stream; the absence of larval Creek Chubs in our samples, despite the presence of adults, suggests that the adults were transients and unable to find suitable spawning habitat. Adult Bluegills and Green Sunfish were observed at one site (the Cunningham Barn site) but no larval sunfish were collected at that site. Larval sunfish are susceptible to light traps (e.g., Floyd et al. 1984b; Gregory and Powles 1985) and their absence in light trap samples at the Cunningham Barn site suggests that there was no spawning habitat in that pond and adults observed in previous surveys were transients. Finally, Banded Sculpins were common at the type locale (the only species other than Barrens topminnows at that site), but no larval sculpins were collected. Hartmann (1994) did not report capturing any Banded Sculpin larvae in light traps set in a small Tennessee stream, but noted that adults were common. Likewise, Floyd et al. (1984b) also failed to capture Banded Sculpin larvae in light traps in a stream where juvenile and adults were present. These observations might suggest that larval sculpins are not phototaxic. Another possible explanation for their absence in our samples is that Banded Sculpins spawn in late winter or early spring (Etnier and Starnes 2001); larvae may have grown and assumed a demersal existence (and been less likely to swim into a floating trap) by the time we deployed our traps in early May.

Efficacy of Light Traps and Future Research Needs

Sponaugle and Cowen (1996) and others have noted the taxonomic biases associated with using larval light traps; namely, a species must be positively phototaxic and display directional swimming at the larval stage in order to be sampled. However, all gears have biases associated with them, and lighted traps provide important information on the supply of larval forms of those species susceptible to capture in that type of gear (Thorrold 1992). In our study and others, larval Flame Chubs, Fringed Darters, and Western Mosquitofish were susceptible to light traps, and the presence of larval Barrens Topminnows at some sites where subsequent recruitment could not be documented provided an important clue regarding the critical period in the early life history of that imperiled species (Goldsworthy and Bettoli 2006). The capture of numerous larval Flame Chubs and Fringed Darters in the light traps (and adults of both species in other gears) indicated that recruitment was occurring in those populations despite the presence of numerous mosquitofish juveniles and adults. It is unknown why those two species (Flame Chubs and Fringed Darters) and not Barrens Topminnows are able to thrive in spring habitats of the Barrens Plateau region. The population dynamics and behavior of Western Mosquitofish and native fishes, especially at the early life history stage, need to be examined more closely to determine the mechanisms that allow some species, but not others, to persist in the presence of dense populations of invasive Western Mosquitofish. On a related topic, Laha and Mattingly (2006) discussed the differential influence of temperature on the growth rates of Barrens Topminnows and Western Mosquitofish (and the conditions under which the former species might coexist with the latter) and the thermal ecology and bioenergetics of Western mosquitofish have been described (e.g., Chipps and Wahl 2004). Unfortunately, similar data do not exist for Fringed Darters and Flame Chubs to potentially explain why those species persist in spring pools of the Barrens Plateau region in the presence of the invasive Western Mosquitofish.

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Table 1. Species collected by larval light traps deployed at nine spring pools in the Barrens Plateau region of Tennessee, May-September 2004.

Species	N	Range in total length (mm)
Etheostoma crossopterum	128	5.8 - 21.5
Forbesichthys agassizii	1	8.0
Fundulus julisia	10	7.2 - 24.0
Gambusia affinis	168	9.5 - 56.0
Hemitremia flammea	43	6.2 - 29.7

Table 2. Presence of adult and larval individuals of nine fish species at nine spring sites in the Barrens Plateau region of middle Tennessee where larval fish were sampled using lighted larval traps. Open circles indicate that adults were collected in seine and electrofishing samples preceding larval sampling; filled circles indicate that larval and adult forms were both collected.

Site	*Gambusia affinis*	*Hemitrema flammea*	*Etheostoma crossopterum*	*Forbesichthys agassizi*	*Fundulus julisia*	*Semotilus atromaculatus*	*Lepomis cyanellus*	*Lepomis macrochirus*	*Cottus carolinae*
Clayborne 1	○	●	●	●	○	○			
Clayborne 4[1]					●				
Clayborne 6	●	●	○	○	●				
Clayborne 7	●	●	○	○	○				
Sain Upper	●	●	●		○				
Sain Lower	●	○	●		○				
Cunningham Barn	●		●		○		○	○	
Cunningham Lower	●	●	○		○	○			
Type Locale[2]					●				○

[1] This small, man-made pool was isolated from all nearby pools.

[2] A concrete barrier erected at the outfall of this spring pool prevented any colonization by stream fishes residing in downstream reaches.

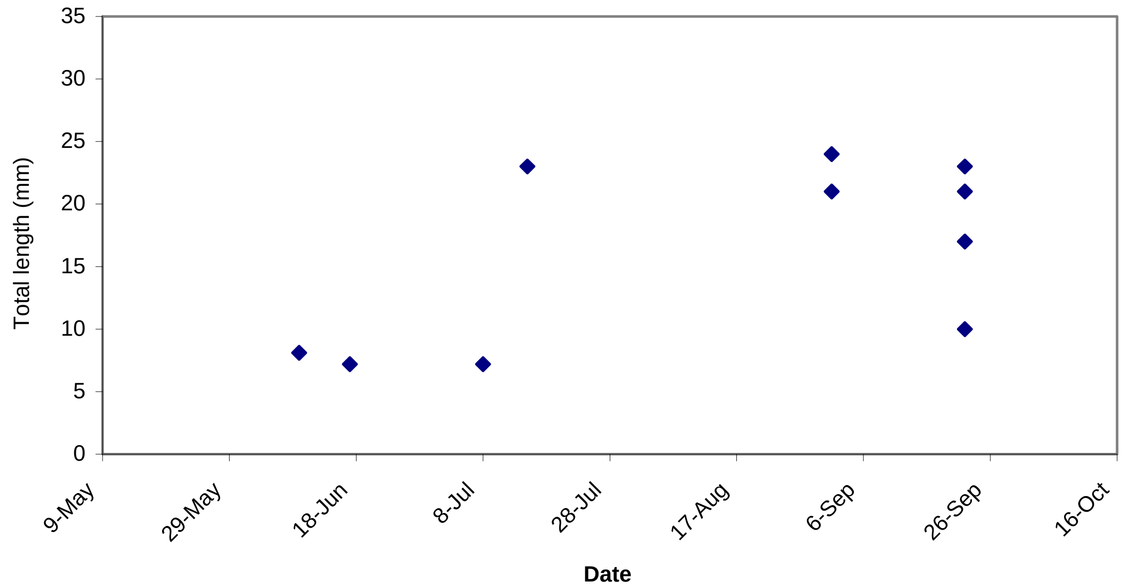

Figure 1. Total length and date of capture for Barrens Topminnows captured at three sites in light traps deployed weekly or biweekly between May and September 2004 in the Barrens Plateau region of middle Tennessee.

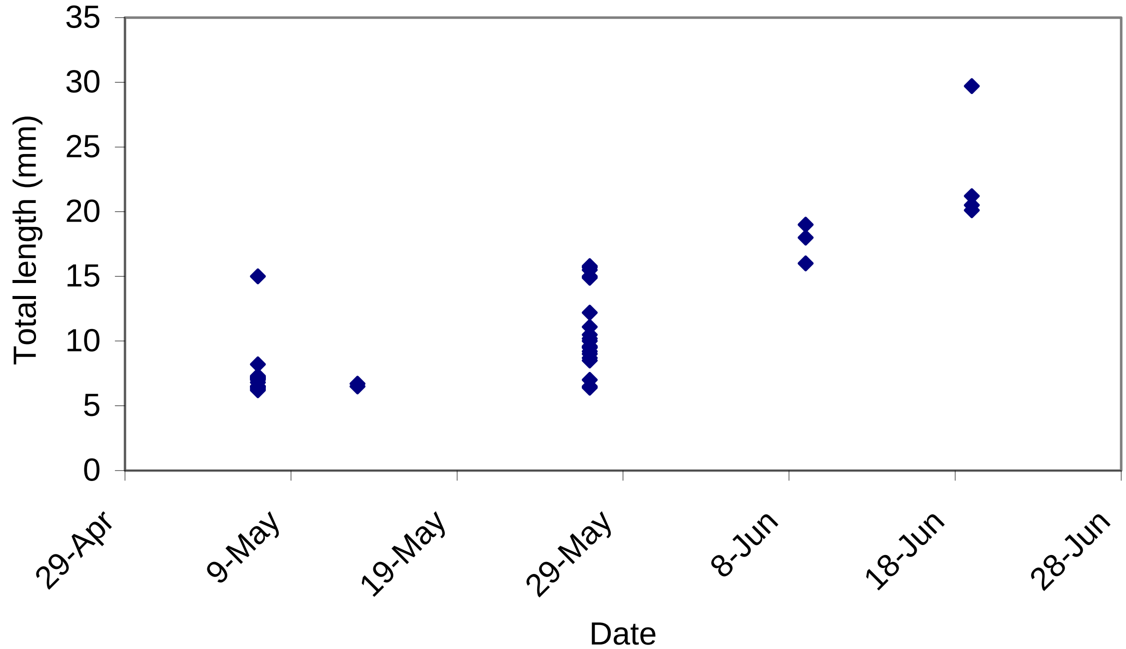

Figure 2. Total length and date of capture for Flame Chubs captured at five sites in light traps deployed weekly or biweekly between May and September 2004 in the Barrens Plateau region of middle Tennessee.

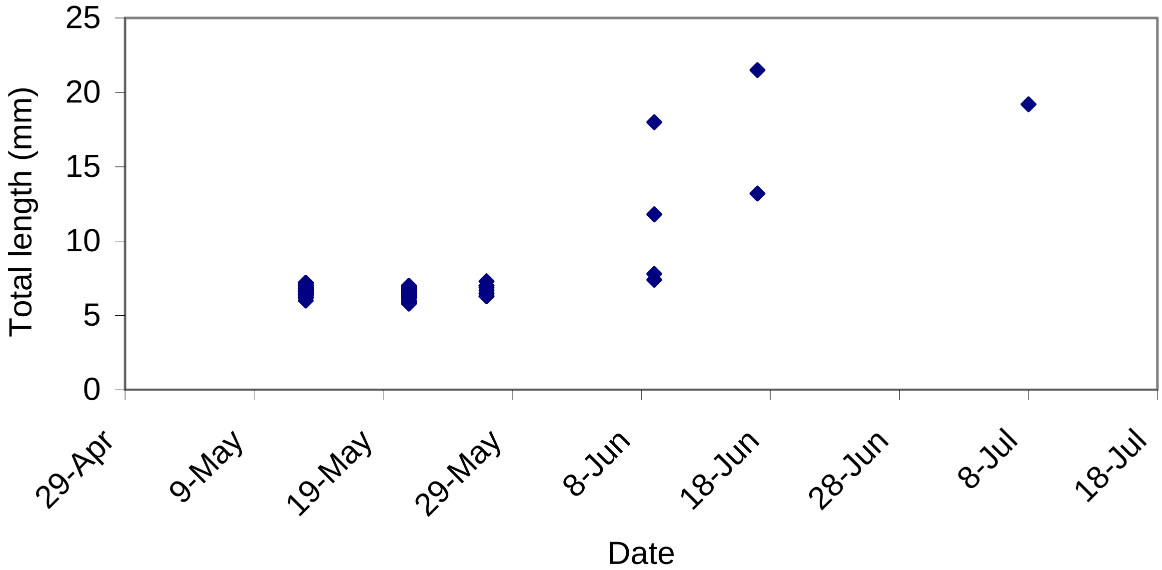

Figure 3. Total length and date of capture for Fringed Darters captured in light traps deployed weekly or biweekly between May and September 2004 at the upper Sain site in the Barrens Plateau region of middle Tennessee.

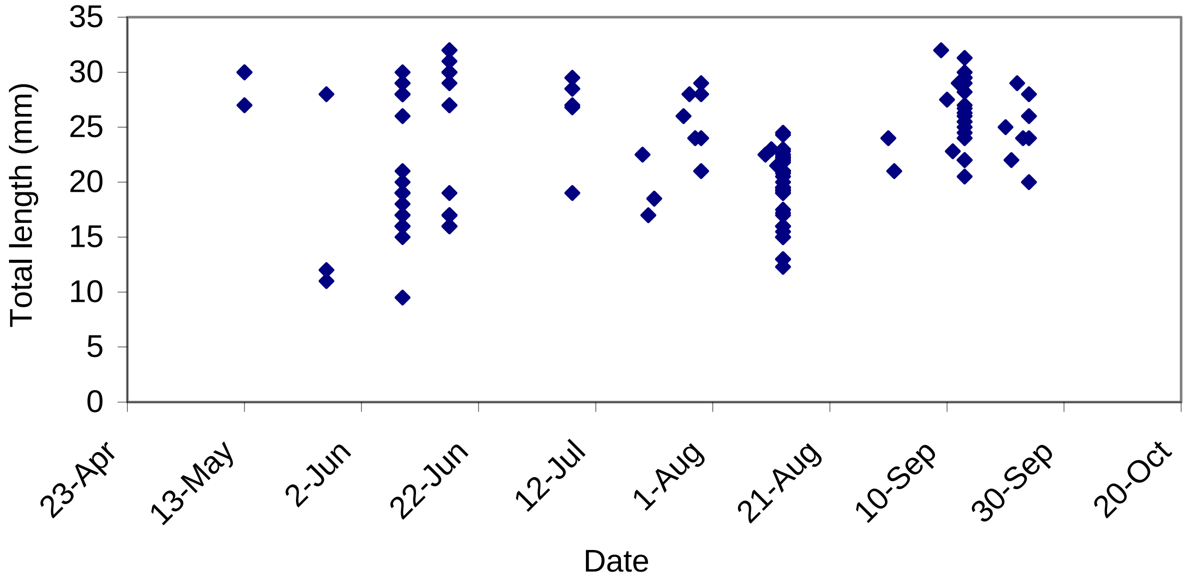

Figure 4. Total length and date of capture for Western Mosquitofish captured at six sites in light traps deployed weekly or biweekly between May and September 2004 in the Barrens Plateau region of middle Tennessee.