The Fate of Stocked Barrens Topminnows *Fundulus julisia* (Fundulidae) and Status of Wild Populations




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By

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Executive Summary

1. Barrens topminnow *Fundulus julisia* populations have declined precipitously since the species was described in 1982. Propagation and reintroductions have been the primary means of recovery since 2001, but the reintroductions have been generally unsuccessful in creating self-sustaining populations.

2. Biotic and abiotic factors affecting 17 stocked Barrens topminnow populations were examined from 2003 to 2005 and the status of wild populations was described. Populations of stocked and wild topminnows and introduced-exotic Western mosquitofish *Gambusia affinis* were estimated using the Zippin removal-depletion technique. Lighted larval traps were deployed at eight reintroduction sites and the type locale to determine whether topminnows could reproduce in the presence of mosquitofish. The thermal environment and aquatic flora were also described at reintroduction sites.

3. The density of mosquitofish at reintroduction sites ranged from zero to 66 fish per m^2. Annual mortality of stocked Barrens topminnows ranged from 45 to 100%. Annual mortality of stocked topminnows was not related to mosquitofish density, nor the minimum, maximum, or average temperatures recorded at each site.

4. The adjusted mean weights (i.e., robustness) of Barrens topminnows did not differ in the presence or absence of mosquitofish, suggesting interspecific competition for food was not occurring. Similarly, mean growth rates of stocked Barrens topminnows were unrelated to mosquitofish density.

5. Although Barrens topminnow reproduction was observed at one site harboring mosquitofish, recruitment (i.e., wild age-0 topminnows collected in the fall) was limited to sites without mosquitofish.

6. Mosquitofish were present at a spring-fed farm pond that supports one of four wild populations of Barrens topminnows and mosquitofish recently invaded a second site harboring wild Barrens topminnows. One of the four wild populations (i.e., Type Locale) has been threatened in the past by drought.

7. The findings of this study and concurrent laboratory studies support the hypothesis that mosquitofish predation on larval or juvenile Barrens topminnows was the primary mechanism in failed reintroductions and is the greatest threat to reintroduced and wild populations.

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COVER PHOTOS

Clockwise from upper left: a cohort of propagated topminnows marked with an elastomer tag and ready for stocking; Summitville Pond (the Type Locale for the Barrens topminnow); a larval topminnow collected in a light trap at the Type Locale; Cory Goldsworthy and Chad Holbrook preparing to seine the barn pond at the Cunningham property.

INTRODUCTION

Due to limited distribution and scarcity of undisturbed habitats, the Barrens topminnow *Fundulus julisia* is considered one of the most critically endangered fishes in eastern North America (Williams and Etnier 1982). The species is endemic to the Barrens Plateau region of Middle Tennessee and confined to three watersheds – the Duck River, Elk River, and Caney Fork River (Figure 1). The Tennessee Valley Divide separates these watersheds; streams and creeks in the Caney Fork River drainage flow into the Cumberland River system and streams and creeks in the Duck River and Elk River drainages flow into the Tennessee River system. The Barrens Plateau is made up of chert rock and its soil derivatives, which supports limited tree life. Softer Mississippian limestones below create many springs that emanate from numerous aquifers (Etnier and Starnes 2001) and provide habitat for spring-associated fishes including spring cavefish *Forbesichthys agassizii*, flame chubs *Hemitremia flammea*, and southern redbelly dace *Phoxinus erythrogaster*. Spring habitats have been altered during development of farmlands and nurseries, which has limited the Barrens topminnow to a few isolated locales (Rakes 1989). Prior to 1993, Barrens topminnows were known to exist at 20 locations; by 1994, seven of these populations remained and only four wild Barrens topminnow populations were known to exist by 2005.

The first Barrens topminnows were collected on March 26, 1937, when L.F. Miller was conducting pre-impoundment surveys for the Tennessee Valley Authority in the Duck River system (Williams and Etnier 1982). These specimens were catalogued as the whiteline topminnow *Fundulus albolineatus,* which were only known from specimens collected in 1889 in Big Spring, Huntsville, Alabama; the species is considered extinct (Rakes 1995). In 1966, J.D. Williams discovered Barrens topminnows at a new site called Summitville Mountain Spring (in the Cumberland River system) in Coffee County, Tennessee. Distinct species status was given in 1982 and this site was designated the type locale of the species (Williams and Etnier 1982).

Though proposed for listing in the late 1970s, the Barrens topminnow has never been afforded protection under the Endangered Species Act. Federal listing was initially proposed on 30 December 1977 (United States Fish and Wildlife Service [USFWS]

1977); however, changes in the designation of critical habitat in 1978 resulted in the withdrawal of 1,850 proposals, including the proposal for the Barrens topminnow (USFWS 1978). On 27 July 1979 the proposal was resubmitted (USFWS 1979) and Drs. Howell and Stiles of Samford University offered to propagate and stock topminnows for $15,000 (B. Bingham, USFWS, personal communication). A public hearing was held in Manchester, TN, on 30 August 1979, but public outcry and the failure by the USFWS to fully implement the 1978 amendments to the Endangered Species Act (e.g., adequate public notification, coordination with local officials, complete public involvement, and adequate biological and economical data) necessitated withdrawal (B. Bingham, USFWS, personal communication). The Barrens topminnow was officially removed from the proposed list on 30 December 1979 and is currently listed as a species of management concern. The Tennessee Wildlife Resources Agency (TWRA) listed the Barrens topminnow as state-endangered on 12 June 1975 under the Tennessee Nongame and Endangered or Threatened Wildlife Conservation Act of 1974. However, several new Barrens topminnow populations were discovered in the early 1980s and the species was subsequently downlisted to threatened status.

In 1983, the USFWS and the TWRA funded surveys to gain information on Barrens topminnow abundance and distribution. Fourteen populations were discovered in the Caney Fork River drainage and one was discovered in the Elk River drainage; however, several historic populations in the Duck River drainage and West Fork of Hickory Creek had been extirpated (Etnier 1983). The TWRA attempted to establish two populations in the Duck River in 1983, but those attempts failed. Population declines and extirpations were observed in the 1980s and 1990s, which prompted additional surveys in the mid-1990s (Rakes 1996). Rakes (1996) concluded that the number of Barrens topminnows had declined from 4,500-5,000 adults at 14 localities in 1983 to only a few hundred adults at seven localities by 1995.

In 2000, Barrens topminnows were known to exist at only two locations, both on private property, and neither population was considered stable. One population was threatened by drought and the other declined precipitously since the 1980s due to habitat degradation by cattle (Rakes 1996). In 2001, in an effort to preclude listing under the Endangered Species Act, a task force (Barrens Topminnow Working Group) comprised of state, federal, and non-profit agencies was created to work in cooperation with landowners to protect wild populations and use propagation and stocking to establish at least five populations each in the Duck River, Elk River, and Caney Fork River watersheds. Brood stocks were collected from wild populations and fish were propagated at Conservation Fisheries, Inc., in Knoxville, TN, and the Tennessee Aquarium in Chattanooga, TN. Strange and Lawrence (2002) recommended managing each drainage population separately through planned reintroductions, being careful not to reduce genetic diversity within populations by releasing fish from other stream systems.

In 2001-2002 the Working Group stocked 1,267 Barrens topminnows in the Barren Fork River and Hickory Creek watersheds of the Caney Fork River system to establish new populations and identify factors regulating their persistence; however, the results were generally negative (Johnson 2004). In 2002, two populations of Barrens topminnows were discovered in the McMahan Creek watershed (Barren Fork River system), but the stability of these populations was questionable. One site occurred behind a new housing subdivision and the other occurred on an active cattle farm adjacent to a major highway.

Competition with introduced-transplanted mosquitofish *Gambusia affinis* has been implicated in the decline of Barrens topminnows. The term *introduced-transplanted* is defined as a plant or animal moved outside its native range, but within a country where it naturally occurs (Shafland and Lewis 1984). Mosquitofish have been indiscriminately stocked around the world as mosquito-control agents to the detriment of native fishes, especially in spring ecosystems (Courtenay and Meffe 1989). Ehrlich (1986) described eight characteristics of highly successful invasive species, of which mosquitofish possess seven: abundance in original range, polyphagous, short generation times, a single female can colonize, broad physiological tolerances, closely associated with man, and high genetic variability. The only characteristic of a successful invasive species not possessed by mosquitofish is large body size. Courtenay and Meffe (1989) discussed how specialized reproduction and high aggression levels contribute to the success of mosquitofish as an invader. Mosquitofish are livebearers and produce multiple broods throughout the warmer months. A single mature mosquitofish can produce 3-4 broods each spawning season, each ranging from several dozen to several hundred precocious offspring (Pflieger 1975; McDowall 2000). In contrast, fecundity of wild Barrens topminnows does not exceed 300 eggs (Rakes 1989) and their offspring are true larvae.

Female mosquitofish can store sperm from one copulation event for up to eight months and fertilize an entire year's broods. The aggressive nature of mosquitofish contributed to the elimination or decline of threatened or endangered species such as the Railroad Valley springfish *Crenichthys nevadae* in Nevada, the least chub *Iotichthys phlegethontis* in Utah, the endangered Gila topminnow *Poeciliopsis occidentalis* in Arizona, and the California newt, *Taricha torosa* (Galat and Robertson 1992; Gamradt and Katz 1996; Fuller et al. 1999; Mills et al. 2004).

The objectives of this study were to determine: (1) whether mortality, growth, and body condition of stocked Barrens topminnows varied with mosquitofish density; (2) whether Barrens topminnows can reproduce and produce juvenile recruits in the presence of mosquitofish; (3) the status of wild populations of wild Barrens topminnows.

Stocking Sites (clockwise from upper left): Vervilla Pool #3; Collier springhead; Pocohantas spring pool; excavated pool in Marcum property spring run; excavated pools at Clayborne site; upper Murphy springhead and excavated pool.

STUDY AREA DESCRIPTION AND TAGGING PROTOCOLS

Most reintroduction efforts have focused on properties in the Hickory Creek watershed in the headwaters of the Caney Fork River system (Table 1). Five properties were located on West Fork Hickory Creek and two were on the main branch of Hickory Creek. One property was on the South Prong of the Barren Fork River (Caney Fork River system) and one was on Sink Creek, a tributary to the Caney Fork River. The last two properties were on Carroll Creek in the Duck River system. Only one of 11 properties (Vervilla) was not private property. The 11 properties we studied represented most of the properties that were stocked with topminnows by the Working Group in 2003-2005. These specific properties were chosen based on past sampling experiences and logistics; sites that were difficult to sample quantitatively were avoided, as were sites where ready access could not be guaranteed.

Before stocking, all hatchery-reared Barrens topminnows were anaesthetized using MS-222, measured for total length, and injected with Visible Implant Fluorescent Elastomer (VIE) developed by Northwest Marine Technology, Inc. The VIE tags were placed anterior or posterior to the left or right of the dorsal fin to created distinct marks for each cohort and study site. Four colors (red, green, yellow, orange) and nine tag locations (i.e., left posterior; right posterior; left-posterior and right-anterior; etc.) were used to distinguish among stocked cohorts. Amber glasses and a portable UV light source were used as an aid in identification of tagged individuals.

Mapping was conducted using a plane table and optical alidade to obtain surface areas of most stocking sites. Sites void of mosquitofish were not mapped. Temperature data loggers (Onset Corporation) were placed at sampling locations in 2003 and 2004 and retrieved in February 2004. A line-transect method was used to conduct vegetation surveys at each reintroduction site in late summer (July - September) 2003 or 2004. Transects were set perpendicular to stream flow and presence or absence of aquatic vegetation was recorded at each 1.0 m or 0.5 m interval, depending on the length and width of the site (McMahon et al. 1996). Coverage was estimated visually when it appeared to be greater than 95% or less than 5%.

Reintroduction Sites

Little Hickory Creek

Clayborne Property. This site consists of seven small pools at a springhead (Figure 2), four of which were stocked and examined in this study: Clayborne 3, 4, 6, and 7. Pools 1, 2, and 5 were not stocked during this study (although pool 1 was sampled for larval fish; see below) and no individuals or cohorts stocked into these three pools prior to this study were collected. Clayborne 3 was excavated in the spring run, measures 49 m^2, and was stocked with fifty topminnows in August 2003. Clayborne 4 measures 16 m^2 and was excavated above the floodplain of the spring system, is completely isolated from the other pools, and was void of fish prior to its initial stocking in 2001 (n = 10). This same pool was stocked again on 10 April 2002 (9 fish) and 3 June 2003 (12 fish). Pools 6 and 7 were excavated in June 2003. Both pools are situated in the floodplain and measure 59 and 46 m^2, respectively. On 27 August 2003, 88 fish were released into Clayborne 6 and 78 fish were released into Clayborne 7. Submersed aquatic vegetation was scarce (< 5% coverage) in all pools. Resident fishes in this complex of pools (except pool 4) included fringed darters *Etheostoma crossopterum,* creek chubs *Semotilus atromaculatus*, spring cavefish, mosquitofish, and flame chubs (Johnson 2004).

Sain Property. This site is immediately downstream of the Clayborne site. Clayborne 3 drains into the upper Sain pool (51 m^2), which drains into the lower Sain pool (50 m^2), about 30 m downstream. A small spring flows into the lower pool. Aquatic vegetation coverage was less than 5% in both pools. The pools at this site were stocked twice in 2003 with a total of 110 Barrens topminnows. Resident fish fauna included mosquitofish, flame chubs, and fringed darters (Johnson 2004).

Cunningham Properties. Two spring systems on the Cunningham property were stocked with Barrens topminnows. The most upstream site, noted as the Cunningham barn property, is a spring-influenced, excavated pool with a surface area of 191 m^2. In summer it is usually covered with a layer of duckweed *Lemna* spp., filamentous algae, and pondweed *Potamogeton* spp. (> 95% coverage), which recedes in winter. Fifty Barrens topminnows were stocked at this site in August 2003. Resident fish fauna included green sunfish *Lepomis cyanellus*, bluegill *L. macrochirus*, mosquitofish, and fringed darters (Johnson 2004). The downstream property (designated Cunningham Lower) consists of two smaller pools (68 and 56 m^2) excavated by the USFWS in 2003. These pools are spring-influenced and connect to the mainstream of Little Hickory Creek by a spring run. Both pools are prone to large infestations (> 95% coverage) of *Myriophyllum* sp. and filamentous algae. Between August 2003 and June 2004, 558 Barrens topminnows were released into these pools. Resident fishes included largemouth bass *Micropterus salmoides*, bluegill, mosquitofish, and fringed darters (Johnson 2004).

Murphy Property. This site is the most downstream site on Little Hickory Creek and consists of a springhead that forms a spring run, which empties into Little Hickory Creek. A small pool (10 m^2) was excavated in 2002 adjacent to the spring run to provide slack-water habitat for the 367 Barrens topminnows stocked between August 2001 and June 2004. A dense infestation (> 95% coverage) of pondweed occupies the site much of the year. Resident fishes included telescope shiners *Notropis telescopus*, bluntnose minnows *Pimephales notatus*, banded sculpin *Cottus carolinae*, mosquitofish, flame chubs, spring cavefish, largemouth bass, and bluegill (Johnson 2004).

<u>Hickory Creek</u>

Ramsey Property. This site is located on a cattle farm in the town of Viola, Tennessee, and consists of two connected pools measuring 432 m^2 and 100 m^2, which were excavated by the USFWS in 2004. Aquatic vegetation coverage reached 68% in 2004 with *Zannichellia palustris, Ludwigia palustris*, and *Nasturtium officinale* being the dominant species. The site was seined on 6 January 2005 and resident fishes included striped shiners *Luxilus chrysocephalus*, northern studfish *Fundulus catenatus*, mosquitofish, creek chubs, and flame chubs. Three cohorts totaling 617 topminnows were stocked in May, June, and November 2004.

Vervilla Property. This site, located on land owned by the USFWS, is the most downstream stocking site on Hickory Creek. Six pools ranging from 9 m^2 to 82 m^2 were excavated on a small spring run that discharges into Hickory Creek. Native grasses were planted in the riparian zone and the perimeter was fenced to exclude off-road vehicles.

The pools are all prone to dense blooms (> 95% coverage) of filamentous algae. Two cohorts totaling 373 Barrens topminnows were stocked between August 2003 and June 2004. Resident fishes included golden shiners *Notemigonus crysoleucas*, bluntnose minnows, fringed darters, mosquitofish, and flame chubs (Johnson 2004).

Caney Fork River

Herndon Property. Located in southeastern DeKalb County, Tennessee, this site consists of a large spring pool and spring run that flows into Sink Creek, a tributary to the Caney Fork River. Aquatic vegetation was scarce (< 5% coverage) in the spring pool. The spring pool was stocked in July 2004 (n = 175) and November 2004 (n = 105). The site was sampled with a seine net on 2 December 2004 and resident fishes included flame chubs, creek chubs, southern redbelly dace, green sunfish, and bluegill.

Barren Fork River

Bridges Property. This site, which consists of a shallow spring run with riffles, runs, and pools with scant (< 5% coverage) aquatic vegetation, flows into an unnamed tributary of Mud Creek in northeastern Coffee County, Tennessee. Twenty-two Barrens topminnows were released at this site in May 2004. The site was sampled with a seine net on 10 January 2005 and resident fishes included central stonerollers *Campostoma anomalum,* redband darters *Etheostoma luteovinctum,* creek chubs, flame chubs, southern redbelly dace, spring cavefish, banded sculpin, and fringed darters.

Duck River

Marcum Property. Located on private property near Tullahoma, Tennessee, this site consists of a spring discharging into a concrete trough, which drains into a spring run that empties into a pond. A concrete barrier isolates the small pond from a small reservoir (Lake Tullahoma), and the small pond was void of resident fishes. To improve topminnow habitat, the USFWS excavated a series of pools between the concrete trough and the small pond. Afterwards, the landowners cleared trees and underbrush above the spring run and runoff from a severe thunderstorm filled all but one of the excavated pools with gravel. The small pond also received a heavy load of sediment from that same storm. Barrens topminnows were stocked into the remaining excavated pool in May 2004 (n = 30) and July 2004 (n = 91), when vegetation coverage was 72%.

Collier Property. This site, located on a small, unnamed tributary to Lake Tullahoma, consists of two pools excavated around multiple springheads. Watercress and filamentous algae covered 50% of the pools when 50 Barrens topminnows were stocked in May 2004. A seine survey conducted on 13 November 2004 collected stonerollers, green sunfish, and bluegills.

Wild Populations

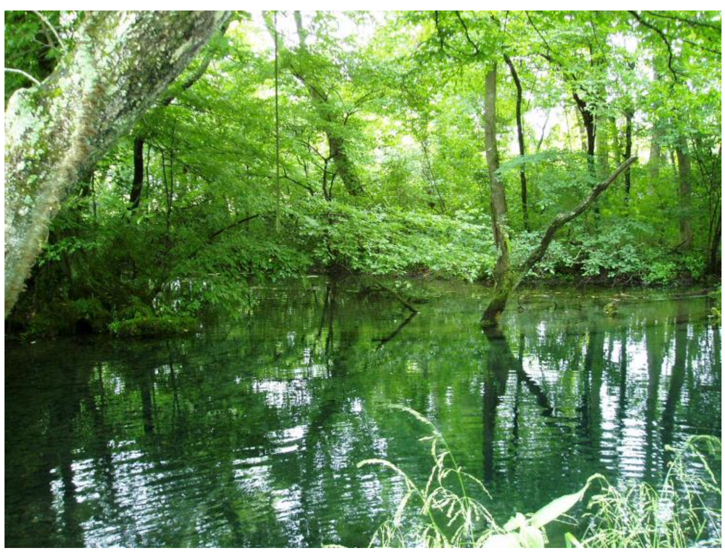
Pedigo Property. Lewis Creek, a tributary to Witty Creek in the Barren Fork River watershed, flows through an active cattle farm and consists of riffle habitat with gravel substrate and intermittent slackwater pools with scant aquatic vegetation. Barrens topminnows were known to exist at this locale, but the population had never been sampled. On 2 March 2005 a seine survey collected Barrens topminnows, flame chubs, rosyside dace *Clinostomus funduloides*, central stonerollers, northern studfish, bluegill, banded sculpin, and mosquitofish. Mosquitofish were previously restricted to the lower reach of the Pedigo site due to a short (~ 0.5 m) drop in the streambed that served as a partial barrier to upstream fish movement, but heavy flooding in the winter of 2004-2005 permitted upstream invasion by mosquitofish.

Type Locale. The Type Locale is a spring emanating from a cave located about 100 m north of Tennessee Highway 55 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 rainbow trout *Oncorhynchus mykiss*. The trout dispersed downstream, but the pool acted as a refuge for Barrens topminnows and the dam prevented mosquitofish invasion (Williams and Etnier 1982). Filamentous algae was abundant in the pool. This site is susceptible to drought and in 1980 and 1981 Barrens topminnows were captured and moved to indoor holding facilities until water levels in the pool returned to normal (Rakes 1995). A seine survey on 22 April 2004 collected Barrens topminnows and banded sculpin.

Pond Spring. Pond Spring is an inundated sinkhole (~ 0.25 hectare) with multiple springs (Rakes 1989). The outflow of Pond Spring creates a tributary to Bradley Creek in the Elk River watershed in southeastern Coffee County, Tennessee. The pond is surrounded by pastures and was fenced off to exclude cattle. A seine survey conducted on 2 March 2005 collected mosquitofish, bluegill, and flame chubs. Common carp *Cyprinus carpio* were sighted but not collected and pondweed and filamentous algae were abundant throughout the pond.

Counting, measuring, and weighing topminnows at the Clayborne property.

Deploying a light trap at one of the Clayborne pools.



The Herndon stocking site in the headwaters of the Caney Fork River watershed.

METHODS

Data Collection

A seine net (1.8 m x 6.0 m, 3.1-mm mesh) was used to sample reintroduction sites between November 2004 and January 2005. Catch data were used to estimate Barrens topminnow and mosquitofish population sizes using the Zippin removal-depletion method (Zippin 1958; Platts et al. 1983). At least two and no more than four seine hauls were taken; sampling was terminated if no Barrens topminnows were caught. We sampled in the winter months when vegetation coverage at each site would be at its lowest and seining would be easiest. Mosquitofish in each seine haul were preserved in 10% formalin and returned to the laboratory and counted. Barrens topminnows were counted, measured for total length (mm), weighted (0.1 g), sexed, and assigned to a cohort based on tag color and location.

Larval Barrens topminnows were sampled using light traps modified from a Tennessee Valley Authority (TVA) prototype (Hartman 1994). Seven sites stocked during this study were selected for sampling based on their proximity to one another (to reduce travel costs) and to sample a wide range of vegetation coverages (< 5% to near 100% coverage) and presumed mosquitofish densities (from zero to very high densities). An eighth site (Clayborne 1) that was stocked with 138 topminnows in 2001-2003, but was not stocked during this study, was also sampled for larval fish, as was the Type Locale. Thus, larval fish were sampled at nine sites. Light traps were best suited for presence or absence studies (Kelso and Rutherford 1996) and we did not attempt to relate larval catch rates to number of stocked topminnows or estimated topminnow densities. The main body of the trap consisted of a 10.2-cm wide section of 27.9 cm (inside diameter) PVC pipe with about 1/3 of the circumference removed for an entrance (Hartman 1994). The entrance consisted of 5-mm thick clear acrylic plastic angled inward to create a 5-mm wide entrance slit. The width of this slit was subsequently reduced to 3 mm using 2-mm thick acrylic sheets to prevent adult Barrens topminnows and mosquitofish from entering the trap. Photochemical light sticks were placed into each trap, and traps were deployed overnight at each of the nine sites weekly between

May and June 2004. In a laboratory trial using a 720-liter aquarium, six of nine Barrens topminnows (20-25 mm TL) entered the light trap overnight. Traps were deployed bimonthly between June and September 2004. These dates corresponded with water temperatures (15-20^{o} C) considered optimal for Barrens topminnow spawning (Rakes 1989; Johnson 2004). When the traps were retrieved the following morning the contents of each trap were washed through a small mesh net and fixed in 10% formalin solution, returned to the laboratory for processing, and then transferred to ethanol. Barrens topminnow larvae were subsequently counted and measured for total length. Propagated larval and juvenile Barrens topminnows obtained from the Tennessee Aquarium in Chattanooga, Tennessee, were used as an aid in identifying wild-caught larval topminnows.

Three of the four wild populations were sampled in 2004 or 2005 using a seine net (1.8 m x 6.0 m, 3.1-mm mesh). All Barrens topminnows were externally sexed and measured for total length. The presence of mosquitofish was also recorded.

Data Analysis

Catch-depletion data were analyzed using the Zippin removal-depletion procedure and MicroFish 3.0 software to estimate population sizes (Van Deventer and Platts 1985). If removal patterns failed to produce a population estimate, or minimum capture probabilities (determined by MicroFish 3.0) did not exceed 0.2, the total number of fish captured was used as the population estimate (White et al. 1982; Dunham et al. 2002). The instantaneous mortality rate (Z) of Barrens topminnows was calculated as

$$Z = (\log_e N_t - \log_e N_{t+1}) / \Delta t \quad ,$$

where N_t is the number of fish stocked at the beginning of an interval, Δt is the days-post-stocking, and N_{t+1} is the number of fish at the end of the interval (i.e., population estimate). Annual interval mortality (A, %) was calculated as

$$A = 1 - e^{-Z*365} \quad .$$

Movements of Barrens topminnows out of a stocked site would inflate mortality estimates at that site; we assumed survival of fish that dispersed downstream was nil. Linear regression techniques were used to explain the variation in interval mortality of Barrens topminnows as a function of mosquitofish density and the minimum, maximum, and average temperatures at each site.

Instantaneous growth rates (G, %-d^{-1}) were calculated as:

$$G = \left(\log_e \overline{TL_t} - \log_e \overline{TL}_{t+1}\right) / \Delta t$$

where $\overline{TL}_t$ and $\overline{TL}_{t+1}$ are the mean total lengths of fish when they were stocked and recaptured, and Δt is the interval in days. Instantaneous growth rates were calculated over short intervals (< 200 days) and long intervals ($\geq$ 200 days) whenever at least three fish of a particular cohort were recaptured at any site.

Fisher's exact test was used with 2 x 2 contingency tables to test whether Barrens topminnow reproduction and recruitment were associated with mosquitofish presence or absence. Irregular sampling with a seine revealed the presence of topminnows and mosquitofish in the Clayborne 1 pool (which was not subjected to catch-depletion sampling) as late as November 2003 (Bettoli et al. 2004); therefore, we assumed that mosquitofish were present when we sampled larval fishes in that pool in 2004. Reproduction was defined as the capture of larval Barrens topminnow in light traps. Recruitment was defined as the presence of wild (untagged) age-0 Barrens topminnows in autumn or winter seine collections.

The robustness of Barrens topminnows in the presence or absence of mosquitofish was compared using analysis-of-covariance (ANCOVA). The ANCOVA model included a response variable (weight), an intercept (β_0), and two independent variables: a covariate (length) and a dummy variable that represented the effect of mosquitofish presence on Barrens topminnow weight. Differences in regression intercepts would indicate differences in robustness among populations (Bolger and Connolly 1989). The assumption of equal slopes ($P > 0.05$) was tested using an F-test prior to performing the analysis-of-covariance of adjusted mean weights.

VIE-tagged topminnows ready for release.

Seining the Cunningham Barn site to obtain a Zippin population estimate of topminnows and mosquitofish.

RESULTS

Reintroduced Topminnows

Annual mortality of stocked Barrens topminnows ranged from 45% to 100%; 23 of 28 cohorts experienced annual mortality rates over 95% (Table 2). No topminnows were captured at seven stocking sites. Although no topminnows were collected at Clayborne 3 on 12 November 2004 (Table 3), two topminnows stocked in that pool in August 2003 were collected in Clayborne 6 and Clayborne 7; thus, the annual mortality rate for that cohort was 93% (not 100%). Similarly, no topminnows were collected in the lower Sain pool on 12 November 2004, but two of the topminnows stocked there in June 2003 were collected in November 2004 in the upper Sain pool; thus, annual mortality for that cohort was 94% (not 100%). The depletion sampling yielded declining, or zero, catches of individual cohorts at all sites except the Ramsey site. This site was one of the largest sites, and the irregular bottom contour and aquatic vegetation made seine sampling difficult. We did not deplete the last two cohorts stocked into the Ramsey site when it was sampled on 6 January 2005 and we used the total catch of each cohort as a population estimate; thus, we probably overestimated mortality of those two cohorts.

Mosquitofish were collected at 12 of the 17 sites (Table 2) and their densities ranged from 0 to 66 fish per m^2. Annual mortality of Barrens topminnows was not related to mosquitofish density ($F = 0.04$; df = 1, 26; $P = 0.844$), or the simultaneous effects of the minimum, maximum, and average temperatures ($F = 0.81$; df = 3.5; $P = 0.541$).

Of the nine sites sampled with light traps, larval Barrens topminnows were collected at three sites: Clayborne 4 (n = 2 larvae), Clayborne 6 (n = 1), and the Type Locale (n = 7). Clayborne 4 and the Type Locale were devoid of mosquitofish, but mosquitofish were present in Clayborne 6. Larval Barrens topminnows collected at Clayborne 4 and Clayborne 6 were the offspring of stocked fish; whereas, fish collected from the Type Local were produced by wild Barrens topminnows. The first larval Barrens topminnow collected measured 8 mm in total length and was captured at the Clayborne 4 pool on 9 June 2004. On 8 July 2004, another larval Barrens topminnow (7 mm) was collected at that site; therefore, reproduction was deemed successful and sampling ceased at Clayborne 4. Barrens topminnow larvae collected at Clayborne 6 (8 July 2004) and the Type Locale (15 July 2004) measured 7 mm and 10 mm, respectively. Six age-0 Barrens topminnows were also collected at the Type Locale on 15 July (23 mm), 3 September (24 mm and 21 mm), and 24 September (21 mm, 23 mm, and 17 mm). Recruitment (i.e., the presence of wild age-0 topminnows) was observed at the Collier site on 13 November 2004; therefore, reproduction had occurred earlier in the year and this site was included in subsequent analyses.

The remaining six sites where no larval Barrens topminnows were collected (Clayborne 1; Cunningham barn; Cunningham 2 and 3; Sain upper and lower) all harbored mosquitofish at varying densities (Figure 3). Larval Barrens topminnows were collected at all sites devoid of mosquitofish; whereas, only one larval topminnow was collected at a site with mosquitofish; thus, a significant association existed between mosquitofish absence and Barrens topminnow reproduction ($P = 0.033$) and recruitment ($P = 0.050$).

Slopes of the $\log_{10}$(length)-$\log_{10}$(weight) regression lines for Barrens topminnows in the presence and absence of mosquitofish were similar ($F = 0.15$; df = 3, 286; $P = 0.697$; Figure 4). The adjusted mean weights of Barrens topminnows did not differ ($F = 1.70$; df = 2, 287; $P = 0.193$), which suggests that interspecific competition for food was not occurring. Because mosquitofish did not affect Barrens topminnow condition, lengths and weights were pooled to define the length-weight relationship. The linear relationship between length (31-90 mm TL) and weight (0.2-6.1 g) of reintroduced Barrens topminnows was best represented by the model:

$$\text{Log}_{10}\,(\text{weight}) = -5.408 + 3.14\ \text{Log}_{10}\,(\text{TL}) \qquad (n = 290;\ P < 0.0001;\ r^2 = 0.91).$$

Instantaneous growth in length varied more than three-fold among cohorts of Barrens topminnows recaptured 49 to 182 days post stocking (Table 4). There was no linear relationship between mean instantaneous growth and mosquitofish density ($F = 1.48$; df = 1,11; $P = 0.249$). Barrens topminnows grew fast at sites with high densities of mosquitofish (e.g., Cunningham barn site) and at sites lacking mosquitofish (e.g., Clayborne 4). Conversely, growth was slow at some sites with mosquitofish (e.g., Clayborne 7) and without mosquitofish (e.g., Collier).

Wild Topminnows

Visual analysis of length-frequency distributions identified three, possibly four, age classes of wild Barrens topminnows at three sites (Figure 5). At the Type Locale, no fish were collected between 54 mm and 76 mm TL, suggesting a failure of the 2002 year class, which made the presumptive age-1 year class (32-52 mm TL) and presumptive age-3 year class (78-90 mm TL) more easily discernible. An age-4 year class may also have been present because the two largest fish measured 98 mm TL, considerably larger than the next largest fish.

At Pond Spring, all of the Barrens topminnows measured between 30 mm and 48 mm TL and they were presumably all age-1 fish from the 2004 year class. Filamentous algae and pondweed hindered sampling at that site and an adequate representation of the population age-structure was probably not achieved.

Distinguishing year classes in the length-frequency distribution at the Pedigo site was more difficult due to the presence of what appeared to be three consecutive year classes and substantial overlap in their lengths. Based on mode locations and the assumption that lengths were normally distributed within each age group, fish between 26 mm and 44 mm TL were presumed to be age-1 and fish between 46 mm and 78 mm TL were presumed to be age-2. Seine sampling at the Pedigo site in May 2004 found no mosquitofish in the reach upstream of a ~0.5-m drop in the streambed; however, flooding in the winter of 2004-2005 permitted upstream invasion by mosquitofish, which were collected in that same reach in March 2005. Most (75%) of the Barrens topminnows collected at the Pedigo site in March 2005 were captured in an isolated, slackwater side channel with no direct connection to the main channel of the creek during base flow.

Side channel at the Pedigo site, where most of the wild Barrens topminnows were collected in 2005.

A large male Barrens topminnow collected at the Type Locale in 2004.

A swarm of mosquitofish at the lower Cunningham stocking site.

DISCUSSION

In a concurrent laboratory study, large mosquitofish inflicted substantial damage to the fins of adult Barrens topminnow but mortality was nil (Laha 2004), which may explain the lack of a relation between Barrens topminnow mortality and mosquitofish density in the present study. Although mosquitofish probably did not kill any stocked adult Barrens topminnows, repeated negative interactions may have caused topminnows to emigrate from the pools and springheads where they were stocked. The displacement of one species from preferred habitat by another, more aggressive species such as mosquitofish has been documented for other imperiled fishes (e.g., least chub, Mills et al. 2004; plains topminnow *Fundulus sciadicus,* Whitmore 1997).

We assumed that survival of any topminnows that emigrated from stocking sites was nil; if stocked topminnows emigrated in large numbers and survived in new habitats, the mortality rates we reported would have been inflated. One site in particular where mortality rates may have been inflated was the Marcum site, where topminnows moving downstream of the excavated pool in which they were stocked would have entered a small pond that was fishless (except for topminnows stocked there in 2003). Irregular surveys of habitats downstream of stocking sites in 2003 and 2004 captured two tagged topminnows that had emigrated from their respective stocking sites (Johnson 2004); thus, downstream movements by stocked topminnows has been documented. However, those two topminnow recaptures were probably the exception rather than the rule. For instance, we collected no topminnows in an electrofishing survey in 2004 of 10 isolated pools in a 2.5-km reach of the stream draining the Sain, Clayborne, and Cunningham stocking sites, where 1,495 Barrens topminnows were stocked over three years. Stocked Barrens topminnows emigrating downstream from most stocking sites (except the Marcum site) would have encountered a suite of potential predators such as largemouth bass and green sunfish in atypical topminnow habitats (i.e., streams and rivers). Similarly, if topminnows continued to move downstream of the pond at the Marcum site, they would have entered Lake Tullahoma, a small reservoir harboring many potential predators. The naiveté of stocked fish would have contributed to high mortality rates, which has been observed for other hatchery-reared species (e.g., rainbow trout, Bettinger and Bettoli 2005). Thus, the assumption that survival of emigrating topminnows was nil appears valid in the absence of compelling contrary data.

We observed scant evidence of reproduction and no evidence of recruitment by Barrens topminnows at reintroduction sites where mosquitofish were present, most likely due to predation by mosquitofish on topminnow larvae. The negative effects of mosquitofish on native fish assemblages have been well documented (Myers 1967; Courtenay and Meffe 1989; Lydeard and Belk 1993; Belk and Lydeard 1994; Rincon et al. 2002). Courtenay and Meffe (1989) described anatomical structures such as strong, conical teeth and short gut lengths and documented predation on other fishes as evidence that mosquitofish predation was the primary mechanism in the decline or extinction of native species. Recent aquarium studies confirm that mosquitofish aggression and predation can eliminate larvae and fry of native species, including Barrens topminnows. Mills et al. (2004) reported that no larval least chubs survived in enclosures with high mosquitofish densities. More importantly, Laha (2004) reported that no Barrens topminnow larvae survived in aquaria experiments when the relative density of mosquitofish to topminnows was 1:1, which was substantially lower than the lowest relative density observed in sites sampled with larval light traps in this study. Johnson (2004) documented natural reproduction of Barrens topminnows at the Vervilla site prior to the invasion of mosquitofish in 2001 and 2002, but subsequent sampling in 2003 and 2004 collected no age-0 Barrens topminnows.

The lack of recruitment after mosquitofish invasion at the Vervilla site, the *ex situ* aquaria studies, and the results of the larval sampling in this study provide strong indirect evidence that predation by mosquitofish on topminnow larvae was the primary mechanism responsible for Barrens topminnow recruitment failure. The ability of adult Barrens topminnows to persist and grow to large sizes at some sites where mosquitofish densities were extremely high (e.g., Cunningham Barn site) is evidence that adult topminnows can coexist with mosquitofish, although their offspring cannot.

Recruitment failure can create positive feedback loops that negatively affect a population and can lead to extinction (Hallerman 2003). Gilpin and Soule` (1986) refer to this process as the R (for recruitment) extinction vortex - a positive feedback process that drives a population towards extinction. Barrens topminnow populations would be especially vulnerable because the short-lived spawning stock reproduces only once or twice in a lifetime (Rakes 1989). Successive years of recruitment failure would lead to a smaller spawning stock, which would reduce the number of offspring and produce an even smaller spawning stock the following year. The process continues to extinction or until a catastrophic event eliminates the diminished population. The loss of many Barrens topminnow populations in the past four decades is undoubtedly linked to the invasion of mosquitofish throughout the Barrens region and predation by mosquitofish on larval topminnows at a critical period in the recruitment process.

Barrens topminnow robustness did not differ in the presence or absence of mosquitofish, suggesting interspecific competition for food resources was not occurring. Adult Barrens topminnows also displayed good growth at some sites despite dense populations of mosquitofish. When mosquitofish appear and native fishes disappear, competition is usually asserted by default, although no experimental evidence exists to prove competition caused the replacement (Courtenay and Meffe 1989). A more plausible explanation for the replacement of Barrens topminnows, and one supported by the results of this study and others, is predation by mosquitofish on topminnow larvae and juveniles (Meffe 1985; Courtenay and Meffe 1989; Mills et al. 2004).

The age structures of the wild populations provided insight into their current status, but in the absence of historical data it cannot be determined whether current year-class strengths are high, low, or the norm. The apparent absence of age-2 fish at the Type Locale means persistence at that site hinges on successful reproduction by the two remaining age classes.

The length-frequency distribution of Barrens topminnows at the Pedigo site represented the first age-structure analysis (albeit crude) for that population and our age class designations are open to interpretation. Observing length-frequency distributions through time would help define age groups in that population. Year-class strength appeared to be strong among all age classes at the Pedigo site; however, long-term persistence is doubtful. Mosquitofish invaded the Pedigo site in 2004 and most of the topminnows were observed in an ephemeral side channel. Thus, the Pedigo population currently faces two threats that have imperiled the species elsewhere: drought and mosquitofish. A representative sample of the Pond Spring population was not collected, and historical data do not exist; therefore, we do not know whether the population has remained stable or declined in the presence of mosquitofish at that site.

An effective barrier to mosquitofish invasion at the Type Locale.

These ledges downstream of the Pedigo site, which harbors one of four known wild populations of Barrens topminnows, did not prevent mosquitofish from invading the site during flood events in 2004.

CONCLUSIONS

The present study and results from previous studies provide compelling evidence that mosquitofish were responsible for suppressing the establishment of new, self-sustaining Barrens topminnow populations. The most likely mechanism would appear to be direct predation by mosquitofish on larval and juvenile topminnows. We subscribe to the views held by Conant (1988) and others that reintroduction programs for imperiled species will not succeed if the factor(s) causing their imperilment still exist in the landscapes slated for the reintroductions. If future reintroductions are scheduled to occur in small springheads such as those stocked in the present study, they should be restricted to sites free of mosquitofish and with fish barriers in place.

The ability of wild Barrens topminnows to persist at the Pond Spring site in the presence of mosquitofish deserves further scrutiny and may provide insight into why the reintroduction program elsewhere has generally been unsuccessful to date. No sampling data exists to indicate whether topminnows are more or less abundant now compared to when they were first observed at Pond Spring in the 1980s, although visual observations in recent years suggest that they are much less abundant now (P. Rakes; Conservation Fisheries, Inc., personal communication). Regardless of their present status at Pond Spring, the persistence of topminnows for at least several decades in the presence of mosquitofish has always been intriguing. Topminnows may be able to coexist with mosquitofish at Pond Spring because it is larger by several orders of magnitude than the typical springheads and spring runs where Barrens topminnows have been stocked, and where wild populations currently exist. Pond Spring’s large size provides distinctly different microhabitats, with different thermal characteristics, such as broad, shallow shorelines and open water habitats with strong spring upwellings in the middle of the pond, which may allow for habitat partitioning by the two species.

Wild populations should be sampled annually to monitor their dynamics and persistence. In particular, recruitment of Barrens topminnows at the Pedigo site should be scrutinized following the 2004 invasion of the site by mosquitofish. Determining whether or not missing year classes are the norm at the Type Locale should also be a research priority.

Finally, we agree in principle with Schute et al. (2005) when they caution that reintroduction efforts should not be abandoned prematurely if recruitment is not immediately documented. However, the species they reintroduced included small, cryptic, benthic fishes (e.g., smoky madtom *Noturus baileyi*; duskytail darter *Etheostoma percnurum*), which were difficult to observe and hard to collect. In the present and previous studies, we failed to observe recruitment (in the presence of mosquitofish) of a species that inhabits the surface of the water column in most seasons and can achieve lengths in excess of 90 mm total length. In addition, male topminnows are brightly colored and the adults of both sexes are clearly visible to the naked eye. After four years of field observations, we do not think we are premature in concluding that it is unlikely reintroduced Barrens topminnows will establish self-sustaining populations when stocked into habitats occupied by mosquitofish, or susceptible to invasion by mosquitofish.

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Table 1. Reintroduced and wild populations of Barrens topminnows sampled in 2003-2005.

Property	Watershed	County
	Stocked Populations	
Bridges	Barren Fork River	Coffee
Clayborne[1]	West Fork Hickory Creek	Coffee
Murphy	West Fork Hickory Creek	Coffee
Cunningham Barn	West Fork Hickory Creek	Coffee
Cunningham Lower[1]	West Fork Hickory Creek	Coffee
Sain[1]	West Fork Hickory Creek	Coffee
Ramsey	Hickory Creek	Warren
Vervilla[1]	Hickory Creek	Warren
Herndon	Caney Fork River	DeKalb
Marcum	Duck River	Coffee
Collier	Duck River	Coffee
	Wild Populations	
Pedigo	Barren Fork River	Cannon
Pond Spring	Elk River	Coffee
Type Locale	West Fork Hickory Creek	Coffee

[1] – Properties with multiple stocking sites

Table 2**.** Mean total length at stocking, annual interval mortality (%) of stocked Barrens topminnows, percent aquatic vegetation coverage, and mosquitofish density at Barrens topminnow reintroduction sites. Each site was sampled on one date between November 2004 and January 2005 to estimate population size of Barrens topminnows (and subsequent cohort mortality rates) and mosquitofish density.

Site	Date Stocked	Number Stocked	Mean Length at Stocking (mm)	Annual Mortality (%)	Mosquitofish Density (fish/m^2)
Bridges	5/27/2004	22	52.1	100	0.0
Clayborne 3	8/27/2003	50	42.4	92.95	2.7
Clayborne 4	6/3/2003	12	33.1	61.65	0.0
Clayborne 6	8/27/2003	88	43.0	44.57	16.1
Clayborne 7	8/27/2003	78	45.4	86.28	8.4
Collier	5/27/2004	50	50.4	98.04	0.0
Cunningham Barn	8/27/2003	50	33.1	89.12	66.3
Cunningham 2	8/27/2003	100	42.4	100	21.3
	12/18/2003	41	44.2	100	21.3
	5/27/2004	154	43.9	99.97	21.3
Cunningham 3	8/27/2003	100	41.4	100	14.8
	12/18/2003	36	45.8	100	14.8
	5/27/2004	127	37.4	99.11	14.8
Herndon	7/8/2004	175	38.1	100	0.0
	11/18/2004	105	43.1	100	0.0
Marcum	5/27/2004	30	51.9	98.23	0.0
	7/8/2004	91	41.4	97.79	0.0
Murphy	8/27/2003	50	44.2	100	33.0
	5/27/2004	189	39.0	100	33.0
Ramsey (Viola)	5/27/2004	238	38.0	99.96	0.4
	7/14/2004	270	38.2	99.78	0.4
	11/18/2004	109	41.7	99.99	0.4
Sain Upper	8/27/2003	60	43.3	85.70	3.5
Sain Lower	6/3/2003	50	44.1	93.93	8.1
Vervilla 3	8/27/2003	50	42.7	100	12.7
	5/27/2004	136	43.3	100	12.7
Vervilla 4	8/27/2003	50	43.6	100	4.9
	5/27/2004	137	43.3	100	4.9

Table 3. Number of wild and stocked Barrens topminnows (*F.j.)* and mosquitofish (*G.a.*) collected in seine hauls at 17 sites during the 2004-2005 sampling season. Zippin population estimates (N) are listed for those species and sites where the population was depleted and minimum capture probabilities exceeded 0.2 (see text for explanation).

			Seine Haul					
Site	Date	Species	1	2	3	4	Total	N
Bridges	10-Jan-05	*F.j.*	0	0	-	-	0	-
		G.a.	0	0	-	-	0	-
Clayborne 3	12-Nov-04	*F.j.*	0	0	0	-	0	-
		G.a.	130	-	-	-	130	-
Clayborne 4 †	12-Nov-04	*F.j.*	18	6	2	-	26	26
		G.a.	0	0	0	-	0	-
Clayborne 6	12-Nov-04	*F.j.*	36	10	0	-	46	46
		G.a.	590	302	57	-	949	1,003
Clayborne 7	12-Nov-04	*F.j.*	6	0	-	-	6	-
		G.a.	353	33	-	-	386	389
Collier †	13-Nov-04	*F.j.*	6	4	3	0	13	16
		G.a.	0	0	0	0	0	-
Cunningham Barn	2-Dec-04	*F.j.*	2	1	0	-	3	-
		G.a.	8407	2803	964	-	12,174	12,656
Cunningham 2	6-Jan-05	*F.j.*	1	0	-	-	1	-
		G.a.	954	227	-	-	1,181	1,251
Cunningham 3	6-Jan-05	*F.j.*	7	1	-	-	8	-
		G.a.	888	107	-	-	995	1,009
Herndon	2-Dec-04	*F.j.*	0	0	-	-	0	-
		G.a.	0	0	-	-	0	-
Marcum	5-Nov-04	*F.j.*	5	13	7	6	31	-
		G.a.	0	0	0	0	0	-

Table 3. Continued.

			Seine Haul					
Site	Date	Species	1	2	3	4	Total	N
Murphy	12-Jan-05	*F.j.*	0	0	0	-	0	-
		G.a.	93	90	40	-	223	330
Ramsey (Viola)	6-Jan-05	*F.j.*	13	14	10	6	43	64
		G.a.	191	97	30	8	326	333
Sain Upper	12-Nov-04	*F.j.*	3	1	1	-	5	-
		G.a.	183	25	15	-	223	225
Sain Lower	12-Nov-04	*F.j.*	0	0	-	-	0	-
		G.a.	250	49	-	-	299	310
Vervilla 3	13-Nov-04	*F.j.*	0	0	-	-	0	-
		G.a.	154	30	-	-	184	190
Vervilla 4	13-Nov-04	*F.j.*	0	0	-	-	0	-
		G.a.	164	98	-	-	262	400

† Population estimate includes the offspring of stocked Barrens topminnows.

Table 4. Mean instantaneous growth rates (G) for cohorts of Barrens topminnows in which at least three recaptures occurred within 200 days (short term) and beyond 200 days (long term) post stocking.

Site	Date Stocked	Mean TL(mm) at Stocking	Recapture Date	Mean TL (mm) at Recapture	Number Collected	Days Post Stocking	G (% per day)
			Short Term				
Clayborne 7	8/27/2003	45.4	11/25/2003	49.7	62	90	0.101
Cunningham Lower	8/27/2003	42.4	12/11/2003	49.9	12	106	0.154
Sain Upper	8/27/2003	43.3	11/4/2003	48.4	53	69	0.160
Clayborne 6	8/27/2003	43.0	11/25/2003	50.2	57	90	0.171
Clayborne 3	8/27/2003	42.4	11/4/2003	48.1	32	69	0.183
Sain Lower	6/3/2003	44.1	11/4/2003	60.9	18	154	0.211
Cunningham Barn	8/27/2003	33.1	1/29/2004	58.0	42	155	0.363
Clayborne 4	6/3/2003	33.1	12/2/2003	65.0	7	182	0.371
Collier	5/27/2004	50.4	11/13/2004	64.5	8	170	0.145
Marcum	5/27/2004	51.9	11/5/2004	82.0	5	162	0.283
Marcum	7/8/2004	41.4	11/5/2004	57.7	26	120	0.277
Ramsey	7/14/2004	38.2	1/6/2005	53.2	14	176	0.188
Ramsey	11/18/2004	41.7	1/6/2005	45.8	27	49	0.190
			Long Term				
Clayborne 6	8/27/2003	43.0	11/12/2004	57.2	43	443	0.064
Clayborne 7	8/27/2003	45.4	11/12/2004	56.8	5	443	0.051
Sain Upper & lower	9/3/2002	43.1	11/25/2003	71.9	7	448	0.114
Cunningham Barn	8/27/2003	33.1	12/2/2004	64.3	3	463	0.144
Clayborne 4	6/3/2003	33.1	11/12/2004	84.7	3	528	0.178
Cunningham Lower	5/27/2004	37.4	1/6/2005	58.1	7	224	0.198
Sain Lower	6/3/2003	44.1	11/12/2005	78.0	3	893	0.064

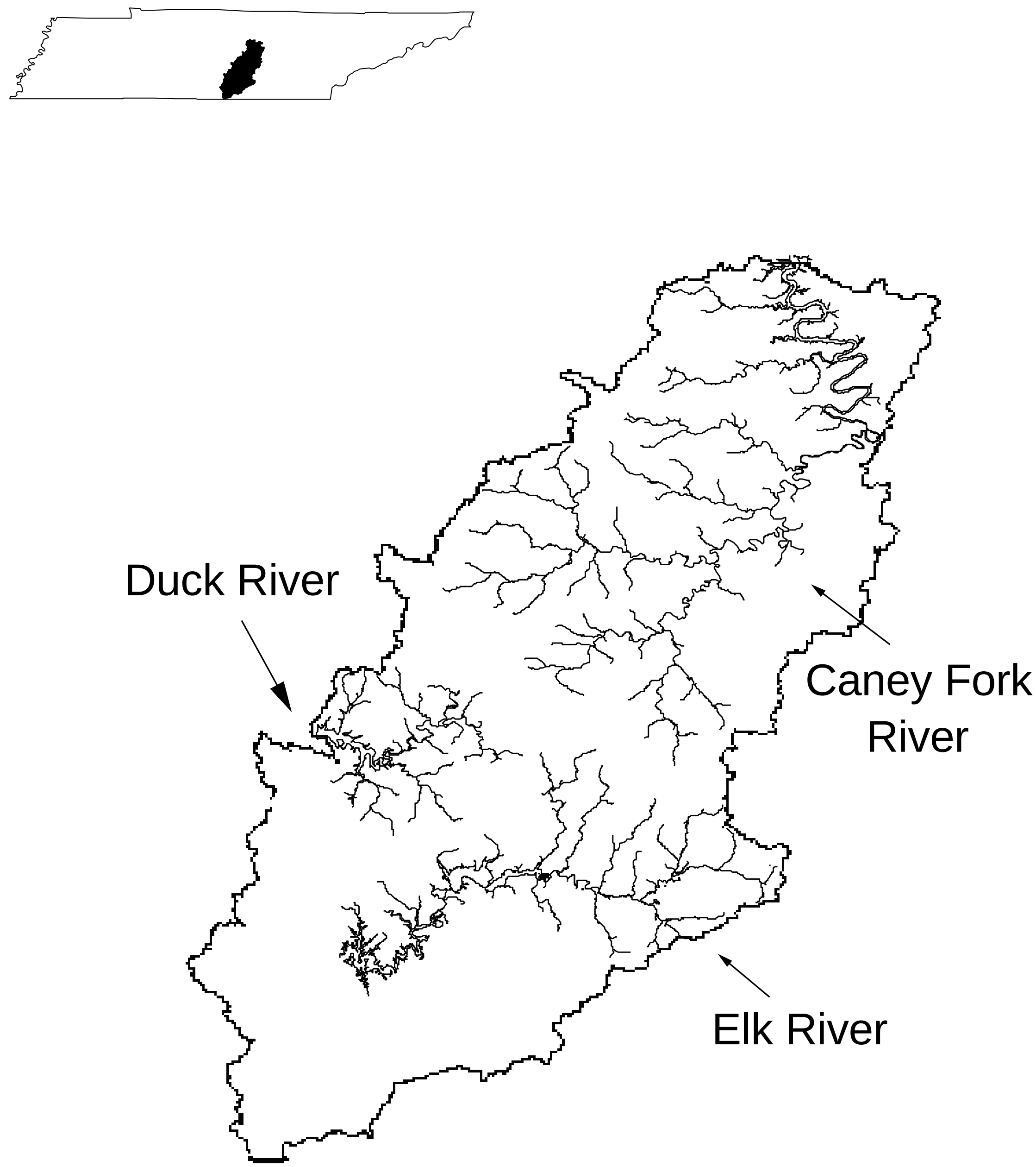
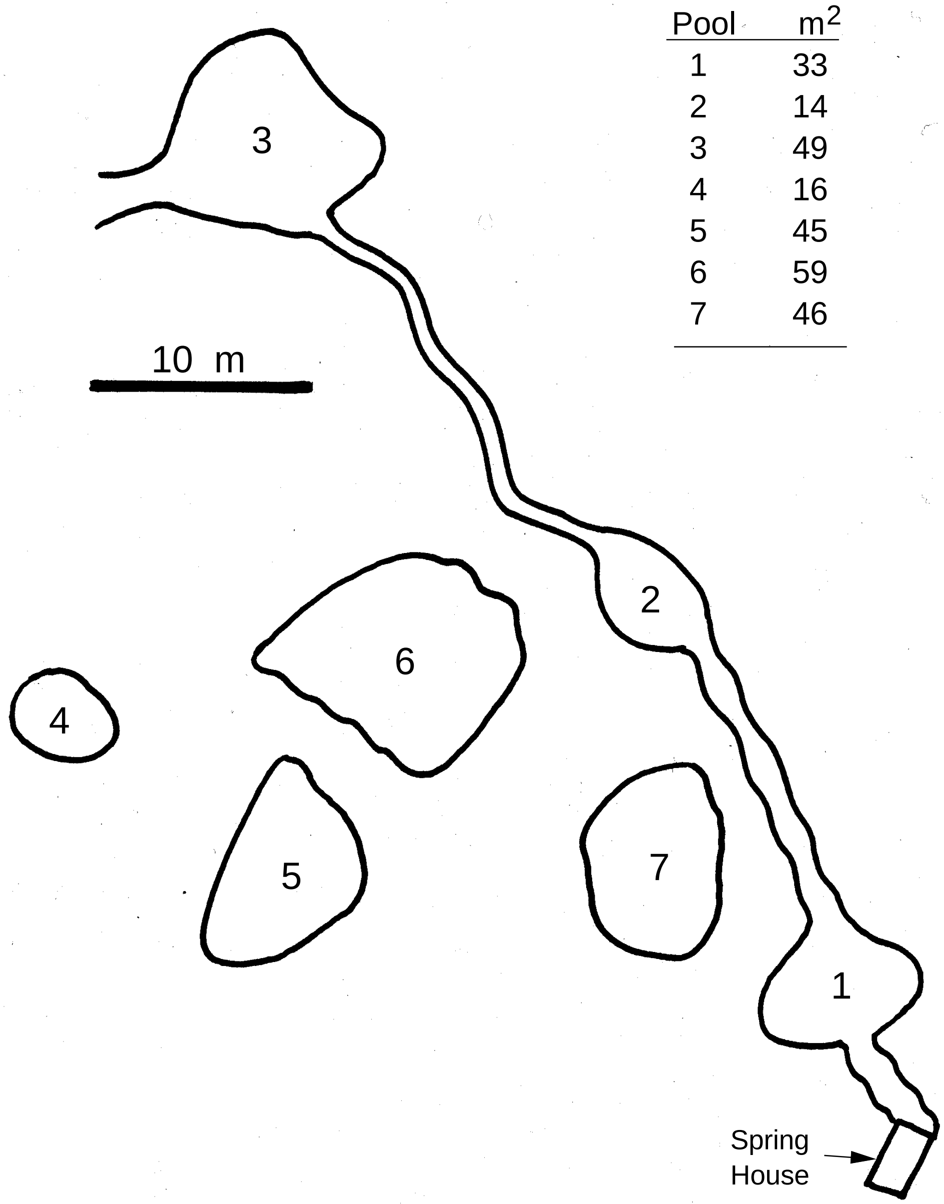


Figure 1. The Caney Fork River, Duck River, and Elk River drainages on the Barrens Plateau.



Pool	m^2
1	33
2	14
3	49
4	16
5	45
6	59
7	46

Figure 2. Map of the Clayborne pools, January 2004 (from Bettoli et al. 2004). Pool 4 was above the floodplain and isolated from all other pools, even during heavy rain events. Pool 3 drained into the upper Sain pool.

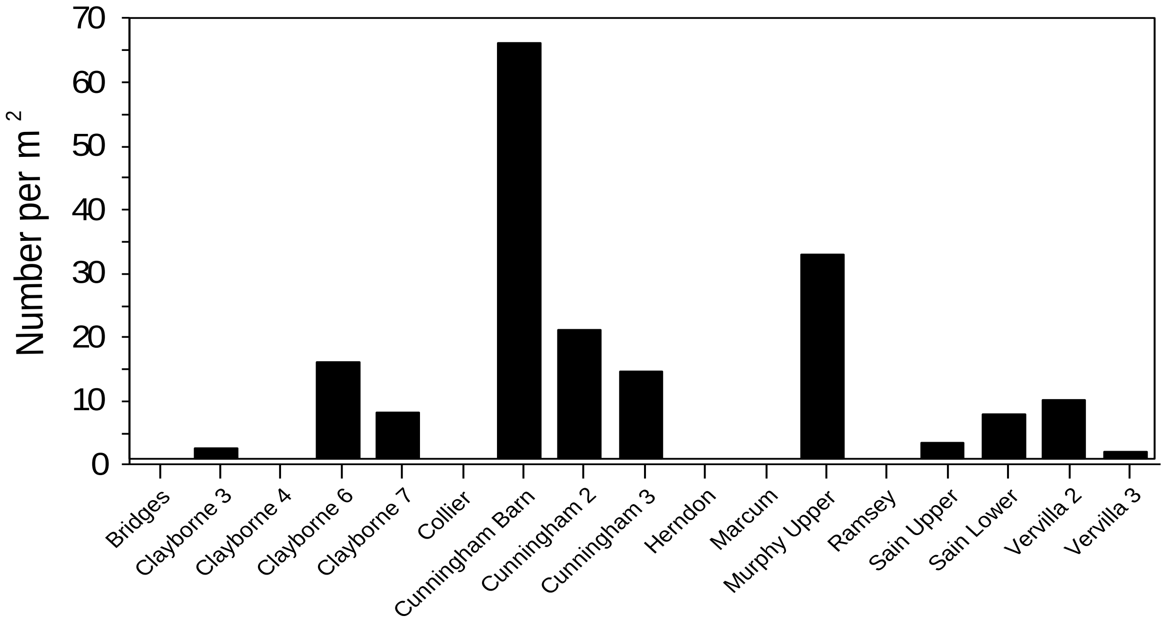


Figure 3**.** Mosquitofish density at 17 sites stocked with Barrens topminnows in 2003-2004.

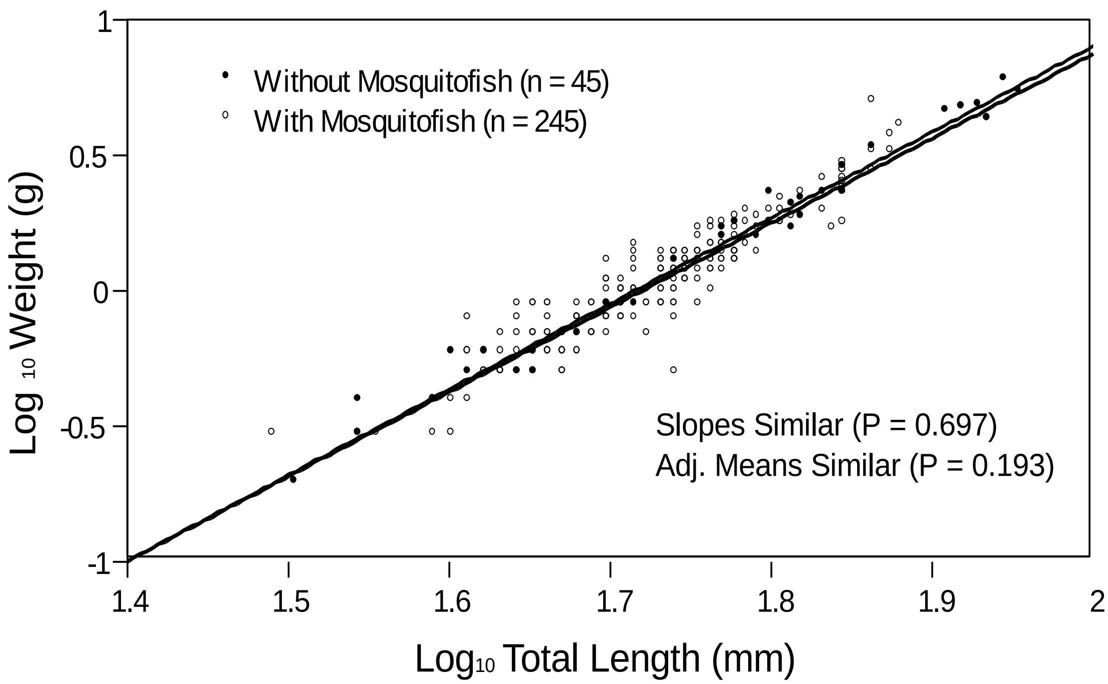


Figure 4**.** Log_{10}(length) versus log_{10}(weight) of reintroduced Barrens topminnows collected at sites with (open circle) and without (solid circle) mosquitofish during 2003-2005.

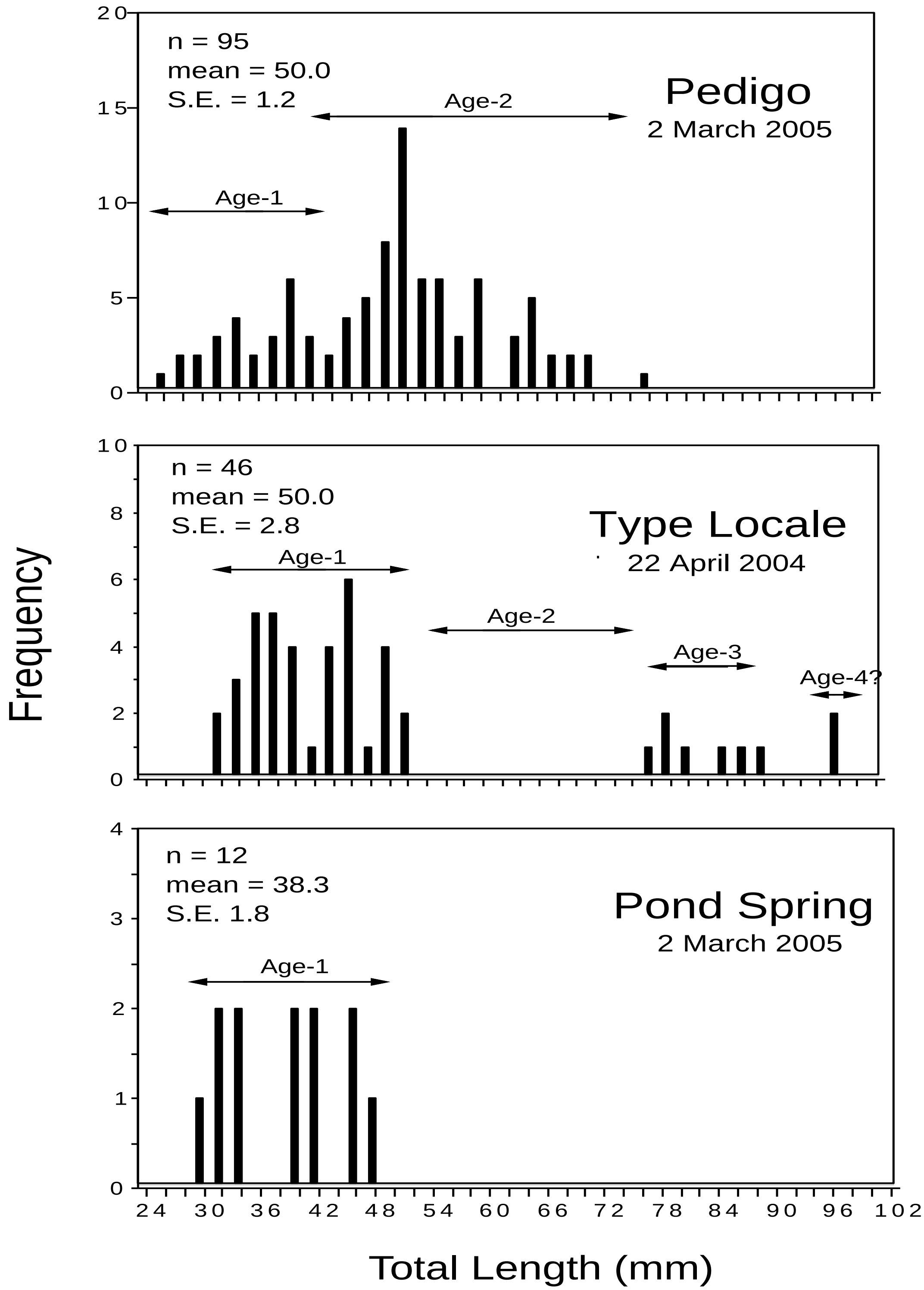


Figure 5. Length-frequency distributions of wild Barrens topminnows collected at three sites. Presumptive ages are indicated.

APPENDIX I

Miscellaneous Field Notes Pertaining to Sampling Activities at Barrens Topminnow Stocking Sites Not Discussed in this Final Report

Hancock Property

The Hancock site was one of the original topminnow stocking sites (first stocked in 28 September 2001) and the fate of 480 topminnows stocked there was discussed in Bettoli et al. (2004). We sampled this site on 2 December 2003 using a seine net and electrofishing gear. In one seine haul we collected one tagged female topminnow (85 mm TL), which was stocked on 10 April 2002, or 601 days post-stocking (DPS). Also collected in the seine haul were 551 flame chubs and one creek chub. Seining proved difficult because of lush vegetation and stumps; therefore, we used the seine as a blocknet where the spring pool joined Little Hickory Creek and electrofished the site using a Smith-Root backpack DC electrofisher. We made two passes with the electrofisher and most of the fish were captured when we pulled in the blocknet. Two tagged topminnows were collected in the first electrofishing pass (both 45 mm TL; stocked August 2003, or 97 DPS). Other fishes collected included green sunfish (n = 3), banded sculpin (n = 11), flame chubs (n = 160) and mosquitofish (n = 2). In the second electrofishing pass we collected another topminnow (36 mm TL; stocked June 2003, or 182 DPS), 35 flame chubs, 33 banded sculpins, two green sunfish, and one central stoneroller. The blocknet contained four Barrens topminnows: two were stocked on 3 June 2003 (182 DPS) and two were stocked on 27 August 2003 (97 DPS). Flame chubs (n = 35), green sunfish (n = 2), and one central stoneroller were also collected in the blocknet.

Crook Property

The spring run at the Crooks property was divided into upper and lower sites , which were separated by a culvert. The lower site was stocked with three cohorts of topminnows (n_{total} = 433 fish) in 2003 (Bettoli et al. 2004); the upper site was stocked with two cohorts in 2003 (n_{total} = 157 fish). This site was sampled with a backpack DC electrofishing unit on 18 December 2003. The upper site (above the road culvert) was electrofished for 433 seconds and one Barrens topminnow was collected (45 mm TL), which was stocked on 27 August 2003 (97 DPS). We also collected rosyside dace (n = 10), central stonerollers (n=10), banded sculpins (n = 117), and flame chubs (n = 21). The spring run below the road culvert was electrofished for 1,964 seconds and three topminnows were collected: one male (54 mm TL) and one female (43 mm TL) were stocked on 27 August 2003 (113 DPS) and one female (43 mm TL) was stocked on 3 June 2003 (198 DPS). Subsequent sampling of the Crooks Property did not occur because of difficulties we had in contacting the two landowners (e.g., ownership of the land surrounding the upper site changed after fish were stocked; the gate to the lower site was subsequently padlocked).

Gipson Site.

This is a potential stocking site located on a small tributary to the Elk River in northeast Franklin County, TN. On 5 November 2004, a 100-m section of stream was sampled for resident fishes using a backpack DC electrofishing unit. We collected 28 creek chubs, 10 central stonerollers, three fantail darters *Etheostoma flabellare*, two mosquitofish, and one pumpkinseed sunfish *Lepomis gibbosus*.

Farris Springs.

This is a potential stocking site located in central Franklin County. The flow from the springhead creates a small tributary to Dry Creek, which flows into Tim's Ford Lake. The resident fish fauna was surveyed on 5 November 2004 using a backpack DC electrofishing unit. We collected four flame chubs, two central stonerollers, and numerous salamanders and tadpoles. The absence of mosquitofish makes it a highly desirable stocking location.

Pocohontas Site

This was one of the original sites chosen by the Working Group to undergo habitat improvements (including cattle exclusion) and receive propagated topminnows. Bettoli et al. (2004) discussed the fate of 135 topminnows stocked in 2001 and 2002. We conducted an aquatic vegetation survey on 14 October 2003. Eight species of aquatic plants covered 91% of the spring pool and run connecting the springhead to Pocohontas Branch Creek. We did not sample fishes at this site in 2004 or 2005.

Witty Creek.

This was another of the original sites chosen by the Working Group; 146 topminnows were stocked in 2001 and 2002. We surveyed the aquatic vegetation at this site on 9 September 2003. Five species of aquatic vegetation covered 62% of the spring run. We did not sample fishes at this site in 2004 or 2005.

Lower Murphy Site.

A visual survey was conducted at the lower Murphy site on 14 October 2003. Eight Barrens topminnows were observed and based on the VIE tags we could see, they were probably stocked 3 June 2003 (right-posterior and left-anterior red tag). This site has proved to be one of the most difficult sites to sample of all the sites stocked since 2001. It is heavily vegetated and the margins of the pool are thick mud, which makes walking or wading nearly impossible.

Lower Marcum Site

As described in earlier in the description of Reintroduction Sites, the large pond at the end of the spring run on the Marcum Property was stocked with 122 topminnows on 27 August 2003. We were unable to sample that site because of extensive vegetation; however, as late as May 2004 we observed topminnows in that pond, which was fishless when stocked and is separated from Lake Tullahoma by an impassible fish barrier.

Floating Minnow Trap

We briefly investigated whether a modified, floating minnow trap would catch topminnows. We deployed it at the Lower Murphy site throughout the day on 25 August 2003, but only flame chubs and mosquitofish entered the trap. We fished the trap all day at the Clayborne 4 pool on 26 August 2003 (pictured below) and collected no fish.

APPENDIX II

Summary of all Barrens Topminnows stocked between August 2001 and November 2004.

Table A1. Stocking summary for Barrens topminnows by watershed, site, and stocking date, 2001-2004. The fate of topminnows stocked at sites marked with an asterisk is unknown.

Watershed/Site	Tagging Location	Number Stocked	Tag Color	Date Stocked	Total Stocked
BARREN FORK					
Pocahontas	Right and Left Anterior	45	Red	8/30/2001	135
	Right and Left Anterior	90	Green	9/3/2002	
Witty Creek	Left Anterior	64	Red	8/30/2001	146
	Left Anterior	82	Green	9/3/2002	
Bridges	Right Anterior	22	Orange	5/27/2004	22
Herndon	Left Anterior	175	Orange	7/8/2004	280
	No tag	105	n/a	11/18/2004	
Green Brook Pond*	Elastomer(Right Anterior)/Calcein	71	Green	11/18/2004	283
(Smithville)	Calcein	53	n/a	11/18/2004	
	Elastomer(Left Anterior)/Calcein	159	Yellow	12/17/2004	
DUCK RIVER					
Marcum	Left Posterior	122	Yellow	8/27/2003	243
	Left Posterior	30	Orange	5/27/2004	
	Right Anterior	91	Orange	7/8/2004	
Collier	Left Posterior	50	Yellow	5/27/2004	50
ELK RIVER					
Gipson*	Left Posterior	181	Orange	11/16/2003	181
Farris*	Left Posterior	200	Orange	11/16/2003	200
HICKORY CREEK					
Vervilla	Left Anterior	45	Red	8/22/2001	516
	Left Anterior	50	Yellow	4/10/2002	
	Left Anterior	48	Green	9/3/2002	
	Left Anterior	100	Orange	8/27/2003	
	Left Posterior	273	Yellow	5/27/2004	

Table A1. Continued.

Clayborne Isolated (Pool 4)	Right and Left Posterior	10	Red	8/22/2001	31
	Right and Left Posterior	9	Yellow	4/10/2002	
	Right and Left Posterior	12	Red	6/3/2003	
Clayborne Middle (Pool 5)	Right Posterior	29	Red	8/22/2001	53
	Right Posterior	24	Yellow	4/10/2002	
Clayborne Spring (Pool 1)	Left Posterior	28	Red	8/22/2001	138
	Left Posterior	35	Yellow	4/10/2002	
	Left Posterior	25	Green	9/3/2002	
	Left Posterior	50	Red	6/3/2003	
Clayborne 3	Left Posterior	50	Yellow	8/27/2003	49
Clayborne 6	Left Anterior	88	Yellow	8/27/2003	88
Clayborne 7	Right Anterior and Posterior	78	Red	8/27/2003	75
Sain	Right and Left Anterior	56	Green	9/3/2002	166
	Right and Left Anterior	50	Red	6/3/2003	
Sain Upper	Right and Left Anterior	60	Yellow	8/27/2003	
Cunningham Upper (Barn)	Right Anterior and Left Posterior	28	Yellow	4/10/2002	78
	Right Anterior and Left Posterior	50	Red	8/27/2003	
Cunningham Lower #1	Right Anterior and Left Posterior	80	Green	9/3/2002	333
	Right Anterior and Left Posterior	100	Red	6/3/2003	
	Right Anterior and Left Posterior	50	Yellow	8/27/2003	
	Right Anterior and Left Posterior	103	Orange	12/18/2003	
Cunningham Lower #2	Left Posterior	100	Yellow	8/27/2003	295
	Left Posterior	41	Orange	12/18/2003	
	Left Posterior	154	Green	5/27/2004	
Cunningham Lower #3	Right Posterior	100	Green	8/27/2003	263
	Right Posterior	36	Yellow	12/18/2003	
	Right Posterior	127	Orange	5/27/2004	

Table A1. Continued.

Murphy (Upper)	Right Anterior	29	Red	8/22/2001	367
	Right Anterior	50	Yellow	4/10/2002	
	Right Anterior	49	Green	9/3/2002	
	Right Anterior	50	Orange	8/27/2003	
	Right Posterior and Left Anterior	189	Green	5/27/2004	
Murphy (Lower)*	Right and Left Posterior	84	Green	9/3/2002	510
	Right Posterior and Left Anterior	76	Green	11/26/2002	
	Right Posterior and Left Anterior	204	Red	6/3/2003	
	Right Posterior and Left Anterior	146	Yellow	8/27/2003	
Hancock	Right and Left Anterior	57	Red	9/28/2001	872
	Right and Left Anterior	55	Yellow	4/10/2002	
	Right and Left Anterior	57	Green	9/3/2002	
	Right Posterior	62	Green	11/26/2002	
	Right Posterior	156	Red	6/3/2003	
	Right Posterior	93	Yellow	8/27/2003	
	Left Posterior	392	Green	5/27/2004	
Crooks Lower*	Right Anterior and Right Posterior	191	Red	6/3/2003	629
	Right Anterior and Right Posterior	150	Green	8/27/2003	
	Right Anterior and Right Posterior	92	Orange	12/18/2003	
	Right Anterior and Right Posterior	196	Yellow	5/27/2004	
Crooks Upper*	Right Anterior and Right Posterior	49	Green	8/27/2003	1,284
	Right Posterior	108	Yellow	12/18/2003	
	Left Posterior	201	Red	5/27/2004	
	Left Anterior	278	Yellow	7/14/2004	
	Right Anterior	648	Green	11/18/2004	
Ramsey Viola	Left Anterior	238	Green	5/27/2004	617
	Left Posterior	270	Orange	7/14/2004	
	Left Anterior	109	Red	11/18/2004	
Ramsey Hickory*	Right Anterior	232	Yellow	7/14/2004	329
	Right Anterior	97	Red	11/18/2004	
Grand Total					8,233