Growth, Body Condition, Reproduction and Survival of Stocked Barrens Topminnows, Fundulus julisia (Fundulidae)

Author(s): Cory A. Goldsworthy and Phillip W. Bettoli
Source: The American Midland Naturalist, 156(2):331-343. 2006.
Published By: University of Notre Dame
DOI: 10.1674/0003-0031(2006)156[331:GBCRAS]2.0.CO;2
URL:
http://www.bioone.org/doi/full/10.1674/0003-0031%282006%29156%5B331%3AGBCRAS%5D2.0.CO%3B2

Am. Midl. Nat. 156:331–343

Growth, Body Condition, Reproduction and Survival of Stocked Barrens Topminnows, Fundulus julisia (Fundulidae)

CORY A. GOLDSWORTHY
Tennessee Cooperative Fishery Research Unit, Tennessee Technological University, Cookeville 38505

AND

PHILLIP W. BETTOLI[1]
U.S. Geological Survey, Tennessee Cooperative Fishery Research Unit, Tennessee Technological University, Cookeville 38505

Abstract.—We documented the fate of 29 cohorts of propagated Barrens topminnows *Fundulus julisia* stocked as juveniles and adults ($n_{total} = 2770$ fish) into 17 springheads and small ponds in middle Tennessee in 2003 and 2004. Annual mortality rates were calculated after estimating the number of individuals of each cohort remaining 1–18 mos after fish were stocked. Lighted larval fish traps were deployed at seven reintroduction sites and the Type Locale to determine whether topminnows could reproduce in the presence of the introduced-transplanted Western mosquitofish *Gambusia affinis.* At stocking sites harboring mosquitofish (n = 12), their density ranged from 0.4 to 66.3 mosquitofish per m^2. Annual mortality of stocked Barrens topminnows ranged from 45 to 100% and 24 cohorts experienced annual mortality greater than 95%. Mortality was not related to mosquitofish density or the mean size at stocking. The robustness of Barrens topminnows did not differ in the presence or absence of mosquitofish, suggesting that interspecific competition for food was not occurring. Larval Barrens topminnows were collected at two reintroduction sites and the Type Locale, but juvenile recruits were produced only at sites lacking mosquitofish. The findings of this study, and concurrent laboratory studies, support the hypothesis that mosquitofish predation on larval Barrens topminnows was the primary mechanism in failed reintroductions and is the greatest threat to wild and reintroduced populations of this imperiled species.

Introduction

Due to limited distribution and the scarcity of undisturbed habitats, the Barrens topminnow *Fundulus julisia* has been 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 watersheds. The soft Mississippian limestone geology of the Barrens region creates many springs that emanate from numerous aquifers (Etnier and Starnes, 2001), which provides 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 occurred at 20 locations; by 1994 seven of these populations remained and by 2005 only four wild Barrens topminnow populations were known.

[1] Corresponding author: Telephone: (931) 372-3086; e-mail: pbettoli@tntech.edu

The first Barrens topminnows were collected in 1937 in the Duck River watershed in south central Tennessee (Williams and Etnier, 1982), although they were originally catalogued as the whiteline topminnow *Fundulus albolineatus*, a species now thought to be extinct. In 1966 Barrens topminnows were discovered at a new site called Summitville Mountain Spring (in the Cumberland River system). 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 in 1977; however, changes in the designation of critical habitat in 1978 resulted in the withdrawal of many proposals, including the proposal for the Barrens topminnow. The Tennessee Wildlife Resources Agency (TWRA) listed the Barrens topminnow as state-endangered in 1975. Fourteen new populations were discovered during surveys in 1983 and the species was subsequently downlisted to threatened status.

The 1983 surveys revealed that several historic populations had been extirpated (Etnier, 1983). Population declines and additional extirpations were observed in the late 1980s and early 1990s, which prompted additional surveys. Rakes (1996) concluded that the number of Barrens topminnows had declined from 4500–5000 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. In 2002 two new populations of Barrens topminnows were discovered in the McMahan Creek watershed (Caney Fork River system). One site occurred behind a new housing subdivision; the other was on a cattle farm adjacent to a major highway.

In an effort to preclude listing under the Endangered Species Act, a task force (Barrens Topminnow Working Group, BTWG) comprised of state and federal agencies and non-profit organizations was created in 2001. The goal of the BTWG was to work with private 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, Tennessee, and the Tennessee Aquarium in Chattanooga, Tennessee. The BTWG stocked 1267 Barrens topminnows in 2001–2002 into the Barren Fork River and Hickory Creek watersheds of the Caney Fork River system; however, the results were not encouraging in terms of survival and natural reproduction by stocked fish (Johnson, 2004).

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 *et al.*, 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 new habitats, 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 aggressive behavior contribute to the success of mosquitofish as an invader. Mosquitofish are livebearers and produce multiple broods throughout the warmer months. A mature mosquitofish can produce 3–4 broods each spawning season and each brood can range from several dozen to several hundred precocious offspring (Pflieger, 1975). In contrast, the annual fecundity of wild Barrens topminnows probably does not exceed 300 eggs (Rakes, 1989) and their offspring are true larvae. Mosquitofish contributed to the elimination or decline of imperiled species such as the threatened Railroad Valley springfish *Crenichthys nevada*, the least chub *Iotichthys phlegethontis*, the endangered Gila topminnow *Poeciliopsis occidentalis* and the California newt *Taricha torosa* (Galat and Robertson, 1992; Gamradt and Katz, 1996; Fuller *et al.,* 1999; Mills *et al.,* 2004).

Our primary objective was to determine whether Barrens topminnows could survive, reproduce and create self-sustaining populations in the springs and pools where they were stocked, many of which contained mosquitofish. Secondary objectives included estimating growth and mortality rates for stocked cohorts and determining whether those rates varied with mosquitofish density or the mean size at stocking. A final objective was to determine whether the robustness (*i.e.,* body condition) of stocked topminnows varied with mosquitofish density.

STUDY AREAS

Most reintroduction efforts have focused on properties in the Hickory Creek watershed in the headwaters of the Caney Fork River system of middle Tennessee (Fig. 1). Five properties were located on Little Hickory Creek and one was 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 in the Duck River system, one on Carroll Creek and one on an unnamed tributary to Ovoca Lake. Only one of these properties (Vervilla) was not private property. These 10 properties were a subset of 16 properties stocked by the BTWG task force since 2001. The sites chosen for intensive sampling in this study occurred on properties where landowners granted us unrestricted access and where the habitat was conducive to sampling.

The Clayborne property in the Little Hickory Creek watershed consisted of seven small pools at a springhead, four of which were included in this study: Clayborne 3, 4, 6 and 7. Pools 1, 2 and 5 were not stocked during this study and no individuals or cohorts stocked into these three pools prior to this study were collected. Clayborne 3 was excavated in the spring run, measured 49 m^2 and was stocked with 50 topminnows in August 2003. Clayborne 4 measured 16 m^2 and was excavated above the floodplain of the spring system, was 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 were situated in the floodplain and measured 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 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).

The Sain property was immediately downstream of the Clayborne property. Clayborne 3 drained into the upper Sain pool (51 m^2), which drained into the lower Sain pool (50 m^2) about 30 m downstream. A small spring also flowed into the lower pool. Aquatic vegetation coverage was scarce 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).

The Cunningham property had two separate spring systems that were stocked with Barrens topminnows. The most upstream site, noted as the Cunningham barn site, was a spring-influenced excavated pool with a surface area of 191 m^2. The pool was usually covered with a thick layer of duckweed *Lemna* spp., filamentous algae and pondweed

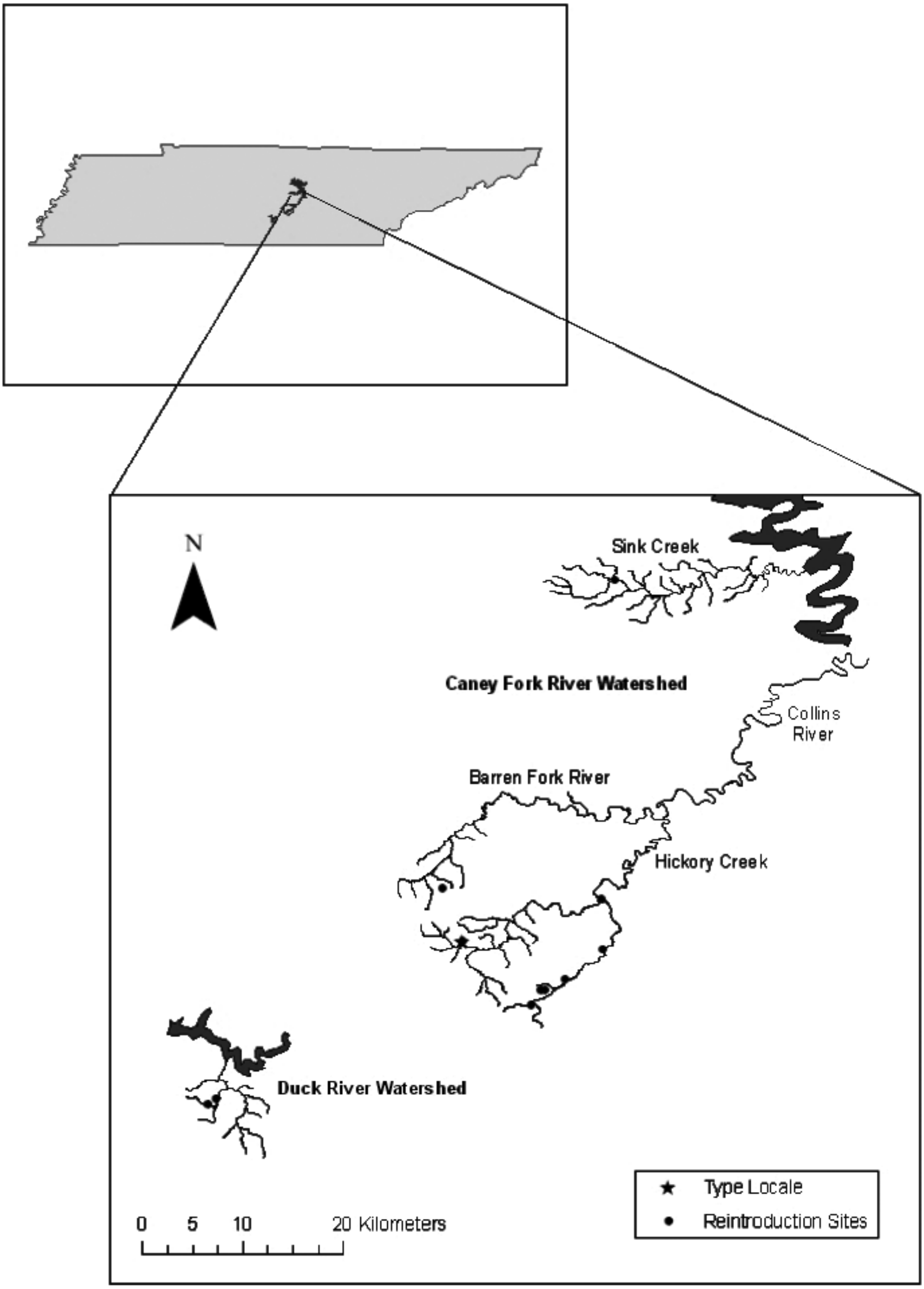

FIG. 1.—The Caney Fork River and Duck River watersheds on the Barrens Plateau in middle Tennessee. The 10 properties, representing 17 stocking sites, where Barrens topminnows were stocked are indicated

Potamogeton spp., which receded in the fall. 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 consisted of two smaller pools (68 and 56 m^2) excavated by the USFWS in 2003. Both of these pools were spring-influenced and connected to the mainstream of Little Hickory Creek by a spring run. Both pools were prone to large infestations of *Myriophyllum* sp. and filamentous algae. Between August 2003 and May 2004, 558 Barrens topminnows were released into these pools. Resident fishes included largemouth bass *Micropterus salmoides*, bluegill, mosquitofish and fringed darters (Johnson, 2004).

The Murphy property consisted of a springhead that formed a spring run, which emptied into Little Hickory Creek. A small pool (10 m^2) was excavated in 2002 adjacent to the spring run to provide slackwater habitat for the 239 Barrens topminnows stocked between August 2003 and May 2004. A dense infestation of pondweed occupied the site throughout 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).

The Ramsey property was located on a cattle farm and consisted of two pools (432 m^2 and 100 m^2), which were excavated by the USFWS in 2004. Aquatic vegetation (*Zannichellia palustris*, *Ludwigia palustris* and *Nasturtium officinale*) was abundant. 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, July and November 2004.

The Vervilla property was located on land owned by the USFWS, and was the most downstream stocking site on Hickory Creek. Six pools ranging in size 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 were all prone to blooms of filamentous algae. Two cohorts totaling 373 Barrens topminnows were stocked into two pools between August 2003 and May 2004. Golden shiners *Notemigonus crysoleucas*, bluntnose minnows, fringed darters, mosquitofish and flame chubs inhabited the site (Johnson, 2004).

The Herndon property consisted of a large spring pool and spring run that flowed into Sink Creek, a tributary to the Caney Fork River. Aquatic vegetation was scarce 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.

The Bridges property consisted of a shallow spring run with riffles, runs and pools with scant aquatic vegetation, which flowed into an unnamed tributary of Mud Creek in the Barren Fork River watershed. 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.

The site on the Marcum property (Duck River watershed) where topminnows were stocked consisted of a spring discharging into a concrete trough, which drained into a spring run that emptied into a pond. A concrete barrier isolated the pond from an adjoining reservoir, Lake Tullahoma and the pond was fishless when stocked with topminnows. The USFWS excavated a series of pools between the concrete trough and the small pond to improve the habitat for topminnows. The first Barrens topminnows ($n = 122$) were stocked into the pools and pond in August 2003. Afterwards, runoff from a severe thunderstorm filled all but one of the excavated pools with gravel. More Barrens topminnows were stocked into the remaining pool in May 2004 (n = 30) and July 2004 (n = 91). The pond experienced dense blooms of duckweed each summer.

The Collier property was located on a small, unnamed tributary to Lake Tullahoma, and consisted of two pools excavated around multiple springheads. Watercress and filamentous algae were abundant in each pool when 50 Barrens topminnows were stocked in May 2004. A seine survey conducted on 13 November 2004 collected stonerollers, green sunfish and bluegill.

METHODS

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, and 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 (*e.g.,* left posterior; right posterior; left posterior and right anterior double tag) were used to distinguish stocked cohorts. Amber eyeglasses and a portable UV light source were used as an aid in identification of tagged individuals. Mean total lengths (TL) of the 29 cohorts ranged from 33 to 52 mm total length when they were tagged.

A seine net (1.8 m × 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). At least two and no more than four seine hauls were taken at each site and sampling was terminated if no Barrens topminnows were caught. 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), weighed (0.1 g), sexed and assigned to a cohort based on tag color and location.

Larval Barrens topminnows were sampled at seven of the reintroduction sites using light traps modified from the design of Hartman (1994). The light was provided by a green photochemical light stick. The main body of the trap consisted of a 10-cm wide 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. In a laboratory trial using a 720-liter aquarium, six of nine Barrens topminnows (20–25 mm TL) in the aquarium entered the light trap overnight; thus, we assumed that the trap would be sufficient for determining the presence/absence of larval Barrens topminnows. Traps were deployed overnight weekly in May and June 2004 and every 2 wk in July, August and September 2004. When the traps were retrieved the following morning the contents of each trap were washed through a 100-micron 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 larval topminnows.

Larval light traps were also deployed at the Type Locale at the same time the reintroduction sites were sampled in order to confirm that the traps would capture larval topminnows if they were present. We assumed that larval topminnows would be present because the Type Locale population had persisted for at least several decades in the absence of mosquitofish; thus, reproduction and recruitment were routine events in that population.

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 (Dunham *et al.*, 2002).

The instantaneous mortality rate (Z) of Barrens topminnows was calculated as

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

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

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

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 negligible. Linear regression techniques were used to explain the variation in interval mortality of Barrens topminnows as a function of mosquitofish density and mean size at stocking.

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 $\overline{TL}_{t+1}$ were 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 d) and long intervals (≥200 d) whenever at least three fish of a cohort were recaptured at any stocking site.

Fisher's exact test was used with 2 × 2 contingency tables to separately test whether Barrens topminnow reproduction and recruitment were associated with mosquitofish presence or absence at sites sampled with light traps. Reproduction by a population was defined as the capture of at least one larval Barrens topminnow in a light trap. Recruitment in the populations sampled with light traps was defined as the presence of wild age-0 topminnows in catch-depletion seine samples. Reproduction was assumed and recruitment was confirmed at sites not sampled with light traps if wild age-0 topminnows were collected in catch-depletion seine samples; such sites were subsequently included in the statistical analysis of whether mosquitofish absence/presence was linked to topminnow reproduction and recruitment. Data on the Type Locale population (*i.e.,* reproduction and recruitment occurred in the absence of mosquitofish) were also included in the statistical analyses.

The robustness of Barrens topminnows at sites with and without mosquitofish was compared using analysis-of-covariance (ANCOVA). The ANCOVA model included a response variable (weight), an intercept and two independent variables: a covariate (length) and a dummy variable that represented the effect of mosquitofish presence on Barrens topminnow weight. The assumption of equal slopes ($P > 0.05$) was tested using an F-test prior to performing the test of adjusted mean weights.

RESULTS

Annual mortality of stocked Barrens topminnows ranged from 45% to 100%; 24 of 29 cohorts experienced mortality rates over 95% (Table 1). Mosquitofish were collected at 12 of the 17 reintroduction sites; densities where they occurred ranged from 0.4 to 66 fish per m^2 (Table 2). Annual mortality was not related to mosquitofish density ($F = 0.07$; $df = 1, 27$; $P = 0.796$) or mean size at stocking ($F = 1.52$; $df = 1, 27$; $P = 0.228$).

TABLE 1.—Mean total length at stocking, annual interval mortality of stocked Barrens topminnows, mosquitofish density and number of larval topminnows collected at reintroduction sites. Each site was sampled on one date between November 2004 and January 2005 to estimate population size of Barrens topminnows and mosquitofish density. An asterisk denotes sites sampled with larval fish traps in 2004

Site	Date stocked	Number stocked	Mean length at stocking (mm)	Annual mortality (%)	Mosquitofish density (fish/m2)	Number of larval topminnows collected
Bridges	5/27/2004	22	52.1	100.00	0	—
Clayborne 3	8/27/2003	50	42.4	96.02	2.7	—
Clayborne 4*	6/3/2003	12	33.1	61.65	0	2
Clayborne 6*	8/27/2003	88	43.0	44.57	16.1	1
Clayborne 7*	8/27/2003	78	45.4	86.28	8.4	0
Collier	5/27/2004	50	50.4	98.04	0	—
Cunningham Barn*	8/27/2003	50	33.1	89.12	66.3	0
Cunningham 2*	8/27/2003	100	42.4	100.00	21.3	0
	12/18/2003	41	44.2	100.00	21.3	—
	5/27/2004	154	43.9	99.97	21.3	—
Cunningham 3	8/27/2003	100	41.4	100.00	14.8	—
	12/18/2003	36	45.8	100.00	14.8	—
	5/27/2004	127	37.4	99.11	14.8	—
Herndon	7/8/2004	175	38.1	100.00	0	—
	11/18/2004	105	43.1	100.00	0	—
Marcum	8/27/2003	122	43.8	100.00	0	—
	5/27/2004	30	51.9	98.23	0	—
	7/8/2004	91	41.4	97.79	0	—
Murphy	8/27/2003	50	44.2	100.00	33.0	—
	5/27/2004	189	39.0	100.00	33.0	—
Ramsey	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	0
Sain Lower*	6/3/2003	50	44.1	96.57	8.1	0
Vervilla 3	8/27/2003	50	42.7	100.00	12.7	—
	5/27/2004	136	43.3	100.00	12.7	—
Vervilla 4	8/27/2003	50	43.6	100.00	4.9	—
	5/27/2004	137	43.3	100.00	4.9	—

Of the eight 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 lacked mosquitofish, but mosquitofish were present in the Clayborne 6 pool. 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 (8 mm TL) was collected on 9 June at the Clayborne 4 pool; the last larval topminnow was collected at the Type Locale (24 mm TL) on 24 September 2005. Juvenile recruitment (*i.e.,* the presence of wild age-0 topminnows) was observed at the Collier site on 13 November 2004; therefore, larval fish were produced earlier in the year and the data from this site, which was not sampled with larval traps, were included in subsequent analyses.

The five sites where no larval topminnows were collected by the light traps (Cunningham Barn, Cunningham 2 and 3 and Sain upper and Sain lower) all harbored varying densities

TABLE 2.—Number of Barrens topminnows (*F.j.)* and mosquitofish (*G.a.*) collected in seine hauls at 17 sites during 2004–2005. Zippin population estimates (N) are listed for those species and sites where the population was depleted and minimum capture probabilities exceeded 20%

Site	Date	Species	Seine haul 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	1003
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	—	—	1181	1251
Cunningham 3	6-Jan-05	*F.j.*	7	1	—	—	8	—
		G.a.	888	107	—	—	995	1009
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	—
Murphy	12-Jan-05	*F.j.*	0	0	0	—	0	—
		G.a.	93	90	40	—	223	330
Ramsey	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 age-0 offspring of stocked Barrens topminnows

of mosquitofish (Table 2). Larval topminnows were collected at two sites lacking mosquitofish and were assumed to be present at the Collier site, which also lacked mosquitofish; whereas a single larval topminnow was collected at one site that harbored mosquitofish. Thus, there was an association between mosquitofish absence and Barrens topminnow reproduction ($P = 0.048$).

At the nine sites where we reported the presence/absence of larval topminnows (seven reintroduction sites sampled with light traps; Type Locale; Collier site), wild topminnow recruits were collected at only the three sites devoid of mosquitofish (Type Locale, Collier and Clayborne 4). No topminnow recruits were collected at the remaining six sites harboring mosquitofish; thus, there was also an association between mosquitofish absence and Barrens topminnow recruitment ($P = 0.012$).

The absence of mosquitofish did not guarantee the production of recruits by Barrens topminnows where stocked fish persisted. For instance, no topminnow recruits were observed at the Marcum site, which was devoid of moquitofish (Table 2). Larval fish traps were not deployed at this site and it is unknown whether any reproduction occurred.

The robustness of Barrens topminnows did not vary among sites with and without mosquitofish. Slopes of the $\log_{10}$length-$\log_{10}$weight regression lines for Barrens topminnows in the presence and absence of mosquitofish were similar ($F = 0.15$; $df = 3, 286$; $P = 0.697$) and the adjusted mean weights of Barrens topminnows did not differ ($F = 1.70$; $df = 2, 287$; $P = 0.193$).

Instantaneous growth in length varied more than three-fold among 13 cohorts of Barrens topminnows recaptured 49 to 182 days post stocking. The number of recaptures per cohort ranged from 3 to 43 fish and mean short-term growth was 0.22% $–d^{-1}$ (SE = 0.02). 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; 0.36% $–d^{-1}$) and at sites lacking mosquitofish (*e.g.,* Clayborne 4; 0.37% $–d^{-1}$). Conversely, growth was slow at some sites with mosquitofish (*e.g.,* Clayborne 7; 0.12% $–d^{-1}$) and without mosquitofish (*e.g.,* Collier; 0.15% $–d^{-1}$). We recaptured 3 or more fish from four cohorts that had been at large for 443 to 528 d; their long-term growth averaged 0.11% $–d^{-1}$ (SE = 0.03).

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 adult topminnows outright, repeated negative interactions may have caused some topminnows to emigrate from the pools and springheads where they were stocked. The displacement of one species from its preferred habitat by a stronger competitor such as mosquitofish has been documented for other imperiled species (*e.g.,* least chub, Mills *et al.*, 2004).

The topminnow stocking sites we studied were selected, in part, based on whether it would be possible to effectively sample them; we avoided sites stocked by the BTWG task force that were deep (<1.5 m) or could not be seined because of complete vegetation coverage or innumerable stumps. Although topminnows may have been present at sites where we did not collect them, such sites were small (10–50 m^2) and amenable to seining; thus, concluding they were absent from those sites after two or more seine hauls is not unreasonable.

We assumed that survival of any topminnows that emigrated from stocking sites was negligible; if stocked topminnows emigrated in large numbers and survived in new habitats, the mortality rates we reported would have been positively biased. In fact, irregular surveys of habitats downstream of stocking sites in 2002 and 2003 captured two tagged topminnows that had emigrated from their respective stocking sites (Johnson, 2004). However, those two topminnow recaptures were probably the exception rather than the rule. For instance, an electrofishing survey in 2004 of a 2.5-km reach of Little Hickory Creek draining the Sain, Clayborne, and Cunningham stocking sites, where 1495 Barrens topminnows were stocked over three years, yielded no topminnows. Stocked Barrens topminnows emigrating downstream from stocking sites would have encountered a suite of potential piscine predators such as largemouth bass and green sunfish in atypical topminnow habitats (*i.e.,* streams and rivers). The naiveté of stocked fish would also have contributed to high mortality rates, which has been observed for other hatchery-reared species (*e.g.,* rainbow trout *Onchorhynchus mykiss,* Bettinger and Bettoli, 2005).

We observed scant evidence of reproduction and no evidence of recruitment by Barrens topminnows at reintroduction sites where mosquitofish were present. The negative effects of mosquitofish on native fish assemblages have been well documented (Courtenay and Meffe, 1989; Lydeard and Belk, 1993; Belk and Lydeard, 1994; Rincon *et al.*, 2002). Courtenay and Meffe (1989) concluded that mosquitofish predation was the primary mechanism in the decline or extinction of native species based on anatomical structures of mosquitofish and numerous studies that documented mosquitofish predation on other fish species. Recent aquarium studies confirmed that mosquitofish predation could 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 the present 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.

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 gradual disappearance of Barrens topminnows, and one supported by the results of this study and mosquitofish studies elsewhere (*e.g.,* Meffe, 1985; Courtenay and Meffe, 1989), is predation by mosquitofish on the early life history stages of topminnows.

In conclusion, the lack of recruitment after mosquitofish invaded the Vervilla site, the ex situ aquaria studies and results of our larval and juvenile topminnow sampling 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 high is evidence that adult Barrens topminnows can coexist with mosquitofish, although their offspring cannot.

We agree with Conant (1988) and others that reintroduction programs for imperiled species will not succeed if the factor(s) causing their imperilment still exist in the landscape. If future reintroductions are slated to occur in small springheads such as those stocked in the present study, they should be restricted to sites free of mosquitofish and with barriers in place, if necessary, to prevent subsequent invasions.

We also agree in principle with Shute *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 study, we failed to observe recruitment (in the presence of mosquitofish) by a species that inhabits the surface of the water column during the growing season, achieves lengths in excess of 80 mm total length, and in which the males are brightly colored and clearly visible to the naked eye. After 4 y of field observations and the stocking of over 4000 propagated fish, we believe it is time to abandon any reintroductions of propagated topminnows into habitats harboring mosquitofish.

Acknowledgments.—Funding and support for this project was provided by the Tennessee Wildlife Resources Agency, the U.S. Fish and Wildlife Service, The Nature Conservancy of Tennessee, the Center for the Management, Utilization and Protection of Water Resources at Tennessee Technological University and the U.S. Geological Survey. This field research was possible only with cooperation of the organizations and personnel that constituted the Barrens Topminnow Working Group, particularly Conservation Fisheries, Incorporated; the Tennessee Aquarium, Dale Hollow National Fish Hatchery and Wolf Creek National Fish Hatchery. We thank all of the landowners who graciously provided access to their land and springs. This manuscript benefited from reviews of an earlier draft by H.T. Mattingly, M. Allen and two anonymous reviewers.

LITERATURE CITED

BELK, M. C. AND C. LYDEARD. 1994. The effects of *Gambusia holbrooki* on a similar sized, syntopic poeciliid, *Heterandria formosa* – competitor or predator. *Copeia,* **1994**:296–302.

BETTINGER, J. M. AND P. W. BETTOLI. 2002. Fate, dispersal, and persistence of recently stocked and resident rainbow trout in a Tennessee tailwater. *N. Am. J. Fish. Manage.,* **22**:425–432.

CONANT, S. 1988. Saving endangered species by translocation: are we tinkering with evolution? *Bioscience,* **38**:254–258.

COURTENAY, W. R., JR. AND G. K. MEFFE. 1989. Small fishes in strange places: a review of introduced Poeciliids, p. 319–331. *In:* G. K. Meffe and F. F. Snelson, Jr. (eds.). Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, New Jersey.

DUNHAM, J. B., B. S. CADE AND J. W. TERRELL. 2002. Influences of spatial and temporal variation on fish-habitat relationships defined by regression quantiles. *Trans. Am. Fish. Soc.,* **131**:86–98.

EHRLICH, P. 1986. Which animal will invade?, p. 79–95. *In:* H. A. Mooney and J. A. Drake (eds.). Ecology of Biological Invasions of North America and Hawaii. Springer-Verlag, New York.

ETNIER, D. A. 1983. Status report for the Barrens topminnow (*Fundulus julisia* Williams and Etnier). Unpublished report to U.S. Fish and Wildlife Service. 27 p.

——— AND W. C. STARNES. 2001. Fishes of Tennessee. The University of Tennessee Press. Knoxville. 681 p.

FULLER, P. L., L. G. NICO AND J. D. WILLIAMS. 1999. Nonindigenous fishes introduced into inland waters of the United States. American Fisheries Society, Special Publication 27, Bethesda, Maryland. 613 p.

GALAT, D. L. AND B. ROBERTSON. 1992. Response of endangered *Poeciliopsis occidentalis sonoriensis* in the Rio Yaqui drainage, Arizona, to introduced *Gambusia affinis. Environ. Biol. Fish.,* **33**:249–264.

GAMRADT, S. C. AND L. B. KATZ. 1996. The effect of introduced crayfish and mosquitofish on California newts (*Taricha torosa*). *Conserv. Biol.,* **10**:1155–1162.

HARTMAN, M. A. 1994. Nocturnal microhabitats of larval and juvenile fish in Shoal Creek, Tennessee. Unpublished M.S. Thesis, Tennessee Technological University, Cookeville. 53 p.

JOHNSON, A. B. 2004. Monitoring introductions of the Barrens topminnows. Unpublished M.S. Thesis, Tennessee Technological University, Cookeville. 64 p.

LAHA, M. 2004. Impacts of invasive mosquitofish on the imperiled Barrens topminnow: a laboratory study. Unpublished M.S. Thesis, Tennessee Technological University. 77 p.

LYDEARD, C. AND M. C. BELK. 1993. Management of indigenous species impacted by introduced mosquitofish: an experimental approach. *Southwest. Natur.,* **38**:370–373.

MCMAHON, T. E., A. V. ZALE AND D. J. ORTH. 1996. Aquatic habitat measurements, p. 83–120. *In:* B. R. Murphy and D. W. Willis (eds.). Fisheries Techniques, 2nd ed. American Fisheries Society, Bethesda, Maryland. 732 p.

MEFFE, G. K. 1985. Predation and species replacement in American southwestern fishes: a case study. *Southwest. Natur.,* **30**:173–187.

MILLS, M. D., R. B. RADER AND M. C. BELK. 2004. Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia,* **141**:713–721.

PFLIEGER, W. L. 1975. The Fishes of Missouri. Missouri Department of Conservation, Columbia. 343 p.

RAKES, P. L. 1989. Life history and ecology of the Barrens topminnow, *Fundulus julisia* Williams and Etnier (Pisces, Fundulidae). Unpublished M.S. thesis, University of Tennessee, Knoxville. 65 p.

———. 1996. Status survey and review with recommendations for management of the Barrens topminnow for Arnold Engineering Development Center, Coffee and Franklin Counties, including surrounding areas. Final Report to The Nature Conservancy, Nashville.

RINCON, P. A., A. M. CORREAS, F. MORCILLO, P. RISUENO AND J. LOBON-CERVIA. 2002. Interactions between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *J. Fish Biol.,* **60**:1–26.

SHAFLAND, P. L. AND W. M. LEWIS. 1984. Terminology associated with introduced organisms. *Fisheries,* **9**(4):17–18.

SHUTE, J. R., P. L. RAKES, AND P. W. SHUTE. 2005. Reintroduction of four imperiled fished in Abrams Creek, Tennessee. *Southeast. Natur.,* **4**:93–109.

VAN DEVENTER, J. S. AND W. S. PLATTS. 1985. A computer software system for entering, managing, and analyzing fish capture data from streams. Research Note INT-352, U.S. Forest Service, Intermountain Research Station., Ogden, Utah. 12 p.

WILLIAMS, J. D. AND D. A. ETNIER. 1982. Description of a new species, *Fundulus julisia,* with a redescription of *Fundulus albolineatus* and a diagnosis of the subgenus *Xenisma* (Teleostei: Cyprinodontidae). *Occasional Papers of the Museum of Natural History, University of Kansas,* **102**:1–20.

ZIPPIN, C. 1958. The removal method of population estimation. *J. Wildlife Manage.,* **22**:82–90.

SUBMITTED 18 JULY 2005 ACCEPTED 22 MARCH 2006