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Life History Traits of the Tennessee Dace (Phoxinus tennesseensis) in Northeast Tennessee

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ABSTRACT.—We examined life history traits of *Phoxinus tennesseensis* (Tennessee dace), a globally vulnerable (G3) upper Tennessee River drainage endemic, in 2 populations in Northeast Tennessee. The spawning season lasted from Apr. to Jun. Spawning occurred over the nests of *Semotilus atromaculatus* (creek chub) and *Campostoma anomalum* (central stoneroller), where large spawning aggregations of dace gathered over host species nests. *Phoxinus tennesseensis* populations have 4 size classes that are indicators of age classes. The mean life span of *P. tennesseensis* is about 2 y with 1st y fish constituting a majority of the population. Fecundity samples indicate that females produce 398 to 721 ova that were 0.9 to 1.5 mm in diameter. *Phoxinus tennesseensis* has the lowest fecundity when compared to closely related congeners and inhabits the smallest headwater streams that frequently experience reduced or no-flow conditions. These characteristics probably contribute to its very limited distribution and small population sizes, and make the few remaining populations very vulnerable to environmental disturbances.

INTRODUCTION

The Tennessee dace, *Phoxinus tennesseensis*, is a small minnow (Cyprinidae) with a limited distribution in eastern Tennessee and extreme southwestern Virginia (Etneir and Starnes, 1993; Jenkins and Burkhead, 1994). Originally, *P. tennesseensis* was thought to be a variant of *P. oreas* (mountain redbelly dace), but these species are morphologically distinct (Starnes and Jenkins, 1988). *Phoxinus tennesseensis* is regarded as a member of a monophyletic group that includes *P. cumberlandensis*, *P. oreas*, and *P. saylori* (Skelton, 2001). There are seven North American *Phoxinus* species and some have extremely limited distributions. *Phoxinus cumberlandensis* has a limited distribution and is classified as endangered (Etnier and Starnes, 1993) and *P. saylori* appears to have a distribution that includes only a few streams (Skelton, 2001).

Phoxinus tennesseensis often inhabits spring-fed, first order streams that are typically shaded by woody vegetation. Stream habitat includes silt and fine gravel pools and undercut banks with woody debris and other material serving as cover (Starnes and Jenkins, 1988; Hamed and Alsop, 2005). There are only 62 known populations of this species (Shute, 2001). A large percentage of *P. tennesseensis* populations in Tennessee are found in jeopardized spring and seepage areas (Etnier and Starnes, 1991). Many populations have apparently become extirpated as recent surveys have failed to find *P. tennesseensis* in 55% of Northeast Tennessee streams previously inhabited (Hamed and Alsop, 2005). Because of its limited distribution, the fish is listed as G3 (globally vulnerable to extirpation and extinction) and S3 (state vulnerable to extirpation and extinction) (Tennessee Division of Natural Heritage, 2001), and ''in need of management'' in Tennessee (Tennessee Wildlife Resource Agency, 2000). The species is listed as ''endangered'' in Virginia (Virginia Game and Inland Fisheries, 1989).

Little has been published on *P. tennesseensis*, especially with respect to life history parameters. However, much of the spawning behavior, age structure, and growth are likely to be similar to other *Phoxinus* species where lifespans are generally limited to a maximum of three years, fecundities range from 724 to 2872 mature ova per female, and age class structure is dominated by one and two year old fish (Starnes and Starnes, 1981). Other *Phoxinus* species do not construct their own nest but use the nest of another minnow (Etnier and Starnes, 1993) and like its congeners, *P. tennesseensis* has been presumed to be a nest associate spawner and has been observed over the nest of *Campostoma anomalum* (Starnes and Jenkins, 1988; Jenkins and Burkhead, 1994). Data from this study provides baseline life history information for *P. tennesseensis*. These data may have utility for management of this species as well as other *Phoxinus* species such as *P. saylori*, or others not yet described (Skelton, 2001).

MATERIAL AND METHODS

The purpose of this study is to describe the spawning behavior, nest site selection, ova production, and age class structure of *P. tennesseensis*. Two Sullivan County, Tennessee streams in the South Holston River drainage, Timbertree Branch in the Arcadia community (82°26′28″N, 36°35′40″W) and Trinkle Creek in the city of Bristol (82°12′16″N, 36°34′41″W) have the highest density of *P. tennesseensis* in Northeast Tennessee and were chosen as our study sites (Hamed and Alsop, 2005). The streams are spring-fed, first order streams with silt bottomed pools and small riffle areas flowing through a mixed-deciduous forest. Tributaries to Trinkle Creek become dry in the autumn (Aug.–Oct.) and only a few pools retain water during these times. Timbertree Branch exhibited reduced flow during the same period. Pools in both streams had undercut banks, root masses suspended in the water, and woody debris along the edges. Most root masses were growing from sycamore (*Platanus occidentalis*) or green ash (*Fraxinus pennsylvanica*). Other common streamside vegetation included spotted jewelweed (*Impatiens capensis*) and great rhododendron (*Rhododendron maximum*).

Samples of *P. tennesseensis* were collected from Trinkle Creek on a monthly basis from Dec. 1999 to Nov. 2000, with additional collections made during the spawning season (14 total samples). Samples were collected using seine nets (10 × 1.5 m) and dip nets (45 cm pocket) with a 3.5 mm mesh netting (Tennessee Wildlife Resource Agency Permit issued to MKH and FJA). An 800 m section of stream was sampled by repeated seine hauls through pools until fish were no longer captured. Other habitat types along the 800 m section were sampled with dip nets. This sampling method enabled a greater capture of juvenile fish because dip nets could be used to sample root masses, debris, and vegetation where juveniles were often found. Standard length (SL) and pectoral fin length were measured to the nearest 0.1 mm with dial calibers and the fish were then released. Size (age) classes were estimated from histograms based on monthly standard length measurements.

Twenty dace from Trinkle Creek and 20 from Timbertree Branch were collected during spawning aggregations and data from these collections were pooled. A limited number of fish were sacrificed because of the rare status of the species and permit restrictions. Body color was documented from the sampled fish. Pectoral fin length was measured to investigate the possibility of sexual dimorphism in this character. Specimens were sacrificed with tricaine methanesulfonate (MS-222) at a concentration of 2 g/L, fixed in a 10% formalin solution and dissected (E.T.S.U. Committee on Animal Care permit P000901). Mature ova from females were counted and measured to the nearest 0.1 mm in diameter. A two-sample *t*-test ($\alpha = 0.05$) was used to compare mean standard length and ova production of dace from Timbertree Branch and Trinkle Creek. Linear regression was employed to examine the relationship between fecundity and standard length and between pectoral fin length and sex.

Spawning behavior of *P. tennesseensis* was observed over the nests of *Semotilus atromaculatus* (creek chub) on 7 May 2000 and over the nests of *C. anomalum* (central stoneroller) on 5 May 2001 in Timbertree Branch. Observation periods ranged from 30 min to 1.5 h. Observations from stream banks with a pair of 8 × 40 binoculars and video recordings allowed for detailed descriptions of spawning behaviors. The number of *P. tennesseensis* at each nest and interactions with the nest building species were documented. The length, width, and depth of nests were measured to the nearest 0.5 cm after cessation of spawning. Water temperature was also recorded at each nest after spawning was completed. Eggs from the nests were brought to the laboratory and hatched, and juveniles were fed brine shrimp (*Artemia salina*) and raised until adult characteristics were observed to ensure correct identification of the eggs.

RESULTS

LIFE HISTORY

A total of 1099 fish were collected during the study. Length frequency distributions were used to determine size classes, and have been successfully employed in previous studies of other *Phoxinus* species to determine age classes (Settles and Hoyt, 1976; Starnes and Starnes, 1981). Age determination by scale annuli examination was attempted but proved to not be useful for *P. tennesseensis*. Scale annuli were equally spaced and showed no sign of slower growth during winter. Length frequency distributions were used to determine size classes, and are thought to be representative of age class structure in *P. tennesseensis*.

Standard length frequency histograms for *P. tennesseensis* from the Trinkle Creek population suggest that there are four size classes. Sept. and Dec. (Fig. 1) revealed the clearest size class distinctions because of the presence of young-of-the-year individuals. The size classes as estimated from the Dec. sample were: size-0 fish 15–30 mm SL; size-1 fish 31–45 mm SL; size-2 fish 46–56 mm SL; size-3 fish 57+ mm SL. A majority of fish were in size class 1 during spawning. There was noticeable mortality by Dec. with very few size class 2 fish observed. By Dec. less than 1% of the population was found in size classes 2 and 3. After hatching was completed and young fish had at least one month of growth, size class 0 fish composed 27.0% of the population in Sept. and 51.2% in Dec. The largest individual dace collected was 65.0 mm SL collected from Timbertree Branch.

SPAWNING BEHAVIOR

Spawning of *P. tennesseensis* was observed in Timbertree Branch where adults achieved breeding colors by Apr. 4, 2000 and Apr. 7, 2001. The pectoral, pelvic, and anal fins were sulfur yellow while the flanks and operculum were geranium as described by Smithe (1975). Initially, males were colored more vividly than females, but as the spawning period progressed the sexes were colored equally.

Chasing behavior was observed on Apr. 24, 2000, and May 1, 2001. During this behavior, males followed a single female throughout pool and run areas. Frequently, as many as 20 males would follow a single female, where males formed a straight line with the snout of one male just behind the caudal fin of the preceding male. Males did not pass each other in these spawning aggregations.

On May 7, 2000 *P. tennesseensis* were observed over nests of *S. atromaculatus* that had been constructed 4 d prior. The spawning area was 5 m below a large pool that dace and stonerollers had inhabited throughout the winter. Water temperature during spawning was 21°C with the spawning area being shaded. In the pool both *S. atromaculatus* and *Rhinichthys atratulus* (blacknose dace) were common, but only *R. atratulus* were present in the immediate spawning area.

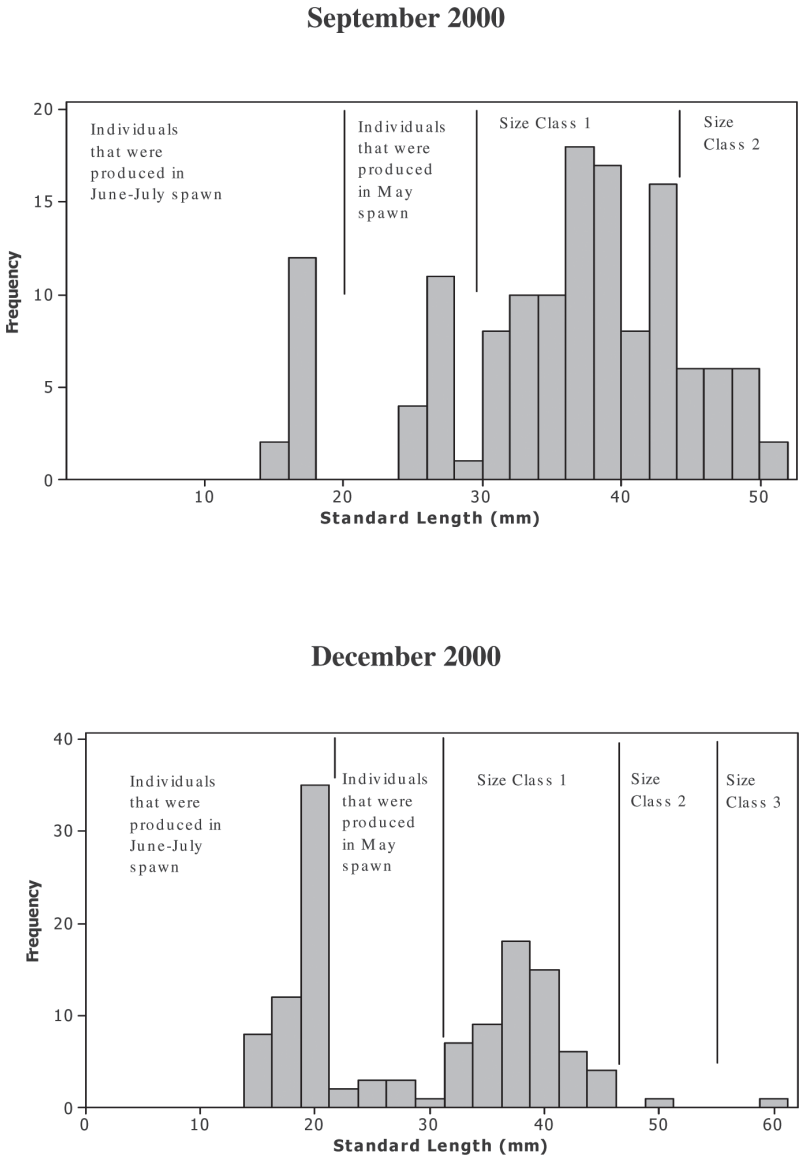

FIG. 1.—Size class distributions of *P. tennesseensis* in Trinkle Creek of Sullivan County, Tennessee during Sept. (n = 137) and Dec. (n = 159)

There were four nests of *S. atromaculatus* within a 5 m diam area of a gravel run. One of these four nests appeared to be preferred by *P. tennesseensis* with 57.5% of spawning individuals using that nest (Table 1). Nests of *S. atromaculatus* were 3.5 to 5.5 cm deep ($\bar{x}$ = 4.4, 95% CI = 2.74–6.02), 15.0 to 18.0 cm wide ($\bar{x}$ = 16.0, 95% CI = 13.75–18.25), and 15.0 to 20.0 cm long ($\bar{x}$ = 17.3, 95% CI = 13.72–20.78) (Table 1).

TABLE 1.—Dimensions of seven minnow nests of two species used by *P. tennesseensis* during spawning, and numbers of dace in each spawning aggregation

Species	Date	Width	Length	Depth	# of dace
S. atromaculatus	5/7/00	15.0 cm	18.0 cm	5.0 cm	46
S. atromaculatus	5/7/00	18.0 cm	20.0 cm	3.5 cm	17
S. atromaculatus	5/7/00	15.0 cm	16.0 cm	5.5 cm	9
S. atromaculatus	5/7/00	16.0 cm	15.0 cm	3.5 cm	8
C. anomalum	5/5/01	18.0 cm	27.0 cm	15.0 cm	56
C. anomalum	5/5/01	17.0 cm	22.0 cm	16.0 cm	9
C. anomalum	5/5/01	23.0 cm	28.0 cm	12.0 cm	18

Rhinichthys atratulus were swimming throughout the run prior to spawning, and many *P. tennesseensis* followed *R. atratulus* through the abandoned chub nests. *Phoxinus tennesseensis* spawning began with two males stopping and holding their positions over the nest. Females swam through the nest repeatedly for intervals of about 30 s. These swim-through events continued sporadically for approximately 30 min. At 3:47 PM a female stopped over the nest, and two male dace swam around her in a pattern similar to a barrel roll with the female being in the center. Within 10 s, 28 males approached from several areas of the run and nearby pool. The dace formed a large aggregation, causing the water to appear yellow from the coloration of the fins. The fish remained in the aggregation for 11 s. After the group dispersed two dace remained over the nest until 5:45 PM when they retuned to the original pool.

Phoxinus tennesseensis chased away several *R. atratulus* and other species that swam through the nest after spawning by the dace was completed. However, *Etheostoma simoterum* (snubnose darter) were not chased and paused over the nest. The darters were not observed feeding on ova, but were present in the nest substrate.

On Apr. 25, 2001, three nests under construction by *C. anomalum* were found in Timbertree Branch. Three days after construction *Notropis rubricroceus* (saffron shiners) were observed spawning over these nests. On May 5, 2001, at 2:15 PM, *P. tennesseensis* were observed spawning over the same nests of *C. anomalum*. The spawning site, which received full sun, was a gravel run 7 m downstream from a large pool and 150 m downstream from the spawning site observed in 2000. The water temperature in this area was 19°C at the time of spawning.

Three of four nests within a 5 m section of the run were used by *P. tennesseensis*. The unused nest was relatively small and still under construction. Male *C. anomalum* guarded all of the nests. Nests of *C. anomalum* were 17.0 to 23.0 cm deep ($\bar{x}$ = 19.3, 95% CI = 9.16–19.50), 12.0 to 16.0 cm wide ($\bar{x}$ = 14.3, 95% CI = 11.35–27.32), and 22.0 to 28.0 cm long ($\bar{x}$ = 25.7, 95% CI = 17.68–33.65) (Table 1).

At least 68 *P. tennesseensis* were observed at the spawning site where the dace moved from the downstream pool to a run with stoneroller nests. As dace entered the spawning site they jumped out of the water, possibly catching small insects or as a part of their spawning behavior. As a group of dace congregated over a nest, *C. anomalum* moved to defend the nest. Subsequently, a much larger group of dace moved to another nest of *C. anomalum* that had been abandoned. Male and female dace entered the abandoned nest together. The ratio of males to females was 5:1 based on observations of breeding coloration. Once over the nest, a spawning mass formed as the males pressed the female into the nest. On some occasions *P. tennesseensis* spawned while the stonerollers were over the nest.

On several occasions a male *C. anomalum* placed its snout into the nest and was observed moving small pebbles, but did not appear to be consuming eggs. Larger male stonerollers

TABLE 2.—Standard length of individual female *P. tennesseensis* and the number and size of ova produced by each female in two streams in Northeast Tennessee

Standard length of female (mm)	Stream	Number of ova	Size of ova (mm) range (mean ± SD)
42	Timbertree	496	0.9–1.3 (1.11 ± 0.07)
42	Timbertree	508	0.9–1.3 (1.17 ± 0.08)
46	Timbertree	541	0.9–1.2 (1.14 ± 0.08)
47	Timbertree	607	1.0–1.4 (1.25 ± 0.12)
48	Timbertree	635	1.0–1.3 (1.24 ± 0.09)
48	Timbertree	650	1.0–1.5 (1.28 ± 0.09)
51	Timbertree	671	0.9–1.5 (1.27 ± 0.14)
54	Timbertree	714	1.1–1.5 (1.31 ± 0.09)
56	Timbertree	721	1.1–1.5 (1.30 ± 0.09)
43	Trinkle Creek	398	0.9–1.2 (1.12 ± 0.06)
43	Trinkle Creek	461	0.9–1.3 (1.10 ± 0.11)
45	Trinkle Creek	435	0.9–1.2 (1.12 ± 0.09)
46	Trinkle Creek	427	0.9–1.3 (1.13 ± 0.09)
46	Trinkle Creek	460	1.0–1.3 (1.15 ± 0.10)
47	Trinkle Creek	549	1.1–1.5 (1.16 ± 0.09)
48	Trinkle Creek	563	1.1–1.4 (1.19 ± 0.10)
49	Trinkle Creek	580	1.0–1.5 (1.25 ± 0.19)
51	Trinkle Creek	571	1.2–1.5 (1.26 ± 0.07)
53	Trinkle Creek	601	1.0–1.5 (1.30 ± 0.11)

seemed to chase conspecifics more frequently than they chased *P. tennesseensis* during spawning. Spawning finished at 3:35 PM, and the dace returned to an upstream pool.

FECUNDITY

Nine female *P. tennesseensis* from Timbertree Branch and 10 from Trinkle Creek were collected and examined for the number of mature ova. Ova completely filled each female's body cavity. Females contained both mature and undeveloped ova, but only mature ova and ova close to maturity were counted. Ova counted were 0.9 to 1.5 mm in diameter and total numbers ranged from 398 to 721 ($\bar{x}$ = 556, 95% CI = 510.1–603.2) (Table 2). In *P. tennesseensis*, fecundity is strongly correlated with size (Fig. 2, $r^2 = 0.70$, P = 0.000). The number of ova produced by Timbertree Branch females (N = 9) was significantly greater than the number of ova produced by Trinkle Creek females (N = 10) (t = 3.02, P = 0.008).

Overall, the standard length of the dace examined ranged from 42 to 56 mm ($\bar{x}$ = 48.1, 95% CI = 46.3–49.9). Standard lengths of *P. tennesseensis* from Trinkle Creek ranged from 43 to 53 mm ($\bar{x}$ = 47.1, 95% CI = 44.78–49.42) and the number of ova ranged from 398 to 601 ($\bar{x}$ = 504.5, 95% CI = 450.7–558.3). Standard length of *P. tennesseensis* from Timbertree Branch ranged from 42 to 56 mm ($\bar{x}$ = 49.2, 95% CI = 45.94–52.50) and the number of ova ranged from 496 to 721 ($\bar{x}$ = 615.9, 95% CI = 576.1–691.9). A two-sample *t*-test showed no significant difference between the standard lengths of dace in Timbertree Branch and Trinkle Creek (t = 0.59, P = 0.57). However, the ability to detect a difference between these two populations with the sample sizes available was very low ($1 - \beta = 0.068$).

SEXUAL DICHROMATISM AND DIMORPHISM

Pectoral fins of young dace did not appear to be sexually dimorphic; however, males over 50 mm appeared to have longer and more rounded fins than females of the same size

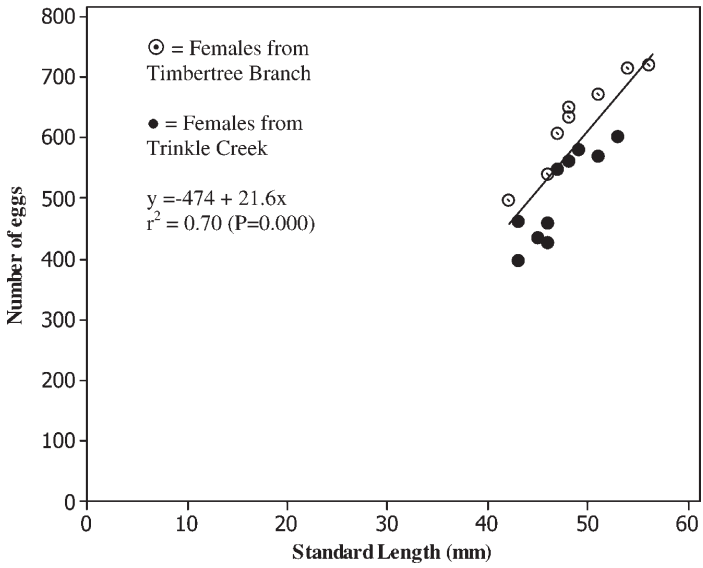

FIG. 2.—Regression of number of eggs on standard length in Timbertree Branch and Trinkle Creek females in Sullivan County, Tennessee ($r^2 = 0.70$, P = 0.000)

(Fig. 3). In adult *P. tennesseensis*, standard length is strongly correlated with length of the pectoral fin in both sexes (males $r^2 = 0.96$, P = 0.002; N = 6; SL $\bar{x}$ = 48.33, 95% CI = 42.96–53.70; pectoral fin $\bar{x}$ = 8.75, 95% CI = 8.14–9.36 and females $r^2 = 0.98$, P = 0.005, N = 5; SL $\bar{x}$ = 47.30, 95% CI = 35.51–59.09; pectoral fin $\bar{x}$ = 8.04, 95% CI = 6.74–9.34) (Fig. 3).

DISCUSSION

Phoxinus tennesseensis populations have four size classes that are suggestive of age classes. The age classes were inferred from the size classes in length frequency histograms from Sept. and Dec. collections (Fig. 1). Most other *Phoxinus* species have only three age classes, and females often comprise a larger percentage of third year fish than males (Settles and Hoyt, 1976; Starnes and Starnes, 1981). One possible explanation for the additional year of life is the relative absence of piscivorous fish species in *P. tennesseensis* habitat. Trinkle Creek had no large piscivorous fish and Timbertree Branch had only *Ambloplites rupestris* (rock bass). Some *P. tennesseensis* live in relatively larger streams, with piscivorous fish. Laurel Creek in Virginia not only has *A. rupestris*, but also *Oncorhynchus mykiss* (rainbow trout), *Salmo trutta* (brown trout), and *Salvelinus fontinalis* (brook trout). These species presumably consume *P. tennesseensis* and may contribute to the absence of the fourth size class in this population (Moran *et al.*, 2000). Most other species of *Phoxinus* inhabit streams that would be expected to have several piscivorous fish species.

Size classes often exhibited two peaks within each class. Size classes 0 and 1 in particular were characterized by two distinct peaks. This bimodality appears to be the result of two distinct spawning periods that are separated by four to six weeks. *Phoxinus erythrogaster* exhibits a similar spawning strategy (Settles and Hoyt, 1978). Size class data and the presence of breeding coloration in some fish during late Jun. also indicated this later spawning period, but spawning was not directly observed. Fish hatched from the first spawning of the year were 7 to 10 mm SL longer than those hatched from the apparent later second spawning.

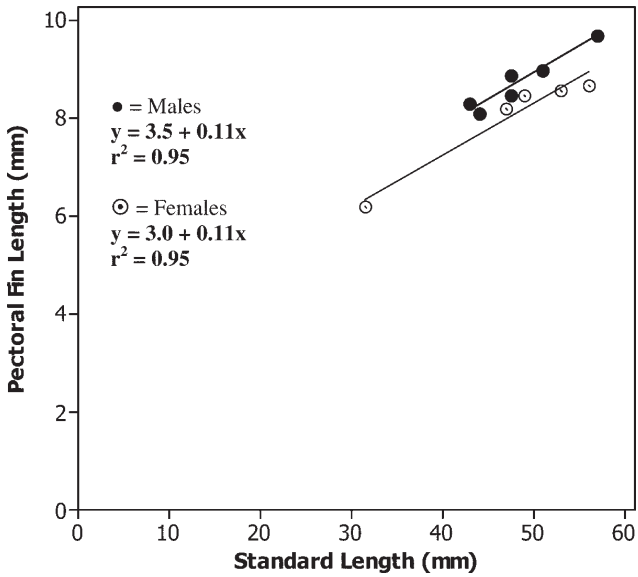

FIG. 3.—Regression of pectoral fin length on standard length of males (r^2 = 0.92, P = 0.002, n = 6) and on standard length of females (r^2 = 0.95, P = 0.005, n = 5) from Timbertree Branch in Sullivan County, Tennessee

Length frequency histograms show that the majority of individuals of *P. tennesseensis* in Northeast Tennessee populations are comprised of size classes 0 and 1 during the spawning period. During Sept., size class 2 fish comprise a maximum of 15.0% of the population. Some of the size class 1 fish that are the products of the previous year's late Jun.–Jul. spawning do not reproduce during their first year because they have not matured sufficiently. These late maturing individuals are unlikely to survive long enough to spawn more than once in their lifetime, if at all. Because size class 1 fish comprise by far the largest reproductive class of the dace populations in this study, any barrier to successful spawning by this cohort could have strong negative effects on population viability.

The range of standard length for age/size classes in *P. tennesseensis* was very similar to *P. cumberlandensis*. However, *P. cumberlandensis* reached a much larger size by the end of the second year with a maximum standard length of 57 mm. *Phoxinus tennesseensis* reached only 49 mm SL by the end of their s y. The largest and oldest age class (3) of *P. cumberlandensis* is the same standard length (65.5 mm) as *P. tennesseensis* age/size class 4 (Starnes and Starnes, 1981). *Phoxinus tennesseensis* attains the same size as *P. cumberlandensis*, but an additional year of growth appears to be needed. This slower growth may be caused by lower productivity in the very small headwater streams that are subject to low flow and drying during late summer and early fall.

Phoxinus tennesseensis spawning was observed in detail on two occasions. This species is a nest associate spawner found to use nests of *S. atromaculatus* and *C. anomalum*. *Phoxinus tennesseensis* had been previously observed over nests of *C. anomalum* but spawning was not observed (Jenkins and Burkhead, 1994). Other *Phoxinus* species are known nest associate spawners. *Phoxinus cumberlandensis* spawns over nests of *S. atromaculatus* and *C. anomalum*, and *P. erythrogaster* and *P. saylori* use the nests of *C. anomalum* (Pflieger, 1997; Skelton, 2001). *Phoxinus oreas* has been observed to spawn over nest of *Nocomis leptocephalus* (Jenkins and

Burkhead, 1994). However, *P. tennesseensis* were not observed over nests of *Nocomis* species in this study. Only Timbertree Branch was inhabited by *Nocomis* species (*Nocomis micropogon*), but none were observed spawning. It seems likely that *P. tennesseensis* will spawn over nests of *Nocomis* species if nests are present. If minnow nests are not present, some *Phoxinus* species are thought to use shallow riffle areas in streams (Starnes and Starnes, 1981), but *P. tennesseensis* was not observed in breeding coloration over riffle areas at any time in this study. It is thought that *Phoxinus* species receive several benefits from being a nest associate spawner, including physical protection provided by the nest and parental care by the host (Johnston, 1994). A dependence on the nests of other species has been shown to be associated with species rarity and this association may contribute to the rarity of *P. tennesseensis*. Several minnow species that are protected on a state or federal level are nest associate spawners (Johnston, 1999).

Prior to spawning *P. tennesseensis* exhibited chasing behavior similar to other species of *Phoxinus*. *Phoxinus* species often engage in communal spawning over nests of *C. anomalum* where one or a few females may be surrounded by as many as 50 males (Starnes and Starnes, 1981; Jenkins and Burkhead, 1994). In some species, such as *P. cumberlandensis* and *P. oreas*, swarming behavior begins with only a few males attending a female, followed by additional males joining to create a large mass (Starnes and Starnes, 1981; Etnier and Starnes, 1993).

Among *Phoxinus* species the ability to adjust to the presence or absence of the host minnow has only been observed in *P. tennesseensis*. A suite of spawning behaviors was exhibited by *Phoxinus tennesseensis* that was associated with the presence or absence of a nest building minnow. The actual act of spawning is the same if the host is present or absent. However, events leading to spawning vary based on the presence or absence of the host species. One observation of spawning behavior at a nest occupied by the host species showed that spawning was initiated within a few seconds of the nest being vacated by the host. A small group of *P. tennesseensis* entered the nest and were chased by the host, which removed the host from the nest and allowed the main group of dace to spawn. A separate observation of a nest with the host absent, found only a few dace converging around the host nest, but after a period of several minutes additional individuals (mainly males) joined the spawning aggregation simultaneously.

Most *Phoxinus* species breed from Apr. to Jun., and *P. erythrogaster* has two distinct spawning periods during that time period (Settles and Hoyt, 1978). *Phoxinus tennesseensis* breeds from Apr. to Jul., and size class histograms indicate the presence of two spawning periods. Water temperatures ranged from 12.5° to 25.3°C during spawning by other *Phoxinus* species (Starnes and Starnes, 1981; Jenkins and Burkhead, 1994). Water temperatures during spawning of *P. tennesseensis* in this study ranged from 19° to 21°C. Spawning occurred in the afternoon on clear days, as has been observed in other *Phoxinus* species.

Phoxinus tennesseensis was observed to leap out of the water one hour prior to and after spawning. Numerous small, flying insects were within 1 to 4 cm of the water surface. It is not known if the dace were feeding on the insects or if the behavior is part of spawning, but *P. erythrogaster* has been observed to behave similarly before and after spawning in apparent feeding related behavior (Phillips, 1969).

Phoxinus tennesseensis has the lowest fecundity among its closely related congeners and this probably contributes to its relatively limited distribution and low population numbers. Ova produced by female *P. tennesseensis* ranged from 398 to 721. The fecundity of *P. tennesseensis* is considerably less than for the federally threatened *P. cumberlandensis*, which produces 724 to 2872 ova (Starnes and Starnes, 1981). *Phoxinus erythrogaster* produced 140 to 681 ova (Settles and Hoyt, 1978). However, only the mature ova were counted in that study, and *P. erythrogaster* is thought to produce as many as 1000 ova per spawning season (Etnier and Starnes, 1993).

Both *P. erythrogaster* and *P. cumberlandensis* females of older age classes produce more ova (Settles and Hoyt, 1976; Starnes and Starnes, 1981) and female *P. tennesseensis* follow this trend. Larger and presumably older females produced more ova than smaller, younger females. Female dace from Timbertree Branch had a mean fecundity of 634 ova, compared to females from Trinkle Creek that had a significantly lower mean fecundity of 504 ova ($t = 3.02$, $P = 0.008$). The difference in the fecundity could be a result of the smaller size and lower productivity of Trinkle Creek, which dries in the fall with only shallow pools remaining. Fewer resources available to female dace in Trinkle Creek could have contributed to significantly fewer ova produced. *Phoxinus tennesseensis* in smaller streams like Trinkle Creek may be more vulnerable to events that disrupt spawning or destroy ova compared to populations in relatively larger, more stable and productive streams.

When female dace were examined, most ova were mature. Immature ova were a clearly smaller sized group based on diameter. Diameters of mature ova from *P. tennesseensis* ranged from 0.9 to 1.5 mm, with larger individuals seeming to produce larger ova. The size range of *P. tennesseensis* ova is similar to *P. erythrogaster* (0.67 to 1.26 mm in diameter) and *P. cumberlandensis* (0.82 and 1.36 mm in diameter) (Settles and Hoyt, 1978; Starnes and Starnes, 1981). Due to collection permit restrictions, only a limited number of dace were removed from each stream, and fecundity samples were not taken after the peak spawning season or in late winter. However, it appears that undeveloped ova overwinter at a small size and then develop in the spring prior to the spawning season.

The ability to distinguish male from female *P. tennesseensis* in field conditions is important for the successful management of this species. Other *Phoxinus* exhibit potential sexual dimorphism in pectoral fin lengths (Settles and Hoyt, 1978). After the spawning season has begun coloration is not a reliable indicator of sex, but males have more rounded pectoral fins compared to females that have narrower and more pointed fins. These differences can only be used to distinguish among individuals in size class 2 and 3. A similar dimorphism has also been observed in *P. phoxinus* (Frost, 1943).

Stream size and productivity appear to have strong influences on the life history characteristics of *P. tennesseensis* populations. *Phoxinus tennesseensis* exhibits several life history traits that are similar to other *Phoxinus* species, but the fecundity of *P. tennesseensis* is considerably less than that of other *Phoxinus* species. Most reproducing *P. tennesseensis* are size/age class 1 fish, and very few are likely to live more than two years. Most individual fish will only spawn once in their lifetime. The low reproductive potential observed in *P. tennesseensis* may be of particular importance in this rare species because any disruptions of reproduction caused by environmental stochasticity and anthropogenic disturbance could have consequential negative effects on individual populations. Limiting potential anthropogenic disturbances to *P. tennesseensis* populations, particularly during spawning periods, should be a priority in managing this globally vulnerable species.

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