Rodman, J.E. 1991. A taxonomic analysis of glucosinolate-producing plants. Part 2: Cladistics. Systematic Botany 16:619–629.

Schmidt, G.J., and E.E. Schilling. 2000. Phylogeny and biogeography of *Eupatorium* (Asteraceae: Eupatorieae) based on nuclear ITS sequence data. American Journal of Botany 87:716–726.

Sennblad, B., M.E. Endress, and B. Bremer. 1998. Morphological and molecular data in phylogenetic fraternity: The tribe Wrightieae (Apocynaceae) revisited. American Journal of Botany 85:1143–1158.

Simmons, M.P., V. Savolainen, C.C. Clevinger, R.H. Archer, and J.I. Davis. 2001. Phylogeny of the Celastraceae inferred from the phytochrome B, rbcL, atpB, 26S nuclear ribosomal DNA and morphology. Molecular Phylogenetics and Evolution 19:353–366.

Song, B.H., X.Q. Wang, F.Z. Li, and D.Y. Hong. 2001. Further evidence for paraphyly in the Celtidaceae from the chloroplast gene matK. Plant Systematics and Evolution 228:107–115.

Spangler, R.E. and R.G. Olmstead. 1999. Phylogenetic analysis of Bignoniaceae based on the cpDNA gene sequences rbcL and ndhF. Annals of the Missouri Botanical Garden 86:33–46.

Swensen, S.M., B.C. Mullin, and M.W. Chase. 1994. Phylogenetic affinities of Datiscaceae based on an analysis of nuclear sequences from the plastid rbcL gene. Systematic Botany 19:157–168.

Wagstaff, S.J., and M.I. Dawson. 2000. Classification, origin and patterns of diversification of *Corynocarpus* (Corynocarpaceae) inferred from DNA sequence. Systematic Botany 25:134–149.

Wen, J., G.M. Plunkett, A.D. Mitchell, and S.J. Wagstaff. 2001. The evolution of Araliaceae: A phylogenetic analysis based on ITS sequence of nuclear ribosomal DNA. Systematic Botany 26:144–167.

Westoby, M., M.R. Leishman, and J.M. Lord. 1995. On misinterpreting the "phylogenetic correction." Journal of Ecology 83:531–534.

Zar, J.H. 1974. Biostatistical Analysis, 2nd Edition. Prentice Hall, Englewood Cliffs, NJ. 718 pp.

2004 SOUTHEASTERN NATURALIST 3(2):289–296

Notes on the Life History and Demographics of the Savannah Lilliput (*Toxolasma pullus*) (Bivalvia: Unionidae) in University Lake, NC

Shane D. Hanlon[1,*] and Jay F. Levine[2]

Abstract - The savannah lilliput (*Toxolasma pullus*, Bivalvia: Unionidae) is the only member of its genus represented along the mid-Atlantic slope. The rarity, limited range, and declining status of this species have caused concern among resource managers for its conservation. Little is known about the life history of *T. pullus*; such information is necessary for recovery of the species. We conducted a fish host trial and examined population demographics of *T. pullus* from University Lake, NC. *Toxolasma pullus* appears to be a long-term brooder, brooding into August. Hybrid bluegill (*Lepomis macrochirus* x *L. cyanellus*) are suitable hosts for *T. pullus*, however, other *Lepomis* species may also serve as hosts. The sex ratio of the population was 1:1. Most specimens of *T. pullus* were between 4 and 6 years old; the oldest specimen was 9 years of age. Predation by muskrats and raccoons may be an important source of mortality in University Lake.

Introduction

The savannah lilliput (*Toxolasma pullus*, Conrad 1838) is one of the smallest Atlantic drainage unionids, and is the only representative of its genus on the mid-Atlantic slope. This species is found primarily in mud and sand substrates in slow moving shallow water near the banks of streams and ponds (Johnson 1970). *Toxolasma pullus* occurs from the Altamaha River system in Georgia north to the Neuse River system in North Carolina (Johnson 1970). In North Carolina, the savannah lilliput was reported as rare in the Catawba River drainage of the Cooper-Wateree-Santee River system, the New Hope River drainage of the Cape Fear River system, and the Neuse River system (Johnson 1967). Presently, *T. pullus* is considered extirpated from the Neuse and Waccamaw River basins (Adams et al. 1990). University Lake, Orange County, harbors the only known stable population in North Carolina.

Because of its rarity, the savannah lilliput was listed as state threatened in North Carolina in 1990 and elevated to state endangered status

[1]U.S. Fish and Wildlife Services, Southwestern Virginia Ecological Services Field Office, 330 Cummings Street, Abingdon, VA 24210. [2]Department of Farm Animal Health and Resource Management, College of Veterinary Medicine, University of North Carolina, 4700 Hillsborough Street, Raleigh, NC 27606. *Corresponding author - shane_hanlon@fws.gov.

Hanlon Levine 2004

in July 2002. Resource managers have proposed reintroducing artificially-propagated juvenile *T. pullus* into various locations within its historic range. However, little is known about the life history of *T. pullus*; such information is necessary for propagation and successful management and recovery of this species. Our objectives were to determine the period of gravidity, identify fish hosts, and examine the demographics of *T. pullus* in University Lake, NC.

Methods and Materials

Study site

University Lake (86 ha, constructed 1932) is located in Orange County, NC, just southwest of Carrboro (35°53'47"N, 79°05'33"W) and receives water from a 78 km^2 watershed consisting of 5 major tributaries. Water level is regulated by the Orange County Water and Sewer Authority, and, with the exception of a small recreational area, the shoreline is completely forested and protected from foot and vehicle traffic. Maximum depth of the reservoir is 8 m, and the substrate consists of a mixture of clay, silt, sand, mud, gravel, and cobble. The reservoir supports a fish community of over 20 species and a mussel community consisting of 4 species: *T. pullus*, *Utterbackia imbecillis* (Say 1829), *Elliptio complanata* (Lightfoot 1786), and *Pyganodon cataracta* (Say 1817).

Gravid period

We assessed the period of gravidity of *T. pullus* on 8 sample dates between late-April and mid-September, 2001 (Table 1). We collected mussels by snorkeling, water scope, and grubbing (tactile search) along the shoreline. We measured length of all mussels using Vernier calipers, and examined females for gravidity. We determined sex of each specimen based on shell morphology; females are subquadrate in shape and show evidence of marsupial swelling in the posterio-ventral portion of the shell (Fig. 1). We determined gravidity by gently prying the valves

Table 1. Sex and gravidity of the savannah lilliput (*Toxolasma pullus*) from University Lake, Orange County, NC, in 2001.

Date	Live mussels observed		% females gravid
	Males	Females	
April 27	2	2	50
April 30	1	3	67
June 4	2	6	100
June 21	1	3	67
July 19	5	12	100
July 26	39	29	90
August 9	7	26	92
September 13	22	6	0

open slightly to examine the gills. After examination, we released all mussels back to the lake. Five gravid mussels were collected on April 30 (2) and August 9, 2001 (3) to extract glochidial and determine their developmental state. Developmental state was determined by the presence of embryos and glochidia, as well as glochidial activity.

Fish hosts

We conducted a fish-host identification trial following Zale and Neves (1982). We collected 2 gravid mussels from University Lake on April 30, 2001. We transported the gravid mussels to the laboratory and immediately flushed the gills using a 12 gauge hypodermic needle and water-filled 25 mL syringe. We tested for viability of glochidia by exposing them to a weak saline solution; viable glochidia snapped shut rapidly when exposed to the solution. After harvest of glochidia, we returned mussels to the lake. Since *T. pullus* brood a relatively low number of glochidia, we combined glochidia from the female mussels in order to conduct the trial.

Our ability to obtain multiple fish species was limited; therefore, only two species were used for our fish host trial. We purchased largemouth bass (*Micropterus salmoides*) (7–25 cm in length) and hybrid bluegill (*Lepomis macrochirus* x *L. cyanellus*) (10–20 cm in length) from commercial suppliers and held them in a closed recirculating system at the College of Veterinary Medicine, North Carolina State University. We fed fish daily with pellet food.

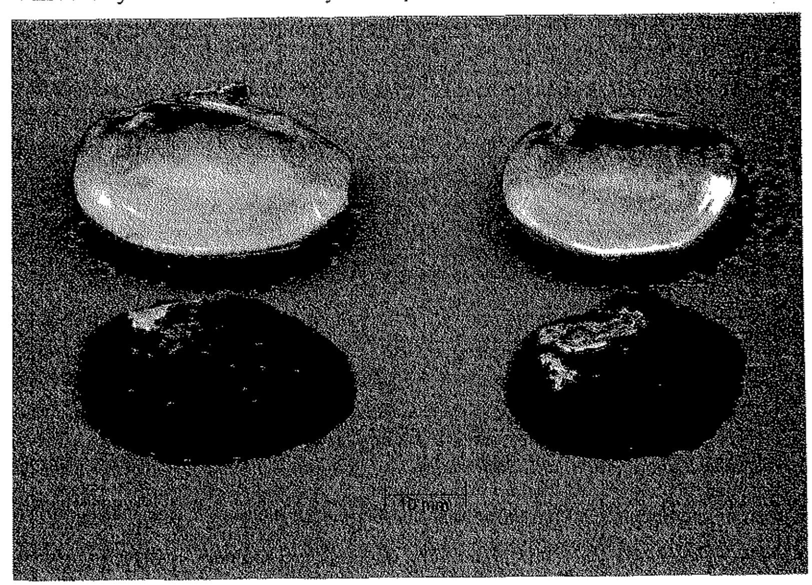

Figure 1. Savannah lilliput (*Toxolasma pullus*) collected from University Lake, Orange County, NC. Specimens on left and right are male and female, respectively.

We pipetted glochidia directly onto the gills of each fish, then rinsed fishes in water and examined gills for successful attachment of glochidia. After exposure, we placed fishes in 75 L glass aquaria with a recirculating water supply at 23 °C. Individual aquaria contained a single fish species.

We siphoned the bottom of each aquarium every 2 to 4 d with a flexible hose and concentrated material on a 120 µm mesh nylon sieve. We examined collected material with a stereo microscope and recorded the number of live juveniles. We continued siphoning aquaria until 5 days after the last juveniles were found. We considered a fish species a host for *T. pullus* if glochidia successfully developed to the juvenile stage.

Population demographics

We collected a total of 181 pairs of shells from muskrat middens and the shoreline of University Lake. We measured valve length, height, and width, and determined the sex of each individual based on shell morphology. We randomly selected 20 specimens of each sex for thin sectioning.

External growth rings in *T. pullus* were indistinguishable because of the dark satin-like periostracum of this species. Therefore, we used thin-sectioning procedures (Clark 1980, Neves and Moyer 1988), to estimate ages. We determined age by using a stereomicroscope to count growth rings that originated in the umbo and extended to the periostracum. We considered rings that did not originate in the umbo or extend to the periostracum to be false annuli (Neves and Moyer 1988, Rogers et al. 2001). The first year growth ring was eroded in most specimens. Therefore, we added 1 y to the age of each specimen based on the location of the age-1 growth ring visible on our youngest specimens. Currently, no data exist to validate age rings we judged to be annuli in this species. However, we assumed that the regular patterns of observed growth rings corresponded to annual patterns of growth in this relatively stable, lentic environment.

We examined shell length frequency distributions and used a one-way analysis of variance (ANOVA) to compare shell lengths and ages of females and males of live and relic specimens. We performed all statistical analyses with the Minitab statistical software package (Minitab Inc., State College, PA). We deposited shell material at the North Carolina State Museum of Natural Sciences, Raleigh, NC.

Results

Period of gravidity

A total of 167 live *T. pullus* were found during the study. Gravid females were found from April 27 to August 9, 2001 (Table 1). Only the posterior portion of the outer demibranchs served as marsupia for this

species. Fully charged marsupial gills were milky-white and inflated, and resembled the knuckles of a closed fist. No female mussels were gravid on September 13, 2001.

Most gravid mussels we examined in late July and early August had only partially inflated marsupia that were substantially less distended than marsupia of individuals examined in April–June. Broods of gravid mussels collected on April 30 were fully developed with very active glochidia (quick and frequent snapping of the valves). Conversely, gravid mussels we collected in August had immature broods, mostly consisting of embryos and a few glochidia that responded slowly to saline solution.

Fish hosts

Glochidia extracted from 2 gravid mussels on April 30 were fully developed and responsive to a saline solution. Mean (mm ± SE) shell length, height, and hinge length of glochidia (n = 5) was 206 ± 2, 226 ± 2, and 108 ± 2, respectively. Glochidia transformed successfully on hybrid bluegill, but did not transform on largemouth bass. Seventy-seven fully metamorphosed juveniles were recovered from aquaria containing hybrid bluegill between 15 and 22 days post-infection. No additional juveniles were recovered from hybrid bluegill after 22 days.

Population demographics

The sex ratio of *T. pullus* did not differ from 1:1 for either collected shell remains (94M, 87F; $\chi^2 = 0.290$, $p < 0.590$) or live animals (50M, 60F, $\chi^2 = 0.909$, $p < 0.340$). Males were significantly larger than females in both samples (shell remains: mean length of males (mm ± SE) = 31.4 ± 0.3, females = 25.9 ± 0.3, $F = 171.8$, $p < 0.0001$; Live animals: males = 34.2 ± 0.5, females = 28.3 ± 0.4, $F = 85.7$, $p < 0.0001$). Sex of the smallest specimen (7.0 mm) could not be determined from shell morphology and

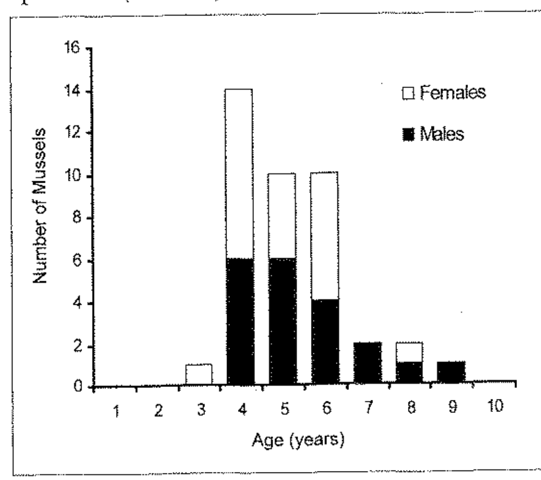

Figure 2. Age distribution of males and females of the savannah lilliput (*Toxolasma pullus*) determined from shell thin-sections.

therefore may have represented a sexually immature individual. This individual was attached to a live adult male by a byssal thread.

Ages of *T. pullus* ranged from 3 to 9 years old (Fig. 2). Average age of *T. pullus* was 5.2 years for males and 4.7 years for females, and did not differ between sexes (ANOVA, $p < 0.237$). For both sexes combined, 85% of individuals were 4–6 years old. Mean shell lengths ($\pm$ SE) of thin-sectioned males and females were 31.17 ± 0.57 mm and 25.93 ± 0.69 mm, respectively.

Discussion

Period of gravidity

Our results suggest that *T. pullus* is a longterm brooder, like its congeners *T. cylindrellus*, *T. lividus*, and *T. parvus* (Baker 1928; Ortmann 1919, 1921). Like many lampsilines, glochidial release of *T. pullus* appears to occur over a protracted period of time. Given the reduced distention of the marsupia we observed in specimens collected in July and August, we speculate most females had released the majority of their brood by mid-summer. However, the undeveloped embryos we extracted from 3 specimens in August suggests that either not all females in the population were successful in complete fertilization of their brood, or egg deposition for the next brooding cycle occurred earlier for these individuals. The later scenario suggests low brooding synchrony within the population.

Fish hosts

Hybrid bluegill appear to be suitable hosts for *T. pullus*. It is likely that other *Lepomis* species are also suitable hosts. Several species of *Lepomis* have been confirmed as hosts for other species of *Toxolasma*, including: *T. lividus* on longear sunfish (*L. megalotis*) and green sunfish (*L. cyanellus*) (Jenkinson 1982); *T. parvus* on green sunfish, orangespotted sunfish (*L. humilis*) and warmouth (L. *gulosus*) (Fuller 1974); and *T. texasensis* on bluegill (*L. macrochirus*) and warmouth (Stern and Felder 1978).

Population demographics

Based on our observations, the sex ratio of the *T. pullus* population in University Lake was 1:1. However, without a thorough subsurface survey of the population, this ratio cannot be confirmed. In a population of *Epioblasma torulosa rangiana*, adult females represented 60% and 20% of the population, based on surface and subsurface surveys, respectively, and females were more susceptible to predation due to conspicuous reproductive behavior (Smith et al. 2001).

Female *T. pullus* may be smaller than males due to the higher energetic cost of producing eggs compared to sperm. Similar differences in male and female size have been reported for the tan riffleshell, *Epioblasma florentina walkeri* (Rogers et al. 2001).

Our oldest specimen was estimated to be 9 years old. Compared to other freshwater mussels that exceed 100 years (Hendelberg 1960, Ziuganov et al. 2000), *T. pullus* is a relatively short-lived species. This is important when considering the sensitivity of the University Lake population of *T. pullus* to predation.

Neves and Odom (1989) suggest that predation poses a serious threat to endangered mussel populations that have been reduced to isolated locations. Twenty eight percent of their studied populations of the endangered shiny pigtoe (*Fusconaia cor*) were consumed by muskrats over a 7-year period. Predation of mussels in University Lake may be substantial. Based on our collected shell remains, it is clear that muskrats selected mussels of reproducing cohorts. Larger mussels are probably easier to detect and are an optimal food source in terms of foraging efficiency for muskrats (Neves and Odom 1989). Most of the fresh shells we collected were found during low-water periods and very few shells were found when water levels were high, indicating that the incidence of predation is greatest when water levels in the lake drop. Mussels are probably most vulnerable to predation during this time because they may be easier to detect and their route of escape to deeper water maybe hindered by physical obstructions on the receding shoreline. As a result, the fate of the *T. pullus* population existing in University Lake may depend strongly on the management of water levels in the lake and the size of the resident muskrat population. Because *T. pullus* is a short-lived species, successful annual recruitment will be critical in sustaining a healthy population.

Acknowledgments

We appreciate and thank Robert Glosson, Rachel Monschein, and the Orange County Water and Sewer Authority for providing information and access to University Lake. Special thanks to John Holland and Morgan Raley for their field and technical assistance. This project was funded in part by the U.S Fish and Wildlife Service and the State of North Carolina.

Literature Cited

Adams, W.F., J.M. Alderman, R.G. Biggins, A.G. Gerberich, E.P. Keferl, H.J. Porter, and A.S. Van Devender. 1990. A report on the conservation status of North Carolina's freshwater and terrestrial molluscan fauna. N.C. Wildlife Resources Commission, Raleigh, NC. 246 pp.

Baker, F.C. 1928. The fresh water Mollusca of Wisconsin. Part II. Pelecypoda. Bulletin of the Wisconsin Geological and Natural History Survey 70:1–495.

Clark, G.R. 1980. Study of molluscan shell structure and growth lines using thin sections. Pp. 603–606, *In* D.C. Rhoads and R.A. Luts (Eds.). Skeletal Growth in Aquatic Organisms. Plenum Press, New York, NY.

Fuller, S.L.H. 1974. Clams and mussels (Mollusca: Bivalvia). Pp. 215–273, *In* C.W. Hart and S.L.H. Fuller (Eds.). Pollution Ecology of Freshwater Invertebrates. Academic Press, New York, NY.

Hendelberg, J. 1960. The fresh-water pearl mussel, *Margaritifera margaritifera* in the British Isles. Journal of Conchology 17:195–204.

Jenkinson, J.J. 1982. Cumberlandian Mollusk Conservation Program. Pp. 95–103, *In* A.C. Miller (Ed.). Report of Freshwater Mollusks Workshop. U.S. Army Engineer Waterways Experimental Station, Vicksburg, MS. 185 pp.

Johnson, R.I. 1967. *Carunculina pulla* (Conrad), an overlooked Atlantic drainage unionid. The Nautilus 80(4):127–131.

Johnson, R.I. 1970. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic Slope Region. Bulletin of the Museum of Comparative Zoology 140(6):263–449.

Neves, R.J., and S.N. Moyer. 1988. Evaluation of techniques for age determinism of freshwater mussels (Unionidae). American Malacological Bulletin 6:179–188.

Neves, R.J., and M.C. Odom. 1989. Muskrat predation on endangered freshwater mussels in Virginia. Journal of Wildlife Management 53:934–941.

Ortmann, A.E. 1919. A monograph of the naiades of Pennsylvania. Part III: Systematic account of the genera and species. Memoirs of the Carnegie Museum 8, 384 pp.

Ortmann, A.E. 1921. The anatomy of certain mussels from the Upper Tennessee. The Nautilus 34:81–91.

Rogers, S.O., B.T. Watson, and R.J. Neves. 2001. Life history and population biology of the endangered tan riffleshell (*Epioblasma florentina walkeri*) (Bivalvia: Unionidae). Journal of the North American Benthological Society 20:582–594.

Smith, D.R., R.F. Villella, and D.P. Lemarie. 2001. Survey protocol for assessment of endangered freshwater mussels in the Allegheny River. Journal of the North American Benthological Society 20(1):118–132.

Stern, E.M., and D.L. Felder. 1978. Identification of host fishes for four species of freshwater mussels (Bivalvia: Unionidae). American Midland Naturalist 100:233–236.

Ziuganov, V., E. San Miguel, R.J. Neves, A. Longa, C. Fernández, R. Amaro, V. Beletsky, E. Popkovitch, S. Kaliuzhin, and T. Johnson. 2000. Life span variation of the freshwater pearl shell: A model species for testing longevity mechanisms in animals. Ambio 29:102–105.

Zale, A.V., and R.J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Canadian Journal of Zoology 60:2535–2542.