A quantitative assessment of the reproductive biology of *Cyclonaias tuberculata* (Bivalvia: Unionidae)

Thomas M. Haggerty, Jeffrey T. Garner, George H. Patterson, and Lannis C. Jones, Jr.

Abstract: Two hundred and thirty-three purple wartyback unionids (*Cyclonaias tuberculata*) were collected approximately monthly over a 31-month period from Kentucky Reservoir (Tennessee river mile 201.3), Tennessee, between August 1988 and February 1991. An equal sex ratio and only one case of hermaphroditism were discovered. Histological examinations showed that spermatogenesis and oogenesis occurred throughout the year except during late summer and early fall. "Typical" spermatogenesis was most evident between May and July. Spawning occurred between early spring (March–April) and late summer (August). Brooding variation among females was shown by the presence of embryos in the suprabranchial chambers and gills between early April and late August. Brooding was short term, as indicated by mature larvae being found in the outer demibranchs between early July and late August. Full demibranchs were never found, possibly indicating that the study took place during years of poor reproductive performance.

Résumé : Deux cent trente-trois Mulettes verruqueuses (*Cyclonaias tuberculata*) ont été récoltées dans des échantillons à peu près mensuels recueillis au cours d'une période de 31 mois dans le réservoir Kentucky (Tennessee River mile 201.3) au Tennessee, d'août 1988 à février 1991. Un rapport mâles:femelles égal à 1 et un seul cas d'hermaphrodisme ont été enregistrés. L'examen histologique des mulettes a révélé que la spermatogenèse et l'ovogenèse se produisent pendant toute l'année, sauf à la fin de l'été et au début de l'automne. C'est en mai et en juillet que la spermatogenèse est le plus « typique ». La ponte a lieu du début du printemps (mars–avril) à la fin de l'été (août). Chez les femelles, la variation du statut reproducteur se manifeste par la présence d'embryons dans la chambre supra-branchiale et dans les branchies entre le début d'avril et la fin d'août. L'incubation est de courte durée, puisque des larves à maturité sont déjà présentes dans les hémibranchies externes entre le début de juillet et la fin d'août. Nous n'avons jamais observé d'hémibranchies remplies à capacité, ce qui semble indiquer que la performance reproductrice a été faible au cours des années qu'ont duré l'étude. [Traduit par la Rédaction]

Introduction

The general reproductive cycle for unionids was well documented during the twentieth century (Lefevre and Curtis 1910; Coker et al. 1921). This work provided the foundation for more detailed research on the reproductive biology of individual species (e.g., Matteson 1948; van der Schalie and van der Schalie 1963; Yokley 1972*a*; Smith 1976, 1978; Zale and Neves 1982; Yeager and Neves 1986; Weaver et al. 1991). Investigations of gametogenic cycles of unionids have detailed temporal changes in the makeup of gonadal tissue. In males, temporal variation in the numbers of the different types of germ cells (i.e., spermatogonia, spermatocytes, spermatids, spermatozoa, and atypical cells) has been noted. In females, temporal changes in the production and growth of oocytes have been observed. Although these types of changes in gonadal tissue composition are inherently quantifiable, no quantitative assessments have been done on unionids. Qualitative evaluations of gametogenesis typically categorize the "phase" or "state" of the gonadal tissue of a specimen at the time it was sacrificed (Dinamani 1974). Although qualitative methods appear adequate for summarizing the annual gametogenic cycle, quantitative methods should give a more detailed picture of the activities within the gonadal tissue.

Quantitative methods can also show intraspecific variation in a population within and among years and allow for more accurate comparisons to be made among populations. Recent research (Heard 1975; Lewis 1985) indicates that intraspecific variation in the reproductive strategies of freshwater bivalves

Received May 19, 1994. Accepted October 6, 1994.

T.M. Haggerty,[1] J.T. Garner,[2] G.H. Patterson,[3] and L.C. Jones, Jr.[4] Department of Biology, University of North Alabama, Florence, AL 35632-0001, U.S.A.

[1] Author to whom all correspondence should be addressed.
[2] Present address: Alabama Game and Fish Division, P.O. Box 366, Decatur, AL 35602, U.S.A.
[3] Present address: Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37235, U.S.A.
[4] Present address: Apt. B1, 2251 Helton Drive, Florence, AL 35630, U.S.A.

may exist. Reproductive biology studies throughout a species' range are needed to better understand the causes of this variation.

The primary objectives of this research were to quantify the periods of gametogenesis, gamete release, and larval development and release over a 31-month period in a population of purple wartyback unionids (*Cyclonaias tuberculata*). The purple wartyback is found throughout the Mississippi drainage, Lake St. Clair and Lake Erie drainage, and Ohio drainage (Ortmann 1919; Burch 1973). Although it is fairly common throughout its range, only one study dealing with its reproductive biology has been reported (Jirka and Neves 1992). This study was qualitative, followed only one annual cycle, and was conducted on a population from a different physiographic region than our study.

Study area

Specimens were collected from the Tennessee River between Tennessee river miles (TRM) 200.0 and 201.3 (88°18′N, 35°06′W). This stretch of the river is located in the headwaters of Kentucky Reservoir, Hardin County, Tennessee, downstream of Pickwick Dam (TRM 206.7). The river is approximately 0.4 mi (1 mi = 1.609 km) wide at the study area. The water level in Kentucky Reservoir ranges from 107.7 to 109.4 m above mean sea level at low and high pools, respectively (Tennessee Valley Authority 1974). The flow in this stretch of river can be swift to barely perceptible depending on the amount of water being released from Pickwick Dam. The river bottom is primarily gravel with extensive areas of sand and a fine layer of silt coating the bottom surfaces in areas of slow flow.

Materials and methods

The first 6–21 individuals were collected by hand, using SCUBA, on approximately a monthly basis throughout the year. Only sexually mature individuals were used in our study. The sample period began 3 August 1988 and ended 3 February 1991. Weather and water conditions inhibited collecting for some months. Specimens were returned to the laboratory in insulated coolers containing river water. No evidence of aborted conglutinates was noted. After fixation in 10% formalin, transverse sections from the central portion of the visceral mass were dehydrated, cleared, infiltrated with paraffin, embedded in paraffin, sectioned (6 μm), mounted on glass slides, and stained with hematoxylin and eosin using methods described in Humason (1979).

Spermatogenesis was quantified using methods similar to those used by Jones et al. (1986). Five cell types found in the male gonadal tissue were quantified along a transect through the approximate center of each of 10 acini per specimen under 1000× magnification with a light microscope. The transect was defined by the path of an eyepiece pointer across the acinus. The slide was moved in one direction and each cell touching the pointer tip was identified and tallied. The five cell types were distinguished using a combination of size, shape, intensity of staining, and position of the acinus. The cell types were defined as follows: spermatogonia: round to oval, found along or near the acinus wall, 10–16 μm in diameter; spermatocytes, round or oval, 6–8 μm in diameter, often found in groups of similarly stained cells (various stain intensities were encountered, ranging from deep blue to mottled); spermatids: round, grading to oval and "bullet-shaped" as spermiogenesis occurred, 3–5 μm in diameter or length (depending on the stage of spermiogenesis), typically darkly stained; spermatozoa: bullet-shaped, oblong, with one end rounded and the other flat, a single flagellum protruding from the flat end (typically could not be seen at 1000× unless many were clumped together), typically lightly stained; and multinucleated inclusions (= sperm morulae): consist of 1–8 masses of decondensed DNA surrounded by a thin layer of cytoplasm and differentiated into spermatozoa that are indistinguishable from those produced by typical spermatogenesis (Kotrla 1989). The transformation of multinucleated inclusions into spermatozoa is called atypical spermatogenesis. No distinction was made between primary and secondary spermatocytes. For detailed descriptions of the cell types found during spermatogenesis see Dinamani (1974) and Peredo and Parada (1984).

Oogenesis was quantified by approximating the average oocyte diameter as well as the average number of oocytes per follicle. Oocytes were measured along a transect across the entire section of tissue. Only those oocytes in which the plane of the section passed through the nucleus were measured. Two measurements (to the nearest micrometre) were made on each oocyte, one along the longest axis and another along a second axis perpendicular to the first axis at its midpoint. The sum of the two measurements was then divided by 2. Thirty oocytes were measured in each specimen. The number of oocytes per follicle was determined by counting the number of egg sections in the first 30 follicles that were touched by the eyepiece pointer as the slide was moved in one direction on the microscope stage. During 1990 and 1991, freshly sacrificed specimens were examined with the unaided eye, as well as under low magnification, to determine the presence or absence of embryos of glochidia in the marsupia.

Temporal effects on spermatogenesis and oogenesis were analyzed by one-way ANOVAs using a general linear models procedure (SAS Institute Inc. 1982). Scheffé's test was used to compare means. Results were termed significant only if $P < 0.05$.

Results

Male reproductive cycle

Fifty-two percent of the 233 *C. tuberculata* that were collected and sectioned were males and 48% were females. This was not statistically different from a 1:1 sex ratio (χ^2 test, $P > 0.05$). One individual was hermaphroditic.

Spermatogenesis showed the same annual cycle throughout our study, and no between-years differences were found for any of the spermatogenic cell types (Scheffé's test, $P > 0.05$; Fig. 1). Significant differences among collection dates were found for all the cell types ($P < 0.0001$).

Spermatogenic activity was observed during most of the year. However, the least amount of activity was seen during late summer and early fall, during the transition from one annual cycle to the next (Fig. 1*a*). During this period the acini were dominated by multinucleated inclusions (>50% of the total cell count; Fig. 1*f*). In the fall, typical spermatogenesis began anew and by early winter there were significantly more germ cells than in late summer and early fall

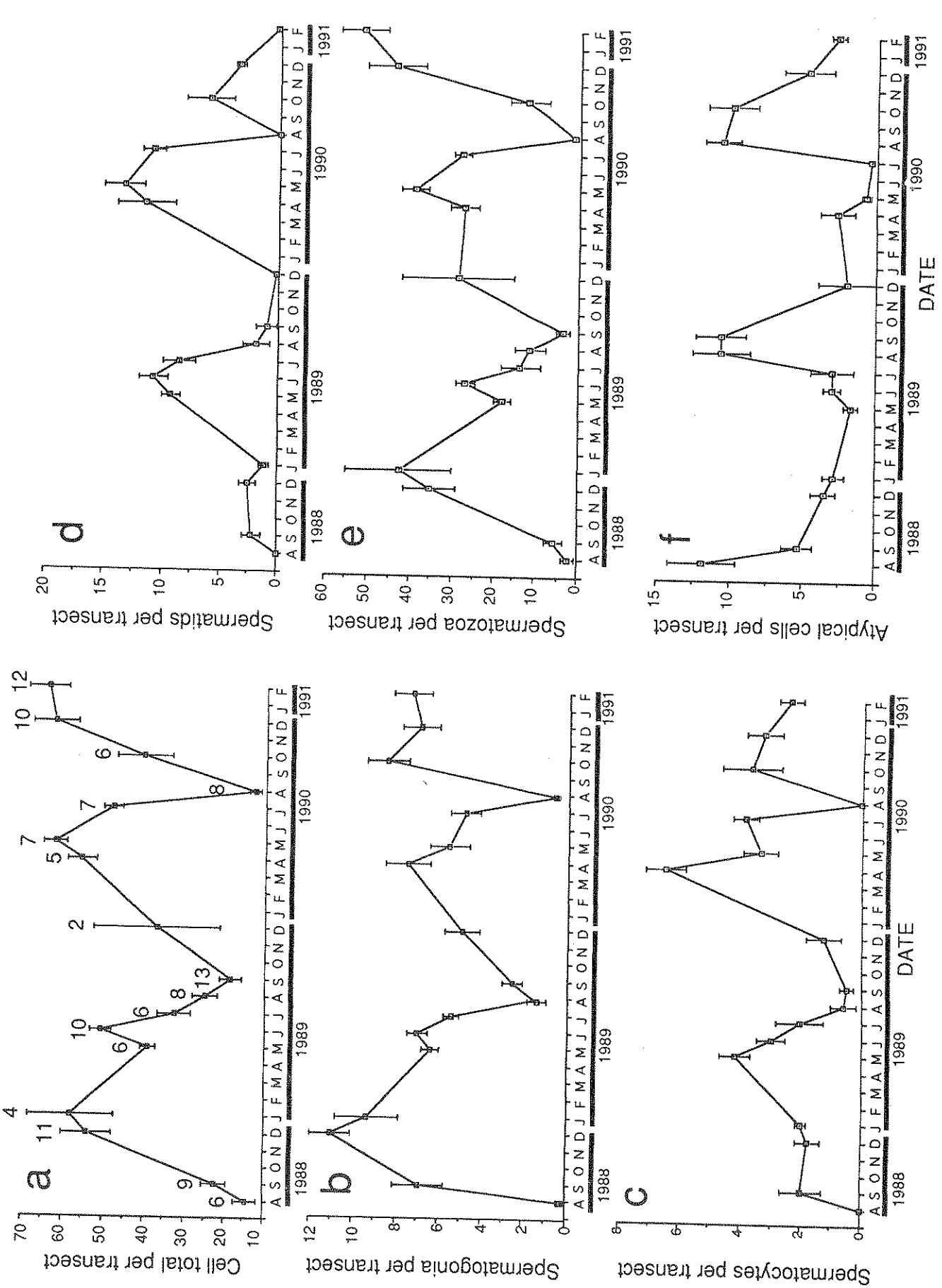
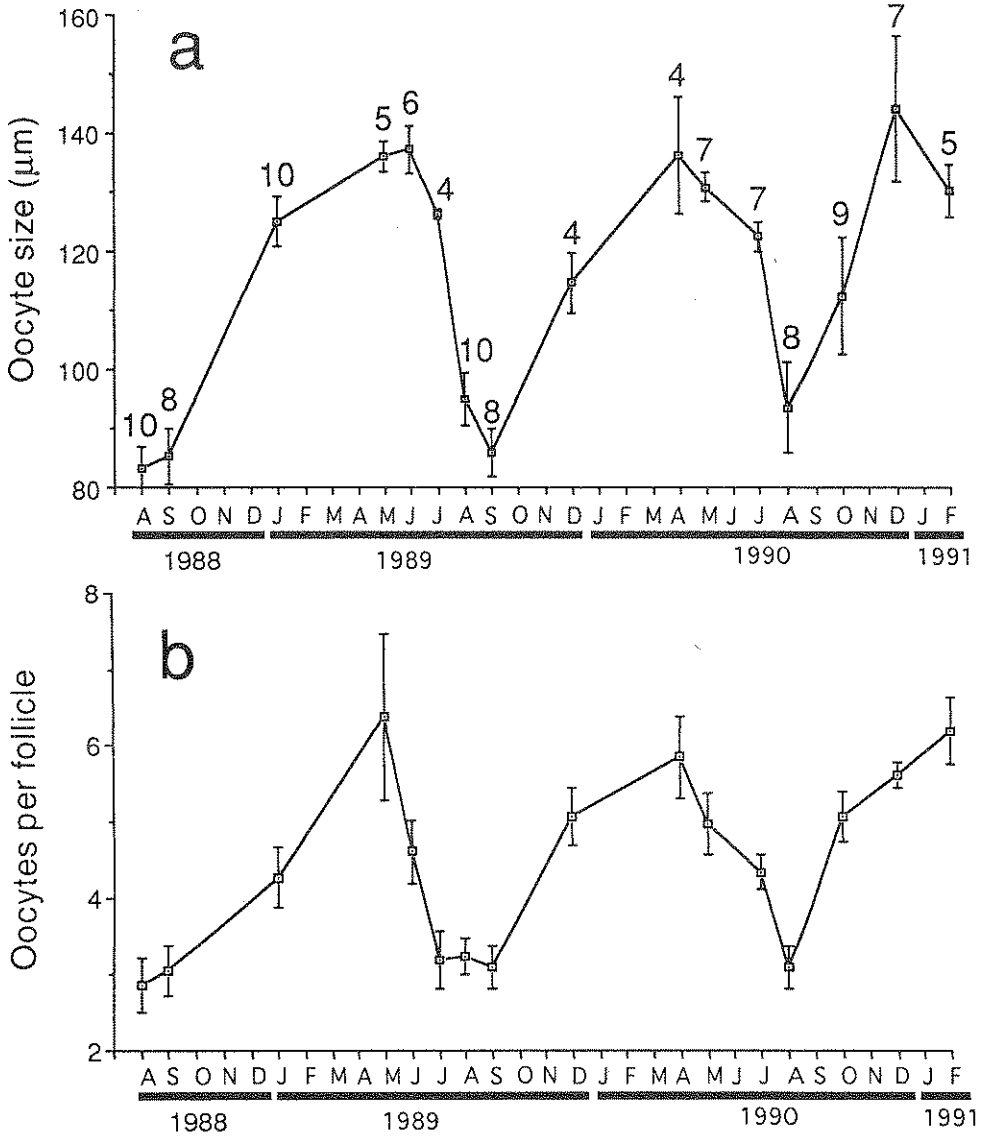


Fig. 1. Mean (±1 SE) numbers of total spermatogenic cells (*a*), spermatogonia (*b*), spermatocytes (*c*), spermatids (*d*), spermatozoa (*e*), and multinucleated inclusions (*f*) per acinus in each transect between August 1988 and February 1991. The numbers above the means show the number of individuals examined.

Fig. 2. Mean (±1 SE) oocyte size (*a*), and number of oocytes per follicle (*b*) between August 1988 and February 1991. The numbers above the means show the number of individuals examined.



(e.g., December 1990 and February 1991 than in August 1988, 1989, and 1990, and September 1988 and 1989; Scheffé's test, $P < 0.05$; Fig. 1*a*). Numbers of spermatogonia reached their peak in midwinter and declined somewhat in spring, but remained relatively constant through most of the summer; they were lowest in August. Numbers of spermatogonia were significantly greater from approximately October through February than in late summer and early fall (e.g., December 1988, January 1989, October 1990, and February 1991 compared with August 1988, 1989, and 1990; Scheffé's test, $P < 0.05$; Fig. 1*b*). Spermatocyte numbers peaked in April, and counts in April 1990 were significantly greater than in August, September, and December 1988, August and September 1989, and August 1990 (Scheffé's test, $P < 0.05$; Fig. 1*c*). Maximum spermatid counts occurred in spring and midsummer (e.g., June 1989, and April, May, and July 1990) and were significantly greater than counts in late summer, fall, and winter (e.g., August, September and December 1988, January, February, August, and September 1989, and August and December 1990; Scheffé's test, $P < 0.05$; Fig. 1*d*). Numbers of spermatocytes and spermatids decreased sharply during late July, reaching their lowest points at approximately the same time as those of spermatogonia. There was less difference between low and high counts in spermatocytes and spermatids than in spermatogonia. From the initial accumulation of spermatozoa in the fall, numbers remained relatively high through winter, spring, and early to mid summer. Numbers were significantly greater in December 1988, February 1989, and May, July, and December 1990 than in September 1989 and August 1990 (Scheffé's test, $P < 0.05$; Fig. 1*e*). Release of spermatozoa began in late winter or very early spring and continued through midsummer (July).

Female reproductive cycle

Ovum production began during late summer and early fall as oocytes emerged from the follicle wall. During the initial stages of their development they were attached to the follicle

wall by cytoplasmic stalks. Developing oocytes were at their smallest average diameter during that period. Oocytes of individuals collected in August and September 1988 and 1989 and August 1990 were significantly smaller than those of individuals collected in January and May 1989 April, May, and December 1990 (Scheffé's test, $P < 0.05$; Fig. 2*a*). Oocyte size peaked between midwinter and spring.

Oocyte abundance in the follicles was lowest during late summer and early fall. Egg number was positively and significantly correlated with egg size ($r = 0.61$, $P < 0.0001$). The numbers of oocytes per follicle differed significantly among collection dates ($F = 8.0$, $P < 0.0001$). For example, individuals collected in May 1989 and February 1991 had significantly more eggs than individuals collected in August and September 1988, September 1989, and August 1990 (Scheffé's test, $P < 0.05$; Fig. 2*b*).

Of the 38 females examined before fixation in 1990, 18 (47%) showed evidence of fertilization (i.e., the presence of embryos or glochidia) between 4 April and 25 August. Embryos were found in the suprabranchial chambers of 75% of the females examined on 4 April ($n = 4$) and 11% of those examined on 3 July ($n = 9$). Embryos were found in the outer gills of 44% of females examined on 29 May ($n = 9$), 57% on 20 July ($n = 7$), and 14% on 25 August ($n = 7$). Mature glochidia were found in females collected on 1 July (11%; $n = 9$), 20 July (29%, $n = 7$), and 25 August (29%; $n = 7$).

Discussion

Like van der Schalie (1970) and Jirka and Neves (1992), we found *C. tuberculata* to be dioecious. However, sporadic hermaphroditism is well documented among dioecious species (van der Schalie 1970; Heard 1975; Kat 1983) and we found one hermaphroditic individual (0.3%; $n = 354$) in our study. Although the cause of hermaphroditism is unclear, an unbalanced sex ratio, a low population density (Bauer 1987), and parasitism (Kat 1983) have been suggested as possible causes. However, our study population had an equal sex ratio, a relatively high density (Yokley 1972*b*), and showed no evidence of internal parasites. Jirka and Neves (1992) reported a male-dominated sex ratio (1.6:1; $n = 90$) from a stretch of the New River in West Virginia and Virginia.

Typical and atypical spermatogenesis showed the same annual pattern during our study. Typical spermatogenesis occurred throughout most of the year. After a short period of reduced activity in late summer, germ cells within the acini began to increase. Accumulation of spermatozoa in the acini resulted from this activity. This fall accumulation appears to be the result of a combination of typical and atypical spermatogenesis. Multinucleated inclusions were prevalent in the spermatogenic acini during the late summer and fall, and some differentiated into spermatozoa (Heard 1975; Kotrla 1989; personal observation). However, because these spermatozoa formed by ameiotic means (Kotrla 1989), their function is unclear.

Jirka and Neves (1992) also encountered fall gametogenesis in *C. tuberculata* as well as *Elliptio dilatata* and *Tritogonia verrucosa* in the New River, Virginia and West Virginia. They stated that this occurrence may be due to effects of the Bluestone Dam on the water temperature. It is unlikely, however, that our study population was exposed to any temperature-regulating mechanisms. Others have reported a similar pattern of spermatogenesis (Yeager and Neves 1986; Garner 1993).

During typical spermatogenesis, the peaks of production of spermatogonia, spermatocytes, and spermatids were staggered, with spermatogonia peaking in midwinter, spermatocytes in spring, and spermatids in early summer. These events led to the peak sperm-producing period of late spring through midsummer. However, no further accumulation of spermatozoa occurred during that period, as was evidenced by a decrease in total cell number (total cell number peaked in midwinter following the autumn production of sperm). These factors indicate the extended nature of male spawning, with concurrent spermatogenesis in *C. tuberculata*.

Spawning synchrony was evident between the sexes. Oocyte size and number per follicle were highest in spring, when males were producing their highest numbers of sperm. Both sexes showed a period of reduced activity during late summer. The spring and summer spawning period seen in *C. tuberculata* in this study is expected of short-term brooders (Bruenderman and Neves 1993; Heard 1975; Matteson 1948; Weaver et al. 1991; Yeager and Neves 1986; Yokley 1972*a*).

Ortmann (1919), Utterback (1915), Lefevre and Curtis (1910), Gordon and Layzer (1989), and Jirka and Neves (1992) reported *C. tuberculata* as being a spring and summer brooder (i.e., short-term brooder or tachytictic) and our results concur. Although *C. tuberculata* is a short-term brooder, the actual period of spawning extended from early spring through late summer (5 months). Females with full marsupia were never encountered, but the reasons for this are unclear. Perhaps the population was sampled during years of low reproductive success. Miller and Payne (1988) found evidence of yearly variation in recruitment rates in unionids. This variation could be correlated with river discharge levels. Dilution of sperm during years of high discharge may negatively affect fertilization rates. Conversely, concentration of sperm during drought years, when water levels are low, may enhance fertilization success. During the sampling period of this investigation, precipitation levels were above normal (National Oceanic and Atmospheric Administration climatological data). Additional research is needed to examine the effects of water depth and flow rate on unionid reproduction.

In summary, we present a quantitative approach to the study of the gametogenic cycles in unionids. Although qualitative methods are more practical than quantitative ones for studying the reproductive cycles of unionids, they are more subjective and the results may be difficult to interpret, compare, and analyze statistically. A series of comparative quantitative studies on selected species, representing varying aspects of the Unionidae (e.g., systematic and distributional), may increase our knowledge of the reproductive biology of this group of organisms. The quantitative methods used in our study revealed that in *C. tuberculata* typical spermatogenesis occurred throughout most of the year but was most evident between May and July. Atypical spermatogenesis predominated in late summer and early fall, and this may contribute significantly to the accumulation of spermatozoa in the fall and winter. Oogenesis followed a pattern similar to typical spermatogenesis. Spawning of the sexes was synchronized and occurred between early spring (March–April) and late

summer (August). Females were short-term brooders, embryos or larvae being found in the outer gills between May and late August.

Acknowledgments

We thank P. Yokley, Jr. for his encouragement and support, T. Richardson and P. Kittle for improving the manuscript, and A. Gooch and Z. Bowen for their assistance in collecting specimens. The University of North Alabama provided equipment, library assistance, and computer time.

References

Bauer, G. 1987. Reproductive strategy of the freshwater pearl mussel *Margaritifera margaritifera*. J. Anim. Ecol. **56**: 691–704.

Bruenderman, S.A., and Neves, R.J. 1993. Life history of the endangered fine-rayed pigtoe *Fusconaia cuneolus* (Bivalvia: Unionidae) in the Clinch River, Virginia. Am. Malacol. Bull. **10**: 83–91.

Burch, J.B. 1973. Freshwater unionacean clams (Mollusca: Pelecypoda) of North America. *In* Biota of freshwater ecosystems: identification manual. Vol. 11. U.S. Environmental Protection Agency, Washington, D.C.

Coker, R.E., Shira, A.F., Clark, H.W., and Howard, A.D. 1921. Natural history and propagation of fresh-water mussels. Bull. U.S. Bur. Fish. **37**: 77–181.

Dinamani, P. 1974. Reproductive cycle and gonadal changes in the New Zealand rock oyster *Crassostrea glomerata*. N.Z. J. Mar. Freshwater Res. **8**: 39–65.

Garner, J.T. 1993. Reproductive cycle of *Quadrula metanevra* (Unionidae) in Kentucky Reservoir, Tennessee River, Hardin Co., Tennessee. M.S. thesis, University of Alabama, Huntsville.

Gordon, M.E., and Layzer, J.B. 1989. Mussels (Bivalvia: Unionidae) of the Cumberland River system: review of life histories and ecological relationships. U.S. Fish Wildl. Serv. Biol. Rep. No. **89**(15).

Heard, W.H. 1975. Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda: Unionidae). Malacologia, **15**: 81–103.

Humason, G.L. 1979. Animal tissue techniques. 4th ed. W.H. Freeman and Co., San Francisco.

Jirka, K.J., and Neves, R.J. 1992. Reproductive biology of four species of freshwater mussels (Mollusca: Unionidae) in the New River, Virginia and West Virginia. J. Freshwater Ecol. **7**: 35–44.

Jones, H.A., Simpson, R.D., and Humphrey, R.D. 1986. The reproductive cycles and glochidia of freshwater mussels (Bivalvia: Hyriidae) of the Macleay River, northern South Wales, Australia. Malacologia, **27**: 185–202.

Kat, P. 1983. Sexual selection and simultaneous hermaphroditism among the Unionidae (Bivalvia: Mollusca). J. Zool. (1965–1984), **201**: 395–416.

Kotrla, M.B. 1989. Evidence suggestive of ameiotic spermatogenesis in unionid bivalves. Am. Zool. **29**: 148A. [Abstr.]

Lefevre, G., and Curtis, W.C. 1910. Reproduction and parasitism in the Unionidae. J. Exp. Zool. **9**: 79–115.

Lewis, J.B. 1985. Breeding cycle of the freshwater mussel *Anodonta grandis* Say. Can. J. Zool. **63**: 2477–2478.

Matteson, M.R. 1948. Life history of *Elliptio complanatus* (Dillwyn, 1817). Am. Midl. Nat. **40**: 690–723.

Miller, A.C., and Payne, B.S. 1988. The need of quantitative sampling to characterize size demography and density of freshwater mussel communities. Am. Malacol. Bull. **6**: 49–54.

Ortmann, A.E. 1919. Monograph of the naiades of Pennsylvania. Part III: systematic account of the genera and species. Mem. Carnegie Mus. **8**: 1–384.

Peredo, S., and Parada, E. 1984. Gonadal organization and gametogenesis in the fresh-water mussel *Diplodon chilensis chilensis* (Mollusca: Bivalvia). Veliger, **27**: 126–133.

SAS Institute Inc. 1982. SAS user's guide: statistics. SAS Institute Inc., Cary, N.C.

Smith, D.G. 1976. Notes on the biology of *Margaritifera m. margaritifera* (Lin.) in central Massachusetts. Am. Midl. Nat. **96**: 252–256.

Smith, D.G. 1978. Biannual gametogenesis in *Margaritifera margaritifera* (L.) in northeastern North America. Bull. Am. Malacol. Union Inc. 1978: 49–53.

Tennessee Valley Authority. 1974. Quality of water in Kentucky Reservoir. Tennessee Valley Authority, Water Quality Branch, Chattanooga.

Utterback, W.I. 1915. The naiades of Missouri. Am. Midl. Nat. **4**: 97–152.

van der Schalie, H. 1970. Hermaphroditism among North American freshwater mussels. Malacologia, **10**: 93–112.

van der Schalie, H., and van der Schalie, A. 1963. The distribution, ecology, and life history of the mussel, *Actinonaias ellipsiformis* (Conrad), in Michigan. Occas. Pap. Mus. Zool. Univ. Mich. No. 633. pp. 1–17.

Weaver, L.R., Pardue, G.B., and Neves, R.J. 1991. Reproductive biology and fish hosts of the Tennessee clubshell *Pleurobema oviforme* (Mollusca: Unionidae) in Virginia. Am. Midl. Nat. **126**: 82–89.

Yeager, B.L., and Neves, R.J. 1986. Reproductive cycle and fish hosts of the rabbit's foot mussel, *Quadrula cylindrica strigillata* (Mollusca: Unionidae) in the upper Tennessee River drainage. Am. Midl. Nat. **116**: 329–340.

Yokley, P. 1972*a*. Life history of *Pleurobema cordatum* (Rafinesque 1820) (Bivalvia: Unionidae). Malacologia, **11**: 351–364.

Yokley, P. 1972*b*. Freshwater mussel ecology, Kentucky Lake, Tennessee: May 1, 1969 – June 15, 1972. Project 4-46-R, Tennessee Game and Fish Commission, Nashville.

Zale, A.V., and Neves, R.J. 1982. Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. Freshwater Invertebr. Biol. **1**: 17–28.