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Reproductive Cycle of Quadrula metanevra (Bivalvia: Unionidae) in the Pickwick Dam Tailwater of the Tennessee River

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ABSTRACT.—*Quadrula metanevra* were collected monthly from Pickwick Dam tailwater (Tennessee River mile 201.3), Tennessee, between July 1988 and June 1990. A total of 227 specimens were examined. The population consisted primarily of dioecious individuals (98%) and had an unequal sex ratio (1.5 female: 1 male). Histological examinations showed that typical spermatogenesis began in autumn (September), increased in spring (March–April), and continued until midsummer (July). Atypical spermatogenesis predominated during July and August, and may have increased the number of sperm produced in autumn and winter. Oogenesis followed a pattern similar to that of typical spermatogenesis, with smallest average oocyte size observed in August. Gamete release occurred between late winter-early spring (March) and midsummer (July). Brooding females were found between late March and July.

INTRODUCTION

Basic knowledge of the reproductive biology of a species is fundamental to understand its life history. Generalities of reproduction in the Unionidae (Bivalvia: Unionoidea) h been understood since the late 19th and early 20th centuries (Sterki, 1898; Lefevre a Curtis, 1910; Coker *et al.*, 1921). Tachytictic species are short-term brooders that ret glochidia (*i.e.*, larvae) for a relatively short period (*e.g.*, 3–6 wk, Gordon and Layzer, 19 *but see* Weaver *et al.*, 1991). Bradytictic species are long-term brooders that hold their g chidia for an extended period, typically from fall until the following spring and sumr (*but see* Sterki, 1898; Zale and Neves, 1982a, b).

Although most unionids have been classified as either tachytictic or bradytictic, otl aspects of their reproductive biology (*e.g.*, gametogenic cycle and period of fertilizatic are not well known (Zale and Neves, 1982a, b; Gordon and Layzer, 1989). Further, rec investigations indicate that not all species of unionids can be clearly classified as tachyti or bradytictic (Heard, 1975; Smith, 1978; Gordon and Layzer, 1989; Gordon and Sm 1990; Woody and Holland-Bartels, 1993) and that intraspecific differences may exist amc populations (van der Schalie, 1970; Lewis, 1985).

The objectives of this research were to quantify the periods of gametogenesis, gam release and brooding activity over a 24-mo period in a population of *Quadrula metane* (Unionidae). *Quadrula metanevra* is found from the northern portions of the Mississi drainage S to the Tennessee, Arkansas (Burch, 1973), and Alabama rivers (J.T.G., p obs.). Although it is relatively common throughout much of its range, no detailed study

its reproductive habits has been published. Additionally, quantitative assessments of unionid reproductive biology are few and in-depth analyses should help determine the ultimate causations of life history traits in the Unionidae. *Quadrula metanevra* is commercially harvested and information concerning its reproductive biology is also of value to resource managers.

STUDY AREA

Specimens were collected from the Tennessee River between river miles (TRM) 200.0 and 201.3 (88°18'N, 35°06'W) in Hardin County, Tennessee, just downstream of Pickwick Dam (TRM 206.7). At the study site the river was approximately 0.6 km wide. Specimens were collected at depths from 4.5–9.0 m and the current velocity was highly variable depending on discharge from Pickwick Dam. Substratum consisted primarily of gravel, with some sandy areas.

MATERIALS AND METHODS

Specimens were collected by hand using SCUBA, approximately monthly between 28 July 1988 and 17 June 1990 (24 mo). Weather and flood conditions prevented sample collection during some months (*see* Fig. 1). A sample consisted of the first 15 individuals that were encountered per sample date. All specimens were ≥50 mm in length. Samples were transported to the University of North Alabama laboratory alive in insulated containers of river water.

Soft tissues were cut from the shells and fixed in 10% formalin. Transverse sections of the visceral mass were paraffin-sectioned (6 μm), mounted on glass slides and stained with hematoxylin and eosin using methods described in Humason (1979).

Cell quantification methods were similar to those of Jones *et al.* (1986) and Haggerty *et al.* (1995). For spermatogenesis, cells were identified and counted if they touched the X axis of an eyepiece reticule that was moved across the approximate center of 10 acini (*i.e.*, epithelial sacs within the visceral mass where gametogenesis occurs) per male specimen using a light microscope and 1000× magnification. Five males were randomly selected and evaluated per sample date. Cells were identified as spermatogonia/spermatocytes, spermatids, spermatozoa and multinucleated inclusions based on size, shape, intensity of staining and position in the acinus. Multinucleated inclusions (*i.e.*, sperm morulae) consist of 1–8 masses of decondensed DNA surrounded by a thin layer of cytoplasm (Kotrla, 1989). They differentiate into spermatozoa (*i.e.*, atypical spermatogenesis) that are indistinguishable from those produced by typical spermatogenesis (*i.e.*, those differentiating from spermatids). Descriptions in Dinamani (1974) and Peredo and Parada (1984) helped with identification of male germ cells.

Oogenesis, formation and maturation of ova, was quantified by measuring the diameters of oocytes along a transect across the entire section of gonad. Fifty oocytes in each of five randomly selected specimens were measured. Only those oocytes in which the plane of the section passed through the nucleus were measured. A ruled eyepiece reticule was used for measurement. Transects were run along the Y axis of the reticule cross hairs. A transect was defined as the width equal to 10 units of measure on each side of the Y axis. Any oocyte falling totally or partially within the transect was measured along its longest axis.

To determine brooding period, gills were grossly examined for presence of glochidia or embryos. In 1989, we were not able to quantify the percentage of gravid females because they aborted conglutinates (*i.e.*, packets of glochidia) during transfer from river to laboratory. In 1990, three-prong clamps were used to hold valves of collected mussels together to prevent abortion of embryos and larvae. Only 1990 brooding results are presented.

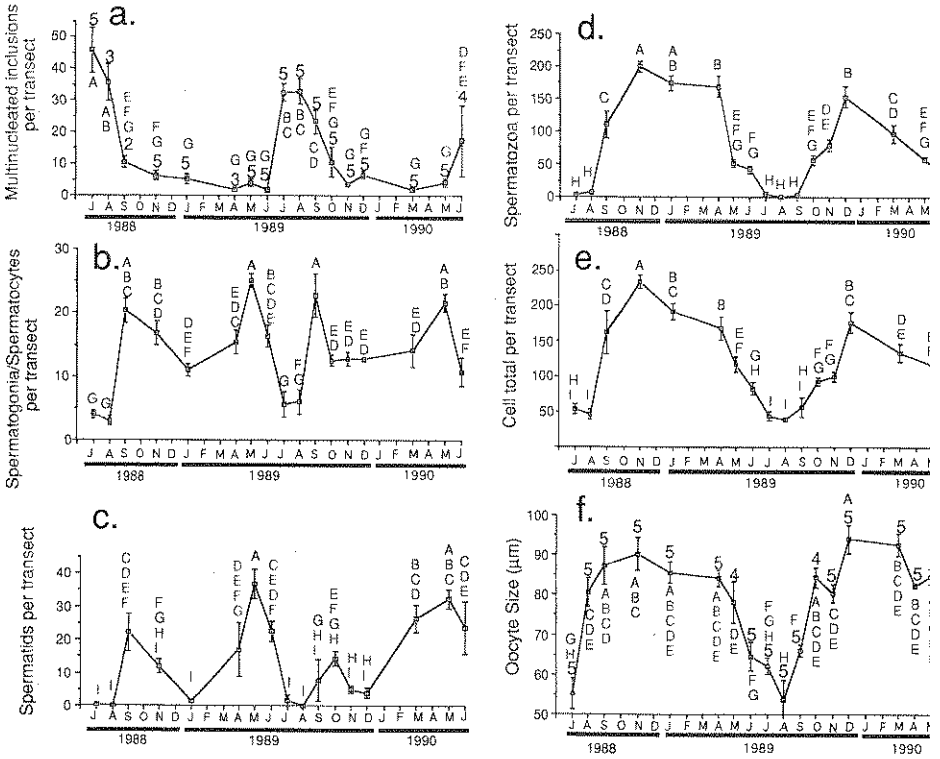

FIG. 1.—Mean (±1 SE) numbers of multinucleated inclusions (a) spermatogonia/spermatocytes; (spermatids; (c) spermatozoa; (d) and total spermatogenic cells; (e) per transect per acinus betwe July 1988 and June 1990. Mean (±1 SE) oocyte size during the same time period; (f) the numb above the means show the number of individuals examined. Sample dates that do not share any of t same letters were significantly different ($P < 0.05$). Months with missing values indicate those mon for which data were unavailable due to unfavorable diving conditions

Temporal effects on spermatogenesis and oogenesis were analyzed by one-way ANOV (SAS Institute Inc., 1982). Duncan's multiple-range test was used to compare means. Resu were termed significant only if $P < 0.05$.

RESULTS

Sex ratio and hermaphroditism.—Of 227 *Quadrula metanevra* examined during this stu 58% were females, 40% were males and four individuals (2%) were monoecious, giving sex ratio of 1.5:1 (female to male). This was significantly different from a 1:1 sex ratio (Goodness-of-fit test, $P < 0.05$). Gonads of the hermaphrodites consisted primarily of m tissue, with small numbers of female acini per section.

Spermatogenesis.—Spermatogenesis followed the same basic pattern over both years (F 1) and significant differences among collection dates were found for all cell types (P 0.0001). During late summer and early autumn, multinucleated inclusions dominated t acini (Fig. 1a). In September and October, onset of typical spermatogenesis was indicat by a significant increase in spermatogonia/spermatocytes (*e.g.*, August 1989 compared

September 1989, Fig. 1b). Spermatids also appeared at this time (Fig. 1c). These cell formed a thick band around the periphery of each acinus and spermatozoa began to ac cumulate in the lumina of acini (Fig. 1b–d). This was accompanied by a decrease in mul tinucleated inclusions (Fig. 1a). By November, the decrease in multinucleated inclusion was significant (*e.g.*, July 1988 compared to November 1988). Also in November, number of spermatozoa had increased significantly compared to July and August (*e.g.*, July and August 1989 compared to November 1989). Numbers of spermatogonia/spermatocytes and spermatids were significantly lower in winter, as compared to autumn (*e.g.*, September 198 compared to January 1989, Fig. 1b–c), due to their differentiation into spermatozoa, which significantly increased (Fig. 1d). A significant decrease in spermatozoa numbers was ob served between winter and spring (*e.g.*, January 1989 compared to May 1989, Fig. 1d). A acini began to empty of spermatozoa produced during autumn and early winter, typica spermatogenesis increased. This was marked by a significant increase in spermatogonia, spermatocytes and spermatids (*e.g.*, January 1989 compared to May 1989, Fig. 1b–c) to level similar to those seen in autumn. Gametogenic activity continued until July, as indicated b the presence of spermatogonia/spermatocytes and spermatids (Fig. 1b–c). However, sper matozoa accumulated only during autumn (Fig. 1d). Also, total cell count significantly de creased from January through July (*e.g.*, January 1989 compared to July 1989, Fig. 1e) These two factors suggest that spermatozoa were released as they were produced during spring and summer. In July, multinucleated inclusions again became the dominant cell type

Oogenesis.—Oogenesis occurred throughout most of the year and the annual cycle dif fered little between years (Fig. 1f). Size of oocytes differed significantly among collection dates ($F = 15.3$, $df = 17$, $P < 0.0001$). During late summer (*i.e.*, July or August) developing oocytes were at their smallest size (Fig. 1f). Oocyte size increased significantly during the autumn (*e.g.*, August 1989 compared to October 1989, Fig. 1f). Oocytes emerged from the acinus walls attached to cytoplasmic stalks. As oocytes grew, they eventually broke away from the acinus wall. During the early stages of oogenesis, small, spherical structures were presen in the gonadal tissues. These structures disappeared as oocyte size increased which sug gested a nutritive function. Oocyte size decreased significantly between spring and lat summer (*e.g.*, April 1989 compared to August 1989, Fig. 1f).

Brooding.—During 1990, embryos and mature glochidia were found in marsupia from late March through July. Marsupia were located in both inner and outer gills. The per centages of females with embryos and/or glochidia in marsupia were: March—67% (4/6) April—58% (11/19), May—53% (16/30), June—53% (9/17) and July—14% (1/7).

DISCUSSION

Sexuality and sex ratio.—In the study population, 2% of the typically dioecious *Quadrul metanevra* was hermaphroditic. These results are similar to those found for other popula tions of dioecious unionids (Heard, 1979, *but see* Downing *et al.*, 1989). Studies have show both male-biased (Yokley, 1972) and female-biased (Dudgeon and Morton, 1983) sex ratio in unionids.

Gametogenesis.—Typical spermatogenesis and oogenesis began in autumn and continue until midsummer (Fig. 1b–f). A possible benefit of starting gamete production in autumn is that it allows individuals to take advantage of high nutrient resources that may be availabl during that period. The Tennessee Valley Authority (1974) found elevated chlorophyll level in Kentucky Reservoir during autumn. By starting gamete production at that time, exces food resources can be channeled toward gamete production. An early start in gametogenes may be critical to insure that a sufficient number of spermatozoa are released in the spring when water levels are often high and the chances of fertilization reduced due to sperm

dilution and increases in the flow rate (Haggerty *et al.*, 1995). Gamete release in the spri may be important with regard to fish host relationships (*see* below). Some females in c study had embryos in different stages of development, which may indicate a low fertilizati rate (Matteson, 1948; Gordon and Smith, 1990). This condition has been observed in males of other tachytictic species (Lefevre and Curtis, 1910; Matteson, 1948; Yokley, 19 Yeager and Neves, 1986). Population demographic studies show that annual recruitm varies greatly (Miller and Payne, 1988; Payne and Miller, 1989). Such variation might dicate that some years may have lower fertilization rates than others.

A proportion of the initial accumulation of spermatozoa in the autumn may have result from the differentiation of multinucleated inclusions (Heard, 1975; Kotrla, 1989; per. ob: However, the significance of gamete production by ameiotic means remains unclear (Hea 1975).

Gamete release.—Based on a drop in the number of spermatozoa, a decline in egg si and the presence of eggs and glochidia in the marsupia, the gamete release period in t study population extended from at least March through July (Fig. 1). A proximate cause gamete release may be water temperature (Yokley, 1972). *Cyclonaias tuberculata,* a sympati short-term brooder, releases gametes during the same period (Haggerty *et al.*, 1995). T species' gametogenic cycle was quantified in a similar way. Qualitative studies of tachytic species indicate that lengths of gamete release periods vary (Yokley, 1972; Yeager and Nev 1986; Weaver *et al.*, 1991; Jirka and Neves, 1992).

The failure to find individuals in various stages of gamete release (*e.g.*, spent individu and very ripe individuals) for the spring and early summer collection dates suggest lengthy gamete release in all individuals, rather than an asynchronous gamete rele among individuals. The possible cost of a lengthy gamete release period in *Quadrula tanevra* is that a considerable amount of energy is used to make gametes. The bene however, is a longer annual period to produce offspring. Again, this behavior may be vantageous when gamete release occurs in the spring and early summer when water c ditions are often varied. A shorter period in the spring or early summer could mean t all of an individual's gametes would be released at a time when water levels and/or fl rates may not favor fertilization.

An additional advantage of late winter to midsummer gamete release is that it allow shorter brooding period. Glochidia of species that release gametes from late winter throu midsummer mature in time to be discharged during the same year, with a likelihood of fi host infestation that is still relatively high (*see* below). Females that release gametes late the summer (*i.e.*, bradytictic brooders) usually retain their glochidia until the followi spring. These females must survive the autumn and winter before they are able to rele their progeny.

Brooding and glochidia release.—The individuals of our study had a tachytictic brood reproductive pattern that is in accord with the results of Lefevre and Curtis (1910) a Utterback (1915). Like most other tachytictic and many bradytictic brooders (Lefevre a Curtis, 1910, *but see,* Sterki, 1898; Neves and Widlak, 1988; Woody and Holland-Barte 1993), *Quadrula metanevra* were gravid during the spring and early summer months. G chidial release during this period is not surprising since its reported fish hosts (*i.e., Lepo cyanellus, L. macrochirus* and *Stizostedion canadense,* Gordon and Layzer, 1989) are migr ing to breeding grounds, actively feeding, building nests and spawning at this time (Etni and Starnes, 1993). It is assumed that discharge of glochidia at this time of host fish activ optimizes reproductive success and offspring dispersal. Although it is unclear how glochic are discharged by female *Q. metanevra,* they do produce conglutinates (Lefevre and Curt

1910; pers. obs.) that may attract the host fish (Chamberlain, 1934; Kat, 1984, Jones *et al.*, 1986) and increase the potential of host infestation (Neves and Widlak, 1988).

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