

Primary Research Paper

Reproductive phenology in *Megaloniaias nervosa* (Bivalvia: Unionidae) in Wheeler Reservoir, Tennessee River, Alabama, USA

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Abstract

Two hundred and sixty-nine specimens of *Megaloniaias nervosa* (Rafinesque, 1820) were collected approximately monthly from the Wheeler Reservoir (Tennessee River mile 298), Alabama, USA, between July 1995 and June 1997. Microscopic and gross examinations of gonadal tissue and marsupia were used to determine the phenology of gamete production, spawning, brooding, and glochidia discharge. In addition, 11 females were collected in October 2001 to obtain estimates of brood size. Little reproductive activity was observed through most of the year, culminating in a bout of activity in late summer and early autumn. In males, spermatozoa were seen only in individuals collected in August and September. In females, oocytes developed and grew quickly during the same period and all oocytes were released to the marsupia over a relatively short period, showing a high degree of spawning synchronicity with males. Glochidia quickly matured and were present in both inner and outer demibranchs until December. The average number of glochidia per female was $740,379 \pm 72,854$ (95% CI). The reproductive cycle observed differed from those reported for other members of the Ambleminae and was more similar to members of Lampsilinae. This suggests that the lengths of the gametogenic and spawning periods may be related to the timing of fertilization, rather than to the length of the brooding period or to the species phylogeny.

Introduction

Unionids have internal fertilization and females retain (i.e. brood) their embryos and larvae (i.e. glochidia) within gill chambers called marsupia (McMahon & Bogan, 2001). Two basic brooding patterns have long been recognized in unionids and have been used to help classify freshwater bivalves (Ortmann, 1919; Heard & Guckert, 1970). Some species retain their glochidia for a relatively short period of time (i.e. tachytictic or short-term brooders), while others retain their glochidia for a longer period (bradytictic or long-term brooders). Recent quantitative assessments of unionid reproductive biology suggest that lengths of the gametogenic, spawning, and brooding periods may be

related to seasonal timing of these reproductive activities (Haggerty et al., 1995; Garner et al., 1999; Haggerty & Garner, 2000). For example, bradytictic species tend to have relatively short (usually a few weeks) gamete production and spawning periods in late summer and early autumn. Tachytictic species, however, have relatively long gamete production periods that can begin in autumn and continue into the following summer, and have spawning periods that last almost as long. If we are to better understand the ultimate causations of these disparate reproductive habits in unionids, further studies that investigate the seasonal timing of reproductive activities are needed.

Megaloniaias nervosa (Rafinesque, 1820) is the largest North American unionid, and is found in a

variety of lotic and lentic habitats, usually in medium to large rivers and reservoirs (Gordan & Layzer, 1989; Parmelee & Bogan, 1998; personal observation). It occurs through much of the Mississippi River Basin, as well as many drainages of the Gulf Coast (Parmelee & Bogan, 1998; personal observation).

Megaloniaias nervosa becomes sexually mature around 8 years of age (Woody & Holland-Bartels, 1993). It is a member of the Ambleminae, which typically have a tachytictic brooding strategy. The reported autumn and winter brooding of *M. nervosa* has long been considered idiosyncratic among amblemines (Howard, 1915; Utterback, 1915), which has led to confusion concerning its brooding classification. For example, Utterback (1915) considered *M. nervosa* tachytictic, whereas, Heard and Guckert (1970) and Parmelee & Bogan (1998) considered it bradytictic. A more recent investigation considered the species late tachytictic (Woody & Holland-Bartels, 1993).

Because of its relatively unique brooding habits, *M. nervosa* serves as an excellent species for examining the relationships among the lengths of various reproductive activities (e.g. gametogenesis, spawning, and brooding) with their time of occurrence and with a species phylogeny. Being a tachytictic brooder and an amblemine, does *M. nervosa* also have the relatively long gametogenic and spawning periods that have been reported for other tachytictic brooders and amblemines (Haggerty et al., 1995; Garner et al., 1999)? Or, since the species appears to spawn in late summer and autumn, like a bradytictic species or lampsiline, does it have relatively short gametogenic and spawning periods, even though it is considered an amblemine? Our research attempts to answer these questions.

A previous study of *M. nervosa* (Woody & Holland-Bartels, 1993) supports the association between late summer and autumn spawning with relatively short gamete and spawning periods. However, the more quantitative approach taken in our study is less subjective and allows for comparisons with previous studies that used similar methods (Haggerty et al., 1995; Garner et al., 1999; Haggerty & Garner, 2000). Also, since intraspecific differences in life history may occur among populations (Heard, 1998), studies at different latitudes and among different faunal provinces are of value. Further, since fecundity and

productivity are important components of a species' life history, we also present an estimate of brood size for *M. nervosa*.

Materials and methods

The study site was located in overbank habitat of the Round Island Creek embayment in Wheeler Reservoir, Tennessee River, Limestone County, Alabama, USA (Tennessee River mile 298; 87 ° 3' N, 34 ° 4' W). Water depths at the site ranged from 2.0 m to 4.5 m. Substrata consisted of mud, gravel and/or *Corbicula fluminea* (Müller, 1774) shells and water was generally turbid with visibility less than 0.5 m. Specimens were collected by hand, while diving with surface air supply, approximately monthly between 19 July 1995 and 24 June 1997. Two samples were collected during some months to give better details of spawning time. A sample consisted of at least 15 specimens. Specimens were sacrificed in the field by cutting anterior and posterior adductor muscles with a knife and placing them into a 20 l bucket of buffered 10% formalin.

A transverse section was cut from the visceral mass of each specimen and embedded in paraffin. Thin sections (6 µm) were made with a microtome, mounted on glass slides and stained using hematoxylin and eosin methods described in Humason (1979). Methods similar to those of Haggerty et al. (1995) and Garner et al. (1999) were used to quantify gametogenesis. Spermatogenesis was quantified by identifying and counting germ cells along transects across ten follicles (= acini, epithelial sacs within the visceral mass in which gametogenesis occurs). A light microscope at 1000× was used. An eyepiece pointer, moved along the X-axis of a mechanical microscope stage, was used for the transects. All cells touching the tip of the pointer were identified and counted. Cells were identified as spermatogonia/spermatocytes, spermatids, spermatozoa and spermatogenic cysts (i.e. atypical cells of Coe & Turner, 1938; sperm morulae of Yokley, 1972) based on size, shape, intensity of staining and position within the follicle. Spermatogenic cysts consisted of variable clusters of decondensed DNA surrounded by a thin layer of cytoplasm (Kotrla, 1988). Transects ran through the approximate centers of the largest follicles in a tissue section. Descriptions in

Dinamani (1974) and Peredo & Parada (1984) helped with identification of male germ cells.

Two methods were used to quantify oogenesis. Oocyte development was quantified by measuring diameters of oocytes along transects across the entire gonad section. Thirty oocytes in each specimen were measured. Only those oocytes in which the plane of the section passed through the nucleus were measured, using a light microscope with a ruled eyepiece reticle. Magnification of 100 \times was used. Transects were run along the *Y*-axis of the reticle crosshairs. A transect was defined as the width equal to ten units of measure on each side of the *Y*-axis. Two measurements were made on each oocyte falling totally or partially on the transect. The first measurement was along the longest axis of the oocyte. The second measurement was made perpendicular to the first measurement at its widest point. The second method of oogenesis quantification was determining the number of oocytes per follicle. This was accomplished by counting the number of egg sections in the first 30 intact follicles that were touched by the eyepiece pointer as the slide was moved in one direction on the microscope stage. Temporal changes in gametogenesis were analyzed by one-way ANOVA's (SAS Institute Inc., 1982). Duncan's multiple-range test was used to compare means. Results were termed significant only if $p < 0.05$.

To determine the period of glochidial brooding, gills of females were examined grossly at 7–10 \times using a dissecting microscope. Stage of glochidial development was determined from samples of marsupial content. A multicellular mass enclosed in a fertilization membrane was defined as an embryo. A glochidium was considered mature when it was no longer membrane bound. Samples were taken medially, and from near the anterior and posterior ends of the left lateral demibranch, with three samples (proximal, medial and distal) taken from each area, for a total of nine samples of marsupial content per gravid specimen. Marsupial content was examined in water on a depression slide at 40 \times using a light microscope.

Brood size estimates were determined by approximating the number of embryos present in the gills of 11 females that were collected on 16 October 2001. Numbers of larvae were determined by modifying the methods of Jupiter & Bryne (1997). The brooding gills were cut from the

female and all glochidia were removed from the gills by washing with distilled water. Embryos and wash were placed into a beaker and distilled water was added to make a final volume of 500 ml. A small magnetic stir bar was used to suspend embryos uniformly and twelve 100 μ l aliquot samples were taken from the 500 ml embryo suspension. Each 100 μ l embryo sample was placed on a depression slide, a digital image was made, and NIH Image software (version 1.62) was used to count all embryos within the sample.

Results

Spermatogenesis and spawning

Most individuals sampled were dioecious ($n = 122$), with only one functional male having a few female follicles. Significant differences among collection dates were found for all cells types ($p < 0.0001$, Fig. 1). Meiotic production of spermatozoa occurred over a relatively short period of time in late summer (i.e. 2 months). Spermatogonia/spermatocyte numbers increased significantly from late July to late August, then decreased sharply from late August to late September (Duncan's test, $p < 0.05$, Fig. 1A). Spermatid and spermatozoa numbers showed a similar temporal pattern, but spawning (i.e. sharp decrease in number of spermatozoa in follicle) continued through late September and was completed by early October (Fig. 1B and 1C). Spermatogenic cysts were prevalent during all months except those during which spermatogenesis occurred (late summer and early autumn; Fig. 1D).

Oogenesis and spawning

Significant differences among collection dates were found for both egg size and egg number ($p < 0.0001$, Fig. 2, $n = 146$). Oocytes were difficult to find within the follicles from early October to late June. A significant increase in number and size of oocytes occurred between late June and late September (Duncan's test, $p < 0.05$; Fig. 2). Spawning (i.e. movement of oocytes into marsupia) occurred between late August and early October as indicated by the significant decrease in size and number of oocytes during that time period (Duncan's test, $p < 0.05$). By early October the follicles were again empty and returned to an inactive state.

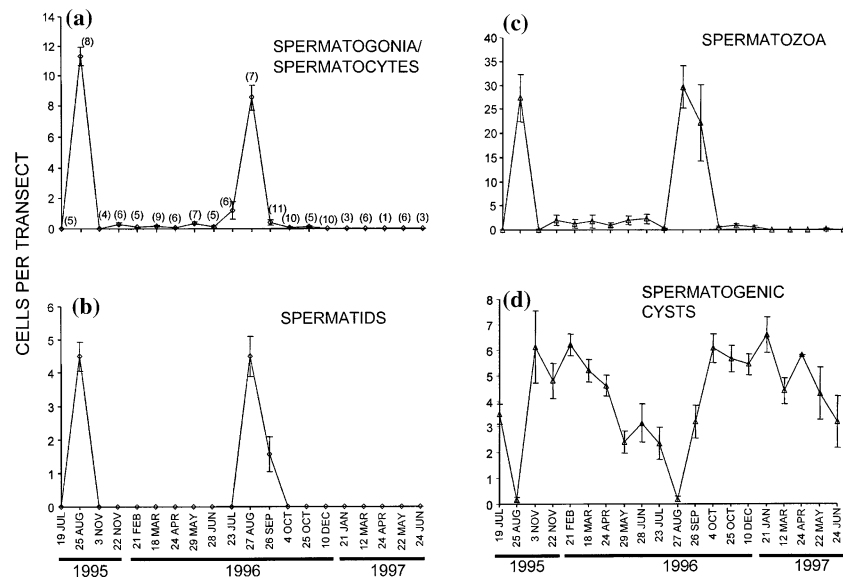


Figure 1. Mean (± 1 SE) numbers of spermatozoa (A), spermatids (B), spermatozoa (C), and spermatogenic cysts (D), in each transect between 19 July 1995 and 24 June 1997. Number of individuals examined is in parenthesis in A.

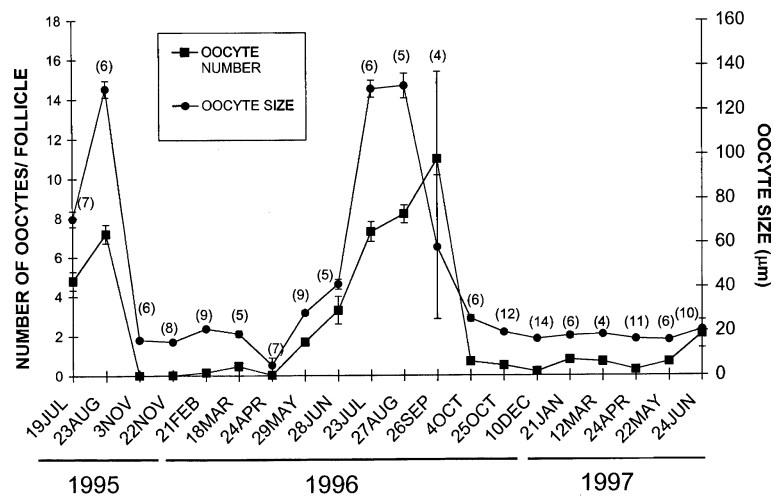


Figure 2. Mean (± 1 SE) oocyte size and number of oocytes per follicle between 19 July 1995 and 24 June 1997. Number of individuals examined is in parenthesis.

Glochidium development, brooding, brood size, and discharge

Embryos were brooded in all four demibranchs of females collected in late September (80%; 4/5) and early October (100%; 6/6). Glochidia were present in females collected in late October (92%; 11/12), November (89%; 17/19), and early December (25%; 4/16). Of the eleven gravid females [Length = 137 ± 4.8 (SD)mm] that were collected

in early October 2001, the average number of glochidia per female was $740,379 \pm 72,854$ (95% CI).

Discussion

Although our study of *M. nervosa* showed the same basic reproductive pattern that has been found by others (Utterback, 1915; Woody and

Holland-Bartels, 1993), we did find evidence that variation in the specific timing of reproductive events can occur among populations. For example, in a more northern, Mississippi River population (Woody & Holland-Bartels, 1993), males with spermatids were reported in May and most males contained spermatozoa by July. In our population, males with spermatids and spermatozoa were not seen until August. Further, the more northern females were gravid in August and September with a few gravid females in October and November (Woody & Holland-Bartels, 1993), whereas, we found gravid females from September through early December.

Variation in the specific timing of reproduction was also reported in another species of unionid [*Potamilus alatus* (Say, 1817)] that was collected from similar locations (Holland-Bartels & Kammer, 1989; Haggerty & Garner, 2000). For *P. alatus* we suggested variation in water temperature and fish host availability accounted for the population differences that were noted. We suspect similar causations may be responsible for the variation reported in this study.

Although *M. nervosa* has a relatively short brooding period, which is typical of amblemines, it does not have the relatively long gamete production and spawning periods than has been found in other amblemines (Haggerty et al., 1995; Garner and Haggerty, 1999). Instead, *M. nervosa* has relatively brief gamete production and spawning periods similar to those of other species that spawn in late summer and autumn (e.g. Haggerty & Garner, 2000), primarily lampsilines, which tend to be bradyctic. This suggests that the lengths of gamete production and spawning periods are not related to brooding period length and are not constrained phylogenetically, but are related to the timing of gamete production and spawning. That is, those species that spawn in late winter, spring and summer (e.g. typically amblemines) will have relatively long gamete production and spawning periods, whereas, those species that spawn in late summer and autumn (e.g. typically lampsilines and *M. nervosa*, an amblemine) will have shorter gamete production and spawning periods. Although Haggerty & Garner (2000) proposed that the temporal pattern of gamete production and spawning may be helpful characters in discerning phylogenetic relationships among unionids, the

results of this study suggests they may actually be poor indicators of such relationships in this group.

As we have proposed elsewhere, varying climatic conditions between spring and summer may serve as the ultimate causations for the varying reproductive strategies seen among spring/early summer spawners versus late summer/autumn spawners (Garner & Haggerty, 1999; Haggerty & Garner, 2000). Spring climate conditions often lead to the flooding of streams and rivers, which increases flow rates that may lower fertilization probabilities for individuals that spawn at that time. Alternatively, individuals producing gametes and spawning in late summer and autumn may find water conditions more favorable for fertilization, which would increase their likelihood of producing zygotes (Zale & Neves, 1982). The possible tradeoff of late summer and autumn spawning, however, is that it may lead to the development of glochidia at a less than optimal time for infesting fish hosts (Kat, 1984). In anodontines and lampsilines, gill modifications (e.g. conversion of water tubes into separate, isolated brooding chambers) occur during reproduction and may allow for some plasticity in glochidia release (Richard et al., 1991). *M. nervosa* females have no such gill modifications for brooding (Davis & Fuller, 1981) and therefore may be constrained to release their glochidia soon after they are developed in autumn or early winter. However, *M. nervosa* appears to be a host fish and habitat generalist (Watters, 1994; Garner & McGregor, 2001), which may allow them to achieve a relatively high infestation rate (Bauer, 1994), while still taking advantage of the fertilization benefits of late summer and autumn spawning. In addition, the use of four demi-branches to help produce approximately 750,000 glochidia may help maximize host contact and attachment (McMahon & Bogan, 2001), and the large size of *M. nervosa* glochidia, relative to other amblemines (Davis & Fuller, 1981) may shorten the time needed for metamorphosis (Bauer, 1994).

In conclusion, our Tennessee River population of *M. nervosa* carried out reproductive activities over a 5 month period. Sperm and egg production peaked in late August and spawning occurred synchronously between the sexes and relatively soon after gamete production between late August and early October. Embryos first appeared in

marsupia in late September and glochidia were found in females collected in late October through early December. The average brood size was approximately 750,000. These data indicate that in our population, *M. nervosa* has relatively short-term gamete and spawning periods, as well as a short-term autumn/early winter brooding period.

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