Haggerty Garner 2000

Invertebrate Reproduction and Development, 38:1 (2000) 35–41
Balaban, Philadelphia/Rehovot
0168-8170/00/$05.00 © 2000 Balaban

Seasonal timing of gametogenesis, spawning, brooding and glochidia discharge in *Potamilus alatus* (Bivalvia: Unionidae) in the Wheeler Reservoir, Tennessee River, Alabama, USA

THOMAS M. HAGGERTY[1*] and JEFFREY T. GARNER[2]
[1]*Department of Biology, University of North Alabama, Florence, Alabama 35632, USA*
Tel. +1 (256) 765-4432; Fax +1 (256) 765-4430; email: thaggert@unanov.una.edu
[2]*Division of Wildlife and Freshwater Fisheries, Alabama Department of Conservation and Natural Resources, PO Box 366, Decatur, Alabama 35602, USA*

Received 30 March 2000; Accepted 26 June 2000

Summary

One hundred sixty-eight specimens of *Potamilus alatus* (Say, 1817) were collected approximately monthly from the Wheeler Reservoir (Tennessee River mile 298), Alabama, USA, between February 1995 and March 1996. Microscopic and gross examinations of gonadal tissue and marsupia were used to determine seasonal timing of gamete production, spawning, brooding and glochidia discharge. All individuals examined were dioecious. Spermatogenesis occurred between July and August. Spermatozoa numbers peaked in late August and male spawning occurred over a 2–3-week period in late August and early September. Oogenesis and female spawning had patterns similar to spermatogenesis and male spawning. Brooding of glochidia occurred between September and April. Glochidia discharge occurred in April and possibly early May, and coincided with the onset of the spawning season of the reported fish host, *Aplodinotus grunniens* (Rafinesque, 1819). The temporal patterns of gamete production and spawning in *P. alatus* of the subfamily Lampsilinae are in sharp contrast to members of the subfamily Ambleminae that have been similarly studied. This suggests that detailed studies of gamete production and spawning may be helpful in understanding unionid phylogeny and character evolution.

Key words: *Potamilus alatus*, reproductive cycle, spawning, Unionidae, glochidia, brooding, Bivalvia

Introduction

North American unionids are ovoviviparous, freshwater bivalves that produce specialized larvae (i.e., glochidia) that are typically obligate ectoparasites on the fins and/or gills of fishes (McMahon, 1991). Glochidia are held (i.e., brooded) in gill demibranch chambers (i.e., marsupia) until the time of discharge, which occurs primarily in spring and summer to optimize dispersal (Kat, 1984; but see Neves and Widlak, 1988). Some taxa retain their glochidia for short periods and discharge them soon after spawning occurs (short-term brooders). Others hold their glochidia over winter and discharge them the following

*Corresponding author.

spring and/or summer (long-term brooders; Lefevre and Curtis, 1910; Gordon and Layzer, 1989; but see Neves and Widlak, 1988).

Considerable attention has been focused on the brooding (i.e., incubation) period of unionids, which has long been important in taxonomy of the group (Ortmann, 1919; Heard and Guckert, 1970). However, recent phylogenetic work suggests that length of brooding period is homoplastic (Lydeard et al., 1996). Further, intraspecific variation in the brooding period can occur (Heard, 1975; Lewis, 1985). While brooding time is an important trait of unionid life histories, temporal patterns of gamete production and spawning are also important and may be less evolutionarily labile. Current unionid classification is ambiguous (Parmalee and Bogan, 1998), and additional information concerning reproduction in specific taxa may help our understanding of unionid phylogeny and character evolution. Further, although gamete production and spawning are important aspects in the life history of unionids, few studies have quantified them in detail (Zale and Neves, 1982a; Gordon and Layzer, 1989).

Potamilus alatus is a reported long-term brooder that is in the subfamily Lampsilinae (Ortmann, 1919; but see Davis and Fuller, 1981 and Lydeard et al. 1996). It is widespread in eastern North America, being found throughout the Mississippi River system, as well as in the St. Lawrence River system and parts of the Canadian Interior Basin (Parmalee and Bogan, 1998). Although aspects of the reproductive biology of *P. alatus* have been studied (Ortmann, 1919; Holland-Bartels and Kammer, 1989), detailed information concerning its reproduction at more southerly latitudes is lacking. Further, studies that allow for interpopulation comparisons of the seasonal timing of reproductive activities in unionids are few and are of value (Lewis, 1985). The purpose of this research was to quantify, in detail, seasonal timing of gamete production, spawning, brooding, and glochidia discharge in a Tennessee River population of *P. alatus*.

Materials and Methods

The study site was located in an overbank habitat of the Round Island Creek embayment in the Wheeler Reservoir, Tennessee River, Limestone County, Alabama, USA (TRM 298; 34°4′ N, 87°3′ W). Water depths at the site ranged from 2.0 m to 4.5 m. Substrata consisted of mud, gravel and/or *Corbicula fluminea* (Muller, 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 26 February 1996 and 12 March 1997. Two samples were collected during some months to give better details of spawning time. A monthly sample consisted of at least 15 specimens. An effort was made to collect approximately equal numbers of each sex, based on sexual dimorphism of shell characters, but during some months we found it difficult to find females. 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 hemotoxylin 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 1,000× 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 multi-nucleated inclusions (i.e., atypical cells of Coe and Turner, 1938; sperm morulae of Yokley, 1972) based on size, shape, intensity of staining and position within the follicle. Multi-nucleated inclusions consisted of variable clusters of decondensed DNA surrounded by a thin layer of cytoplasm (Kotrla, 1989). Transects ran through the approximate centers of the largest follicles in a tissue section. Descriptions in Dinamani (1974) and Peredo and 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× was used. Transects were run along the *Y* axis of the reticle cross-hairs. 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 within 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.

To determine the period of glochidial development and brooding, gills of females were examined grossly at 7–10× using a dissecting microscope. The period of glochidial development was determined from samples of marsupial content. A multi-cellular mass enclosed in a fertilization membrane was defined as an embryo. A glochidium shaped like an ax-head (Lefevre and Curtis, 1910) and enclosed by a fertilization membrane was considered immature, whereas a mature glochidium was no longer membrane bound. Samples were taken from anterior, medial and posterior marsupia on the left gill demibranch, with three samples (proximal, medial and distal) taken from each marsupium, for a total of nine samples of marsupial content per gravid specimen. Marsupial content was examined in water on a depression slide at 40× using a light microscope. Glochidial release was determined by the presence of a perforation (i.e. "rent", Sterki, 1911) in the ventral margin of a marsupium.

Temporal changes in gametogenesis were analyzed by one-way ANOVAs (SAS Institute Inc., 1982). Duncan's multiple-range test was used to compare means. Results were termed significant only if $P<0.05$.

Results

Spermatogenesis

All individuals examined were dioecious. Significant differences among collection dates were found for all of the cell types ($P<0.0001$, Fig. 1). Typical spermatogenesis (i.e., meiotic production of spermatozoa) occurred over a relatively short period of time (i.e., approximately 2 months). A significant increase in spermatogonia/spermatocytes occurred between late June and late July, peaked in early August, but returned to lower numbers by the end of August (Duncan's test, $P<0.05$; Fig. 1A). Spermatid numbers also peaked in early August and most spermatids underwent spermiogenesis during that month, which lead to a significant increase in the number of spermatozoa by the end of August (Duncan's test, $P<0.05$; Fig. 1B and 1C). Multi-nucleated inclusions dominated the follicles during months when typical spermatogenesis was inactive (September through June). The number of multi-nucleated inclusions remained relatively constant until August when they decreased significantly compared to late July (Duncan's test, $P<0.05$; Fig. 1D). Male spawning occurred between the last week of August and the second week of September as indicated by a significant decrease in spermatozoa in the follicles (Duncan's test, $P<0.05$; Fig. 1C). Multi-nucleated inclusion numbers increased significantly along transects between late August and early September as spawning occurred (Duncan's test, $P<0.05$; Fig. 1D).

Oogenesis

Oocytes were present in the gonadal tissues throughout the year, but significant differences in size and number of oocytes per follicle occurred among collections dates ($P<0.0001$, Fig. 2). Oocytes remained relatively small throughout most of the year (i.e., between early September and late June), but a significant increase in size occurred between late June and late August (Duncan's test, $P<0.05$; Fig. 2A). Spawning (i.e., movement of oocytes into marsupia) was indicated by a significant decrease in oocyte diameter between late August and early September (Duncan's test, $P<0.05$; Fig. 2A). The number of oocytes per follicle showed a pattern similar to the oocyte diameter results (Fig. 2B). The number of oocytes per follicle peaked in August and decreased significantly between the end of August and the second week of September, indicating that oocytes moved out of the follicles during that time (Duncan's test, $P<0.05$; Fig. 2B).

Glochidial development, brooding, and release

Embryos were found in the marsupia of all females collected in September ($n=5$). No unfertilized eggs were found. Immature glochidia were present in marsupia of all females collected in early October ($n=4$). Morphologically mature glochidia were found in marsupia collected from late October through late April. Percentages of brooding females were as follows: late October, 86% (6/7); December, 100% (7/7); January, 100% (2/2); March, 100% (9/9) and April, 75% (3/4). Evidence of glochidial release (i.e., a perforation in the distal end of each marsupium) was found in all females collected in April ($n=4$) and May ($n=1$). Repair of marsupial perforations occurred between May and June.

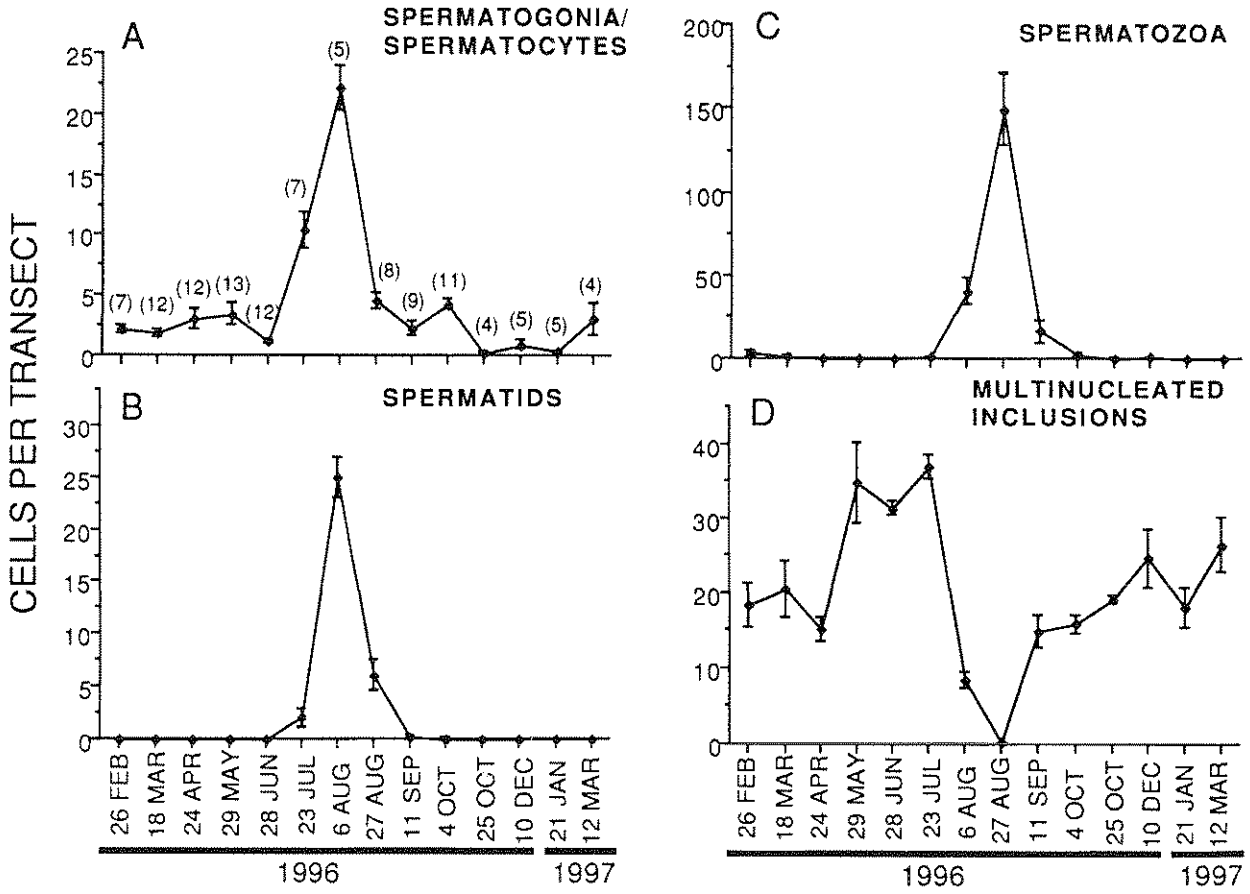

Fig. 1. Mean (±1 SE) numbers of spermatogonia/spermatocytes (A), spermatids (B), spermatozoa (C), and multi-nucleated inclusions (D), in each transect between February 1996 and March 1997. Number of individuals examined is in parenthesis in A.

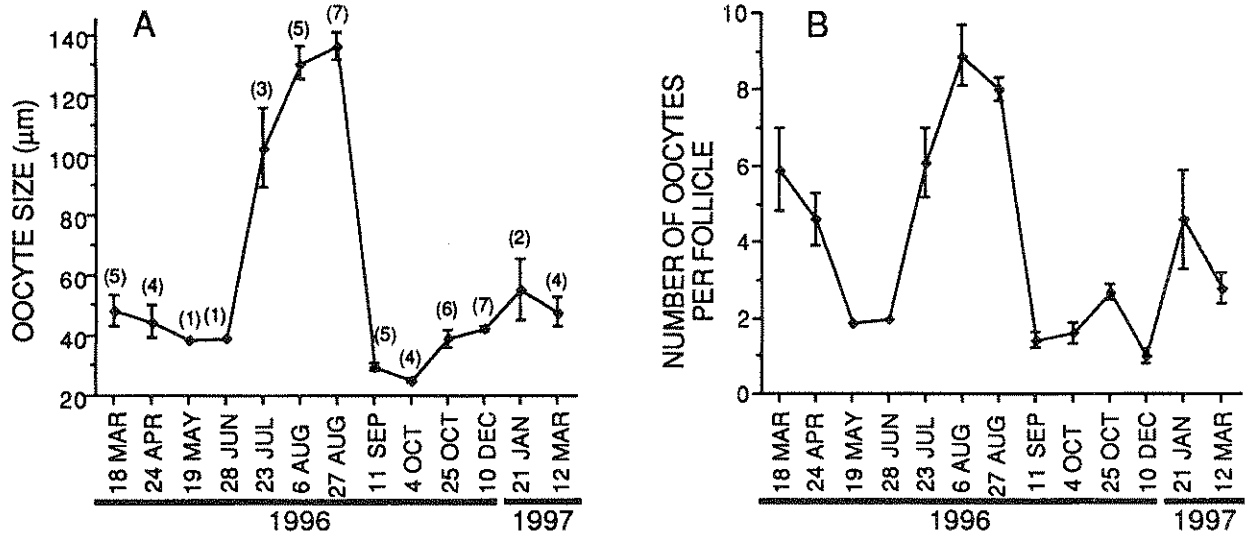

Fig. 2. Mean (±1 SE) oocyte size (A), and number of oocytes per follicle (B) between March 1996 and March 1997. Number of individuals examined is in parenthesis in A.

Discussion

The males in our study population carried out typical spermatogenesis during summer and early autumn (i.e., between July and early September). Production of multi-nucleated inclusions overlapped typical gamete production during June and August. As multi-nucleated inclusion numbers decreased between late July and August, spermatozoa reached their highest numbers. This suggests that multi-nucleated inclusions may play a roll in spermatozoa production (Coe and Turner, 1938). Holland-Bartels and Kammer (1989) also reported summer sperm production in a population of *P. alatus* in the upper Mississippi River (i.e., LaCrosse, Wisconsin). However, onset of spermatogenesis was almost 2 months earlier than what we found in the Wheeler Reservoir population of *P. alatus*.

The development of oocytes in our study population showed a temporal pattern similar to that of the spermatozoa. Oocyte growth occurred during summer months (i.e., between June and August). Egg size peaked in early August, which was about 2 months later than what was found in the upper Mississippi River population (Holland-Bartels and Kammer, 1989). The sharp decrease in number of sperm and oocytes between late August and early September, as well as the decrease in oocyte size, indicated that spawning occurred during that period and was synchronous between the sexes. Holland-Bartels and Kammer (1989) observed that spawning occurred in their upper Mississippi River population in late July and early August.

The gamete production period observed in the Wheeler Reservoir population of *P. alatus* is similar to that reported for some other lampsilines (Zale and Neves, 1982a; Holland-Bartels and Kammer, 1989). As expected, the short period of sperm production (approximately 2 months) observed in *P. alatus* was in stark contrast to those of some short-term brooders of the Ambleminae (i.e., *Cyclonaias tuberculata*, *Quadrula metanevra*) that have been studied in a similar manner (Haggerty et al., 1995; Garner et al., 1999). In those species, individuals began gamete production in the fall and continued until mid-summer. The shorter gamete production period found in some lampsilines may indicate that less energy is required to make gametes for maximum fitness. If fewer resources are needed for optimal gamete production, additional resources may be utilized for growth and survival-enhancing behaviors (e.g., movement). Some lampsilines have been reported to grow faster (Lefevre and Curtis, 1910; but see Kesler and Downing, 1997) and are more active (Waller et al., 1999) than some amblemines. Further, a reduction in reproductive effort may allow lampsilines to utilize habitats that are less stable and have fewer available food resources. This may explain why this subfamily dominates many small stream and headwater habitats (Weaver et al., 1991; Haag and Warren, 1998).

Length of spawning period of *P. alatus* was also similar to that reported for other lampsilines. Although seasonal timing of spawning appears to vary among lampsilines (summer–autumn), the duration of the spawning is relatively brief (2–3 weeks) and very synchronous between sexes (Zale and Neves, 1982a; Holland-Bartels and Kammer, 1989). Like the gametogenic period, the duration of the spawning period of *P. alatus* contrasted greatly with those found in some amblemines, which often begin spawning in early spring and continue until mid-summer (Haggerty et al., 1995; Garner et al., 1999). A possible benefit of summer–autumn spawning is that water conditions may be more favorable (e.g., low flow) for fertilization (Zale and Neves, 1982a), with fewer gametes needed to maximize fertilization. Support for this idea comes from reports of unfertilized eggs and embryos of different ages in the marsupia of amblemines (Matteson, 1948; Yokley, 1972; Garner et al., 1999), but not lampsilines (Zale and Neves, 1982a; pers. observ.). A possible cost of delayed gamete production and spawning, however, is that glochidia mature at a time that is often not optimal for dispersal, and young therefore need to be brooded longer.

Embryos were first found in the marsupia in early September. This is later than what was found in upper Mississippi River *P. alatus* (Holland-Bartels and Kammer, 1989). In that population, embryos/glochidia were found in early August. Ortmann (1919) reported a female with eggs in late June in Pennsylvania, but mature glochidia were not found until late August. In our study, morphologically mature glochidia were present in the marsupia by late October, indicating that development of *P. alatus* glochidia can occur within at least a 2-month period. This concurs with developmental times reported for other lampsilines (Zale and Neves, 1982a).

Evidence of glochidia discharge (i.e., presence of perforations on distal ends of marsupia) was not seen until April, indicating that embryos/glochidia can remain in the marsupia for at least 8 months. Assuming that glochidia primarily exit the marsupia through ventral perforations, the glochida discharge period for the Wheeler Reservoir population was primarily in

April and possibly early May. This timing of glochidia discharge coincides with the spawning movements of the freshwater drum (*Aplodinotus grunniens*, Rafinesque, 1819) (Pflieger, 1975; Mettee et al., 1996), the only reported fish host for *P. alatus* (Fuller, 1974). Longer periods of glochidia discharge have been reported for some other lampsilines (Zale and Neves, 1982a, 1982b).

The timing of glochidia discharge in our study population of *P. alatus* differed from those reported from higher latitudes. Both Ortmann (1919) and Holland-Bartels and Kammer (1989) reported glochidia discharge periods from late May through early July in Pennsylvania and Wisconsin. This was later than the discharge period observed in the Wheeler Reservoir even though gamete production began earlier in the upper Mississippi River population (Holland-Bartels and Kammer, 1989). This is not surprising since intraspecific variation in life histories is not uncommon in mollusks (McMahon, 1991; Johnson and Brown, 1998). The differences between these populations of *P. alatus* suggest that conditions at higher latitudes (e.g., colder temperatures) may affect metabolic activities and that longer periods are needed between gamete production and glochidia discharge. Temperatures have been shown to affect bivalve metabolic rates (McMahon, 1991). Further, the optimal dispersal times may differ between populations because temperature differences associated with latitude lead to variation in host fish spawning times.

Conclusions

Our research quantified the temporal pattern of gamete production and spawning for a population of *P. alatus* in a large, southern river in North America. Although seasonal timing varied, our population showed the same basic gamete production and spawning pattern that has been reported for other lampsilines (Zale and Neves, 1982a; Trdan, 1981; Holland-Bartels and Kammer, 1989) and a more northerly population of *P. alatus* (Holland-Bartels and Kammer, 1989). Gamete production occurred over a short period during summer (2 months) and a very brief (2–3 weeks) synchronous spawning followed in late summer. Little gametogenic activity was seen while glochidia were being brooded. The observed lampsiline reproductive pattern was in sharp contrast to the relatively long gamete production and spawning periods reported for some amblemines (e.g., Haggerty et al., 1995; Garner et al., 1999). This suggests that the temporal pattern of gamete production and spawning may be useful characters in diagnosing natural groups of unionids.

Acknowledgments

A very special thanks goes to P. Yokley, Jr. for initiating our interest in freshwater mussels. The assistance of J. Johns, K. Chong, J. Allen, and C. Otsuka in the laboratory is greatly appreciated. Thanks to P. Kittle and T. Richardson for improving the manuscript. The University of North Alabama provided equipment, supplies and library assistance. The research was partially supported by a College of Arts and Sciences Research Grant from the University of North Alabama to TMH.

References

Coe, W.R., and Turner, H.J. Jr., Development of the gonads and gametes in the soft-shell clam (*Mya arenaria*). J. Morph., 62 (1938) 91–111.

Davis, G.M. and Fuller, S.L.H., Genetic relationships among recent Unionacea (Bivalvia) of North America. Malacol., 20 (1981) 217–253.

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

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

Garner, J.T., Haggerty, T.M., and Modlin, R.F., Reproductive cycle of *Quadrula metanevra* (Bivalvia: Unionidae) in the Pickwick Dam tailwater of the Tennessee River. Am. Midl. Nat., 141 (1999) 277–283.

Gordon, M.E. and Layzer, J.B., Mussels (Bivalvia: Unionidae) of the Cumberland River system: review of life histories and ecological relationships. U.S. Fish and Wildlife Service, Biological Report No. 89, 1989.

Haag, W.R. and Warren, M.L., Jr., Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Can. J. Fish. Aquat. Sci., 55 (1998) 297–306.

Haggerty, T.M., Garner, J.T., Patterson, G.H. and Jones, L.C., Jr., A quantitative assessment of the reproductive biology of *Cyclonaias tuberculata* (Bivalvia: Unionidae). Can. J. Zool., 73 (1995) 83–88.

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

Heard, W.H. and Guckert, R.H., A re-evaluation of the recent Unionacea (Pelecypoda) of North America. Malacologia, 10 (1970) 333–355.

Holland-Bartels, L.E. and Kammer, T.W., Seasonal reproductive development of *Lampsilis cardium*, *Amblema plicata plicata* and *Potamilus alatus* (Pelecypoda:

Unionidae) in the upper Mississippi River. J. Fresh. Ecol., 5 (1989) 87–92.
Humason, G.L., Animal Tissue Techniques, 4th ed. W.H. Freeman, San Francisco, 1979.
Johnson, P.D. and Brown, K.M., Intraspecific life history variations in the threatened Louisiana pearlshell mussel, *Margaritefera hembeli*. Freshwater Biol., 40 (1998) 317–329.
Kat, P.W., Parasitism and the Unionidae (Bivalvia). Biol. Rev., 59 (1984) 189–207.
Kessler, D.H. and Downing, J.A., Internal shell annuli yield inaccurate growth estimates in the freshwater mussels *Elliptio complanata* and *Lampsilis radiata*. Freshwater Biol., 37 (1997) 325–332.
Kotrla, M.B., Evidence of ameiotic spermatogenesis in unionid bivalves. Am. Zool., 29 (1989) 148A.
Lefevre, G. and Curtis, W.C., Studies on the reproduction and artificial propagation of fresh-water mussels. Bull. U.S. Bureau. Fish., 30 (1910) 105–201.
Lewis, J.B., Breeding cycle of the freshwater mussel *Anodonta grandis* Say. Can. J. Zool., 63 (1985) 2477–2478.
Lydeard, C., Mulvey, M. and Davis, G.M., Molecular systematics and evolution of reproductive traits of North American freshwater unionacean mussels (Mollusca: Bivalvia) as inferred from 16S rRNA gene sequences. Phil. Trans. R. Soc. Lond. B, 351 (1996) 1593–1603.
Matteson, M.R., Life history of *Elliptio complanatus* (Dillwyn, 1817). Am. Midl. Nat., 40 (1948) 690–723.
McMahon, R.F., Mollusca: Bivalvia. In: Ecology and Classification of North American Freshwater Invertebrates, J.H. Thorp and A.P. Covich (eds.), Academic Press, San Diego, 1991, pp. 315–399.
Mettee, M.F., O'Neil and P.E., Pierson, J.M., Fishes of Alabama and the Mobile Basin. Oxmoor House, Birmingham, Alabama, 1996.
Neves, R.J. and Widlak, J.C., Occurrence of glochidia in stream drift and on fishes of the upper North Fork Holston River, Virginia. Am. Midl. Nat., 119 (1988) 111–120.
Ortmann, A.E., Monograph of the naiads of Pennsylvania. Part III: systematic account of the genera and species. Mem. Carnegie Mus., 8 (1919) 1–384.
Parmalee, P.W. and Bogan, A.E., The freshwater mussels of Tennessee. University of Tennessee Press, Knoxville, Tennessee, 1998.
Peredo, S. and Parada, E., Gonadal organization and gametogenesis in the fresh-water mussel *Diplodon chilensis chilensis* (Mollusca: Bivalvia). Veliger, 27 (1984) 126–133.
Pflieger, W. L., The fishes of Missouri. Missouri Department of Conservation, 1975.
SAS Institute, Inc., SAS User's Guide: Statistics. SAS Institute, Cary, North Carolina, 1982.
Sterki, V., Notes on the anatomy and physiology of the Unionidae. Ohio Nat., 11 (1911) 331–334.
Trdan, R.J., Reproductive biology of *Lampsilis radiata siliquoidea* (Pelecypoda: Unionidae). Am. Midl. Nat., 106 (1981) 243–248.
Weaver, L.R., Pardue, G.B. and Neves, R.J., Reproductive biology and fish hosts of the Tennessee clubshell *Pleurobema oviforme* (Mollusca: Unionidae) in Virginia. Am. Midl. Nat., 126 (1991) 82–89.
Waller, D.L., Gutreuter, S. and Rach, J.L., Behavioral responses to disturbance in freshwater mussels with implications for conservation and management. J. N. Am. Benthol. Soc., 18 (1999) 381–390.
Yokley, P., Life history of *Pleurobema cordatum* (Rafinesque, 1820) (Bivalvia: Unionacea). Malacologia, 11 (1972) 351–364.
Zale, A.V. and Neves, R.J., Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. Freshwater Invert. Biol., 1 (1982a) 17–28.
Zale, A.V. and Neves, R.J., Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Can. J. Zool., 60 (1982b) 2535–2542.