Notes on the Biology and Morphology of *Margaritifera hembeli* (Conrad, 1838) (Unionacea: Margaritiferidae)

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ABSTRACT

The freshwater pearl mussel *Margaritifera hembeli* is found only in the Red River basin and a few nearby drainages in Louisiana. Though of concern to conservationists because of its declining numbers, *M. hembeli* remains virtually unknown with respect to its anatomy and biology. The species contains all the anatomical characters that typify margaritiferid species. The sexes are separate and the gonads show a definite seasonality in activity. Gametogenesis is pronounced in specimens collected in the fall, followed by degeneration of reproductive tissues in the late winter through to late spring. It is concluded, on the basis of observed gonadal activity, that oviposition and spawning take place between late November and late January. Characters are evident in the morphology of the visceral nervous system and the stomach of *M. hembeli* that clearly distinguish *M. hembeli* from *M. marrianae* and other eastern North American margaritiferid species. A distinct relationship between *M. hembeli* and *M. marrianae*, however, is suggested by the mutual occurrence of lateral hinge teeth and a corrugated surface of the posterior portion of the shell. Due to the lack of knowledge of the anatomy and biology of other margaritiferid species, especially those living in Asia, it is premature to suggest relationships between *M. hembeli* and other described margaritiferid species, particularly those with lateral hinge teeth.

Key words: *Margaritifera*; anatomy, reproduction, North America.

INTRODUCTION

The North American freshwater mussel *Margaritifera hembeli* (Conrad, 1838) was once believed to comprise two geographically discontinuous populations taxonomically linked by vague similarities of the shell. Johnson (1983) separated the two populations taxonomically by describing the Alabama group as *M. marrianae*, thus restricting the *M. hembeli* group to Louisiana. His description included characters of the shell only, principally the degree of sculpturing on the shell surface and the shape of the ventral shell margin. Additional conchological differences between the Louisiana and Alabama populations were noted by Smith (1983) who pointed out dissimilarities in the mantle-shell attachment scars on the inner nacreous surface.

Margaritifera hembeli probably had a more extensive range in the Red River drainage as indicated by museum records, particularly a specimen in the American Museum of Natural History (AMNH 193786) from the Red River in Arkansas. During the present century, however, the range has contracted considerably due to deteriorating environmental conditions. The present range is limited to the Bayou Teche drainage (Vidrine, 1985) and a single stream in the Red River drainage. The drastic reduction in range has elicited concern from the federal government, which has provided protection for the remaining populations (Stewart, 1988).

Despite increased concern for *M. hembeli*, very little is known about this species other than the characteristics of its shell. Ortmann (1912) provided a brief description of the anatomy of *M. "hembeli,"* but the specimens upon which he based his description came from Alabama and, therefore, are appropriately referred to *M. marrianae*. Hence the anatomy of *M. hembeli* remains undescribed, and nothing is known about its biology. The present study provides some information on gonadal activity and details of the anatomy of the stomach and nervous system. Comparisons are made with other North American margaritiferid species, including *M. marrianae*, as studied by Smith (1979a, 1980, 1986, and unpublished).

MATERIALS AND METHODS

A total of 43 partially or completely relaxed, preserved, specimens were studied. All were collected on various dates from 1973 to 1986 from Brown Creek, Gardner, Rapides Parish, Louisiana. Specimens had been fixed in 10% formalin, washed in water, and stored in 50% isopropyl alcohol. Five specimens lacked information on date of collection and were utilized for dissection purposes only. The remaining lots were used for histological investigations of gonadal activity and sexual characteristics, as well as for anatomical studies. The collection dates and numbers of specimens used in the study of gonadal activity were as follows: October 1, 1973 (1 specimen); October 5, 1974 (4 specimens); February 22, 1975 (8 specimens); March 28, 1975 (4 specimens); April 25, 1975 (3 specimens); June 21, 1975 (2 specimens); March 30, 1986 (5 of 16 specimens).

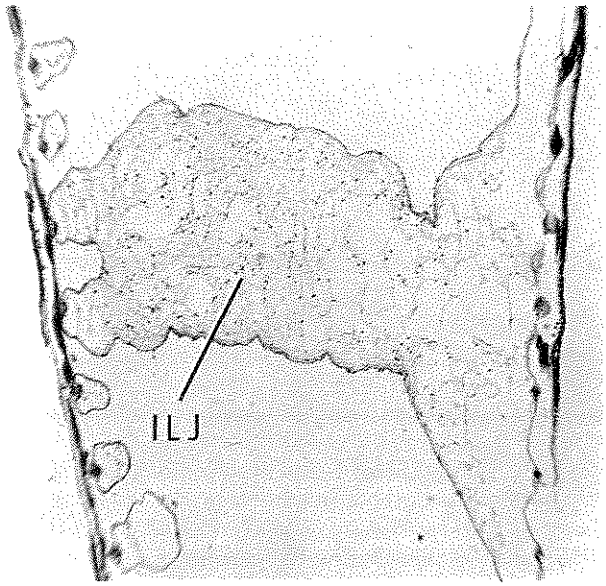


Figure 1. Photomicrograph of a transverse section through the gill of *Margaritifera hembeli* showing the interlamellar junction (ILJ), 30 ×.

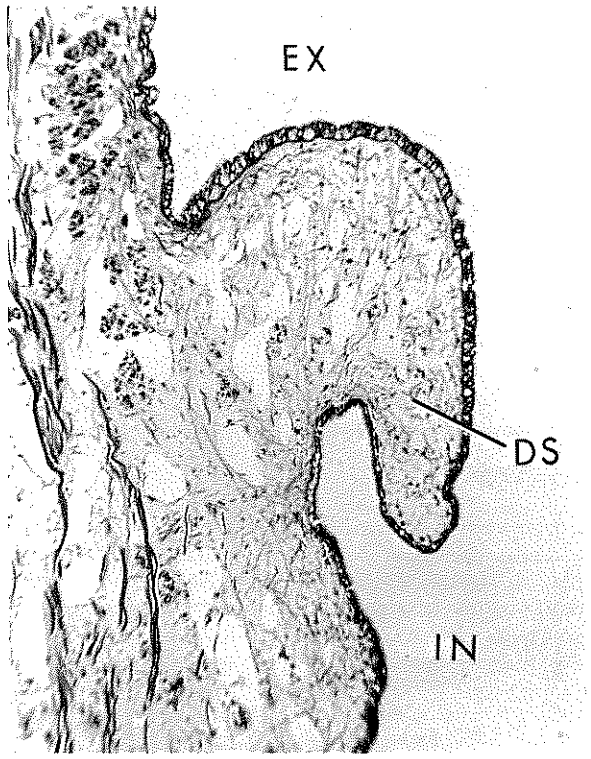


Figure 2. Photomicrograph of a transverse section through the posterior portion of the mantle of *Margaritifera hembeli* showing the partially contracted diaphragmatic septum (DS), which separates the exhalent (EX) and inhalent (IN) chambers, 80 ×.

Portions of the viscera of each specimen were infiltrated with paraffin, sectioned at seven micrometer thickness, and stained with Ehrlich's hematoxylin and eosin. Some sections were stained in a picro-ponceau connective tissue stain following the method described in Humason (1979:147). At least five slides were prepared for each specimen. The barren gills of two specimens and a portion of the posterior mantle lobe of one specimen were also sectioned in a similar manner and stained with picro-ponceau connective tissue stain.

Dissections were undertaken on the stomach and visceral nervous system of eight specimens. Three specimens were investigated for gross morphology of the gills, nervous system, and excretory system. The method of dissection and exposition of specific internal organs, and the terminology used to describe various organ components, follows Smith (1980, 1986).

All material relevant to this study has been cataloged in the invertebrate collections of the Museum of Zoology (Nos. MO. 1643–1645), University of Massachusetts, Amherst, Massachusetts.

RESULTS

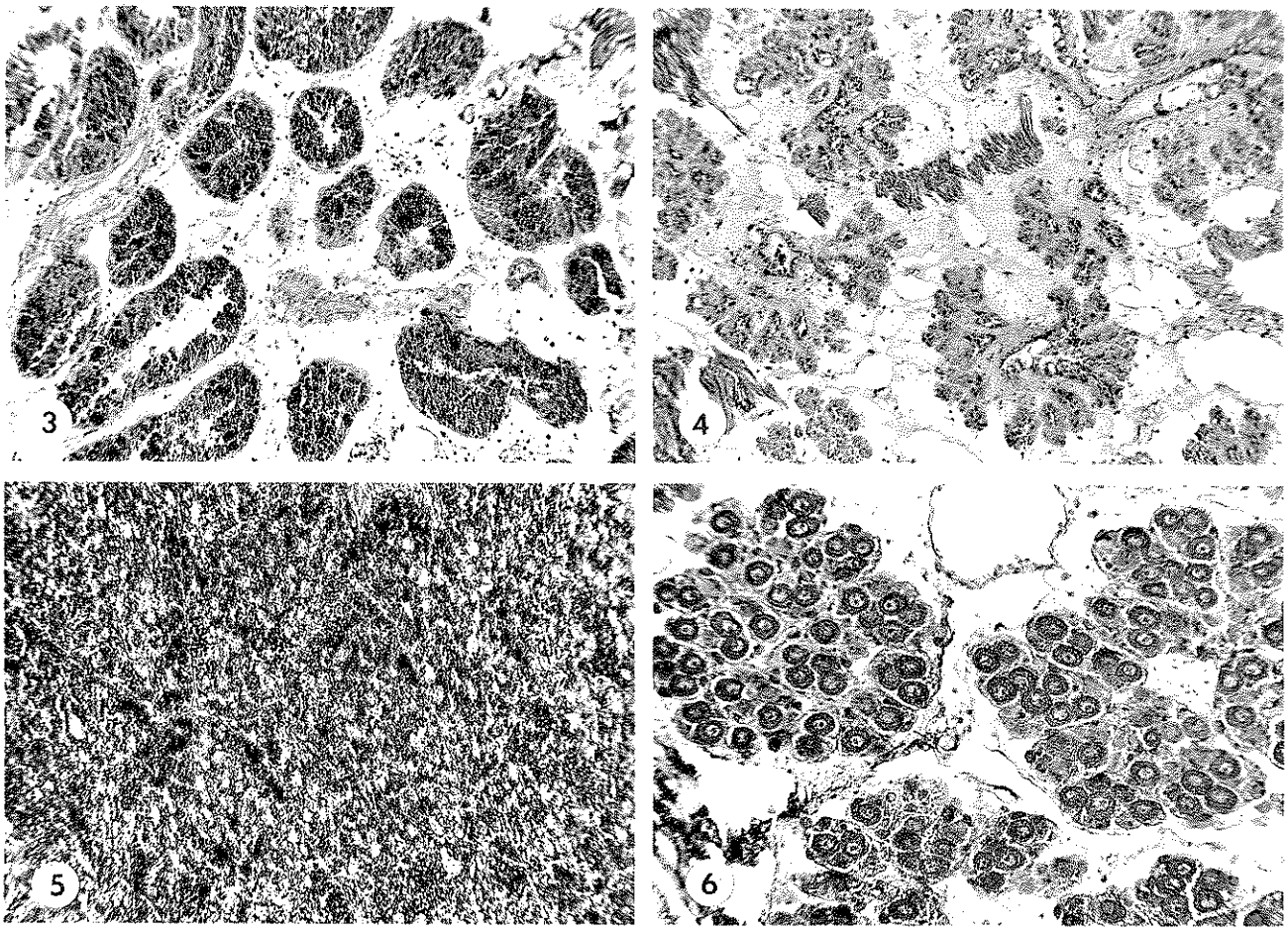
Gross Anatomy

Anterior and posterior adductor muscles subequal, foot musculature and associated pedal and retractor muscles well developed. Cerebral and pleural ganglia fused, kidney with both glandular and non-glandular chambers, renal pore and gonopore closely set but clearly separate. Labial palps falcate and large, gills or demibranchs lamellar, inner gill larger than outer gill, both inner and outer gills free from mantle posterior to pallial line. Both lamellae of each gill held together by solid, separate interlamellar junctions (figure 1, ILJ), lined with squamosal epithelium and composed of loose connective tissue and fine fibers that are more muscular appearing than collagenous (in *M. margaritifera*, see Smith, 1979a). Interlamellar junctions for the most part arranged in oblique rows in typical margaritiferine fashion (Ortmann, 1912; Smith and Wall, 1983: fig. 1b), similar to gills of *M. marrianae* (Ortmann, 1912:235). Gill junctions are somewhat patternless along lower margin and anterior and posterior extremities of each gill plate.

Mantle lobes free all around, no indication of division of exhalent region of mantle margins into separate anal and supra-anal apertures. Inhalent and exhalent chambers separated posteriorly by diaphragmatic septa (figure 2, DS), and though not observed in the living animal, are presumed to function similar to *M. margaritifera* (Smith, 1980). Inhalent margin of mantle with densely pigmented papillae, exhalent region pigmented, with crenulate margin.

Gonadal Activity and Sexuality

All animals examined histologically were sexually mature, including several small specimens ranging in shell length from 49 to 69 mm (believed to be from 6 to 9



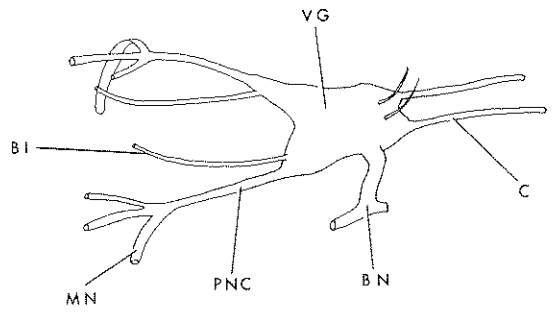
Figures 3–6. Photomicrographs of histological sections through male and female gonads of *Margaritifera hembeli*. **3.** Gonad of a male specimen of *M. hembeli* collected in February, following spawning of gametes, 65 ×. **4.** Gonad of a female specimen collected in February following spawning, 65 ×. **5.** Gonad of a male specimen of *M. hembeli* collected in October and showing male gametes which have filled the entire gonadal stroma, 100 ×. **6.** Gonad of a female specimen of *M. hembeli* collected in October and containing fully developed ova, 100 ×.

years old on the basis of shell annuli). This would suggest that *M. hembeli* matures at an earlier age than North American *M. margaritifera* (Smith, 1979b). No evidence of hermaphroditism was observed.

Although no gravid females were among the available specimens, a specific reproductive cycle was indicated by the gonads of sectioned specimens. Animals collected in February, March, and April showed characteristic post-spawning features (figures 3, 4) including partial or complete occlusion of gonadal acini by granules, presumed pycnotic cells, and unspawned gametes in various stages of development or cytolysis. Animals collected in June showed little change from April specimens indicating that complete resorption of reproductive tissues, corresponding to an undifferentiated stage of non-reproductive activity, apparently does not occur. By early October, gonadal activity is resumed and sex cells, including the latter stages of spermatogenesis and oogenesis, are abundant within all observed acini (figures 5, 6). It is therefore hypothesized that the oviposition of eggs into marsupial demibranchs and the spawning of male gametes takes place sometime between late November and late December with release of larvae occurring in late December or January. Based on examined specimens, there is no evidence that production of glochidial larvae takes place at any other part of the year.

Visceral Nerve Anatomy

In *M. hembeli*, the first pallial bifurcation (figure 7, BI) of the posterior nerve is well anterior of the mantle nerve separation, usually arising from the visceral ganglion itself at a point near the origin of the posterior nerve cord (figure 7, PNC). Some variation exists in the position of this bifurcation relative to the visceral ganglion. The overall pattern differs from that observed in other eastern North American margaritiferids including *M. marrianae*, in which the first bifurcation is normally posterior of the visceral ganglion, but similar to that observed in *Cumberlandia monodonta* (Smith, 1980, unpublished data). Moreover, in both *M. hembeli* and *M. marrianae*, the accessory nerve, which is typically present in *M.*



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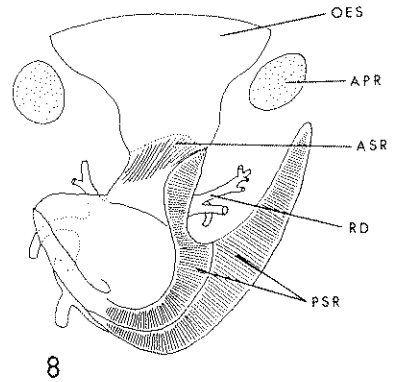

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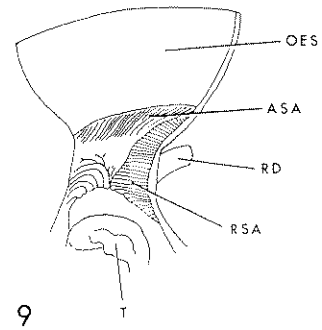

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Figures 7–9. Anatomy of the visceral nervous system and stomach of *Margaritifera hembeli*. **7.** Visceral ganglion (VG) of *M. hembeli*, 20 ×. **8.** Morphology of the stomach roof of *M. hembeli*, 5 ×. **9.** Morphology of the stomach floor of *M. hembeli*, 5 ×. APR, anterior protractor muscle; ASA, anterior sorting area; ASR, anterior sorting area of roof; BI, first pallial bifurcation; BN, branchial nerve; C, commissure to cerebropleural ganglion; MN, mantle nerve; PNC, posterior nerve cord; OES, esophagus; PSR, posterior sorting area; RD, right duct; RSA, right side sorting area; T, major typhlosole.

margaritifera and *C. monodonta* (Smith, 1980), is almost always absent, being observed only once in *M. marrianae* (Smith, unpublished data).

Stomach Anatomy

In addition to the posterior sorting area, which is typical of other eastern North American margaritiferids, the stomach roof of *M. hembeli* contains a well developed anterior sorting area and a continuation of the posterior sorting area extending along the right side of the stomach roof and separated from the anterior sorting area by a ridge (figure 8).

The floor of the stomach interior (figure 9) is characterized by an anterior sorting area that is somewhat similar to *M. marrianae* (Smith, 1986: fig. 4b); however, the right side sorting area of *M. hembeli* is completely unlike *M. marrianae* in that a distinct platform is absent in *M. hembeli* and the sorting ridges run primarily laterally, rather than in the anterior-posterior pattern seen in *M. marrianae*. A groove sets off the right side sorting area from the anterior sorting area in *M. hembeli* and the morphology of the stomach floor sorting areas of *M. hembeli* can be considered more similar to *M. margaritifera* in overall appearance than to other eastern North American species.

DISCUSSION

As demonstrated by anatomical investigations of the visceral nervous system and the stomach (this study), and conchological differences discussed elsewhere (Johnson, 1983; Smith, 1983), *M. hembeli* and *M. marrianae* clearly represent distinct lineages within the genus *Margaritifera*. Although both species have lateral teeth, these teeth are also present in *M. auricularia* (southern Europe) and *M. laosensis* (southeast Asia). Lateral teeth may therefore represent structures that have arisen separately in different "stocks" of margaritiferid species, or may be symplesiomorphies indicative of an ancestral relationship.

The presence of a corrugated surface of the posterior slope of the shell (weakly expressed in *M. hembeli*) and the close geographical proximity of these two species are the strongest lines of evidence indicating a relationship between them. Nevertheless, the pattern of visceral nerve bifurcation and stomach morphology in *M. hembeli* and *M. marrianae* show interspecific variation as great as that observed between either of these two species and *M. margaritifera* or *C. monodonta*. Biochemical data of the sort developed for *M. margaritifera*, *C. monodonta*, and *M. hembeli* by Davis and Fuller (1981) is not available for *M. marrianae*, precluding a comparison of genetic distances among the four species. However, the available biochemical data (Davis and Fuller, 1981), combined with anatomical information presented in this study and elsewhere (Smith, 1980, 1986), clearly supports the concept that the North American species of the Margaritiferidae have been isolated from one another for a considerable period of time (Smith, 1976).

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LITERATURE CITED

Conrad, T. A. 1838. Monography of the family Unionidae, or naiades of Lamarck (fresh water bivalve shells) of North America, Numbers 10, 11. Philadelphia, PA, p. 81–102.

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

Humason, G. L. 1979. Animal tissue techniques, 4th ed. W. H. Freeman & Co., San Francisco, 661 p.

Johnson, R. I. 1983. *Margaritifera marrianae*, a new species of Unionacea (Bivalvia: Margaritiferidae) from the Mobile-Alabama-Coosa and Escambia River systems, Alabama. Occasional Papers on Mollusks (Harvard University) 4:299–304.

Ortmann, A. E. 1912. Notes upon the families and genera of the najades. Annals of the Carnegie Museum 8:222–365.

Smith, D. G. 1976. The distribution of the Margaritiferidae: a review and a new synthesis. Bulletin of the American Malacological Union 1976:42 (abstract).

Smith, D. G. 1979a. Marsupial anatomy of the demibranch of *Margaritifera margaritifera* (Lin.) in northeastern North America (Pelecypoda: Unionacea). Journal of Molluscan Studies 45:39–44.

Smith, D. G. 1979b. Sexual characteristics of *Margaritifera margaritifera* (Linnaeus) populations in central New England. Veliger 21:381–383.

Smith, D. G. 1980. Anatomical studies on *Margaritifera margaritifera* and *Cumberlandia monodonta* (Mollusca: Pelecypoda: Margaritiferidae). Zoological Journal of the Linnean Society 69:257–270.

Smith, D. G. 1983. On the so-called mantle muscle scars on shells of the Margaritiferidae (Mollusca: Pelecypoda), with observations on mantle-shell attachment in the Unionoida and Trigonioida. Zoologica Scripta 12:67–71.

Smith, D. G. 1986. The stomach anatomy of some eastern North American Margaritiferidae (Unionoida: Unionacea). American Malacological Bulletin 4:13–19.

Smith, D. G. and W. P. Wall. 1983. The Margaritiferidae reinstated: a reply to Davis and Fuller (1981), "Genetic relationships among Recent Unionacea (Bivalvia) of North America." Occasional Papers on Mollusks (Harvard University) 4:321–330.

Stewart, J. H. 1988. Endangered and threatened wildlife and plants; final endangered status for the Louisiana pearl shell "Margaritifera hembeli." Federal Register 53:3567–3570.

Vidrine, M. F. 1985. Fresh-water mussels (Unionacea) of Louisiana; a zoogeographical checklist of post-1890 records. The Louisiana Environmental Professional 2:50–59.