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**SEXUAL DIMORPHISM IN THE INFLATED HEELSPLITTER,  
POTAMILUS INFLATUS (LEA, 1831) (UNIONIDAE: LAMPSILINAE),  
AND OBSERVATIONS ON ITS REPRODUCTIVE BIOLOGY**

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**Sexual dimorphism in the inflated heelsplitter, Potamilus inflatus (Lea, 1831) (Unionidae: Lampsilinae), and observations on its reproductive biology.**

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ABSTRACT

Potamilus inflatus demonstrates a unique sexual dimorphism in which males are considerably larger than females. Additional characteristics which differ between the sexes are shell shape and nacre color. Shell shape differs between the sexes in that females have slightly more rounded, inflated posterior margins. The shell nacre of females is bluish-white in color, while that of males is purple. Females of the species sexually mature in their first year of life. The species may complete two reproductive cycles/year. Extreme size dimorphism between the species, as well as a uniquely toothed glochidia support the taxonomic validity of Potamilus inflatus (Lea 1831).

## INTRODUCTION

Potamilus inflatus (Lea, 1831) is found in the Amite River, Louisiana, and the lower Tombigbee and Black Warrior rivers, Alabama, and is listed as a threatened species by the U.S. Fish and Wildlife Service (1990). Though P. inflatus was described in 1831, no information on its reproductive biology or observations of gravid females or glochidia have been reported. The absence of such basic information appears to have gone unnoticed until 1990, when USFWS biologists began searching for information on P. inflatus glochidia in an attempt to resolve a suspicion that it is a southern ecomorph of Potamilus ohioensis or Potamilus alatus. During routine surveys between 1990-1993, USFWS biologists made field examinations of what were considered “mature adults” (generally specimens greater than 100 mm in shell length). The mussels were gently pried open to determine sex (i.e., differentiation of the outer gill as a marsupium) and gravidity. No females were identified, or gravid specimens found until June 1993, when a full marsupium was noticed in a 35 mm (shell length) individual. Closer examination of all size classes in the June 1993 collections revealed the majority of smaller specimens (< 90 mm) to be gravid, while continued examination of specimens greater than 90 mm revealed no gravid individuals. Such extreme size differentiation between the sexes is unknown in the Unionidae.

Sporadic hermaphroditism has been reported for both Potamilus alatus and P. ohioensis (van der Schalie 1980), and sex reversal in Anodonta had been considered by Heard (1975). Our preliminary field observations of small “juvenile” females and larger “mature” males suggested that P. inflatus might undergo protogeny, defined by Hoagland (1984) as the case in which an organism matures as a female, then becomes male. The presence of a few non-gravid “juveniles”, however, suggested that the considerable size differences between male and female P. inflatus might be a sexually dimorphic character. This paper summarizes the results of additional collections and observations, as well as histological examinations of gonadal tissues, from two populations of P. inflatus that were conducted between 1993-1995 in an attempt to determine sexual and reproductive strategies in the species.

## MATERIALS AND METHODS

Four collections were made using SCUBA from a large meander bend in the Black Warrior River, RM 327.8, Tuscaloosa County, Alabama. Potamilus inflatus were found throughout the the area and from a variety of depths (1-7 meters) and bottom types, including silt, mud, sand, and sandy gravel. All collections were qualitative. On 10 June 1993, 26 P. inflatus were collected and examined for gravidity. Six gravid females and one nongravid (112 mm) individual were retained for dissection. On 14 October 1993, 10 P. inflatus were collected and examined. Three large individuals (>100 mm) and four smaller specimens (<100 mm) were kept for dissection. On 5 August 1994, 18 P. inflatus were collected and examined. One larger specimen (118 mm) and seven smaller specimens (< 100 mm) were retained for dissection. On all dates, specimens not kept for dissection were returned to the river. On 27 June 1995, an additional 12 live P. inflatus were collected from the Black Warrior River study site. Their position in the substratum was noted and the specimens were sexed, measured, and returned to the river.

On 22 September 1993, 16 P. inflatus were examined from a reach of the Amite River, between Denham Springs and Watson, Livingston Parish, Louisiana. All were found by sight or touch in shallow water in a sand or sandy gravel substratum. Three individuals were retained for dissection. On 2 August 1994, 17 specimens were collected from the Amite River using SCUBA between Port Vincent and U.S. Interstate 12, East/West Baton Rouge Parish, Louisiana, and preserved for dissection. Potamilus inflatus were found at depths of 0.5-5 meters, in primarily mud or silt bottoms. An additional 14 alcohol preserved specimens, collected from the Amite River in 1986, were included in the study.

Tissues were removed from the shell and fixed for several days in 10% formalin, then preserved in 70% ethanol. Cross sections of tissue were removed from the approximate center of the visceral mass. Tissues were infiltrated with paraffin, and thin sections (5 $\mu$ m to 7 $\mu$ m) were stained using either PAS with malachite green and hematoxylin counterstains or standard hematoxylin/eosin procedures (Humason 1979). Tissues were examined with a compound microscope under various magnifications. Sex was determined by the types of germ cells present

in the gonad. Once the sex had been determined for an individual, the entire section was systematically scanned to search for germ cells characteristic of the opposite sex.

A single female collected during the June 10, 1993, sample contained fully mature glochidia. A small sample was sent to the Illinois Natural History Survey for SEM photography.

## RESULTS

Of 66 P. inflatus from the Black Warrior River visually examined for gravidity in situ, 21 were determined to be female by the presence of distinct marsupia. Females ranged in size from 29 mm to 94 mm in shell length (SL), and averaged 60mm SL. Males ranged from 38-118 SL, with an average of 97 mm SL. Glochidia from all six gravid females retained in the 10 June 1993 sample had developed the ax-head shape, typical of Potamilus. The marsupia of one female in this sample were brown in coloration and found to contain mature glochidia, while the marsupia in the five other females were creamy white in color, and their glochidia were still enclosed in embryonic membranes. In the October, 1993, sample three females were collected with glochidia enclosed in embryonic sacs present in their white marsupia. No glochidia were found in five females examined during the August, 1994, sample, however the marsupia were distinctly swollen and contained elongated, bivalved, embryonic larvae. In the seven gravid females examined on 27 June 1995, the marsupia were brown in coloration, and approximately 1/4-1/2 of the anterior demibranchs had been discharged. It is presumed these animals were in the process of releasing mature glochidia.

No female P. inflatus were collected from the Livingston Parish site on the Amite River on 22 September 1993. Four females were collected from the East/West Baton Rouge Parish site on 2 August 1994. All four had distinct marsupia with early embryos. A single female with immature glochidia was present among the 14 specimens collected on 2 October 1986.

Forty-nine specimens of P. inflatus were examined histologically. Five of these were unusable due to poor preservation or staining. No asexual or hermaphroditic intermediate stages were encountered among the specimens examined. All specimens contained typical germ cells for

either male or female (Figure 1). The histological examination confirmed field sexing, with no additional females identified.

Histological confirmation of sex supported additional aspects of sexual dimorphism in shell morphology. These included bluntly rounded or slightly truncate posterior margins in females, more pointed posteriors in males; and bluish-white nacre color in females, purple nacre in males. There also appeared to be differential growth rates between the sexes, with external shell growth annuli of males appearing considerably more widely separated than in females, however, annuli beyond the first are often indistinct, particularly in the male shells.

Examination of the glochidia of P. inflatus revealed them to be distinct within the genus Potamilus, and with a character unique within the Unionidae. The glochidia are intermediate in size (height approximately 200um), axe-head shaped and ligulate, and with small lateral hooks (Figure 2). The dorsal margin is slightly curved, and the ventral margin is strongly curved. As in other Potamilus, the ventral margin is sculptured with fine micropoints, however, the ventral margin of the glochidial shells are also produced into up to 10 strong, triangular, incurved teeth of varying size (Figure 3). Such glochidial armature is unlike any reported from the Unionidae.

## DISCUSSION

Results from this study indicate that sex-specific size differences in P. inflatus represent sexual dimorphism and not protogyny. Histological examination of P. inflatus gonadal tissues revealed no intersex specimens, i.e., hermaphroditic or asexual. Though the sample size was relatively small (N=49) due to the threatened status of P. inflatus, we expected to find some indication of sex change if the species was protogynous.

Sexual dimorphism has been recognized in the shells of some species of the Unionidae since at least 1834 (Kirtland 1834). Such dimorphism is typically expressed in characters of shell shape, and is related to the nature of the species reproductive strategy. For example, shell growth in females may become distorted as shell layers are added around the distended marsupia during the brooding period. Males, which do not brood offspring, are typically less inflated posteriorly than females. The longer the brooding period, the greater the disparity of shell shape between the

sexes. Most Lampsilinae, to which P. inflatus belongs, are bradytictic, with a relatively brief spawning period, followed by an extended brooding period. Thus the most dramatic sexual dimorphism is seen in this subfamily.

The dimorphic shell shape common to the lampsilines was found in P. inflatus, and although the differences were subtle, they were distinct even in the smallest specimens observed (29 mm gravid female, 38 mm male). Apart from the early sexual maturity of the species reflected in shell shape, the most outstanding aspect of sexual dimorphism was in shell size, with males growing considerably larger than females (Figure 4). Size dimorphism has been previously reported in unionids, specifically in Potamilus alatus (Ortmann 1919), but not to the degree exhibited by P. inflatus.

It has been shown that female fecundity is strongly related to age and size in some marine mollusks (Hoagland 1978), and such is presumed to be the case in the Unionidae, although no studies quantifying this relationship in unionids could be found. It is likely that a reduced size in female P. inflatus would be accompanied by a decrease in fecundity. Sacrifice of fecundity for reduced body size would be disadvantageous without some compensation in life history or reproductive strategy. Our observations suggest that such compensation is in the form of early sexual maturity, and possibly through iteroparity, or multiple brood production.

Early sexual maturity is evidenced in P. inflatus by the gravidity of the smallest females encountered during the study. The collection of gravid females bearing 0-1 annulus indicates females mature during their first year of life.

Observations on glochidial stage and their release in the Black Warrior River population of P. inflatus support a multiple cohort hypothesis. Of the gravid females examined during early June 1993, only one contained mature glochidia. All others collected on this date contained distinctly axe-head shaped glochidia enclosed in embryonic sacs. Females collected during October 1993 also contained distinct glochidia in sacs. The three females retained for dissection from the early August 1994 sample, however, contained early stage embryos, i.e., ligulate and bivalved, but without the characteristic axe-head shape. These observations suggested that glochidial release was occurring in late June or early July. This was confirmed by the late June, 1995, observation of seven partially discharged, gravid females containing mature glochidia. The

relatively short period (just over one month) between glochidial release and newly gravid females supports observations (Zale and Neves 1982) that production and maturation of eggs and sperm are synchronized in lampsilines, with spawning occurring over a short period.

Typically, lampsiline glochidia mature in the fall, shortly after spawning, and are retained in the marsupia until the following summer (Lefevre and Curtis, 1910a, 1910b; Ortmann, 1919). The presence of late embryonic glochidia in P. inflatus just prior to spawning may indicate the production of at least one other generation during the year. If this is not the case, the glochidia of P. inflatus are held overwinter as immatures, a condition not known by us to occur in any other unionid species.

The first annulus, an indication of the first year growth, is usually quite distinct in the shell of both sexes of P. inflatus. In specimens we have collected, there is great variation in length of the first annulus among specimens that are two or more years old. First annulus length ranged from 20-48 mm in females, and 20-63 mm in males. Obviously, such differences in growth can be attributed to a variety of factors (e.g., individual variation, habitat, food, etc.), however, a biannual reproduction should result in a two cohorts within a single years offspring exhibiting variation in first year growth. For example, individuals resulting from a winter release would be subject to long encystment periods (temperature related), and a long first growing season, while a summer cohort would have a shorter growing season. Such differences in growth period should be reflected in the length of the first annulus. Although our sample is too small to indicate a significant bimodal pattern, the wide variation observed in first year growth rates may be related to biannual reproduction in the species.

Based on the observations presented above, we believe the early maturity and possible multiple cohort production in P. inflatus are adaptations to, or causes of, the extreme size dimorphism observed between the sexes of this species. Such adaptations may be compensatory for the loss of fecundity that would accompany a reduction in size of the female. On the other hand, energy requirements resulting from early maturity and multiple cohort production by females of the species could have resulted in the size differences noted between the sexes.



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Figure 1: Cross sections of Potamilus inflatus gonadal tissues (male on top and female on bottom).

Figure 2: Lateral view of the glochidium of Potamilus inflatus.

Figure 3: Inner view of the ventral margin of a Potamilus inflatus glochidial valve.

Figure 4: Sexual dimorphism in the shells of the Black Warrior River population of Potamilus inflatus. Total length (TL) of pictured males (on left) ranges from 38-130 mm; note their pointed posterior margins. TL of pictured females (on right) ranges from 35-94 mm; note their blunt posterior margins. The 94 mm female is the largest specimen known of the sex.