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Determination of the fish host
of the Inflated Heelsplitter
Potamilus inflatus (Lea 1831)

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Abstract - The inflated heelsplitter, *Potamilus inflatus* was once found in many river systems in the southeastern United States, but is now restricted to three rivers: the Amite and Pearl rivers in Louisiana and the Black Warrior river in Alabama. In 1990, the U. S. Fish and Wildlife Service listed *P. inflatus* as a threatened species because of its diminished range and potential threats to its continued survival in those rivers where it still occurs (Federal Register 1990).

A survey of the fishes of the Black Warrior river was undertaken in order to determine the fish host of the inflated heelsplitter, with the understanding that this knowledge would be integral to the recovery and de-listing of this distinctive mussel. Seven hundred and seventy five individuals representing 31 species of fishes were examined; mussel glochidia were found on 10 individual fishes representing 9 species. *P. inflatus* glochidia were only found infecting the freshwater drum (*Aplodinotus grunniens* (Rafinesque)). In addition, observations were made of spawning behavior of a female *P. inflatus*

INTRODUCTION

Potamilus inflatus (Lea, 1831) is a distinctive mussel which inhabits large rivers in the southeastern United States. The historical range of *P. inflatus* has decreased markedly in the last decade prompting concern about the conservation status of this organism. Little is known about the natural history of *P. inflatus*; such information is critical to effective conservation and species management. A critical stage in the development of all unionid mussels is the attachment and encystment of the glochidia larvae on a suitable fish host. Our goal in this study was to identify the fish host(s) of *P. inflatus* in the Black Warrior river.

The inflated heelsplitter is an oval, moderately inflated bivalve. The shell is thin and the periostracum varies from an iridescent straw-yellow, with fine rays in small individuals to a dark brown in the larger ones. The umbonal cavity is shallow and the nacre varies from light to dark purple. The left valve contains two thin blade-like lateral teeth and a single similarly shaped pseudo-cardinal, the right valve contains a single larger lateral tooth as well as a single pseudo-cardinal tooth similar in size and shape to that in the left valve. The most conspicuous aspect of the shell is

the large wing on the posterior portion of the dorsal margin. The wing comprises at least $\frac{1}{3}$ of the total height of the shell and unlike other members of *Potamilus* gently curves from its highest point down the posterior slope. In addition, there is also a smaller anterior wing which is often bent or broken in larger individuals. Examination of a limited number of specimens found during this study indicated sexual dimorphism in adult size, with females rarely attaining two thirds the shell length of males.

one-third

how many?

The inflated heelsplitter was known historically from the Amite and Tangipahoa Rivers in Louisiana, the Pearl River and Tombigbee Rivers in Mississippi, and the Black Warrior, Coosa and Tombigbee Rivers in Alabama (Hurd, 1974; Stern, 1976; Hartfield, 1988). Presently it is limited to the lower and middle reaches of the Amite River and Pearl River in Louisiana and in the Black Warrior River between the Demopolis Lock upstream to just below the Oliver Lock and Dam, Tuscaloosa Co., Alabama (U. S. Fish and Wildlife Service, 1992).

Reproduction in the inflated heelsplitter is similar to that of the ~~majority of~~ ^{the other} mussels in the family Unionidae. Prior to fertilization the eggs of the female pass into the suprabranchial chamber and then into the water tubes of the gills. It is in the water tubes that the eggs are fertilized. The developing embryos are then retained in the modified portion of the gill called a marsupium. Members of the genus *Potamilus* are long-term breeders; the eggs are fertilized in the summer and the embryos are not released for almost an entire year. Like other unionids, members of *Potamilus* possess a glochidium larvae which is temporarily parasitic on a fish host. After attachment to a host the glochidium is encysted as the tissue of the fish grows to cover it. It is during this stage, which can last from 10 to 190 days that the juvenile mussels develop their adult shell and anatomy that will enable them to begin life as a filter feeding member of the infaunal community. While all species of freshwater mussels do not appear to be host specific, members of the genus *Potamilus* appear to parasitize the freshwater drum (*Aplodinotus grunniens*) exclusively (Surber, 1913, Wilson, 1916, Cummings *et al.*, 1990). A single exception was reported by Surber (1913) who found glochidia of *P. ohiensis* on several white crappie (*Pomoxis annularis* (Rafinesque)).

encysted of this?

reference

?

The objective of this study was to determine the fish host(s) of *Potamilus inflatus* which is ~~federally designated as a threatened species~~ (U.S. Fish and Wildlife Service, 1990). Such information may prove useful in determining reasons for the decline in the distribution of this species, and suggest appropriate measures for species management and recovery.

METHODS

Glochidia of *Potamilus inflatus* are axe-head shaped, having a narrow dorsal margin and body that is expanded broadly along the ventral margin. They most closely resemble the glochidia of two congeners, *Potamilus capax* (Green) and *Potamilus ohioensis* (Rafinesque) (Surber 1913, Cummings *et al.* 1990). A survey was conducted from June 27 through July 28 1995 in the Black Warrior River at 12 Mile Rock, (river mile 327.3), Tuscaloosa Co. Alabama. One collection was made at Choctaw Bend, Greene Co., Alabama, in the river bend way below the Warrior Dam (between river mile 262 and 261) on 14 July 1995 (Fig. 1). Both sites were selected because those portions of the river were known to contain populations of *P. inflatus*. It was thought that by concentrating our efforts in these areas when female mussels were releasing glochidia we would increase the likelihood of encountering fish infected with *P. inflatus* glochidia. A preliminary survey on June 27, 1995 of adult mussels at 12 mile rock indicated that female *P. inflatus* were already releasing glochidia.

A general description of the study area can be found in Williams *et al.* (1992). Collections of fish were made twice a week ~~every week for the duration of the study.~~ Several collection methods were employed and included experimental gill netting, seining, and hook and line. ~~Fishes collected~~ were preserved in the field in a solution of 10% formalin and examined for the presence of glochidia on the fins and gills using a dissecting microscope. Gills harboring glochidia were isolated and placed in a solution of 70% ethanol for later identification using light microscopy. Gills containing *P. inflatus* glochidia were placed in a solution of 10% trypsin to clear the tissue. Illustrations of glochidia were made using a dissecting scope.

Observations were made daily on a single gravid female clam ~~mussel~~ collected on June 27 1995. The clam was kept in a 10 gallon aquarium

for what?

would you know this

for X weeks

for how long?

how does one illustrate with a dissecting scope?

is this important?

containing river water and sediment for a period of four weeks ~~and~~ afterwards ~~was returned to the river.~~ Glochidia recovered from the ~~live~~ spawning female *P. inflatus* and glochidia from a preserved female *P. purpuratus* were prepared for scanning electron and light microscopy for comparative purposes. The glochidia were removed from the gills, digested in 1% trypsin, and transferred to 20% ethanol. They were then air dried and mounted for examination. Measurements of glochidia were made using an ocular micrometer. No attempt was made to identify the other glochidia found during this study.

~~live and temp~~ (Place Figure #1 Here)

RESULTS

During the preliminary survey, live *P. inflatus* were found in mud and sand overlain by silt in approximately 4-5 m³ of water. Subsequent ~~encounters with live *P. inflatus* made while collecting fishes~~ observations of less than 2 m³ of water.

~~infectious~~ A total of 755 fishes representing 30 species were collected and examined for glochidial infection (Table 1). Common and scientific names of fishes follow Robins *et al.* (1991). The number of fish species examined represented 61% of those reported by Mettee *et al.* (1989) between the Oliver Lock and Dam and the Warrior Lock and Dam (Fig. 1). All glochidia were ~~found~~ attached to or encysted in the gills; no glochidia were observed on the fins of any fishes examined. Ten of the 755 specimens were infected with glochidia and one of the 37 specimens of *Aplodinotus grunniens* was infected with glochidia identified as *Potamilus inflatus*. The drum was collected on 10 July 1995, and it is unknown how long the glochidia were attached to the gills.

(Place Table 1 Here)

The only other species of *Potamilus* occurring in the Black Warrior River is *Potamilus purpuratus* (Lamarck) (Williams *et al.*, 1992). The glochidia of these two species are readily distinguishable from each other. The glochidia of *P. inflatus* are small, ~~avg~~ ^{avg} length 0.188 mm (s.d. = .01 mm, n=10); and axe-head shaped possessing large lanceolate hooks on the anterior and posterior edges of both valves. Between these large hooks are a number of smaller bifurcate hooks. A row of small tooth-like projections is present ventral to the hooks, with some extending onto the

~~scientific illustration or photomicrograph~~ is needed.

base of the hooks (Fig 2a,b). The glochidia of *P. purpuratus* are twice as large, averaging 0.371 mm in length (s.d. = .001 mm, n=10) and are elongate and strap-like; they also possess large lanceolate hooks on the anterior and posterior margins of the valves but lack the smaller bifurcate hooks found on *P. inflatus*. The small tooth-like projections are also present on the ventral margins of both valves (Fig. 2c,d). The ~~*Potamilus inflatus*~~ glochidia were well encysted in the gill tissue of their host (Fig. 3). The axe-head shape, hooked teeth and minute adult valves were clearly visible.

The gravid female *Potamilus inflatus* that was retained for observation completely buried ^{itself} within ^{2 hr} two hours of its placement in the aquarium. The animal remained completely buried for the duration of the study. No siphoning or other behavior was noted, and the only indication of the position of the ^{mussel} clam was a slight depression in the sand. On July 16, 1995, an eyedropper was used to siphon water and substrata from the depression. Examination of the water revealed individual glochidia ^{and} as well as strings of glochidia loosely bound in a mucous-like substance. Glochidia were recovered until the last week in July, at which time the animal began to siphon continuously.

(Place Fig.# 2 Here)

(Place Figure #3 Here)

DISCUSSION

Confirmation of the freshwater drum as the host of *Potamilus inflatus* in the Black Warrior River has some ramifications for efforts to de-list the inflated heelsplitter. Although female *Potamilus inflatus* were actively discharging glochidia when the study began, only one of the 37 freshwater drum collected (2.7 %) was infected with their glochidia. Low infection rates might be due to low numbers of host fish or alternatively depressed numbers of gravid female mussels. Freshwater drum are widespread and appear to be relatively common in the Black Warrior River (Mettee *et al.*, 1989) indicating that the first scenario is unlikely. Quantitative assessments of *P. inflatus* in the Black Warrior are in progress and should provide information concerning female abundance and recruitment of juvenile mussels.

Previously reported infection rates for other members of *Potamilus* are comparable to those found in this study. Surber (1913) indicates that only 2% of the *A. grunniens* in his survey were infected with the glochidia of *P. ohiensis*. Weiss and Layzer's (1995) examination of glochidial infestations of the fishes of the Barren River in Kentucky found the number of infected fish for *P. alatus* was 3%. The 1990 survey by Cummings *et al.* of the glochidial host of *P. capax* found that 6 of the 8 *A. grunniens* collected (75%) were infected with glochidia of *P. capax*. When reported the total number of fishes infected with *Potamilus* glochidia in these studies is small, which may indicate that the low percentage of infected fishes ~~observed~~ are a natural component of this species life history.

Currently the mode of transmission of glochidia from mussel to host in *Potamilus inflatus* is not known. Observations of the single female ~~clam~~ *mussel* indicate that glochidia are not released into the water but at the sediment water interface and not all at once but gradually over several days. North American mussels display a variety of strategies for infecting host fishes. Some produce "lures" of one kind or another (Kraemer, 1970) so that the host fish, by taking the "bait" initiates infection, while others broadcast glochidia into the water. All modes of infection require relatively close contact between the gravid mussel and the host fish as glochidia are poor swimmers. Preliminary observations ~~based upon this study~~ indicate that *P. inflatus* release glochidia at or near the sediment-water interface. While freshwater drum are common in a river system they may not be locally abundant in stretches of the river inhabited by mussels. Changes in the river flow due to impoundment may have altered the habitat so that drum now rarely encounter the mussels and subsequently do not become infected. Swingle (1953) found a significant decrease in the abundance of freshwater drum following river impoundments, and hypothesized that flowing water was necessary for reproduction of the fish. Subsequent infection of freshwater drum appears to rely on the chance encounter of glochidia on the river bottom. These facts highlight the importance of maintaining appropriate habitat for *A. grunniens* in areas known to contain large numbers of *P. inflatus*. As the freshwater drum is known to be the host of many other species of unionids (Surber, 1913; Hoggarth, 1988) regular monitoring of *A. grunniens* stocks may prove useful for designing a recovery plan for *P. inflatus* as well as providing information regarding

lab
aquarium
only
(no current)

Speculation

potential host abundance for *P. inflatus* and other unionids in the Black Warrior river.

Speculation

The effects of barge traffic on the river particularly in those areas where populations of *P. inflatus* are known to occur should be determined. While conducting this survey we observed rapid fluctuations in the water level during the day presumably due to lock activity. During times when ~~of~~ the water was low the water temperature increased noticeably. These periods of increased water temperature could result in low oxygen levels which may adversely affect mussels in that area. Recovery of the Inflated Heelsplitter requires that recruitment of new individuals also takes place; this aspect of the reproductive cycle is dependent on the existence of adequate substrate onto which the glochidia can settle. For this reason populations of *P. inflatus* should be monitored to determine if recruitment of larvae is indeed taking place as outlined in the *P. inflatus* recovery plan (U. S. Fish and Wildlife Service 1992).

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Table 1 List of fishes reported from the Black Warrior River between the William Bacon Oliver Lock and Dam and the Armisted I. Selden Lock and Dam (Mettee et al. 1989), With Numbers of Each Species Examined for this Survey.

Species	N	Glochidia
Lepisosteidae		
<i>Lepisosteus oculatus</i>	8	-
<i>Lepisosteus osseus</i>	-	-
Amiidae		
<i>Amia calva</i>	-	-
Clupeidae		
<i>Alosa chrysochloris</i>	17	-
<i>Dorosoma cepedianum</i>	49	-
<i>Dorosoma petenense</i>	59	-
Esocidae		
<i>Esox niger</i>	-	-
Cyprinidae		
<i>Cyprinella venusta</i>	29	-
* <i>Cyprinus carpio</i>	1	-
<i>Hybopsis winchelli</i>	-	-
<i>Macrhybopsis storeriana</i>	9	-
<i>Notropis atherinoides</i>	13	-
<i>Notropis candidus</i>	75	-
<i>Notropis edwarddraneyi</i>	213	-
<i>Notropis texanus</i>	1	-
<i>Opsopoeodus emiliae</i>	-	-
<i>Pimephales vigilax</i>	47	-
Catostomidae		
<i>Carpiodes cyprinus</i>	5	-
<i>Carpiodes velifer</i>	11	+
<i>Ictiobus bubalus</i>	2	-
<i>Moxostoma erythrurum</i>	-	-
<i>Moxostoma poecilurum</i>	-	-
Ictaluridae		
<i>Amieurus natalis</i>	-	-
<i>Ictalurus furcatus</i>	2	-
<i>Ictalurus punctatus</i>	17	+
<i>Pylodictus olivaris</i>	-	-

Reduse

Aphrododeridae		
<i>*Aphrododerus sayanus</i>	1	-
Belonidae		
<i>Strongylura marina</i>	5	-
Fundulidae		
<i>Fundulus olivaceus</i>	-	
Poeciliidae		
<i>Gambusia affinis</i>	1	-
Atherinidae		
<i>Labidesthes sicculus</i>	2	-
Moronidae		
<i>Morone chrysops</i>	-	
<i>*Morone mississippiensis</i>	14	+
<i>Morone chrysops xM. saxatilis</i>	1	-
Centrarchidae		
<i>Lepomis macrochirus</i>	22	+
<i>Lepomis megalotis</i>	36	+
<i>Lepomis microlophus</i>	21	+
<i>Lepomis punctatus</i>	-	
<i>Micropterus punctulatus</i>	20	+
<i>Micropterus salmoides</i>	1	-
<i>Pomoxis annularis</i>	1	-
<i>Pomoxis nigromaculatus</i>	-	
Percidae		
<i>Percina shumardi</i>	-	
<i>Percina vigil</i>	-	
Sciaenidae		
<i>Aplodinotus grunniens</i>	37	+
Total	755	

List includes Fishes caught at Choctaw Bend on 14 July 1995: *Cyprinella venusta* (5), *Notropis atherinoides* (3), *N. edwardraneyi* (5), *Ictalurus punctatus* (1), *Labidesthes sicculus* (1), *Lepomis macrochirus* (1), *L. megalotis* (9), *L. microlophus* (4), *Micropterus punctulatus* (5), *M. salmoides* (1).

* Indicates fishes not reported by Mettee *et al.*, 1989.