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REPRODUCTIVE BIOLOGY AND HOST FISHES OF FOUR UNIONIDS FROM THE LAKE PONTCHARTRAIN BASIN, LOUISIANA, U.S.A.

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ABSTRACT

Host fishes, fecundity estimates, and gravid periods were identified for four species of freshwater mussels from the Lake Pontchartrain basin, Louisiana. Two of the mussel species have broad distributions both in the Mississippi River and elsewhere in Louisiana: *Villosa lienosa* (Conrad, 1834) and *Lampsilis teres* (Rafinesque, 1820). The other two species have more restricted distributions: *Quadrula refulgens* (Lea, 1868) and *Lampsilis ornata* (Conrad, 1835). *Lampsilis ornata* is listed as a species of concern in Louisiana. Of the 23 species of fishes tested as potential hosts, we found 4 previously unknown hosts for *Villosa lienosa*: *Lepomis megalotis*, *Lepomis humilis*, *Lepomis microlophus* and *Lepomis cyanellus*, and confirmed 2 previously documented: *Lepomis macrochirus* and *Micropterus salmoides*. *Villosa lienosa* was gravid from April until June and had an estimated fecundity of 38,562 + 3,073 glochidia/female. For *Lampsilis ornata* we established a host relationship with *Luxilus chrysocephalus*, and confirmed *Micropterus salmoides* as a host. *Lampsilis ornata* was gravid from February until April and had a fecundity estimate of 451,214 + 27,239 glochidia/female. *Lampsilis teres* was gravid from April until September and had a fecundity estimate of 407,333 + 24,727 glochidia/female. We confirmed three hosts for *L. teres*: *Micropterus salmoides*, *Pomoxis annularis*, and *Lepomis humilis*, and identified two new hosts: *Lepomis microlophus* and *Notropis venustus*. Only a single *Quadrula refulgens* was found gravid in late June and its fecundity was estimated at 32,450 glochidia and a host was identified as *Pylodictis olivaris*.

KEY WORDS mussels, host fish, Lake Pontchartrain

INTRODUCTION

Freshwater mussels (Unionidae) are among the most endangered aquatic animals in North America (Williams et al., 1993; Neves et al., 1998; Lydeard et al., 2004; Strayer, 2008). Their loss from lotic ecosystems could have considerable effects on ecosystem health and function, because they often provide food resources and physical structure for other macro-invertebrates (Vaughn et al., 2004; Howard & Cuffney, 2003) and are important in lotic nutrient cycling (Vaughn et al., 2008). Within the Lake Pontchartrain Basin of Louisiana there are 32 species of unionids (Stern 1976) with around 17% without identified fish hosts (Oesch, 1995; Howells et al., 1996; Keller & Ruessler, 1997; Watters et al., 2009). Understanding these host relationships is important because host diversity is a strong predictor of mussel diversity (Watters, 1992; Haag & Warren, 1998; Vaughn & Taylor, 2000; Strayer, 2008) and is also an important factor in dispersal, propagation (Newton et al., 2008; Strayer, 2008), and mussel recruitment (Newton et al., 2008).

In vivo host fish determination techniques have been described in several studies (Howard, 1916; Coker et al., 1921; Penn, 1939; Cope, 1959; Hove & Neves, 1994; Watters, 1994; Hove et al., 2000; Yeager & Saylor, 2007). The success of determining the host is based, at least in part, on knowledge of the natural history of the species. The complexity of some unionid life histories makes determination of their hosts difficult. Complicating factors include brooding period length, percentage of population that is gravid, and whether the mussel is a host fish specialist (Farris & Van Hassel, 2007).

The host fish is thus a critical component of the mussel's natural history and is required knowledge for successful conservation. We conducted host fish trails and collected natural history data on four species of mussels: the little spectacle case *Villosa lienosa* (Conrad, 1834), yellow sandshell *Lampsilis teres* (Rafinesque, 1820), purple pimpleback *Quadrula refulgens* (Lea, 1868), and southern pocketbook *Lampsilis ornata* (Conrad, 1835). For each species, we present data on their gravid period, fecundity, and host suitability. These abundant species are found in many of the larger rivers (*Lampsilis teres*, *Lampsilis ornata*, and *Quadrula refulgens*) or in the smallest drainages that support unionid species (*Villosa lienosa*) in the Lake Ponchartrain Basin, Louisiana. The identification of additional host fishes and data on reproductive biology should aid in future studies detailing important environmental influences on mussels, including a state species of concern, *L. ornata* (Gregory, 2009).

METHODS

Gravid females of all unionids were collected by hand from the Lake Pontchartrain Basin (Fig. 1) in the spring and summer 2008-2010 and transported immediately to the laboratory for host trials. Specimens were collected from the Amite, Tickfaw, and Tangipahoa rivers. All specimens were inspected in the field for engorged marsupia. Females were transported to the lab in aerated coolers. Mussels were isolated in glass aquaria with sand substrate and re-circulating river water.

Glochidia were obtained from gravid females by two methods: 1) direct removal and 2) using expelled glochidial packets. Direct removal involved puncturing the marsupial gills with a 20 gauge needle. Glochidia were then flushed from gills into a Petri dish using a squirt bottle filled with tank water. The second method involved the use of expelled glochidial packets from some of the *Villosa lienosa* individuals. In both cases, the glochidia obtained were held in suspension in beaker of 50 - 100 ml of water with use of a stir rod. Each female had a single 3 ml aliquot of suspended glochidia and water removed for fecundity counts. The 3 ml samples were counted with a dissecting scope at 50X and corrected for the exact volume of water used to keep glochidia in suspension. Viability of the glochidia was tested by exposing a subsample to NaCl. Glochidia were considered viable if > 90% of the subsampled glochidia snapped shut.

The glochidia were then transferred to the fish by direct placement onto the fish's gills. Before infestation with glochidia, fish were anaesthetized with MS-222 (tricane methanesulfonate, trade name Finquel™). The anaesthetized fish had 3 ml of a glochidial water solution injected into their mouths and flushed across the gills. In preliminary experiments with *Lampsilis ornata*, the fish were instead exposed to the glochidia in a heavily aerated beaker. This passive infection method was ineffective and the direct transfer method was therefore used.

Following infestation, the fish were immediately placed into individual 26.5 L aerated tanks. Each tank was kept at 23º C and nitrogenous waste maintained at < 0.2 mg/l through the infestation period. The tank bottom was siphoned twice a week to check for juvenile mussels and to replace existing water with fresh, de-chlorinated water. Between 11-19 L were siphoned each time. All siphoned water was filtered through an 87 µm mesh to retain juveniles or rejected glochidia and filtrates were examined with a 50X dissecting microscope during which all glochidia and juveniles (dead or alive) were counted. Juveniles were characterized by noting movement, or the presence of adductor muscles and a foot. Experiments were terminated after 40 days, if no juveniles were found, or one week after the last juveniles were collected from a tank.

Fish species selected for potential host tests were collected in the same streams as the mussel species. Fishes were sampled in either 1) wadeable streams (Tickfaw and Tangipahoa rivers) with a backpack electrofishing unit (Smith-Root model 15), or 2) from the larger Amite river with an electro-fishing boat, emphasizing fish habitat along banks of the river. The fishes were stored in aerated coolers for transport back to the laboratory. All fishes used in experiments were housed, handled, and disposed off according to departmental and university guidelines. Young individuals of species were preferred for host trails as they were less likely to have developed any immunity to unionid infections. Fishes were held in 3,029 L raceways for no less than a month to prevent accidental introduction of wild glochidia to test tanks. All fishes used in host trails had their gill inspected for glochidial infection before use. Previously known host fishes of mussels were determined from recent literature (Oesch, 1995; Howells et al., 1996; Keller & Ruessler, 1997; Watters et al., 2009).

RESULTS

Twelve gravid *Lampsilis teres*, seven *Lampsilis ornata*, twelve *Villosa lienosa*, and one *Quadrula refulgens* were found. Although *Quadrula refulgens* populations were surveyed for gravid females through two seasons of field work, only a single female was found gravid in late June, and was used to test for host suitability.

Of the twenty-three species of fishes tested as potential hosts (Table 1), we found four previously unknown hosts for *Villosa lienosa*: *Lepomis megalotis*, *Lepomis humilis*, *Lepomis microlophus* and *Lepomis cyanellus*, and confirmed two already documented hosts: *Lepomis macrochirus* and *Micropterus salmoides*. For *Lampsilis ornata*, we established a mussel-host relationship with *Luxilus chrysocephalus*, and confirmed M. salmoides as a host. We confirmed three hosts for *Lampsilis teres*: M. salmoides, *Pomoxis annularis*, and L. humilis, and established two new fish hosts: L. microlophus and Notropis venustus. For *Quadrula refulgens*, a mussel-host-fish relationship was established with *Pylodictis olivaris*.

Brooding period

Lampsilis teres was gravid from April until September and is considered a bradytictic brooder. *Lampsilis*

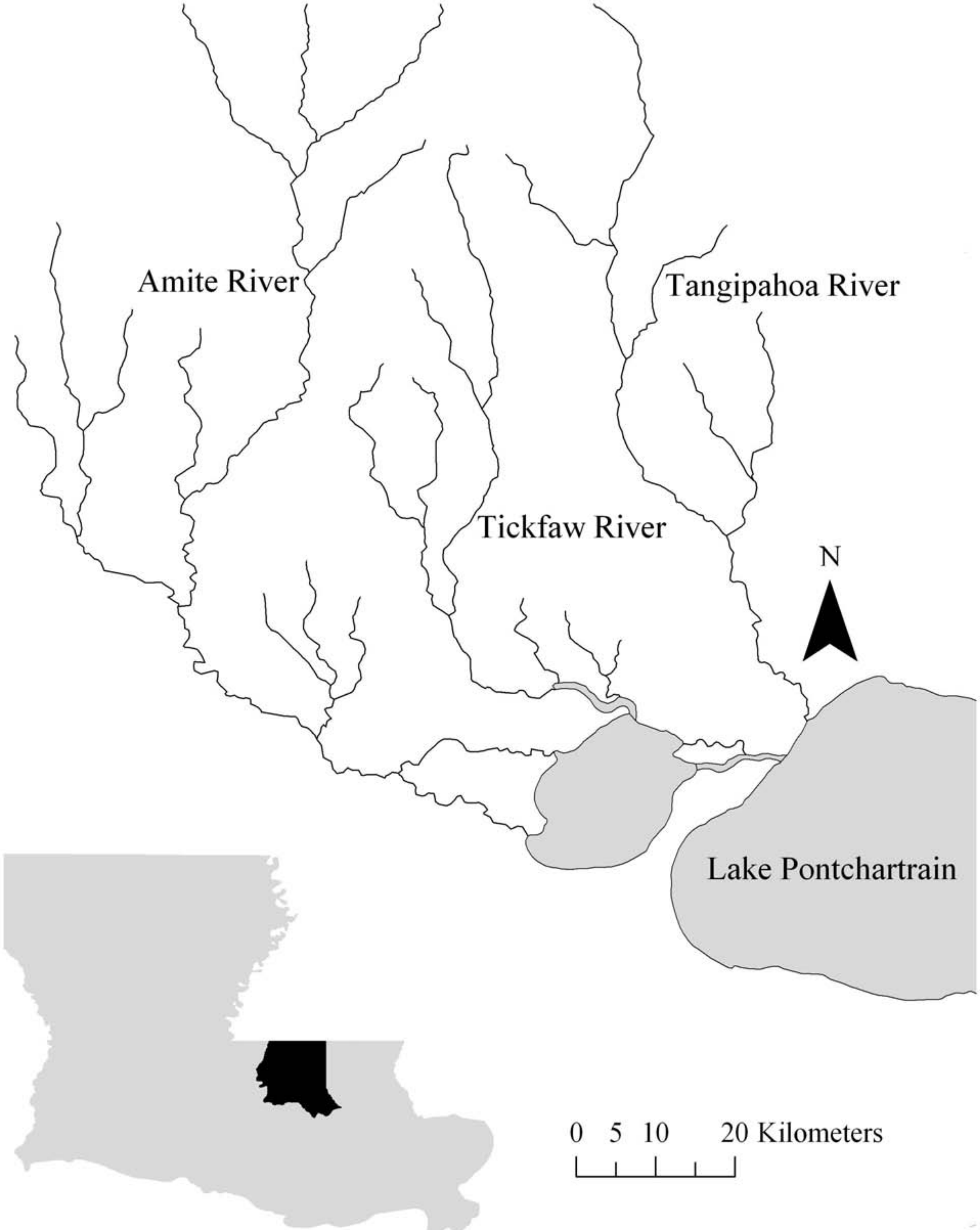

FIGURE 1

Rivers in the Lake Pontchartrain Basin sampled for fishes and mussels for host fish trials.

ornata was gravid from late February until April and is a tachytictic brooder. *Villosa lienosa* was considered in the literature (Keller & Ruessler, 1997) a long term brooder, but was only found gravid in our study from April until June. The single *Quadrula refulgens* gravid female was found in late June, and the species is considered to be a tachytictic brooder.

Fecundity estimates

Lampsilis teres had an average fecundity of 407,333 glochidia with a standard error of +24,727 glochidia for the 12 females that averaged 113.25 mm in shell length. *Lampsilis ornata* had a fecundity of 451,214 +27,239 glochidia for seven females that averaged 89.12 mm. The 12 *Villosa lienosa* averaged 48.15 mm had a fecundity estimate of 38,562 +3,073 glochidia. The single *Quadrula refulgens* was 48.5 mm in length and had an estimated 32,450 glochidia.

DISCUSSION

Understanding the complex reproductive cycle of unionids can be critical to their management and represents a major barrier to their conservation (Yeager & Saylor, 2007). Lack of suitable host fishes may limit the reproductive and dispersal ability of unionids within drainages. We have identified suitable hosts for four species of mussels from the Lake Pontchartrain Basin, Louisiana.

We categorized *Lampsilis teres* as a host generalist because five species, from two families were identified as proper hosts. L. teres is listed in the literature using over a dozen hosts from five families (Keller & Ruessler, 1997; Watters et al., 2009). We classified Villosa lineosa as a specialist on the Centrarchidae family using six species within this family. *Quadrula refulgens* and *Lampsilis ornata* were specialists, with only one and two hosts, respectively. *Lampsilis ornata* was classified as a specialist, because it was tested on 16 species of fish in our study, and 15 species in Haag and Warren's (2003) study. *L. ornata* used only two species as potential hosts from 26 species from nine families used in the two studies. *Micropterus salmoides* was also a host, which was also verified by Haag and Warren (2003), but *Luxilus chrysocephalus*, was a poor host, because it had only nine juveniles metamorphosed. Even though the two hosts are from different families, we believe that *L. ornata* can still be classified as a specialist.

Lampsilis teres and *Villosa lienosa* were gravid for long periods, whereas *Quadrula refulgens* and *Lampsilis ornata* had shorter brooding periods. *Villosa lienosa* is generally considered a long-term brooder, but was only found gravid during the early spring. Several *V. lienosa* aborted glochidia or eggs during transport, or the following day after being housed in the laboratory. Other species did not abort glochidia, suggesting *V. lienosa* are less tolerant of stress. If *V. lienosa* responds to disturbance by aborting glochidia, temperature stress during midsummer could lead to loss of reproduction in the fall.

Only a single gravid female was found of *Quadrula refulgens*, although the species is one of the most abundant mussels in the Amite River, LA. We suggest that this species may be a short term brooder that only broods glochidia for a few weeks. Quadrula species are long lived (Haag, 2009), including Q. refulgens (W. Daniel, unpublished data) and may not reproduce every season. The short brooding period and sporadic reproduction make finding gravid Q. refulgens difficult.

Both of the *Lampsilis* species (*ornata* and *teres*) were relatively fecund compared to *Villosa lienosa* and *Quadrula refulgens*. Thus, we found considerable variation among mussel species in brooding patterns, host selectivity and fecundity. Further studies will allow us to better categorize these mussel species as to their reproductive tactics and life history, and help resource managers better conserve these populations, especially the locally rare *L. ornata*.

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TABLE 1

Fish species tested as a host for each unionid. Numbers represent the total number of live juveniles recovered from host fish. X represents a trial with no juveniles produced. *indicates a previously known host fish.

Family/ Common Name	Scientific Name	*L. ornata*	*V. lienosa*	*L. teres*	*Q. refulgens*
Aphredoderidae					
Pirate Perch	*Aphredoderus sayanus*	X	X	X	
Catostomidae					
Blacktail redhorse	*Moxostoma poecilurum*	X		X	
Centrarchidae					
Bluegill	*Lepomis macrochirus*	X	**119, 76***	X*	X
Green sunfish	*Lepomis cyanellus*	X	**17**	X*	
Largemouth bass	*Micropterus salmoides*	**49, X***	**145***	**154***	
Longear sunfish	*Lepomis megalotis*	X	**266, 176**	X	
Orangespot sunfish	*Lepomis humilis*	X	**72**	**68***	
Shadow bass	*Ambloplites ariommus*		X		
Warmouth	*Lepomis gulosus*	X	X	X*	
White Crappie	*Pomoxis annularis*	X		**28***	
Redear Sunfish	*Lepomis microlophus*		**4**	**60, 37**	
Cyprinidae					
Striped shiner	*Luxilus chrysocephalus*	**9**	X		
Golden shiner	*Notemigonus crysoleucas*	X	X	X	
Blacktail shiner	*Cyprinella venustus*			**12**	
Cyprinodontidae					
Blackstripe topminnow	*Fundulus notatus*	X		X	
Ictaluridae					
Yellow bullhead	*Ameiurus natalis*	X	X	X	X
Tadpole madtom	*Noturus gyrinus*	X	X	X	
Brown madtom	*Noturus phaeus*		X	X	
Flathead catfish	*Pylodictis olivaris*				**28**
Percidae					
Banded darter	*Etheostoma zonale*		X	X	
Blackbanded darter	*Percina nigrofasciata*		X		
Poeciliidae					
Mosquitofish	*Gambusia affinis*	X		X	
Sailfin Molly	*Poecilia laptipinna*	X			