EVIDENCE OF EXTENDED MARINE PLANKTONIC LARVAL DEVELOPMENT IN FAR-UPSTREAM POPULATIONS OF THE RIVER SHRIMP MACROBRACHIUM OHIONE (SMITH, 1874) FROM THE MISSISSIPPI RIVER

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ABSTRACT
Coastal populations of Macrobachium ohione (Smith, 1874) have been shown previously to be amphidromous, i.e., with adults living in fresh water but with marine larval development. Larval delivery to coastal estuaries in far-upstream populations seemed unlikely because of the distances involved. Therefore, we tested the hypothesis of freshwater larval development in far-upstream populations from the Mississippi River, near Vicksburg and Greenville, Mississippi. We compared the molting success of newly hatched stage-1 (non-feeding) to stage-2 (first feeding) zoeae when exposed to fresh water and salt water (15 ppt) treatments. In addition we also tested the duration of time larvae spent in fresh water or salt water prior to major larval mortality or larval molting. In all freshwater treatments, stage-1 larvae failed to molt to stage 2; in contrast, molting success in saltwater treatments was ~99% and after 5-6 d of exposure to salt water all surviving larvae molted to stage 2. In freshwater treatments, there was a significant decline in larval survivorship after 3-5 of exposure to fresh water. Larval survivorship declined below 50% after 5 d of “freshwater drifting.” These results suggest that far-upstream populations of M. ohione require saline environments to complete larval development. Alternate hypotheses (long-distance hatching migrations of females to and from the sea, inland brine springs producing low salinity larval nurseries, upstream population sinks) are proposed to explain the former existence of far-upstream populations.

KEY WORDS: amphidromy, freshwater shrimp, larval development, migrations, Palaemonidae
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INTRODUCTION
Shrimp in the infraorder Caridea are predominantly marine species; however, a majority of shrimp found in Atyidae and many species of Palaemonidae (especially the genus Macrobachium) have adapted to living in fresh water (Bauer, 2004; De Grave et al., 2008; De Grave and Fransen, 2011). Within Palaemonidae, especially Palaemonininae, “freshwaterization” has occurred because of various alterations to the ancestral marine planktonic larval development (Jalihal et al., 1993; Mashiko and Shy, 2008). Within Macrobachium, the most speciose genus in Palaemonidae, Jalihal et al. (1993) describe three forms of larval development. Two of these forms, abbreviated larval development and direct larval development, have been suggested to be adaptations to an exclusively freshwater life cycle (Jalihal et al., 1993; Bauer, 2004, 2011a, b). The third form of development, believed to be the ancestral condition, requires larvae to progress through planktonic larval stages in salt water and is observed in marine and amphidromous (Jalihal et al., 1993; Wowor et al., 2009; Bauer, 2011a, b).

Amphidromy is a life cycle that requires migrations between the river and the sea because juvenile and adults mature, live, and breed in fresh water, but larval development is marine (McDowall, 1988, 1992). This life history is common among fish, shrimp (Atyidae, Palaemonidae, Xiphocarididae), and snails (Neritina) found on tropical and subtropical islands of the Caribbean and Indo-Pacific (McDowall, 1988, 2007, 2010; Holmquist et al., 1998; Blanco and Scatena, 2005; McRae, 2007; Kikkert et al., 2009; Röller-Lara and Wehrtmann, 2011). Studies report that larvae from amphidromous shrimp, e.g., atyids and species of Macrobachium from the Caribbean, passively drift to the sea with river currents after being hatched upstream in a matter of days (Hunte, 1978; March et al., 1998). However, there are some populations of carideans such as Cryphiops caementarius (Molina, 1782), Macrobachium amazonicum (Heller, 1862), M. malcomsonii (Milne Edwards, 1844), and M. rosenbergii (De Man, 1879) that are found in large continental rivers systems at distances > ~100 km~1000 km which would require extensive drifting to the brackish or marine water sources needed for larval development (Hartmann, 1958; Ibrahim, 1962; Ling, 1969; Magalhães, 1985; Magalhães and Walker, 1988).

In the United States within the Mississippi River System (MRS), Macrobachium ohione (Smith, 1874) were once found in densities large enough to support local fisheries throughout the shrimp’s distribution which ranged from the Gulf of Mexico north into the upper Mississippi and lower Ohio River (Fig. 1) (McCormick, 1934; Gunter, 1937, 1978; Hedgpeth, 1949; Huner, 1977; Bowles et al., 2000). Gunter (1978) explained the extensive distribution of M. ohione by hypothesizing that far-upstream populations were sustained because new shrimp are recruited from within their home
population, i.e., freshwater larval development. This implies that larval development in upstream populations differs from the marine planktonic larval development of coastal populations, i.e., they have evolved abbreviated larval development or direct larval development (Dugan, 1971; Dugan et al., 1975; Bauer and Delahoussaye, 2008; Rome et al., 2009). A similar situation has been observed in *M. amazonicum*, in which some populations >1000 km upstream in the Amazon River no longer required brackish or marine water for larval development, yet coastal populations remain amphidromous (Magalhães, 1985; Magalhães and Walker, 1988; Anger and Hayd, 2010). Although these shrimp have small eggs and large clutch sizes, a characteristic of extend marine larval development (Mashiko, 1990; Walsh, 1993; Bauer, 2004), larvae from these upstream populations were reported to complete their larvae development in the slack waters (flood plain) of the main river channel, areas with an abundance of plankton (Magalhães, 1985). These conditions were hypothesized to allow the larvae to maintain their position upriver, i.e., prevent from being washed out to sea, in a habitat with planktonic larval food supply (Magalhães, 1985).

In contrast, females from both upriver populations and coastal populations of *M. ohione* in the MRS have small eggs and large clutch sizes (RTB, personal observation), but several studies have suggested that *M. ohione* throughout its distribution in the MRS are amphidromous, which supports the hypothesized need for marine water for larval development (Gunter, 1937; Truesdale and Mermilliod, 1979; Bauer and Delahoussaye, 2008; Olivier and Bauer, 2011). However, Rome et al. (2009), reported that populations of *M. ohione* from the Atchafalaya River, Louisiana, USA (AR) experienced reductions in the success of larvae molting to the first feeding stage (stage-2 zoea) depending on the duration of the larvae’s exposure to fresh water. The optimal larval drift hypothesis suggests that the critical molt from stage-1 larva to stage-2 larva is optimized when larvae drift in fresh water is no longer than 1-3 d prior to reaching a salinity of at least ~6 ppt (Rome et al., 2009). This suggests that the amount of time larvae remain in fresh water prior to reaching the sea is both crucial and limited. Given the limited time that larvae can drift in fresh water, populations *M. ohione* that once existed >1500 km and those populations that are currently found >700 km upstream of the Gulf of Mexico in the MRS were and are faced with the problem of successfully delivering larvae to the sea.
According to the optimal larval drift hypothesis (Rome et al., 2009), populations farther upstream than 3 days drifting distance from the sea will contribute little to the next generation unless: 1) gravid females migrate downstream to hatch larvae within close proximity of the sea (Bauer and Delahoussaye, 2008; Olivier and Bauer, 2011), or 2) upstream populations have evolved adaptations for freshwater larval development (Gunter, 1978; Magalhães, 1985). This study tests the hypothesis that M. ohione is now extensively distributed far upstream in the MRS (and in the past even more so) because planktonic larval development occurs entirely in fresh water (Gunter, 1978).

MATERIALS AND METHODS

Study Site and Collections

All shrimp used in this study were taken by traps or trawls from the Mississippi River (MR) at Vicksburg, Mississippi (near 32°20′01″N, 90°54′10″W) and Greenville, Mississippi (near 33°24′05″N, 91°06′48″W) approximately 740 km and 892 km upstream of the MR river mouth, respectively, from June to August in 2010 and from July and September in 2011. Shrimp were transported to the laboratory at the University of Louisiana Lafayette in river water with light aeration. Females were obtained in the gravid condition or became gravid while housed with males from the same upstream river location while in the laboratory.

Larval Development Experiments

A carbon-dechlorinating water tank system was used to provide the fresh water used on the water tables and in the larval experiments. The water temperature on the table was maintained at 24°C with a 13 hours light:11 hours dark photoperiod. While on the water table, gravid females (with embryos) were contained in 5 L perforated containers (prehatching buckets). Females with near-hatching embryos (see Bauer and Delahoussaye, 2008) were further isolated into non-perforated containers with fresh water obtained from the water table. An airstone was added to these hatching buckets to provide gentle aeration. Hatching buckets were checked daily for newly hatched larvae, and larvae were harvested and larval observations were initiated on the morning of hatching.

Typically in caridean shrimp species that exhibit extended planktonic larval development (see Type I developmental pattern in Jalihal et al., 1993; Bauer, 2004), larvae hatch out as stage-1 zoeae (sessile eyes, only 3 pairs of thoracopods, natatory thoracic exopods, no pleopods, and no free uropods on the tail fan) and then proceed through 5-10 or more larval instars in which appendages are gradually added, i.e., anamorphic development. In shrimp that display abbreviated larval instars in which appendages are gradually added, i.e., anamorphic development. In shrimp that display abbreviated larval development or direct larval development, the larvae will hatch out in a more advanced larval state, undergoing as few as 2 or 3 larval stages, or in a juvenile/postlarval state (see Type II and III developmental patterns in Jalihal et al., 1993; Bauer, 2004). All of the 22 reproductive females used in this study provided ~80 larvae, such that ~40 larvae from each female were available for each treatment (freshwater and saltwater). All the larvae used hatched with stage-1 characteristics typical of caridean larvae with extended larval development. After a female hatched a brood, the positively phototactic larvae were harvested by illuminating one side of the hatching bucket with a table lamp in a dark room. While viewing the larvae under a dissecting microscope, stage-1 zoeae were then transferred using a small plastic pipette with a diameter of ~3-4 mm into one of two culture dishes containing either fresh water (0 ppt) or salt water (15 ppt; optimal salinity for larval development of M. ohione; see Dugan, 1975; Bauer and Delahoussaye, 2008). The 44 culture dishes were then placed inside an incubator set at 28°C and a light:dark photoperiod of 13 hours:11 hours. Light aeration was provided to the culture dishes via an external electrical aerator fitted with glass Pasteur pipettes (1 mm diameter tip).

All dishes in both the freshwater and saltwater treatments were checked daily to determine if molting and/or mortality had occurred. Stage-2 zoeae were identified by the presence of stalked eyes (Bauer, 2004; Bauer and Delahoussaye, 2008); in stage-1 zoeae, the eyes are sessile. In the daily checks, all dead stage-1 and newly molted stage-2 larvae were removed from the culture dishes and counted. Stage-2 larvae were fixed in a mixture of 10% seawater/formalin (10 parts formaldehyde and 90 parts 15 ppt salt water). This was done to allow later verification that larvae that were considered to have molted were truly stage-2 zoeae. After checking for mortalities and larval molters, the water in the treatment dishes was changed. In both freshwater and saltwater treatments, one-half the volume of the water was removed and replaced daily with new fresh water or salt water respectively. The salinity of the saltwater treatments were checked and adjusted accordingly to ensure that the salinity remained 15 ppt. All observations were continued until all larvae within the each culture dish either died or molted to stage 2.

Data on time to molt from stage 1 to stage 2 and mortality data (as time to death) were analyzed using survival analysis (Allison, 1995). Proportional hazard analysis was initially used to determine whether there was a “petri dish” effect (variability among petri dishes within a treatment, e.g. as a consequence of differences in position among dishes in the incubator) or “female” effect (variation in females from which larvae were harvested) results of subsequent statistical tests. It was determined that neither petri dish nor female of larval origin had a statistically significant effect on the observed outcomes. Consequently, the effect of petri dish and female identity were removed from the analysis, such that data were now analyzed by individual. Proc. Lifetest (SAS Ondemand, 2012) was then used to test the null hypothesis that there was no difference in molting success from stage-1 to stage-2 larvae between the freshwater and saltwater treatments. The same statistical test was also used to test the null hypothesis that there was no difference in mortality between the freshwater and saltwater treatment groups.

RESULTS

All of the 782 M. ohione stage-1 larvae failed to molt to stage 2 in the freshwater treatments. In contrast, 98.8% of all the larvae (N = 883) from the saltwater treatments survived and molted to stage 2 prior to the termination of the experiment. Furthermore, all larvae that molted to stage-2
larvae in the saltwater treatments were typical stage-2 zoae of carideans with extended larval development. In saltwater treatments, an increase in molting from stage-1 larvae to stage-2 larvae occurred between days 3 to 5 with the median of molting occurring on day 4 (Fig. 2). The null hypothesis of no difference in molting success in freshwater and saltwater treatment was rejected (Wilcoxon $\chi^2_1 = 905.6$, $P < 0.0001$).

Qualitatively, larvae remaining in freshwater treatments begin to swim less actively, experienced a reduction in phototaxis, and an increase in external fouling after approximately 4 days post-hatching. The first major decline in larval survival was observed at day 3 and continued to decline rapidly through day 5, after which mortality continued at a slower rate (Fig. 3). Larval survival approached 50% by day 4 (51%). Three percent of larvae survived up to 10 days in fresh water, but larval survivorship was 0% by day 11. In contrast, larval survivorship in the “seawater” treatment was very high with <2% total mortality prior to the critical molt to the first feeding zoea. The null hypothesis of no difference in larval survivorship in freshwater and saltwater treatment was rejected (Wilcoxon $\chi^2_1 = 558.0$, $P < 0.0001$).

**DISCUSSION**

Gunter (1978) contended that the populations of *M. ohione* from the Ohio and upper Mississippi River were incapable of migrating to and from the Gulf of Mexico. Furthermore, it was suggested that migrations were not needed because saline environments were not a necessity for larval development in upstream populations (Gunter, 1978). Until now little work has been done to test this hypothesis, especially with far-inland populations of *M. ohione*. In this study, larvae taken from populations outside of optimal larval drifting distance (Rome et al., 2009; Olivier and Bauer, 2011) failed to molt from stage-1 to stage-2 zoae in the freshwater treatment. However, larvae cultured in salt water molted to stage 2 typical of the first postmolt zoeal larva of carideans with extended marine larval development. The more advanced larvae of abbreviated larval development or post-larvae/juveniles of direct development (Jalihal et al., 1993; Bauer, 2004) were not observed. These results indicate that far-upstream populations of *M. ohione* in the Mississippi River do require salt water for larval development. How the larvae from these populations from far-upstream populations reach saline waters remains open to debate.

During 2010 and 2011 sampling of females, surface current velocities obtained near Vicksburg, Mississippi (MS) and Greenville, MS ranged from 0.1 km h$^{-1}$ to 3.8 km h$^{-1}$. At these river speeds it would take approximately 8-308 days and 10-372 days (based on 24 hour of larval drift) to reach the Gulf of Mexico from Vicksburg, MS and Greenville, MS respectively. The optimal larval drift hypothesis (based on results from Rome et al., 2009) proposed that the ideal larvae drift time in fresh water should be no longer than 3 days. Thus, embryo-bearing females from far-upstream populations do not have the luxury of hatching larvae into the current as observed in other amphidromous shrimp on islands in which populations live closer to the sea (Hunte, 1978; Jalihal et al., 1993; March et al., 1998). Results from this study (~50% mortality after 4 days of freshwater drifting) support this hypothesis because larvae from upstream populations would have little chance of molting to stage 2 if arriving to the sea at the observed river velocities. However, a downstream hatching migration by embryo-bearing females to the coast or within the optimal drift distance can feasibly allow successful molting of larvae from stage 1 to stage 2 (Bauer and Delahoussaye, 2008; Olivier and Bauer, 2011). This suggests that within the 1-2 year life expectancy of the shrimp (Truesdale and Mermilliod, 1979), female shrimp from the far-upstream populations would have to conduct long-distance migrations in both directions, first upstream as juveniles coming from the sea after larval development and then as adult females migrating back to the sea to hatch larvae close to the sea.

Source/sink dynamics (Pulliam, 1988; Dunning et al., 1992) may account for the existence of far-upstream popu-
lulations of *M. ohione*. Such processes have been proposed for amphidromous fishes in Hawaii and New Zealand, in which the source populations act as “feeders” to supply recruits to remote isolated islands or to “down-current” island populations through expatrial dispersal of larvae (McDowall, 2007, 2010). In this scenario, growth to successful reproduction occurs in the source but not the sink population, which exists only because of recruitment from the source population. For example, McRae (2007) showed that the far-upstream populations of amphidromous gobies in Hawaiian streams might be explained by source/sink dynamics. Juvenile fishes coming from the sea disperse far upstream but larvae produced by the resultant upstream population are not capable of surviving the long trip downstream to the sea. Similarly, recruitment into the formerly abundant far-upstream populations of *M. ohione* may have come only from juveniles produced by coastal populations. Due to limits on the amount of time the non-feeding stage-1 larvae can drift in freshwater (optimally, 1-3 days; Rome et al., 2009), drifting larvae from females of far-upstream populations would not have been able to reach the Gulf of Mexico in time to continue successful development. According to this hypothesis, far-upstream populations only existed because offspring of females from downstream populations were able to migrate to upstream habitats within the MRS. However, because of present human impacts on juvenile migration (Bauer, 2011b; studies in progress), such far-upstream migration and recruitment no longer occurs. Source/sink dynamics may still support the upstream populations currently observed.

Bauer and Delahoussaye (2008) proposed an alternate hypothesis to explain far-upstream populations of *M. ohione*. Naturally occurring brine springs near or along the MRS provided low-salinity larval nurseries for upstream populations. *Macrobrachium ohione* has been recorded from as far inland as: Sebastian County, Arkansas; St. Louis, Missouri; Shawneetown, Illinois; in Indiana at Channelton and Lawrenceburg; and in Ohio near Scioto and Lawrence Counties (Hedgpeth, 1949), all of which are localities within close proximity of known brine springs (see Brown, 1980; Hansen, 1995). It is hypothesized that during periods of high water, i.e., naturally high river stages during the late winter and early spring season, which is concurrent with the beginning the shrimp’s reproductive season, flood waters would mix with seepage from these springs and produce salinities high enough to promote larval development. Along the Red River, bordering Oklahoma and Texas, similar brine seepages have been responsible for salinities near 2 ppt (Baldys and Hamilton, 2003). Although not optimal, a salinity of 2 ppt will allow hatching larvae to molt to stage 2 even though the proportion of a brood doing so is highly reduced (Rome et al., 2009). This evidence suggests that some saline springs in the vicinity of rivers where *M. ohione* was once found can produce salinities that could support low salinity larval nurseries. In contrast, water quality data from the early 1900s reported salinities far less than 1 ppt from northern tributaries of the MR and Ohio River that occur near brine springs, e.g., Scioto River (Clarke, 1924). Salinities that low will not stimulate larval development.

Nevertheless, if these areas were being used as larval nurseries, they are now no longer accessible due of the increased degree of anthropogenic river modification and control. For example, along the Ohio River and Red River there are a series of dams that *M. ohione* larvae and/or reproductive females would encounter prior to reaching saline springs. Such dams have been shown to act as barriers to downstream larval drift to the sea as well as to upstream juvenile migrations of amphidromous shrimp on Caribbean islands (Holmquist et al., 1998; Benstead et al., 1999; March et al., 2003). Juveniles and adults of *M. ohione* have also been observed to lack the ability to bypass dams (Horne and Beisser, 1977; TJO, personal observation). This suggests that these shrimp would be excluded from the areas near the salines. Floodwalls and levees would additionally serve as a barrier because those structures prevent the natural flooding needed to create the low salinity nurseries. Furthermore, along the Red River and its tributaries, government agencies have been in charge of removing naturally occurring salts from the river system to improve water quality (Red River Authority, 1997; United States Army Corps of Engineers Tulsa District, 2002).

The goal of the current study was to address the question of larval development in far-inland populations. Our results show that current upstream populations do require the sea for larval development. Regardless of how the once far-upstream historical populations were able to obtain the salt water needed for larval development or continued to persist without it, the current populations seem unable to follow suit. Since the 1930-1940s, those far-upstream populations have disappeared from the upper MRS (Taylor, 1992; Conaway and Hrabik, 1997; Bowles et al., 2000; Barko and Hrabik, 2003). The disappearance of these populations coincides with the extensive modifications (implementation of control structures such as levees, dike fields, dams, and revetments) that the MR has experienced since the early part of the 20th century (Baker et al., 1987; Shields, 1995; DuBowy, 2010). The far-upstream populations apparently no longer exist (Bowles et al., 2000; Barko and Hrabik, 2003) as a direct result of the modifications that the MRS has undergone. Further research is still needed to investigate how river control structures affect the life history of the shrimp, specifically concerning their migration (upstream and downstream). In addition, knowledge about other aspects of their biology, such as genetic variability and dispersal among *M. ohione* populations (MR and AR, other Gulf coast rivers), upstream migratory capabilities, habitat selection, and life expectancy are also lacking. More information in these areas can aid in management of current populations of *M. ohione* as well as restoration of former populations in the MRS and other coastal river systems. In addition, this research may contribute to a better understanding of how other amphidromous shrimp populations function in far-inland rivers and streams.

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