

REPRODUCTIVE ECOLOGY OF THE AMERICAN HORSESHOE CRAB *LIMULUS POLYPHEMUS* IN THE INDIAN RIVER LAGOON: AN OVERVIEW

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ABSTRACT: *Horseshoe crabs, Limulus polyphemus, typically inhabit estuaries with pronounced tides that influence the timing of reproduction. However, many of the cues known to mediate spawning and larval hatching are absent in the Indian River Lagoon (IRL) since much of the area is microtidal. This review summarizes our recent studies of the reproductive ecology of L. polyphemus in the IRL that focused on the potential impact of microtidal conditions on the timing and frequency of mating, spawning, and larval hatching and abundance. Extensive surveys conducted throughout the northern region of the IRL indicated that spawning occurs year-round but is aperiodic. Throughout the sampling period, larval abundances were low, despite the fact that environmental conditions were suitable for their survival and development. Peaks in larval abundance were independent of spawning events but were linked to changes in water level. Laboratory experiments indicated that conditions associated with periods of high water, including inundation and mechanical agitation, may facilitate hatching, resulting in short-term peaks in larval abundance. In general, the reproductive ecology of L. polyphemus in the IRL differs markedly from populations in tidal areas. However, results indicate that spawning and larval hatching are cued by environmental conditions that are similar to those associated with tidal changes.*

Key Words: *Limulus polyphemus*, horseshoe crab, Indian River Lagoon, reproductive activity, larval development, salinity tolerance, survival, temperature tolerance, hatching cues

THE American horseshoe crab, *Limulus polyphemus* (Linnaeus) typically inhabits estuaries and coastal areas with pronounced tides. Tidal rhythms generally synchronize the timing of spawning and larval hatching (Cohen and Brockmann, 1983; Rudloe, 1985; Shuster and Botton, 1985; Botton et al., 1988; Penn and Brockmann, 1994). However, horseshoe crabs also occur in estuarine and coastal habitats that lack regular tidal changes and periodic shoreline inundation. Coastal lagoons have characteristically low tidal amplitudes because of the restrictive effects of inlets and passes on tidal exchange (Smith, 1990). Horseshoe crab adults and juveniles are common in the Indian River Lagoon (IRL) despite the fact that it is virtually tideless (tidal amplitudes <5 cm) with changes in water level that are largely the result of wind forcing and freshwater input (Smith, 1993). Although the reproductive biology of *L. polyphemus* in tidal systems along the northeast and Gulf Coast of the United

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States has been studied extensively (see Shuster et al., 2004 for review), little is known about populations that inhabit microtidal areas.

Ecological Role of Horseshoe Crabs in the IRL—*Limulus polyphemus* is an important component of the IRL ecosystem, providing food for numerous species, including the threatened loggerhead sea turtle which feed on adults (Botton and Ropes, 1987). Migrating shorebirds and many species of fish feed on horseshoe crab eggs and larvae. The plowing action of adults during feeding promotes species diversity, richness, and abundance by aerating the bottom substrate, affecting infaunal community structure (Botton, 1984). Due to the important role that *L. polyphemus* plays in the estuarine and coastal ecosystems, the decline in numbers over the past twenty years in the IRL may have a serious impact on biodiversity in the IRL and may serve as an indicator of profound environmental disturbance in the IRL.

Status of Horseshoe Crabs in the IRL—There has been a widespread decline in the abundance of *L. polyphemus* in the last 20 years throughout its range, mostly as a consequence of overfishing for use as eel and whelk bait (Shuster, 2001). In a long-term study of horseshoe crabs in Delaware Bay, an increase in abundance began in the 1970s and continued with 923,000 adults reported in the 1990 spawning survey (Shuster, 2001). Since the adult population was increasing, the expectation was that it would continue, however the population fell to an average of 422,000 during the 1990s (Shuster, 2001). Since there was no other obvious cause for the decline, heavy harvesting for bait appears to have been the cause (Shuster, 2001). Horseshoe crabs are also harvested for pharmaceutical use. *Limulus* Amebocyte Lysate (LAL) is a medical product found in horseshoe crab blood that is used to detect contamination in injectable drugs and medical devices (Berkson and Shuster, 1999; Swan 2001). In the case of LAL, all horseshoe crabs are bled and returned to the water.

Preliminary investigations suggest that the IRL population is geographically and behaviorally distinct from other U.S. populations and may be in decline (Provancha, 1997). While *L. polyphemus* is not currently categorized as “threatened”, there is a rising concern that the abundance of *L. polyphemus* in the IRL has decreased significantly over the past twenty years. Recent netting efforts targeting juvenile loggerhead and green sea turtles in Mosquito Lagoon resulted in significantly lower horseshoe crab bycatch per unit effort than in 1979 (Provancha, 1997). In these surveys, *L. polyphemus* were common in the northern Indian River, while densities in Mosquito Lagoon ranged from 0–4 animals per 800 m², with 0 being most common (Provancha, 1997). Earlier (1978–79) sampling efforts in the IRL were largely qualitative, yet researchers noted that nets deployed in Mosquito Lagoon were regularly weighted down by large numbers of *L. polyphemus* (Provancha, 1997). Differences in these reports suggest that horseshoe crab populations in the IRL declined significantly from 1979–1993.

While quantitative data on the *L. polyphemus* population in the IRL is limited, in 1979 Applied Biology Incorporated (ABI) conducted one of the few studies that involved horseshoe crabs in the Indian River. The study examined the potential environmental impact of water intake canals of two power plants on the Indian River, the Florida Power and Light Cape Canaveral Plant (FPL) and the Orlando Utilities Commission Indian River Plant (OUC). As part of the study, researchers quantified the number of horseshoe crabs that became trapped on the impingement screens of the intake canals. At the FPL Plant, a total of 39,097 horseshoe crabs were retained on the impingement screens during the 12-month study (Applied Biology Inc., 1980). At the OUC Plant, a total of 53,121 horseshoe crabs were retained (Applied Biology Inc., 1980). The Applied Biology Inc. report (1980) also provided results of other studies completed at the FPL and OUC plants that found that in 1975, an estimated 69,662 horseshoe crabs were retained at the FPL plant and 104,000 were retained at the OUC plant (Applied Biology Inc., 1980). Comparison of the results of the 1979 study with those obtained in 1975 indicates a nearly 50% decrease in horseshoe crab density (Applied Biology Inc., 1980). Whether the change in abundance represents natural variation in population size or a significant decline is unknown.

A major die-off occurred in July 1999 where an estimated 100,000 adult *L. polyphemus* died in the northern portion of the Indian River and the southern portion of Mosquito Lagoon (Scheidt and Lowers, 2001). No other species were affected which would occur if low oxygen or other water quality issues such as pollution inputs or algal blooms were the cause of the horseshoe crab die-off. The die-off suggested that conditions in the IRL may no longer be favorable for the survival of horseshoe crabs and represented a significant event that may be an indicator of an imminent decline in the horseshoe crab population in the IRL. The ramifications of a die-off of adults of this magnitude may not be fully displayed right away because of a lag time that is due to their long maturation period and maximum age (Shuster, 2001). Up to ten years after a large die-off of the adult population occurs the recruitment of new spawners remains relatively high due to the former number of spawners, but ultimately the juvenile year classes are greatly reduced due to fewer spawners which results in fewer young adults being added to the spawning population (Shuster, 2001).

The *L. polyphemus* population in the IRL presents a unique opportunity to study the life history patterns of a population in a microtidal system that lacks the tidal cues found in other areas where horseshoe crabs have been studied. Many of the life history strategies, such as spawning, nest location, and the timing of larval release are influenced by environmental factors such as tide height and shoreline inundation. In the IRL, the life history strategy of *L. polyphemus* may be very different than that of other populations due to the lack of tidal cues. Thus, there is a need to understand the factors that influence spawning, larval development, and hatching of horseshoe crabs in a microtidal system. The IRL also experiences high water temperatures and hypersaline conditions during the spawning season. These extreme environmental

conditions may influence embryonic development, hatching success, and larval survival. This overview summarizes our work that used a combination of field observations and laboratory experiments to characterize the reproductive ecology of spawning, larval development and hatching in a system that lacks the usual lunar and tidal cycles that are present throughout most of its range.

Spawning and Larval Abundance—Adult and larval surveys conducted at 21 sites in Indian River Lagoon system (Fig. 1) from February 1999 to August 2000 (See Ehlinger et al., 2003 for detailed methodology) indicates that adult spawning activity in the IRL varies seasonally with peaks in the frequency of spawning pairs occurring in the late winter/early spring (Ehlinger et al., 2003; Figs. 2 and 3). Spawning activity is episodic and lacks any apparent lunar or semi-lunar periodicity. Periods of increased spawning are often separated by long intervals (3–4 wks) in which no mating occurs (Figs. 2 and 3). Larvae are collected in plankton samples from late spring to early summer (Fig. 3). However, larval densities are most likely unrelated to spawning activity since the time interval (lag) between peaks in adult and larval densities (≈ 8 weeks; Fig. 3; Ehlinger et al., 2003) exceeds the duration of embryonic development (≈ 4 weeks; Rudloe, 1979; Penn and Brockmann, 1994). Spawning activity among the 21 sites in the IRL is not synchronized since peaks occur at different sites at different times. Moreover, the presence of larvae is not always linked to the occurrence of spawning adults due to the fact that larvae are found at sites where spawning pairs were rarely observed.

Different environmental and hydrologic variables are found to be important in predicting the presence of adults and larvae in the IRL (Ehlinger et al., 2003). A seasonal pattern for adults is indicated with a decrease in temperature/salinity leading to an increase in the probability of adult presence. However, the presence of spawning adults is not associated with any other environmental variable that might serve as a synchronization cue. The variables to predict the presence of larvae in the IRL are different than those used to predict adults. The two variables that predict the presence of larvae are associated with beach morphology and water level. The presence of larvae is associated with periods of high water when beach width and salinity levels are low. When the beach width/salinity decreased (i.e., the water level increased), the likelihood of larval presence increased and when beach slope decreased, the likelihood of larval presence increased.

Tolerance of Embryos and Larvae to Temperature and Salinity—The ability of horseshoe crab embryos to tolerate temperature and salinity conditions in the IRL was tested by rearing artificially fertilized eggs under different temperature and salinity combinations (see Ehlinger and Tankersley, 2004 for detailed methodology). Results indicated that both high temperatures and salinities have a significant effect on the rate and success of embryonic development (Ehlinger and Tankersley, 2004). At 35 and 40°C, eggs fail to hatch after 75 days at all salinities (30–60 psu; Ehlinger and Tankersley, 2004).

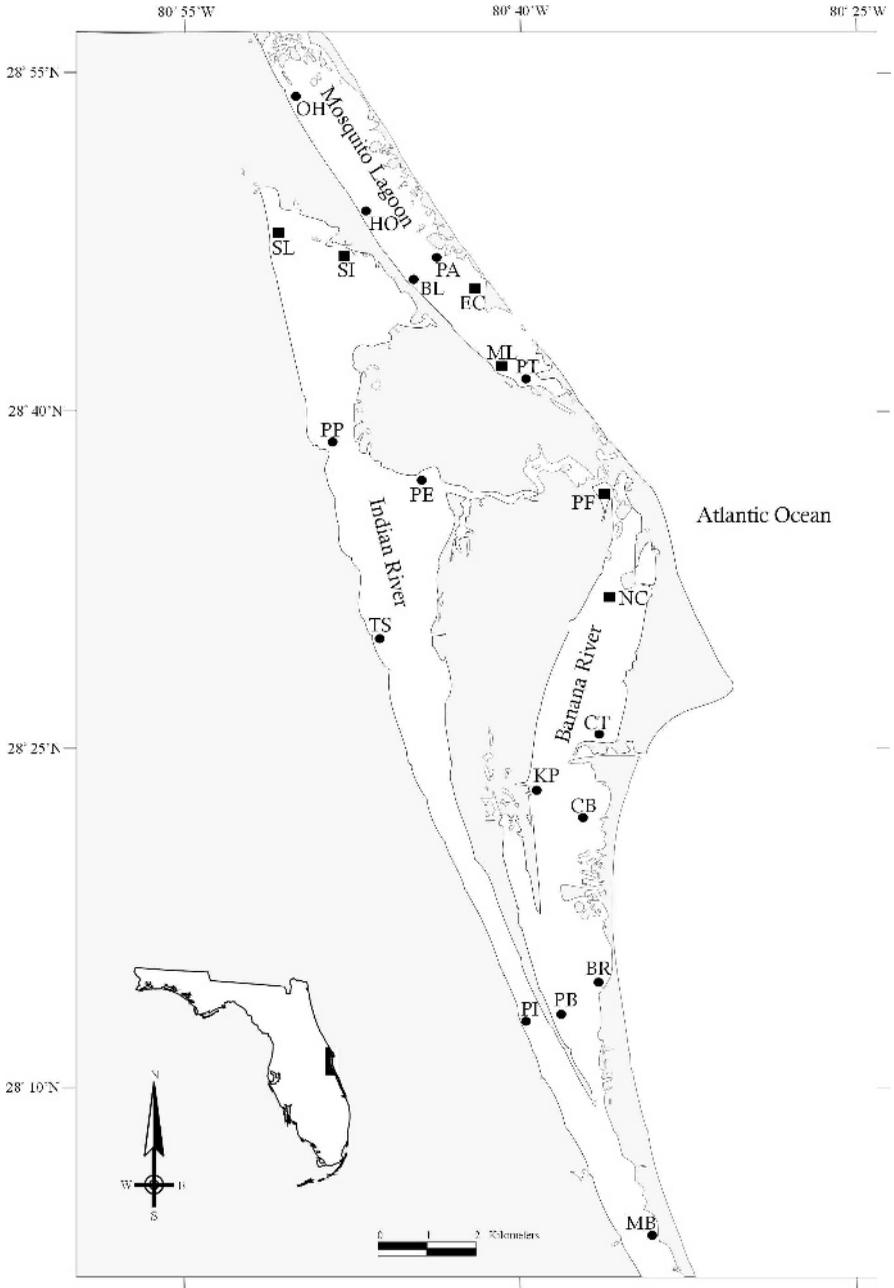


FIG. 1. Study sites in the Indian River Lagoon system. Six study sites (■) were sampled during Phase I of the study from February 1998–December 1999 for adult *Limulus polyphemus*. Twenty-one study sites (■ and ●) were sampled during phase II of the study from February–August 2000 for adult and larval *Limulus polyphemus*. Redrawn from Ehlinger et al., 2003.

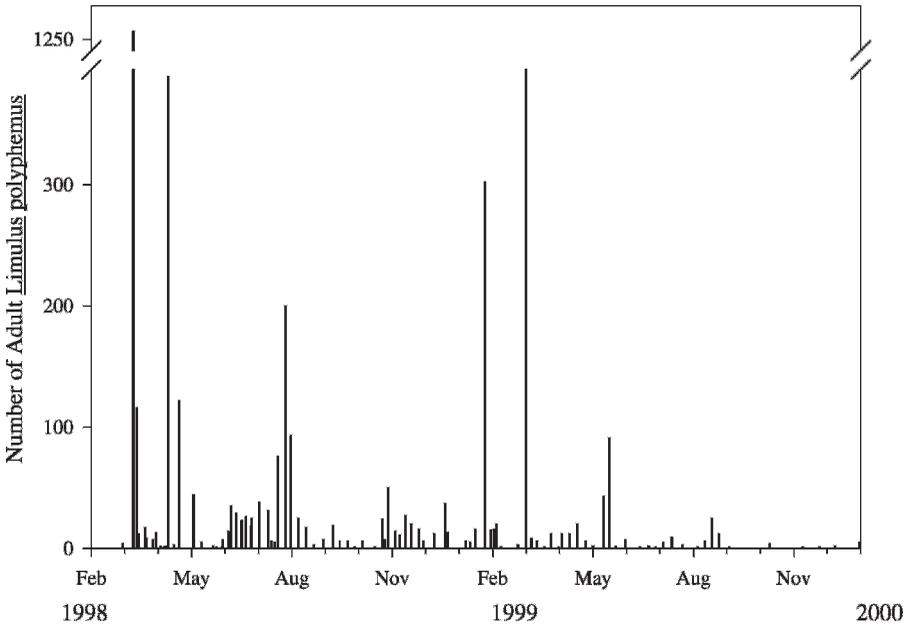


FIG. 2. Temporal pattern of spawning by *Limulus polyphemus* during Phase I (February 1998–December 1999) of this study. Redrawn from Ehlinger et al., 2003.

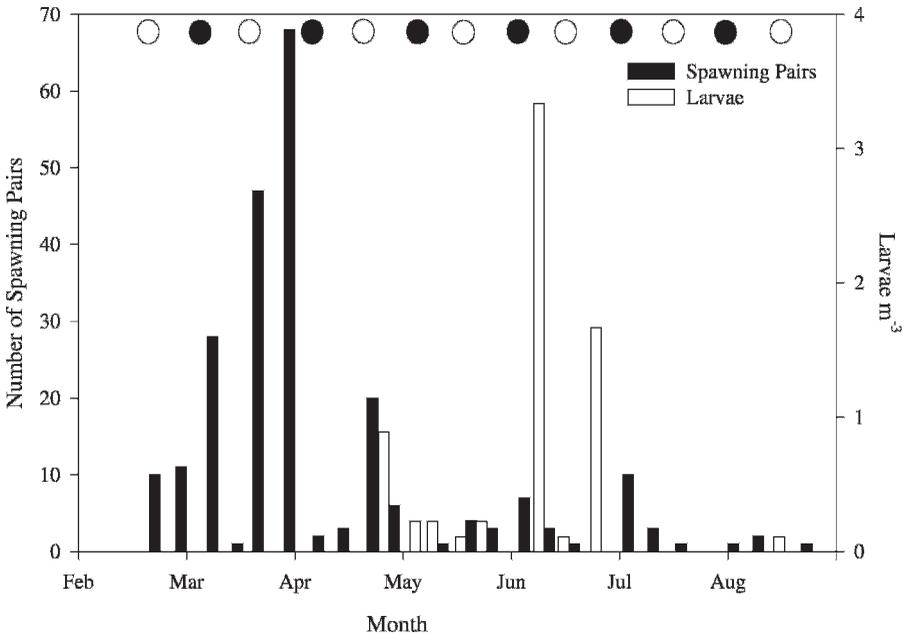


FIG. 3. Temporal pattern of spawning and larval hatching of *Limulus polyphemus* during Phase II (February–August 2000) of this study. Open circles represent full moons and closed circles represent new moons. Redrawn from Ehlinger et al., 2003.

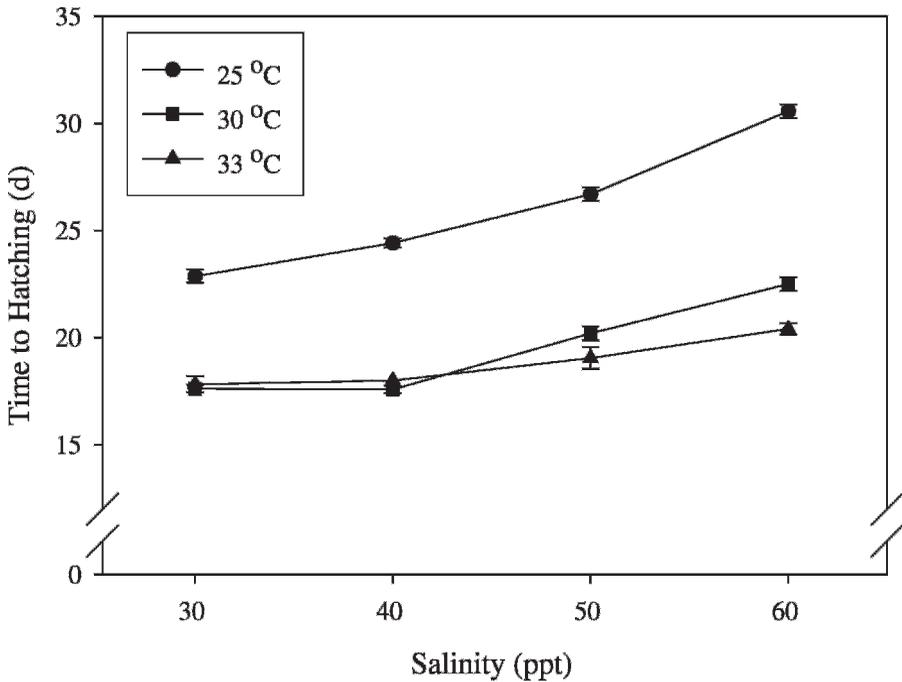


FIG. 4. Mean (\pm SE) number of days from fertilization to hatching of *Limulus polyphemus* embryos at 25°C, 30°C, and 33°C and at 30, 40, 50, and 60 ppt. No hatching occurred in any of the test salinities at 35°C and 40°C. N = 30 for each trial. Redrawn from Ehlinger and Tankersley, 2004.

Normal development and hatching occur at all other temperatures (25–40°C). Temperature and salinity also had a significant effect on time to hatching (Fig. 4). Embryonic development takes significantly longer at 25°C compared to 30 and 33°C at all test salinities (Fig. 4). At 25°C, time to hatching increases significantly as the salinity increases (Fig. 4). Embryos maintained at 30 and 33°C and 30 and 40 psu have similar hatching rates, but hatching is delayed significantly in more hypersaline conditions (50 and 60 psu; Fig. 4). The optimal temperature and salinity conditions for development is 30–33°C and 30–40 psu (Ehlinger and Tankersley, 2004).

Experiments designed to test *Limulus polyphemus* larval tolerance of different salinities found that trilobite larvae are able to survive for at least 30 days at salinities ranging from 10 to 70 psu (see Ehlinger and Tankersley, 2004 for detailed methodology). Mortality only occurs in the very extreme salinities of 5, 80 and 90 psu (Fig. 5). Comparisons of the survivorship curves among salinity treatments indicate that survivorship is only significantly reduced when larvae were maintained at 90 psu (Fig. 5). Time to 50% mortality (TM₅₀) is \approx 16.0 days in 90 psu (Ehlinger and Tankersley, 2004).

Hatching Triggers—Embryos hatch into trilobite larvae in approximately 28 days and move toward the surface of the substrate to enter the water when

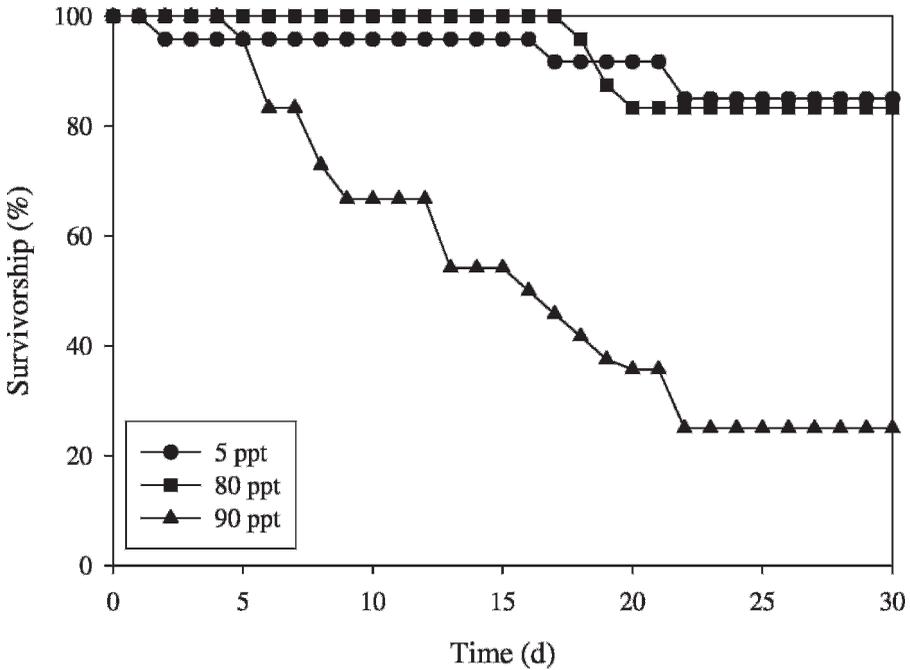


FIG. 5. Kaplan-Meier survivorship curves for *Limulus polyphemus* larvae subjected to salinities ranging from 5 to 90 ppt for 30 days. All larvae survived in salinities from 10–70 ppt. N = 24 for each trial. Redrawn from Ehlinger and Tankersley, 2004.

inundated during nocturnal high tides near the time of full moon (Rudloe, 1979; Penn and Brockmann, 1994). Given the diverse range of shoreline inundation patterns that occur throughout its range, the hatching of *L. polyphemus* eggs may be facilitated by an environmental trigger that synchronizes larval hatching with water levels on the beach, especially in microtidal areas. If an environmental cue associated with inundation triggers hatching of *L. polyphemus* larvae, then larvae will hatch and emerge when conditions are favorable for entry into the water column increasing their chance of survival. This is especially important in microtidal systems because inundation of the beach is aperiodic. To test this hypothesis, developing embryos were exposed to different treatments that mimicked conditions experienced during periods of high water (hydration, agitation, and osmotic shock; Ehlinger and Tankersley, 2003). When exposed to hydration and agitation conditions that simulate inundation at high tide, hatching levels of Stage 21 embryos increase significantly relative to levels in control treatments (Fig. 6; Ehlinger and Tankersley, 2003). For all experimental treatments (hydration, agitation, hydration and agitation, and hydration with agitation and sand), most hatching occurs within the first 2 h following exposure (Fig. 6). Most embryos (>65%) in the treatments involving agitation hatch during the exposure period (Fig. 6). Hatching levels in the hydration only

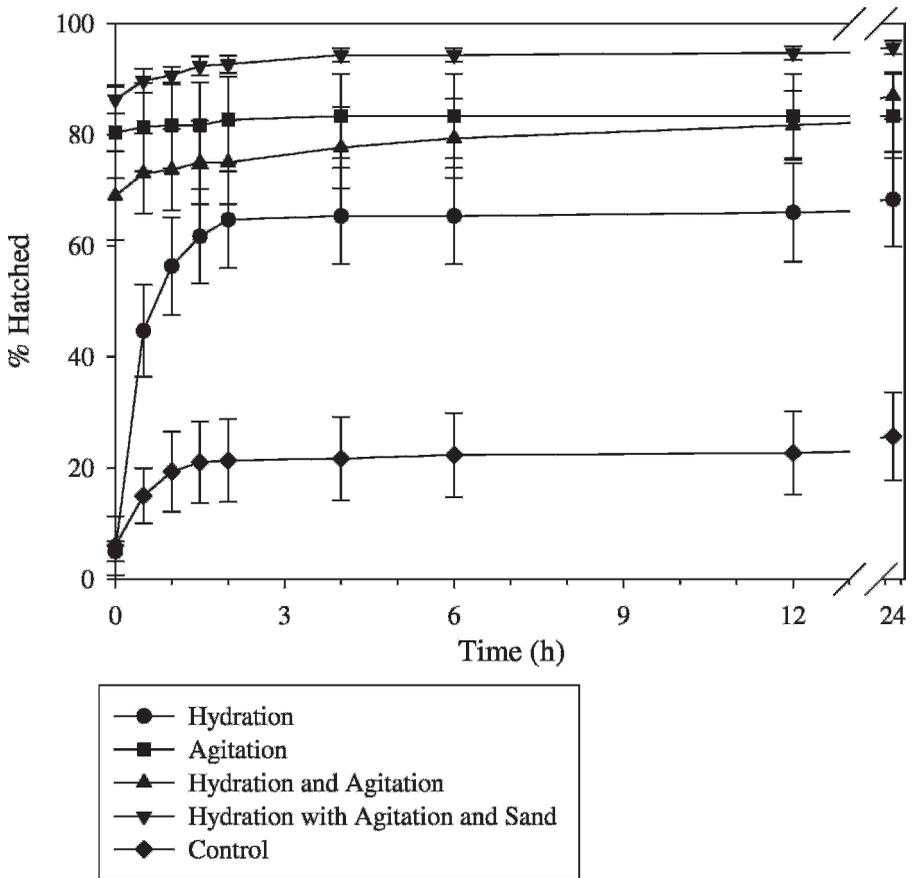


FIG. 6. Percentage ($\bar{X} \pm SE$) of hatched larvae over 24 h following exposure to one of the four hydration and agitation treatments for 30 embryos (N = 10). Embryos in control conditions were maintained in rearing conditions (moist paper towels) and were not inundated with seawater or agitated by shaking. Redrawn from Ehlinger and Tankersley, 2003.

treatment increase rapidly following exposure, but are significantly lower than the other treatments (agitation, hydration and agitation, and hydration with agitation and sand). At the end of the monitoring period, hatching levels are significantly higher for embryos exposed to hydration and agitation with sand than for all other treatments. These results support the field findings of Rudloe (1979) who found that peaks in larval abundance in the plankton occur when localized storms with strong onshore winds coincided with high tide, producing unusually heavy surf. Botton and Loveland (personal communication) also found that peaks in larval abundance occur during periods of rough surf.

When embryos are placed in isoosmotic seawater (60 psu), hatching increases sharply and levels off at 40% after 2 h (Fig. 7; Ehlinger and Tankersley, 2003). Hatching levels are significantly higher than in the treatment in which embryos received no inundation. Hatching significantly

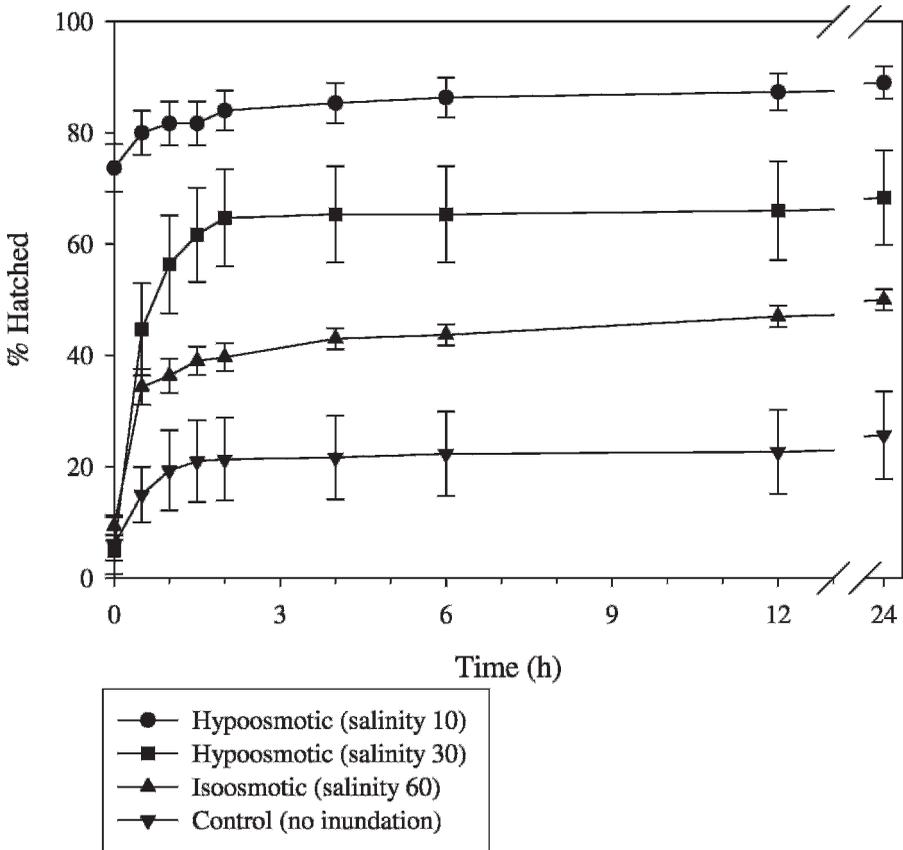


FIG. 7. Percentage ($\bar{X} \pm SE$) of hatched larvae over 24 h after exposure to osmotic shock for 30 embryos ($N = 10$). Two hypoosmotic treatments were tested (salinities of 10 and 30) and an isoosmotic treatment (salinity of 60). Embryos in control conditions were maintained on moist paper towels and were not placed in seawater. ($N = 300$). Redrawn from Ehlinger and Tankersley, 2003.

increases when embryos are placed in hypoosmotic seawater of 10 and 30 psu (Fig. 7). Hatching rates in both hypoosmotic treatments (10 and 30 psu) are significantly greater than levels in the isoosmotic treatment (Ehlinger and Tankersley, 2003). In all treatments, most hatching occurs within the first 2 h following exposure (Fig. 7).

DISCUSSION—In areas with regular tides, spawning by *L. polyphemus* is controlled by an endogenous oscillator that is entrained by external cues associated with the tides (Rudloe, 1979; 1980; Barlow et al., 1986). However, in areas where tidal changes are negligible and high water occurs due to wind forcing, *L. polyphemus* does not appear to respond to localized external cues. Instead, spawning in the IRL is aperiodic and unpredictable (Figs. 2 and 3). In another microtidal system, St. Joseph Bay, FL, horseshoe crabs spawn when

triggered by changes in water level (Rudloe, 1985). However, spawning in the IRL is not triggered by environmental cues.

Compared to more temperate areas, such as Delaware Bay, where spawning occurs from April through July (Shuster and Botton, 1985), horseshoe crab spawning in the IRL is protracted and occurs year-round. This is consistent with latitudinal differences in seasonal temperatures, with spawning generally beginning earlier and ending later along the Gulf coast of Florida (Rudloe and Herrnkind, 1976; Shuster, 1979; Rudloe, 1980; Barlow et al., 1986). However, peaks in spawning in the IRL occur in the spring, which is consistent with other populations in Delaware Bay and on the Gulf Coast of Florida (Figs. 2 and 3; Rudloe, 1980; Barlow et al., 1986; Penn and Brockmann, 1994). Larvae generally appear in the plankton three to four weeks after peaks in spawning (Rudloe, 1979; 1980; Penn and Brockmann, 1994). In the IRL, larvae are present in the plankton in May and June, approximately eight weeks after peak periods of spawning (Fig. 3). This substantial lag between spawning and the appearance of larvae in the water column indicates that hatching may be decoupled from spawning. This decoupling is most likely due to the lack of regular inundation of nests by tidal changes in water level.

In areas with periodic tides, larvae hatch and emerge from the nest only when it is inundated during nocturnal high tides associated with full moons (Rudloe, 1979). In the IRL, larvae were more common in the plankton during periods of high water. This is similar to other marine arthropods, such as *Carcinus maenas* and *Rhithropanopeus harrisi*, which exhibit tidal and lunar rhythms in areas with significant tides, but lack the similar rhythms in areas without significant tidal changes (Naylor, 1960; Cronin and Forward, 1979; 1983). The paucity and irregularity of shoreline inundation in the IRL may contribute to the low densities of larvae found in the plankton. In our sampling, the maximum density was 4 larvae m^{-3} (Fig. 3) compared to over 700 larvae m^{-3} reported in plankton tows on the Gulf Coast of Florida (Rudloe, 1979). Without regular inundation, larvae may be stranded in the upper beach and unable to leave the nest and enter into the water column. Low larval densities in the IRL may also be attributed to more intense predation or the fact that egg densities may be lower.

Horseshoe crab nests are typically located 10–20 cm below the sediment surface at the high water line. Thus, eggs, embryos, and larvae are exposed to wide and rapid fluctuations in temperature and salinity (Penn and Brockmann, 1994). In microtidal areas shoreline inundation is irregular and *L. polyphemus* nests may not be submerged for months. In the IRL, conditions in the shallow waters where horseshoe crabs nest have been documented to reach temperatures and salinities as high as 45°C and 55 psu, respectively (Ehlinger and Tankersley, 2004). *Limulus polyphemus* embryos and larvae can tolerate and successfully develop and hatch in temperatures up to 35°C and in salinities as high as 60 psu (Figs. 4 and 5). Thus, the high salinities experienced by developing embryos and larvae in the IRL do not account for the low larval

densities found, but the high temperatures may account for the low larval densities. Physiologically tolerant embryos and larvae, which are capable of surviving variable temperature and salinity regimes, may be a factor that has contributed to the success of *L. polyphemus* over time.

The presence of larvae in the IRL is associated with periods of high water. Laboratory studies indicate that larvae are triggered to hatch when they are exposed to environmental cues that simulate tidal inundation (Ehlinger and Tankersley, 2003). Hatching is facilitated by hydration, agitation and osmotic shock (Figs. 6 and 7). In tidal systems, larvae hatch and leave nests when inundated at high tide. Thus, the environmental cues that facilitate hatching occur with a regular periodicity, which increases the chances that larvae will be released into the water column (Rudloe, 1979). In microtidal systems, hatching is triggered by environmental cues that simulate hatching. During embryonic development in the nests, the perivitelline fluid within the eggs becomes hyperosmotic to the surrounding water, most likely due to desiccation (Sekiguchi, 1988). Thus, when the nest is finally inundated, the eggs experience an osmotic shock, which triggers hatching (Fig. 7). Hatching is facilitated by environmental cues that occur when nests are inundated by either the tide or by wind driven (forced) changes in water level, thus releasing larvae from the nest when they have the greatest chance of entering the water column. The patchiness of larvae in the IRL may be due to the fact that the nests are inundated aperiodically, resulting in the observed decoupling between peaks in larval abundance and spawning (Ehlinger et al., 2003).

The *Limulus polyphemus* population in the IRL differs in spawning and larval hatching patterns from populations in tidal areas due to the absence of the usual tidal zietgebers. The IRL lacks tidal changes in environmental conditions (salinity, temperature, turbulence) that often serve as synchronization cues and triggers for behaviors such as spawning, hatching, and vertical migration. Adult spawning becomes asynchronous and larvae hatch when they are inundated, resulting in peaks in larval abundance during periods of high water. Consequently, methodologies used to assess population size and the reproductive success of *L. polyphemus* in tidal systems are not as effective in the IRL. Because of the asynchronous spawning and lack of tidally related synchronization cues, the spatial and temporal distribution of adult and larval horseshoe crabs in the IRL is patchy, making it difficult to accurately assess the density and structure of the population.

The *L. polyphemus* population in the IRL also differs in the abundance of adults. The density of spawning adults ranges from 10–60 m⁻² on the Gulf Coast of Florida (Cohen and Brockmann, 1983; Rudloe, 1985) to over 100 m⁻² in Delaware Bay (Shuster and Botton, 1985; Barlow et al., 1986), but the maximum density in the IRL is 6 m⁻² and rarely exceeds 1 m⁻² for most sampling dates (Fig. 2). This difference may be due to the low number of adults or to the absence of synchronized spawning.

Another difference is that horseshoe crabs inhabiting the IRL may be a resident population with little migration into or out of the lagoon. There is no

indication that horseshoe crabs in the IRL are migrating between microtidal areas within the lagoon and tidal areas along the coast and near inlets. Horseshoe crabs in the IRL may remain in the IRL year-round and may not migrate offshore to the continental shelf as they do in many of the northern portions of their range (Botton and Ropes, 1987). In the northern portion of the IRL, horseshoe crabs are approximately 50 km from the closest inlet leading to the Atlantic Ocean. The inlets are small and there is limited exchange between the IRL and the coastal waters. There is little evidence that spawning occurs near the five inlets that link the IRL to the Atlantic Ocean and adults and juveniles have been observed in the Lagoon year-round. Thus, the majority of the population probably remains in the IRL. These results are consistent with the findings of Saunders et al. (1986) who reported significant genetic differences between northern and southern populations in the United States. The isolation of the Indian River Lagoon horseshoe crabs from other populations may contribute to this genetic diversity.

Possible reasons for the decline or small population size of horseshoe crabs in the IRL include changes in water quality, loss of habitat, and increased human harvest. A 188% increase in the human population adjacent to the IRL from 1970 to 1990 resulted in significant decline in water quality, an increase in the prevalence of anoxic sediments, and altered sediment composition along the shoreline (Woodward-Clyde, 1994). The build up of muck and anoxic sediments along the shoreline as a result of increased runoff may have reduced areas of optimal spawning habitat. The inability of eggs to survive in anoxic sediments supports the suggestion that spawning horseshoe crabs do not nest on beaches with anoxic sediments (Botton et al., 1988). Creation of impoundments around the IRL in the 1960s drastically reduced horizontal and vertical diversity of the shoreline suitable for spawning and nesting. Declines in habitat quality and quantity probably resulted in a significant reduction in the reproductive success of *L. polyphemus*. Another factor for the decline may be increased human harvest for biomedical and pharmaceutical research and bait. The extent of human harvest of *L. polyphemus* in Florida has not been documented, however, harvesters have been seen hauling away truckloads of adult crabs from the Indian River (J. Provanca; Dynamac Corp.; personal communication).

Due to aperiodic spawning in the IRL, methodologies used to assess population size in tidal systems are not as effective in microtidal systems, since population censuses are conducted to coincide with full and new moon periods (Smith et al., 2002). The *L. polyphemus* population in the IRL differs in spawning and larval hatching patterns from populations in tidal areas due to the absence of the usual tidal zeitgebers. Larvae hatch when they are inundated, leading to peaks in larval abundance during periods of high water. While *L. polyphemus* in the IRL are capable of spawning and normal embryonic development, further research of the *Limulus polyphemus* population in the IRL is needed to obtain a more accurate measure of population size,

assess habitat quality and determine if the population is a true resident population.

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