

# Ecology of Horseshoe Crabs in Microtidal Lagoons

G.S. Ehlinger and R.A. Tankersley

**Abstract** The American horseshoe crab, *Limulus polyphemus*, typically inhabits estuaries and coastal areas with pronounced tides that have a significant influence on their physiology, behavior, and ecology. Most notably, changes in water level associated with the tides have been shown to underlie temporal and spatial patterns in spawning. However, horseshoe crabs also inhabit areas that lack significant tides and associated cues that are known to mediate reproduction. Our investigations of the potential impact of conditions in microtidal lagoon habitats on the timing and frequency of spawning of *L. polyphemus* adults and the development and survival of embryos and larvae have focused on populations inhabiting the Indian River Lagoon (IRL), a large, shallow estuary along the east coast of Florida, USA. Extensive surveys of adult activity along beaches indicated that spawning occurs year-round but is aperiodic. Larval abundances in the plankton are low and more closely associated with changes in water level than adult spawning activity. Laboratory experiments indicated that embryos and larvae are able to tolerate extreme salinities and temperatures that occur in temperate and tropical microtidal lagoons in the summer. Embryos complete development and molt at salinities below 60, yet fail to develop at temperatures  $\geq 35^{\circ}\text{C}$ . Larvae tolerate salinities of 10–70, but survivorship declines significantly at more extreme salinities (i.e., 5, 80, and 90). Results indicate that both temperature and salinity influence the rate of development, but only the extremes of these environmental factors have an effect on survival. In general, the reproductive ecology and early life history of *L. polyphemus* in microtidal lagoons differ markedly from those documented for populations inhabiting tidal areas. This difference is likely due to extreme salinity and temperature conditions during summer reproductive periods and the lack of tidally related synchronization cues for spawning and regular, periodic beach inundation.

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## 1 Introduction

The American horseshoe crab *Limulus polyphemus* (Linnaeus) is a common inhabitant of the sublittoral zone of estuaries throughout the Atlantic and Gulf coasts of North America. Throughout much of its geographic range, spawning and larval hatching occur synchronously with tidal and lunar cycles (Rudloe 1979, 1980, 1985, Botton et al. 1988). Details of their reproductive biology are best known for populations inhabiting Delaware Bay and the Gulf Coast of Florida, USA (Cohen and Brockmann 1983, Rudloe 1985, Shuster and Botton 1985, Botton et al. 1988, Penn and Brockmann 1994). In these areas, mating occurs in the spring and early summer when females migrate to shallow areas during spring tides to spawn on sandy beaches (Cohen and Brockmann 1983, Rudloe 1985, Shuster and Botton 1985, Botton et al. 1988, Penn and Brockmann 1994). Peak spawning generally occurs near the time of high tide during new and full moons and appears to be under endogenous control (Rudloe 1980, Cohen and Brockmann 1983, Barlow et al. 1986). Eggs develop in the sand, hatching into trilobite larvae approximately 24–28 days after fertilization (Botton and Loveland 2003). Most trilobites emerge and enter the water column when inundated during spring tides (Rudloe 1979, 1980, Jegla and Costlow 1982, Laughlin 1983, Sugita 1988, Penn and Brockmann 1994). Newly hatched trilobite larvae exhibit a diurnal vertical migration pattern, with larvae remaining buried during the day and entering the water column at night (Rudloe 1979, 1980).

Timing of spawning and larval hatching of horseshoe crabs varies among areas with different tidal regimes (Cohen and Brockmann 1983, Rudloe 1985, Shuster and Botton 1985, Botton et al. 1988, Penn and Brockmann 1994). While seasonal and tidal patterns in spawning have been well documented for *L. polyphemus* inhabiting areas with semi-diurnal tides (Shuster 1982, Rudloe 1985, Barlow et al. 1986, Shuster et al. 2004), horseshoe crabs along the Gulf Coast of Florida that experience diurnal tides exhibit a spawning pattern that is consistent with local tides (Rudloe 1979, 1980, 1985, Cohen and Brockmann 1983). However, horseshoe crabs also occur in microtidal lagoons that lack regular tidal changes and periodic shoreline inundation.

*Limulus polyphemus* generally occurs in estuaries where the salinity ranges from 5 to 34. While *L. polyphemus* densities are highest in euhaline areas near the mouths of estuaries, they also inhabit oligohaline regions (Shuster 1982). Although adult and juvenile *L. polyphemus* live in subtidal benthic habitats, embryonic and early larval development occurs in intertidal areas. Adult *L. polyphemus* nest near the waterline in the mid- to upper intertidal zone and deposit up to 20,000 eggs 10–25 cm below the sediment surface (Shuster and Botton 1985, Brockmann 1990, Penn and Brockmann 1994). Horseshoe crabs deposit their eggs in the mid to upper beach where conditions are optimal for egg development since the sediments at higher elevations are warmer and drier and those at lower elevations are often anoxic (Penn and Brockmann 1994). Nests are laid in sandy areas that are regularly inundated in tidal systems, but

have variable frequencies and periods of inundation in microtidal areas (Rudloe 1985, Penn and Brockmann 1994). Nevertheless, because of the location of the nests on the beach, *L. polyphemus* embryos and larvae are potentially exposed to larger fluctuations in temperature and salinity than those experienced by subtidal adults. During low tide, nests may be exposed to freshwater during periods of rain and experience rapid changes in temperature as a result of heating of the beach by sunlight. Thus, developing embryos are expected to be capable of tolerating rapid and wide fluctuations in environmental conditions.

Adult horseshoe crabs also inhabit microtidal lagoons that are characterized by a much broader salinity range (5–55) due to shallow conditions and high rates of evaporation and freshwater input (Pritchard 1967, Robertson 1970, Shuster 1982, Botton et al. 1988, Ehlinger et al. 2003). In estuarine habitats of New England and the mid-Atlantic region, low salinities caused by snow melt and freshwater run-off are more common than high salinity conditions. Therefore, most studies conducted on the effects of salinity on embryonic development have focused on tolerance of hypoosmotic stress. Optimal salinity range for larval development is between 20 and 30 (Jegla and Costlow 1982, Laughlin 1983, Sugita 1988). Development time of embryos to the larval stage (trilobite larva) increases at salinities above and below this range (Jegla and Costlow 1982, Laughlin 1983, Sugita 1988). Temperature has also been found to affect the rate of embryonic development and the duration of posthatch intermolt stages, with the optimal temperature for development ranging between 25 and 30°C (Jegla and Costlow 1982).

Placement of *L. polyphemus* nests in the upper littoral zone of sandy beaches affords developing embryos protection from aquatic predators, with most surviving to hatching (Rudloe 1979). However, this nesting pattern creates problems for the emergence and dispersal of larvae into the aquatic environment. Embryos hatch into trilobite larvae in approximately 28 days and emerge into the water when inundated during nocturnal high tides near the time of full moon (Rudloe 1979, Penn and Brockmann 1994). However, because of the wide geographic distribution of *L. polyphemus*, tidal conditions experienced by developing embryos vary significantly. In microtidal lagoons, the frequency of nest inundation varies throughout the species' range and is not always predictable. Given the diverse range of shoreline inundation patterns, hatching of *L. polyphemus* eggs may be facilitated by environmental triggers that help synchronize larval hatching with water levels on the beach, especially in nontidal 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 plankton.

In tidally dominated estuaries and coastal habitats, periodic (tidal) changes in water level help maintain optimal conditions for horseshoe crab embryonic development (Penn and Brockmann 1994). However, in shallow microtidal estuaries, submergence of nesting areas on the beach depends on freshwater input and wind forcing. Therefore, spawning and nesting activities may be restricted to periods when wind events result in significant increases in water

level. Moreover, the lack of regular beach inundation may cause conditions within the sediments to be unsuitable for embryonic development, hatching, or larval emergence. Thus, the paucity of tidally related synchronization cues and regular beach inundation is hypothesized to have a significant impact on the mating activities of adult crabs and the development and hatching patterns of larvae.

Our investigations of the potential impact of conditions in microtidal lagoons on the timing and frequency of spawning of *L. polyphemus* adults and the development and survival of embryos and larvae have focused on populations inhabiting the Indian River Lagoon complex (IRL). The IRL consists of three shallow (1–3 m deep) sub-basins, the Indian River, Banana River, and Mosquito Lagoon, that extend approximately 250 km parallel to the Atlantic coast of Florida (Smith 1987, De Freese 1991). Although significant tidal changes occur in the immediate vicinity of the five inlets that link the IRL to the ocean, most of the system is virtually tideless (tidal amplitudes < 5 cm), with wind forcing and freshwater input responsible for changes in water level (Smith 1993).

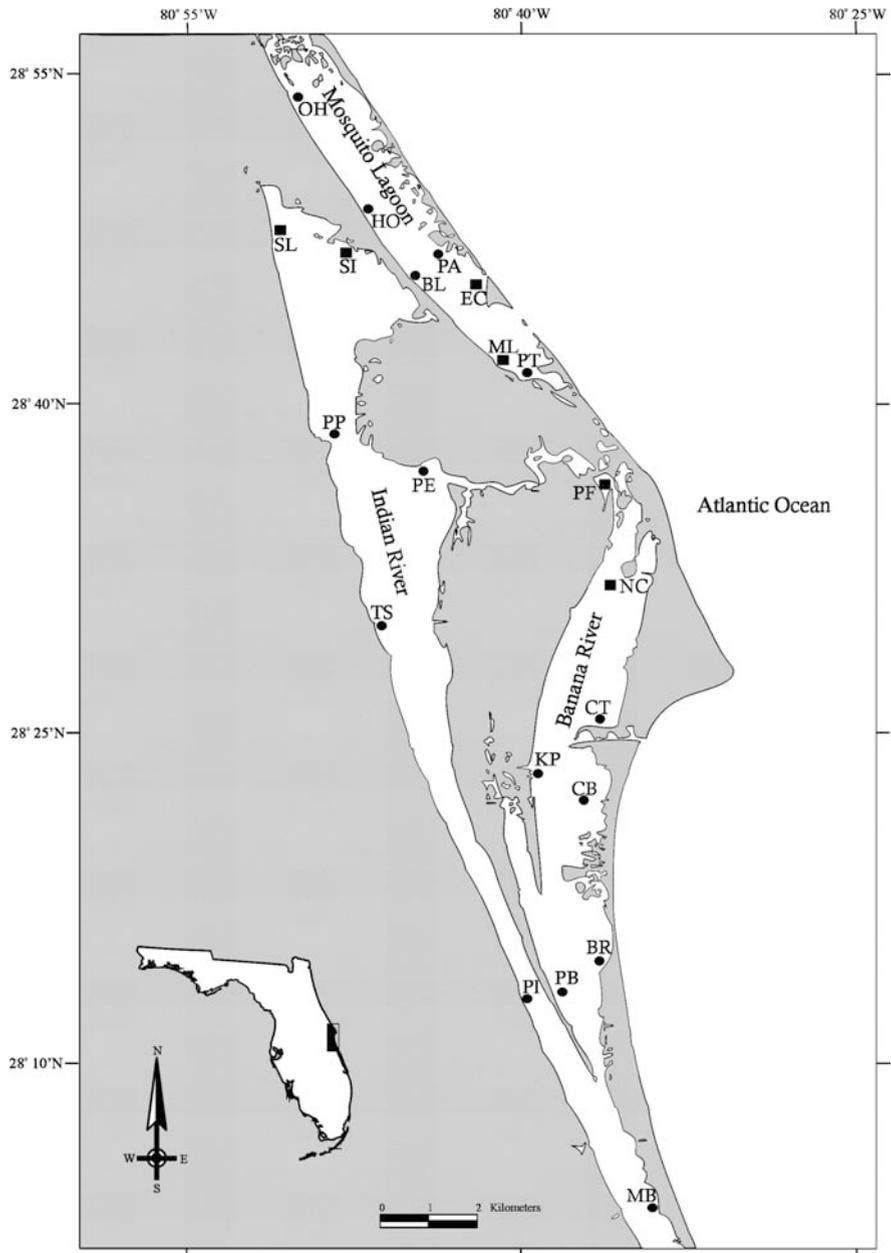
Like many microtidal lagoons, the IRL experiences temperature and salinity conditions that are more extreme than those found in most estuaries (Pritchard 1967, Robertson 1970). Temperature and salinity values as high as 45°C and 55, respectively, have been reported in the IRL during summer spawning periods (IRLNEP 1994). These extreme environmental conditions may influence embryonic development, hatching success, and larval survival. This chapter summarizes the results of a series of studies investigating the reproductive ecology and early life history of *L. polyphemus* in the microtidal IRL. The studies focus on the potential impact of microtidal conditions on the timing and frequency of spawning and embryonic and larval development in extreme temperature and salinity conditions.

## 2 Methods and Results

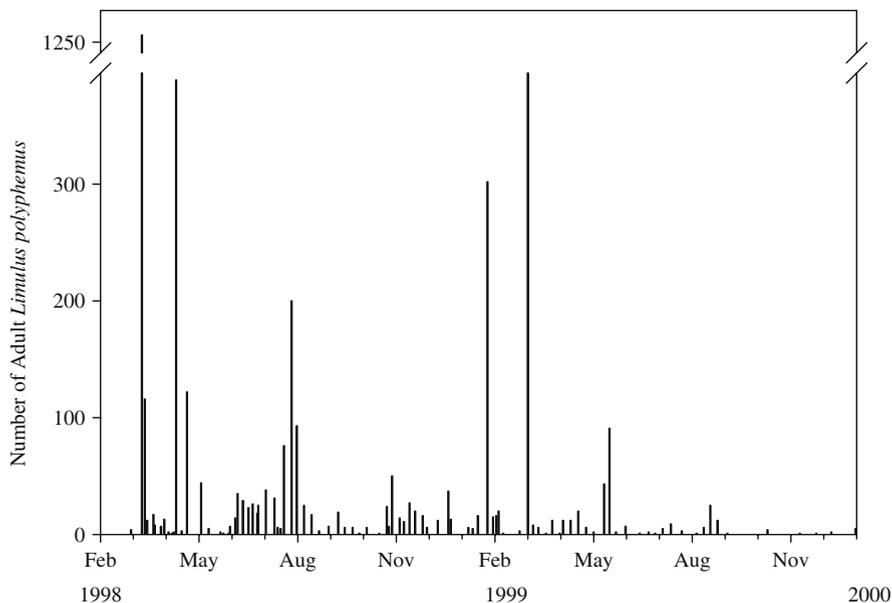
### 2.1 Spawning and Larval Abundance

Adult and larval surveys conducted at 21 sites in the IRL (Fig. 1) from February 1999 to August 2000 (see Ehlinger et al. 2003 for detailed methodology) indicated that adult spawning activity varies seasonally with peaks in the abundance of spawning pairs occurring in the late winter/early spring (Ehlinger et al. 2003, Figs. 2 and 3). During the study period, short episodic peaks in spawning were often separated by long intervals (3–4 weeks) in which no mating occurred (Figs. 2 and 3). Spawning is therefore aperiodic and lacks any apparent lunar or semi-lunar periodicity.

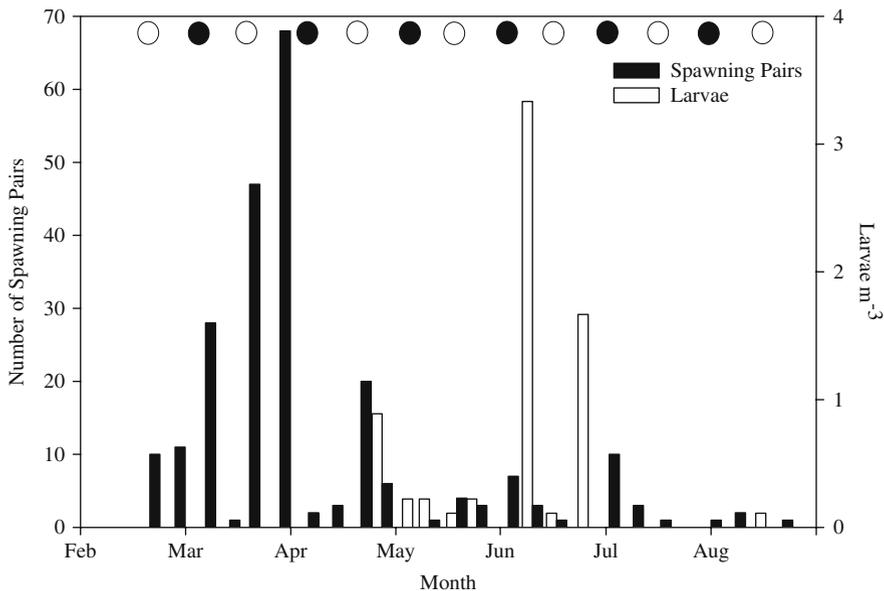
Larvae were collected in plankton samples from late spring to early summer, but were only found at 12 of the 21 sites (Fig. 3). In general, larval abundance was unrelated to spawning activity since the time interval (lag) between peaks in



**Fig. 1** Study sites in the Indian River Lagoon system. Six study sites (■) were sampled during Phase I of the study from February 1998 to December 1999 for adult *Limulus polyphemus*. Twenty-one study sites (■ and ●) were sampled during phase II of the study from February to 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)



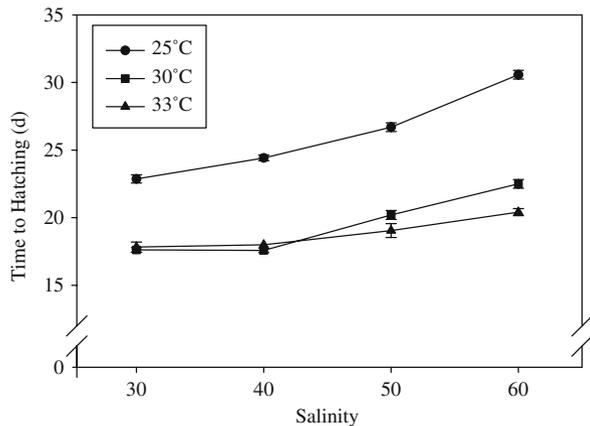
**Fig. 3** Temporal pattern of spawning and larval hatching of *Limulus polyphemus* during Phase II (February–August 2000) of this study. *Open circles* represent full moons and *closed circles* represent new moons. Redrawn from Ehlinger et al. (2003)

adult and larval densities ( $\approx$  8 weeks, Fig. 3; Ehlinger et al. 2003) exceeded the duration of embryonic development ( $\approx$  4 weeks, Rudloe 1979, Sekiguchi 1988, Penn and Brockmann, 1994). Moreover, spawning activity throughout the IRL is not synchronized since peaks occur at different sites at different times. The distribution and abundance of larvae is not always linked to the spatial distribution of spawning adults since larvae were frequently found at sites where spawning was rarely observed.

Attempts to correlate the abundance of adults and larvae with environmental and hydrologic variables indicated that spawning and larval hatching appear to be triggered or associated with different cues (Ehlinger et al. 2003). For adults, seasonal abundances were associated with a decrease in temperature/salinity. However, the presence of spawning adults is not associated with any other environmental variable that might serve as a synchronization cue. Conversely, the presence of larvae is associated with periods of high water when beach width and salinity levels are low.

## 2.2 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 tested (salinities of 30–60; 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

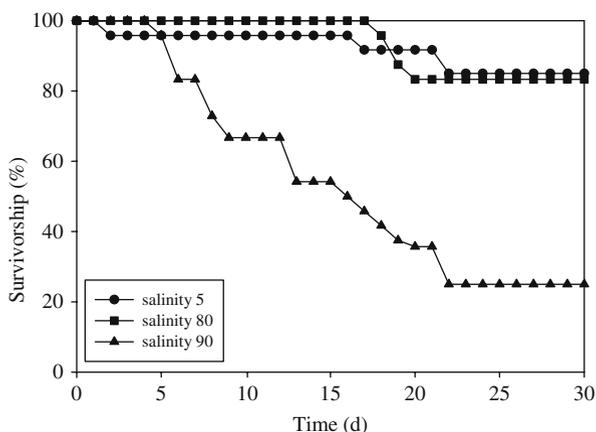


**Fig. 4** Mean ( $\pm$  SE) number of days from fertilization to hatching of *Limulus polyphemus* embryos at 25, 30, and 33°C and at salinities of 30, 40, 50, and 60. No hatching occurred in any of the test salinities at 35 and 40°C.  $N = 30$  for each trial. Redrawn from Ehlinger and Tankersley (2004)

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 salinities of 30 and 40 have similar hatching rates, but hatching is delayed significantly in more hypersaline conditions (salinities of 50 and 60, Fig. 4). The optimal temperature and salinity conditions for development is 30–33°C and 30–40, respectively (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 (see Ehlinger and Tankersley 2004 for detailed methodology). Mortality only occurs in the very extreme salinities of 5, 80, and 90 (Fig. 5). Comparisons of the survivorship curves among salinity treatments indicate that survivorship is only significantly reduced when larvae are maintained at a salinity of 90 (Fig. 5). Time to 50% mortality (TM<sub>50</sub>) is ≈16.0 days in a salinity of 90 (Ehlinger and Tankersley 2004).

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

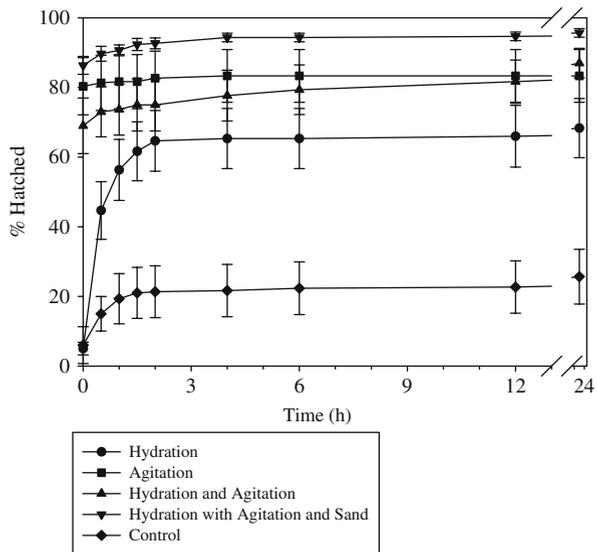


### 2.3 Hatching Triggers

Embryos hatch into trilobite larvae in approximately 28 days and move toward the surface of the substrate to enter the water when inundated during nocturnal high tides near the time of full moon (Rudloe 1979, Penn and Brockmann 1994). Given the diverse range of tidal patterns that occur throughout its range, we hypothesized that the release of *L. polyphemus* larvae from nests in the sand may be facilitated by one or more environmental triggers that serve to synchronize hatching with periods of high water on the beach. If an environmental cue associated with inundation triggers hatching, then larvae will hatch and emerge when conditions are favorable for entry into the water column, thereby increasing their chance of survival. This is especially important in microtidal systems since inundation of the beach is aperiodic. To test this hypothesis, developing

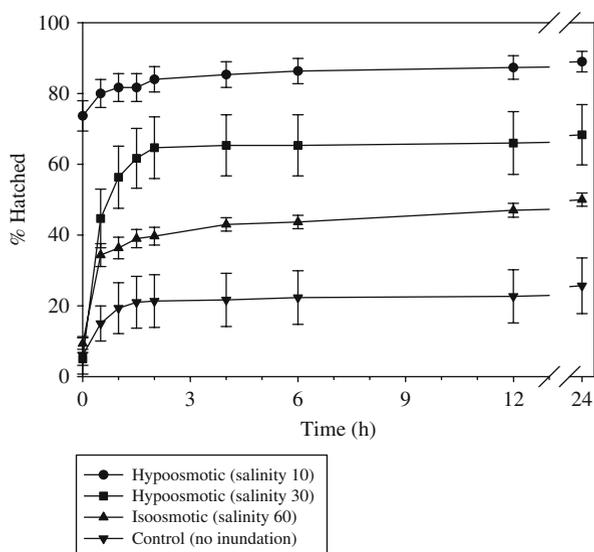
embryos were exposed to different treatments that mimicked conditions experienced during periods of high water (hydration, agitation, and osmotic shock; Ehlinger and Tankersley 2003). When exposed to conditions representative of high tide in the field, hatching levels of late-stage (Stage 21) embryos increase significantly relative to levels in control treatments (Fig. 6; Ehlinger and Tankersley 2003). For all treatments tested (hydration, agitation, hydration and agitation, and hydration with agitation and sand), most hatching occurred within the first 2 h following exposure (Fig. 6). Most embryos (>65%) in the treatments involving agitation hatched during the exposure period (Fig. 6). Hatching levels in the hydration-only treatment increased rapidly following exposure, but were 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 were 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 coincide with high tide, producing unusually heavy surf. Botton and Loveland (2003) also found that peaks in larval abundance occur during periods of rough surf.

**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)



When embryos are placed in isoosmotic seawater (salinity of 60) relative to their perivitelline fluid, 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 is facilitated when embryos are exposed to seawater that is hypoosmotic to their perivitelline fluid. Ehlinger and Tankersley (2003) found that the perivitelline fluid of embryos collected from the Banana River, FL, and Delaware Bay, NJ,

**Fig. 7** Percentage ( $\bar{X} \pm \text{SE}$ ) 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)



was hyperosmotic to the surrounding seawater and porewater collected at the same time, thus, when inundated, embryos experience a hypoosmotic shock. Hatching significantly increased when embryos were placed in hypoosmotic seawater with a salinity of 10 and 30 (Fig. 7, Ehlinger and Tankersley 2003). In all treatments, most hatching occurred within the first 2 h following exposure (Fig. 7) which would time hatching with inundation of the nest and allow the larvae to enter the water column.

## 2.4 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 microtidal lagoons where tidal changes are negligible and high water occurs due to wind forcing and freshwater input, *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.

Larvae generally appear in the plankton 3–4 weeks after peaks in spawning (Rudloe 1979, 1980, Penn and Brockmann 1994). During our sampling period in the IRL, larvae were present in the plankton in May and June, approximately 8 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 tidal areas, larvae hatch and emerge from the nest when it is inundated during nocturnal high tides associated with full moons (Rudloe 1979) or when there is a strong onshore wind that creates wave action (Botton and Loveland 2003). Hatching and emergence from the nest appears to be triggered by environmental stimuli in microtidal systems since the appearance of larvae in the plankton was associated with periods of high water. This pattern is consistent with the results of Rudloe (1979) who found that larvae exhibit strong lunar and diel patterns in hatching and emergence in areas where nests are inundated by high tides associated with full moons. This pattern is also consistent with Botton and Loveland (2003) who found that the abundance of planktonic horseshoe crab larvae in lower Delaware Bay was most closely linked with strong onshore winds that generated wave heights above 30 cm. This is similar to other marine arthropods, such as *Carcinus maenas* and *Rhithropanopeus harrisi*, which exhibit tidal and lunar rhythms in tidal areas 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  $\text{m}^{-3}$  (Fig. 3) compared to over 700 larvae  $\text{m}^{-3}$  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.

Horseshoe crab nests are typically located 10–20 cm below the sediment surface at the high water line. Thus, developing embryos and larvae are often 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, respectively (Ehlinger and Tankersley 2004). *Limulus polyphemus* embryos and larvae develop and hatch in constant temperatures up to 35°C and in salinities as high as 60 (Figs. 4 and 5). *Limulus polyphemus* populations inhabiting the IRL experience higher temperatures and salinities than those in the more northern portion of its range. Therefore, populations inhabiting the IRL may be able to withstand higher temperatures and salinities, as a result of acclimatization, resulting in slightly higher optimal ranges for development and growth (30–33°C and salinities of 30–40). Botton et al. (2006) found that horseshoe crabs have adapted to living in a thermally stressful environment by maintaining a high baseline level of cellular stress proteins such as heat shock protein 70 (Hsp70). They found that there was a slight elevation of Hsp70 only among heat-shocked trilobite larvae in a 6 h recovery treatment and that Hsp70 levels did not differ significantly between control and heat-shocked embryos, thus intertidal embryos and larvae do not have to synthesize inducible Hsps when stressful temperatures are encountered (Botton et al. 2006). This is advantageous because embryos and

larvae encounter regular heat shocks due to diurnal and tidal temperature changes. 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.

The ability of *L. polyphemus* embryos and larvae to tolerate a wide range of salinities is an important adaptation to the extreme conditions in microtidal lagoons. Tolerating such a wide range of conditions may be an advantage in species that live in a highly variable, unpredictable environment (Anger 1991). Physiologically tolerant embryos and larvae, which are capable of surviving extreme temperature and salinity regimes, may be a factor that has contributed to the evolutionary success of *L. polyphemus*.

In the IRL, larvae occur in the plankton during periods of high water. Laboratory studies indicate that larvae are triggered to hatch when they are exposed to environmental cues that are associated with inundation (Ehlinger and Tankersley 2003). Although larval hatching in *L. polyphemus* occurs in the absence of external stimuli (Jegla 1979), hatching is facilitated when embryos are subjected to environmental conditions associated with periods of high water, including hydration, agitation, and osmotic shock (Figs. 6 and 7). Hatching in response to these environmental cues is likely adaptive since it would increase the likelihood that they would hatch and emerge from the nest during periods of inundation, thereby preventing them from being stranded. 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 lagoons, hatching is triggered by environmental cues that are associated with inundation. 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 inundated, the eggs experience an osmotic shock, which triggers hatching (Fig. 7). Increased hatching levels in *L. polyphemus* exposed to only hydration supports the findings of Rudloe (1979) that *L. polyphemus* embryos enter the water column immediately when the nest is inundated. During periods of high water, the nests would be inundated which would facilitate hatching and larval release from the nest, leading to a greater presence of larvae in the plankton. Increased hatching when hydrated is particularly important in microtidal and nontidal systems where shoreline inundation is unpredictable and controlled by wind forcing, not changes in water level associated with tides (Ehlinger et al. 2003). If the nest is briefly inundated, *L. polyphemus* larvae would hatch and emerge into the water column during periods of high water, thus preventing stranding in the nest and problems of nest release in a nontidal system. Similar responses to mechanical stimulation have been reported for other species which undergo development in intertidal areas. Waves and mechanical agitation that simulate the action of the rising tide trigger larval hatching in the capelin, *Mallotus villosus* (Frank and Leggett 1981) and in the isopod *Excirolana chiltoni* (Enright 1965). The inconsistency of planktonic 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).

Patterns of spawning and larval hatching in *Limulus polyphemus* populations in microtidal lagoons differ from those in tidal areas due to the absence of tidally associated zeitgebers. 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 reproductive periodicity of *L. polyphemus* in tidal systems are ineffective in microtidal lagoons. Because of the asynchronous spawning and lack of tidally related synchronization cues, the spatial and temporal distribution of adult and larval horseshoe crabs in microtidal lagoons may be patchy, making it difficult to accurately assess the density and structure of the population.

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## References

- Anger K (1991) Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Grapsidae). *Mar Ecol Prog Ser* 72:103–110
- Barlow RM, Powers K, Howard H, Kass L (1986) Migration of *Limulus* for mating: Relation to lunar phase, tide height, and sunlight. *Biol Bull* 171:310–329
- Botton ML, Loveland RE, Jacobsen TR (1988) Beach erosion and geochemical factors: Influence on spawning success of horseshoe crabs (*Limulus polyphemus*) in Delaware Bay. *Mar Biol* 99:325–332
- Botton ML, Loveland RE (2003) Abundance and dispersal potential of horseshoe crab (*Limulus polyphemus*) larvae in the Delaware Estuary. *Estuaries* 26:1472–1479
- Botton ML, Pogorzelska M, Smoral L, Shehata A, Hamilton MG (2006) Thermal biology of horseshoe crab embryos and larvae: A role for heat shock proteins. *J Exp Mar Biol Ecol* 336:65–73.
- Brockmann HJ (1990) Mating behavior of horseshoe crab, *Limulus polyphemus*. *Behavior* 114:206–220.
- Cohen JA, Brockmann HJ (1983) Breeding activity and mate selection in the horseshoe crab, *Limulus polyphemus*. *Bull Mar Sci* 33:274–281.
- Cronin TW, Forward RB Jr (1979) Tidal vertical migration: An endogenous rhythm in estuarine crab larvae. *Science* 205:1020–1022.
- Cronin TW, Forward RB Jr (1983) Vertical migration rhythms of newly hatched larvae of the estuarine crab, *Rhithropanopeus harrisi*. *Biol Bull* 165:139–153.
- De Freese DE (1991) Threats to biological diversity in marine and estuarine ecosystems of Florida. *Coast Manage* 19:73–101.
- Ehlinger GS, Tankersley RA, Bush MB (2003) Spatial and temporal patterns of spawning and larval hatching by the horseshoe crab, *Limulus polyphemus*, in a microtidal coastal lagoon. *Estuaries* 26:631–640.

- Ehlinger GS, Tankersley RA (2003) Larval hatching in the horseshoe crab, *Limulus polyphemus*: Facilitation by environmental cues. *J Exp Mar Biol Ecol* 292:199–212.
- Ehlinger GS, Tankersley RA (2004) Survival and development of horseshoe crab (*Limulus polyphemus*) embryos and larvae in hypersaline conditions. *Biol Bull* 206:87–94.
- Enright JT (1965) Entrainment of a tidal rhythm. *Science* 147:864–867.
- Frank KT, Leggett WC (1981) Wind regulation of emergence times and early larval survival in capelin (*Mallotus villosus*). *Can J Fish Aquat Sci* 38:215–223.
- Indian River Lagoon National Estuary Program (1994) Biological resources of the Indian River Lagoon. IRL National Estuary Program, Melbourne FL.
- Jegla TC (1979) The *Limulus* bioassay for ecdysteroids. *Biol Bull* 156:103–114.
- Jegla TC, Costlow JD (1982) Temperature and salinity effects on developmental and early posthatch stages of *Limulus*. In: Bonaventura J, Bonaventura C, Tesh S (eds) *Physiology and Biology of Horseshoe Crabs*. Alan R. Liss Inc., New York, pp 103–113.
- Laughlin R. (1983) The effects of temperature and salinity on larval growth of the horseshoe crab *Limulus polyphemus*. *Biol Bull* 164:93–103.
- Naylor E (1960) Locomotory rhythms in *Carcinus maenus* (L.) from nontidal conditions. *J Exp Biol* 37:481–488.
- Penn D, Brockmann HJ (1994) Nest-site selection in the horseshoe crab, *Limulus polyphemus*. *Biol Bull* 187:373–384.
- Pritchard DW (1967) What is an estuary: physical viewpoint. In: Lauff GH, (ed) *Estuaries*. American Association for the Advancement of Science, Washington, DC, pp. 1–157.
- Robertson JD (1970) Osmotic and ionic regulation in the horseshoe crab *Limulus polyphemus* (Linnaeus). *Biol Bull* 138:157–183.
- Rudloe AE (1979) Locomotor and light responses of larvae of the horseshoe crab, *Limulus polyphemus*. *Biol Bull* 157:494–505.
- Rudloe AE (1980) The breeding behavior and patterns of movement of horseshoe crabs, *Limulus polyphemus*, in the vicinity of breeding beaches in Apalachee Bay, Florida. *Estuaries* 3:177–183.
- Rudloe AE (1985) Variation in the expression of lunar and tidal behavior rhythms in the horseshoe crab, *Limulus polyphemus*. *Bull Mar Sci* 36:388–395.
- Sekiguchi K (1988) *Biology of horseshoe crabs*. Science House, Tokyo.
- Shuster CN Jr. (1982) A pictorial review of the natural history and ecology of the horseshoe crab, *Limulus polyphemus*, with reference to other Limulidae. In: Bonaventura J, Bonaventura C, Tesh S (eds) *Physiology and Biology of Horseshoe Crabs*. Alan R. Liss Inc., New York, pp 1–52.
- Shuster CN, Botton ML (1985) A contribution to the population biology of horseshoe crabs, *Limulus polyphemus* in Delaware Bay. *Estuaries* 8:363–372.
- Shuster CN, Brockmann HJ, Barlow RB (eds) (2004) *The American Horseshoe Crab*. Harvard University Press, Cambridge.
- Smith, NP (1987) An introduction to the tides of Florida's Indian River Lagoon. I. Water Levels. *Fla Sci* 50:49–61.
- Smith NP (1993) Tidal and wind-driven transport between Indian River and Mosquito Lagoon, Florida. *Fla Sci* 56:235–246.
- Sugita H (1988) Environmental adaptations of embryos. In: Sekiguchi K, (ed) *Biology of horseshoe crabs*. Science House, Tokyo, pp 195–224.