Reference: *Biol. Bull.* **206:** 87–94. (April 2004)

Survival and Development of Horseshoe Crab (*Limulus polyphemus*) Embryos and Larvae in Hypersaline Conditions

GRETCHEN S. EHLINGER* AND RICHARD A. TANKERSLEY

Florida Institute of Technology, Department of Biological Sciences, 150 W. University Blvd., Melbourne, Florida 32901

Abstract. The horseshoe crab *Limulus polyphemus* spawns in the mid- to upper intertidal zone where females deposit eggs in nests below the sediment surface. Although adult crabs generally inhabit subtidal regions of estuaries with salinities from 5 to 34 ppt, developing embryos and larvae within nests are often exposed to more extreme conditions of salinity and temperature during summer spawning periods. To test whether these conditions have a negative impact on early development and survival, we determined development time, survival, and molt cycle duration for *L. polyphemus* embryos and larvae raised at 20 combinations of salinity (range: 30–60 ppt) and temperature (range: 25–40 °C). Additionally, the effect of hyperosmotic and hypoosmotic shock on the osmolarity of the perivitelline fluid of embryos was determined at salinities between 5 and 90 ppt. The embryos completed their development and molted at salinities below 60 ppt, yet failed to develop at temperatures of 35 °C or higher. Larval survival was high at salinities of 10–70 ppt but declined significantly at more extreme salinities (*i.e.,* 5, 80, and 90 ppt). Perivitelline fluid remained nearly isoosmotic over the range of salinities tested. Results indicate that temperature and salinity influence the rate of crab development, but only the extremes of these conditions have an effect on survival.

Introduction

Estuaries are physiologically challenging habitats for organisms because of the temporal and spatial variation in environmental conditions. Among environmental variables, salinity and temperature are two factors that especially influence the survival and growth of marine invertebrates (Kinne, 1970, 1971). Salinity influences many physiological functions and is therefore important in regulating the distribution of estuarine and marine organisms. Estuarine species are generally euryhaline and eurythermal and, therefore, more tolerant of widely ranging temperatures and salinities than marine species (Costlow *et al.,* 1966; Laughlin and French, 1989; Goncalves *et al.,* 1995). Because the range of conditions that an organism can survive may change throughout development (Kinne, 1970, 1971; Charmantier *et al.,* 1988), ontogenetic differences in temperature and salinity tolerance often enable larvae, juveniles, and adults to inhabit different habitats or regions of estuaries (Charmantier *et al.,* 1988).

The American horseshoe crab *Limulus polyphemus* (L.) occurs in estuaries along the east coast of North America, where the general salinity range is 5 to 34 ppt. Although its densities are highest in portions of the estuary with higher and variable salinities, the species also inhabits regions with lower salinities (Shuster, 1982). The adults and juveniles live in subtidal benthic habitats, but embryonic and early larval development occurs in intertidal areas. Females dig nests near the waterline in the mid- to upper intertidal zone and deposit up to 20,000 eggs per clutch 10–25 cm below the sediment surface (Shuster and Botton, 1985; Brockmann, 1990; Penn and Brockmann, 1994). Peak spawning occurs in the late spring to early summer (Cohen and Brockmann, 1983; Barlow *et al.,* 1986), generally near the time of high tide during new and full moons (Rudloe, 1980; Cohen and Brockmann, 1983; Barlow *et al.,* 1986). Eggs are laid in sandy areas that are regularly inundated in tidal systems and have variable frequencies and periods of inun-

Received 4 August 2003; accepted 13 January 2004.

* To whom correspondence should be addressed. Current address: Florida Fish and Wildlife Conservation Commission, Jacksonville Field Laboratory, 6134 Authority Ave, Jacksonville, FL 32221. E-mail: Gretchen.Ehlinger@fwc.state.fl.us

dation in nontidal areas (Rudloe, 1985; Penn and Brockmann, 1994). Because the nests are located on the beach, the embryos and larvae may be exposed to fluctuations in temperature and salinity that are greater than those experienced by adults in subtidal areas. During low tide, nests may be exposed to freshwater during rainfall and to rapid changes in temperature when the beach is heated by the sun. Thus, developing embryos might be expected to tolerate rapid and wide fluctuations in temperature and salinity. Alternatively, they may be protected from changes in external conditions by the perivitelline fluid, the fluid inside the inner egg membrane, which may buffer embryos from changes in external conditions, especially salinity.

Adult horseshoe crabs also inhabit lagoons and coastal embayments with a much broader salinity range (5–55 ppt) due to shallow conditions and relatively high rates of evaporation and freshwater input (Pritchard, 1967; Robertson, 1970; Shuster, 1982; Botton *et al.,* 1988; Ehlinger *et al.,* 2003). One such habitat is the Indian River Lagoon (IRL) located along the east coast of Florida, USA. The IRL consists of three shallow (1–3 m) sub-basins, the Indian River, Banana River, and Mosquito Lagoon, that extend about 250 km parallel to the Atlantic coast (Smith, 1987; De Freese, 1991). Although appreciable 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; Smith, 1993). Despite the presence of spawning adults, densities of *L. polyphemus* larvae in the IRL are low compared to levels in tidally dominated habitats such as Delaware Bay and the Gulf coast of Florida (Rudloe, 1979; Ehlinger *et al.,* 2003; M. L. Botton, Fordham University, pers. comm.). One possible cause of this low larval abundance is physiological stress. A recent study of the spawning and reproductive behavior of horseshoe crabs inhabiting the IRL indicates that water temperatures and salinities during the spawning season reach levels as high as 45 °C and 55 ppt, which may surpass the tolerance limits of the embryos or prevent larval development and hatching (Ehlinger, 2002).

In estuarine habitats of New England and the mid-Atlantic region, low salinities caused by snow melt and freshwater runoff are more common than high salinities. Therefore, most studies on the effect of salinity on embryonic development have focused on hypoosmotic stress and tolerance to low salinities. Previous studies indicate that the optimal salinity for the development of horseshoe crab embryos is between 20 and 30 ppt (Jegla and Costlow, 1982; Laughlin, 1983; Sugita, 1988). Temperature also affects the rate of embryonic development and the duration of posthatch intermolt stages, with the optimal temperature for development ranging from 25 °C to 30 °C (Jegla and Costlow, 1982). Laughlin (1983) concluded that the effect of salinity is secondary to that of temperature, since the duration of larval development was similar among salinity treatments but differed significantly among temperature treatments.

No published studies have examined the tolerance of *L. polyphemus* embryos to salinities higher than 40 ppt. The objectives of this study were to determine the effect of high temperatures and salinities on embryonic and larval development and to determine the salinity tolerance of *L. polyphemus* larvae. The effect of hyperosmotic shock on the osmotic concentration of the perivitelline fluid within the eggs was also examined. The results indicate that *L. polyphemus* embryos and larvae can tolerate a wide range of salinities (30–60 ppt), but they are more sensitive to high temperatures (≥35 °C).

Materials and Methods

Adult specimens of *Limulus polyphemus* were collected by hand during the spawning season (February–May 2002) from the Indian River Lagoon, Florida. Crabs were obtained from two sites: (1) Pineda Causeway, Banana River (28°12′33″N, 80°38′12″W) and (2) Peacock Pocket, Indian River (28°39′41″N, 80°43′45″W). The crabs were maintained in the laboratory in a recirculating fiberglass tank (2.7 m × 1.7 m × 1 m) containing natural seawater (temperature 20–23 °C; salinity 30 ppt). For all experiments, eggs were fertilized by artificial insemination, and the embryos were cultured in the laboratory according to standard procedures (Brown and Clapper, 1981; Sekiguchi, 1988). Sperm collected from males by manual stimulation of the genital operculum were diluted with filtered seawater to make a 10% (vol/vol) sperm solution. Eggs collected from females by direct extraction from the ovaries were washed several times with 5-μm filtered seawater, placed in petri dishes (diameter 8.5 cm; height 1.4 cm) containing 50 ml of filtered seawater, and fertilized with 1 ml of the sperm solution. The eggs were incubated with sperm for 1 h and then rinsed with filtered seawater to remove excess sperm. To determine whether fertilization was successful, a subset of the eggs was stained with a solution of 0.1% neutral red and observed under a dissecting microscope for signs of cleavage and gastrulation (Sekiguchi, 1988). The developmental stage of the embryos was determined using the classification scheme of Sekiguchi (1988).

Effect of high temperature and salinity on embryonic and larval development

To determine whether high temperature and salinity influence the rate and success of embryonic development and the duration of the larval stage following hatching, fertilized eggs were reared under one of 20 combinations of salinity and temperature (salinities: 30, 40, 50, 60 ppt; temperature: 25, 30, 33, 35, 40 °C). Thirty eggs were placed in individual wells (1.5 cm diameter × 1.5 cm depth) of a multiwell tissue culture dish containing 3 ml of filtered natural sea-

water at each temperature-salinity combination. For each combination, 10 dishes were placed in a thermostatically controlled incubator (Precision Scientific, Winchester, VA) to maintain a constant temperature. To avoid temperature and osmotic shock, all eggs were fertilized at 25 °C and 30 ppt, and the temperature and salinity were increased by 5 °C and 5 ppt each day until the target treatment combination was reached. Seawater was changed every other day, and dishes were checked daily for the presence of newly hatched larvae and juveniles. Eggs that showed no sign of development after 75 days were excluded from the analysis. The effects of temperature and salinity on the time to hatching and molting to the first juvenile instar were determined using a two-factor analysis of variance (ANOVA; SYSTAT 10.0, SPSS Inc.). If the overall analysis indicated significant treatment effects, comparisons among treatment levels were conducted using Tukey pairwise comparisons (SYSTAT 10.0).

Salinity tolerance of larvae

To determine the salinity tolerance of *L. polyphemus* larvae, embryos were reared at 30 °C and 30 ppt until they hatched to the trilobite larva stage. Within 24 h of hatching, 24 larvae were placed in individual wells (1.5 cm diameter × 1.5 cm depth) of a multiwell culture dish containing 3 ml of filtered seawater at salinities of 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 ppt. Larval survival was monitored daily for 30 days. Larvae were considered dead when they were inactive (no leg or book-gill movement) and unresponsive to mechanical stimulation. Time to death (in days) or time of molting to the first juvenile instar (in days) was recorded. Survivorship curves for larvae in each of the salinity treatments were constructed using the product-limit method (Kaplan-Meier method: Muenchow, 1986; Kleinbaum, 1996). Survival functions were compared among treatments using the Mantel log-rank test (χ^2 approximation, SYSTAT 10.0, SPSS Inc.). The same analysis was used to compare the duration of the larval stage, with time to molting as the dependent variable. Larvae that were alive but had not molted after 30 days of exposure were treated as right-censored observations in the analysis.

Osmotic concentration of the perivitelline fluid

To determine the effect of changes in external salinity on the osmotic concentration of the perivitelline fluid surrounding developing embryos, *L. polyphemus* eggs reared at 25 °C and 30 ppt were transferred, after the fourth embryonic molt, to one of 10 test salinities (5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 ppt). The osmotic concentration of the perivitelline fluid of a subset of randomly selected eggs (n = 10) from each salinity treatment was determined at 0, 0.5, 1, 2, 4, and 6 h following exposure. Thus, a total of 60 eggs (10 eggs × 6 time exposures) were tested at each combination of salinity and temperature. Perivitelline fluid within the egg was collected by using the tip of a micropipette to carefully tear the egg's outer membrane and then to draw up 10 μl of perivitelline fluid. Osmolarity was determined with a vapor pressure osmometer (Vapro model 5520, Westcor Inc., Logan, Utah) calibrated with standards of 290 and 1000 mmol kg^{-1}. The effect of the salinity of the external medium on the osmolarity of the fluid over time was determined using a two-factor analysis of variance (salinity and time as factors; SYSTAT 10.0, SPSS Inc.). Comparisons between experimental treatments and the control (*i.e.,* 30 ppt) were conducted using *a priori* directed contrasts (SYSTAT 10.0).

Results

Effect of high temperature and salinity on embryonic and larval development

Both high temperatures and salinities significantly affected the success of embryonic development in *Limulus polyphemus.* At 35 °C, eggs developed to embryonic stage 20, where the embryo is enclosed by a clear membrane and the legs and gills are visible (Sekiguchi, 1988); but at all salinities, these embryos failed to hatch to trilobite larvae after 75 days. At 40 °C, eggs showed no signs of development after 75 days at all salinities. Development and hatching were normal at all other temperatures. Temperature and salinity also had a significant effect on time to hatching (Fig. 1, F = 52.02, df = 6, 202, $P < 0.001$). At all test salinities, embryonic development took longer at 25 °C than at 30 and 33 °C (Tukey pairwise comparisons, Fig. 1, Table 1). At 25 °C, time to hatching increased significantly as the salinity increased (Fig. 1, Table 1). Embryos maintained at 30 °C and 33 °C and 30 and 40 ppt had similar hatching

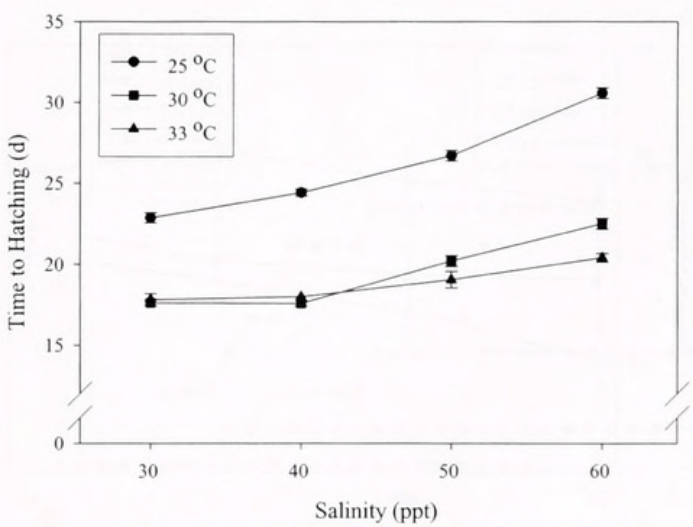

Figure 1. Mean (±SE) number of days from fertilization to hatching of *Limulus polyphemus* embryos at 25 °C, 30 °C, and 33 °C and at 30, 40, 50, and 60 ppt. No hatching occurred in any of the test salinities at 35 °C and 40 °C. n = 30 for each trial.

Table 1

Matrix of pairwise comparison probabilities (Tukey test) for days to hatching for Limulus polyphemus *embryos*

		T/S											
T/S		25/30	25/40	25/50	25/60	30/30	30/40	30/50	30/60	33/30	33/40	33/50	33/60
	n =	27	29	30	26	26	27	25	28	27	29	27	25
25/30		1.00											
25/40		<0.01	1.00										
25/50		<0.01	<0.01	1.00									
25/60		<0.01	<0.01	<0.01	1.00								
30/30		<0.01	<0.01	<0.01	<0.01	1.00							
30/40		<0.01	<0.01	<0.01	<0.01	**0.97**	1.00						
30/50		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.00					
30/60		**0.03**	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	1.00				
33/30		<0.01	<0.01	<0.01	<0.01	**0.86**	**1.00**	<0.01	<0.01	1.00			
33/40		<0.01	<0.01	<0.01	<0.01	**0.46**	**1.00**	<0.01	<0.01	**1.00**	1.00		
33/50		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.00	
33/60		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	1.00

T = temperature (°C); S = salinity (ppt); n = sample size. Bold type indicates pairwise comparisons that are not statistically significant.

rates (Table 1), but hatching was delayed significantly in more hypersaline conditions (50 and 60 ppt, Fig. 1). Optimal temperature and salinity conditions for development to stage 21 were 30–33 °C and 30–40 ppt.

The duration of the trilobite larva stage (time from hatching to molting to the first juvenile instar) decreased significantly with increasing temperature ($F = 3.79$, df = 6, 202, $P < 0.001$, Fig. 2), yet was similar for all salinity treatments ($F = 0.22$, df = 6, 202, $P = 0.64$). Larval stage duration was shortest at 30–33 °C and was significantly longer at 25 °C (Tukey test, Fig. 2, Table 2). Development times were similar for larvae maintained at 30 °C and 33 °C (Fig. 2, Table 2).

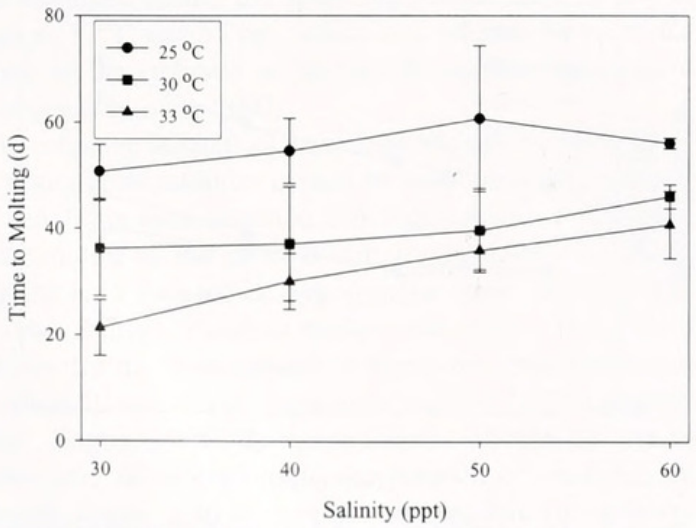

Figure 2. Mean (±SE) time to molting to the first juvenile stage (in days) of *Limulus polyphemus* at 25 °C, 30 °C, and 33 °C and 30, 40, 50, and 60 ppt. Sample sizes are provided in Table 2.

Salinity tolerance of larvae

All *L. polyphemus* trilobite larvae survived for 30 days at salinities ranging from 10 to 70 ppt; they died only in the extreme salinities of 5, 80, and 90 ppt (Fig. 3). Comparisons of the survival curves among salinity treatments indicated that survival was significantly reduced relative to control levels only when larvae were maintained at 90 ppt ($\chi^2 = 33.0$, df = 2, $P < 0.01$, Fig. 3). The time to 50% mortality (TM_{50}) was ≈16.0 days in 90 ppt. Salinity also had a significant effect on molting rate of larvae to the first juvenile instar ($\chi^2 = 12.1$, df = 4, $P < 0.01$, Fig. 4). Larval stage duration increased at salinities above and below 30 ppt (Fig. 4). Molting did not occur after 30 days at the most extreme salinities tested (≤10 ppt or ≥70 ppt).

Osmotic concentration of the perivitelline fluid

Salinity significantly affected the osmotic concentration of the perivitelline fluid of *L. polyphemus* eggs (Fig. 5, $F = 176.81$, df = 9, 45, $P < 0.01$). When developing embryos were placed in hypoosmotic solutions (5, 10, and 20 ppt), the osmolarity of the perivitelline fluid decreased significantly within 0.5 h of exposure (Fig. 5). After the first hour, fluid osmolarity leveled off and remained relatively constant for the remainder of the experiment (Fig. 5). At 30 ppt, the osmolarity of the perivitelline fluid did not change significantly throughout the exposure period ($F = 0.098$, df = 9, 45, $P = 0.925$, Fig. 5). When embryos were exposed to more hyperosmotic conditions (>30 ppt), the fluid osmolarity increased significantly within the first 0.5 hour and initially reached levels that were slightly above that of the bathing medium (Fig. 5). However, the osmolarity de-

Table 2

Matrix of pairwise comparison probabilities (Tukey test) for time to hatching to the first juvenile instar for Limulus polyphemus *larvae*

	T/S											
T/S	25/30	25/40	25/50	25/60	30/30	30/40	30/50	30/60	33/30	33/40	33/50	33/60
n =	26	27	26	23	24	25	22	25	24	27	24	24
25/30	1.00											
25/40	**1.00**	1.00										
25/50	**0.68**	**0.12**	1.00									
25/60	**0.98**	**0.38**	**1.00**	1.00								
30/30	**0.04**	**0.04**	<0.01	<0.01	1.00							
30/40	0.01	0.01	<0.01	0.01	**1.00**	1.00						
30/50	**0.02**	**0.02**	0.01	<0.01	**0.97**	**1.00**	1.00					
30/60	**0.03**	0.01	**0.04**	<0.01	**0.08**	**0.39**	**0.73**	1.00				
33/30	<0.01	<0.01	<0.01	<0.01	**0.06**	**0.09**	**0.39**	**0.05**	1.00			
33/40	<0.01	<0.01	<0.01	<0.01	**0.08**	**0.13**	**0.06**	**0.07**	**0.77**	1.00		
33/50	<0.01	<0.01	<0.01	<0.01	**0.49**	**0.10**	**0.10**	**0.09**	**0.08**	**0.86**	1.00	
33/60	<0.01	<0.01	<0.01	<0.01	**0.49**	**0.93**	**1.00**	**1.00**	**0.11**	**0.25**	**1.00**	1.00

T = temperature (°C); S = salinity (ppt); *n* = sample size. Bold type indicates pairwise comparisons that are not statistically significant.

creased after 1 h and remained at levels slightly above that of the bathing medium (Fig. 5). Similar changes in the osmotic concentration of the perivitelline fluid occurred when eggs were exposed to salinities above 40 ppt. Yet the magnitude of the change and its duration increased with increasing hyperosmotic shock (Fig. 5). After 6 h, the perivitelline fluid of all embryos was nearly isoosmotic with the bathing medium (Fig. 6).

Discussion

In estuaries, *Limulus polyphemus* is exposed to rapid fluctuations in salinity, particularly in intertidal areas where embryos and larvae undergo development. The results of the current study support earlier reports that *L. polyphemus* embryos and larvae are remarkably hardy and able to withstand the fluctuating and often harsh environmental conditions of intertidal areas (Jegla and Costlow, 1982; Palumbi and Johnson, 1982; Sugita, 1988; Botton *et al.*, 1988). Although both embryos and larvae completed development in hypersaline conditions, time to hatching and metamorphosis was delayed at salinities above 40 ppt (Fig. 1). Posthatching development was not affected by salinity (Fig. 2). Optimal salinities for development were between 30 and 40 ppt, which differs slightly from previous reports of optimal values between 20 and 30 ppt (Jegla and Costlow, 1982; Laughlin, 1983; Sugita, 1988). This difference may

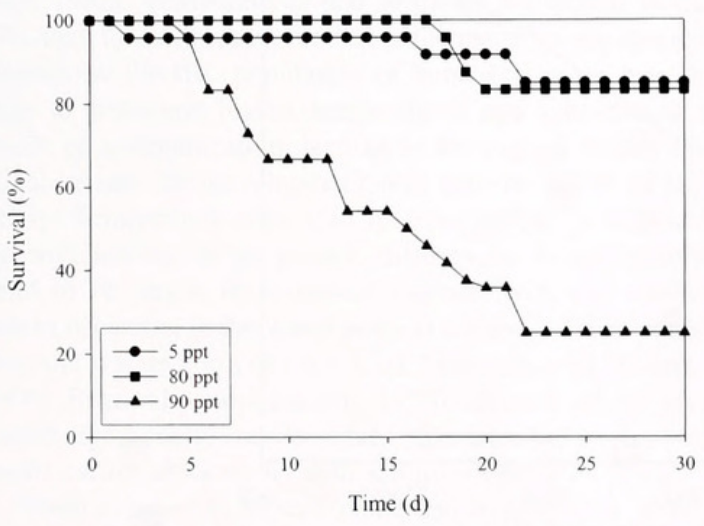

Figure 3. Kaplan-Meier survival curves for *Limulus polyphemus* larvae subjected to salinities ranging from 5 to 90 ppt for 30 days. All larvae survived in salinities from 10 to 70 ppt. *n* = 24 for each trial.

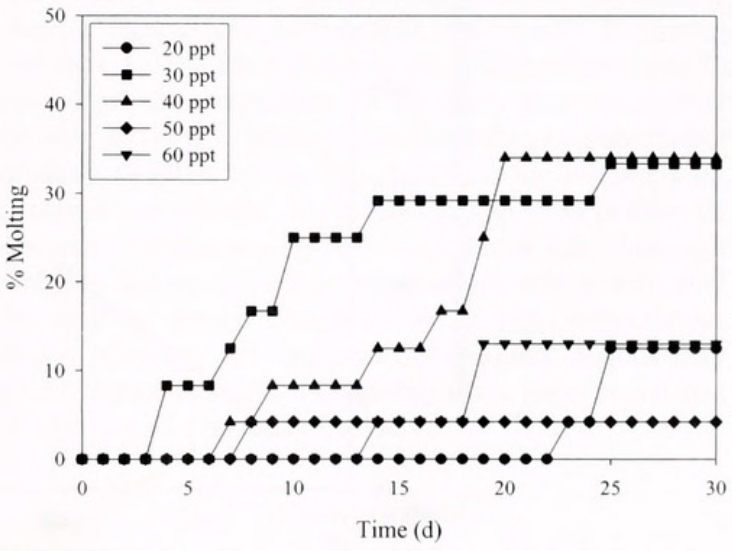

Figure 4. Kaplan-Meier curves for time until molting for *Limulus polyphemus* larvae subjected to salinities ranging from 5 to 90 ppt for 30 days. *n* = 24 for each trial.

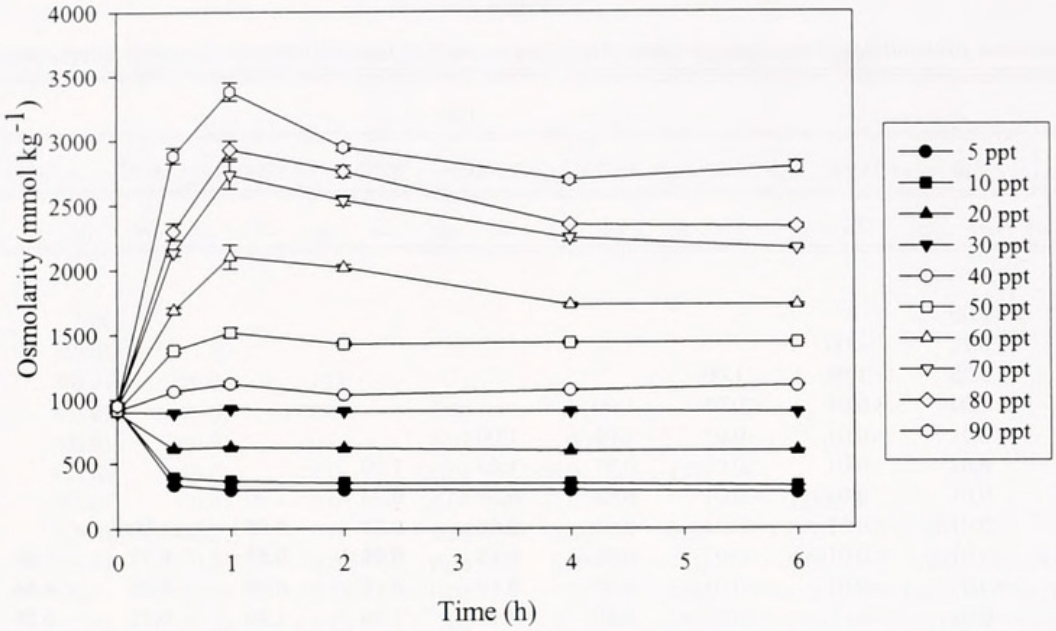

Figure 5. Mean (±SE) osmotic concentration of the perivitelline fluid at 0, 0.5, 1, 2, 4, and 6 h after exposure to salinities ranging from 5 to 90 ppt.

be the result of acclimation by the adults to the extreme salinity conditions found in the IRL compared to other estuaries because it is a nontidal, shallow lagoon.

The results of the current study indicate that eggs and embryos of *L. polyphemus* are more sensitive to high temperatures than to high salinities (Figs. 1 and 2). Optimal temperatures for development were 30–33 °C, yet temperatures 35 °C and above were lethal to embryos and adversely affected larval growth and development. This differs slightly from previous studies in which lower temperatures produced optimal development (25–30 °C: Jegla and Costlow, 1982; Laughlin, 1983; Sugita, 1988). Another difference is that hatching did not occur at temperatures above 33 °C in our study, whereas other researchers reported hatching at temperatures up to 35 °C (Jegla and Costlow, 1982; Laughlin, 1983).

Temperature tolerance in *L. polyphemus* varies with life-history stage: older stages are better able to withstand ex-

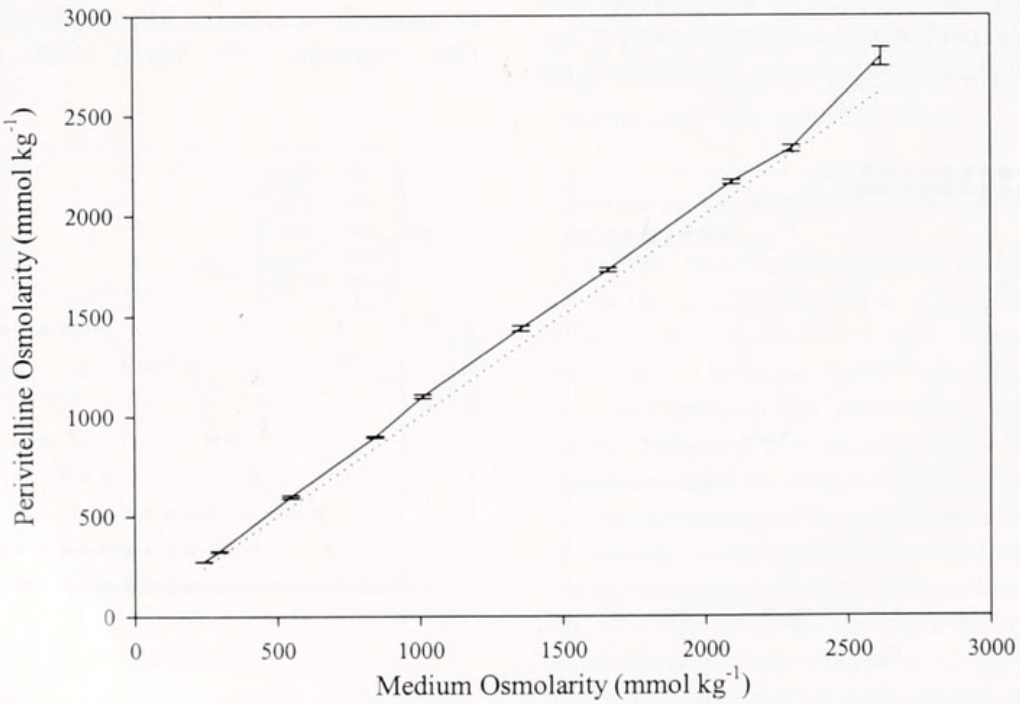

Figure 6. Variations in the perivitelline osmolarity of *Limulus polyphemus* eggs as a function of the bathing medium following 6 h of exposure. Values are means (±SE). The dashed line is the isoosmotic line.

treme temperatures (Fraenkel, 1960; Jegla and Costlow, 1982; Laughlin, 1983). In the current study, embryos could not tolerate temperatures above 33 °C (Fig. 1). Reynolds and Casterlin (1979) found that juveniles were tolerant of temperatures from 15 °C to 40 °C. The lethal temperature for 1-h exposure is 44 °C for adults, although they can survive more than 72 h at 40 °C (Fraenkel, 1960). Thus, adults are more tolerant than embryos to high temperatures since embryos failed to develop and hatch at temperatures of 35 °C.

Salinity tolerance also varies with life-history stage. Embryos developed, hatched, and molted to the first juvenile instar at 60 ppt (Figs. 1 and 2), and larvae survived at salinities from 10 to 70 ppt (Fig. 3). Juveniles (12th instar) are able to withstand salinities of 12 ppt for several days, but as salinity decreases, mortality increases, and survival time decreases (Reynolds and Casterlin, 1979). Adults can withstand direct transfer from 25 ppt to 13 ppt with no adverse effects, but transfer to 6 ppt causes swelling of the limb joints and gills (Robertson, 1970). Our results indicate that larvae are more tolerant of sudden hyposalinity shock than adults and juveniles. This may be due to ontogenetic differences that enable embryos and larvae to tolerate the rapid fluctuations in salinity that typically occur in intertidal nesting areas. These results are consistent with studies of other estuarine and marine arthropod species that found that tolerance to a wide range of salinities is greater in the larval stage than in the adult. For example, the larvae of the coastal crabs *Armases ricordi* and *A. roberti* are tolerant of a wider range of salinities than the adult stages and, as a consequence, have different habitats (Diesel and Schuh, 1998). The larvae of the Chinese mitten crab *Eriocheir sinensis* also has a much wider salinity tolerance than the juveniles and adults (Anger, 1991).

Temperature and salinity have been found to affect the physiology and growth of *L. polyphemus* (Jegla and Costlow, 1982). Temperatures and salinities are higher in the IRL than in the northern portion of the range for the species. Therefore, the IRL population of horseshoe crabs may be able to withstand higher temperatures and salinities as a result of acclimatization, leading to the slightly higher optimal ranges for development and growth found in this study. Temperature may also have an effect on size and growth, leading to geographic differences in size. Adults tend to be larger in temperate regions, with the smaller adults occurring in the warm tropical waters of Yucatan and the cold waters north of Cape Cod, Massachusetts (Shuster, 1979; Reynolds and Casterlin, 1979). Shuster (1979) also found significantly smaller adults that matured one or two molts earlier at locations with salinities below 18 ppt.

When exposed to hyperosmotic and hypoosmotic stress, the perivitelline fluid contained within the outer membrane of *L. polyphemus* embryos changed rapidly and became nearly isoosmotic to the surrounding medium (Figs. 5 and 6). Since the osmotic concentration of the perivitelline fluid changes with the surrounding medium, the perivitelline fluid does not buffer developing embryos from changes in external salinity. Partial regulation of perivitelline fluid osmolarity may be a common trait among members of the Xiphosura, since Sugita (1988) reported similar results for embryos of *Tachypleus tridentatus*, the Japanese horseshoe crab. Sekiguchi (1988) found that the osmotic concentration of the perivitelline fluid of *L. polyphemus* and *T. tridentatus* embryos bathed in high-salinity waters changed during the exposure period, attaining a slightly higher value than the surrounding medium. Sekiguchi (1988) also found that inorganic ions pass freely through the inner egg membrane. Thus, osmoactive substances secreted by the embryo, but which cannot pass through the inner egg membrane, are most likely responsible for the slightly higher osmolarity of the perivitelline fluid relative to the outer medium. Since the perivitelline fluid of the eggs conforms osmotically to the surrounding medium, one would expect the egg volume to change initially, and then return to its original level once the egg has reached the osmolarity of the surrounding medium. Ehlinger (2002) found that the volume of eggs exposed to salinities from 5 to 90 ppt changed over a 6-h exposure period: in general, volume decreased in hyperosmotic solutions and increased in hypoosmotic solutions. These results differ from those reported by Jegla and Costlow (1982), who found that egg volume did not change conspicuously when exposed to salinities of 10 and 40 ppt.

The wide salinity tolerance of embryos and larvae of *L. polyphemus* is an important adaptation to the extreme conditions in the intertidal nursery habitat. Such variability in tolerance may be an advantage in species that live in a highly variable, unpredictable environment (Anger, 1991). Embryos and larvae of *L. polyphemus* are exposed to and can tolerate a much wider range of salinities than juveniles and adults. This enables the embryos to survive and hatch in shallow lagoons and embayments with rapidly fluctuating salinities. Larvae are able to tolerate salinities from 20 to 70 ppt, which they experience in intertidal areas where they develop and molt to larger stages before they migrate farther offshore (Rudloe, 1979). This tolerance for extreme temperatures and salinities is particularly important in nontidal estuarine systems and lagoons, such as the IRL. Although embryos and larvae of *L. polyphemus* are able to withstand the high salinities experienced in the IRL, temperatures during summer spawning and development periods may exceed tolerance limits, thus leading to the lower abundance of embryos and larvae in the IRL.

Acknowledgments

Research supported by National Park Service Grant No. CA518099049. We thank Canaveral National Seashore, Merritt Island National Wildlife Refuge, and NASA/

Kennedy Space Center for access to the collection sites. We thank Drs. M. Botton, M. Bush, E. Irlandi, and J. Lin for their comments on an early version of this manuscript. We are grateful to M. Mota, E. Reyier, and D. Scheidt for their assistance in collecting adult crabs, A. Brenner and K. Smolarek for their assistance in the lab, and Dr. J. Grimwade for use of laboratory equipment. We thank two anonymous reviewers for their critical review of this manuscript.

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