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# High resolution tracking of adult horseshoe crabs *Limulus polyphemus* in a New Hampshire estuary using fixed array ultrasonic telemetry

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**Abstract** While several studies have documented the large-scale, seasonal movements of horseshoe crabs, little is known about their fine-scale, daily movement patterns. In this study we used a fixed array ultrasonic telemetry system to track the movements of 12 male and 16 female horseshoe crabs in the Great Bay estuary, New Hampshire. Data were obtained during the mating season, as well as during the remainder of the summer and fall, in the years 2005-2008. During the mating season animals were often, but not always, active during the high tides when they were approaching and leaving the spawning beaches. On average, both males and females approached mating beaches during 33% of the high tides they experienced and they most often made the transition from being inactive to active during the last two hours of an incoming tide. From April-October horseshoe crabs were significantly more active during high tide periods vs low tide periods, with no clear preference for diurnal vs nocturnal activity. After the mating season ended horseshoe crabs continued to move into shallower water at high tide and then return to deeper water at low tide. Observations by SCUBA divers suggest that during these excursions into the mudflats horseshoe crabs were digging pits in the sediment while foraging for food. Thus, the tidal rhythm of activity that has been so well documented during the mating season probably persists into the fall, and primarily involves foraging activities [*Current Zoology* 56 (5): 599–610, 2010].

**Key words** Telemetry, Tracking, Movements, Mating, Foraging, Estuary

Every year, typically around the late spring and early summer, horseshoe crabs emerge from the deeper waters and spawn in large numbers on selected beaches along the east coast of the United States. As a result of this dramatic activity, their unique and interesting mating behavior has been the subject of many investigations (Lockwood, 1870; Shuster, 1950; Rudloe, 1980; Cohen and Brockmann, 1983; Barlow et al., 1986; Brockmann, 1990, 2003), some of which are summarized in this volume. However, while a great deal is known about many aspects of *Limulus* reproductive activities, the behaviors they express in deeper water, when they are not as easily observed, are poorly understood. The paucity of data about horseshoe crab behavior during most of their lifetime is due, primarily, to the challenges inherent in the investigation of any marine species; it is almost impossible to observe them for long periods of time underwater.

In recent years, more and more windows into the lives of marine species have opened as a result of advances in video and telemetry instrumentation. In particular, acoustic telemetry has emerged as an extremely valuable tool for studying the small- and large-scale movements of a wide range of mobile marine species (Wolcott and Hines, 1990; Freire and Gonzalez-Gurriaran, 1998; Clark et al., 1999; Golet et al., 2006; Scopel et al., 2009). Large-scale and seasonal movements are typically investigated with telemetry using one of the following two methods: 1) scientists on boats find animals equipped with acoustic transmitters using appropriate handheld hydrophones and receivers and then note the apparent location of the animal; 2) an array of receivers are distributed in an area where tagged animals are located and whenever one of these animals passes close enough to a receiver it logs the time and identity of the animal. Both these techniques have provided very valuable information about a range of marine species, including horseshoe crabs, and two papers in this volume demonstrate the utility of this approach (James-Pirri, 2010; Schaller et al., 2010).

For finer scale investigations of the movements of marine species, such as *Limulus polyphemus*, it is

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necessary to use a fixed array of hydrophones and receivers, such as the VEMCO VRAP system (Amirix Inc, Halifax, Nova Scotia), to triangulate animal locations (Golet et al., 2006; Scopel et al., 2009). This approach makes it possible to obtain positional fixes of animals equipped with appropriate transmitters, at ~1 min intervals, with a resolution approaching 1–2 meters. Importantly, with this approach, it is possible to continuously monitor the movements of horseshoe crabs, both during the day and during the night, as long as the animal in question remains within the detection range of the fixed array (Watson et al., 2009). During this study we used this approach to begin addressing questions about the patterns of movements horseshoe crabs typically express in the Great Bay estuary, NH, throughout the year.

In the earliest studies of horseshoe crab movements, scientists used tag recapture methods to study seasonal movements and their tendency to repeatedly return to the same beach to spawn in the same season or subsequent seasons (Baptist et al., 1957; Rudloe, 1980; James-Pirri et al., 2005; Swan, 2005; Leschen et al., 2006). They discovered that while some animals made very large excursions to very deep water in the late summer and fall, most animals were recaptured close (within several kms) to their original tagging locations. More recent investigations have taken advantage of telemetry technology to demonstrate that many horseshoe crab populations have a limited annual range and likely remain within the same cove, bay, or estuary during the entire year (Kurz and James-Pirri, 2002; Brousseau et al., 2004; Moore and Perrin, 2007; Watson et al., 2009; James-Pirri, 2010; Schaller et al., 2010). In addition to shedding light on their large-scale movement patterns, telemetry has also been used effectively to begin to address some of the long-standing questions about finer scale movements. Specifically, Brousseau et al. (2004) used a combination of radio and acoustic tags to investigate the movements of female horseshoe crabs during a 5 day period centered on the new moon and within the mating season in Delaware Bay. They found that females: 1) spawned repeatedly, often on the same beach; 2) moved away from the mating beach an average of 299 m during low tide and; 3) rapidly moved out of the area within a few days after the new moon. This investigation, along with the others summarized above, all provided glimpses of the many advantages of biotelemetry for investigating the behaviors of aquatic species, like horseshoe crabs, that cannot be readily observed for long periods of time underwater.

In this investigation, our focus was not on the large-scale, seasonal movements of horseshoe crabs, but rather we aimed to determine the movement patterns they were expressing on a daily basis and on a local scale. In particular, a major goal of this study was to determine the types of biological rhythms horseshoe crabs exhibited in their natural habitat. Previous studies of horseshoe crab mating behavior in the field (Rudloe, 1980; Cohen and Brockmann, 1983; Barlow et al., 1986; Brockmann, 1990, 2003), and locomotor activity in the laboratory (Chabot et al., 2007; Chabot and Watson, 2010), have provided very clear evidence that horseshoe crabs often express a tidal rhythm of activity. Moreover, it has been demonstrated that the expression of this tidal rhythm is the result of an endogenous clock system that is strongly influenced by the changes in water depth associated with fluctuating tides (Chabot et al., 2008; Watson et al., 2008). Therefore, one hypothesis this study was designed to test was that horseshoe crabs would express a tidal rhythm of locomotion during all the months of the year when they were active (April–October). However, horseshoe crabs also possess an endogenous circadian clock that modulates visual sensitivity (Barlow, 1983; Watson et al., 2008, 2009) and in the laboratory some animals express a daily, rather than a tidal, pattern of locomotion (Chabot et al., 2007). While data from our laboratory indicates that most horseshoe crabs, whether expressing a tidal or daily pattern of locomotion, tend to prefer to be most active in the day, there is also evidence indicating, at least during mating season in some areas like Cape Cod, MA, that most animals approach the mating beaches during the new and full moons at night (Barlow et al., 1986). Therefore, another working hypothesis that we aimed to test in this study was that horseshoe crabs express a daily rhythm of locomotion in the field, with a tendency towards diurnal activity.

## 1 Materials and Methods

### 1.1 Animals and Transmitters

In 2005–2008 we equipped a total of 13 males (mean carapace width 14.4 ± 0.27 cms; mean ± *SEM* used here and throughout paper) and 25 females (18.9 ± 0.32 cms) with ultrasonic transmitters (V13-1L coded, R256 or 64K, 69kHz, transmit intervals ranging from 15–30 sec, Vemco Inc., Halifax, Canada; 36 mm long, 6 g in water; output=147 db; battery life=~400–500 days; Table 1). Most (25) transmitters were inserted into the sub-frontal, axial triangulate area, on the anterior-most portion of the ventral surface of the prosoma. A total of 13 transmitters were attached to the dorsal carapace of the horseshoe

**Table 1 Horseshoe crabs tracked in 2005–2008. The "days" column refers to the number of complete days of data that were obtained from each animal**

| Pinger ID | Sex | Size (cms) | Date Tagged | Days | Consecutive Days |
|---|---|---|---|---|---|
| 10 | M | 14.3 | 10/5/05 | 3 | 3 |
| 11 | F | 18.0 | 6/12/07 | 6 | 6 |
| 12 | M | 12.5 | 6/22/07 | 6 | 4 |
| 13 | F | 22.0 | 6/22/07 | 1 | 1 |
| 21 | F | 21.0 | 5/31/06 | 3 | 2 |
| 22 | F | 20.5 | 6/1/06 | 3 | 2 |
| 23 | M | 15.3 | 6/1/06 | 2 | 2 |
| 24 | F | 21.0 | 6/6/06 | 2 | 1 |
| 25 | F | 19.6 | 6/15/06 | 5 | 2 |
| 26 | F | 19.7 | 6/13/06 | 2 | 2 |
| 27 | F | 19.3 | 6/6/06 | 6 | 3 |
| 28 | F | 19.1 | 6/13/06 | 5 | 2 |
| 29 | F | 19.0 | 6/13/06 | 3 | 2 |
| 31 | F | 17.7 | 6/16/06 | 2 | 2 |
| 33 | F | 19.7 | 6/16/06 | 2 | 1 |
| 35 | F | 18.3 | 6/20/96 | 2 | 1 |
| 36 | F | 19.4 | 6/20/06 | 2 | 1 |
| 37 | F | 19.0 | 6/29/06 | 2 | 2 |
| 39 | M | 13.5 | 6/21/06 | 1 | 1 |
| 70 | F | 18.5 | 6/25/06 | 1 | 2 |
| 71 | M | 16.0 | 6/14/07 | 7 | 2 |
| 72 | M | 15.0 | 6/12/07 | 4 | 2 |
| 73 | M | 13.5 | 6/10/07 | 12 | 3 |
| 74 | M | 14.0 | 6/20/07 | 5 | 3 |
| 75 | M | 14.0 | 6/14/07 | 3 | 3 |
| 76 | M | 14.8 | 6/10/07 | 2 | 1 |
| 50832 | M | 15 | 6/21/08 | 24 | 5 |
| 50833 | M | 16.5 | 6/13/08 | 10 | 6 |

crabs using a combination of superglue, epoxy and duct tape. More females were equipped with transmitters than males for two reasons. First, they were larger so the transmitters were likely to have less of an influence on their activity and it was easier to attach them externally or insert them internally. Second, because males often remain attached to females for long periods, we were concerned that data obtained from males might actually represent the movements of the females to which they were attached. To avoid this problem in 2007 and 2008 we glued and taped shut the appendages they use to clasp females.

The majority (30) of horseshoe crabs used in this study were captured near a mating beach adjacent to the Jackson Estuarine Laboratory (JEL), fitted with transmitters, and immediately released at the same location. In contrast to many mating beaches in the southern end of the *Limulus* range, the density of mating animals is much lower in the Great Bay estuary. For example, in 2010 the mean number of animals counted during the peak two weeks of the mating season in May and June was 0.2 horseshoe crabs per sq. meter and in 2006 it was slightly higher (Watson et al., 2009). The remainder (8) of the horseshoe crabs were captured in lobster traps in the fall of 2005 and released at the same location, about 0.5 kms south of JEL.

### 1.2 High Resolution Tracking

Adult horseshoe crabs equipped with transmitters were tracked using an array of three buoys (VRAP system, Amirix Inc., Halifax, Canada; VRAP=Vemco Radio Acoustic Positioning). Each buoy is equipped with a hydrophone that detects the acoustic signal produced by the transmitters and a radio that transmits data to a receiver on land. A computer connected to the receiver uses proprietary software to triangulate the position of a given transmitter based on data received from all three buoys. The three buoys were typically positioned ~ 100-300 m apart and each buoy was capable of detecting a V13-1L ultrasonic transmitter if it was within ~ 300 m. Individual transmitters were identified by the unique code they produced and transmitted signals every 25–40 sec. Based on data provided by Vemco, and our own previous studies, we estimated that the system has an accuracy of ~1-2 meters if conditions are optimal and animals are near the center of the array. Some transmitters also served as temperature and depth sensors and these data were also transmitted to the shore station. We have also used this system to track lobsters along the coast of NH and more details about it can be found in Golet et al. (2006) and Scopel et al. (2009).

Most data were collected from animals located in a small cove adjacent to the UNH Jackson Estuarine Laboratory (JEL) where horseshoe crabs mate each year (Fig. 1). The remainder of the data were obtained from various locations within the Great Bay estuary, primarily in the same Little Bay region of the estuary were JEL is located. The accuracy of the data obtained from the VRAP system was influenced by the depth of the water, the location of animals relative to the array, movements of the VRAP buoys due to changing tides and ambient noise due to boats and waves. Therefore, data were filtered to remove points that were considered artifacts using some of the same methods employed by Golet et al. (2006). For example, it was clear when a particular point was an artifact because horseshoe crabs are unlikely to go from one point, to another, and back at speeds faster than 30 m/min. These types of artifacts were most pronounced when animals were outside of the array or when they were in very shallow water while spawning.

The major factor limiting the amount of data obtained from a given animal was the tendency of horseshoe crabs to move away from the detection distance of the three buoys in the hydrophone array, which was on the order of 0.4 km from the center of the array triangle. Because animals had a slight tendency to remain for several days near the spawning beach where they were first found, the majority of data presented in this manuscript were collected during the months of May and June near JEL. During the remainder of the year the VRAP array was repeatedly moved to areas where horseshoe crabs were residing and data were collected from these animals until they moved to a new location. Overall, an average of 3.93 ± 0.86 days of high resolution VRAP data were obtained from 28 of the 38 animals that were tracked, and all of the data presented in this paper are based on these 28 animals (Table 1).

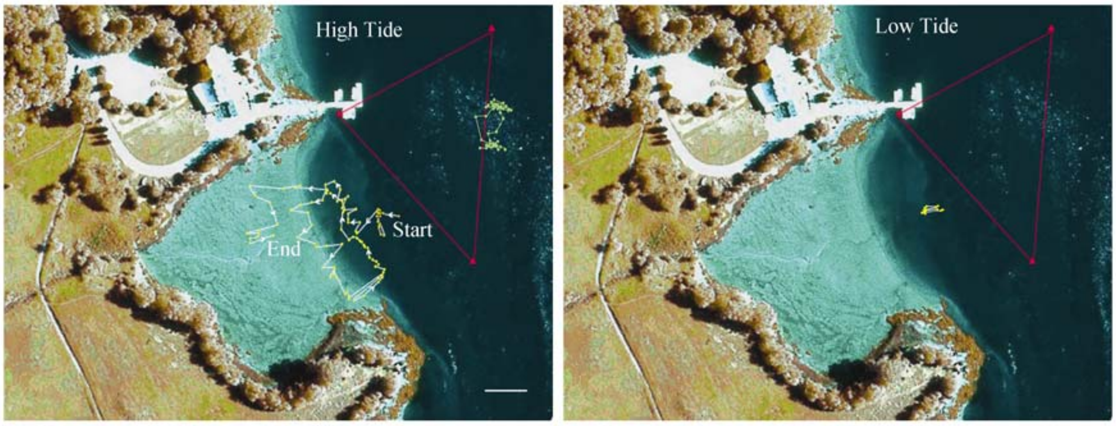

**Fig. 1 Movements of a male horseshoe crab during a 5 hr period of time when the tide was either high (left; peak high at 11:50 AM; data shown start at 12:30 PM and end at 5:30 PM) or low (right; peak low was at 7:30 AM; data shown start at 7:00 AM and end at noon)**

Each point represents one positional fix obtained from the VRAP tracking system. The points have been superimposed on an infrared image of the cove that was colorized to look more “natural”. The red lines connect each of the three VRAP buoys used to track the horseshoe crabs. In the left panel the light green dots and lines represent the position of the reference transmitter during the same time period (not shown in right panel). Note how it expresses apparent movement, even though it was stationary, because buoy movements due to the tides and waves generate small movement artifacts. The white scale bar on the left panel represents 20 m.

### 1.3 Calculations of distance traveled

Distance traveled per hour was calculating by measuring the distance traveled between one x, y positional fix and the next and then adding all these distances to give a total distance traveled for each one hr time period. Unfortunately, the data obtained using this approach was quite variable for two reasons. First, whenever a buoy moved, even if the animal being tracked did not, it appeared as if the animal moved because the position of the animal does, in fact, change relative to the buoys. Second, because the system is not perfectly accurate, even if an animal is not moving, each successive positional fix will be in a slightly different location. As a result of these types of errors, if many positional fixes are obtained each hr, even if an animal is not moving, it will appear as if has traveled some distance. When possible, we corrected for these errors, especially the buoy movements, by subtracting the apparent distance traveled by a reference transmitter that was at a fixed point in the array, from the distance traveled by the animal in question. When reference pinger data were not available a different method was used to reduce the apparent distance traveled by stationary animals. With this method only the x, y coordinates at the beginning of every hr were used for calculating distance traveled so the linear distance traveled in one hr was calculated, not the total distance. In other words, only one vector between subsequent x, y positions, one hr apart, was used, rather than adding up many vectors per hr. While this method underestimated the total distance traveled per hr, it more clearly showed movement trends and periods of inactivity.

Because of the inherent small artifacts associated with this form of telemetry, it is difficult to determine if an animal is meandering slowly, or sedentary. Therefore, rates of locomotion were only determined when it was very obvious that animals were moving at a consistent pace, from one area to another. The initial position and final position of the animal was then used to calculate the distance traveled and rate of locomotion.

### 1.4 Analysis of activity relative to tides, night and day

A major goal of this study was to determine the types of biological rhythms that are expressed by freely moving horseshoe crabs in their natural habitat. Due to the issues related to calculating distance traveled, summarized above, and gaps in the data due to animals moving in and out of the detection area of the VRAP array, we turned to “observations” of animal movements for this analysis. VRAP files were replayed and the digital tracks were visualized on a computer monitor using the VRAP software. Observers noted periods of time when animals were clearly active (moving in a given direction at a normal walking rate), as well as periods when they were clearly inactive (repeated fixes in a very small area that appeared to occur in random directions). Then, for each animal the percent of time that it was active during high tides in comparison to low tides, and daytime vs nighttime was determined.

A similar approach was used to test the hypothesis that horseshoe crabs approach breeding beaches at every high tide during the mating season. For this analysis, tracks of animals were replayed and visualized, along with data that were simultaneously collected from a depth sensor in the same cove. This made it possible to very accurately determine the relationship between the depth changes associated with the tides and *Limulus* movements. For the first analysis, for each animal, the number of high tides during which tracking data were available were recorded, and then the percentage of these potential tides when they actually approached the beach was calculated (% beach approaches). This analysis was only carried out during the mating season, when animals were approaching the beaches to spawn. For the second analysis, the goal was to test the hypothesis that freely behaving horseshoe crabs exposed to natural tidal fluctuations would begin periods of locomotion at a particular phase in the tide cycle. Observers watched playbacks of animal movements and identified times when animals initiated long bouts of activity (periods of time when animals were continuously active for > 2 hrs) after long periods of inactivity (> 2 hrs). They then calculated the phase of the tide cycle when this activity was initiated. The rising tide went from 0 (low tide)-100% (high tide) and, for this analysis, we divided it into three time periods (0%–33%, 34%–66%, 67%–100%). Likewise, the falling tide was also divided into three equivalent time periods. Data were expressed as the percentage of animals that initiated their bouts of activity during any of these time periods.

### 1.5 SCUBA

On ~ 30 occasions pairs of SCUBA divers engaged in the following activities: 1) searching for an animal equipped with an ultrasonic transmitter using an underwater receiver/hydrophone system; 2) collecting animals for laboratory studies or; 3) surveying tidal flats at high tides to observe horseshoe crabs. Certain inferences and conclusions presented in this paper are based on observations made during these dives.

## 2 Results

As expected, during mating season horseshoe crabs were most active during the high tides. Horseshoe crabs typically approached the same spawning beach where they were initially equipped with transmitters during at least one subsequent high tide (Fig. 1, left panel; more approach data will be presented later in the results). When they were clearly moving in a consistent direction, either approaching the beach or moving laterally and parallel to the beach, they moved at a mean rate of 4.85 ± 0.54 m/min (291 m/hr; *n*=21 animals), although maximal rates of walking occasionally exceeded 10 m/min (600 m/hr; Figs. 2,3). In contrast to their elevated periods of activity during high tides, they were typically inactive during low tides (Fig. 1, right panel; mean speeds during low tide are difficult to determine due to the artifacts described in the methods and below). These periods of inactivity were typically, but not always, spent in slightly deeper water, on the edge of the channel, where there was a dramatic change in water depth from ~ 3 m to 8 m, at low tide (Fig. 1, right panel). On at least three occasions, animals spent the entire low tide period buried in the mud, on an exposed tidal flat, and then became active again with the incoming tide.

While there was a clear tendency to be most active during high tides, the animals we tracked were rarely active during at least three successive high tides. One, out of a total of four (all were males with claspers glued shut), of these exceptions is illustrated in Figure 3. This animal was a male that was tagged and released in June and subsequently expressed elevated levels of activity during most of high tides. In contrast, most of the *Limulus* we tracked expressed inconsistent patterns, “skipping” high tides and remaining inactive throughout one to several complete high tide cycles (Fig. 2), and then becoming active during a subsequent high tide.

To test the hypothesis that horseshoe crabs approached appropriate beaches during every high tide during the mating season we examined the movements of 18 animals for which we had tracking data covering at least 4 high tide periods during the mating season. In total, these 18 (7 males, 11 females) animals experienced 190 high tides in May–June of 2006–2007, and the mean duration of the bouts of activity expressed by these animals, during this time period, was 6.2 hrs. These 18 animals only approached the spawning beach during 30.5% of the possible high tides they experienced (58 of 190 high tides; range 1–9 approaches per animal). Females approached the beach on 31.4% (27 of

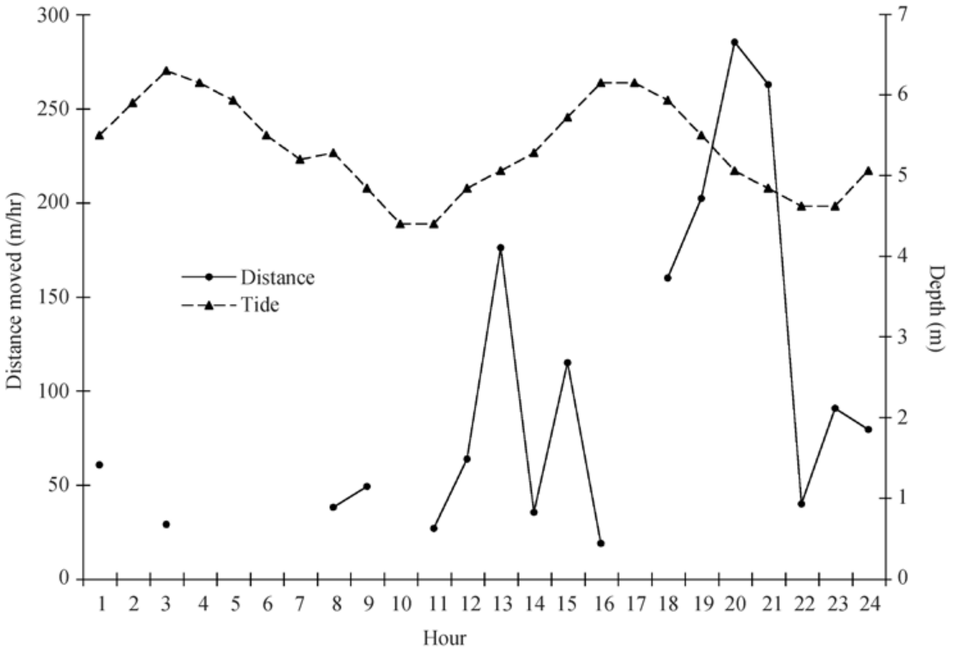

**Fig. 2 Distance traveled by a male horseshoe crab in comparison with the depth changes associated with changing tides**

Movement values are missing for some hours. Distance traveled per hr values have been adjusted by subtracting the apparent distance traveled by the horseshoe crab per hr, from the apparent distance traveled by a stationary transmitter. Note that this horseshoe crab was not active during the night (1-5 AM) high tide and then initiated activity during the rising tide in the day (5 AM–21:00), and remained active even while the tide was receding. This is a clear example of “skipping” (from the 4th to the 7th hour of observation) that is similar to what has been observed in the laboratory (Chabot et al., this volume).

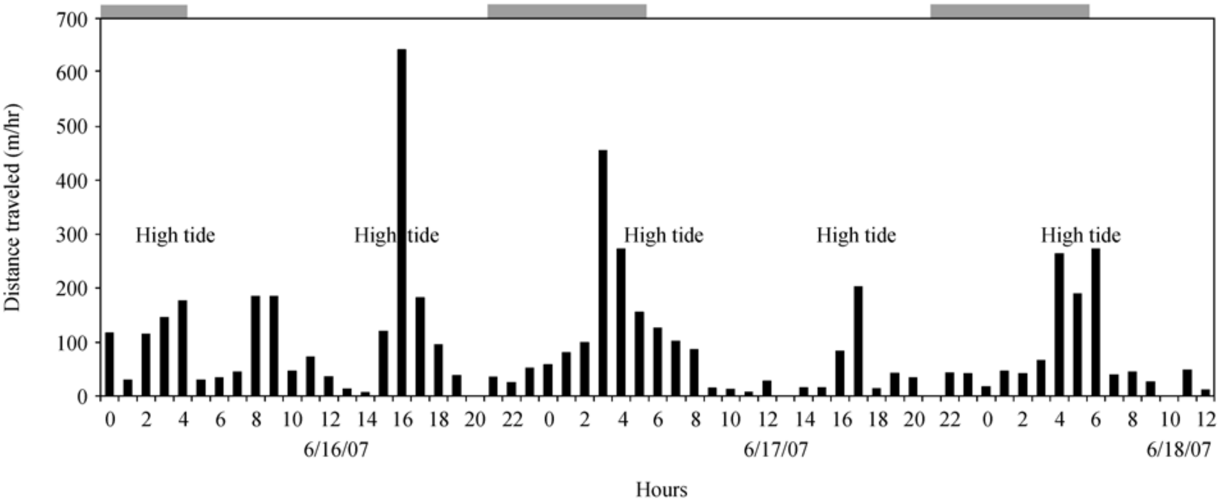

**Fig. 3 Activity of a male horseshoe crab in June of 2007, illustrating consistent increases of activity during high tides**

The "linear" distances traveled by this animal were calculated somewhat differently from those depicted in Fig. 2. In this case, as described in the methods, only the x,y values obtained at the end of each hour were used to eliminate potential errors that arise when numerous positional fixes throughout the hour are summed. Night hours are depicted by the gray bars on the top of the graph and peak high tides are also noted.

86) and males on 29.8% (31 of 104) of the potential high tides they experienced. When comparing the mean approaches between males and females there was no statistically significant difference between the proportion of tides when beach approaches took place (*P*=0.3189; Mann-Whitney *U*-test; *n*=11 females and 7 males). Horseshoe crabs of both sexes approached mating beaches significantly more during the day high tide (mean 2.3 ± 0.3, *n*=18; *P*=0.01; Mann-Whitney *U*-test) than the night high tide (0.9 ± 0.5, *n*=18). Overall, out of the 58 times when animals approached the beach at high tide, 70.6 ± 9.0% of the approaches took place during a daytime high tide.

One goal of this study was to determine if horseshoe crabs have a tendency to be more active during high tides vs low tides, and the night vs the day. In contrast to the analyses above, these bouts of activity were not restricted to instances when they were approaching a beach to, presumably, mate. First, we identified 2617.4 hrs when 27 animals were clearly moving during the month of June in 2006 and 2007. On average, these bouts of activity lasted 4.5 ± 0.6 hrs (max=11.1 hr, min=0.8 hr). Then we determined the proportion of these bouts of activity that took place during high tides vs low tides (3 hrs before and after the peak of each), and day vs night (excluding the 30 min on either side of dawn and dusk). Animals were significantly more active during high tides than low tides (Fig. 4A; *P*=0.0035, Mann-Whitney U-test; 17 of 27 animals more active at high tide), but there was no difference in their preference for day vs night activity (*P*=0.09; 16 of 27 more active at night).

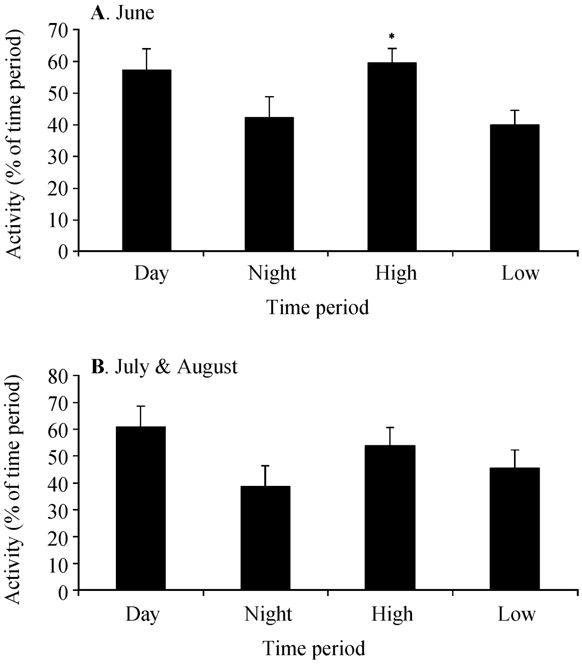

**Fig. 4 The percentage of time that horseshoe crabs were active during the day vs night and high vs low tides in June (A) or July and August (B)**

Data for June were derived from 27 animals in 2006 and 2007 and data for the other months came from 10 different animals in 2007. While the trends were consistent from one time period to the other, the only statistically significant difference was between high and low tides in June.

To determine if this pattern was unique to the spring mating period, data from July and August were also analyzed in the same manner. In this case we used 636.4 hours of data, from 10 animals, that contained bouts of activity lasting, on average, 2.5 ± 0.4 hrs. There was no significant difference between the amount of time animals were active during high vs. low tides (Fig. 4B; *P*=0.53, Mann-Whitney *U*-test; 50% more active at high tide) or the day vs. the night (*P*=0.06; 70% more active in the day).

Previously it has been shown that horseshoe crabs have an endogenous circatidal clock that can be entrained to small changes in water depth (Chabot et al., 2008; Watson et al., 2008). Therefore, we were interested in determining when during a typical tidal cycle animals in the field became active. For this analysis we examined the movements of 15 horseshoe crabs that expressed clear increases in activity that were initiated at some point during a given tide cycle during the mating season in June of 2006. The tide cycle was divided into 6 time periods as described in the methods; three when the tide was coming in, and three when the tide was going out. While most increases in activity took place in the latter third of the incoming tide, when the water depth had increased the most, and perhaps the currents were the strongest, animals went from being inactive, moving < 15 m/hr, to active, moving more than 50 m/hr, during all phases of the tide cycle (Fig. 5). Or put another way, the average time after peak low tide when horseshoe crabs initiated bouts of activity that lasted at least 30 min (mean=4.59 ± 1.2 hrs in duration) was 6.31 ± 1.17 hrs (~ the time of peak high tide; *n*= 56 bouts of activity obtained from 12 different animals).

After breeding season, in the summer and fall, horseshoe crabs remained very active and moved up and down the estuary, over a range of several km (Watson et al., 2009). In general, they resided along the interface between the deep channels (10–20 m) and much shallower mud flats (0–4 m), and often stayed in the same general area for 1–2 weeks before moving to a new location. In one instance we successfully tracked an individual horseshoe crab for 13 days in July, during which time it covered an area of ~ 500 m along the shore and 200 m out from the shore (a portion of which is shown in Fig. 6 below). Interestingly, some animals continued to express periods of elevated activity during high tide

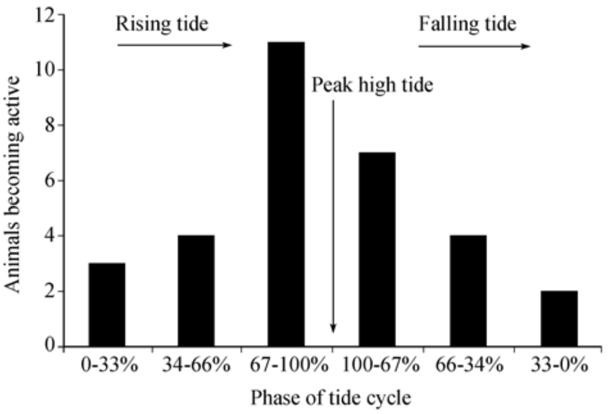

**Fig. 5 Phase of the tide cycle during which male and female horseshoe crabs initiated activity after a period of quiescence**
Data (32 observations) were obtained in June of 2006, from 11 different animals.

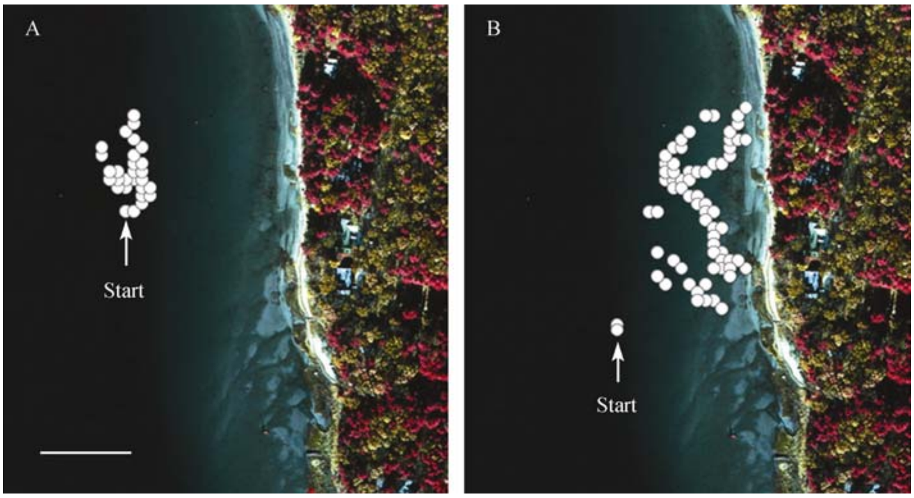

**Fig. 6 Movements of a male horseshoe crab, in July, in the Great Bay estuary**
A. Positions during 4 hrs of low tide in approximately 7 m of water. Points were not obtained at consistent time intervals, but were generally about 5 min apart. Artifacts have been removed, some points fell directly on top of each other, and fewer positional fixes are obtained during low tides when animals are buried in the sediment; thus, fewer points are depicted than one might expect. B. Positions during 4 hrs of high tide, demonstrating possible foraging movements in shallow water. Positions are overlaid on an infrared image taken from an airplane. Scale bar=100 m. Apparent movements at low tide are partially due to buoy movements (see Methods and Discussion).

periods during the summer and fall even though they were not approaching the beach to mate (Fig. 6). This pattern of activity was very similar to what we documented during the mating season, except they did not move all the way to the water's edge. Observations by SCUBA divers indicated that horseshoe crabs were burying in the sediment, digging pits, and most likely foraging for food during these excursions into shallower areas. Divers also reported, during all seasons, that a number of horseshoe crabs continued to be in pairs, with males attached to females.

## 3 Discussion

While telemetry has previously been used very successfully to investigate the movements of horseshoe crabs (Kurz and James-Pirri, 2002; Brousseau et al., 2004; Moore and Perrin, 2007), this is the first study to use an automated fixed array system to obtain continuous fine scale data concerning *Limulus* locomotion, habitat use and patterns of activity. Specifically, in this study, it was possible to: 1) continuously quantify the locomotor activity of animals and determine if their patterns of activity coincided with daily cycles of light and dark or tides; 2) determine where animals resided relative to depth contours and; 3) measure how fast they moved and exactly when they initiated and terminated bouts of activity. We found that: 1) there was a tendency to be most active during the high tides, but no preference for night vs daytime activity; 2) they consistently moved from the edge of channels into shallow water either to breed or to carry out other activities, such as foraging, during the times of the year when they were not mating and; 3) they often expressed very clear bouts of activity during which they walked at rate of 100–200 m/hr for 4–5 hrs at a time. These are questions that have been difficult to address in the past using visual observations or more traditional manual tracking systems and we are optimistic that future studies with this technology will be able to reveal even more about horseshoe crab behavior in their natural habitat.

The manual tracking systems that have been used to study the movements of horseshoe crabs and other aquatic species in the past have two major disadvantages. First, manual tracking is very time consuming and data are only obtained intermittently when researchers are able to spend significant amounts of time in the field. Second, positional fixes have only a limited resolution and thus it is both difficult to know exactly where animals are located and whether they are moving or not. For example, it would be difficult with a manual system to determine if the horseshoe crabs shown in figures 1 and 6 in this manuscript were moving or not. The fixed array VRAP system used for this investigation overcomes those drawbacks and thus makes it possible to more precisely address questions about horseshoe crab behavior in their natural habitat. However, while the fixed array system has many advantages over other related types of tracking systems, there are limitations that should be considered. First, because the buoys move with the tides and the waves, and each positional fix that is obtained has some degree of error, these errors can accumulate when trying to calculate distance traveled over some time period. For example, during one 24 hr period (6/19/08) a horseshoe crab that was tracked in the same area shown in Figure 1 moved an average of 163.3 ± 23.1 m/hr (max.=136.2 m/hr, min.= 37.3 m/hr) while the reference transmitter apparently moved 18.2 ± 1.4 m/hr (max.=31.7, min.=7.6). Therefore, while it is fairly simple to subtract the apparent movement of the reference transmitter from the much larger movement of these animals, it is very difficult to determine if an animal is truly quiescent. Second, the system can only accurately track animals that are within a fairly confined area because all three hydrophones must hear each coded transmission in order to triangulate the position of the animal. Thus, in our hands, it was difficult to track any given animal for many days in a row, because most horseshoe crabs we tracked only remained in one place for a few days. Third, there was considerable variability in the number of positional fixes that were obtained during different phases of the tide cycle. For example, the lowest number of positional fixes obtained from one subject was during peak low tide (3 fixes) and the maximum gathered during peak high tide was 57. This difference was due to both the difficulty detecting and triangulating data in shallow water and the fact that horseshoe crabs often buried themselves during low tide periods and this considerably reduced the effective transmission distance of the acoustic transmitters. Finally, because the buoys run on batteries, data are lost when any one of the batteries runs low, and this can cause a gap in the data. Recently, with the development of tags that actually transmit data (acceleration, depth, temperature), stationary listening receivers (VR2s by Amirix; SURs by Sonotronics) and methods for converting listening station data into continuous tracks of animal movements, it will be possible to overcome some of these limitations and thus learn a great deal more about the behavior of mobile marine invertebrates such as *Limulus polyphemus*.

When horseshoe crabs are mating in the spring and summer, it is quite easy to observe their approaches to particular beaches during the rising tide to mate in very shallow water at high tide. However, our knowledge of their full suite of activities during this time of year is somewhat limited by the fact that it is difficult to observe the behavior of individual animals continuously and, for obvious reasons, night observations are somewhat limited. Biotelemetry allows us to extend our understanding of *Limulus* behavior during this time of year by making it possible to learn about what they are doing when they are not readily observed near the water edge at high tide. For example, our data demonstrated a tendency for horseshoe crabs to move away from mating beaches to relatively deeper water (3–5 m in this location) during low tide, often remaining in one location throughout the low tide (Fig. 1). Brousseau et al. (2004), also found that the females they tracked moved away from the mating beaches at low tide, but at their study site in Delaware Bay females moved much further (mean of 299 m) from the mating beaches at low tide. This is likely due to the fact that they had to move further to reach deeper water at their study site. Our data also indicated that most animals did not approach the beach at every high tide, but only on 30.5% of the high tides they experienced while they were being tracked. This value is somewhat less than was reported by Brousseau et al. (2004). One possible reason for this discrepancy is that they tracked only large gravid females that were probably initiating their first bout of spawning, while some of our animals might have engaged in several spawning events prior to being tracked and were therefore less motivated.

Males almost always outnumber females at mating beaches and it has been proposed that the male bias is the result of several factors, reviewed in Brockmann and Smith (2009), including the fact that females only visit mating beaches several times in a season while males return more often (Rudloe, 1980; Leschen et al., 2006). However, in this study, we found that there was no significant difference between the number of times males vs females approached mating beaches during the available high tides. Therefore, we favor the hypothesis put forth by Brockmann and Smith (2009), that the sex ratio differences on mating beaches result from the fact that only a small portion of the available females in the population mate at a given time, while males are engaged in mating activities throughout the mating season (Cohen and Brockmann, 1983; Brockmann, 2003). As a result, individual females may approach the beach just as many times as males, which is consistent with our data, but not every female in the population would be engaged in mating, resulting in the skewed sex ratios observed at most spawning beaches.

The historical view that horseshoe crabs are most abundant on mating beaches during the full moon (night) high tides, and the discovery by Barlow and his colleagues that horseshoe crabs increase the sensitivity of their eyes at night (Barlow, 1983), have led many to believe that there is a strong tendency for horseshoe crabs to be most active at night (for example on page 361 of Rudloe, 1981; Barlow et al., 1986). However, both our laboratory studies and field studies indicate that horseshoe crabs have a preference to be most active during the day (Chabot et al., 2007; 2008; Watson et al., 2008; Chabot and Watson, 2010), or during high tide periods (Chabot et al., 2007; 2008; Watson et al., 2008; Chabot and Watson, 2010). Moreover, some of the animals that express a tidal rhythm show a preference for activity during the daytime high tide (Fig. 1, 2). In this study, in June, animals expressed a significant preference for approaching mating beaches in the day, and a trend towards being most active in the day. While this trend to be more active in the day was also observed during the remainder of the summer, it was not statistically significant. It is not obvious why different populations of horseshoe crabs differ so much in terms of their diurnal vs nocturnal preferences, and why individual horseshoe crabs express such a variety of activity patterns. One possibility is that, because of their daily fluctuations in visual sensitivity (Barlow, 1983), they do not experience large differences in perceived ambient illumination and thus changes in light levels do not modulate their activity as much as some other animals. However, it seems that in both the lab and the field animals are strongly influenced by light, based on evidence of “masking” (one form of masking is where, because of a strong tendency for animals to be influenced by light or dark, or tides, they appear to express a rhythm even when they do not have an endogenous clock controlling that rhythm). For example, horseshoe crabs might skip night high tides when one might expect them to be more active. A preference for day vs night activity might also have to do with the apparent risk of predation by shore birds, with smaller animals tending to avoid being active during the day. Interestingly, this plasticity also appears to extend to juveniles. In Florida juveniles appear to favor locomotion in the day (Rudloe, 1981), while in New England they appear to be more active at night (Borst and Barlow, 2002; unpublished data). In summary, while it is clear that some horseshoe crabs express clear daily patterns of activity, both the way these rhythms are modulated by light and the reason that different life history stages and populations tend to be express one type of rhythm or another, remain to be elucidated.

In the laboratory, under constant conditions, many horseshoe crabs express a tidal pattern of activity that is driven by endogenous clocks (Chabot et al., 2007). Moreover, this endogenous rhythm is capable of being synchronized and entrained by small changes in water depth that are delivered with a timing that is similar to the natural tides (Chabot et al., 2008; Watson et al., 2008). This suggests that in locations such as the Great Bay estuary most animals can sense the natural changes in water depth associated with the tides and synchronize their activity to the tides. We tested this hypothesis recently and found that it was, in fact, the case (Watson et al., 2009; Chabot et al., 2010; Chabot and Watson, 2010). In addition, we found that animals that were unable to sense the changes in water depth because they were secured under a floating dock that went up and down with the tides, failed to express a tidal rhythm of activity (Watson et al., 2009; Chabot et al., 2010 and this volume). These observations suggest that freely behaving horseshoe crabs in their natural habitat can synchronize their activity to the natural tides and initiate their activity early in the tidal cycle after the water had changed depth by only a meter or less (in the laboratory entrainment only required a 20–40 cm change in depth). However, while animals were most active during the high tide portion of the tidal cycle, there was a tremendous variability in terms of the phase of the cycle when they were active. Specifically, many did not initiate their activity as soon after the tide began to rise as expected (Fig. 5), many even initiated their bouts of activity during the falling phase of the tide cycle (which is consistent with some data from some of our previous studies in the laboratory; unpublished data) and most did not express increased activity during sequential high tides; instead expressing a “skipping” pattern (see Chabot and Watson, this volume). Therefore, in their natural habitat, many other factors must be influencing the likelihood that they will express a tidal pattern of locomotion. As a result, despite the regularity and magnitude of the natural tidal fluctuations, twice-per-day tidal rhythms of activity tend to be less robust than those expressed in the laboratory.

One major factor that is likely to have a very strong influence on the timing and location of horseshoe crab locomotion is foraging behavior, the availability of prey, and the duration of time animals remain satiated after consuming prey. A number of animals that were successfully tracked during the late summer and early fall months, when mating was not taking place, were most active during high tides and moved all over tidal flats where the depth was ~ 1–3 meters at high tide (Fig. 6). Observations of these animals by SCUBA diving indicated that they were digging frequent pits in the sediment, which was likely related to foraging for food (see Lee, 2010). Furthermore, we suggest that after successfully consuming prey, horseshoe crabs bury in the sediment in slightly deeper water, and remain there until they have fully digested and assimilated their prey, which might take days. For example, prior to the presumptive bout of foraging activity illustrated in Fig. 6, which occurred during the non-breeding season, this same horseshoe crab was inactive for 4 straight days, during both high and low tides. We have also observed long periods of quiescence in animals after they are fed in the laboratory (unpublished observations). If this is, in fact, typical, it might be one reason why the patterns of activity expressed by freely moving horseshoe crabs in their natural habitat are not as consistent as those observed in the laboratory, which typically involve animals that are not fed (Chabot et al., 2007, 2008; Watson et al., 2008).

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