The Relationship Between Small- and Large-scale Movements of Horseshoe Crabs in the Great Bay Estuary and *Limulus* Behavior in the Laboratory

Winsor H. Watson III
Susanne Y. Schaller
Christopher C. Chabot

Winsor H. Watson III (Susanne Schaller)
Zoology Department
University of New Hampshire
Durham, NH 03824
email: Win@unh.edu

Christopher C. Chabot
Department of Biological Sciences
Plymouth State University, Plymouth, NH 03264
email: chrisc@mail.plymouth.edu

Abstract: The overall goal of our research program is determine the short- and long-term patterns of horseshoe crab (*Limulus polyphemus*) movements in the Great Bay estuary and then seek an understanding of the endogenous and exogenous processes that give rise to these patterns. Small- and large-scale movement data were obtained from 27 horseshoe crabs tracked using ultrasonic telemetry for at least a year. During mating season animals were most active during high tides, but they did not increase their activity or approach mating beaches during every high tide. During the remainder of the year tidal or daily patterns of activity were less evident, and the extent of their movements gradually decreased as water temperatures dropped in the late fall and winter. During the spring, when water temperatures exceeded 10°C, tagged animals moved several km up into the estuary into shallow water (< 4 m) one month prior to spawning. A similar temperature threshold was also evident in laboratory experiments, with little rhythmic behavior expressed at temperatures below 11°C. Mating activity lasted approximately one month and was followed by a period of high activity. In the fall, most animals moved downriver into deeper water, where they remained during the colder months. Thus, the majority of *Limulus* exhibited a similar seasonal pattern of movement, remaining within a 3 km stretch of the estuary. In the laboratory, animals expressed both daily and tidal rhythms of locomotion. Those with daily rhythms were more likely to be diurnal than nocturnal, but both tendencies were evident. The clock involved in modulating their locomotory activity appears to be separate from the clock controlling their circadian rhythm of visual sensitivity. When animals were exposed to "artificial tides", created by changing water depth every 12.4 hrs, they expressed clear tidal rhythms of activity that were synchronized to the imposed tides. Similar data were obtained from horseshoe crabs in running wheels placed in the estuary. However, if the running wheels were attached to a floating dock, so water depth did not change with the tides, the horseshoe crabs were primarily diurnal. Thus, while endogenous biological clocks are capable of controlling many aspects of horseshoe crab locomotion, the actual patterns manifested in the field are strongly influenced by the water depth changes associated with the tides, as well as light levels and seasonal changes in water temperature.

1. Introduction

American horseshoe crabs, *Limulus polyphemus*, offer an interesting and accessible model system for investigating different types of biological rhythms and the environmental factors that influence their expression. Their most well documented behavior, mating, occurs every year, in the early spring and summer, and appears to be very closely associated with natural tidal rhythms (Rudloe 1980; Cohen and Brockmann 1983; Barlow et al. 1986; Brockmann 2003). They are also known to possess a circadian clock that influences the lateral eyes, making them much more sensitive to light at night (Barlow et al. 1980; Kaplan and Barlow 1980; Barlow 1983). Thus, these animals appear to have one or more biological clocks that are capable of keeping track of the circa 12.4 hour tidal cycle as well as the more typical 24 hour daily cycle (and possibly seasons). Our overall objectives during the past several years have been to determine: 1) How far do they move within the estuary and do their movement patterns vary with the seasons in a systematic manner; 2) How much of *Limulus* behavior is under the influence of a tidal clock vs. a circadian clock and; 3) What environmental factors determine which biological rhythm they express during different times of the year?

Throughout our investigations we have used a combination of field and laboratory studies to address the aforementioned questions. Field studies are necessary to establish the "normal" behaviors of horseshoe crabs and develop hypotheses about the factors that influence the expression of different types of behaviors. Laboratory investigations allow us to control some of these factors and thus dissect out the specific mechanisms that give rise to various types of biological rhythms and the environmental stimuli that modulate their expression.

In this short review we will first discuss how ultrasonic telemetry has allowed us to identify the long-term seasonal movement patterns of horseshoe crabs within the Great Bay estuary, N.H. We will then provide data from laboratory experiments that document how, and under what conditions, horseshoe crabs will express both daily and tidal patterns of locomotion. Finally, with the foundation provided by our laboratory data, we

will examine the fine-scale movements of horseshoe crabs in the field and discuss how and why they express particular types of activity at different times of the year.

2. Seasonal movements of horseshoe crabs in the Great Bay estuary

The Great Bay estuary in NH, like most estuaries, it is characterized by large seasonal fluctuations in temperature and salinity (Fig. 1). It has a large population of horseshoe crabs, but very little is known about their movements and habitat preferences within the estuary. It is likely that, like other mobile estuarine species, such as striped bass and lobsters, horseshoe crabs undertake seasonal movements to optimize their survival under these complex conditions. They may also make use of the salinity and temperature gradients within the estuary to guide their movements, in a manner similar to lobsters (Crossin et al. 1998; Jury et al. 1994, 1995, 2000; Watson et al. 1999; Dufort et al. 2001).

2.1 Movements of *Limulus* in the Great Bay estuary

Between the fall of 2005 and the spring of 2007 we tracked twenty-seven horseshoe crabs (6 males, 21 females) as they moved around within the estuary. Each animal was fitted with an acoustic transmitter (VEMCO V13L coded tags, 13 mm diameter, 33 mm long, 6 g in water, battery life > 1 year), either internally (n=15), in the frontal area, or externally (n=12), glued on the dorsal carapace. Animals tagged in the fall of 2005 (n=8) were captured in lobster traps in deeper portions of the estuary, brought to the surface, fitted with transmitters, and then released back in the channel (Fig. 2). In 2006 all animals (n=19) were fitted with transmitters while spawning at a beach adjacent to the UNH Jackson estuarine laboratory.

Horseshoe crabs were tracked using both VR100 receivers with hydrophones and VR2 listening stations (VEMCO Ltd., Halifax, NS, Canada) deployed throughout the estuary. The VR2 listening stations were deployed at the five locations indicated in Figure 1. Each time a horseshoe crab fitted with a coded transmitter moved within range (approximately 300 m) of a VR2, the ID of the animal, time, and date were logged. If animals were fitted with depth/temperature transmitters, those data were also logged. In

December 2006 through March of 2007 animals traveled very little (0.6 ± 0.1 km) and remained in deeper water (range 2.2-22.2 m, median 16.3 m). This tendency to be very inactive in the winter was confirmed using the fixed array telemetry system described in a subsequent portion of this review. We were able to continuously track the same three animals for more than 3 weeks and there was no apparent movement by any of them during this time.

3. Expression of tidal and circadian rhythms in the Laboratory

Limulus reliably express both daily and tidal rhythms of locomotion in the laboratory (Fig. 4, Chabot et al. 2004, 2007). While most animals express either tidal (approximately 12.4 hr) or diurnal (24 hr cycle, active in the day) pattern of activity, some animals were nocturnal. Interestingly, when we monitored the sensitivity of one of the lateral eyes to light (electroretinograms, ERGs), while simultaneously recording locomotor activity, we found that patterns of locomotion were independent of visual sensitivity (Fig. 5; Watson et al. in revision). Thus, the circadian clock that enhances the sensitivity of the eyes at night (Barlow 1983), does not appear to play a role in modulating locomotion.

Locomotion is influenced by a number of factors, including light, changes in water depth, and water temperature. Of these, water depth appears to have the greatest impact. When animals in the laboratory are exposed to artificial "tides", with 12.4 hr changes in water depth of as little as 25 cms, the majority synchronize their activity to the imposed cycles (Fig. 6; Chabot et al. submitted, Watson et al. submitted). Moreover, once the artificial tides are stopped, animals will continue to express a tidal rhythm in phase with the imposed tides, indicating that the artificial tides are entraining an endogenous tidal clock.

The activity cycles of some horseshoe crabs also correlate quite well with changes in light levels (Fig. 4; Chabot et al. submitted; Watson et al. submitted). When exposed to 14:10 LD cycles some animals have a tendency to be most active in the day and some at night (Fig. 4). However, many horseshoe crabs will also spontaneously express tidal rhythms under LD cycles, indicating that under LD conditions tidal rhythms are not completely suppressed. Moreover, on occasion, an animal expressing a tidal cycle will

contrast, the VR100 was used to search the estuary for animals on a weekly basis, from May-October and monthly basis during the colder months. When animals were located, all positions were recorded in GPS coordinates and data were analyzed and mapped using ArcView.

After being tagged in the fall of 2005, most of the horseshoe crabs moved very little during the late fall and winter and were primarily located in the deepest parts of the estuary (10-15 m; Fig. 2). Then, between April 18th and the middle of May, 7 of 8 horseshoe crabs moved upriver into much shallower water (2-4 m) (Fig. 2). The following year (2006-2007) a similar pattern was observed, both with 7 of 8 of the original animals from 2005 and the additional 18 animals tagged in the spring of 2006. They spent the cold months in deeper water, downriver from spawning locations, and then moved up into the estuary 2.3 ± 0.5 km (mean ± SE) in April and May when water temperatures exceeded 10.5°C (Fig. 2). In May of 2007 it was remarkable to observe how, during a short two-week period, all nine of the animals that over-wintered in the lower portion of Little Bay moved at least 1 km upriver. While all animals may have been responding to seasonal changes in ambient light levels, or an internal circannual clock of some type, we favor the hypothesis that increases in activity were triggered by the rapid warming of the water that typically occurs each spring at this time.

Movements up into the estuary in the spring typically resulted in most animals moving into much shallower water about a month prior to spawning (compare positions of animals in Fig. 2, with the depth profile in Fig. 1). Of the 19 animals tagged while spawning in the spring of 2006, 7 returned to the Jackson Estuarine Laboratory (JEL) and likely spawned there, 5 moved past JEL, and the remaining animals were not relocated in the spring. Following spawning in May and June, animals became quite active in the summer (July-August), moving a mean distance of 7.3 ± 1.5 km and residing in a range of depths. It is likely, but not confirmed, that animals are searching for and consuming a great deal of prey during this time of year.

Animals began to decrease their activity in September and this trend continued throughout the fall (Fig. 3). The average distance traveled declined to 3.0 ± 0.6 km in September and October as animals moved into wintering sites, and there was also a tendency to reside in the deeper channels and along the banks of the channels. From

briefly become synchronized to dawn and dusk periods (Chabot et al. 2007; Watson et al., submitted), suggesting that light can even have some influence over the expression of tidal rhythms. The complex ways that light and tides work together to modulate the activity of horseshoe crabs is certainly an area that is worth pursuing in the future.

Horseshoe crabs seem to exhibit different types of activity during different times of the year. They are typically quite inactive in the winter, they mate and express tidal rhythms in the spring and early summer and then they move into deeper water and are fairly active during the remainder of the year (we do not know what types of activity cycles they express during these times of year). We have been trying to determine what environmental cues might cause them to switch from one pattern of activity to another, and the most logical choices are light and water temperature. When horseshoe crabs were exposed to warm water the majority of them expressed tidal rhythms of activity, even if the L:D cycle was 9:15, typical of winter (Chabot et al. in preparation). In contrast, if the water temperature was reduced below 12°C, they did not express a tidal rhythm of locomotion, even if they were exposed to a L:D cycle of 14:10, typical of the spring and summer (see additional data on this subject in section 3.3) This supports the hypothesis, expressed earlier, that in NH when water temperature rises rapidly in the spring and exceeds 10-12°C, horseshoe crabs become quite active and start moving towards areas where they typically breed.

4. Expression of tidal and daily rhythms of activity in the field

While it is generally assumed that horseshoe crabs tend to be more active at night, especially during nighttime high tides, our laboratory data indicated that animals with a daily pattern of activity were just as likely to be diurnal and nocturnal (Chabot et al. 2004, 2007). In order to test this theory in the field one of our first studies involved conducting *Limulus* surveys at two different mating beaches in the Great Bay estuary, in the spring of 2006. We found no statistically significant difference in the number of horseshoe crabs observed mating during the day vs. the night (Fig. 7). In Adam's Point Cove, we observed 24.7 ± 6.1 (mean ± SEM, n=24 days) animals along a 50 m transect during the day and 32.4 ± 6.8 animals during the night (P=0.3119; paired t-test, df=23).

The results from a nearby beach (0.5 km away), were similar (27.5 ± 5.7 *Limulus* during the day and 35.4 ± 9.8 during the night (P=0.4381; df=23). The small tendency for more animals at night than during the day might be due to either the influence of light, or the fact that in this location nighttime high tides tend to be higher than daytime high tides.

4.1 High resolution tracking using ultrasonic telemetry

The primary study site for investigations of small-scale horseshoe crab movements in the field was a cove near the Jackson Estuarine laboratory (Fig. 1). We fitted 18 horseshoe crabs with ultrasonic transmitters while they were mating and then tracked them with a fixed array telemetry system (VRAP, VEMCO Ltd) for as long as they remained in the vicinity of the array. The VRAP system is capable of continuously monitoring the position of horseshoe crabs with an accuracy of approximately1-2 m, depending on the position of the animal relative to the buoy array, noise levels in the area, and habitat (Golet et al. 2006). In this particular situation, the system worked very well, except for a difficulty accurately obtaining positional fixes when horseshoe crabs moved into very shallow (0-1 m) water while mating.

Of the 18 animals tracked in the spring of 2006, we obtained good data from 17 for an average of 2.5 days each (range of 1-6). The majority (14) returned to the same cove to spawn during the high tides subsequent to being fitted with transmitters. However, they did not approach the beach at every high tide. Between tides they returned to deeper water (approximately 5-8 m), but rarely sought the deepest water available (within 100 m of the mating beach the water depth was > 30 m; Fig. 8). Overall, during the mating season, lasting from late May until the end of June, the six animals for which data was available for at least 4 high and 4 low tides, were significantly more active during high tides than low tides (P=0.0079, df=5; Fig. 9A). We also determined, for those same six animals, the percentage of each high or low tide when they were completely inactive. While the trend was to be more inactive during low tides, it was not statistically significant (P=0.1089, df=5; Fig. 9B). Interestingly, while there was a trend to be more active in the day than night, it was not statistically significant (unpaired t-test, P=0.536, df=32; Fig. 9C). Thus, at least during the mating season, our field data confirm our

findings in the laboratory, indicating 1) a strong tendency to be active during high tides and 2) only a weak, if any, influence of a circadian clock in the control of locomotion. Rather, we suggest that the circadian rhythm of eye sensitivity evolved to allow horseshoe crabs to see equally well whether they are active during the day or night. This is particularly adaptive during the mating season, when they must be active during both the day and night if they are driven to breed during each high tide

4.2 The influence of depth and temperature on activity rhythms in the field

During the late fall, winter and early spring when water temperatures were below about 10°C, horseshoe crabs in the field were very inactive (Fig. 3). Then, as the estuarine waters warmed up rapidly in the spring they become much more active and began migrating up into the estuary and into shallower areas. We propose that the combination of increases in water temperature, and moving into shallower water where they can better sense the changes in depth associated with tidal cycles, trigger the expression of a tidal pattern of activity. Our preliminary data (Fig. 10) suggest that tidal rhythms are not expressed in the laboratory below about 11°C (which is consistent with the threshold for increased activity in the spring). When they move into shallow water it both leads to increases in water temperature and larger relative changes in water depth. To test the theory that animals readily express tidal rhythms in shallow water in the field we recently recorded the activity of 8 horseshoe crabs, in running wheels similar to those used in the laboratory, in approximately 3 m of water of water near the Jackson Estuarine Laboratory (Fig. 11). There was a clear tendency for these animals to be active during high tide. To determine if the primary tidal zietgeber entraining animals to the natural tides cycles was changing water depth, or other factors associated with the changing tides, such as fluctuations in salinity or temperature, we also placed animals in running wheels under a floating raft. In that situation the raft went up and down with the tides so there was no change in depth, even though animals were exposed to fluctuations in temperature, salinity and other variables. Under these circumstances, most of the animals ignored the tidal cues and were most active during the day (Fig. 11). Thus, in the field, as well as the laboratory, light, temperature and the changes in water depth typically associated with the

tides all influence both the extent of horseshoe crab movements, and their tendency to express a given pattern of movement. Temperature appears to be permissive, allowing rhythms to be expressed once a certain temperature threshold is exceeded. However, while the temperature in Great Bay changes by as much as 5°C during each tide cycle, these temperature fluctuations do not appear to be sufficient to entrain the tidal rhythm of horseshoe crabs (Fig. 11). In contrast, the depth changes associated with each tide have a profound impact on expression of tidal rhythms. Horseshoe crabs will also synchronize their activity to LD cycles, if tidal cues are weak. Thus, *Limulus* provides a very interesting model system for investigating the way that different environmental signals influence the expression of endogenous rhythms.

Temperature and other variables that change with the tides have also been shown to impact circatidal rhythms in other intertidal species. Temperature changes associated with the tidal cycle are sufficient to entrain several crab species (Williams and Naylor 1969), as are pressure fluctuations for both crabs and fish (Naylor and Atkinson 1972; Abello et al. 1991; Northcutt et al. 1991). Salinity changes appear to be effective for estuarine crabs (*Callinectes sapidus*; Forward et al. 1986), but not for green crabs (Palmer 1995). Finally, periodic agitation is sufficient to entrain tidal rhythms in two species of isopods (Klapow 1972; Hastings 1981). It is interesting to note that a recent paper indicates that agitation cycles are also effective entraining agents for juvenile horseshoe crabs (Ehlinger and Tankersley 2006). In adult *Limulus,* however, water depth changes appear to be more effective than turbulence, temperature or salinity cycles (Chabot et al., in prep). Barlow et al.(1986), proposed that the number of animals mating during any given high tide is proportional to the relative height of that tide. Taken to the extreme, in areas where there is little change in water depth with the tides, such as in the Indian River Estuary in Florida, mating is not very well synchronized to the tides. Furthermore, evidence supporting the view that most mating activity occurs during the new and full moons comes primarily from certain areas in Florida were significant changes in water depth may only occur during this period of the month (Rudloe 1980). Yet, despite all these data, we still know little about how horseshoe crabs, or any marine invertebrates for that matter, detect changes in water depth (Morgan 1984; Macdonald and Fraser 1999; Fraser 2006). This is certainly an area worthy of future investigation.

5. Summary and Conclusions

Horseshoe crabs express a number of biological rhythms in the field and the laboratory. They possess at least two endogenous biological clocks, one tidal and one circadian. The tidal clock influences locomotion and is readily entrained by the depth changes associated with tidal cycles, but not the associated fluctuations in temperature and salinity. The circadian clock controls eye sensitivity, but it does not appear to have an impact on locomotion. Nevertheless, patterns of locomotion are influenced by LD cycles, with some horseshoe crabs expressing nocturnal patterns of behavior, while others are diurnal, both in the laboratory in the field.

The combination of endogenous clocks, and sensitivity to natural stimuli, results in the patterns of activity observed in the field. In the winter, when temperatures are low, animals move very little and they tend to inhabit deeper regions of the estuary. When water temperatures rise above about 10°C in the spring, animals become more active, they tend to move further up into the estuary, and often they migrate into shallower areas adjacent to breeding beaches. In shallow water the natural fluctuations in water depth, associated with the tides, synchronize and entrain the endogenous tidal clock so that animals are not most active at high tide. Following cues that are poorly understood at the present time, they find and approach breeding beaches at high tide. At low tide they move into moderately deep water and are primarily inactive at this time. When mating season is over, activity remains high in the summer and it is not clear what rhythms they express the most. As the water temperature drops in the fall, they move into deeper water to spend the winter. While the our combination of laboratory and field studies has provided some insight into the behavior of horseshoe crabs in their natural habitat, and the internal and external forces that guide this behavior, many questions remain to be resolved.

6. References

Abelló P, Reid DG, Naylor E (1991) Comparative locomotor activity patterns in the Portunid crabs *Liocarcinus holsatus* and *L. depurator*. *J Mar Biol Ass UK* 71:1-10.

Barlow RB (1983) Circadian rhythms in the *Limulus* visual system. *J. Neuroscience* 3:856-870.

Barlow RB, Chamberlain SC, Levinson JZ (1980) *Limulus* brain modulates the structure and function of the lateral eyes. *Science* 210:1037-1039.

Barlow RB, Powers MK, Howard H, Kass L (1986) Migration of *Limulus* for mating: relation to lunar phase, tide height, and sunlight. *Biol. Bull.* 171:310-329.

Brockmann, HJ (2003) Nesting Behavior: A shoreline phenomenon. pp.33-49 In: C.N. Shuster CN Jr, Barlow RB, Brockmann HJ (eds) The American Horseshoe Crab. Harvard University Press, Cambridge. MA.

Chabot CC, Kent J, Watson WH III (2004) Circatidal and circadian rhythms of locomotion in *Limulus polyphemus*. Biol. Bull. 207:72-75.

Chabot CC, Betournay SH, Braley NR, Watson WH III (2007) Endogenous rhythms of locomotion in the American horseshoe crab, *Limulus polyphemus*. J. Exp. Marine Biol. and Ecology 345:79-89.

Chabot CC, Skinner S, Watson WH III (submitted) Tidal cycles of water depth synchronize circatidal rhythms of locomotion in the American horseshoe crab, *Limulus polyphemus*.

Cohen JA, Brockmann HJ (1983) Breeding activity and mate selection in the horseshoe crab, *Limulus polyphemus*. *Bulletin of Marine Science* 33(2):274-281.

Crossin G, Jury SH, Watson WH III (1998) Behavioral thermoregulation in the American lobster, *Homarus americanus*. J. exp. Biol. 201:365-74.

Dufort CG, Jury SH, Newcomb JM, O'Grady DF III and Watson WH III (2001) Detection of salinity by the lobster, *Homarus americanus*. Biol. Bull. 201:424-34.

Ehlinger GS, Tankersley RA (2006) Endogenous rhythms and entrainment cues of larval activity in the horseshoe crab *Limulus polyphemus* J. Exp. Mar. Biol. Ecol. 337:205-214.

Forward RB, Douglass JK, Kenney BE (1986) Entrainment of the larval release rhythm of the crab *Rhithropanopeus harrissii* by cycles of salinity change. *Mar Biol* 90:537-544.

Fraser PJ (2006) Review: Depth, navigation and orientation in crabs: Angular acceleration, gravity and hydrostatic pressure sensing during path integration. *Marine and Freshwater Behavior and Physiology* 39(2):87-97.

Golet WJ, Scopel DA, Cooper AB, Watson WH III (2006) Daily patterns of locomotion expressed by American lobsters (*Homarus americanus*) in their natural habitat. J. Crust. Biol. 26(4):610-620

Hastings MH (1981) The entraining effect of turbulence on the circa-tidal activity rhythm and its semi-lunar modulation in *Eurydice pulchra*. *J Mar Biol Ass UK* 61:151-160.

Jury SH, M.T. Kinnison, Howell WH, Watson WH III (1994) The behavior of lobsters in response to reduced salinity. J.Exp. Marine Biol. and Ecol. 180:23-37.

Jury SH, Howell WH, Watson WH III (1995) Lobster movements in response to a hurricane. Mar. Ecol. Prog. Ser. 119:305-310.

Jury SH, Watson WH III (2000) Thermosensitivity of the American lobster, *Homarus americanus*. Biol. Bull. 199(3):257-264.

Kaplan E, Barlow RB (1980) Circadian clock in *Limulus* brain increases response and decreases noise of retinal photoreceptors. *Nature* 286:393-395.

Klapow LA (1972). Natural and artificial rephasing of a tidal rhythm. *J Comp Physiol* 79:233-258.

Macdonald AG, Fraser PJ (1999) The transduction of very small hydrostatic pressures. Comp. Biochem. Physiol.(A) 122:13-36.

Morgan E (1984) The pressure-responses of marine invertebrates: a psychophysical perspective. *Zoological J. of the Linnean Soc.* 80:209-230.

Naylor E, Atkinson RJ (1972). Pressure and the rhythmic behavior of inshore marine animals. pp 395-415 In: Sleigh M, Alister G. The Effects of Pressure on Organisms. Academic Press, New York.

Northcutt SJ, Gibson RN, Morgan E (1991) On-shore entrainment of circatidal rhymicity in *Lipophrys pholis* by natural zeitgeber and the inhibitory effect of caging. *Mar Behav Physiol* 19:63-73.

Palmer JD (1995) The Biological Rhythms and Clocks of Intertidal Animals. Oxford University Press. New York, NY.

Rudloe A (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.

Watson WH III, Vetrovs A, Howell WH (1999) Lobster movements in an estuary. Marine Biology 134:65-75

Watson, WH III, Bedford L, Chabot CC (submitted) Dissociation between circadian rhythms of visual sensitivity and circatidal rhythms of locomotion in the American horseshoe crab, *Limulus polyphemus*.

Williams BG, Naylor E (1969). Synchronization of the locomotor tidal rhythm of *Carcinus J. Exp Biol* 51:715-725.

7. Figure Legends

Figure 1. The Great Bay estuary. Top left: Location of the estuary (box), which empties into the Gulf of Maine. Top right: Enlarged view of the estuary showing depth contours. Depth values are for low tide. JEL=Jackson Estuarine Laboratory. GB=Great Bay, LB=Little Bay. Bottom panel shows average monthly temperatures and salinities in the estuary, in the vicinity of JEL, in comparison with values obtained along the NH coast.

Figure 2. Seasonal movements of horseshoe crabs in the Great Bay estuary. A. Positions of 7 of the 8 animals (labeled a-g) tagged in the fall of 2005, during the winter of 2005-2006. They are located primarily in the deep channel (see Fig. 1). B. Positions of those same animals in the spring of 2006. Note how they have moved up the estuary into shallow water. C. Locations of 4 of the same animals in winter of 2006-2007. D. Positions of those same animals in spring of 2007.

Figure 3. Net distance moved by horseshoe crabs tagged in 2005 in Great Bay during time periods indicated in 2005-2007. These calculations were made by designating the position of each animal at the beginning of a given time period, such as the spring, as zero. Movements of the animal up the estuary, relative to this position were given positive values. Movements down the estuary, toward the coast, were given negative values. All movements by each animal were totaled to give a net movement per animal and then these values were averaged to obtain a mean for the population.

Figure 4. Three types of activity patterns expressed by horseshoe crabs in the laboratory. All recordings were obtained from male horseshoe crabs walking inside a *Limulus* "running wheel" (Chabot et al. 2004, 2007). Data are double-plotted to facilitate visual recognition of patterns. The light dark paradigm (14:10, LD) is presented on the top of the figure. Right panels - Lomb-Scargle periodogram analyses of respective actogram sections, and the numbers indicate the dominate period. The top panel illustrates a tidal

rhythm, the middle panel a diurnal rhythm, and the bottom panel is data taken from an animal that was nocturnal.

Figure 5. Circadian rhythm of eye sensitivity and tidal rhythm of locomotion recorded simultaneously from the same horseshoe crab in constant dim illumination. Electroretinograms (ERGs) were obtained from one of the two lateral eyes using a clear plastic cup filled with seawater as the active electrode. Light pulses were delivered to the eye every 30 sec, using a green LED glued inside a black plastic cup that was secured over the eye with electrode. This arrangement made it possible to simultaneously record ERGs while animals walked inside a plastic running wheel (details of running wheel in Chabot et al. 2007). Sensors on the wheel enabled us to record wheel rotations. It appears that locomotion is being driven by an endogenous tidal clock while visual sensitivity is under the control of a circadian clock.

Figure 6. The effects of "artificial tides" (water depth cycles) on locomotor activity. LD cycle indicated by black/white bars at top. Periods of time when the water depth changed with a tidal cycle, delivered at periods of 12.4 h and 12.1 h, are indicated by open boxes on the actograms. Water pressure began to increase at the time indicated by the left side of the boxes. Maximum pressure occurred by the time indicated by the right side of the boxes. DD - constant water pressure and constant darkness. Right panels - Lomb-Scargle periodogram analyses of respective actogram sections. Largest peak value above horizontal line of significance ($P>0.01$) indicated by numerical value.

Figure 7. Day and night counts of spawning horseshoe crabs at two different locations. Note that on any given day, the numbers of animals mating at night and during the daytime are either similar, or vary in an unpredictable manner. The drop in counts from 6/2/06-6/13/06 was due, in part, to a drop in salinity as the result of heavy rains.

Figure 8. Activity of a female horseshoe crab during high and low tides. Points were obtained with a fixed array telemetry system approximately every 5 minutes and 2 hours of data are presented in each panel. Points are displayed on an infrared image of the area

around the Jackson Estuarine Laboratory, NH. This animal was fitted with an ultrasonic transmitter on 6/16/06, while spawning at high tide on the beach along the northern, shore of the cove (near where the dock meets the shore). During low tide, around midnight, it was relatively inactive, in about 5 m of water (A). The following high tide (6:40 AM), the next day, it returned to spawn and was much more active (B). Then it moved into deeper water (approximately 7-8 m) and was inactive during the next low tide (C), followed by high activity and spawning during the following high tide (7:30 PM; D). White scale bar in panel A is 20 m.

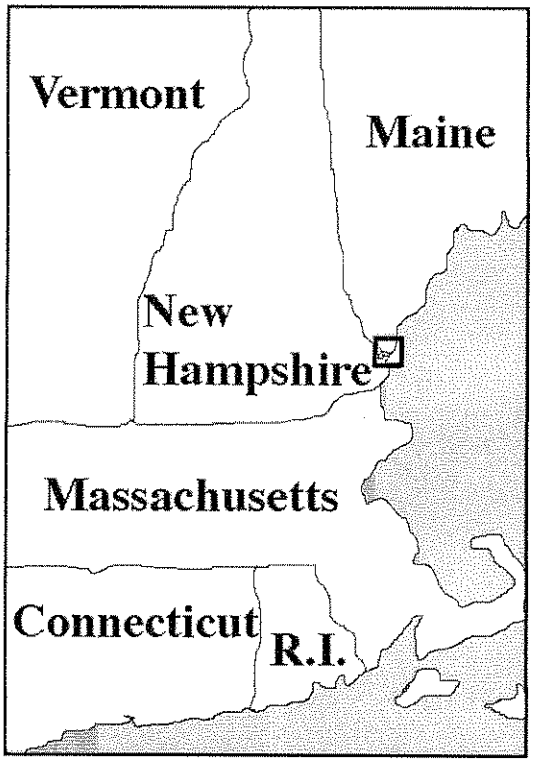
Figure 9. The activity of *Limulus* in the field during different phases of a tide cycle (A & B) and during the day vs. night (C). These data were obtained from analysis of the movements of 17 animals tracked for periods of time ranging from 1-6 days. Positional fixes were obtained about every 5 minutes for each animal. Tracks for individual animals were played back and observers determined the time periods when animals were clearly moving significant distances in a given direction (small movements were not easily distinguishable from background noise due to positioning errors), or clearly inactive. These data were used to calculate the % of each 6 hour high or low tide when animals were either active (A), or inactive (not moving at all, B). Values do not necessarily add up to 100% because there were time periods when movements were ambiguous (approximately 50% of the time in A and B and 35% of the time in C. For the tide analyses (A, B), only animals that were active for at least 4 high and 4 low tides were used (n=6). The day vs. night analyses were carried out with all 17 animals. Differences were statistically significantly different in A, but not B and C.

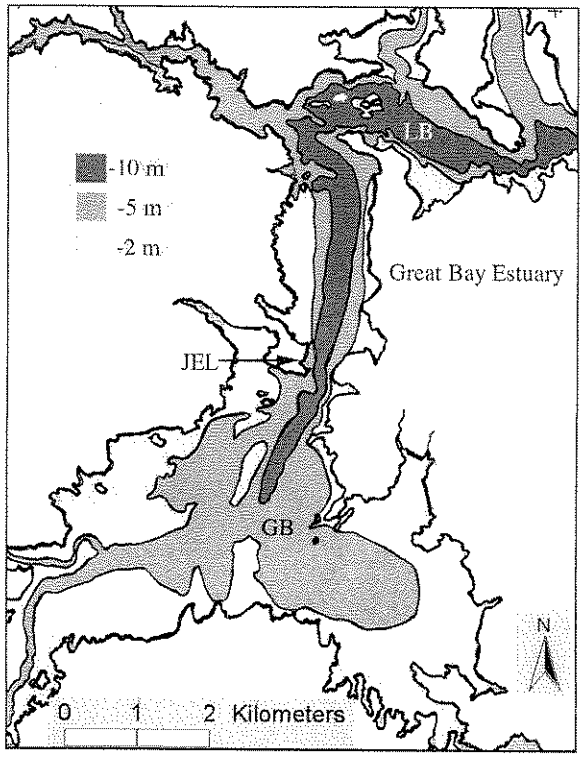
Figure 10. Circatidal rhythms in two different male *Limulus* exposed to three temperatures and a 15:9 (summer) LD cycle. Shaded grey areas in LD bar indicate either increasing or decreasing light intensity. Periodograms and plots are similar to figures four and six. Notice how robust tidal rhythms of activity are only expressed when the temperature is 17°C.

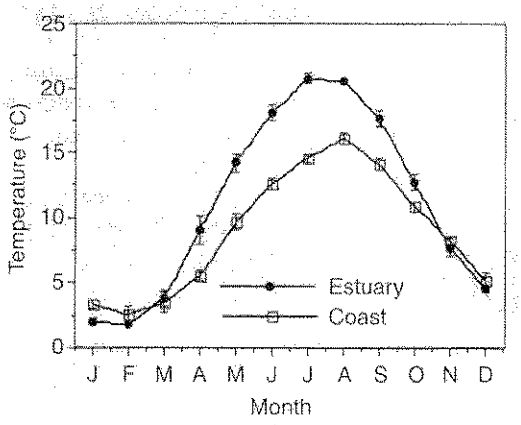
Figure 11. Double-plotted actograms (left panels) of locomotor activity male horseshoe crabs in running wheels in the estuary. A: One set of animals was anchored on the bottom of the estuary, in 4 m of water, and they were subjected to natural tidal pressure changes (approximately 3 m, 300% from low to high). The majority of these animals synchronized their activity to the natural tide cycles. B: The other set of animals were in running wheels secured underneath a floating dock, so they experienced natural tidal cycles, but no change in depth. Black and White bars at the top signify the approximate photoperiod; angled lines in the actogram signify mean high tide. Lomb-Scargle periodograms of the actograms are presented in the right panels. Values indicate highest significant peaks of activity above horizontal line of significance ($P< 0.01$).

Figures

Figure 1.


Vermont
Maine
New
Hampshire
Massachusetts
Connecticut
R.I.


-10 m
-5 m
-2 m
LB
Great Bay Estuary
JEL
GB
N
0 1 2 Kilometers


Temperature (°C)
25
20
15
10
5
0
Estuary
Coast
J F M A M J J A S O N D
Month

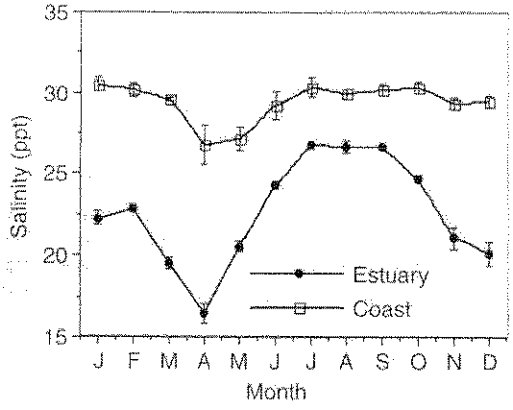

Salinity (ppt)
35
30
25
20
15
Estuary
Coast
J F M A M J J A S O N D
Month

Figure 2.

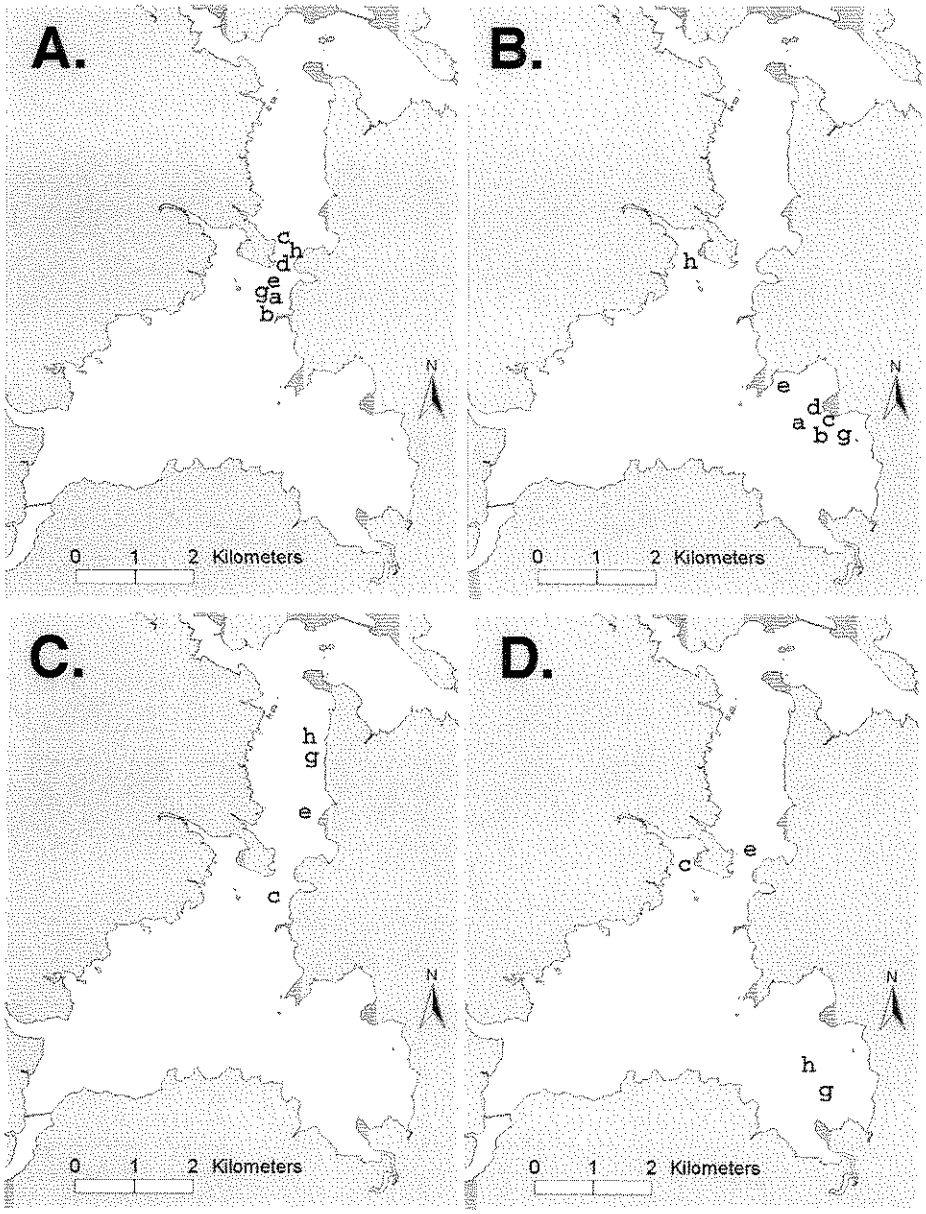


Figure 3.

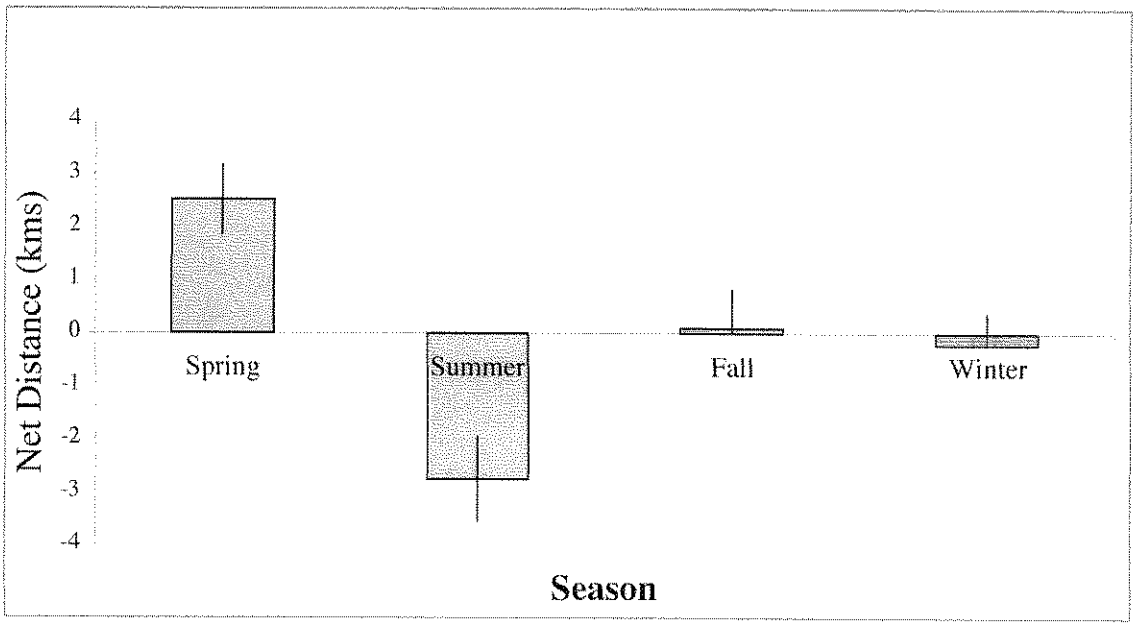

Net Distance (kms)
4
3
2
1
0
-1
-2
-3
-4
Spring
Summer
Fall
Winter
Season

Figure 4.

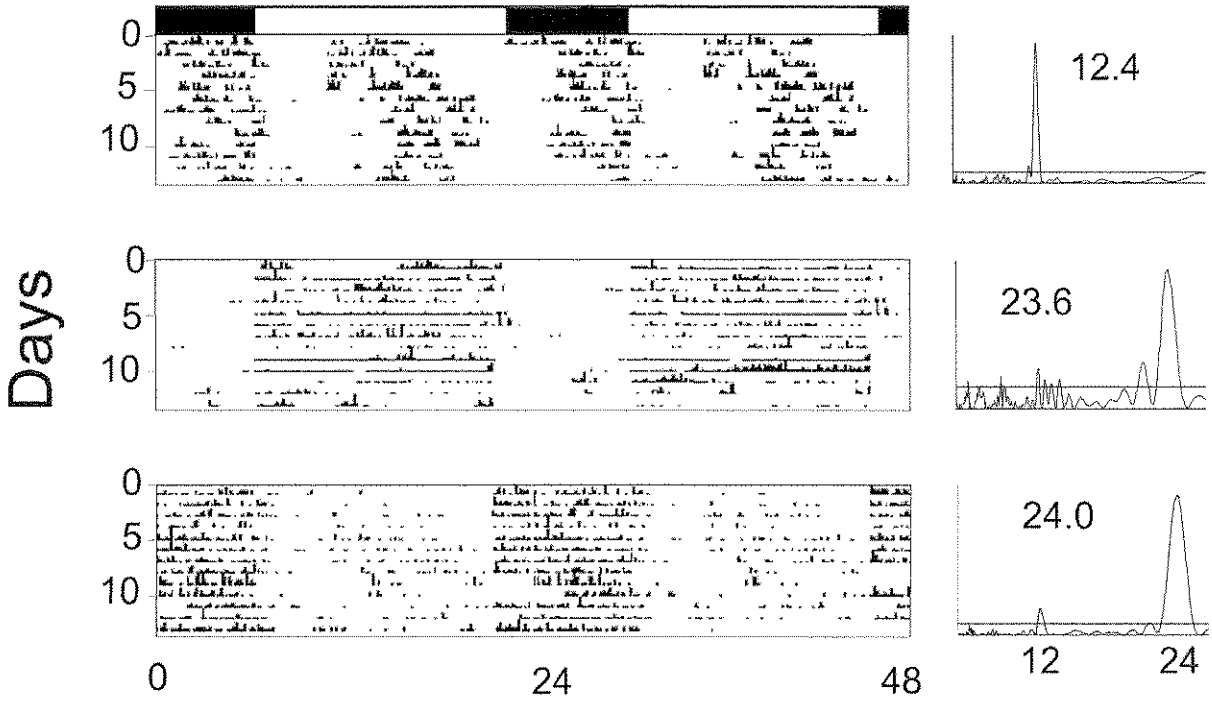


Figure 5.

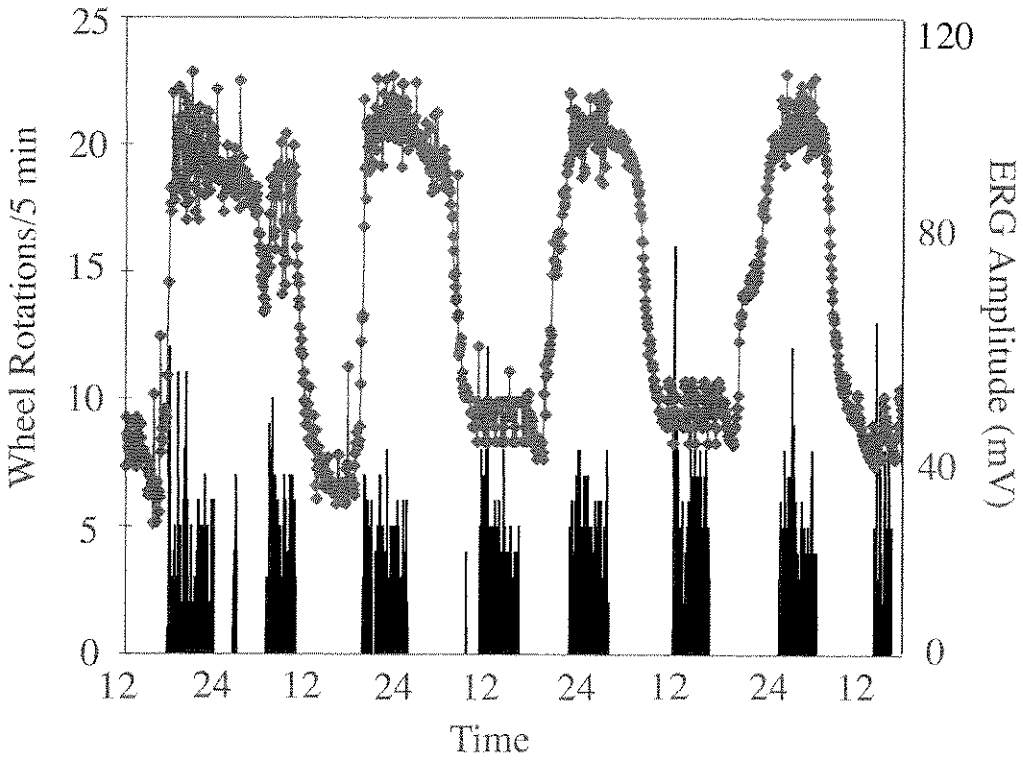

25
20
15
10
5
0
Wheel Rotations/5 min
120
80
40
0
ERG Amplitude (mV)
12
24
12
24
12
24
12
24
12
Time

Figure 6.

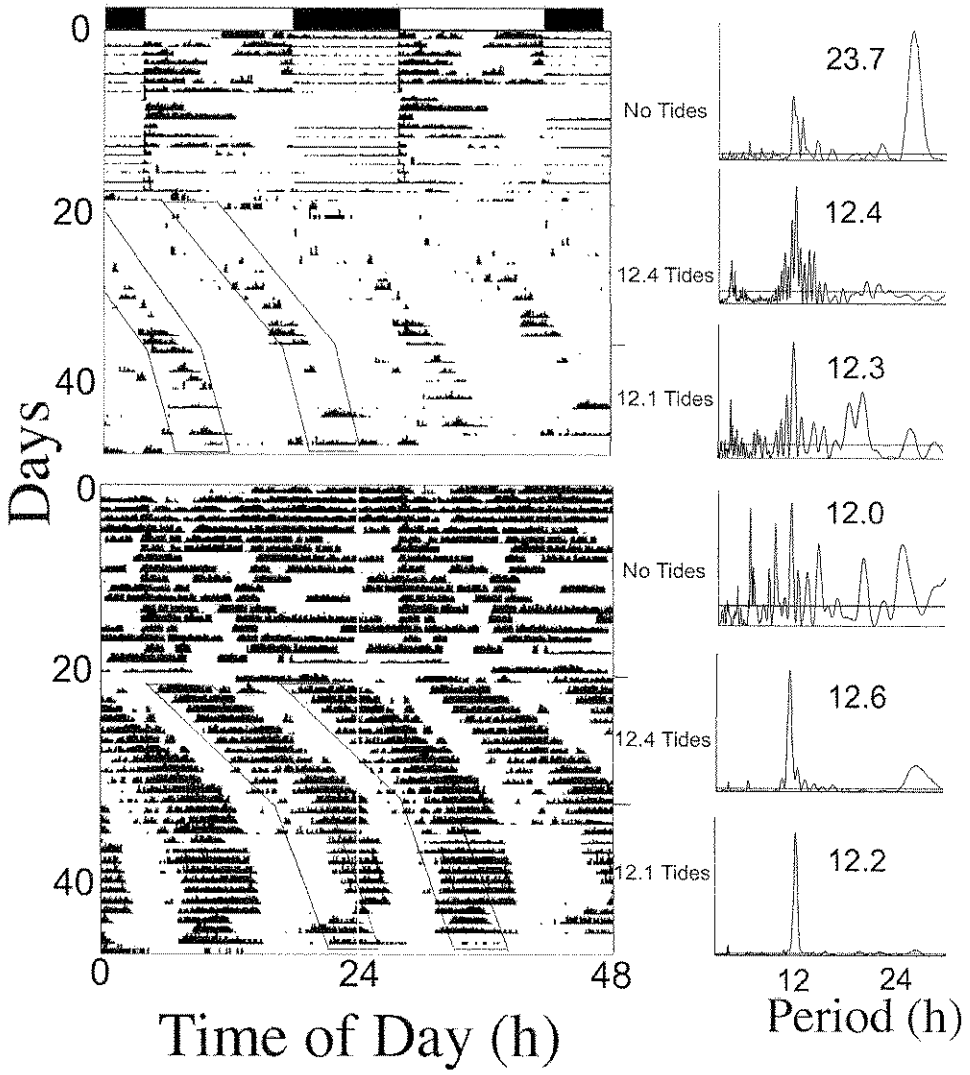

0
20
40
Days
No Tides
12.4 Tides
12.1 Tides
23.7
12.4
12.3
0
20
40
No Tides
12.4 Tides
12.1 Tides
12.0
12.6
12.2
0
24
48
Time of Day (h)
12
24
Period (h)

Figure 7.

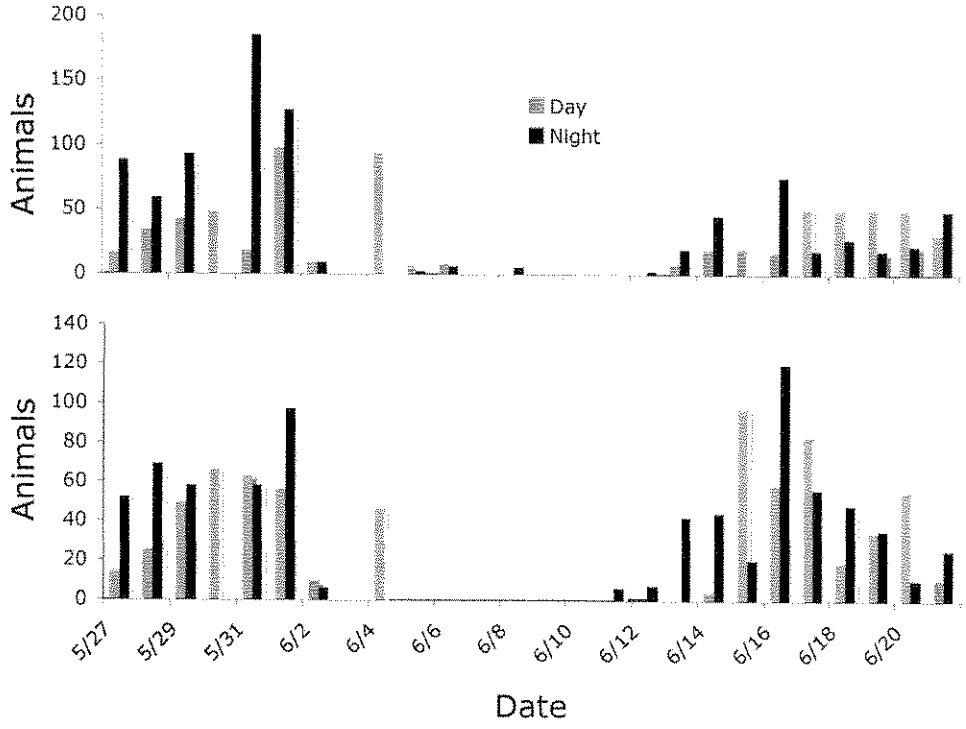

Animals
200
150
100
50
0
Day
Night
Animals
140
120
100
80
60
40
20
0
5/27
5/29
5/31
6/2
6/4
6/6
6/8
6/10
6/12
6/14
6/16
6/18
6/20
Date

Figure 8.

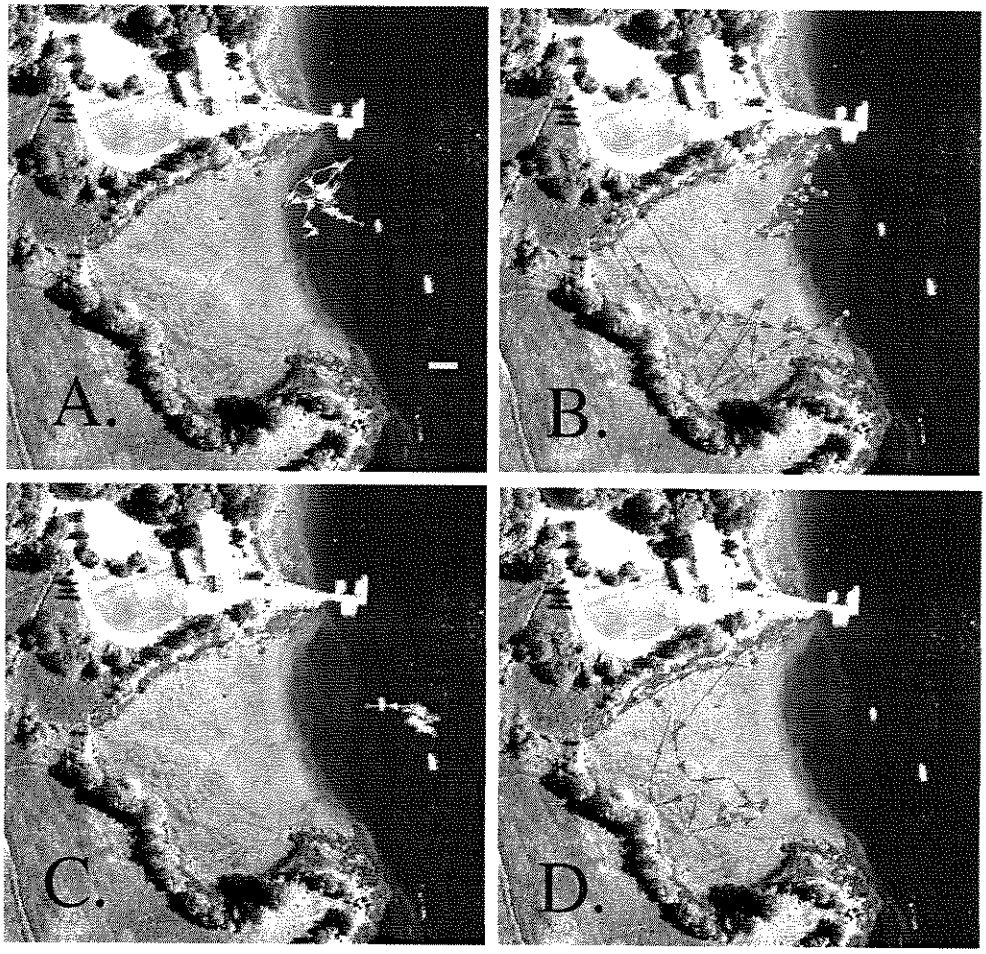


Figure 9.

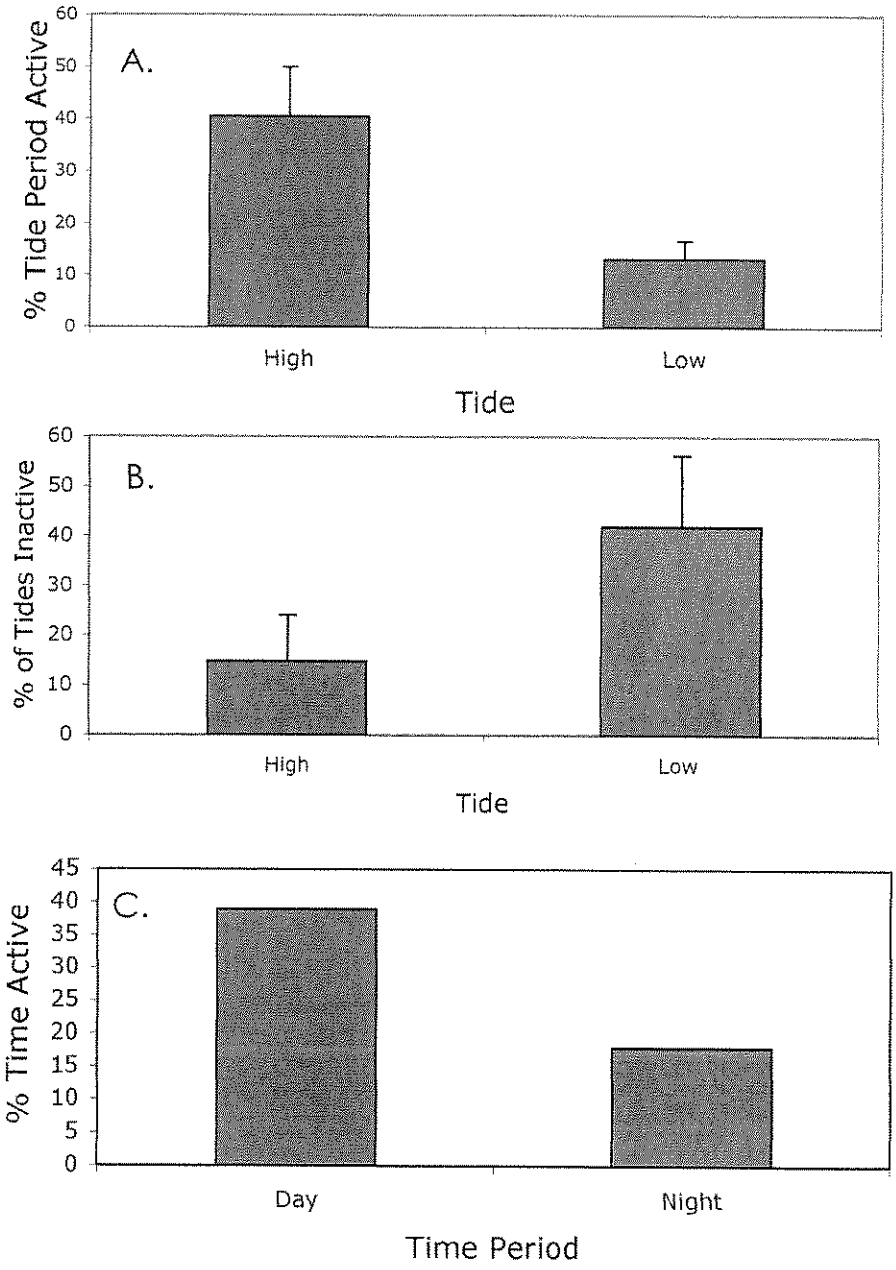

A.
% Tide Period Active
60
50
40
30
20
10
0
High
Low
Tide
B.
% of Tides Inactive
60
50
40
30
20
10
0
High
Low
Tide
C.
% Time Active
45
40
35
30
25
20
15
10
5
0
Day
Night
Time Period

Figure 10.

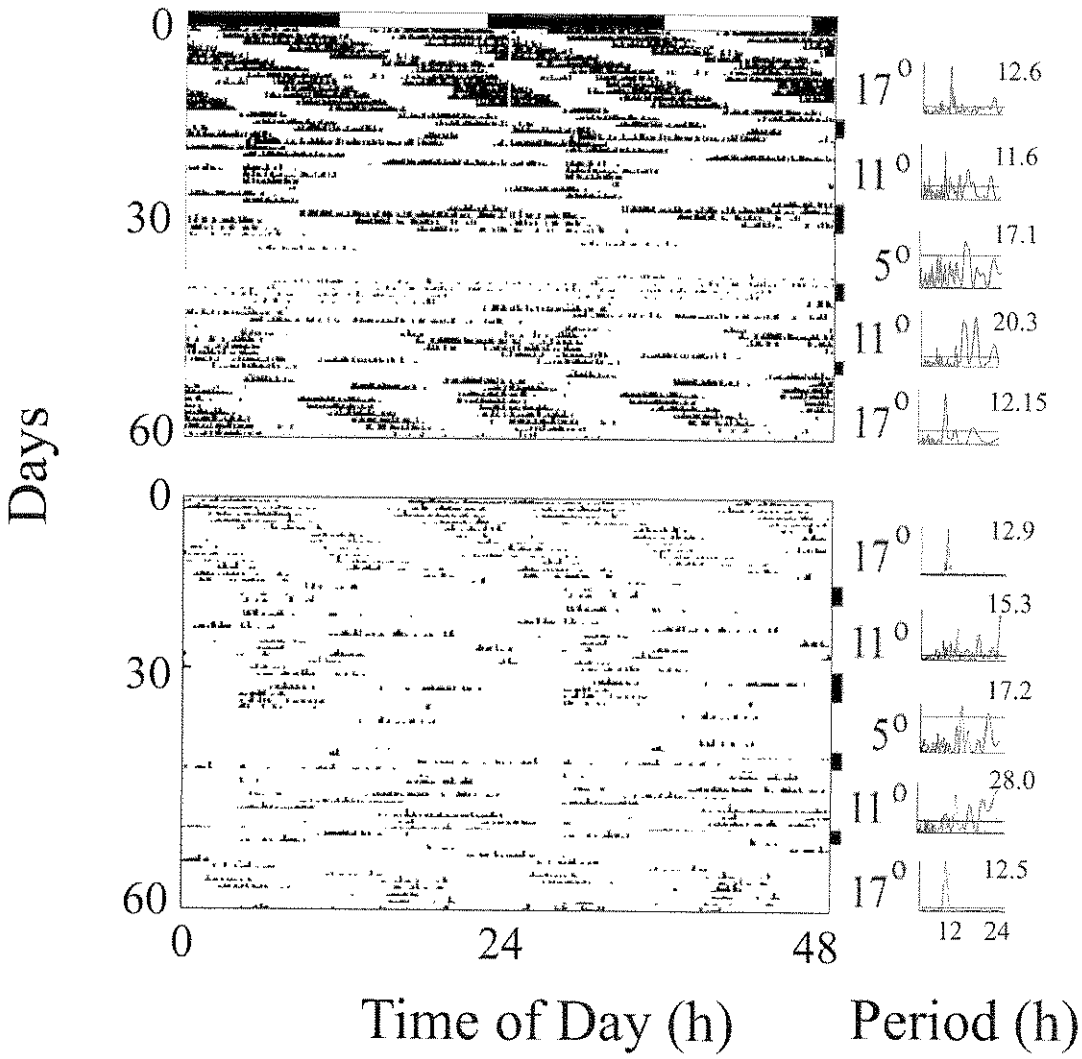
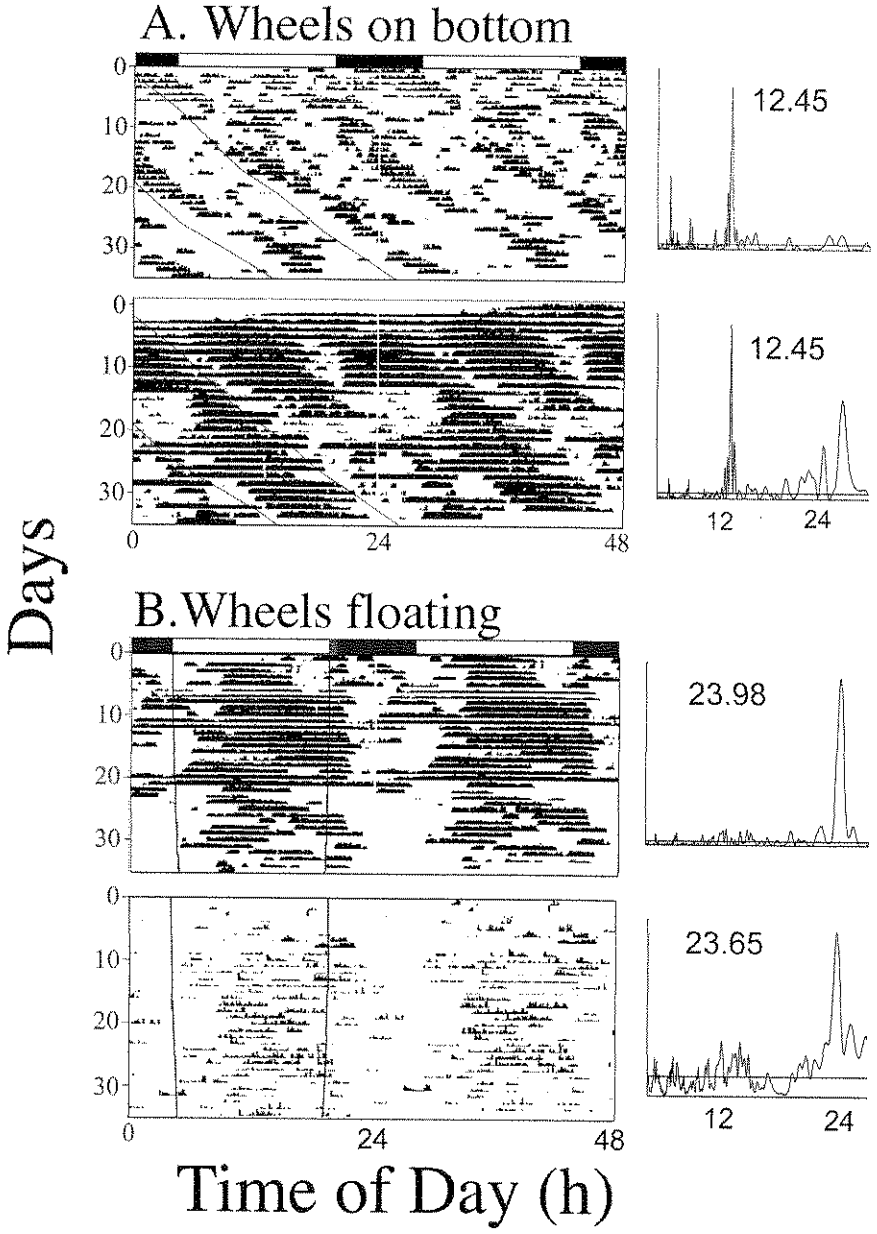

0
30
60
Days
17 0
12.6
11 0
11.6
5 0
17.1
11 0
20.3
17 0
12.15
0
30
60
17 0
12.9
11 0
15.3
5 0
17.2
11 0
28.0
17 0
12.5
0
24
48
12 24
Time of Day (h)
Period (h)

Figure 11.


A. Wheels on bottom
B.Wheels floating
Days
Time of Day (h)
12.45
12.45
23.98
23.65
0
10
20
30
24
48
12