

MIGRATION OF *LIMULUS* FOR MATING: RELATION TO LUNAR PHASE, TIDE HEIGHT, AND SUNLIGHT

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ABSTRACT

In the spring, horseshoe crabs (*Limulus polyphemus*) along the eastern coast of North America migrate toward shore to build nests close to the water's edge. In 1984 the mating season near Woods Hole, Massachusetts, extended from 14 May to 7 July. Mating activity during this period fluctuated with the phase of the moon, the height of the tide, and diurnal changes in daylight. As the moon approached new and full phases, large numbers of animals migrated into the intertidal zone to mate and build nests. They appeared 1–2 h before high tide, and returned to deep water about 2 h after high tide. No mating activity occurred during low tides. The two daily high tides in this region are unequal in height. The inequality is greatest during new and full moons. At these times most animals migrated toward shore on the higher tide, which occurred in the late afternoon and throughout the night. As the moon passed through quadrature the tidal inequality diminished and reversed. Mating activity changed accordingly: shore migration diminished, becoming nearly equal on the two equally high tides, and in several days shifted to the higher high tide. The cue for shifting appears to be the first light of dawn and not the height of the tide itself. We suggest that the migration of *Limulus* involves several sensory modalities including vision.

INTRODUCTION

Much has been learned about vision from research on the horseshoe crab, *Limulus polyphemus*. The classic studies of the *Limulus* lateral eye by H. K. Hartline and his colleagues revealed basic mechanisms of retinal function common to many animals (Hartline, 1969; Ratliff, 1974). More recent studies show that the *Limulus* visual system is an excellent example of the central modulation of retinal sensitivity (Barlow *et al.*, 1977; Barlow, 1983; Barlow *et al.*, 1985). Efferent nerve signals generated by a circadian clock located in the brain increase the sensitivity of the lateral eyes at night. Laboratory studies show that the circadian increases in retinal sensitivity can be detected behaviorally (Powers and Barlow, 1985), and field studies indicate that vision mediates some aspects of mating behavior (Barlow *et al.*, 1982). Further studies of the visual component of *Limulus* mating behavior should enhance our understanding of the functional organization of the visual system (Barlow *et al.*, 1987). As a first step we carried out a detailed investigation of mating activity itself.

Limulus mates in intertidal zones along the eastern coast of North America and along much of the coast surrounding the Gulf of Mexico (Shuster, 1979). These coastal regions are dynamic environments. Their physical characteristics undergo large, periodic changes due to movements of the earth and moon. Although such

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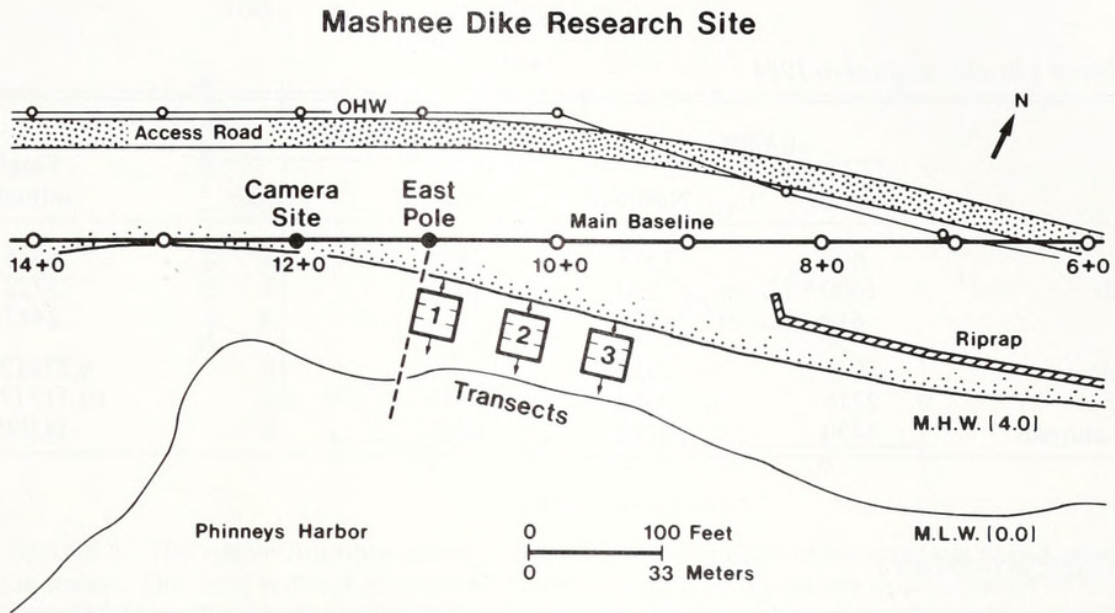


FIGURE 1. Map of the research site on Mashnee Dike. We defined the East Pole as the steel pipe located in 1933 by the U. S. Army Corps of Engineers at $70^{\circ}37'46''\text{W}$ and $41^{\circ}46'34''\text{N}$. Other pipes placed at 100 ft intervals mark the main baseline. All counts were taken in transects 1, 2, and 3 as defined by the shoreline and the East Pole of the main baseline. Broken lines indicate sectors of the transects. The water elevation at mean high water (M.H.W.) is 4.0 feet above mean low water (M.L.W.). The access road on the dike is lined with utility poles that support overhead wires (OHV). The camera site 100 ft from the East Pole was used in a concurrent study of visually guided behavior of *Limulus* (Barlow *et al.*, 1984, 1986).

dramatic fluctuations would appear to produce a forbidding environment, many marine and terrestrial species either inhabit the intertidal zones permanently or migrate into them for important biological activities (review: Neumann, 1981). Many such animals synchronize their activities with the major environmental rhythms of the intertidal zone. Preliminary observations indicated that lunar phase, tide height, and sunlight influence the mating activity of *Limulus* (Howard *et al.*, 1984).

We report here a quantitative study of the immigration of *Limulus* to the tidal zone for mating. We examine the temporal relationships between migratory behavior and the seasonal, lunar, tidal, and daily light cycles.

MATERIALS AND METHODS

We quantified mating activity as the number of animals appearing within the intertidal zone. This definition is based on prior observations that large numbers of adult horseshoe crabs regularly appear along the coastline in conjunction with breeding activities (Schuster, 1957, 1958, 1979; Cavanaugh, 1975; Rudloe and Herrnkind, 1976; Rudloe, 1978, 1979, 1980; Barlow *et al.*, 1982, 1984; Cohen and Brockmann, 1983; Howard *et al.*, 1984). The data were gathered between 13 May and 12 July 1984. Observations were also made throughout the 1985 and 1986 mating seasons.

Research site

Figure 1 shows the location of the research site at Mashnee Dike, Cape Cod, Massachusetts. Mashnee Dike is a narrow strip of sandy beach constructed by the U. S. Army Corps of Engineers in 1933 to connect Mashnee Island with Cape Cod. Permanent landmarks from the original survey include iron pipes that define the main baseline of the survey. One of the pipes, designated the "East Pole," stands about 2 ft. high at longitude $70^{\circ}37'46''$ West and latitude $41^{\circ}46'34''$ North. No houses or artificial lights are located within 0.8 km of the research site.

TABLE I

Number of Limulus counted in 1984

	Pairs		Single		Total animals
	Moving	Nesting	Male	Female	
Near	601	1378	2866	2	6826
Middle	1000	550	2621	1	5722
Far	614	129	958	3	2447
Females	2215	2057	—	6	4,278 (29%)
Males	2215	2057	6445	—	10,717 (71%)
Total animals	4430	4114	6445	6	14,995

Counting procedures

To sample animal numbers in the area, we established three 10 m × 10 m transects along the shoreline, using the East Pole and the water's edge as reference points. This technique is similar to one used by C. M. Cavanaugh (pers. comm.). The west side of Transect 1 (see Fig. 1) was defined by a line perpendicular to the shoreline and passing through the East Pole. The north side of all three transects was defined by the water's edge. The transects were separated from each other by 10 m. Two reflective posts were driven into the sand several meters above mean high water (M.H.W.) along the continuation of the east and west borders of each transect. This allowed us to determine their boundaries for each counting session. The position of the transects thus remained fixed in the longshore direction, shifting along the onshore-offshore direction as the tide flooded and ebbed (Fig. 1).

Most counts were taken around the times of the two daily high tides because previous observations indicated that few animals appeared at this site during low tides (Barlow *et al.*, 1982; Howard *et al.*, 1984). On three occasions we counted animals every half hour around the clock, to confirm that *Limulus* appeared in the intertidal zone only during high tides. At all other times counts were made every half hour for a period of up to 3.5 h beginning about 1 h before predicted high tide.

In each counting session the observer began at the west side of Transect 1 and waded toward the east side counting the animals within the "near" sector, the third of the transect adjacent to the water's edge. Upon reaching the east side, determined by lining up the reflective posts on shore, the observer moved offshore about 4 m, waded toward the west side, and counted the animals in the "middle" sector (the middle third of the transect). The observer then moved to about 8 m offshore, walked across the transect, and counted the animals in the "far" sector (the outer third of the transect; depth < 1 m). This procedure was repeated for Transects 2 and 3 with the entire session requiring about 10 min.

Animals were categorized as they were counted, and the number in each category was noted for the three sectors of each transect. The categories were "moving pairs" (female and male in amplexus and freely swimming), "nesting pairs" (female-male pair had burrowed into the sand), and unpaired "single males." Frequently we noted how many single males were "freely swimming" and how many were associated with a nest ("satellite males"). With this counting technique, no animals were overlooked in the transects during the day. We estimate that less than 5% were overlooked at night. The animals move slowly enough that counting the same animal twice would seldom occur.

Because unpaired females are rarely found on mating beaches (Cavanaugh, 1975;

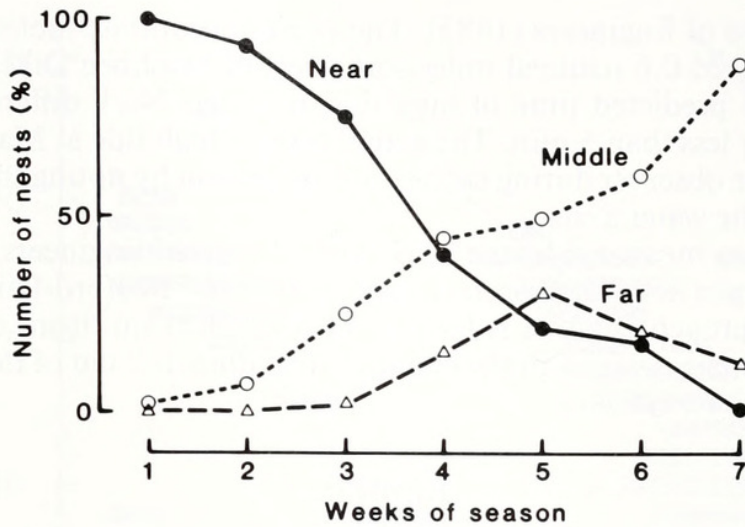


FIGURE 2. The relative number of nests counted in three regions of the transects varied across the mating season. The three symbols give the distribution of nests in the sectors as percentages of the total number of nests counted during a given week. In the early part of the season most animals nested in the near sector of the transects, closest to the water's edge. By the end of the season most animals were nesting in the middle sector. Few nests were ever observed in the far sector of the transects. Sectors are indicated in Figure 1.

R. B. Barlow, Jr., unpub. obs.), we did not examine every free-swimming animal. However when we suspected that a free-swimming animal might be female because of its large size, we picked it up to identify its gender. Only six were females. In several counting sessions in 1985 and 1986 we identified the gender of all unpaired animals and each time found about 0.5% were females.

Tide measurements

To estimate the times of high tides in planning our counting sessions we used the *Cape Cod Canal 1984 Tide Tables*, published by the New England division of the

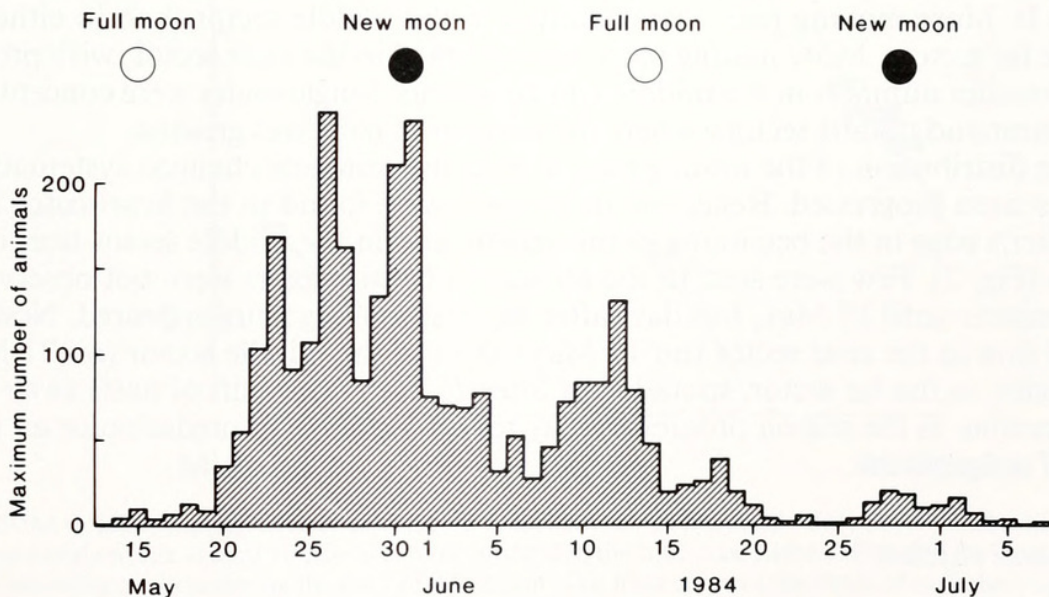


FIGURE 3. Maximum number of *Limulus* counted in the transects each day of the 1984 mating season at Mashnee Dike. The height of each bar shows the largest total number of males and females recorded in a single half-hourly counting session during the solar day. The open and filled circles indicate the full and new moons of 14 and 30 May and 13 and 28 June (Eldridge, 1984).

U. S. Army Corps of Engineers (1983). The most appropriate tables were those for Wings Neck, located 0.6 nautical miles southwest of Mashnee Dike. Except during foul weather, the predicted time of high tide at Wings Neck differed from that at Mashnee Dike by less than 5 min. The actual time of high tide at Mashnee Dike was determined by the observer during each counting session by noting the distance from the East Pole to the water's edge.

Tide height was measured by the U. S. Army Corps of Engineers with a nitrogen gas gauge submerged in the approach channel to the New Bedford-Fairhaven Harbor. Although the approach channel is located 11.6 nautical mi. from our observation site, the tide heights measured in the channel are within 1–2 cm of those at Mashnee Dike (F. Morris, pers. comm.).

RESULTS

Mating season

The mating season of *Limulus polyphemus* at Mashnee Dike in 1984 lasted 55 days, from 14 May to 7 July (Fig. 3). Table I gives the numbers of males, females, and pairs counted in the transects during this period. Because we did not tag individual animals, the values provide information on the relative proportions of animals in the different categories but not their absolute numbers. Absolute values would be influenced by animals that remained in a transect for more than one counting period, moved from one transect to another, or returned to the mating area on subsequent days.

When all transects are considered together the numbers of moving and nesting pairs were approximately equal (2215 and 2057, respectively), and were less than the total number of single males (6445). If each pair is counted as 1 male and 1 female, more than twice as many males (10,717) as females (4,278) were sighted in the transects. For the sessions during which we divided single males into free-swimming and satellite, their ratio was approximately 80:20. As anticipated, very few single females were seen.

Limulus were not uniformly distributed across all three sectors of the transects (Table I). More moving pairs were counted in the middle sector than in either the near or far sectors. More nesting pairs were counted in the near sector, with progressively smaller numbers in the middle and far sectors. Single males were concentrated in the near and middle sectors, where the number of pairs was greatest.

The distribution of the nesting pairs within the transects changed systematically as the season progressed. Relatively more nests were found in the near sector along the water's edge in the beginning of the season, and in the middle sector later in the season (Fig. 2). Few were seen in the far sector. Nesting pairs were not observed in the transects until 19 May, five days after the first moving pairs appeared. Nests appeared first in the near sector (on 19 May), then in the middle sector (on 21 May), and finally in the far sector, sporadically after 26 May. The shift of nests away from the shoreline as the season progresses may reflect a change in predation or an influence of temperature.

Semilunar rhythm

Mating activity was correlated with phases of the moon (Fig. 3). Large numbers of animals were counted each day before and during the new moon on 30 May and around the full moon on 13 June. A smaller peak occurred near the new moon on 28 June. The total number of nests counted in the transects each solar day followed

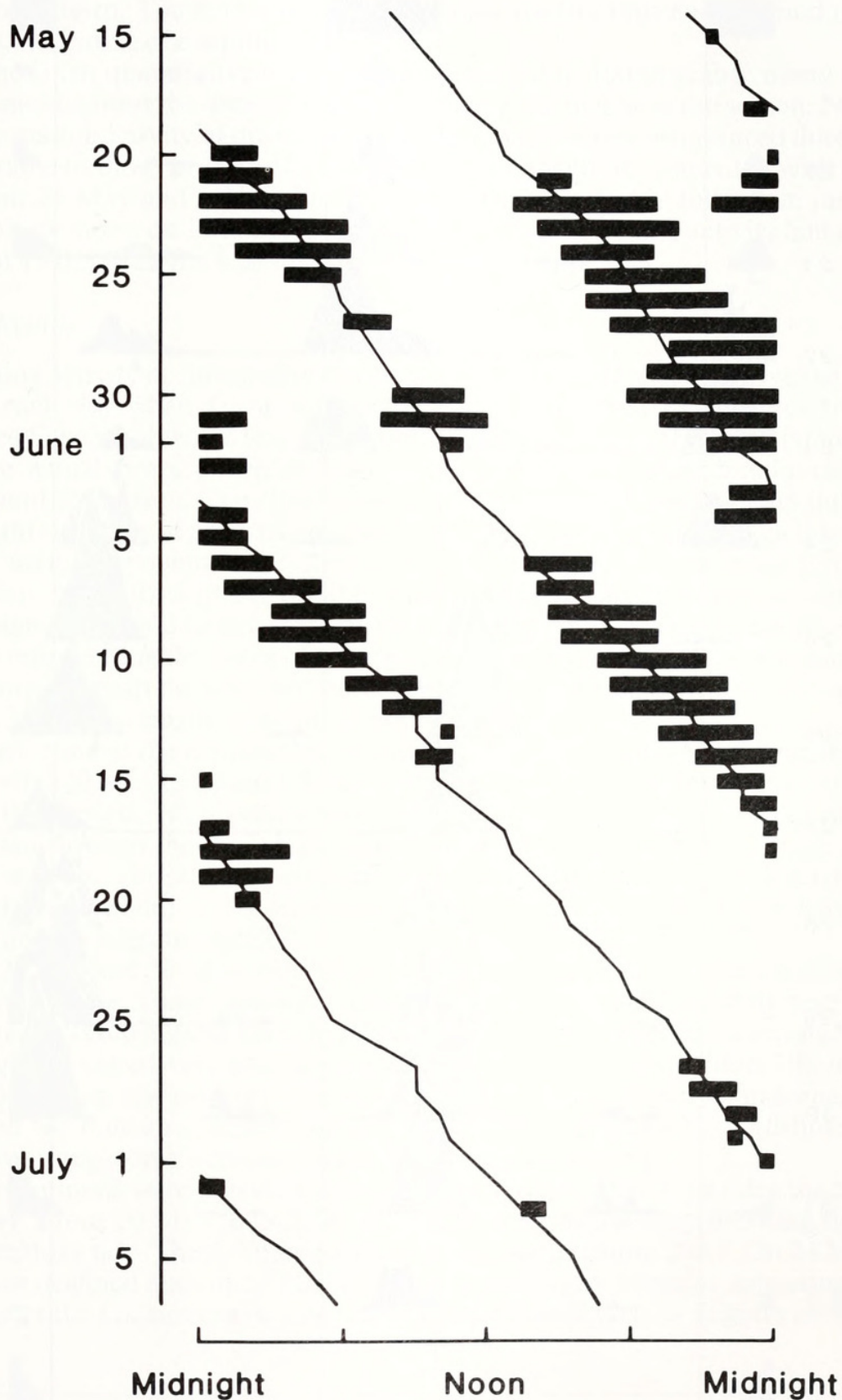
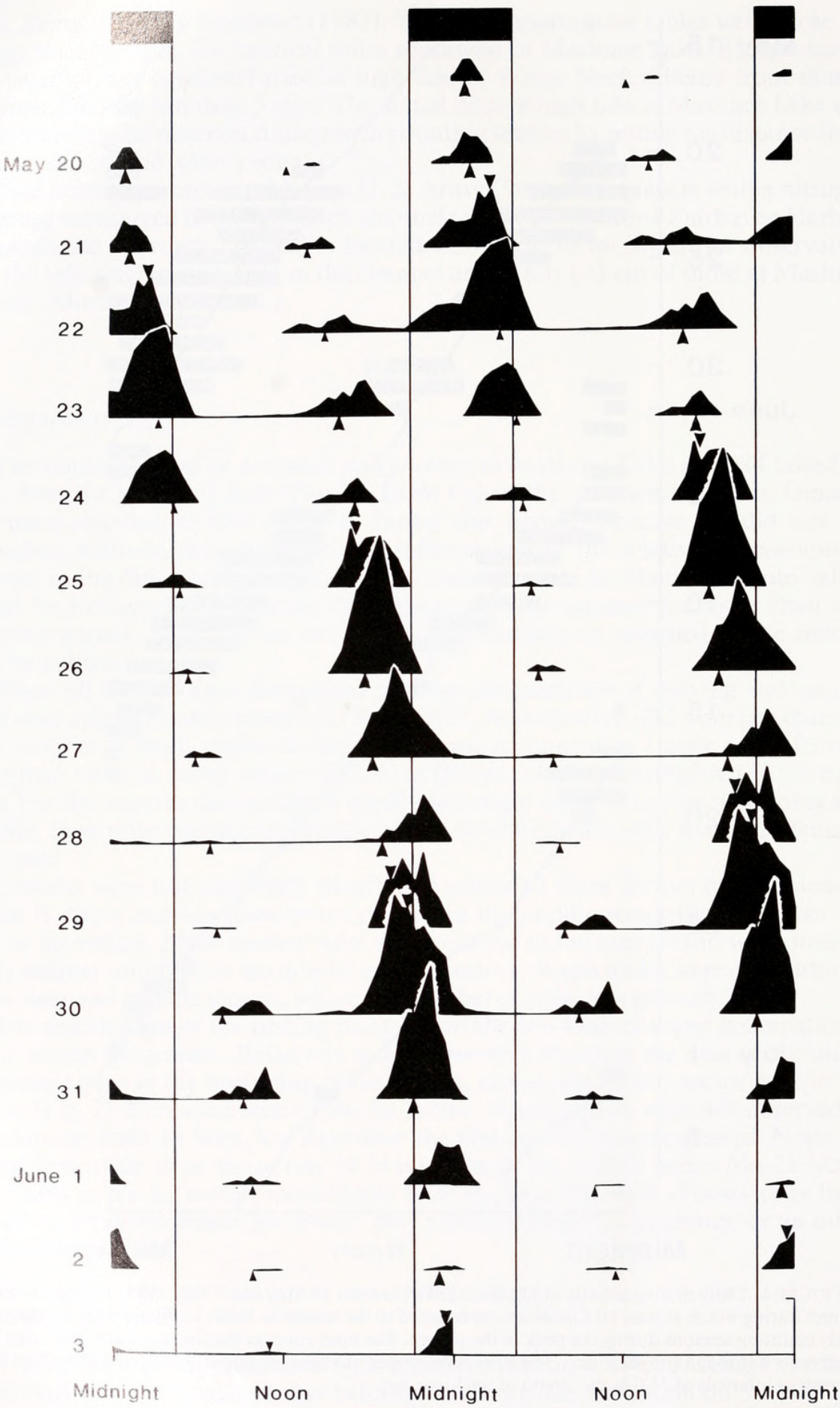


FIGURE 4. Daily mating activity at Mashnee Dike between 14 May and 7 July 1984. The bars indicate the times during which at least 10 *Limulus* were counted in the transects. Refer to Figure 5 for the duration of daily counting sessions during the peak of the season. The lines connect the times of each daily high tide as it advanced through the solar day. The lines have slopes of about 50 min day⁻¹, indicating that each tide crests at intervals of 24.9 h, the length of the lunar day.



the same pattern. The fluctuations in migratory activity thus had a period of about 15 days, indicative of a semilunar rhythm.

A thorough quantitative study was not achieved in 1985 because many animals were removed from the area by fisherman during the height of the season. Nonetheless, the mating activity of the residual population exhibited pronounced fluctuations comparable to those in 1984. The first large peak of activity coincided with the new moon on 23 May and subsequent peaks occurred during the full moon on 3 June and the new moon on 17 June. Again the fluctuation in mating activity had a period of about 15 days and the season lasted for about 50 days.

Tidal rhythm

Mating activity occurred only during high tides (Fig. 4). The bars give the periods during each day when 10 or more animals were observed in the three transects. Mashnee Dike is subjected to a semidiurnal tidal regime, and the diagonal lines connect the actual times at which each of the two tides reached maximum height throughout the season. The close correlation between the lines and bars shows that significant numbers of animals appeared in the transects only at the high tides.

The tidal component in the daily pattern of mating activity persisted throughout the season. A line through the middle of the periods of activity (bars) associated with either high tide would have a slope of about 50 min per day. This equals the slope of the line representing the times of the high tides. Thus, daily episodes of mating activity advance through the solar day along with the high tides each with periods approximating 24.9 h, the length of the lunar day.

Figure 5 shows the distribution of animals during the half month of greatest mating activity (20 May to 3 June). Small vertical arrows indicate the times of high tides during this period. High tides occurred near midnight and noon on 20 May and progressed through the day to noon and midnight respectively by 3 June. Mating activity over this period occurred during both daily high tides with peak activity generally observed about 1–2 h after tidal crests. The animals then began leaving the nesting area to migrate offshore.

On 23, 28, and 30 May we counted animals in the transects every half hour for 30 hours or more. These long-term counts clearly show that no animals were present in the transects during low tides. In addition, underwater observations using SCUBA diving gear revealed very few moving and no buried *Limulus* within 100 m of the shoreline during low tide. Instead, numerous animals, both paired and single, were found on the bottom at depths up to 8 m between 100 and 400 m offshore. Some were swimming, some were stationary, but none were nesting.

More animals were observed during one of the two daily high tides than during the other. From 20 May to 24 May more appeared on the early morning tide than on the midday tide. These differences were large, approaching 200:1. On 24 May, the difference declined and on 25 May it reversed, with more *Limulus* appearing on the afternoon tide. On successive days, most animals continued to migrate to shore on

FIGURE 5. Daily distributions of horseshoe crabs in the transects between 20 May and 3 June 1984. Counts were taken only during the hours marked by baselines. For example, animals were counted around the clock on 22–23 May, 27–28 May, and 30–31 May and near the times of high tides (vertical arrows) on other days. Large arrows indicate the higher daily tides. The flanks of some distributions (about 10%) have been extrapolated when full counts were not available. Note that more animals appeared in the transects during the early morning tides from 19 May to 24 May and during the evening tides after 25 May. Dark bars and vertical lines indicate darkness (see Fig. 9). The data are double-plotted to emphasize the periodicity of the mating activity. Vertical scale equals 100 animals.

this tide as it progressed through the late afternoon and into the night. The animals abandoned the tide only after it moved past dawn on 7 June (not included in Fig. 5). They then shifted to the opposite (afternoon) tide and repeated the cycle. In the following sections we present evidence that these tidal preferences are related to the relative heights of the tides.

Semidiurnal inequality of tide height

On most days during the mating season one tide was clearly higher than the other. Such inequalities in tide height are caused at certain latitudes by the moon's monthly declination north and south of the plane of the ecliptic. This declination produces asymmetries in the system of tide-generating forces such that two tidal waves of different heights occur each day at the northern and southern latitudes whenever the moon is not over the equator (Wylie, 1979). During the spring the pattern of tidal inequality in the Cape Cod area produces maximal differences in the heights of successive tides twice monthly at full and new moons. These diminish over the following two weeks and reverse twice monthly during the first and last quadratures.

Figure 6 shows the actual (top) and predicted (bottom) tide heights at Mashnee Dike from 12 May to 3 June 1984. This period includes the full moon of 15 May, the new moon of 30 May, and the 15-day period covered in Figure 5. One of the two daily tides is indicated by a stippled bar, the other by a black bar. The predicted changes in tides follow a relatively smooth function, whereas the actual changes do not because they are influenced by unpredictable weather conditions. However, both exhibit maximal flood and ebb tides at the full and new moons, with neap tides at the lunar quadrature on 23 May. The daily low tides were nearly equal in height but the high tides were not. The inequality in high tides was greatest during the full moon and minimal near quadrature on 23 May when it reversed and became large again at new moon.

Mating activity on Mashnee Dike was closely related to the magnitude of the inequality between high tides. Figure 7 compares actual tide measurements with mating activity over the 8-day period from 20 to 27 May. On 20, 21, and 22 May, the daily high tides differed in height and more animals were counted within the transects during the higher tides, which occurred in the early morning hours before dawn. This was also the case on 25, 26, and 27 May when the majority of animals again appeared during the higher high tides, which occurred in the afternoon on those days. The animals' preference for the afternoon tide grew as the tidal inequality increased, and continued as this tide progressed through the evening and into the early morning hours.

Limulus did not, however, always prefer the higher high tide. Even though the tidal inequality diminished and reversed, the majority of animals continued to populate the early morning tides, which were slightly lower than the afternoon tides. They switched to the higher afternoon tide on 25 May, three days after tidal reversal. This behavior was repeated during the next tidal cycle, when they again switched to the afternoon high tide three days after reversal of the tidal inequality at the lunar quadrature on 6 June. We believe these exceptional days are significant for understanding factors that control *Limulus* mating activity, and we will consider them in more detail below.

Not only were more animals generally counted during the higher high tides, but the relative number appearing in association with any two consecutive high tides was roughly proportional to the relative difference in the heights of the two tides. This point is illustrated in Figure 8. In general, when the difference in the heights of the two high tides was large, as on 29 May, the degree of preference was also large. When

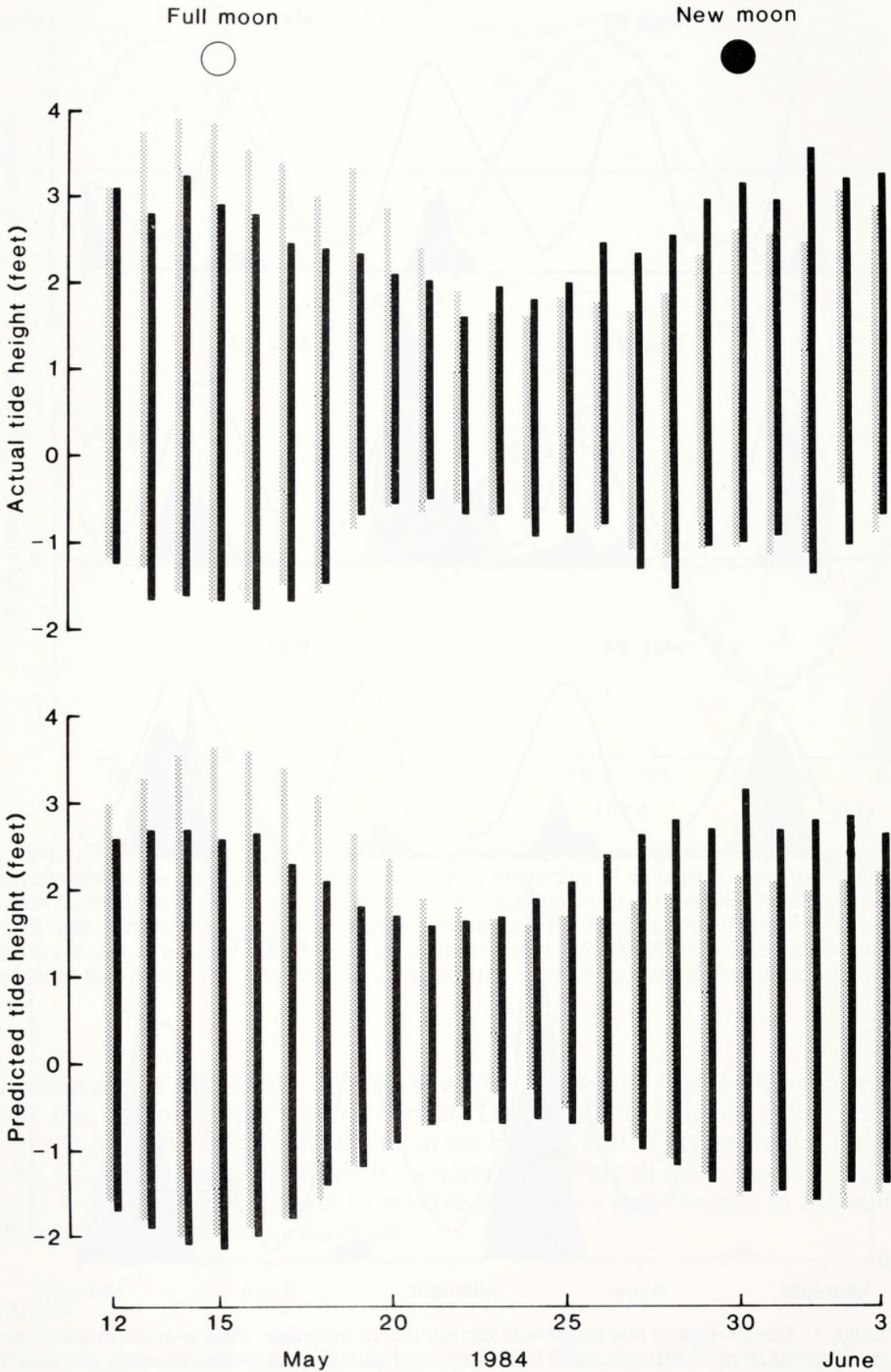


FIGURE 6. Predicted (bottom) and actual (top) heights of tides at Mashnee Dike during the first half of the 1984 mating season (Army Corps of Engineers). One of the two semidiurnal tides is indicated by black bars and the other by stippled bars. The tidal excursions were maximal during full and new moons with one tide (stippled bars) higher during the full moon and the other (black bars) during the new moon. Excursions were minimal during the lunar quadrature on 23 May when the inequality in heights reversed. Note that the heights of the low tides did not differ much over this period.

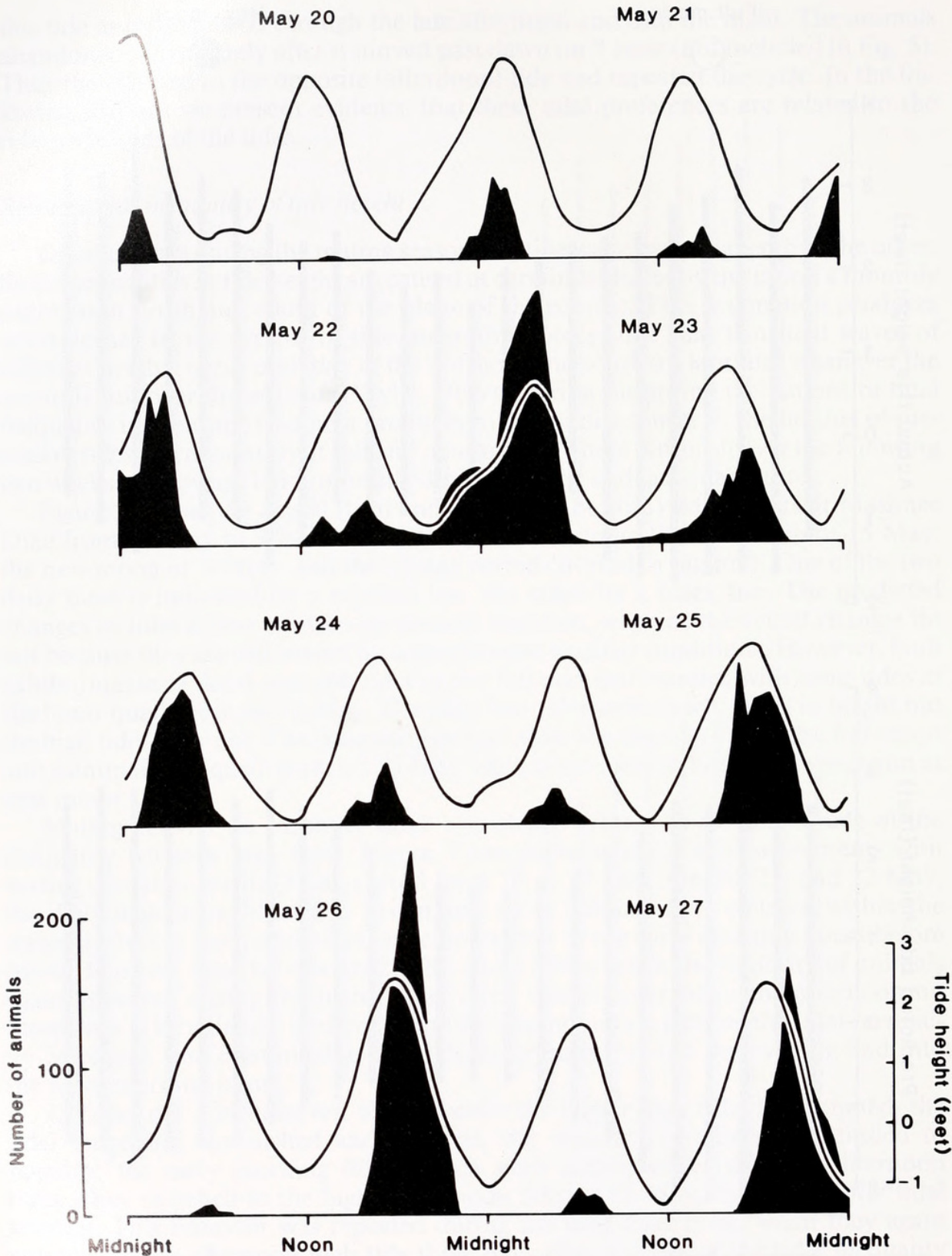


FIGURE 7. Comparison of tide height with the number of horseshoe crabs counted in the transects during the week of 20 to 27 May. From 20 to 22 May more animals appeared on the early morning tide, which was the higher of the two daily tides. On 23 May most animals remained with the early morning tide even though the inequality had reversed and the afternoon tide was higher. This behavior persisted until 25 May, when the animals' tide preference switched to the evening tide.

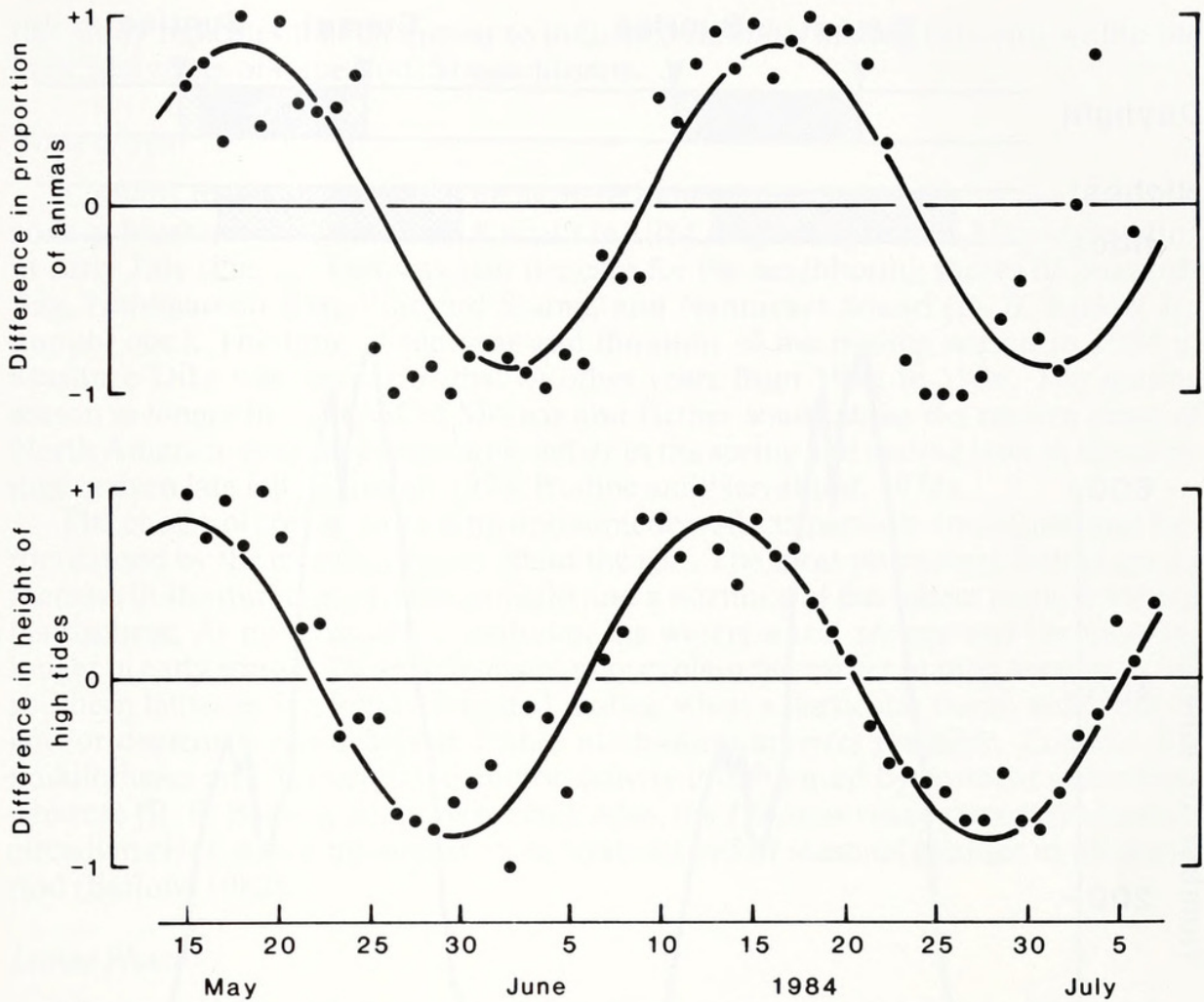


FIGURE 8. The relative proportion of animals observed on a given high tide (top) corresponds to the relative height of the tide (bottom). The difference in proportion of animals is equal to the difference between the total number of animals observed on two semidiurnal high tides divided by the sum observed on both tides. Each point on the bottom graph represents the difference in heights of the pair of semidiurnal high tides in feet. A sinusoid with a period of one lunar month (29.4 days) and an amplitude of 0.85 was fitted to the tidal data by eye. The same function shifted by 3.2 days is plotted in the top half of the figure.

the difference in tide height was small, as on 6 June, the degree of preference was small. But the correlation was not perfect. The sinusoidal function with a period of 29.4 days that describes the tidal data in the bottom half of Figure 8 must be shifted to the right by 3.2 days to fit the behavioral data. This shift implies a phase lag of nearly 3 days between changes in relative tide height and changes in the animals' preference for one tide over the other.

Sunlight

Mating activity was more strongly correlated with the tidal inequality than with light or darkness. For example, from 20 May to 3 June most animals (77%) migrated into the transects during the highest high tides indicated in Figure 5 by large arrows. These tides occurred from 1530 to 0330 h as shown by the stippled bars in Figure 9. Over this 15-day period half of the animals appeared in the transects during light (1530 to 2100 h) and half during darkness (2100 to 0330 h). We conclude that *Limulus* generally prefer to mate on the highest tides regardless of when they occur.

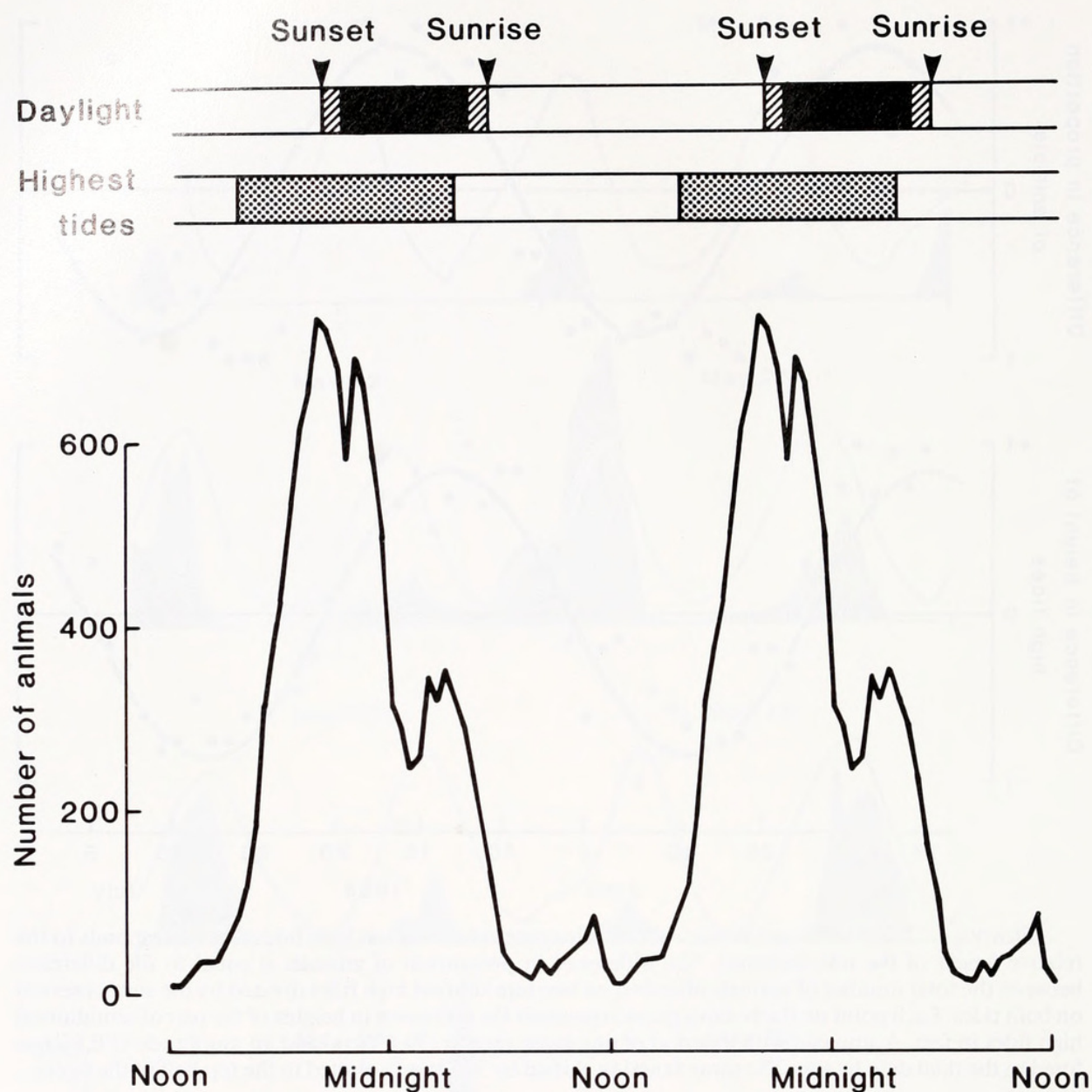


FIGURE 9. Comparison of mating activity with daylight, darkness, and the tidal inequality. The number of animals counted from 20 May to 3 June was plotted as a function of time of day by summing every half hour the counts shown in Figure 5. The data are double-plotted. The times of sunset (2010 h) and sunrise (0510 h) are given at the top with dusk (2110 h) and dawn (0410 h) indicated by cross hatching and darkness by black bars. Because these times changed slightly with each day the data are shown for 27 May, which is in the middle of the period. The stippled bars give the times of occurrence of the higher of the two daily tides over the 15-day period. Note that the peak number of animals was observed before sunset.

DISCUSSION

The mating activity of *Limulus polyphemus* is related to the environmental changes produced by periodic motions of the earth and moon. The yearly rotation of the earth about the sun produces seasonal changes in daylength and in ocean temperature. The daily rotation of the earth about its axis produces diurnal changes in daylight and semidiurnal changes in the levels of most oceans. The monthly rotation of the moon about the earth modulates the tidal flows of the oceans and produces periodic inequalities in the daily tides in the northern and southern hemispheres. All of these astronomical events affect the physical environment of intertidal zones, and

this study indicates that all appear to influence *Limulus* mating behavior within the intertidal zones of Cape Cod, Massachusetts.

Time of year

Limulus mates seasonally. The animals entered and built nests in the intertidal zone at Mashnee Dike for about 8 weeks in 1984, beginning in mid-May and ending in early July (Fig. 3). This was also the case for the neighboring shores of Buzzards Bay, Narragansett Bay, Vineyard Sound, and Nantucket Sound (R. B. Barlow Jr., unpub. obs.). The time of the year and duration of the mating season in 1984 at Mashnee Dike was similar to that of other years from 1982 to 1986. The mating season is longer in the Gulf of Mexico and farther south along the eastern coast of North America, generally beginning earlier in the spring and ending later in the summer or even late fall (Schuster, 1979; Rudloe and Herrnkind, 1976).

The choice of spring for mating undoubtedly reflects periodic environmental factors caused by the earth's rotation about the sun. The most prominent factors are an increase in the duration of daily sunlight and a warming of the waters in the northern hemisphere. At more southern latitudes, the waters warm sooner and the days are longer in early spring. These differences may explain the earlier mating seasons in the southern latitudes if *Limulus* initiated mating when a particular ocean temperature and/or daylength was achieved. Either mechanism appears plausible. *Limulus* is a poikilotherm and its overall locomotor activity is influenced by ambient water temperature (R. B. Barlow, Jr., unpub. obs.). Also, the *Limulus* visual system possesses a circadian clock which maintains an accurate record of seasonal changes in photoperiod (Barlow, 1982).

Lunar phase

Mating activity of a variety of intertidal invertebrates is associated with the lunar syzygies (Klapow, 1972; Enright, 1975; Neumann, 1976; Christy, 1978; Zucker, 1978; Saigusa, 1981). *Limulus* appears to be no exception: mating activity was maximal during new and full moons throughout the season. In both 1984 and 1985, *Limulus* began migrating to the beach after the full moon, in early May. In 1984, mating activity increased significantly in the last week of May as new moon approached, decreased rapidly in early June after the new moon, and then peaked twice again at the full and new moons in June. No activity was detected during the full moon in July. The pattern of mating activity in 1985 was similar in every respect to that in 1984 except that the season began about 10 days earlier in the year. This shift coincides exactly with the earlier appearance of the new moon in 1985.

Two previous studies report that *Limulus* mating activity on the Gulf Coast of Florida was also coordinated with new and full moons. In contrast to our results, both studies found maximal mating activity associated with full moons. Rudloe (1980) counted *Limulus* appearing at Mashas Sand, Florida, between March and November, 1977. Mating apparently began with the new moon of 19 March. Peak numbers of animals were observed in association with high tides between early April and early June. Although daytime tides were monitored throughout, night tides were only monitored from 18 May. Rudloe reported maximal mating during high tides at full moons, with no breeding during neap tides. However, substantial mating also occurred during new moons, especially at the beginning of the season when overall numbers were largest. Cohen and Brockmann (1983) also observed more animals in association with the full moon at Seahorse Bay, Florida, between 12 July and 10 September 1980. The reasons for the differences between our observations and those of Rudloe (1980) and Cohen and Brockmann (1983) are not known.

In the nesting area of Mashnee Dike, unpaired males far outnumber paired males (Table I). This result is consistent with observations in Florida (Rudloe, 1980; Cohen and Brockmann, 1983). As these authors also noted, many males mill about a nesting pair apparently attempting to dislodge the clasping male or to get close enough to deposit sperm where they might be effective. Although in our studies satellite males never dislodged a clasping male (see also Cohen and Brockmann, 1983), they did release sperm around the nesting pair and in the nest itself after the pair had left. Since fertilization is external, sperm release by satellite males may increase the possibility that their sperm will fertilize some eggs.

Tide height

Limulus migrated in great numbers into the intertidal zones during new and full moons, but only on high tides (Figs. 3, 4, 5). None migrated toward shore during low tides. Although significant numbers of animals mated on neap tides at the lunar quadrature, maximal activity was always observed during the highest high tides associated with the new and full moons. Numerous species synchronize their mating activity in the intertidal zone with the tides (for reviews see DeCoursey, 1976; Naylor, 1976; Neumann, 1981). For example, grunion bury fertilized eggs at the high-water mark during spring high tides and the larvae hatch about two weeks later when the next series of spring tides washes the upper beach (see Neumann, 1981). *Limulus* appears to have adapted the same strategy in Florida (Rudloe, 1980). In other areas along the East Coast of North America, however, *Limulus* nests subtidally in up to 1 m of water (see Cohen and Brockmann, 1983). Indeed, we observed nests being built in areas of the intertidal zone that are inundated by high tides twice a day every day during the lunar month (see Table I). The preferred region for nesting moved away from the high-water mark as the season progressed (Fig. 2). The location of nesting sites within the intertidal zone may be related to the relative risks of predation in different geographical regions and seasonal changes in such risks in a given region. In this regard, it is interesting that geographically separate populations of *Clunio*, a marine insect, appear to have adapted to the local conditions of the semimonthly tidal regime (see Neumann, 1976).

Semidiurnal inequality of tide height

Mating activity on Mashnee Dike was directly related to the tidal inequality (Fig. 8). Most *Limulus* individuals (77%) appeared in the transects on the higher high tides which occurred between 1530 and 0330 h (Figs. 5, 9). The greatest mating activity was observed on the highest spring tides in the early evening (1800 to 2400 h) during the new and full moons. In sum, tide height is a powerful predictor of mating activity at Mashnee Dike.

A study along the Gulf Coast of Florida also reported a positive correlation between the number of *Limulus* mating and the highest high tides (Cohen and Brockmann, 1983). However, another Florida study in a nearby area reported the opposite result: more mated on the lower high tides (Rudloe, 1980).

Cavanaugh (1975) reported that *Limulus* mated only after dark at Mashnee Dike. Our study does not support this conclusion. From 20 May to 3 June, half of the animals were counted in the transects during darkness (2100 to 0330 h), and half were counted during light. We conclude that *Limulus* generally favor the highest high tides whether they occur during the day or at night.

Coordination of behavior with the inequality of tide height is not unique to *Limulus*. In an elegant laboratory study, Enright (1972) showed that the endogenous loco-

motor activity of the intertidal isopod *Exciorolana chiltoni* was directly correlated with the height of the daily high tides in its natural habitat along the shores of southern California. The region has a semidiurnal tidal regime of the mixed type, and the endogenous activity of the isopod in isolation was maximal during the higher high tide when the tidal inequality was also maximal. When Figures 1 and 2 of Enright's paper are replotted in the format of Figure 8 of the present paper, their similarity is striking: the ratio of *Exciorolana* locomotor activity on consecutive high tides appears to be directly related to the ratio of tide heights. Activity decreased as the tidal inequality decreased, and increased again as the inequality increased. This circasemilunar rhythm is endogenous in *Exciorolana*, but it may not be in *Limulus*. Under diurnal lighting *Limulus* occasionally exhibits nocturnal locomotor activity in the laboratory, and under constant darkness the locomotor activity sometimes exhibits an endogenous rhythm ($\tau \approx 24$ h) for several days (Barlow and Palfai, 1971; J. Turnbull and R. B. Barlow, Jr., unpub. obs.). We have never observed an endogenous lunar or semilunar rhythm for *Limulus* under laboratory conditions.

How does *Limulus* detect the tidal inequality? A variety of cues are potentially available, particularly when the inequality is large. Indeed, the degree of preference for the highest tides was maximal around the times of maximal tidal inequality. However, as the tidal inequality diminished near lunar quadrature, more animals began to migrate to the intertidal zone during the lower high tides. This behavior would be understandable if the discrimination between the two high tides became progressively more difficult as their heights approached equality. The phase lag of 2–3 days between tidal inequality and animal inequality suggests that *Limulus* persisted in migrating to the beach on alternate high tides until some environmental factor signaled them to shift to the opposite high tide. As discussed below, we believe the signal to shift tides may be the first light of dawn.

Possible role of sunlight

Three aspects of our results suggest that *Limulus* may use only environmental cues to synchronize its shoreward migration with tidal inequalities. (1) As already noted, there is a 2–3 day phase lag between the reversal of the tidal inequality and the shift of animal preference to the opposite high tide (Fig. 7). (2) With few exceptions, the maximum number of animals observed in association with a high tide occurred *after* tide crest (Fig. 5). The exceptional tides were all close to dawn when the animals' preference for one high tide over the other was changing. To see this, compare the early morning distribution of 24 May with the afternoon distribution of 23 May in Figure 5. Note that near dawn the maximum number of animals occurred *before* tide crest. (3) On several occasions we observed a mass exodus of animals from the nesting area as the first light of dawn appeared on the horizon. The rapid movement of animals away from shore was quite unlike the normal decline of mating activity observed on ebbing tides at other times of the day. We suggest that the first light of dawn may serve as a cue for the animals to shift to the opposite high tide after it has become the higher tide.

The *Limulus* visual system is well equipped to detect the first light of dawn. In the early hours of the morning, the animals' visual sensitivity begins to undergo an endogenous transition from a highly sensitive nighttime state to a much less sensitive daytime state (Barlow, 1983). Although visual sensitivity is declining, it can still detect single photons (Kaplan and Barlow, 1976). Another important factor may be the massive turnover of photoreceptor membrane which is triggered at dawn by the first rays of light (Chamberlain and Barlow, 1979, 1984). Within minutes about 70% of the photosensitive rhabdom structure of every photoreceptor cell is torn down, leaving a

small volume of rhodopsin-containing membrane to transduce light, and after one half hour the rhabdom structure is fully reassembled. Although the process of rhabdom renewal is analogous to the outer segment renewal of vertebrate rods and cones (Young, 1976), its function is not understood. However, such a massive metabolic event may disrupt vision, which males use to locate potential mates (Barlow *et al.*, 1982).

The change in migratory behavior near dawn may be triggered by a daily environmental event other than dawn. One possibility is the solar component of the tide, but an analysis of tidal components shows that the solar component does not peak near dawn at Mashnee Dike (R. Gregory-Allen, pers. comm.). The relationship between migratory behavior, dawn, and tide could be further tested by observing a population that nests in an area with a different tidal regime. Several such areas exist near Woods Hole but thus far we have not found one with a mating population of sufficient size.

Our data are consistent with the idea that the animals prefer the highest tides but are incapable of detecting the reversal of tide heights—perhaps because the difference in tide height is smallest when the reversal occurs. Because the tide reversal at Mashnee Dike always occurs before dawn (0300 h), we suggest that the animals stay with this early morning tide until it occurs after dawn, 2–3 days later. Then they shift their shoreward migration to the higher high tide. They continue to follow the afternoon high tide as it progresses through the night until it again occurs after dawn. Such a strategy does not require an endogenous rhythm of the sort detected in the isopod by Enright (1972). However, it does require information about the timing of the two flooding tides. An endogenous circatidal oscillator would provide this information, but studies with other animals indicate that exogenous factors such as water flow and bottom vibration alone are sufficient (Hastings, 1981; Neumann, 1978). It is also possible that *Limulus* possesses an hour-glass timing system which signals the animal at a fixed interval after a preceding event. Such hour-glass timing systems have been detected in other animals (Neumann, 1981). They do not require an endogenous oscillator.

Summary of Limulus mating activity in 1984

Figure 10 summarizes our findings during the 1984 mating season at Mashnee Dike. It shows the heights of the two high tides as “paths” through time. *Limulus* symbols indicate which high tide attracted the majority of animals on any given day.

At the beginning of the season we counted more animals during the higher tide that occurred in darkness. The animals continued to prefer that tide until after the tidal reversal on 23 May, changing to the higher tide on 25 May (Fig. 7). On this day both tides occurred in daylight. Once the animals switched to the afternoon tide they again continued to prefer this tide until after the tidal reversal on 6 June, switching their preference after both high tides occurred during the day. This pattern was repeated following the final tidal reversal of the season on 21 June. The animals thus showed a clear preference for the higher tide: more were seen in association with the higher tide in 44 of 51 observation intervals (lunar days). The seven exceptions occurred after the three reversals in tide height. One interpretation is that the first light of dawn signaled the time to switch to the opposite tide.

A model for the regulation of mating activity in Limulus

From the evidence presented here, and our knowledge about the visual system of *Limulus polyphemus*, we propose the following model for the regulation of mating activity in this species. The initiation of mating activity at the beginning of the season

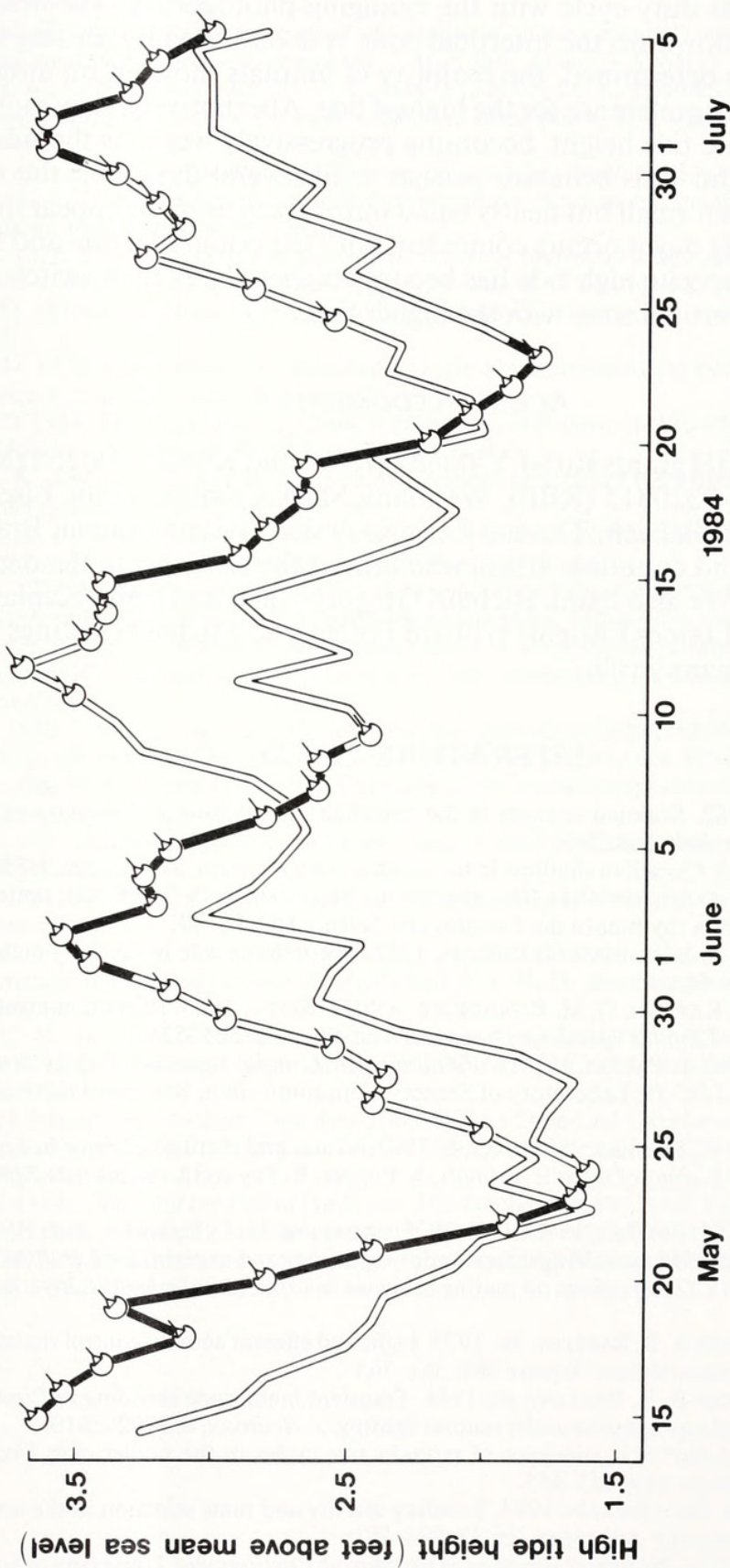


FIGURE 10. Summary of *Limulus* mating behavior during the 1984 season at Mashnee Dike. The height of each of the two daily high tides is tracked through the season by paths. The ordinate gives the tide height and the shade of each path indicates whether the high tide occurred in darkness or in daylight (including dawn and dusk). *Limulus*-shaped symbols mark the tide that was populated by the majority of horseshoe crabs.

is determined by changes in daylength and/or water temperature that occur in the spring. Detection of daylength may involve an endogenous circadian oscillator that continually resets its duty cycle with the changing photoperiod. The first high tide that the animals follow into the intertidal zone is determined by sensing its relative height. Once this is determined, the majority of animals move in on alternate high tides, showing a clear preference for the highest tide. Alternatively, they may continue to try to discriminate tide height, becoming progressively worse as the tides become more equal in height. This behavior persists until several days after the reversal in tidal inequality, when small but nearly equal numbers of animals appear during each high tide. Ultimately dawn occurs coincident with the populated tide and signals the animals that the opposite high tide has become higher. They then switch and begin migrating to the intertidal zone with the higher tide.

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