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Entrainment of the activity rhythm of the mole crab *Emerita talpoida*

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Abstract

The mole crab *Emerita talpoida* migrates with the tide in the swash zone of sand beaches. A circatidal rhythm in vertical swimming underlies movement, in which mature male crabs show peak swimming activity 1–2 h after the time of high tides at the collection site. In addition, there is a secondary rhythm in activity amplitude, in which crabs are maximally active following low amplitude high tides and minimally active following high amplitude high tides. The present study determined the phase response relationship for entrainment of the circatidal rhythm with mechanical agitation and whether the cycle in activity related to tidal amplitude could be entrained by a cycle in the duration of mechanical agitation at the times of consecutive high tides. After entrainment with mechanical agitation on an orbital shaker, activity of individual crabs was monitored in constant conditions with a video system and quantified as the number of ascents from the sand each 0.5 h. Mechanical agitation at the times of high tide, mid-ebb and low tide reset the timing of the circatidal rhythm according to the timing relationship to high tide. However, mechanical agitation during flood tide had no entrainment effect. In addition, a cycle in duration of mechanical agitation entrained the rhythm in activity amplitude associated with tidal amplitude. Both rhythms and entrainment effectiveness over the tidal cycle may function to reduce the likelihood of stranding above the swash zone.

Keywords: Circatidal rhythm; *Emerita talpoida*; Entrainment; Mole crab

1. Introduction

The mole crab *Emerita talpoida* (Say) inhabits the intertidal zone of sand beaches from Harwick, Massachusetts (USA) to the Progreso, Yucatan, Mexico (Williams, 1984). They occur in large aggregations (e.g. White, 1976) that move up the beach on rising tide and down the beach on falling tide in the swash zone as described for a related species *E. analoga* (Cubit, 1969). Our previous study (Forward et al., 2005) determined that all life history stages (megalopae, juveniles, small females, small males, mature males and ovigerous females) have circatidal rhythms in vertical swimming with median free running period lengths near 12.4 h and activity peaks about 1–2 h after the time of high tide at the collection site. The rhythm was not affected by a light:dark cycle.

Tidal cycles in temperature, salinity, hydrostatic pressure, inundation, and mechanical agitation can entrain tidal rhythms (Palmer, 1995). However, among animals living in the swash zone of sand beaches, mechanical agitation is perhaps the dominant environmental cycle for entrainment (Enright, 1965, 1976; Klapow, 1972; Hastings, 1981). Our previous study of *E. talpoida* found that the activity rhythm of mature males could be entrained by a tidal cycle in mechanical agitation (Forward et al., 2005). Among other invertebrates

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inhabiting the swash zones of sand beaches, the effect of mechanical agitation on resetting the timing of a tidal rhythm varied over the tidal cycle and produced a complex phase response curve (e.g. Klapow, 1972; Enright, 1976). The present study investigated the phase response relationship of *E. talpoida*.

Sand beach invertebrates are exposed to semi-diurnal tides on the east coast of the United States with consecutive high tides usually differing in amplitude. Among mature male *E. talpoida*, activity amplitude also varies with tidal amplitude, as crabs show high activity just after the time of the lowest high tide in the field and low activity after the highest amplitude tides (Forward et al., 2005). This pattern was not related to the light:dark cycle. Enright (1963) similarly found that the amplitude of activity within the circatidal rhythm of a sand beach amphipod varies with tidal amplitude. However, in this case activity amplitude oscillated with tidal amplitude. Since the duration of mechanical agitation from waves would vary between high and low amplitude high tides, the present study also considers whether this mechanical agitation cycle could entrain the activity rhythm of *E. talpoida* relative to tidal amplitude. The results indicate that mechanical agitation resets the circatidal rhythm in activity differently at different phase of the tide and entrains the cycle in activity relative to tidal amplitude.

2. Materials and methods

Mature male mole crabs, *E. talpoida* (Say) (13–14 mm carapace length) were collected during the day in the swash zone at Atlantic Beach, North Carolina (USA) from June to November, 2005. This area has semi-diurnal tides. Morphological characteristics were used to sex the animals. Mature males were used for experiments because our previous study indicated that they have a well developed circatidal activity rhythm that can be reset by mechanical agitation (Forward et al., 2005). After collection and during experiments, crabs were maintained in about 32–34 psu seawater at a temperature of 25 °C (June to August) or 22 °C (September to November). To determine the activity rhythm, crabs were placed individually in rectangular Plexiglas columns ($38 \times 55 \times 250$ mm) having about 30 mm of autoclaved sand from the collection site on the bottom and 32–34 psu seawater. Columns had a plastic seal at the top to reduce evaporation and the water was gently aerated throughout the experiments. For experimental crabs, the lights were extinguished at the time of sunset and subsequently held under constant conditions of apparent darkness. This procedure was followed for consistency with our previous study even though we found that the rhythm under constant conditions was no different from that in the presence of a light:dark cycle (Forward et al., 2005).

Crab behavior was observed in apparent darkness for at least 4 tidal cycles with a video system. The background light for viewing the crabs was filtered to near infrared (780 nm). Previous studies have determined that crabs are generally insensitive to light in this region (e.g. Cronin and Forward, 1988). Cameras (Cohu model 4815-3000) were aligned to view multiple columns and behavior was recorded with time-laps video recorders (Panasonic model AGRT 600 A). Although crabs were not fed during the experiments, there was <4% mortality.

There were two general sets of experiments. The first experiment determined the effectiveness of mechanical agitation to reset the timing of the activity rhythms at different phases of the tidal cycle. Crabs were exposed to 14:10 h light:dark cycle (light phase: cool white fluorescent lights, 1.1×10^{15} photons cm^{-2} s^{-1}) phased to the ambient cycle. They were place individually in 200 ml of seawater in 250 ml beakers having about 0.5 cm of sand on the bottom. Beakers were placed on an orbital shaker (New Brunswick Scientific, model G24) and shaken in a sequence of 15 s on followed by 15 s off for 4 h. The middle of the 4 h shaking interval was timed to occur at the times of (1) high tide, (2) mid-ebb tide, (3) low tide, or (4) mid-flood tide at the collection beach. Shaking continued for 4 tidal cycles and times were adjusted each cycle for changes in the times of local tides. In this situation mechanical agitation was applied to both the crab and the sand around the crab. Thus, an effect on the crab could be due to movement of the crab or movement of the sand and the crab. After shaking, crabs were placed individually in the rectangular columns. Monitoring of the activity rhythms began shortly before the beginning of the dark phase when the crabs were placed in constant darkness.

In the columns, crabs either remained in the sand or swam upward toward the surface of the water. They continued to swim at the surface for up to 2–3 min and then returned to the bottom. Behavior was quantified by counting the number of ascents in each 30 min interval. A crab was considered to undergo an ascent if it ascended above the surface of the sand. Since there was agreement between the rhythms in the same conditions, representative figures will be presented along with information about all crabs.

Synchrony between swimming activity (ascents) and the tidal cycle in the field was determined using cross-correlation analysis (MATLAB software; Levine et al., 2002). Tide height relative to mean lower low water

Table 1
The mean time of mole crab activity after the time of high tide in the field upon exposure to an entrainment cycle in mechanical agitation at different times in the tidal cycle

Agitation cycle time	Mean activity time (h)	SE	*n*
High tide	+1.50	0.52	6
Mid-ebb	+4.20	0.44	5
Low tide	+6.17	0.59	6
Mid-flood	+1.10	0.46	10

SE, standard error; *n*, sample size.

(MLLW) was obtained from a tides program (Nautical Software) for Atlantic Beach, NC. Plots of cross-correlation as a function of lag interval (1 lag = 0.5 h) for the first 3–4 cycles of the rhythm were used to compare behavior to tidal stage. Only the first 3–4 cycles were used because the rhythms were very clear over this interval and the effect of a free running period that shifted activity times relative to the tidal times would be reduced. Thus, peaks at positive or negative lag intervals indicated that maximum swimming activity occurred that many hours after (+lags) or before (−lags) high tide at the collection beach. Cross-correlations exceeding the 95% confidence intervals ($\pm 2/\sqrt{N}$) were considered to be statistically significant (Chatfield, 1989).

The second set of experiments was designed to determine whether the change in activity amplitude with tidal amplitude could be entrained by a mechanical agitation cycle. Mature male mole crabs show high activity at the time of the lowest amplitude high tide at the collection site and low activity at the time of the highest amplitude high tide (Forward et al., 2005). In the swash zone, crabs would be exposed to the longest interval of wave action during the high amplitude high tide and the shortest interval of mechanical agitation at the time of the low amplitude high tide. The experimental design was to separate crabs into control and experimental groups immediately after collection. Control crabs were immediately placed individually in the rectangular columns and their activity monitored. Experimental crabs were placed on the orbital shaker and exposed to an agitation cycle of 4 h of agitation centered at the time of lowest high tide and 1 h of agitation centered at the time of the highest high tide in the field. The agitation cycle continued for 4 tidal cycles during which the experimental crabs were exposed to the ambient light:dark cycle (described above). Thereafter, the experimental crabs were placed individually in rectangular columns and their activity was monitored in constant darkness beginning at the onset of the next dark phase.

Three criteria were used to evaluate whether the relationship between activity amplitude and tidal amplitude was entrained by the duration of mechanical agitation at high tide. First, subjectively did the greatest activity of control crabs occur at the time of low amplitude high tides in the field, and did the greatest activity of the experimental crabs occur at the time of the short duration mechanical agitation treatment? Second, each data set was normalized and the mean and standard deviation calculated for values at the first two activity peaks (peak ± 1 h; 5 values). Only the first two peaks were considered because they are representative of the rhythm in activity amplitude. The two means were then compared using a *t*-test for comparison of means to determine (1) whether activity associated with the low amplitude high tide was significantly greater ($p < 0.05$) than activity associated with the high amplitude high tide for the control crabs, and (2) whether activity associated with the short duration mechanical agitation treatment was significantly greater than that associated with the long duration mechanical agitation treatment for the experimental crabs. Statistical differences would indicate there was a rhythm in activity amplitude.

Third, an overall mean and standard deviation were calculated for the normalized activity levels of all control crabs associated with the high and low amplitude high tides. The means associated with the low amplitude tide was compared (*t*-test for comparison of means) to mean activity levels of each experimental crab associated with the short duration agitation treatment. Similarly the control mean activity level associated with the high amplitude high tide was compared to the mean activity of each experimental crab associated with the long duration agitation treatment. The absence of statistical differences

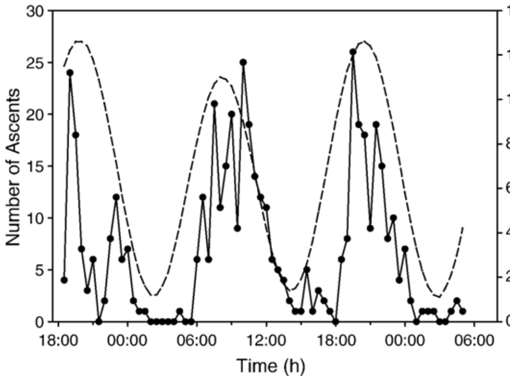

Fig. 1. Number of ascents at 0.5 h intervals (solid line) over time for a mature male mole crab after entrainment to agitation treatments at the time of high tide (±2 h) in the field. The tidal cycle at the home beach is shown for reference (dashed line). The crab was collected on August 4, 2005, exposed to 4 agitation cycles and place under constant conditions on August 6, 2005. The time of maximum swimming activity occurred 0.5 h after the time of high tide in the field.

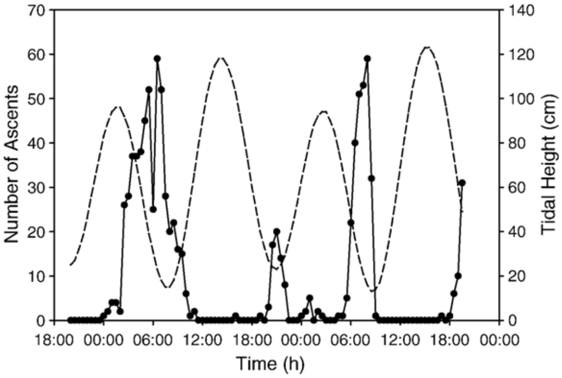

Fig. 2. Number of ascents at 0.5 h intervals (solid line) over time for a mature male mole crab after entrainment to agitation treatments at the time of mid-ebb tide (±2 h) in the field. The tidal cycle at the home beach is shown for reference (dashed line). The crab was collected on July 12, 2005, exposed to 4 agitation cycles and place under constant conditions on July 14, 2005. The time of maximum swimming activity occurred 4.5 h after the time of high tide in the field.

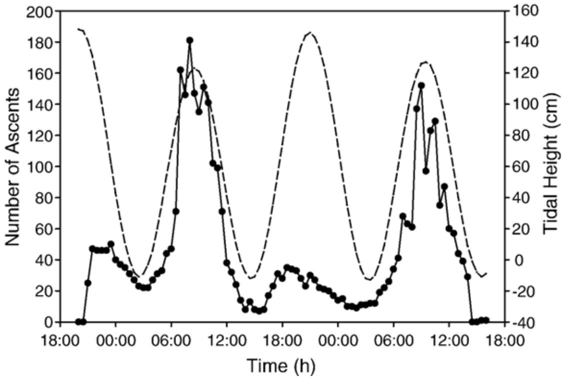

Fig. 4. Number of ascents at 0.5 h intervals (solid line) over time for a mature male mole crab after entrainment to agitation treatments at the time of mid-flood tide (±2 h) in the field. The tidal cycle at the home beach is shown for reference (dashed line). The crab was collected on July 19, 2005, exposed to 4 agitation cycles and place under constant conditions on July 21, 2005. The time of maximum swimming activity occurred 0.5 h after the time of high tide in the field.

would indicate the agitation cycle reset the activity amplitude rhythm.

3. Results

3.1. Entrainment by turbulence at different phases of the tide

After exposure to turbulence treatments, 93% of the crabs were active in the columns and each of these crabs displayed a circatidal rhythm in vertical swimming as described previously (Forward et al., 2005). To evaluate the effect of a turbulence cycle as an entrainment cue, the relationship between the time of maximum swimming activity and the time of high tide in the field was calculated. Since the times for each experimental series were normally distributed, means and standard errors were calculated.

The effect of turbulence for entrainment varied throughout the tidal cycle. When the turbulence treatment occurred at the time of high tide in the field (±2 h), the mean time of peak activity occurred 1.5 h after the time of high tide (Table 1; Fig. 1). Exposure to the turbulence treatment at mid-ebb tide (±2 h) caused the mean time of peak activity to shift to 4.2 h after the time of high tide (Table 1; Fig. 2). Similarly, when crabs

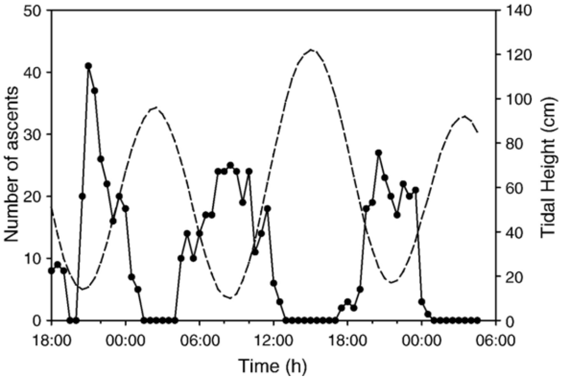

Fig. 3. Number of ascents at 0.5 h intervals (solid line) over time for a mature male mole crab after entrainment to agitation treatments at the time of low tide (±2 h) in the field. The tidal cycle at the home beach is shown for reference (dashed line). The crab was collected on July 26, 2005, exposed to 4 agitation cycles and place under constant conditions on July 28, 2005. The time of maximum swimming activity occurred 7 h after the time of high tide in the field.

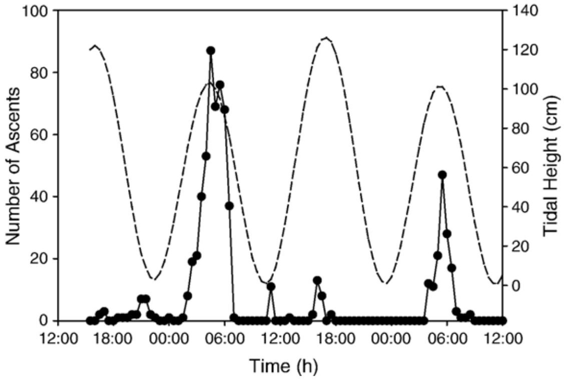

Fig. 5. Number of ascents at 0.5 h intervals (solid line) over time for a mature male mole crab collected on June 1, 2005 and immediately place under constant conditions. The tidal cycle at the home beach is shown for reference (dashed line). The time of maximum swimming activity occurred at the time of the lowest high tide in the field.

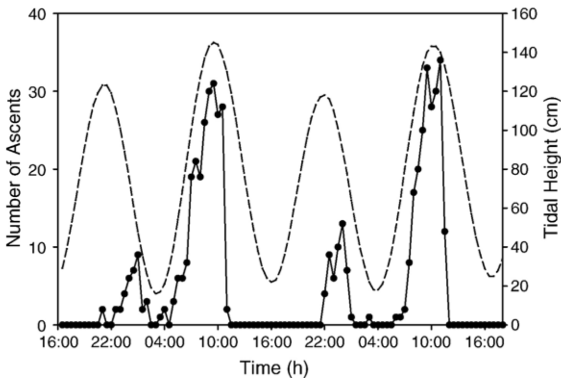

Fig. 6. Number of ascents at 0.5 h intervals (solid line) over time for a mature male mole crab after entrainment to cycle of 4 h of agitation at the time of the lowest high tide and 1 h of agitation at the time of highest high tide in the field. The tidal cycle at the home beach is shown for reference (dashed line). The crab was collected on October 3, 2005 and placed under constant conditions on October 5, 2005. The time of maximum swimming activity occurred at the time of the highest high tide in the field.

were exposed to the turbulence treatment at the time of low tide in the field (±2 h), the mean time of peak activity was 6.17 h after the time of high tide in the field (Table 1; Fig. 3). Surprisingly, when the turbulence treatment was given during the time of mid-flood tide (±2 h), the mean time of maximum activity (Table 1; Fig. 4) occurred at approximately the same time (1.1 h after high tide) as observed for crabs exposed to the turbulence treatment at the time of high tide (e.g. Fig. 1).

3.2. Activity amplitude versus tidal amplitude

The activity of mole crabs varied with tidal amplitude. Seven of the 11 control crabs had a rhythm in which the highest activity amplitude was associated with the lowest high tide in the field and the lowest activity peak corresponded to the highest amplitude high tide (e.g. Fig. 5). The mean activity amplitude of each crab was significantly greater after the low amplitude high tide as compared to that after the high amplitude high tide. Two of the other crabs had the reverse pattern and two crabs were arrhythmic.

Experimental crabs were subjected to a pattern of 4 h of turbulence at the time of the low amplitude high tide in the field and 1 h of turbulence at the time of the high amplitude high tide in the field. The criteria that a crab reset its activity amplitude rhythms were that the highest activity switched to be associated with the time of the short turbulence treatment and highest amplitude high tide in the field. Second, mean activity of the first two peaks was significantly different. Third, mean activity associated with the short duration agitation treatment was not significantly different from mean activity of the control crabs associated with the low amplitude high tide and similarly, mean activity of experimental crabs associated with the long duration agitation treatment was not significantly different from mean activity of control crabs associated with the high amplitude high tide. Eight of the 15 crabs met the criteria for resetting the rhythm in activity amplitude with the agitation cycle (Fig. 6). For the other crabs, 3 retained the original rhythm, 2 had equal amplitude peaks and 2 were arrhythmic.

4. Discussion

A tidal rhythm in vertical swimming underlies movements of the mole crab *E. talpoida* up and down sand beaches in the swash zone (Forward et al., 2005). Under constant conditions, adult male crabs have a circatidal rhythm in which the median time of maximum activity occurs 1.5 h after the time of high tide in the field. Forward et al. (2005) hypothesized that turbulence from mechanical agitation at the time of high tide is an entrainment cue for the endogenous rhythm. The present study was designed to determine the effect of turbulence at different phases of the tidal cycle on resetting the timing of the activity rhythm.

If turbulence at high tide entrains the rhythm, then the time of maximum activity can be predicted from the timing relationship of the laboratory turbulent entrainment events relative to tidal times in the field. Since high tide, mid-ebb, low tide and mid-flood occur at 0, +3, +6 and −3 h relative to the time of high tide, the times of peak activity after the entrainment turbulence treatments at these times were predicted to occur at +1.5, +4.5, +7.5 and −1.5 h relative to the times of high tide. These predictions agree with the results for high tide, mid-ebb tide and low tide entrainment events, as the mean times of peak activity occurred at +1.5, +4.2 and +6.17 h after high tide, respectively (Table 1). The exception was exposure to the turbulence treatment at mid-flood tide. There did not appear to be an entrainment effect, as the mean time of peak activity was +1.1 h (Table 1), which is close to the mean time (+1.5 h) of crabs taken directly from the field (Forward et al., 2005) and not significantly different (*t*-test) from the mean time after exposure to the agitation treatment at the time of high tide (Table 1).

A phase-response curve is typically used to describe the effect of an entrainment cue at different times of the tidal cycle. Among crustaceans, the general trend is that cues given before the activity peak cause an advance in timing of the rhythm and cues given after the peak cause a delay in timing, such that the activity peak is later

(Klapow, 1972; Enright, 1976; Holstrom and Morgan, 1983; Naylor and Williams, 1984). Applying this relationship to adult mole crabs, cues given from high tide through ebb tide to low tide should cause delays in activity and those given from low tide through flood tide to high tide should cause advances. The mole crab showed half of the predictions, as they only delayed the timing of the rhythm in response to cues given from high tide through ebb tide to low tide. The predicted advances in timing when turbulence cues were given during flood tide were not observed.

A possible reason for this response pattern is that entrainment of the activity rhythm only during ebb tide reduces the likelihood of stranding high on the beach. Since consecutive high tides occur at about 12 h 24 min intervals, the normal direction for entraining the rhythm is exposure to a turbulence delay with each tidal cycle. The normal activity peak occurs 1.5 h after the time of high tide. Thus, mole crabs are maximally active during ebb tides which would move them down the beach as the tide recedes. If they responded to an entrainment cue during flood tide, the time of maximum activity would advance and occur at or before high tide. Thus, they would be inactive during ebb tide and become stranded high on the beach, which would increase the threat of predation, desiccation and exposure to adverse temperatures.

The second purpose of the study was to consider the hypothesis that the cycle in the duration of mechanical agitation between high and low amplitude high tides entrains the rhythm in the amplitude of activity peaks. The control crabs clearly showed a rhythm in which high activity occurred just after the time of low amplitude high tides in the field and low activity just after the time of high amplitude high tides. The functional significance of this pattern may be a second mechanism to reduce the likelihood of stranding above the swash zone. If crabs are maximally active just after the highest high tide, they would be distributed over the maximum wave up-rush areas and potentially stranded high in this area during ebb tide. Since the amplitude of the next high tide is lower, the wave up-rush area may not reach crabs stranded high on the beach. Thus the crabs would be stranded for about 24.8 h, the time between consecutive high amplitude high tides. By being maximally active just after the lower amplitude high tides, crabs occur in the wave up-rush zone during all high tides, which would reduce the potential stranding time to 12.4 h.

A cycle in the duration of turbulence entrained the activity amplitude cycle. After exposure to 4 cycles of 4 h agitation at the time of the low amplitude high tide and 1 h at the time of the high amplitude high tide in the field, 62% of the rhythmic crabs had their highest activity at the time of the short turbulence treatment, whereas 23% retained the original rhythm. Thus, the actual time of mechanical agitation serves as an entrainment cue for the phase relationship of the circatidal rhythm in activity relative to tides. In addition, the duration of mechanical agitation establishes a secondary rhythm, in which activity amplitude varies with tidal amplitude.

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