Larval Release Rhythm of the Mole Crab *Emerita talpoida* (Say)

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Abstract. Ovigerous mole crabs *Emerita talpoida* (Say) were monitored in the laboratory to determine if the time of larval release is synchronous and under endogenous control. To determine the time of larval release, ovigerous females were placed under a 14:10 light/dark cycle simulating the ambient photoperiod. Hatching was rhythmic, occurring as a quick burst lasting about 5–15 min shortly after the onset of darkness. An individual mole crab will release batches of larvae for up to three successive nights, suggesting that the rhythm is under endogenous control. Mole crabs monitored under constant low-level red light displayed the same release pattern with hatching occurring near the time of expected sunset, indicating the presence of a circadian rhythm in larval release. To investigate whether the female or the embryos control hatching, a portion of the egg mass (50–100 embryos) was separated from the female. The time of hatching of the detached embryos subjected to either a still or shaken treatment was compared with the hatching time of embryos still attached to the female. Detached eggs in both treatments hatched within 1.5–2 h of the time of the female-attached eggs, which suggests that embryos control the timing of hatching.

Introduction

Rhythmic patterns in the timing of larval release are common among decapod crustaceans and are often synchronized to natural periodic cycles, including moon phase, tide, and time of day (DeCoursey, 1983; Forward, 1987; Morgan, 1995). Most larval release rhythms appear to be under endogenous control, since decapods continue to release their larvae synchronously when placed under constant conditions (e.g., Bergin, 1981; Forward et al., 1982; DeVries and Forward, 1989; Saigusa, 1988; Saigusa and Kawagoye, 1997). For species displaying diel rhythms in release, hatching usually occurs during the night phase, which is considered an adaptation to ensure that larvae are released at times that reduce their vulnerability to visual predators. For species in which larval release is synchronized with the tide, hatching generally occurs near the time of high tide, maximizing the chances of larvae being transported to appropriate nursery areas (Forward, 1987; Morgan, 1995).

Previous studies of intertidal and subtidal crabs living in estuarine areas indicate that the time of larval release depends upon the interaction of the light/dark (LD) and tidal cycles (Forward, 1987; Morgan, 1995). Most crabs release larvae at specific times relative to both tidal and diel cycles, usually near the time of high tide at night. If high tide occurs shortly before sunset, then crabs will delay larval release until the onset of darkness. When high tide occurs after sunset, hatching is closely associated with the time of high tide (Saigusa and Hidaka, 1978; Saigusa, 1981; Bergin, 1981; Christy, 1986; Forward et al. 1986). However, the timing of larval release has been investigated only in crabs that reside inside an estuary or rocky intertidal habitat (Christy, 1986; Forward, 1987; Morgan, 1995). To the best of our knowledge, the timing of larval release has not been investigated for a species living in the surf zone of a sandy beach.

The wind-driven oceanography of the surf zone in a sandy beach environment is much less predictable than that of an estuary influenced by tidal forces. Estuaries and intertidal zones have pronounced tidal changes that are periodic and predictable. In the surf zone, the movement of water above and within the sandy substrate is influenced by surface wave motion, and wave-generated nearshore currents, as well as tide and wind-generated currents (Swart,
1983; McLachlan, 1983), creating a severely physical habit-
itat. These complex physical processes associated with con-
stantly changing tides and sea state make the sandy beach a much
different habitat than an estuary.

The mole crab *Emerita talpoida* (Say) is a ubiquitous part
of the sandy beach habitat throughout its life cycle. In North
Carolina, adult mole crabs mate from the early spring
through the summer and fall months. Females incubate
embryos underneath their abdomen for 2–3 weeks. Larval
release occurs from late January to October (Diaz, 1980).
The larvae develop in nearshore areas, passing through five
to seven zoal stages (Rees, 1959; Diaz, 1974). Postlarvae
(megalopae) recolonize and settle on beaches in June
through July and September through October in North Caro-
lina (Diaz, 1974).

Mole crabs live in and below the swash zone, where the
intertidal zone is under the influence of waves from coastal
waters. Mole crabs maintain position in the swash zones of
sandy beaches by migrating up and down the beach with the
tides (e.g., Cubit, 1969); however, they are distributed
throughout the intertidal zone on the basis of size. Small
individuals (less than 8 mm) are found near the sand-water
interface at the top of the intertidal zone, medium-sized
mole crabs (8 to 15 mm) are located in the center of the
swash zone, and large mole crabs (greater than 15 mm) are
found within the lower half of the active swash zone (White,
1976; Bowman and Dolan, 1985).

Residents of the swash zone, such as mole crabs, may
display a different pattern of larval release than that of
estuarine crabs because they are exposed to different envi-
ronmental factors that may act as entrainment cues for larval
release rhythms. Diel rhythms can be entrained by the daily
LD or temperature cycles (Menaker, 1969). Tidal rhythms
could be entrained by cycles in mechanical agitation (En-
right, 1965), temperature, pressure, or salinity (Naylor,
1996). However, in the surf zone, temperature and salinity
changes are less dramatic, and therefore may not be great
enough to affect or entrain a rhythm in larval release. In
addition, adult mole crabs live in an area that borders the
deeper water where the larvae are transported. Hatchling
at nocturnal tides may not be necessary for larval survival.

Amend and Shanks (1999) examined the timing of larval
release by the mole crab *E. talpoida* by comparing the
frequency that ovigerous females with gray eggs (i.e., eggs
in the late stages of embryonic development) were found in
the field to several physical oceanographic factors. Their
analysis suggested that larval release in *E. talpoida* (mea-
sured as a decrease in the frequency of females with late-
stage eggs) occurred within a day after large wave events.
Their study did not investigate whether an endogenous
timing mechanism is involved in the timing of larval re-
lease.

The goals of the present study was to determine the time
of larval release in ovigerous mole crabs under laboratory
conditions and to examine the phase relationship between
larval release and the environmental cycles (tidal and diel).
The endogenous clock, as evidenced by a free-running
rhythm, becomes apparent only when an individual under
constant conditions in the laboratory displays a rhythm with
a period that is close to but not equal to the corresponding
environmental cycle in the field. If larval release is under
endogenous control, then the actual timing of hatching may
be controlled by the female, the developing embryos, or
both (Forward, 1987). The simultaneous hatching by a
clutch of embryos depends upon the events that synchronize
development and egg hatching (Forward, 1987). Thus, our
study also investigated the site of endogenous control for
the larval release rhythm in *E. talpoida*.

**Materials and Methods**

**Collection and maintenance of animals**

Ovigerous mole crabs *Emerita talpoida* (Say) (17.1–29.8
mm carapace length) were collected during low tide at
Atlantic Beach, North Carolina (34° 42' N; 76° 43' W) from
June through October 2003. This area is an exposed sandy
beach habitat (McLachlan, 1990) that experiences semi-
diurnal tides. Following collection, the developmental stage
of the embryos was initially determined by visual inspec-
tion. The color of the egg mass changes from bright orange
to dark orange, and finally to a translucent gray as the
embryos mature. Egg masses were further classified micro-
scopically on the basis of egg yolk content and eye develop-
ment of the embryos, using the scheme established by
Subramoniam (1982). After collection, mole crabs were
placed in individual glass bowls (20 cm \( \times \) 6.5 cm) contain-
ing filtered (\( > 5 \) \( \mu \)m) seawater (salinity of 35\% ) along with
sand (\( \sim 2 \) cm deep) that was filtered through a 0.5-mm
screen and maintained at 25–27 °C. Ovigerous mole crabs
use the sand to stabilize their position during larval release.
Female mole crabs were observed to remove all of their
embryos if sand was not present in the bowls. Water was
changed daily at random times; sand was changed every
other day. Females with embryos that possessed less than
25% yolk were used to determine the presence of an endo-
genous rhythm in egg hatching. These embryos were
within 2–3 days of hatching. Only females that had egg
masses with embryos that lacked eyespots and possessed
more than 50% yolk content at the time of collection were
used for the entrainment experiments. Embryos in the latter
state are approximately 9–14 days from hatching.

**Solar day rhythm in egg hatching**

Mole crabs whose embryos had less than 25% yolk were
placed under a LD cycle that mimicked the ambient photo-
period. Light (\( \sim 0.76 \times 10^{15} \) photons/cm\(^{-2} \cdot \) s\(^{-1} \)) during
the day phase came from overhead cool white fluorescent
lamps. A low-level red light (GE 25-W red incandescent bulb) was on at all times. Mole crabs were visually monitored every 30 min over a 12-h period, from noon until midnight. Larval release was typically a brief event, lasting 1–5 min. Female mole crabs emerge from the sand and vigorously pump their abdomens while larvae are released. At 30-min intervals, the female was moved to a new bowl so that the number of larvae released could be counted. The mean time of larval release was determined by multiplying the number of larvae observed in each 30-min interval by that interval, taking the sum of these products over all intervals and dividing this sum by the total number of larvae. Thus, mean time was designated as the time of larval release (Forward and Lohmann, 1983).

If eggs remained on a female at the end of the sampling period, then that individual was placed in a glass bowl (20 cm × 6.5 cm) with clean sand and seawater, and the procedure was repeated during the next sampling period. The time of larval release on the first night was compared to the expected phase of the tidal and diel cycles. Since this initial experiment indicated that the time of larval release occurred at the beginning of the dark phase (solar day rhythm) and was not related to tidal times, all future experiments monitored mole crabs around the expected time of egg hatching at the beginning of the dark phase of the LD cycle.

Circadian rhythm in egg hatching

Newly collected mole crabs that were determined to be 4–8 days from larval release were placed under constant conditions (temperature, salinity, and light) at the beginning of the dark phase of the LD cycle. A low-level red light (GE 25-W incandescent bulb) was on at all times. On the day of expected larval release, egg hatching was observed over the predetermined sampling period from 1800 h until midnight. This sampling period began about 2 h before the beginning of the ambient dark phase and was used in all experiments. During this sampling period, mole crabs were transferred every half hour to new glass bowls (20 cm × 6.5 cm) with filtered seawater and clean sand. The number of larvae released within each 30-min interval was counted to determine the mean time of larval release. Typically, most hatching occurred during only one time interval. If eggs remained on the female at the end of the sampling period, then that individual was placed in a new glass bowl with clean sand and seawater, and the procedure was repeated 24 h later. The mean time of larval release was compared to the expected phase of the tidal and diel cycles at the collection site.

Entrainment to a light/dark cycle

To test the hypothesis that larval release in mole crabs is controlled by a circadian clock entrained by the LD cycle, mole crabs were randomly assigned to one of two light treatments. In the first treatment group, mole crabs were subjected to a 14:10 LD cycle (period = 24 h) similar to their ambient photoperiod. In the second treatment group, mole crabs were subjected to a 14:10 LD cycle that was advanced by 12 h relative to the ambient photoperiod treatment. Mole crabs were exposed to the photoperiod treatments for at least 8 d before being placed under constant conditions. The onset of light occurred at 0600 h in the ambient photoperiod treatment and at 1800 h in the advanced photoperiod treatment. Light (≈ 0.76 × 10^15 photons/cm^2 s^-1) was provided by overhead cool-white fluorescent lamps. A low-level red light (GE 25-W incandescent bulb) was on continuously during the LD cycle and under constant conditions.

Embryos from each crab were inspected daily to determine the stage of development. When the egg mass appeared gray, a small sample of eggs (15–25) was removed and examined under a dissecting microscope. Crabs that were expected to release within 48–72 h were moved to constant conditions at the beginning of the dark phase, and the time of larval release was monitored. Only crabs that were placed in constant conditions for more than 42 h prior to larval release were used in the analysis. The mean time of larval release was determined for each crab as described in the previous experiment.

Control of hatching

This experiment was designed to test whether detached eggs can hatch and whether the timing of hatching of detached embryos is synchronous with that of embryos attached to the parent female. Hatching was monitored under three treatment conditions: detached eggs in still water, detached eggs on a mechanical orbital shaker (Eberbach Corp.), and eggs attached to the female. Ovigerous mole crabs with eggs that were expected to hatch within 1–2 days were used. Two groups of about 50–100 eggs were detached from the female and placed in a small beakers containing filtered (> 5 μm) seawater. Females with attached eggs and both groups of detached eggs were placed under constant conditions at the beginning of the dark phase (2000 h) at least 24 h before the predicted time of hatching. Hatching for all three treatments was monitored simultaneously.

Detached eggs were placed on an orbital shaker set at a rate of about 1 cycle/s to mimic the mechanical abdomen pumping of the female mole crab during larval release. Eggs were shaken continuously during the observation time except when the shaker was briefly stopped at half-hour intervals to count and remove swimming zoea by pipette. Any unhatched eggs remaining at the end of each sampling period were maintained under constant conditions, and the procedure was repeated 18 h later.
The females with attached eggs were simultaneously monitored to determine the time of larval release. During the sampling period, the female was transferred to a new bowl of seawater every half hour. The number of larvae released within the 30-min interval was counted. If eggs remained on the female, then the mole crab was kept under constant conditions and the procedure was repeated 18 h later. Ovigerous females and detached eggs were monitored for at least two consecutive nights.

Statistical analysis

The time of larval release was analyzed as the number of larvae released during a 30-min interval. Time series for the observed larval release rhythm of each crab were analyzed for periodicity using a combination of autocorrelation and maximum entropy spectral analysis (MESAS) (Dowse and Ringo, 1989; Tankersley and Forward, 1994). After the autocorrelation coefficients calculated at 30-min intervals (equivalent to the sampling interval) were plotted as a function of lag, period lengths were verified with MESAS according to Levine et al. (2002). Period lengths were averaged from the MESAS calculations for replicate mole crabs.

The synchrony between the time of release and the phase of the tidal and diel cycles was determined using circular statistics (Zar, 1999). Each observation (i.e., mean time of larval release by one mole crab) was converted to a corresponding angular value that indicated the time of hatching relative to the tidal and diel cycles. These angular values were then used to calculate a mean time of release for each experimental group.

Specifically, the LD cycle has a period length of 24 h, or 360°, with a 14-h light phase and a 10-h dark phase. Using circular statistics, each data point was standardized relative to the time of day, with 0° corresponding to 0000 h (midnight), and 300° corresponding to the beginning of the dark phase of the LD cycle (2000 h). Larval release times were used to calculate a mean time of release (equivalent to the mean angle, defined as  a) for each experimental group. A Rayleigh test was used to determine whether the time of hatching differed significantly from a uniform distribution (Zar, 1999). If light was capable of entraining a circadian rhythm, then the mean angles (mean time of larval release) for the ambient LD cycle and the shifted-LD cycle were expected to differ by 180°. In addition, an r value, which is a measure of the concentration, was calculated. The r values can range from 0 to 1, with higher values indicating that the data are concentrated at the same direction (Zar, 1999). Thus, a V-test was used to test the null hypothesis that the mean angle for the ambient photoperiod cycle trials is equal to the mean angle for the trials with a phase-shifted photoperiod cycle + 180° (Zar, 1999). In other words, the V-test compared the ambient photoperiod (observed angle) and the shifted photoperiod experiments (180° + observed angle) to test whether they were statistically different.

For tidal considerations, the time of larval release was compared to the time of high tide (HT) at the collection site on the day hatching occurred. One tidal cycle at the collection site has a period of about 12.4 h, which was converted into 360°. Thus, for the tidal cycle, 0° corresponded to the time of expected HT on the day of release and 180° to the time of low tide. The times of the expected tidal cycles at the collection site were generated using Tides and Currents (Nautical Software, version 2.0). A Rayleigh test was used to determine whether the times of egg hatching differed significantly from a uniform distribution relative to the tidal cycle (Zar, 1999).

To determine if the detached embryos hatch synchronously with embryos attached to the female, the mean time of hatching for detached embryos in both treatments was compared to the mean time of larval release by the female (angle = 0°) using a V-test (Zar, 1999). This test was also used to compare the mean time of hatching by detached embryos in the still-water and shaken treatments.

Results

Hatching when exposed to a light:dark cycle

Successive releases of larvae each night suggest that there is an internal timing mechanism controlling larval release in Emerita talpoida (Fig. 1). Microscopic inspection of developing embryos within the mole crab egg mass suggested that all embryos within the egg mass develop synchronously. Nevertheless, different cohorts hatched on consecutive nights (Fig. 2).

Females subjected to a LD cycle released a large burst of larvae during a 30-min interval, and very few larvae hatched before and after the interval (Fig. 1a). The release of larvae by female mole crabs was confined to a short period at night, and was repeated at a similar time each night for three consecutive nights (Figs. 1a, 2). Hatching always occurred soon after the beginning of the dark phase. A similar pattern was observed when mole crabs were placed under constant conditions (Fig. 1b).

Hatching under constant condition relative to the tidal cycle

The times of larval release for ovigerous mole crabs placed under constant conditions for 4–8 days were random relative to the time of high tide (Fig. 3, r = 0.26, P > 0.05, n = 40). The distribution was not significantly different from a uniform distribution. Thus, no circatidal rhythm in larval release was present. For an example of a circular plot with a significant result, see Weaver and Salmon (2002).
Hatching under constant conditions relative to the light: dark cycle

The same data for the timing of larval release under constant conditions were replotted relative to the LD cycle. Ovigerous mole crabs subjected to constant conditions for 4–8 days prior to the time of predicted larval release expressed a free-running circadian rhythm. Hatching occurred shortly after the time of sunset in the field (Fig. 4). Using circular analyses, the mean time of hatching was 0.09 h after the expected onset of darkness (Fig. 4, \( \bar{a} = 302.5^\circ, r = 0.993, P < 0.001, n = 40 \)). Mole crabs displayed a circadian rhythm in larval release with an average free-running period of 24.3 h (SD = 0.91 h).

Mole crabs collected at the same time but subjected to a LD cycle in the laboratory for 8–14 d that mimicked the natural photoperiod before being transferred to constant conditions released larvae at a mean time of 0.07 h before the beginning of the expected dark phase (Fig. 5, \( \bar{a} = 298.0^\circ, r = 0.995, P < 0.001, n = 37 \)). All hatching occurred within 2 h of the beginning of the expected dark phase.

Figure 1. Typical hatching profile for a single mole crab (Emerita talpoida) under a light/dark cycle (a) and under constant conditions (b). The shaded areas show the light:dark cycle to which crabs were exposed in (a) and the cycle prior to placement in constant conditions in (b).
A separate group of mole crabs was exposed to a 14:10 LD cycle that was advanced by 12 h (relative to the ambient photoperiod treatment) for 8–14 days. Mole crabs subjected to a shifted photoperiod entrained to the altered LD cycle.

Larval release occurred at a mean time of 0.27 h after the time of expected sunset (Fig. 6, $\bar{d} = 126.9^\circ$, $r = 0.987$, $P < 0.001$, $n = 43$). The LD cycle was the synchronizing agent for the circadian rhythm in larval release, since the time of larval release was shifted by the LD cycle. The results of a V-test indicated that the mean time of release for mole crabs in the ambient photoperiod (Fig. 5) was not significantly different from the mean time of release in the shifted photoperiod.
different when the phase shift was taken into account (Fig. 6, \(a + 180^\circ, P > 0.05\)). Thus, mole crabs were entrained by the LD cycle that they experienced in the laboratory.

Hatching of detached eggs

Hatching did not occur if embryos were removed from the female 48 h before the predicted time of larval release (Table 1). A small percentage of detached embryos would hatch if they were removed about 36 h before the predicted release time. Most detached embryos would hatch successfully if removed from the female at about 24 h prior to the predicted release time (Table 1).

Hatching for detached eggs in both the still and shaken treatments was synchronized to the time of larval release by the female (Figs. 7 and 8). Detached eggs in the shaken treatment hatched within 1.5 h of the time of larval release by the female (Fig. 7), with 88% of detached eggs hatching within 0.5 h. The relative time of hatching for embryos in the still-water treatment was also within 1.5 h of the time of release by the female; however, only 64% of the detached eggs hatched within 0.5 h (Fig. 8). The results of a V-test indicated that the mean time of hatching by detached eggs in the still-water treatment was not significantly different from the mean time of hatching for eggs in the shaken treatment (\(u = 1.646, df = 24, P > 0.05\)). Thus, the shaking treatment did not significantly change the mean time of hatching.

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**Table 1**

Percent hatching success of detached eggs from female mole crab removed from the female at various times before the predicted time of hatching

<table>
<thead>
<tr>
<th>Egg treatment</th>
<th>Time before expected hatching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Still water</td>
<td>95% ± 0.81</td>
</tr>
<tr>
<td>Shaken water</td>
<td>96% ± 0.04</td>
</tr>
</tbody>
</table>

Hatching occurred approximately 1.5–2 h before or after larval release by the female (\(n = 25\)). Values are mean ± SD.

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**Figure 5.** Mean times of larval release for ovigerous mole crabs (*Emerita talpoida*) exposed to a 14:10 light/dark cycle and then placed under constant conditions. Shaded areas represent the dark phase of the light regime during the entrainment period (8 to 14 days); white areas represent the light phase on the field. The data show the mean time of release of each mole crab (\(n = 37\)) on the first night of larval release.

**Figure 6.** Mean times of larval release for ovigerous mole crabs (*Emerita talpoida*) after exposure to an altered 14:10 light/dark (LD) cycle and then placed under constant conditions. The onset of darkness (0800 h) was advanced by 12 h relative to the ambient LD cycle treatment (Fig. 5). Shaded areas represent the dark phase of the LD cycle during the entrainment period (8 to 14 days); white areas represent the light phase. The data show the mean time of release of each mole crab (\(n = 43\)).

**Figure 7.** Mean time of hatching of detached eggs from mole crabs (*Emerita talpoida*) in the shaken treatment relative to the timing of larval release by the parent crab. The mean time of hatching by each cohort of detached eggs was plotted relative to the mean time of larval release for the embryos attached to the female at 0 h. Embryos were detached 24 h before the predicted time of larval release for the female (\(n = 25\)).
Embryos detached less than 24 h before larval release hatched at about the same time as eggs attached to the abdomen of ... 24 h before the predicted time of larval release for the female (n/H1100525).

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Figure 8. Mean time of hatching of detached eggs from mole crabs (Emerita talpoida) in the still-water treatment relative to the timing of larval release from the parent crab. The mean time of hatching by each cohort of detached eggs was plotted relative to the mean time of larval release for the embryos attached to the female at 0 h. Embryos were detached 24 h before the predicted time of larval release for the female (n = 25).

Embryos detached less than 24 h before larval release hatched at about the same time as eggs attached to the abdomen of the female (V-test, u = 0.977, df = 24, P > 0.05), suggesting that the “clock” responsible for synchronizing egg hatching is in the developing embryos and not the female.

Discussion

The timing of larval release for Emerita talpoida is precise. Mole crabs synchronized larval release with the onset of darkness during the LD cycle (Figs. 1 and 2). The timing of larval release is under endogenous control, since hatching consistently occurred near the time of expected sunset under constant conditions.

One female mole crab can release batches of larvae each successive night for up to three nights in constant conditions (Fig. 1b), indicating that the timing of larval release is controlled by an endogenous clock. These results are contrary to patterns of larval release found in brachyuran crabs. Most brachyurans release larvae in a very brief event lasting only one night (see Forward, 1987). The persistence of a larval release rhythm in a single individual for a number of consecutive cycles has not been demonstrated previously. Most studies on the timing of larval release consider the rhythm from a population of crabs as the evidence for an endogenous rhythm. This study demonstrates for the first time the persistence of a larval release rhythm in a single individual crab.

Ovigerous female mole crabs exposed to a LD cycle and then placed under constant conditions displayed a circadian rhythm in release (Figs. 4 and 5), in which eggs hatch shortly after the beginning of the expected night phase. The time of larval release changed when female mole crabs with early-stage eggs were exposed to an altered 14:10 LD cycle. When the photoperiod was advanced by 12 h from the ambient photoperiod, the time of hatching shifted by about 12 h (Fig. 6), indicating that the LD cycle is capable of synchronizing the time of larval release and acts as a natural zeitgeber for entraining the circadian rhythm in larval release by E. talpoida.

Nocturnal larval release is common among brachyuran crabs and lobsters. In most cases, the time of larval release for these animals occurs in the first few hours of the night phase (Forward, 1987). Larval release rhythms that are synchronized with the LD cycle could be entrained by the onset of the light or dark phase (Palmer, 1995). The LD cycle similarly entrains the circadian rhythms of the crab Gecarcinus lateralis (Wolcott and Wolcott, 1982), the semi-terrestrial crabs Sesarma haematocheir, Sesarma pictum, and Hemigrapsus sanguineus (Saigusa, 1981, 1986; Saigusa and Kawagoye, 1997), and the subtidal xanthid Rhithropanopeus harrisi (Forward et al., 1986). The lobsters Homarus americanus and Homarus gammarus also display a circadian rhythm in larval release, in which the time of hatching occurs near the time of sunset (Ennis, 1973, 1975; Branford, 1978).

Studies of other decapod species for which larval release times have been determined relative to both the diel and tidal cycles have shown that hatching occurs mainly during the dark phase (e.g., Saigusa and Hidaka, 1978; Moller and Branford, 1979; Saigusa, 1982; Wolcott and Wolcott, 1982; Forward et al., 1986; De Vries and Forward, 1989; Saigusa and Kawagoye, 1997). Larval release occurs at nocturnal high tides for several crab species living in the intertidal zone, including Panopeus herbstii (Morgan, 1995), Catapodius taboganan, Xanthodius sternberghii (Christy, 1986), and Uca pugilator (Bergin, 1981).

Female mole crabs possess a circatidal rhythm in activity (Forward et al., 2005), yet they lack a tidal rhythm in larval release. Our results suggest that the embryos are in control of the timing of larval release. The circatidal rhythm in activity in adult mole crabs can be entrained by mechanical agitation that simulates wave action on a sand beach (Forward et al., 2005). Mole crab embryos may fail to develop a tidal rhythm because they lack the sensory structures to detect mechanical cues during development. However, eye spots are present in mole crab embryos about 12–14 d after fertilization (Subramoniam, 1982), and these develop into compound eyes. Therefore, mole crab embryos are capable of perceiving the LD cycle.

Even though synchronized embryo development probably results from some type of chemical or mechanical communication between the female and the developing
embryos, the results indicate that hatching of detached embryos does not occur randomly, but rather in synchrony with the time of hatching of embryos attached to the female (Fig. 8). Since mole crab embryos have the ability to hatch as viable larvae if detached from a female less than 24 h before the time of larval release, the embryos probably have an endogenous clock. Detached eggs did not hatch as synchronously as the eggs attached to the female, which suggests that the pumping of the female’s abdomen during the hatching process may increase the hatching synchrony.

Although these results suggest that the clock resides in the embryos, the conditions of the experiment do not meet the criteria for demonstrating the presence of an endogenous clock, since the cycle in hatching was only monitored for about 24 h before larval release. A rhythm must persist for a minimum of five cycles in a single individual under constant conditions to qualify as an endogenous rhythm (Menaker, 1969). This requirement was impossible to fulfill for E. talpoida because an egg only hatches once. Thus, our results only suggest the presence of a circadian rhythm in the detached eggs.

Studies of the hatching process for subtidal and intertidal crabs suggest that embryos release enzymes that degrade the inner membrane of the egg case (Forward, 1987; De Vries et al., 1991). These enzymes produce a heterogeneous group of small peptides (< 500 Da) that are used for communication between the female crab and the larvae at the time of hatching (Forward et al., 1987; De Vries and Forward, 1991). Once a few embryos hatch, these peptides are released from the egg and induce larval release behavior, involving vigorous pumping of the abdomen, in the female. This action helps to degrade the membranes of the egg case and results in the synchronized release of larvae (Forward and Lohmann, 1983). Thus, the embryos initiate hatching and the female helps synchronization. The results for mole crabs are consistent with the foregoing hatching sequence.

Mole crabs inhabit the swash zone of a sandy beach, where they are constantly subjected to the mechanical agitation of waves; yet female mole crabs do not rely on tidal cues to time larval release. Instead, mole crabs release larvae near the time of the onset of the dark phase of the LD cycle. Since larval release occurs in the intertidal zone of the sandy beach, mole crabs do not need to depend on the time of high tide to carry larvae away from the adult habitat. The physical wave motions and currents are most likely strong enough to transport larvae away from the shoreline to coastal areas for development. In addition, distribution studies demonstrate that ovigerous females are consistently found submerged in the lower intertidal zone during all phases of the tidal cycle (Cubit, 1969; Diaz, 1974; White, 1976) and therefore do not become stranded in the sand during a low tide. Thus, larvae can be released at sunset regardless of the time of the tide.

Synchronous hatching with respect to the LD cycle enhances the chances of larval survival by ensuring that larval offspring are placed in an appropriate environment at a time when the risk of predation is reduced. One hypothesis proposed for the functional advantages of the observed pattern of larval release suggests that the nighttime release of larvae protects female crabs from being preyed upon by visual predators while releasing their larvae (DeCoursey, 1979; Salmon et al., 1986). In addition, nocturnal egg hatching may be an adaptation to increase larval survival by avoiding planktivorous visual predators (Morgan, 1995; Morgan and Christy, 1995). The synchronous release of larvae at dusk reduces exposure to both diurnal and nocturnal predators because dusk is typically a time when predators switch—diurnal feeders seek shelter and nocturnal feeders emerge. In addition, synchronous egg hatching by a population of mole crabs minimizes the likelihood that the progeny of one female will suffer disproportionately from predation (Paula, 1989).

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