Chapter 3
Daily and Tidal Rhythms in Intertidal Marine Invertebrates

Christopher C. Chabot and Winsor H. Watson III

Abstract Intertidal animals are faced with strong rhythmic environmental signals on both daily and tidal frequencies, and their behavioral output is often complex because these two competing influences affect the endogenous clocks that drive their behaviors. This chapter reviews the nature of the clocks that govern the behaviors in intertidal organisms as well as the inputs and outputs from these clocks. The relative importance of the environmental factors that synchronize these rhythms is discussed, as well as the different types of physiological and behavioral outputs that have been observed. The molecular underpinnings of the circadian clocks are presented as well as the hypothesis that the clocks that drive circatidal rhythms are composed of the same molecular "machinery" used by circadian clocks.

Keywords Circadian • Circalunidian • Circatidal • Clocks • Horseshoe crab

3.1 Overview

There are many reasons one might refer to tidal rhythms as mysterious and enigmatic. First, although the presence of an endogenous timing system controlling tidal rhythms has been known for more than 50 years (Bennett et al. 1957), virtually nothing is known about the underlying physiological/molecular mechanisms that produce this approximately 12.4-h rhythm. Second, this approximately 12.4-h rhythm can vary somewhat in different locations because, although the tides in some areas occur twice daily (semidiurnal), in other areas there are once-daily tides, unequal semidiurnal tides, or even a lack of tides! It is not at all unusual for different populations of the same species to occur in areas that are exposed to all these types of tidal cycles. Finally, perhaps as a result of the underlying timing mechanisms combined with the variety of environmental cues that synchronize the endogenous tidal clocks, circatidal

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rhythms (with periods of about 12.4 h) tend to be less robust than circadian rhythms (periods ~24 h) and thus are more difficult to analyze in the laboratory or field. When all these issues are considered, it is no wonder that Palmer (1995a) warned: "Until one reaches tenure, it would be best not to enter this branch of the field"!

Nevertheless, during the past few decades, significant strides have been made in the understanding of the formal properties of these rhythms, similar to the state of the field of circadian rhythms biology in the early 1970s before the discovery of the *period* gene by Konopka and Benzer (1971). Since that time, the endogenous circadian clock "black box" has been opened and great strides have been made in the understanding of the molecular underpinnings of the circadian clock. Although only hints about the molecular basis of the circatidal clock have emerged thus far, the advent of clear, robust outputs from the clock and the availability of deep sequencing RNA and DNA technologies, makes these discoveries all but certain in the near future.

The aim of this chapter is to provide a summary of what is known about the timing systems, and environmental cues, that control the physiology and behavior of selected marine invertebrates. Because there are several excellent previously published reviews of tidal rhythms, we have chosen to focus our attention on a subset of marine invertebrates that express both daily and tidal rhythms, with an emphasis on the tidal rhythms, because we find this ability particularly enigmatic and thus appropriate for this volume. We have organized the chapter into three sections. First, we present a discussion of the types of behavioral and physiological outputs we use to monitor biological rhythms. Next, we outline the inputs or entraining agents that help to synchronize circatidal clocks to tidal cycles. Last, the formal properties that govern the clocks that control these circatidal rhythms are presented.

3.2 Outputs

Although coastal indigenous peoples have long been aware of the influence of the tides on intertidal organisms, endogenously generated circatidal rhythms were first reported in fiddler crabs by Bennett et al. (1957). As did many circadian biologists of the time, the early researchers used some type of overt behavior as their "window into the clock," because it is relatively easy to measure. Many of these clock pioneers used tilt-cage "actographs" as a way to monitor the activity of these intertidal species.

3.2.1 Laboratory Studies of Tidal Rhythms

Most of the early work on tidal rhythms focused on the European shore crab (known elsewhere around the world as the invasive green crab or green shore crab; Williams and Naylor 1969) and several species of fiddler crabs (*Uca* spp.; Bennett et al. 1957; Palmer 1963). Although these studies paved the way for many future experiments, they also shed light on many of the challenges inherent in studying tidal rhythms. Most importantly, it was clear that the tidal rhythms expressed by these animals were not as robust and persistent in constant conditions as circadian rhythms.

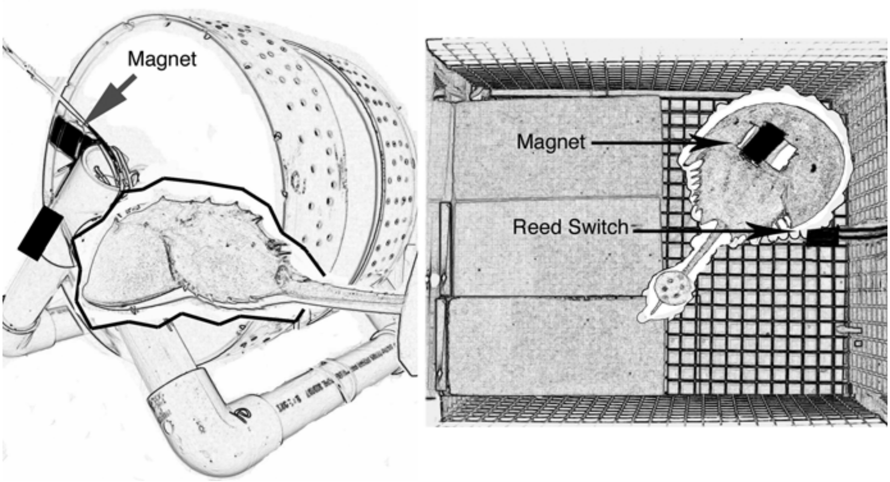

Fig. 3.1 Computer-rendered photos of a *Limulus* “running wheel” (*left panel*) and activity “square” (*right panel*). *Left:* Part of the running wheel has been “cut away” to reveal the position of the animal. Note the circular “tail limiter,” magnetic reed switch (*black rectangle* attached to the running-wheel leg), and magnet (*black rectangle*). *Right:* The ceiling of the square as well as some of the brick “roof” (*left*) has been cut away to reveal the animal. Note the magnet affixed to the dorsal carapace and the magnetic reed switch (*black rectangle at right* of diagram). (Reproduced from Chabot et al. 2007 with permission from Elsevier)

Another challenge with the study of tidal rhythms is developing appropriate methodology for recording the activity of aquatic species for many days at a time. Much of the early work was carried out using the aforementioned tilt cages, but recent technological advances have allowed scientists to use infrared beams, magnetic reed switches, and digital video analysis systems to monitor activity. Each of these techniques has its own set of advantages and disadvantages, and we have used all of them to obtain long-term recordings from both lobsters (Jury et al. 2005) and horseshoe crabs (Fig. 3.1; Chabot et al. 2007).

3.2.1.1 Examples of Tidal Rhythms Expressed by Selected Marine Invertebrates

Circatidal rhythms have been demonstrated in many intertidal invertebrates, including the shore crab *Carcinus maenas* (Naylor 1958), the isopod *Excirolana chiltoni* (Klapow 1972; Enright 1976), and the shrimp *Crangon crangon* (Aladhub and Naylor 1975). Because these investigations were conducted in the laboratory, in constant conditions with respect to tidal cues, the evidence is clear that there are endogenous oscillators that control the circatidal rhythms of locomotion in these species. The presence of such an oscillator(s) would allow these species in their natural habitat to anticipate changes in water depth and currents and adjust their behavior accordingly. Recently, clear endogenous circatidal rhythms have also been documented in

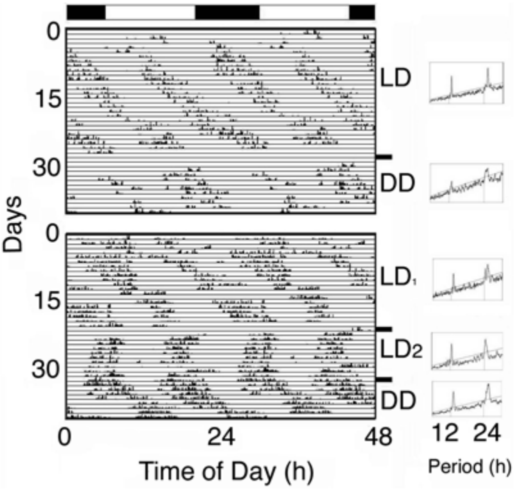

Fig. 3.2 Free-running locomotor rhythms expressed by two individual *Limulus polyphemus* exposed to a 14:10 light–dark (LD) cycle, clearly illustrating tidal patterns of activity (*left panels*). Data are double-plotted to facilitate visual inspection. Chi-square periodogram analyses of sections of the actograms (separated by *horizontal bar*) are presented in the *right panels*. LD$_1$ and LD$_2$ portions of the bottom actogram were analyzed separately to illustrate the different periods exhibited by this animals under this LD cycle [free-running (*Tau* = 12.45 h; LD$_1$) or entrained (*Tau* = 12.0 h, LD$_2$)]. The figure also demonstrates the robust persistence of free-running patterns under constant tidal conditions. (Reproduced from Chabot et al. 2007 with permission from Elsevier)

Limulus polyphemus, the American horseshoe crab (Fig. 3.2; Chabot et al. 2007). In this species, circatidal activity is seen in approximately two-thirds of the animals tested in the laboratory, at virtually all times of the year: summer (Chabot et al. 2004), later summer and fall (Chabot et al. 2007), and winter (Watson et al. 2009), so long as the water temperature is above 11 °C.

Circatidal rhythms of other physiological phenomena have also been observed in intertidal animals and, in general, these parameters are often related to overall activity. For example, a circatidal rhythm of oxygen consumption has been demonstrated in isopods (Marsh and Branch 1979), as well as circatidal rhythms of molting (Abelló et al. 1997), and heart rate (Styrishave et al. 1999) in the shore crab. Locomotion, heart rate, and ventilation activity are all closely correlated in horseshoe crabs (Fig. 3.3) and in sand crabs, *Portunus pelagicus* (Gribble and Broom 1996). The long-term synchrony between rhythms of heart rate and respiration is also supported by electrophysiological studies showing the close coordination of heart rate and gill ventilation in *L. polyphemus* (Watson and Wyse 1978) that occurs during daily activities (e.g., walking, gill cleaning). Interestingly, in the absence of activity, circatidal rhythms of respiration persist in *C. maenas* (Arudpragasam and Naylor 1964; unpublished data), demonstrating that locomotor activity expression is not necessary for the expression of respiratory rhythms. These results suggest that respiration and heart rate rhythms are not simply direct outcomes of locomotor activity rhythms and may indicate control by a common timing mechanism.

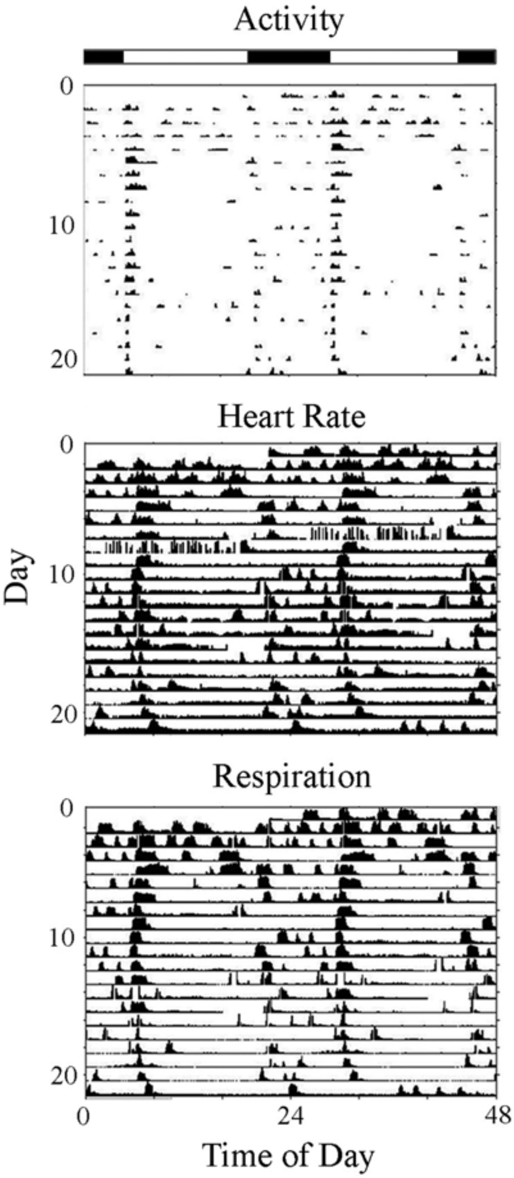

Fig. 3.3 Coordination of rhythms of activity, heart rate, and respiration in an individual *L. polyphemus* maintained for 21 days in a running wheel. The LD cycle (15:9) is indicated by *light/dark bars at the top*. Actograms are double-plotted, with size and position of *black marks* indicating the intensity and timing of activity (*top*), beats per minutes (*middle*), and respiration frequency (*bottom*). Heart rate and respiration were recorded after Chabot and Webb (2008)

3.2.1.2 Daily and Circadian Rhythms in Selected Marine Invertebrates

Time of day also influences the patterns of activity and associated physiological processes expressed by some marine invertebrates. In some cases, such as the color changes in fiddler crabs (Darnell 2012) and transcription and translation in mussels (Connor and Gracey 2011), there is good evidence that these daily rhythms are

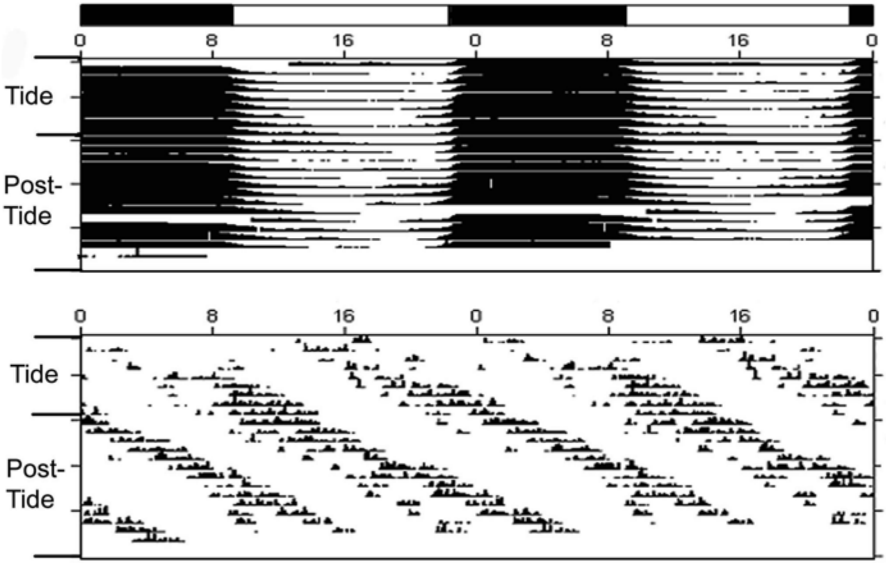

Fig. 3.4 Locomotor activity rhythms can be synchronized to artificial tides and are independent of daily rhythms of visual sensitivity. This animal was first exposed to changing cycles of water depth with a period of 12.4 h, for 8 days; then the imposed tides were turned off and the water level was kept at a constant intermediate depth for an additional 13 days. Although locomotion became entrained to the imposed tides (*bottom panel*), the electroretinogram rhythm remained entrained to the LD cycle (*top panel*). Units for the horizontal axes are hours. The LD cycle is indicated by the *light and dark bars*. (Reproduced from Watson et al. 2008 with permission from The Marine Biological Laboratory)

under the control of an endogenous circadian clock. Similarly, in horseshoe crabs, the sensitivity of their lateral eyes to light is controlled by a circadian clock that is located in the brain (Barlow et al. 1977; Barlow 1983). This change in sensitivity, controlled by input from a circadian clock, is the result of well over a dozen modifications of vision-related physiological parameters, such as dark adaptation (Kass and Berent 1988) and membrane shedding (Chamberlain and Barlow 1979). One might assume that these increases in visual sensitivity at night might serve to enhance the vision of a species that is primarily nocturnal and that the same circadian clock controlling vision would modulate locomotor activity. However, because locomotor activity tends to be expressed in circatidal frequencies (~12.4-h cycles), while eye sensitivity (as measured by electroretinograms) fluctuates with a circadian frequency (~24-h cycles), it is likely that they are controlled by different timing systems (Fig. 3.4; Watson et al. 2008). Thus, it appears that some marine invertebrates contain two different endogenous clocks.

Other life history stages of *L. polyphemus* also express both daily and tidal rhythms. After hatching, the larvae spend approximately 6 days in the water column where they are positively phototactic and nocturnally active (Rudloe 1979). Larvae collected in the field and brought into the laboratory will express a tidal rhythm of activity in constant conditions, becoming active near the start of the expected ebb tide, and can be entrained to 12.4-h cycles of agitation (Ehlinger and Tankersley 2006). Juveniles from both North Carolina (Casterlin and Reynolds 1979) and Massachusetts (Borst and Barlow 2002) are reported to be nocturnal in light–dark (LD) cycles. However, a close examination of the figure from the Borst and Barlow (2002) abstract shows two bouts of activity per 24 h, indicating the presence of circatidal rhythms at this stage as well. In a recent larger study of juvenile horseshoe crab biological rhythms (Dubosky et al. 2013), 63 % of animals exposed to a LD cycle expressed daily rhythms of activity whereas 25 % expressed circatidal rhythms. Of those that displayed a daily rhythm of activity, 47 % preferred to be most active in the day (diurnal activity) and 20 % preferred to be most active at night (nocturnal activity). Interestingly, when the LD cycles were terminated so that animals were in constant darkness (DD), more animals (54 %) began to express a circatidal rhythm, suggesting that exposure to the LD cycle may have "masked" expression of an underlying circatidal clock. Thus, in horseshoe crabs there is an interesting and complex interplay between LD cycles, daily rhythms, and circatidal rhythms, which is addressed in more depth in Sect. 3.3 below.

3.2.2 Biological Rhythms of Marine Invertebrates in Their Natural Habitat

Despite dozens of studies that have addressed activity rhythms of intertidal species, our understanding of the movement patterns displayed by free-living individuals is rather limited, primarily because of the challenges inherent in studying aquatic species. A main limitation is in our ability to obtain continuous data for many days in a row. Nevertheless, there are several examples in which tidal or daily rhythms of activity have been demonstrated. For example, New Zealand mud crabs exhibit circatidal rhythms in situ that are modulated by the solar day (Williams et al. 1985). In green crabs, *C. maenas*, it has been demonstrated that the depth at which animals are held influences the impact of LD cycles on their activity: LD has little or no influence on subtidal individuals (Lynch and Rochette 2007), although it does influence the behavior of individuals that inhabit the intertidal zone (Naylor 1963; Reid and Naylor 1989; Styrishave et al. 2003).

3.2.2.1 Daily and Tidal Rhythms Expressed by Horseshoe Crabs in Their Natural Habitat

It is well known that horseshoe crabs approach mating beaches to spawn during the high tides in the spring and early summer. However, just because groups of animals consistently appear at these beaches at the high tides, it does not mean they are inactive during other portions of the tidal cycle. Using ultrasonic telemetry we have shown that horseshoe crabs express a tidal pattern of behavior during the mating

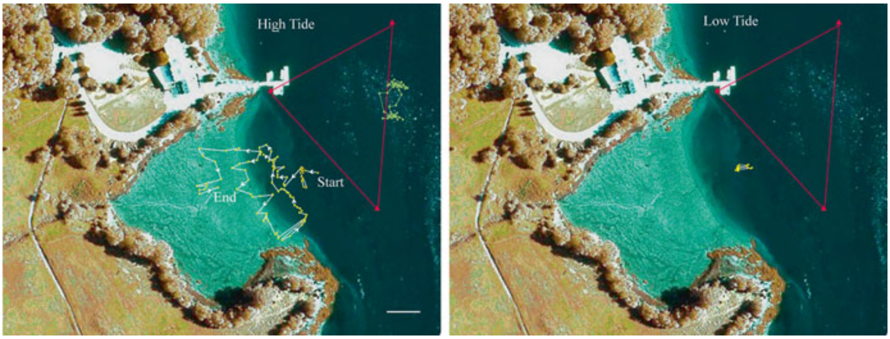

Fig. 3.5 Movements of a male horseshoe crab during a 5-h period when the tide was either high (*left*: peak high at 11:50 a.m.; data shown start at 12:30 p.m. and end at 5:30 p.m.) or low (*right*: peak low was at 7:30 a.m.; data shown start at 7:00 a.m. and end at noon). Each point represents one positional fix obtained from a VEMCO radio-linked acoustic positioning (*VRAP*) tracking system. The points have been superimposed on an infrared image of the cove that was colorized to look more "natural." The *red lines* connect each of the three VRAP buoys used to track the horseshoe crabs. In the *left panel the light green dots and lines* represent the position of the reference transmitter during the same time period (not shown in *right panel*). Note how it appears as if it moved, even though it was stationary, because buoy movements caused by the tides and waves generate small movement artifacts. *White scale bar on left panel* represents 20 m. (Reproduced from Watson and Chabot 2010, with permission from the Editorial Office, *Current Zoology*)

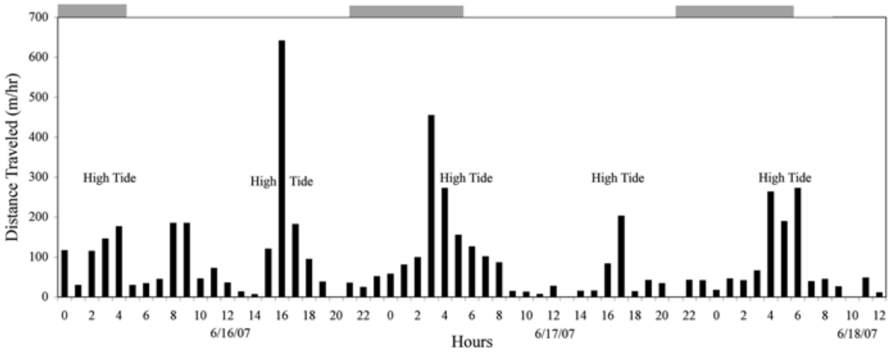

Fig. 3.6 Activity of a male horseshoe crab in June 2007, illustrating consistent increases of activity during high tides. Night hours are depicted by the *gray bars on the top* of the graph and peak high tides are also noted. (Reproduced from Watson and Chabot 2010, with permission from the Editorial Office, *Current Zoology*)

season (Figs. 3.5 and 3.6), and this tendency continued throughout most of the year, from May to October. Observations of these animals using SCUBA (self-contained underwater breathing apparatus) indicated that this tidal rhythm was most likely related to excursions onto intertidal mudflats to feed. Recent studies in the Great Bay estuary have revealed that there is intensive foraging activity by horseshoe crabs in these habitats (Lee 2010). Behavior in the field is also affected by LD cycles: telemetry studies of animals that expressed a tidal rhythm show a preference for activity during the daytime high tide (Watson and Chabot 2010). Similar results have also been seen in animals that are held in recording devices in the same estuary where the telemetry studies were conducted (Chabot and Watson 2010).

3.3 Inputs: Natural Stimuli That Entrain or Synchronize Biological Rhythms

Although light is clearly the most important zeitgeber, or cue, for entraining and synchronizing circadian clocks to natural LD cycles, there are many potential cues that may serve this purpose for tidal rhythms, including temperature, salinity, turbulence, current, turbidity, and depth. The influence of water-level changes on circatidal rhythms has been most thoroughly investigated, and this cue can entrain the activity of the amphipod (*Corophium volutator*; Harris and Morgan 1984), the portunid crab (*Liocarcinus holsatus*; Abelló et al. 1991), and the fiddler crab, *Uca crenulata* (Honeggar 1973), as well as many other species. Agitation or turbulence is an effective entraining agent for the mole crab *Emerita talpoida* (Forward et al. 2007), the amphipod *Synchelidium* spp. (Enright 1963), and the isopod *Eurydice pulchra* (Hastings 1981). The effects of tidal cues have been especially well-studied in *C. maenas*. This species can be entrained to cycles of temperature, inundation (Williams and Naylor 1969), or hydrostatic pressure (Reid and Naylor 1990). *C. maenas* is also one of the very few species in which the relative importance of tidal cues has also been assessed in situ (Styrishave et al. 1999, 2003).

3.3.1 Entrainment by Water Depth Changes and Inundation in Limulus

3.3.1.1 Laboratory Studies

In the laboratory, juvenile and adult horseshoe crabs exhibit endogenous circatidal rhythms that can be entrained to both water-level changes and inundation (Chabot et al. 2008; Dubosky et al. 2013), during both breeding and nonbreeding times of year. Large water-level changes (Fig. 3.7) or inundation cycles are effective entraining signals in this species; small water pressure changes are much less effective. For example, we found clear evidence of entrainment to water-level changes of 0.5 m, whereas depth changes of 0.2 m led to synchronization of activity to the artificial tides, but not clear entrainment (the rhythm did not persist after the artificial tides were stopped; Chabot et al. 2008). Although the “threshold” between 0.2 and 0.5 m is somewhat greater than those reported for *C. maenas* (Naylor and Atkinson 1972) and some planktonic species (0.1 m; Knight-Jones and Morgan 1966), our study appeared to use a more stringent definition of entrainment than the

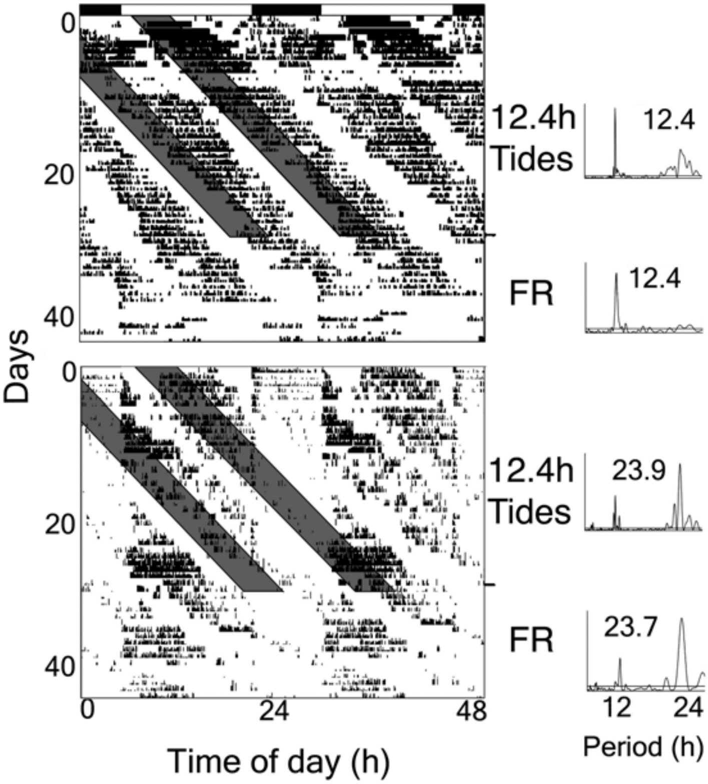

Fig. 3.7 Entrainment of tidal rhythms by artificial tides. These two animals were first exposed to 12.4-h artificial tides (dark parallelograms - high tides), and then the water depth was held constant and the entrained tidal rhythm of locomotion was allowed to free-run (*FR*). The light–dark cycle remained the same throughout the experiment (14:10). (Reproduced from Watson et al. 2008 with permission from The Marine Biological Laboratory)

previous studies. Some in situ results in horseshoe crabs also support the finding that stronger depth change signals increase the percentage of animals that entrain: 40 % of animals exhibited tidal rhythms in deeper water that experienced a 60 % change in water levels during each tide cycle, whereas nearly 80 % of those in running wheels anchored to the bottom in shallower water, and experiencing 300 % water pressure changes, showed tidal rhythms (Chabot and Watson 2010).

3.3.1.2 Rhythms Expressed by *Limulus* in Their Natural Habitat

Given the strong influence of artificial tides on the activity rhythms expressed by horseshoe crabs in the laboratory, one would expect most freely behaving horseshoe crabs to express tidal rhythms in the field. We tested this hypothesis in two different experiments. In the first, we found that most of the animals confined to running

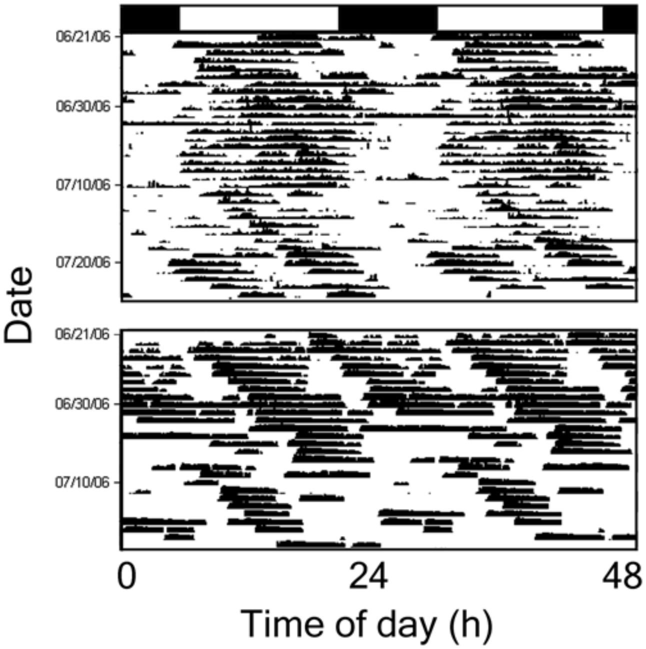

Fig. 3.8 Behavioral activity patterns expressed by two *Limulus* in running wheels that were either attached to a floating raft (*top*) or fixed to the bottom of the estuary (*bottom*). Approximate photoperiod is indicated by *LD bars at top*. Note that the animal attached to the floating raft was primarily diurnal (although with some evidence of tidal rhythmicity during the past 2 weeks) whereas the animal located at the bottom of the estuary was primarily tidal. (Reproduced from Chabot and Watson 2010, with permission from the Editorial Office, *Current Zoology*)

wheels in their natural habitat, where they were exposed to natural tide cycles, synchronized their activity to the tides (Watson et al. 2009; Chabot and Watson 2010; Fig. 3.8). In addition, we found that animals that were unable to sense the changes in water depth, because they were secured under a floating dock that rose and fell with the tides, failed to express a tidal rhythm of activity (Watson et al. 2009; Chabot et al. 2011) and exhibited clear daily rhythms instead. This observation that although factors such as current changes (0–0.2 m/s), temperature changes (Δ5 °C), salinity changes (Δ5 psu), and other physicochemical parameters may influence the behavior of horseshoe crabs, they are generally not sufficient to synchronize tidal rhythms. In the only other comparable field study of which we are aware, water pressure was also the primary tidal cue used by amphipods to synchronize their activity (Enright 1963): animals exposed to water pressure rhythms in situ entrained to the tidal cycles whereas those tethered to a buoy, like our animals tethered to a floating dock, did not. Overall, our data support the hypothesis that water-level changes are the dominant timing cue used by horseshoe crabs to synchronize their activity to the tides (Watson et al. 2009). However, in their natural habitat, when exposed to both tides and natural LD cycles, their rhythms are more complex than we anticipated.

We fitted animals with accelerometers that transmitted activity data to receivers located throughout the estuary so that we could gather activity data from animals that were freely moving in their natural habitat and exposed to all the normal cues. Interestingly, although many of these animals consistently expressed a tidal rhythm of activity, several were more active in the day or the night. Therefore, in their natural habitat, many other factors must be influencing the likelihood that they will express a tidal pattern of locomotion, or a pattern of activity that is the result of a variety of different cues.

The influence of water levels and depth changes on horseshoe crab activity can also be seen in *Limulus* breeding rhythms in the field. Populations that are exposed to tides of at least 0.5 m or so synchronize to the tides (Rudloe 1979, 1980, 1985; Cohen and Brockmann 1983; Barlow et al. 1986), whereas those that experience only microtides (of a few centimeters) do not (Ehlinger et al. 2003). Further, wind action leading to increased or decreased tidal heights significantly affects the number of animals breeding (Brockmann 2003). In addition, several studies have also shown a correlation between the height of the tides and the number of animals breeding, so that, for example, more animals are found on breeding beaches around the new and full moons in Massachusetts (Barlow et al. 1986) and Florida (Rudloe 1980). However, this relationship does not appear to be true at all breeding locations, most likely because different breeding beaches have different slopes, sizes, and sediment types.

3.3.2 The Influence of Temperature, Turbulence, and Salinity on Tidal Rhythms

Other tidal cues can also be effective in entraining or synchronizing tidal rhythms, depending on the species. Salinity changes appear to be effective synchronizing agents for the crabs *Rhithropanopeus harrissii* (Forward et al. 1986) and *C. maenas* (Reid and Naylor 1990), although periodic agitation is sufficient to entrain tidal rhythms in two species of isopods (Klapow 1972; Hastings 1981) and juvenile horseshoe crabs (Ehlinger and Tankersley 2006). Finally, in animals such as mole crabs (Forward et al. 2007), amphipods (Enright 1963), and isopods (Hastings 1981) that inhabit the swash zone, mechanical stimulation appears to be a very important stimulus for entraining rhythms.

In horseshoe crabs, temperature cycles do not appear to be as effective as water-level changes and turbulence cues appear to be less effective than temperature (Chabot et al. 2008). Interestingly, the magnitude of the cue seems to matter, suggesting a threshold effect for temperature as well as for water depth changes. Similar results were found in *Carcinus*: 11 °C cycles are effective entraining agents whereas 4 °C temperature cycles are not (Williams and Naylor 1969).

Artificial currents appear to be even weaker synchronizing agents. Whether in the lab or in running wheels attached to a floating raft in the estuary, horseshoe crabs do not entrain even if exposed to relatively strong currents of 0.2 m/s (Chabot et al. 2008). Interestingly, there is good evidence that larval *Limulus* entrain to cycles of mechanical agitation simulating the turbulence created by the incoming tide (Ehlinger and Tankersley 2006). However, other than during the mating season, juvenile and adult horseshoe crabs rarely inhabit areas where they would encounter significant wave- or wind-induced turbulence, or other types of agitation. Therefore, not surprisingly, the types of cues that are most effective in synchronizing and entraining the tidal rhythms of many marine invertebrates are closely related to their normal habitats and life history strategies. Even within a single species, such as the horseshoe crab, the best entrainment cues depend on both the life history stage and the location where they live.

3.3.3 The Influence of Light on the Expression of Rhythms

Although “The majority of tide-associated rhythms that have been described in different laboratories and different species, have not identified a solar-day influence” (Palmer 1995a), some intertidal animals do appear to be affected by LD cycles. Although many physical factors that fluctuate with the changing tides are likely to entrain or influence endogenous tidal rhythms, LD cycles have an entirely different period. Thus, one would predict that the tidal rhythms of most tidal species would not be affected by the LD cycle (DeCoursey 1983; Palmer 1995a). However, tidal-associated activity in *Sesarma pictum* (Saigusa 1992) and *U. crenulata* (Honeggar 1973) can be phase shifted by LD cycles. In addition, LD cycles appear to cause some transient synchronization of circatidal rhythms in other crustacean species (*Sesarma*, Saigusa 1988; *Hemigrapsus*, Saigusa and Kawagoye 1997).

In horseshoe crabs, we have shown in several studies that LD cycles influence the types of rhythms that horseshoe crabs express and that tidal rhythms can be influenced by LD cycles. Approximately one-third of animals studied in the laboratory exhibit daily rhythms, preferring to be most active during either the night or the day (Fig. 3.9). In several cases the circatidal rhythms expressed by some animals have been entrained by LD cycles and, when released into DD, these rhythms appeared to free run from the point of entrainment (Chabot et al. 2007). We have also observed a transient synchronization in many animals (Fig. 3.10), otherwise known as relative coordination (Johnson et al. 2004), which is indicative of a relatively weak entraining agent influencing an oscillator. Interestingly, beach isopod behavior is also affected by LD cycles, but only if well fed, when they avoid swimming during the day (Reid 1988). It is worth noting that our animals were not fed during the experiments cited here, so hunger could have influenced their propensity to express tidal rhythms.

There appear to be additional effects of LD cycles on circatidal rhythms. In two previous studies, LD cycles seem to allow for more robust circatidal rhythms in the semiterrestrial crab (Saigusa 1992) and the Pacific fiddler crab *Uca princeps* (Stillman and Barnwell 2004). Similarly, we have seen clearer tidal rhythms in LD than DD in horseshoe crabs (Chabot and Watson 2010). Although one would not expect photoperiod to have any effect on circatidal rhythms, because photoperiod is obviously not a strong predictor of tidal phase, light may indirectly act on the underlying oscillators

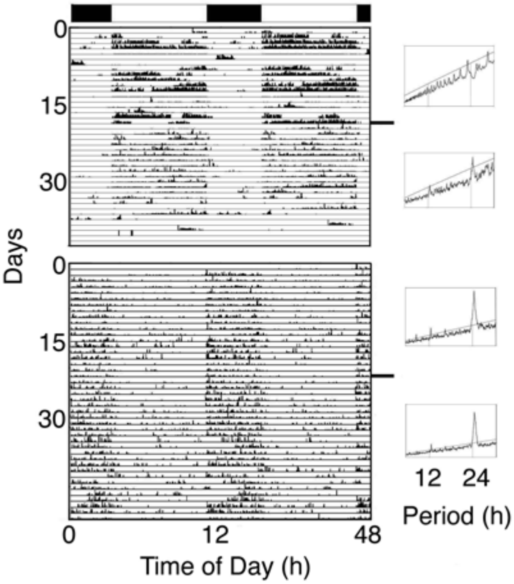

Fig. 3.9 Running-wheel activity of two horseshoe crabs exposed to a 14:10 LD cycle that exhibited a preference ($P < 0.05$) for daytime activity (*top*) or nighttime activity (*bottom*). (Reproduced from Chabot et al. 2007 with permission from Elsevier)

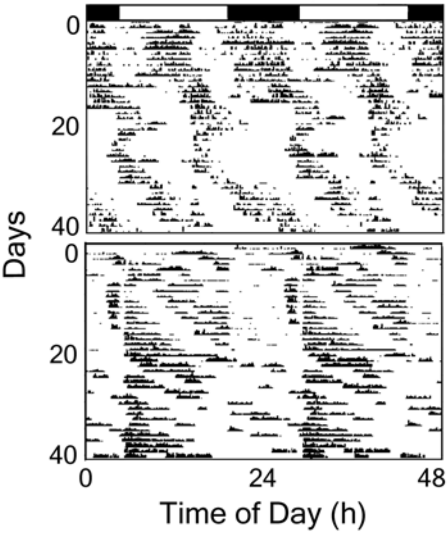

Fig. 3.10 Effects of LD cycles on locomotor activity in two *Limulus*. *Top*: Note the apparent transient synchronization to the LD cycles for the first 20–30 days and the subsequent free-run at approximately day 26. *Bottom*: Note the bouts of tidal activity that free-run through the first few weeks during L but not D. Note also the instant increase in activity upon lights on and the instant cessation of activity at lights off. (Reproduced from Chabot and Watson 2010, with permission from the Editorial Office, *Current Zoology*)

controlling locomotor activity. In particular, the lights-on and lights-off transitions appear to cause a large percentage of animals to begin to move (Fig. 3.10). In mammals, an initiation of activity can induce phase shifts in some species (Wickland and Turek 1991; Gannon and Rea 1995; Buxton et al. 2003). A similar feedback into the clocks that mediate circatidal rhythms may help to explain how light may affect circatidal rhythms. Another working hypothesis is that in bays and estuaries, characterized by turbid water that limits the penetration of light, horseshoe crabs are exposed to tidally fluctuating light levels during the day. In Great Bay, for example, light levels drop by about 1 order of magnitude for each 1 m of depth. Therefore, when horseshoe crabs are in shallow water they are exposed to changes in light levels with each tide cycle, and when they move into very shallow water to mate, the change is even greater. Thus, the clock controlling circatidal rhythms might be sensitive to changes in illumination to provide a redundant tidal zeitgeber.

Overall, it appears that for many species multiple, possibly redundant, cues enable intertidal organisms to synchronize to tidal cycles. Water-level changes seem to be the strongest entraining agent for horseshoe crab activity, but turbulence and temperature appear to contribute to entrainment as well. In a number of marine animals one cue is not sufficient to entrain their behavior, but when presented with a key zeitgeber the entrainment is further enhanced. One species, the amphipod *Corophium volunator*, entrains to cycles of 10 °C but shows greater entrainment when subjected to temperature cycles in conjunction with cycles of immersion (Holmstrom and Morgan 1983). Water pressure and immersion cycles appear to also be important synchronizers in *C. maenas*, and temperature may also serve to enhance entrainment to these cues (Naylor et al. 1971). Similar findings have been observed in other crustacean species as well (Williams and Naylor 1969; Naylor and Williams 1984).

3.3.4 Seasonal Changes in Activity: The Effects of Temperature and Photoperiod

There are few documented seasonal changes in circatidal activity patterns in intertidal invertebrates, and nearly all of these appear to be rhythms of larval release, not locomotion (Thurman 2004). However, the phasing of circatidal rhythms appears to be seasonally adjusted in the amphipod *Corophium volutator* (Harris and Morgan 1984), which the authors attributed to interactions between environmental temperature and endogenous rhythms.

Horseshoe crabs have very clear seasonal changes in behavior that appear to be modulated by temperature. In the spring, in New Hampshire, they become active when the water temperature reaches 8–10 °C or higher (Schaller et al. 2010) and breed at the water's edge during high tides for about a 1-month period in the late spring (Watson et al. 2008). The timing of the breeding season varies somewhat along the East Coast of the United States, with animals mating a little earlier in the year in more southern regions (Barlow et al. 1986; Rudloe 1980; Brockmann and Smith 2009). They remain very active during the remainder of the summer and into the fall (Schaller et al. 2010) and then become more and more sedentary and quiescent when the water temperature drops below about 8 °C (Watson et al. 2009; Watson and Chabot 2010).

Virtually the same pattern of activity occurs in horseshoe crabs exposed to different temperatures in the laboratory, regardless of the LD cycles to which they are exposed. When horseshoe crabs in running wheels were exposed to 17 °C, the majority of them expressed tidal rhythms of activity (Fig. 3.11) (Watson et al. 2009; Chabot and Watson 2010). In contrast, if the water temperature was reduced to

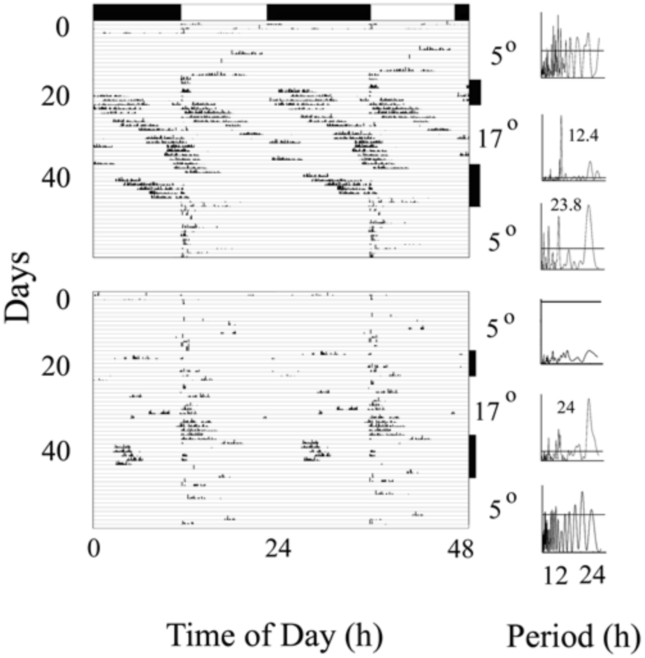

Fig. 3.11 Effects of water temperature on the expression of endogenous circatidal rhythms in two *Limulus* exposed to LD. Periods of increasing or decreasing temperatures are indicated by *black boxes* to the immediate right of the actograms. *Right panels*: Lomb–Scargle periodograms of portions of the activity records as delineated by temperature. *Horizontal line*: level of significance ($P < 0.01$). *Values above line*: highest peak period within the circatidal (10.4–14.4 h) or circadian range (22–26 h). (Reproduced from Chabot and Watson 2010, with permission from the Editorial Office, *Current Zoology*)

11 °C or below, they did not express a tidal rhythm of locomotion, even if they were exposed to an LD cycle of 14:10, typical of the spring and summer. At 4 °C, activity rhythms were completely suppressed and activity levels were greatly attenuated.

3.4 Endogenous Clocks Controlling Circatidal Activity

Although circatidal activity patterns have been documented in many intertidal species, the nature of the clock system that controls these rhythms is somewhat controversial (Palmer 1995b, 1997; Naylor 1996, 1997). An outstanding question in the field of biological rhythms is whether the locomotor activity of intertidal species is driven by two circalunidian clocks (clocks with a free-running periodicity similar to the periodicity of the lunar day, i.e., 24.8 h) or by one circatidal clock. Naylor and colleagues (Reid and Naylor 1989) have hypothesized that intertidal animals have one circatidal clock, with a period of about 12.4 h, that drives behavioral rhythms and one circadian clock which modifies those rhythms (Naylor 1958). Alternatively, the

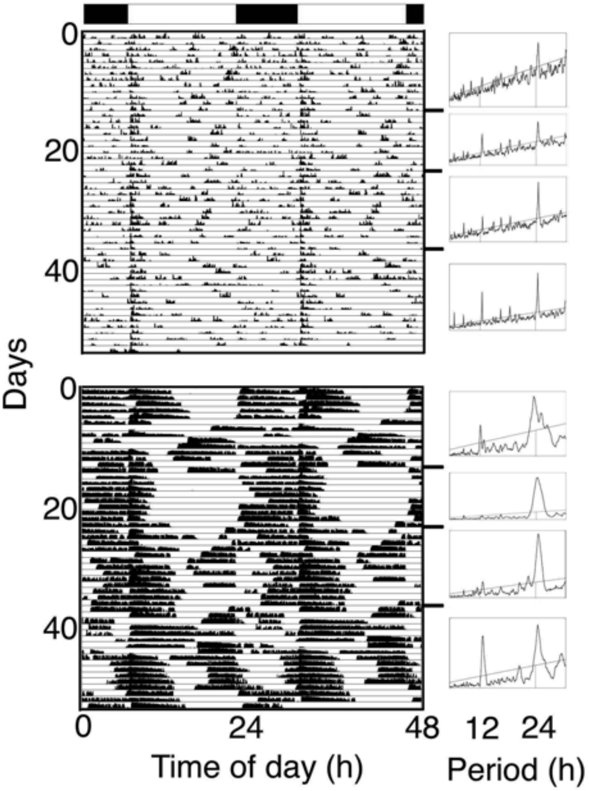

Fig. 3.12 Running-wheel activity of two horseshoe crabs exposed to14:10 LD and exhibiting "masking" (direct effects of lights-on or lights-off) as well as spontaneous changes in *tau* (*bottom panel*). *Top panel:* lights on, increased activity; *bottom panel:* lights off, increased activity. (Reproduced from Chabot et al. 2007 with permission from Elsevier)

circalunidian hypothesis, first proposed by Palmer and Williams (1986), proposes that circatidal rhythms can be best explained by the presence of two circalunidian clocks, each with a period of about 24.8 h (lunidian) and 180° out of phase with one another. Behaviorally, this can lead to major activity bouts every 12.4 h, just like a circatidal clock would. Currently, our data (Figs. 3.12, 3.13) seem to fit the three criteria put forth by Palmer (1997) as part of the circalunidian hypothesis: (1) evidence of two components of rhythms that scan the day at different periodicities (Fig. 3.12, bottom panel); (2) evidence of "skipping," sudden alternations of unimodal and bimodal patterns (Fig. 3.13, top panel, circled; and (3) "splitting," the separation of one component into two components (Fig. 3.12, bottom panel). All three of these criteria were most clearly met by the activity patterns illustrated in these figures (Figs. 3.12 and 3.13). Similar splitting of two "circalunidian components" has also been seen in the locomotor activity of the crab *Helice crassa* (Palmer and Williams 1986). However, the relative rarity of these phenomena in our data suggests that if there are two oscillators governing the timing of locomotion in *Limulus* they are strongly coupled. Thus, although we have some evidence to support the circalunidian hypothesis, convincing evidence supporting a coupled, two-oscillator system (such as is found in trout; Erikson 1973, cited in Pittendrigh 1981; hamsters Pittendrigh and Daan 1976; and *Drosophila* Stoleru et al. 2004) has yet to be obtained.

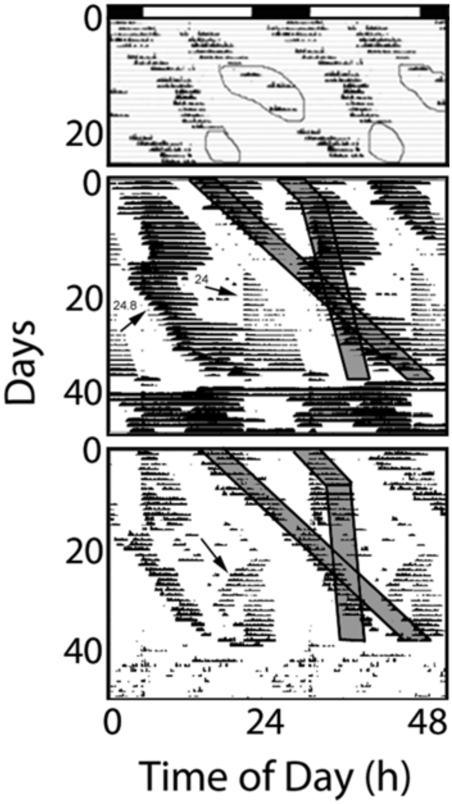

Fig. 3.13 Evidence for the presence of two circalunidian oscillators in *Limulus polyphemus* exposed to LD. *Top panels*: Free-running circatidal activity rhythm shows "skipping" of one of two components (*circled*). *Bottom two panels*: Effects of 12.4 and 12.1 cycles of water pressure on locomotor activity patterns in *Limulus*. Rising water levels indicated by *shaded boxes*: first 8 days, 2 cycles/day delivered each at 12.4 h; next 30 days, 2 cycles/day (one at 12.4 h; other at 12.1 h); remaining 15 days, constant water levels. Note the different periods of each component in the *middle panel* (*two arrows,* values = associated tau). Note also the apparent drift of at least one of the components (*arrow,* tau = 11.6 h) in the *bottom panel* when the 12.1-h tides were initiated. (Reproduced from Chabot and Watson2010, with permission from the Editorial Office, *Current Zoology*)

3.5 Molecular Mechanisms

Much is known about the molecular basis of circadian clocks, but virtually nothing is known about the molecular basis of circatidal clocks. The animal circadian clock has a core molecular mechanism based on the products of four or five genes that undergo a transcriptional and translational rhythm of approximately 24 h (Bell-Pedersen et al. 2005; Hardin 2009). Importantly, these core proteins appear to be conserved in marine invertebrates such as *Aplysia* and *Bulla* (Siwicki et al. 1989), and the lobster (Grabek and Chabot 2012). As it is thought that "evolution is a tinkerer" (Jacob 1977), it is tempting to speculate that the molecular underpinnings of the circadian clock may have been co-opted and modified to create clocks that help intertidal animals to synchronize to tidal cycles. As detailed earlier, one model for how circatidal rhythms might be controlled calls for two circalunidian clocks that keep time in the circadian range (periods of 24.8 h are still well within the circadian range) (Palmer 1995a), but are 180° out of phase, so that each clock could influence the probability that an animal would be active during one of the two high (or low) tides in a day. The presence of two clocks (that beat in this range) has been established in many organisms, including hamsters (Pittendrigh and Daan 1976) and fruit flies (Stoleru et al. 2004). In *Drosophila*, these two clocks couple the animal's activity cycle to dusk and dawn independently, and they are composed of two separate populations of cells in the fly's brain (Stoleru et al. 2004). Therefore, it is not too much of a stretch to imagine that circatidal rhythms might be controlled in the same way, by two circalunidian clocks of about 24.8 h. Efforts are currently underway in several laboratories to investigate this possibility.

3.6 Summary

The rhythms of marine intertidal invertebrates are truly enigmatic. Although many of the animals that inhabit the tidal zone exhibit tidal rhythms, these rhythms often disappear and reappear spontaneously. Although the disappearance of these rhythms in the field can be explained by strong winds, foraging, satiety, large salinity events, etc., the spontaneous disappearance of the rhythms in the lab is certainly enigmatic. The fact that tidal rhythms are not as robust as circadian rhythms may also be explained by comparing the high fidelity of LD cycles to the much lower fidelity of environmental tidal cycles. However, perhaps the observation that "muddies the water the most" (pun intended) is that behavioral tidal rhythms are often modulated, and even synchronized, by LD cycles. Although there are plausible explanations for this synchronization, none of them is completely compelling and a clear explanation of this phenomenon must await genetic and molecular analyses. What is clear is that a number of animal models have emerged to enable researchers to begin to open the "black box" and to identify the molecular gears that keep time in accordance with tidal and solar cycles.

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