Chapter 1
Timing in Tidal, Semilunar, and Lunar Rhythms

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Abstract Tidal, semilunar, and lunar rhythms are particularly pronounced in intertidal organisms, which are affected by strong fluctuations of environmental factors with the tides. For a number of species it has been shown that their tidal, semilunar, or lunar rhythms in behavior and reproduction are not a direct response to the tides, but reflect the action of endogenous biological clocks. This chapter reviews current knowledge on the properties and mechanisms of circatidal and circa(semi)lunar clocks as shown by laboratory experiments and highlights major open questions for future research.

Keywords Biological clock • Chronobiology • *Clunio* • Marine • Tides

1.1 Introduction

The interface between sea and land represents an extremely harsh environment as a result of the strong periodic impact of rising and falling tides (called 'flood' and 'ebb,' respectively), which superimpose the periodic alternations of day and night as well as that of the seasons. It is a fascinating world for any ecophysiologist who is interested in the complex timing adaptations that have evolved in the algae and animals of this intertidal zone.

In contrast to the multitude of experimental studies on daily and photoperiodic timing and on the involvement of circadian clocks in plants, animals, and in humans as well, the first carefully controlled laboratory experiments on tidal or lunar biorhythms did not start until the late 1950s. Since then, a considerable variety of

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Sadly, Dietrich Neumann passed away while he was still working on this chapter. Tobias Kaiser kindly has taken on the responsibility for the chapter and, thanks to his efforts, it has been brought to completion.
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intertidal animals has been examined, mainly with regard to locomotor behavior or reproductive timing. The gradual increase in knowledge in this field of chronobiology has been documented in books and reviews (Bünning 1973; Palmer 1973, 1995; Enright 1975; DeCoursey 1976; Neumann 1981; Naylor 2010). As a selection from the large body of literature, this introductory chapter presents those case studies that give the most detailed and comprehensive information and could be the basis for further research. Biological phenomena correlated with the synodic month of the moon in terrestrial or limnetic biotopes remain unconsidered in this overview.

1.2 General Definitions

1.2.1 Tidal Regimes

On the average, *tidal cycles* have a period of 12.4 h at most coastal locations and estuaries, reflecting the lunar day (24.8 h). It is important to notice that the intertidal range of these *semidiurnal tides* varies in parallel to the phases of the moon. *Spring tides*, with their maximum tidal range, occur every 14.77 days (or half a synodic month of 29.53 days) when the earth, sun, and moon are situated in an approximately straight line and the tide-generating forces of the moon and sun reinforce each other. They occur around days of full and new moons and alternate with *neap tides* of smaller amplitude. The latter occur around the first and the last quarter of the moon when the sun–earth axis and the moon–earth axis form a right angle, so that the tide-generating forces of the sun and moon counteract maximally and the resulting tidal amplitude is at a minimum. The levels of spring high water and spring low water define the extension of the *intertidal zone*.

The semidiurnal tides with their marked situations of high and low water shift from day to day by about 50 min as a result of the delayed meridian transit of the moon on each day. The daily delays of about 0.8 h add up to 12 h during half a synodic month of 14.77 days. As a consequence, any distinct tidal situation (e.g., ‘low water time on a spring tide day’) recurs at the same time of day after half a synodic month (14.77 days). The tides are further influenced by coastline geography, resulting in two important limitations to this general rule. First, this rule is true for each distinct coastal location, but the respective time point will differ between coastal locations; that is, ‘low water time on a spring tide day’ will differ between geographic locations but not at a given geographic location. Second, in places with regular semidiurnal tides this rule is more or less valid for any tidal situation, for example, also for ‘high water on a neap tide day.’ However, at some coasts, coastline geography produces so-called *mixed semidiurnal tides*, which may have interesting daily and seasonal irregularities in the successive levels of high and low waters. Such complex tidal inequalities are mentioned only in context with corresponding biorhythmic adaptations here. In any case, reliable tide tables are available today for nearly all coasts and for any time of the year, so that a chronobiologist who is interested in intertidal timing phenomena does not need to record anew the complex tidal regime of a distinct place; the tide tables allow looking up specific locations where a particular physiological timing adaptation of an intertidal organisms can be expected to be found with regard to part of year, time of synodic month, or time of day.

1.2.2 Intertidal Areas

Intertidal areas occur along the coastline of oceans and their subseas or along the shore of estuaries. They differ in their local maximum *tidal amplitude* (between a few decimeters and far more than 10 m, depending on various geophysical conditions of the sea basin and its shore). Correspondingly, the *spatial extension* of the resulting intertidal zone can be very different. At coasts with strong tidal amplitude, this zone will be relatively small at rocky locations with a steep shore, but considerably larger at, for example, intertidal mudflats, fringing reefs, or tropical mangrove forests. In the Mediterranean Sea, the intertidal area is a relatively narrow zone as a consequence of small tidal amplitudes, meaning that wind from land or sea can more significantly determine the exposure or flooding of this biotope. In any case, intertidal species living between sublittoral and supralittoral areas (for littoral zoning, see Mann 2000) are confronted with the impact of fluctuating physical forces, for example, with strong sea surf at unsheltered coastlines (especially during rising tide), with high solar irradiation during exposure of the intertidal area (especially in subtropical areas during summer), or even with strong water flow and changes of salinity in the range of estuaries.

1.2.3 Intertidal Organisms

Most species that have settled in this intertidal world are of marine origin, such as sessile periphytic algae, sessile and vagile zoobenthos, and littoral fish. Most are physiologically more active during inundation and tolerate exposure to terrestrial-like conditions twice a day, at least in the midlittoral zone. In contrast, some marine crustaceans (such as fiddler crabs), the greenish turbellarian *Symsagittifera roscoffensis* (originally named *Convoluta roscoffensis*), and some genera of terrestrial origin (adults of marine insects and other arthropods) have adapted their activities to intervals when the intertidal biotope is falling dry. In the case of mangroves, even trees have colonized the intertidal zone. Wherever these intertidal organisms may have originated, a reliable timing of some of their physiological features is of considerable selective advantage, may it be in metabolism, color change, locomotor behavior (including migrations), or in development and reproductive timing. At the beginning of experimental studies, the principal question arose as to whether the reliable synchronization of biological events to the very strict conditions of the intertidal habitat is forced by direct responses to distinct tidal or lunar factors or whether these physiological adaptations are actively controlled by endogenous physiological timing mechanisms and their specifically selected tidal or lunar time cues. The general answer is that physiological clocklike mechanisms are involved in the timing of behavior and reproduction time.

1.2.4 Selected Types of Biological Rhythms

Various biological rhythms resulting from evolutionary adaptations can be distinguished in intertidal organisms: (a) *tidal rhythms*, which are correlated with the semidiurnal cycle of tides and shift daily by about 50 min (for exceptions of *diurnal tides* and *mixed tides* at distinct coastlines, see Barnwell 1976), (b) *semilunar rhythms* (more precisely, *semimonthly-lunar* rhythms), which are correlated with the cycle of spring or neap tides (mean period, 14.77 days), and (c) *lunar rhythms* (more precisely, *monthly-lunar* rhythms), which are correlated with every second spring or neap tide event (mean period, 29.53 days).

As already outlined (Sect. 1.2.1), in the case of semidiurnal tides a certain tidal situation reoccurs at approximately the same time of day every 14–15 days (or, in more chronobiological terms, the same *phase relationship* of the daily cycle and the tidal cycle reoccurs every 14–15 days, i.e., in a semilunar cycle). Because of that, it may be advantageous for intertidal species to couple their independent semilunar or lunar timing processes to a daily timing process. An additional timing process comes into play during reproduction events, when the overt semilunar or lunar activity only occurs during a certain season.

1.3 Classic Examples

The nuptial dances of the Pacific palolos are a widely known example of a lunar rhythm in an intertidal invertebrate species. The palolos, which are the sexually mature, posterior part of the bisexual polychaete *Eunice viridis*, are released from the worms on the island of Samoa at about midnight only during neap tide high-water times on days of the waning moon in October or November, when the fringing reefs are flooded but the surf is mild. Their sexual maturation has already begun during the previous months. This predictable temporal occurrence has seasonal, lunar, and daily components (Hauenschild et al. 1968). This highly regular natural phenomenon has found a place in the cultural heritage of the indigenous people of these islands, as the considerable epitokous parts of the worms (full of sperm or eggs) present a festive dish once or sometimes twice a year.

Carefully conducted field observations by natural biologists in the nineteenth and early twentieth century revealed further clear examples of both lunar and tidal rhythms of intertidal species. One of the first may have been discovered by René Chevrel (1894), whose report ‘Sur un diptère marin’ was overlooked for many decades. He observed the tiny, short-lived midge *Clunio* at the coast of Normandy in France and named it *Clunio syzygialis* (later synonymized with *Clunio marinus*) because its reproduction in the lower intertidal zone only occurred between April and October at about the afternoon low-water time on days with spring tides correlated with the 'syzygy' (which is the astronomical term for full and new moon positions, when earth and moon have the same heliocentric ecliptic longitude). Today we know that the female *Clunio* prefer the felt-like substrates of red and brown algae and fine sand on rocks of the lower midlittoral (which is only exposed on these days and which is the best substrate for larval development) for egg deposition. Their development takes from at least 6 weeks to about 1 year (depending on latitude and season); each of the tiny adult midges (1.5–2.0 mm) is only capable of reproduction for about 1 h directly after eclosion (Neumann 1966, 1986).

A much more dramatic semilunar-periodic event is the run of the grunion fish (*Leuresthes tenuis*) on sandy beaches of California. These fish swim to the moist upper beach during nocturnal high tides about every spring tide (on 2–4 successive days) between February and August. The females bury their eggs in the sand and attract mature male fish, which fertilize the eggs. The fertilized eggs develop on the warm beach during the following days (which have a smaller tidal amplitude) until the larvae can hatch when the next spring tide washes the upper beach again (Walker 1949).

In general, tidal activities of intertidal species are more difficult to observe than semilunar or lunar activities, because most of them take place when the terrain is flooded. Examples of exceptions are the locomotor behaviors of fiddler crabs (*Uca* spp.) and the turbellarian *S. roscoffensis*. The latter harbor symbiotic phototrophic algae in their digestive tracts (which give them their characteristic green color) and expose their bodies to sunlight during the ebbing tide when the sandy habitat becomes exposed; they return into the substrate before the next rising tide arrives.

1.4 Tidal Rhythms

1.4.1 First Laboratory Experiments

During the past 50 years a variety of tidal biorhythms have been studied in selected intertidal species kept under controlled laboratory conditions, in absence of tidal influences and nocturnal moonlight. The first persistent free-running tidal biorhythms recorded were the locomotor activity rhythms of individual fiddler crabs, *Uca* (Bennet et al. 1957), and green shore crabs, *Carcinus* (Naylor 1958).

On the basis of these early results, two opposing interpretations were put forward to explain the free-running tidal rhythms: direct influence of weak geophysical influences of the moon (possibly modulated at least in the laboratory) or overt rhythms of an endogenous clockwork, which would (in the field) be controlled by environmental time cues analogous to circadian biorhythms. Using an actograph, Naylor (1960) recorded 'dock crabs' from nontidal docks and 'shore crabs' (*Carcinus maenas*) from the intertidal zone. The 'dock crabs' [kept under 24-h light–dark (LD) cycles] showed mainly nocturnal activity peaks, but in continuous bright or dim light the activity peaks drifted with a period of about 12–13 h for 5 to 6 days. In nontidal aquaria the initial strong tidal rhythm of the 'shore crabs' became less and less pronounced until after 4–8 weeks they behaved quite the same as the ‘dock crabs’ when put in continuous conditions. Naylor concluded that the similar daily drift of the rhythm in both ‘shore crabs’ and ‘dock crabs,’ irrespective of the original tidal or nontidal habitat and of the entrained behavior, reflects an endogenous tidal rhythmicity. In the discussion of a paper on tidal rhythmicity in marine organisms by Fingerman at the now-famous Cold Spring Harbor Symposium of 1960, Enright presented the clear-cut locomotor activity rhythm of groups of the sand-beach amphipod *Synchelidium* from a Californian beach where mixed semidiurnal tides occur and successive tides on any given day can differ considerably in amplitude and period (see Fingerman 1960, discussion). His activity recordings of freshly caught specimens, made at 15- or 30-min intervals under constant conditions (Fig. 1.1), reflected both amplitude and timing of the actual local tide pattern but with a significantly longer period than that of the tidal cycle in the field; the rhythm persisted for the following days until damping. In this context, Enright was presumably the first to use the term “circatidal” for such a free-running tidal pattern, ‘by analogy with circadian’ (and thus opposing speculations on a direct influence of any weak geophysical influences) and ‘in contrast with previous reports of long continued precision of tidal rhythms in other organisms’ (these reports not being confirmed in later studies).

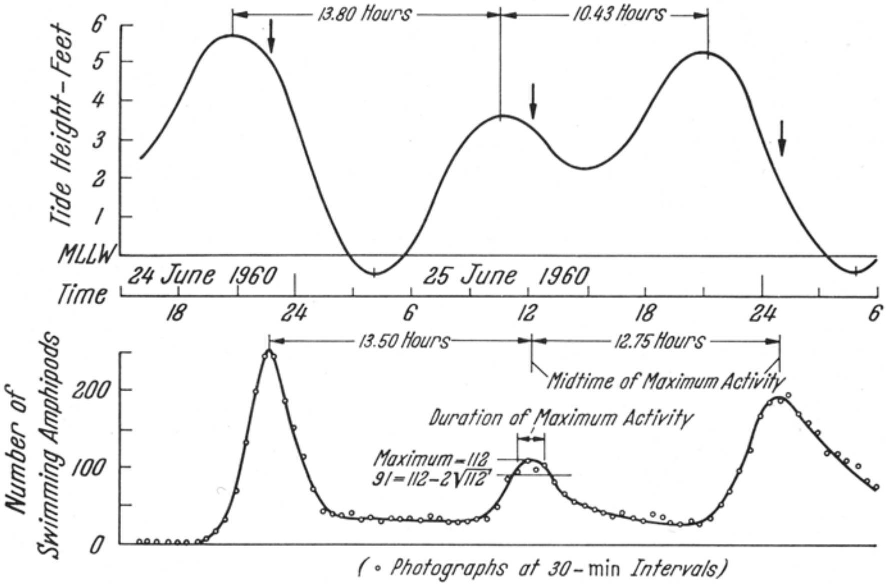

Fig. 1.1 Experiment under constant light and temperature conditions demonstrating the ‘circatidal’ rhythm of freshly collected amphipods (*Synchelidium* sp.) from a Californian sand beach characterized by mixed semidiurnal tides (*upper graph*). Counts of locomotory activity at 30-min intervals (*lower graph*). The height of the activity peaks mirrored the tidal pattern of that day, but by a longer period of 26.25 h, which was then persistent for some weeks. (Data from Enright 1963, Fig. 1; reproduced by permission of Springer-Verlag, Berlin)

1.4.2 Free-Running Rhythms and Entrainment Experiments

Various recording devices were invented for continuous registration of the tidal behavioral activities of groups or of single specimens of marine organisms (cf. Neumann 1981; Palmer 1995). These devices allowed recordings to be made either in 24-h LD cycles, in constant light (LL), or in time schedules of simulated tidal factors as mechanical disturbances of the seawater, changes of hydrostatic pressure or temperature, etc. A high individual variability of the actograms was frequently observed for single specimens: the trend of free-running locomotor rhythms of about 12.5–13 h was superimposed by shorter or longer activity bursts (see Fig. 1.2). Some of the captured specimens did not show any rhythmic regularity. Palmer (1995) presented an overview of all these tide-associated studies, including a compilation of methods for time-series analysis and results on phase setting and entrainment to physical factors. Two different interpretations on these free-running rhythms with regard to an underlying, clearly endogenous pacemaker were put forward: on the one hand, the existence of a *circatidal oscillator* of approximately 12.5-h period, and on the other hand a quasi *circabitidal* 25-h *oscillator*[1], which can

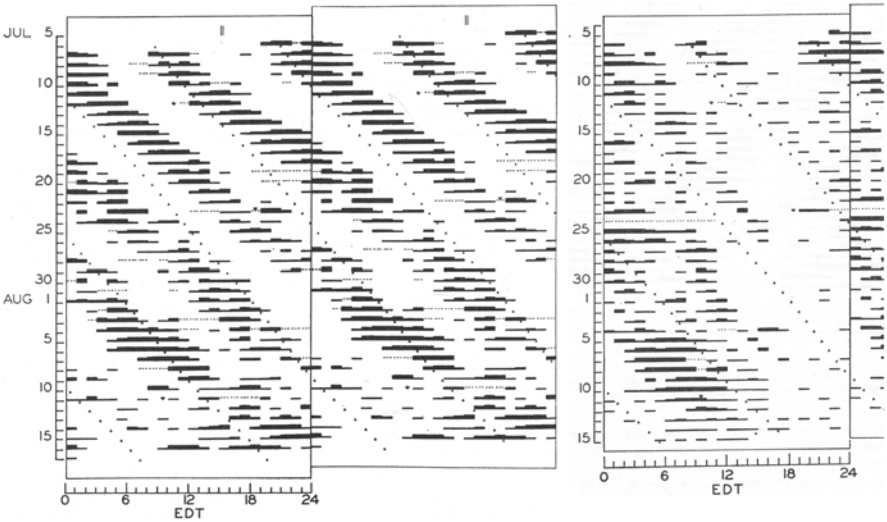

Fig. 1.2 Locomotory activity of two fiddler crabs (*Uca minax*; collected at the Chapoquoit marsh near Woods Hole, MA, USA) in continuous dim light (below 10 lx). The *small points* indicate the times of high tide at the beach. The actograph tracings (*left*: plotted twice) show the duration of activity for each hour, measured as 0, 25, 50, 75, or 100 % of that interval. (Data from Barnwell 1966, Figs. 4 and 5)

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[1] The synonymous term 'lunidian' is avoided in this overview. It correlates these rhythms with the lunar-day rhythm corresponding with the 24.8-h period between two consecutive culminations of the moon, which are the basic cause of the 12.4-h cycle of the tide-producing forces. However, all rhythms of intertidal organisms are primarily responses to the phases of the local tides with all their local modulations. The parallelism with the culmination period of the moon is of secondary importance.

occur in about 180° antiphase with a second one, as observed on coastlines with mixed semidiurnal tides (see Fig. 1.1).

The results of two additional experiments support the existence of an autonomous circatidal pacemaker as seen in green shore crabs (*C. maenas*). Williams and Naylor (1967) reared *Carcinus* larvae to the crab stage in a 24-h LD cycle with no experience of tides; when first subjected to continuous, dim red light they showed a free-running locomotor rhythm of 24–25 h with a peak during subjective night (circadian response), and after an additional chilling period (4 °C for 15 h), a free-running circatidal period of 12–13 h that indicated an inherited period. Reid and Naylor (1989) collected *Carcinus* crabs in winter when they exhibited no overt circatidal rhythmicity in the laboratory; by exposure to hypo-osmotic seawater (7 ppt) during dusk as well as dawn, circatidal rhythms were initiated.

However, two essential characteristics of circatidal actograms differ from those of circadian rhythms in many terrestrial animals: (1) the free-running rhythms often faded after a few days, and (2) additional short phases of both activity and rest occurred in an addition to the dominating circatidal activity pattern and were regarded as ‘noisy’ or ‘ultradian’ activity bursts. Concerning the first characteristic, Naylor hypothesized a multiple clock model, that is, a number of circatidal oscillators that drift out of phase relatively quickly—a hypothesis that cannot be tested without integrated neurophysiological studies. As to the second point, the quasi-stochastic activity bursts may also document inadequate actograph conditions that are too different from the natural habitat, or a specific readiness for direct responses to disturbances by irregularly occurring wind and wave action on the shore (see Sect. 1.4.4).

1.4.3 Further Challenging Studies on Circatidal Pacemakers

Oscillator-driven rhythms such as self-sustained circadian rhythms are characterized (a) by temperature independence of the free-running period, (b) by a restricted range of entrainment to periodic zeitgeber cycles, (c) by phase control with advances or delays in response to effective environmental time cues, and (d) by different effects of strong and weak external time cues (Hoffmann 1969). Some of these properties have already been analyzed in some of the circatidal studies (compare studies cited in Neumann 1981; Naylor 2010). However, the experimental simulation of tidal forces and their temporal patterns is far more difficult than the simulation of 24-h day–night conditions. In general, the previous actograph conditions for individuals or groups of intertidal organisms were often rather simplified and deviated strongly from the species-specific habitat conditions with regard to flooding and sites for feeding and shelter. A radioactive marker technique within small seawater basins with simulated tidal water change was successfully tested in fiddler crabs (Lehmann et al. 1974), so that animals could be studied in a close-to-natural environment without noticeable intervention by the experimenter or data-collecting devices. In any case, it is neither necessary to assume that all intertidal organisms evolved one and the same circatidal (or circabitidal) timing mechanism, nor do these timing mechanisms need to be directly derived from the circadian clock and its genetic inventory.

In conclusion, the free-running records reveal a wide range of unsolved problems. Solving these will require supplementary records of activity and rest within the natural habitat (Green 1970), differentiation between strong and weak times cues for entrainment (Hoffmann 1969, for circadian rhythms), localization of the pacemaker (together with molecular genetic tests), and last but not least, analysis of the perception of tidal time cues.

1.4.4 Noncircatidal Timing Principles

In a study on two fiddler crab species, clear-cut tidal activity rhythms could be evoked under natural-like laboratory conditions (see foregoing) and recorded using a radiomarker technique (Lehmann et al. 1974). These activity rhythms were not subjected to daily modulation when a 24-h LD cycle was added. But under constant conditions, the crabs showed non-circatidal bursts of activity (as already documented by Barnwell (1966) (Fig. 1.2, right panel) and mentioned by many other authors). Neither a preferred duration of activities nor of rest was observable in actograms; the frequency distribution of the durations of both phases could be fitted by negative exponential functions. No significant correlation between one burst and its following rest was found; in other words, the change from an activity burst to rest and from rest to activity was described by a time-independent probability resulting in a stochastic pattern. Under the simulated tidal conditions the frequency distribution of the activity bursts remained unchanged, but the resting periods were lengthened to periods of 7–11 h. The resulting activity 'pattern' was fairly well correlated with the simulated tides, but the correlation was only based on lengthening the resting periods.

An example of tidal hourglass timing was discovered in an artic intertidal population of the 1-h midge *C. marinus*, which reproduces twice a day during midsummer when the range of its sandy beach habitat is exposed and warms up by a few degrees. Although no free-running circatidal rhythms were observed in substrate samples from the beach in the laboratory, in newly bred cultures a small temperature increase resulted in a simultaneous sharp eclosion peak by those pupae that were already pharate (mature) for eclosion and immediate swarming. The peak was followed by a second one about 13 h later, before an irregular eclosion pattern reoccurred after about 18 h. Thus, the tidal rhythm in the field must result from the coincidence of the single 13-h programming (hourglass type) during the preceding ebb and a direct response triggered by the increasing temperature pulse of the actual ebb (Pflüger 1973).

1.5 Circasemilunar and Circalunar Rhythms

1.5.1 First Laboratory Breeding Experiments

Hauenschild (1960) was the first to succeed in demonstrating, in a small-scale experiment, a free-running lunar reproduction rhythm in a laboratory stock of the semelparous Mediterranean polychaete *Platynereis dumerilii*. By superimposing a regular 24-h LD cycle with 5–7 successive nights of simulated moonlight (or bright light) every 30 days, he induced an accumulation of reproducing specimens during certain periods of this artificial moonlight cycle. It was the first evidence of an endogenous, oscillating circalunar clock that controls the development of maturing individuals via neurosecretory cells of the brain. In the years following his experiment it was repeatedly discussed whether one moonlit night would be enough to induce an effect, and what happens in the field when the moonlight zeitgeber signal is disturbed by dense cloud cover. Franke (1985) experimented with the iteroparous Mediterranean polychaete *Typosyllis prolifera* in which the lunar timing can be analyzed not only in entrained groups as in *Platynereis dumerilii*, but in each laboratory reared individual because of the recurring lunar stolonization (up to 18 times). In this context, stolonization is the transformation of the hind part of the body into a specialized reproductive individual (*stolon*) that detaches from the rest of the body for reproduction. Franke showed that for the synchronization of stolonization one or two nights of artificial moonlight is not enough to be effective; additional stimulating nights are needed to induce entrained lunar rhythms (Fig. 1.3). The phase of

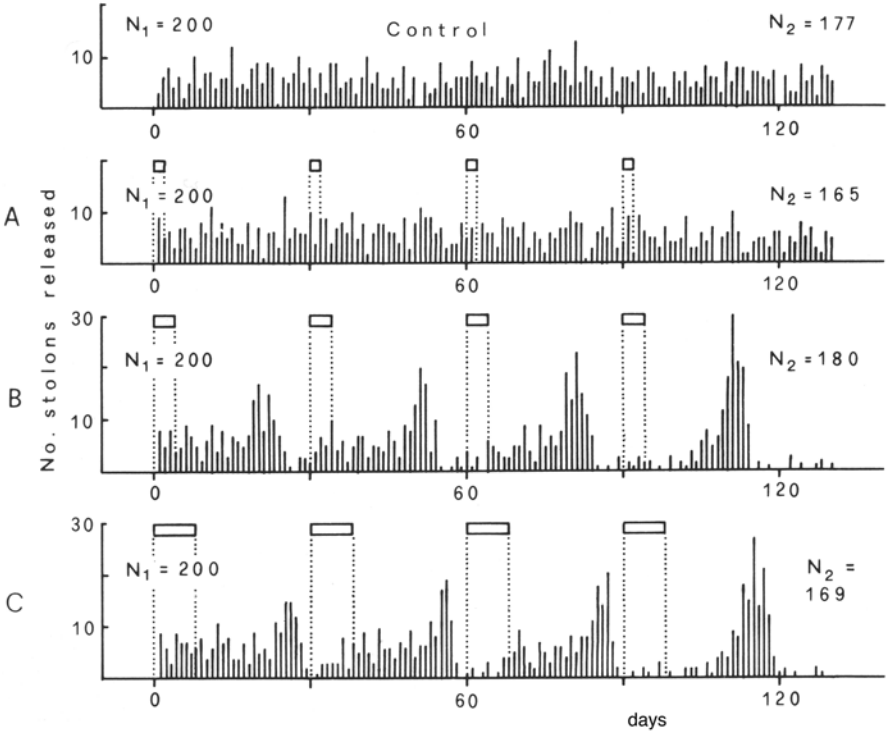

Fig. 1.3 Stolonization, that is, transformation of the hind part of the body into a specialized reproductive individual (*stolon*), which detaches from the rest of the body for reproduction, in laboratory cultures of the polychaete *Typosyllis prolifera* in a 30-day cyclic illumination program with 2–8 successive nights of 0.3–0.5 lx (*horizontal bars*) in initially asynchronous cultures (further conditions, LD 16:8 at 20 °C). (Data from Franke 1985, Fig. 3; reproduced by permission of Springer-Verlag, Berlin)

these rhythms was correlated with the last moonlight night of the laboratory 30-day cycle. Therefore, some cloudiness during the night should be unimportant, particularly if the nocturnal light sensitivity is very high.

1.5.2 *Free-Running Lunar Rhythms*

Further studies on the lunar timing of reproduction events were carried out on the brown algae *Dictyota* (Bünning 1973) and on the 1-h midge *Clunio* (Neumann 1966). The latter turned out to be particularly suited for further laboratory analyses of the properties of both semilunar and lunar rhythms. Strong swarming rhythms of this chironomid insect could be evoked in cultures (dishes of 20 cm diameter with up to about 400 larvae of mixed age structure) by artificial moonlight zeitgeber cycles (mostly four successive nights with about 0.3 lx every 30 days in an otherwise 24-h LD cycle, and this independently of the natural synodic month of the moon; compare Fig. 1.4, left). When cultures synchronized in this way were transferred into continuous conditions of 24-h LD cycles, free-running rhythms were observed for at least 6–8 weeks. In the case of the Normandy stock, the period was 13–14 days, and for a stock from the Basque Coast at the Biscaya Bay it was 26–27 days, corresponding to the semilunar, respective lunar rhythms of their populations in the field (Neumann 1966). In other words, the period of this long-term rhythm is a population-specific property. In the field, it is correlated with the days of spring tides, in the case of

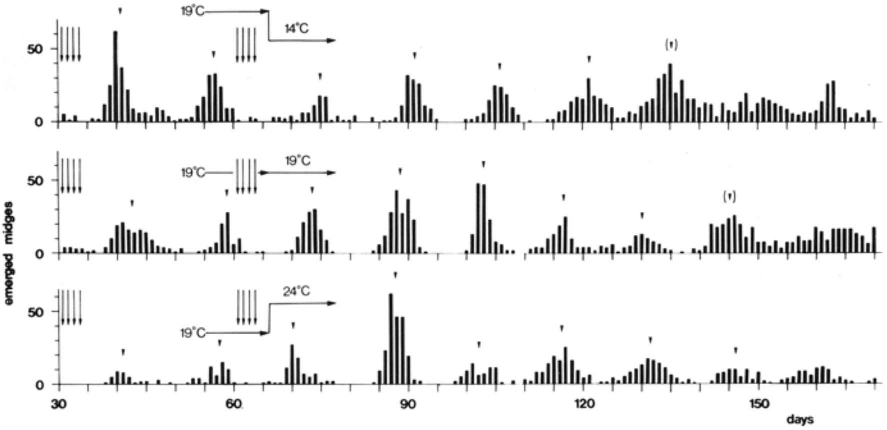

Fig. 1.4 Free-running semilunar emergence rhythms of males in laboratory cultures of the 1-h midge *Clunio tsushimensis* at three different temperatures during light–dark (LD) 12:12 conditions. The semilunar synchronization of the three groups occurred by 30-day illumination cycles of LD 12:12 at 19 °C with artificial moonlight (0.3 lx, *downward arrows*) during nights 1–4 (emergence not plotted until day 31) and nights 31–34 and 61–64. On day 66, groups 1 (*top*) and 3 (*bottom*) were transferred to different temperatures (14 and 24 °C, respectively). *Arrowheads* represent medians of the individual emergence peaks. (Data from Neumann 1988, Fig. 1; reproduced by permission of Springer-Verlag, Berlin)

Normandy with each spring tide during summer around the times of full and new moons (Chevrel 1894), and at the Basque Coast only with new moon spring tides (Neumann 1966). In unsynchronized *Clunio* cultures these free-running lunar rhythms could be also induced by a single treatment with three or four successive nights of 0.3 lx, that is, with no information on any semimonthly or monthly lunar periods, confirming the autonomous physiological property of the period length.

The true nature of the period component was tested in various ways. Three possibilities were considered, as discussed by Bünning (1973): the free-running period could (a) really correspond with a long-term oscillator, or (b) be related to a circadian clock combined with a physiological counter mechanism for about 15 or 30 day–night cycles, or (c) depend on the coincidence of a circadian clock and a circatidal (or circabitidal) clock every 15 (or 30) days ('beat hypothesis'). Corresponding breeding experiments were performed on *Clunio* stocks. Neither in continuous darkness (Neumann 1988) nor in any other sophisticatedly combined LD cycles (Neumann 1976) were the periods of the *Clunio* rhythms disturbed, so that one can conclude that at least the circasemilunar (or circalunar) reproduction rhythm of *Clunio* is characterized by a real long-term cycle as a self-sustained pacemaker with some properties analogous to those of the circadian clock.

1.5.3 Temperature Compensation of the Free-Running Period

Temperature compensation of a reliable timing system is a decisive property of the circadian oscillator and its entrained, overt physiological rhythms. In the case of semilunar rhythms it was tested over an ecophysiologically reasonable temperature range of 14–24 °C in a series of experiments with the subtropical species *Clunio tsushimensis*, which displays a rather clear-cut semilunar rhythm of eclosion in the laboratory (Fig. 1.4). The temperature independence of the free-running period was not significantly different from a Q_{10} of 1.0 (range, 1.03–1.18). In other words, the period of the endogenous circasemilunar clock of *Clunio* that finally triggers the days of reproduction is also temperature compensated. Corresponding results were noticed in the lunar rhythm of *Typosyllis* ($Q_{10} = 1.04$) (Franke 1985).

One has to consider that developmental processes are involved in free-running circasemilunar and circalunar rhythms. Therefore the question arises as to what stage of development represents a physiological 'waiting phase' that is triggered by the endogenous pacemaker, that is, a stage just before the reproduction event or one that perhaps occurs up to a few weeks before. In *Typosyllis* this waiting phase seems to occur directly before the stolonization (Franke 1985), but in *Clunio* this phase already happens about 18 days before as a 'switching point' (Neumann and Spindler 1991). In synchronized *Clunio* cohorts, this physiological 'waiting stage' for the slowly growing larvae occurs at the beginning of the last larval instar, LIV (*Clunio* is characterized by four larval instars, as is true for any other chironomid insect; the developmental time of the first three instars can vary between about 2 and 4 months, or even considerably more when a winter diapause occurs). The imaginal disc development of the final metamorphosis to an adult midge starts with the molting to instar LIV and is easily observable through the transparent larval cuticle (see Neumann and Spindler 1991; Fig. 1.1). In synchronized cultures both growth and further differentiation stop at an early stage in LIV (stages two and three of a total of nine stages until pupation), so that larvae of similar physiological stage accumulate in number. From that point, growth and metamorphosis start again in synchronized cohorts every 15 or 30 days (depending on the physiological race of the respective *Clunio* stock), which is correlated with a short increase in ecdyson titer (Neumann and Spindler 1991). In *C. marinus* (Helgoland stock), this final development before the mature (pharate) pupae is largely independent of temperature (tested here at 11 and 19 °C). Thus, the temperature independence of the free-running rhythm not only reflects the period of the circasemilunar clock but simultaneously demonstrates the temperature-compensated developmental process of final metamorphosis before eclosion (Neumann and Spindler 1991). The final period of metamorphosis until pupation required about 14 days in circasemilunar stocks (Neumann and Spindler 1991; Fig. 1.5) and about 20 days in circalunar stocks (stock Vigo/Spain, Neumann, unpublished data). Thus, the lunar periodically synchronized metamorphosis of *Clunio* larvae draws attention to a previously overlooked phenomenon that may occur in ectothermic animals. How such temperature independence of developmental processes could be endocrinologically controlled is one of the open questions in the field of semilunar and lunar rhythms.

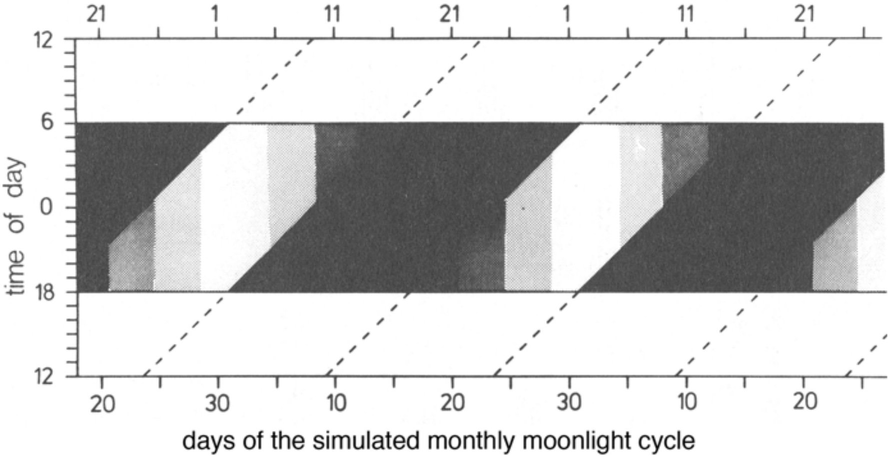

Fig. 1.5 An artificial moonlight regime simulating the 24.8-h period of the lunar day as well as the changing light intensity. The lunar-day period was triggered by a modified synchronous motor-driven 24-h clock; the intensity of small fluorescent tubes was damped stepwise by different *grey* filters to values ranging from 0.3 lx to about 0.07 and about 0.007 lx. (Schedule from Neumann 1985, Fig. 5; reproduced by permission of Springer-Verlag, Berlin)

1.5.4 The Perception of Nocturnal Light as Zeitgeber

The experimental verification that roughly simulated moonlight cycles can entrain these long-term rhythms calls attention to two open issues. (1) In nature, effective moonlight may occur not only during days of full moon but even on many days between the quarters of the crescent and waning moon; furthermore, the effective exposure to moonlight depends on the phases of the tides, with a strong reduction during flooding. (2) The perception of nocturnal light requires a photoreceptor mechanism that should not be disturbed by daylight.

The first problem was examined in a stock of *C. tsushimensis* with the aid of an artificial moonlight program that roughly imitates natural moonlight (Fig. 1.5; Neumann 1985). It simulates the daily 0.8-h shift of moonrise and moonset as well the increasing and decreasing nocturnal light intensity between the quarters of the moon. To further simulate the effect of the tides, the moonlight-like program can be modified by reducing the moonlit time to the hours when the midlittoral would effectively be exposed during low tide, resulting in a very narrow gate of moonlight exposure. Depending on the phase relationship of the local semidiurnal tides to the lunar cycle, the gate may be an early one (in this case, the nocturnal light program was switched on for 3 h just after ‘moonrise’), or a late one (3 h per day just before ‘moonset’), or it was set in the middle between moonrise and moonset. The results are documented in Fig. 1.6. When comparing the effect of ‘four nights of 0.3 lx’ with ‘simulated moonlight’, one can state that (1) the semilunar synchronization was hardly more concentrated in the simulated nocturnal light schedule, and (2) the light stimuli of both series produced the same phase relationship of the entrained rhythm. The other three programs with a reduced gate of simulated moonlight showed no better synchronization of the adult emergence rhythm; obviously the light stimuli were less optimal here. However, there was a clear influence on the phase relationship of the entrained rhythms: a significant advance in the ‘early gate’ program and a delay in the ‘late gate’ program, meaning that the end of these tidally modulated dim light programs determines the phase of the entrained rhythm, as in the case of *Typosyllis*.

Two conclusions could be drawn for the evaluation of entrained semilunar rhythms in the laboratory and in intertidal zones: (a) the daily shift of natural moonlight seems to be of no relevance, and (b) the time schedule of the local tides modulates the effectiveness of moonlight and must have influenced the selected phase relationship between biorhythm and time cue conditions. Comparing different *Clunio* locations along the coastlines where the semidiurnal tides are phase shifted (e.g., Atlantic coast of France and the coast of the English Channel), one has to expect a correcting parameter in the timing mechanism which compensates the phasing effect in the semilunar (or lunar) timing mechanism, so that swarming can occur at each location around the days of the spring tides (Neumann 1985; Kaiser et al. 2011). These evolutionary modifications are treated in detail in Chap. 7.

The second open issue concerns the photoreceptor dilemma: a moonlight receptor system must not be disturbed by sunlight. In experiments on *C. tsushimensis* in

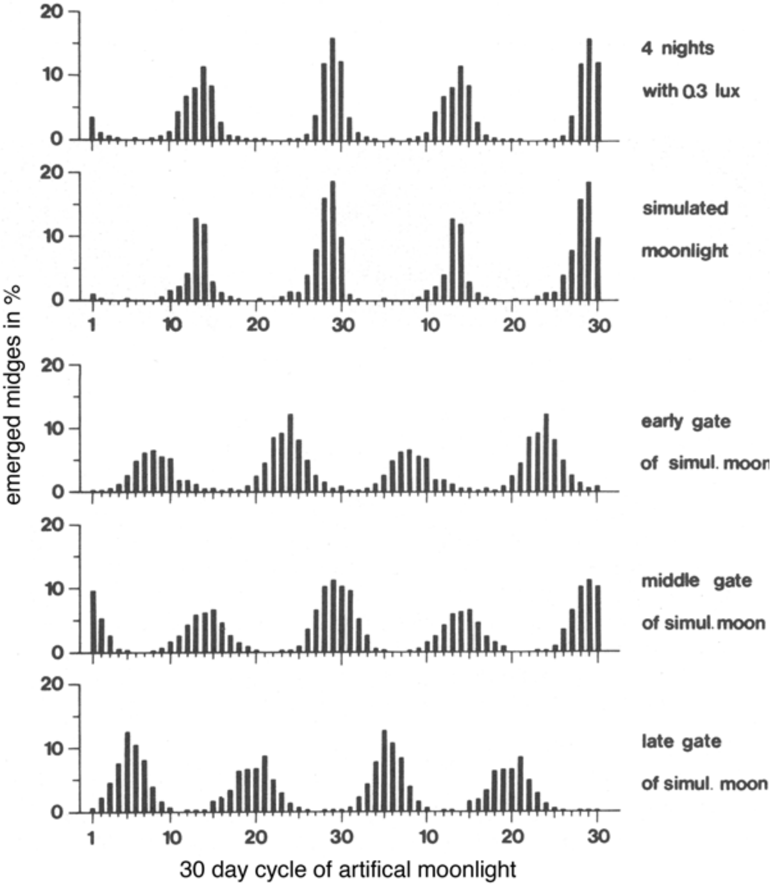

Fig. 1.6 Entrainment experiments with laboratory cultures of *Clunio tsushimensis* in different artificial moonlight programs. *First panel*: Standard laboratory protocol with four nights of artificial moonlight (constant intensity of 0.3 lx). *Second panel*: Simulated natural moonlight regime as shown in Fig. 1.5. *Panels 3–5*: Simulated natural moonlight regime plus additional superposition effect with an semidiurnal tidal regime restricting the changing moonlight intensity to a smaller gate of moonlight exposure, as it occurs in a midlittoral habitat; for details see text. The percentages of emerged midges are summation results of five cultures of three 30-day cycles; they were plotted twice for better visual comparison. The sample size in each experiment was some thousands. (Data from Neumann 1985, Fig. 6; reproduced by permission of Springer-Verlag, Berlin)

which the cultures were exposed to a full-light fluorescent tube for four consecutive nights every 30 days (1,000 lx instead of the 0.3-lx artificial moonlight), the lunar entrainment was even improved. Thus, a "weak-light" receptor for nightly moonlight need not be discussed. As moonlight is simply reflected sunlight and is thus only slighted modified in the range of visible light, it is not possible to distinguish between sunlight and moonlight by wavelength, so that moonlight perception as time cue requires particular physiological properties.

In an experimental series with various *Clunio* stocks that were exposed to different LD cycles (LD 10:10, LD 11:11, LD 12:12, and LD 15:15), the entrainment by the standard 30-day moonlight program turned out well in LD cycles of 22 and 24 h only; in those with shorter or longer LD periods, no semilunar (or lunar) synchronization occurred (Neumann 1989). This finding demonstrates that LD cycles of close to 24 h act as a sort of second time cue condition; in other words, the perception of nocturnal light seems to be embedded in a circadian organization. Outside the range of entrainment of the circadian clock, no synchronization can be caused by moonlight.

In a next experimental series, artificial moonlight was supplied at different times of the day every 30 days over a 4-day period of total darkness (Fig. 1.7). Only ‘moonlight’ presented during the hours of ‘subjective midnight’ was effective. Thus, a circadian clock function must be involved for the perception of the entraining moonlight stimulus. This experiment gives information on the properties of a putative moonlight receptor and thus offers a starting point for an analysis of the photosensory physiology of moonlight perception.

In *Clunio*, studies on nonvisual photoreceptors and other specializations in the larval ocelli have already begun. On the one hand, extraretinal putative photoreceptors cells have been found using both immunohistological as well as ultrastructural methods (with antibodies against ciliary opsins in the larval instars LIII and LIV, which stain cells in the optic lobes, in the intestinal wall, in abdominal ganglia, and below the intersegmental hypodermis; Fleissner et al. 2008a). On the other hand, the larval ocelli show a lunar rhythmic change of shielding pigment transparency so that these photoreceptor cells may function as very sensitive photometers (Fleissner et al. 2008b; Falkenberg et al. 2013). However, no circadian changes were observed in either the extraretinal cells or in the larval ocelli cells, so that a central neural filtering (Nilsson 2009) may be involved in the circadian-modulated perception of moonlight.

1.5.5 Geographic Adaptation: A Tidal Factor as Zeitgeber

Moonlight cannot be a reliable and dependable time cue during summer months in northern areas of the temperate zone because the duration of the night is relatively short and the northern horizon remains relatively bright. Despite this fact, *Clunio* populations display a clear lunar periodicity even in such geographic regions (e.g., Helgoland/North Sea, latitude 54°N; Bergen/Norway, 60°N). In agreement with this environmental situation, *Clunio marinus* stocks from Helgoland (54°N) and Bergen (60°N) could not be entrained by the artificial moonlight cycles. However, a clear-cut semilunar synchronization could be achieved in the Helgoland stock when an artificial 12.4-h tidal cycle was used in combination with the 24-h LD cycle (Fig. 1.8). In the case of the Helgoland stock, the effective tidal stimulus was a mechanical disturbance that lasted continuously for 6 h and which simulated both turbulence and underwater vibrations caused by the surf of the rising flood, with a frequency spectrum of 100–200 Hz and 20–30 dB over the background noise level (Neumann 1978). In this tidal schedule the *Clunio* larvae were exposed to the same

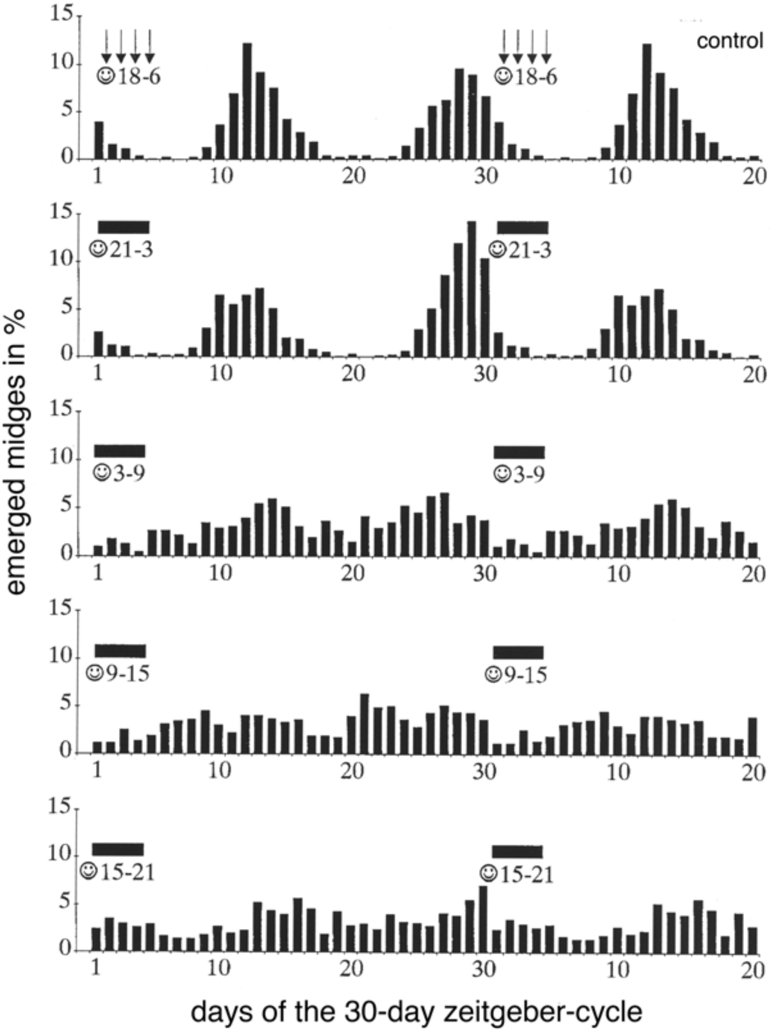

Fig. 1.7 Test on limited nocturnal light sensitivity of *Clunio* larvae (*tsushimensis* stock), as observed in the eclosion of the adults. *Above*: Control in standard program in 24-h LD cycles (*light* 6–18, *darkness* 18–6) and artificial moonlight (see *arrows*). *Below*: Four experiments with 4 days of darkness every 30 days (see *black bar*), but artificial moonlight at different daytimes during the second to fourth 'subjective night' only. Pretreatment in each series: 3 months with the identical program. *Result*: Semilunar entrainment in the second series with moonlight during 21–3 only. (Data from Neumann 1995, Fig. 6; reproduced by permission of Springer-Verlag, Berlin)

daylength and to the same number of hours of mechanical stimulation by tidal turbulence cycles each day, but both conditions had the same phase relationship only every 15 days. The range of 1 of these 15 phase relationships (recurring every 15 days) offers the effective time cue condition of the circasemilunar clock. The Bergen population is localized in sheltered fjords with relatively small tidal

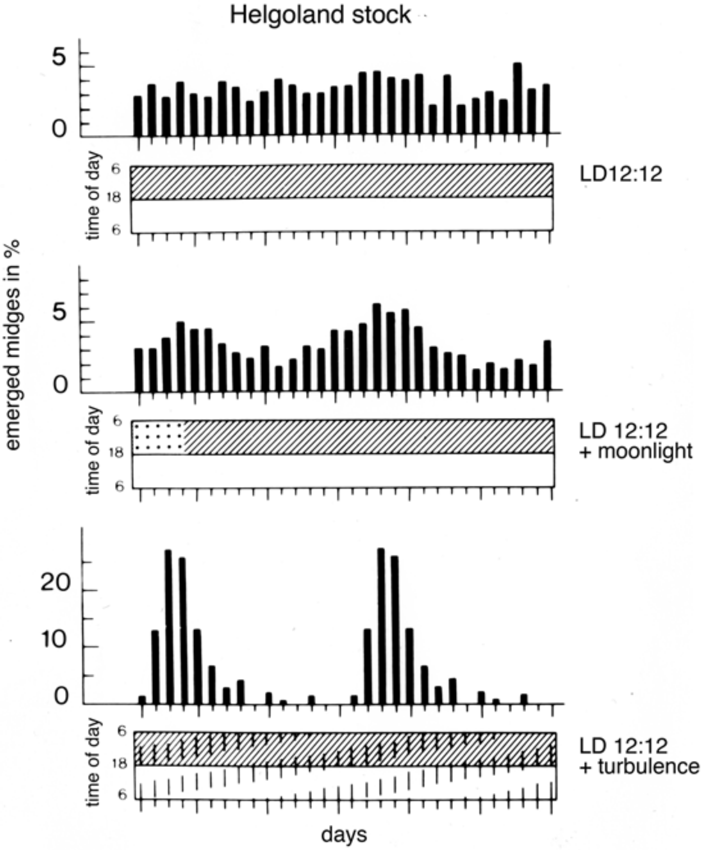

Fig. 1.8 Semilunar entrainment experiments with a northern stock of *Clunio marinus* (Helgoland/North Sea) by artificial moonlight and a simulated cycle of 12.4 h of water disturbance in LD 12:12 (see time schedule below). In the *bottom panel*, *vertical lines* mark the time of "turbulence" in the simulated tidal cycle of water disturbances and its phase relationship with the 24-h LD cycle. (From Neumann 1985, Fig. 3; reproduced by permission of Springer-Verlag, Berlin)

amplitudes and a weak surf; its stock could be entrained by 12.4-h tidal temperature cycles combined with 24-h LD cycles (the amplitude of the temperature cycle was 3–5 °C, which simulated tidal temperature changes of as much as 2 °C during calm weather in the field (Neumann and Heimbach 1984). The effectiveness of these tidal stimuli is also limited by a narrow range of circadian entrainment to LD cycles between 22 and 26 h, so that also here the time cue perception is based on a circadian organization of the *Clunio* larvae (Neumann and Heimbach 1985). Thus, while southern populations of *Clunio* rely on moonlight for entrainment of their circasemilunar timing mechanisms, northern populations (in the absence of reliable moonlight cues in summer) use other time cues for synchronization. This observation demonstrates a perfect adaptation of the circasemilunar timing mechanism and its endogenous semilunar clockwork to reliable time cue conditions.

1.5.6 Daily Timing of Lunar Rhythms

In general, the reproduction time in semilunar and lunar rhythms is not only correlated with the days of spring or neap tides, but also with the time of day when the optimal conditions for swarming or egg deposition occur, particularly at locations with large tidal amplitudes (e.g., at locations where palolo, grunion, and *Clunio* are found). As outlined in the beginning (Sect. 1.2.1), the time of day of a particular tidal phase on a particular day of the lunar cycle (e.g., the 'time of low tide on a spring tide day') can vary along the coastline in response to the influence of coastline geography on the tidal currents. Thus, the optimal ecological situations for semilunar (or lunar) reproduction times occur at different times of day at different locations. Correspondingly different physiological races can be expected to exist and were detected along the coasts of France and southern England. In *C. marinus* populations, the daily timing of eclosion could be identified as a gene-controlled circadian timing mechanism (Neumann 1967) (see Fig. 1.9). The ongoing analysis of daily and lunar phases of the timing mechanisms in various *Clunio* populations is discussed in Chap. 7.

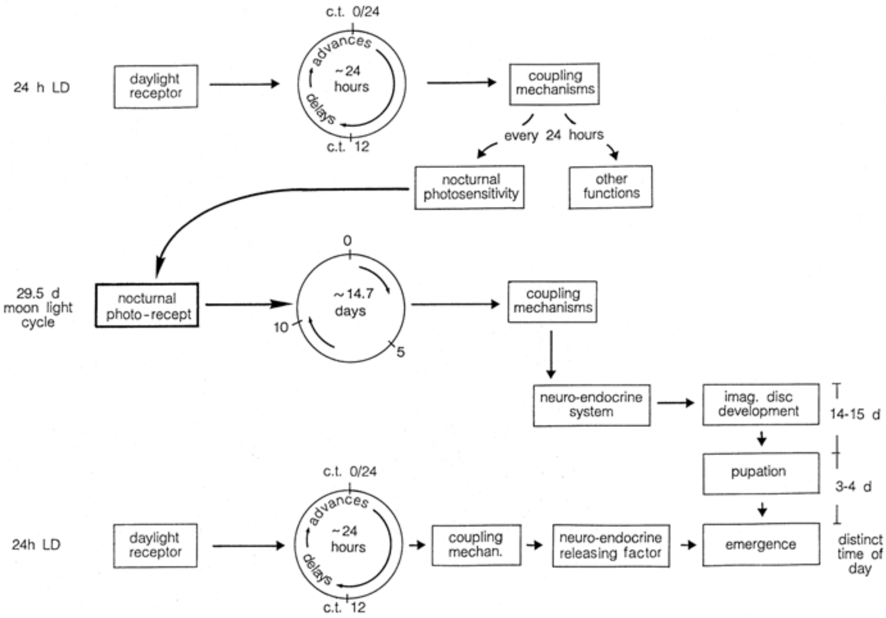

Fig. 1.9 Schematic representation of the physiological components of the semilunar timing mechanism of the intertidal 1-h midge *Clunio* synchronized by 24-h LD cycles and 29.5-day moonlight cycles. *Above*: Circadian clock for the control of nocturnal photosensitivity for perception of moonlight (time cue: 24-h LD). *Middle*: Circasemilunar clock (time cue: nocturnal light of some hours on few days every 30 days) for control of the switching point of the imaginal disc development in early larval stage IV Circadian clock for control of time of day of eclosion in pharate images (*c.t.* times of the circadian oscillator for phases of 'advances' and 'delays'). (Scheme from Neumann 1995, Fig. 7, English version; reproduced by permission of Springer-Verlag, Berlin)

A particular case of daily timing combined with a lunar rhythm occurs in the species *Clunio mediterraneus*. The tidal amplitudes of the European Mediterranean Sea are relatively low, so that spring low-water conditions can be reinforced by continuous offshore wind, and in contrast strongly reduced by onshore wind. In *C. mediterraneus* (Rovinj population), the time of eclosion and swarming correlates with the spring low water in the early morning. This is the time when the tidal amplitude is reinforced by offshore wind in the summer; only then is the upper sublittoral range (which is the most suitable larval habitat) exposed, permitting egg deposition.

1.5.7 Circalunar Clock as Central Component of a Complex Timing Mechanism

The main physiological components of a semilunar periodic timing mechanism as established in the 1-h midge *Clunio* are summarized in Fig. 1.9. The results of the experiments indicate that the circasemilunar (or circalunar) timing during a developmental switching point is coupled to the circadian organization of the organism in two ways. First, nocturnal photosensitivity to moonlight (and potentially sensitivity to any other time cue of the semidiurnal tidal cycle) is under circadian control. Second, daily eclosion time is tuned to the ecologically proper tidal situation, which reoccurs every 15 or 30 days. It may be expected that a corresponding scheme may be also valid for other semilunar or lunar rhythms of species of the intertidal zone.

Particularly important in any such timing mechanism are the components responsible for the ecologically suitable phase relationship of the overt rhythm with the environmental cycles. The general circadian organization seems to be extremely important: (1) in case of the perception of the complex zeitgeber conditions (day–night cycle, moonlight, or tidal factors such as tidally fluctuating turbulence or temperature), (2) regarding the gene-controlled and evolutionarily selected phase relationship between the physical cycles and the controlled physiological process, and (3) in tuning to different zeitgeber conditions (e.g., from photic to mechanical stimuli). The different geographic races arise from genetically different versions of the timing mechanism and offer possibilities of determining timing parameters by genetic analysis (see Chap. 7 by Kaiser, this volume).

Concluding Remarks

Experimental studies on the physiological components of tidal and lunar timing mechanisms are still underway. During the past few decades, important physiological components and properties of endogenous ‘clockworks’ have been revealed through long-term registration of locomotor behavior (in the case of circatidal timing) and by breeding experiments with polychaetes and an intertidal insect and its local physiological races (in the case of lunar rhythms). Many new questions have arisen, such as to zeitgeber effectiveness, zeitgeber perception (especially nocturnal moonlight in contrast to daylight), zeitgeber transfer to the respective internal clock, as well as to gene-controlled components of circadian and circalunar phase relationships. It is still difficult to understand those components of the circadian system that are on the one hand tuned to produce a time-invariant, constant output but on the other hand can be subject to selection by environmental cycles (i.e., must have a certain degree of variation). This apparent antagonism not only applies in the context of semilunar and lunar rhythms but represents an unresolved problem in the study of circadian rhythms in general. The perception of stimulus duration in time cue perception and the identity of the pathways connecting different photic time cue receptors are still open issues in receptor physiology. The population genetics of ecophysiological differentiation along various coastlines offer ideal topics of study in the field of evolutionary biology. Today these open questions on endogenous timing processes can be rendered more precisely, which may eventually allow solving the remaining enigmas in biological timing.

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