An Analysis of the Activity Rhythm
of the Sand Beach Amphipod, *Talorchestia quoyana*

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Summary. 1. The rhythm of locomotor activity in the amphipod *Talorchestia quoyana* has been monitored in populations and individual animals for up to 105 days.

2. Control of the rhythm is dominated by a light sensitive circadian clock.

3. A circatidal clock inhibits activity at projected times of high tide on the beach of collection.

4. The circatidal clock can be entrained by 2 hr pulses of vibration.

5. In long term records of individual activity, there is evidence of a semi-monthly rhythm in total amount of activity. Minimum activity is correlated with spring tides.

6. Various models for the combined activity of the two clocks are discussed.

Introduction

Talorchestia quoyana is an amphipod sandhopper which inhibits sandy beaches. It emerges at night from its temporary burrow along the high tide zone to feed, but at times of high tide when waves wash over this zone, *Talorchestia* burrows once more under the sand. Earlier work has suggested that *Talorchestia* shows an activity rhythm which is light sensitive but which also shows elements of tidal synchronisation (MacIntyre, 1954, 1963; Featherston and MacIntyre, 1957; Broadfoot, 1970). As in *Uca pugnax*, it may be expected that rhythms of some physiological processes, such as oxygen consumption, would be controlled by circadian clock, while locomotor activity would be under the influence of a circatidal as well as a circadian clock. During daylight *Talorchestia* is open to predation, and the action of the waves at high tides on an unburied animal would be equally deleterious.

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A circadian clock dominates the control of the activity rhythm in *Talorchestia* (Benson, 1973). This article provides evidence for this view, and presents an analysis of the possible roles of clocks of non-circadian periodicities in the modification of the locomotor activity rhythm. Properties of clocks are examined in terms of periodicity, type of influence on the rhythmic output, coupling, and Zeitgebers.

Materials and Methods

Large specimens of *Talorchestia quoyana* were selected for experimentation so that the populations used were probably dominated by females. They were collected from Takapuna Beach, Auckland, and transferred in light proof containers to conditions of constant darkness and controlled temperature (20 °C $\pm$ 0.5 °C). Much published work on the rhythms of marine animals has involved populations, but this technique may tend to blur some important inter-individual differences. Other workers have used single individuals, and from their data have selected the behaviour of a small percentage of individuals which display especially precise or informative rhythms. In the present investigation, both techniques have been used.

Populations of animals (15 to 20) were maintained in perspex aquaria or plastic buckets containing 10.0 cm of wet sand. Single individuals were contained in 12.0 cm diameter crystallising dishes, 6.0 cm deep. For some experiments these were half filled with damp sand, with a narrow strip of plastic positioned on the surface along the path of activity detection. Alternatively, the sand was replaced by a solid substrate made up of a disc of expanded polystyrene positioned half way up the dish. In this case the surface was covered by filter paper kept damp from a reservoir of water beneath the disc. The crystallising dishes were covered with clear plastic lids with small ventilation holes (Fig. 1).

Activity was monitored by measurement of frequency of interruption of a deep red light beam which skimmed the surface of the substrate, and was produced by passing tungsten light through a Kodak Wratten 70 filter which transmits from 650 nm upwards, wavelengths to which arthropods are not generally sensitive. Each time the light beam was broken, the event was recorded as an all-or-none spike on the pressure sensitive paper of a Goerz Miniscript event recorder running at 1.0 cm per hr. In parallel with this, events were counted for each 10 min interval by an electronic counting device which punched the coded totals onto computer paper tape. These tapes were then processed in a Burroughs B6700 computer, and the data subjected to various forms of analysis described elsewhere (Lewis and Cassie, in preparation). The aktograms are based on computer-drawn histograms which have been traced and relabelled. For clarity, most are presented as two 24 hr panels; the second panel is a repetition of the first, and is positioned so that Day 2 of the second panel is adjacent to Day 1 of the first. The heights of the histograms are proportional to the rate of sandhopper activity; the scale is arbitrary.

The animal containers were maintained at constant temperature in incubators or a constant temperature room. In the former case any possibility of effects due to vibration of the refrigerating units or of the building were eliminated by suspending the experimental containers from wooden frames by elastic supports.

The light phase of light/dark cycles was provided by tungsten bulbs, and light intensity measurements, in some cases approximate, were made at the substrate surface. There was no feeding, and the very few other experimenter-imposed external disturbances (such as replacing a bulb) were carried out under a red safe light or in darkness. All free-runs were made in constant darkness.

Results

The histograms shown in Fig. 2 and Fig. 3 are examples of free-running activity rhythms of a population of 15 *Talorchestia* and a single individual, respectively. These are typical records with regard to the cirdadian component of control.
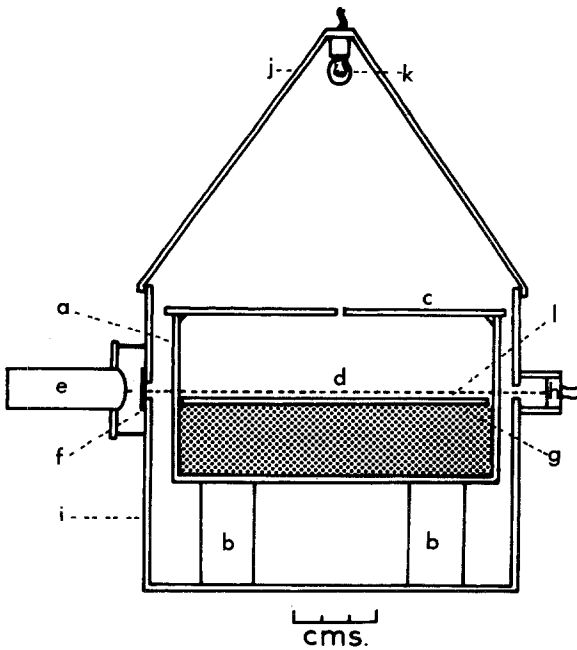
Fig. 1. Sectional diagram of the apparatus for recording locomotor activity of individual sandhoppers. Each animal was contained within a crystallising dish *a*, which was supported on two blocks *b*, and covered by a transparent lid *c* with small ventilation holes. A light beam *d* was projected from a tungsten light source *e* through a red filter *f* onto a light dependent resistor *h*. The dish was half-filled with sand *g* which was covered along the length of the light beam by a narrow strip of plastic *l*. (In an alternative arrangement the substrate consisted of a disc of expanded polystyrene covered with a filter paper kept moist by a reservoir of water below the disc.) The dish was contained by a cylindrical light-proof container *i* which was covered with a conical lid *j* containing a tungsten light source *k*
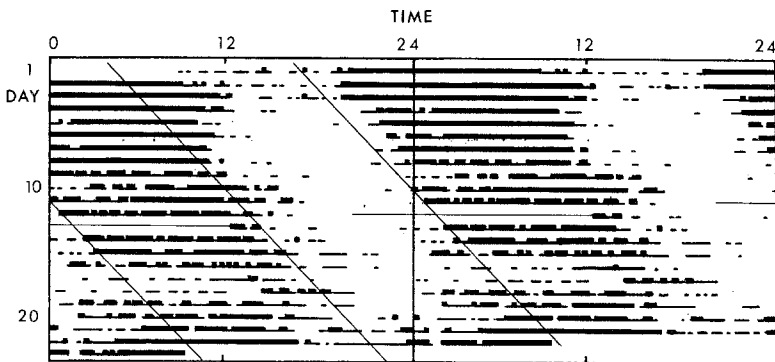
Fig. 2. Activity record of a population of 15 sandhoppers in DD for about 22 days. The thin horizontal line indicates lost data, and the diagonal lines the times of high tide on the home beach
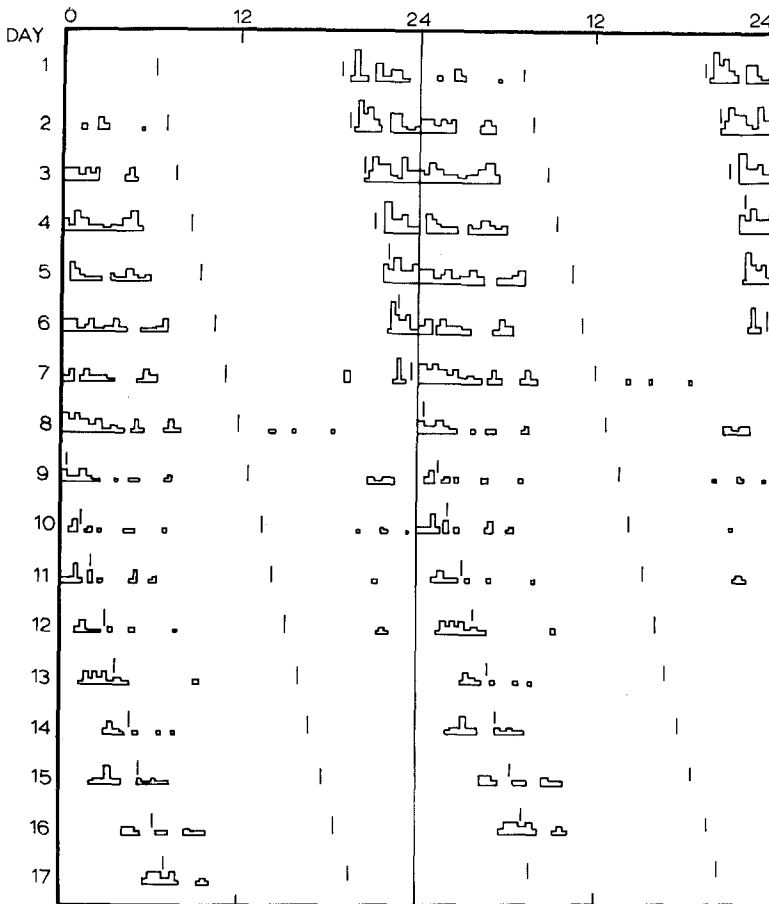
Fig. 3. Activity record of a single sandhopper for 17 days in DD. The times of high tides on the home beach are indicated by the short vertical bars. The substrate was sand

Periodogram analysis shows the average period for the rhythm illustrated in Fig. 2 to be 24.5 hrs and for that in Fig. 3 24.7 hrs. Examination of 18 stable free-running rhythms in single animals reveals a considerable range of period (from 24.1 to 26.3 hrs, with an average of 25.1 hrs), and in a further 4 free-runs there is a distinct change of period, usually during days 7 to 11 (e.g. Fig. 4). Furthermore, the initial phase angle between activity and real time varies among individuals. From these observations of inter-individual variation, greater width of active phase is to be expected in a population (Fig. 2). Although 7 out of 32 records of individual free-running behaviour failed to show a clear rhythm, largely due to a very low level of activity, populations of animals, again as would be expected, invariably exhibited a clear, sustained rhythm.

An examination of the detailed structure of the active phases in DD (Figs. 2, 3, 4) in relation to high tide on the beach of origin reveals a feature which occurs in a significant number of sandhoppers. When the time of high tide
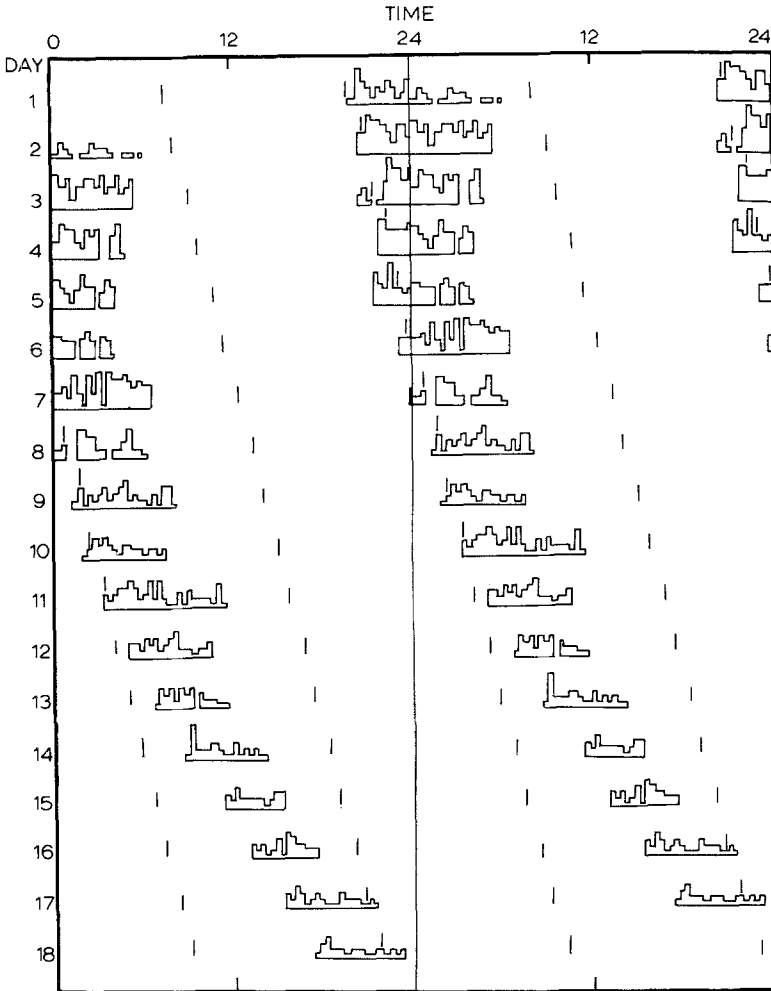
Fig. 4. Activity record of a single sandhopper for 18 days in DD. The times of high tides on the home beach are indicated by the short vertical bars. Note the change in period at about Day 9. The substrate was sand

coincides with the expected time of onset of activity at about subjective sunset, activity appears to be inhibited. In the individuals which show this feature, the period of the overt rhythm as calculated by measuring intervals between succeeding onsets of activity is always close to the twice tidal period of 24.8 hrs for the first 7–11 days, while the period as measured from cessation of activity is always much closer to 24.0 hrs. This is not so clear in the population studies due to the gradual desynchronisation of individuals of the population relative to one another resulting in an increase in the length of the active phase. However, for the population rhythm illustrated in Fig. 2, if the onset for Days 1–3 and 18–23 are taken to reflect the uninhibited circadian output, the circadian period
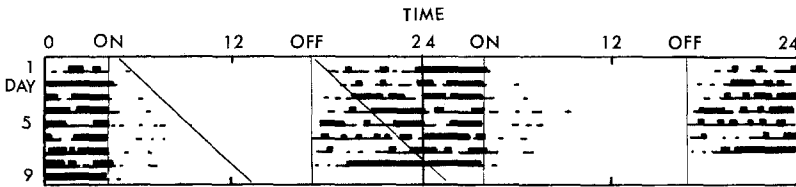
Fig. 5. Activity record of a population of 10 sandhoppers entrained by artificial light cycles (LD 13:11). The times of high tide on the home beach are indicated by the diagonal lines. Note the delay in onset of activity when high tide occurs soon after the times of lights off

is calculated as approximately 24.2 hrs. Due to inhibition of activity at the times of high tides in the intervening days the period appears to be much greater. Advance of the time of cessation of activity can be observed in some records on days when projected high tide coincides with the end of the active phase. This evidence is much less clear due to the greater irregularity of times of activity cessation even in cases where control appears to be purely circadian. The same inhibition of onsets of activity is clearly demonstrated by sandhoppers when entrained by 24 hr light cycles if high tide coincides with the end of the light phase. Delays in the times of onsets of activity also occur in light entrained populations when high tide occurs soon after lights off (Fig. 5).

These observations suggest that there is an inhibitory component of circatidal period involved in the control of the rhythmic output. The period must be circatidal or bimodal circalunadian because such delay of onset still occurs when two successive projected high tide times encounter the active phase of the circadian output. If this inhibition is in fact due to a continuously running clock, it is reasonable to suggest that there should be evidence of inhibition detectable throughout the active phase at the points at which the inhibitory phase of the circatidal clock scans the active phase of the circadian clock. Such, in fact, proves to be the case in a number of animals, but particularly in two cases of individuals entrained to LD cycles (Fig. 6). As time of projected high tide crosses the active phase, activity is considerably reduced. This shows up for both individuals and populations in the form estimates (Figs. 7 and 8) which cancel out random decreases in activity. In both form estimates the circadian inactive and active phases have not been cancelled out due to the shortness of the record (τ_E for the estimate was 24.75 and τ_{FR} for the circadian component was close to 24 hrs. $\tau_{FR}/(\tau_E-\tau_{FR})$ days would be required for complete cancellation, where τ_{FR} = free running period and τ_E = entrained period).

Although not all of the experimental records show evidence of a circatidal component, it seems clear from the examples given here and from other data, that the activity rhythm in DD is under the control of a circadian clock which runs at a period of approximately 24 hrs at least for the first 10 days or so, and that there is a second clock present which tends to inhibit the expression, in terms of locomotor activity, of the output from the circadian clock, with a circatidal period of approximately 12.4 hrs.

As has already been noted, although the average period of stable free-running periods is close to 25 hrs, at least 4 free-runs of individual animals exhibit
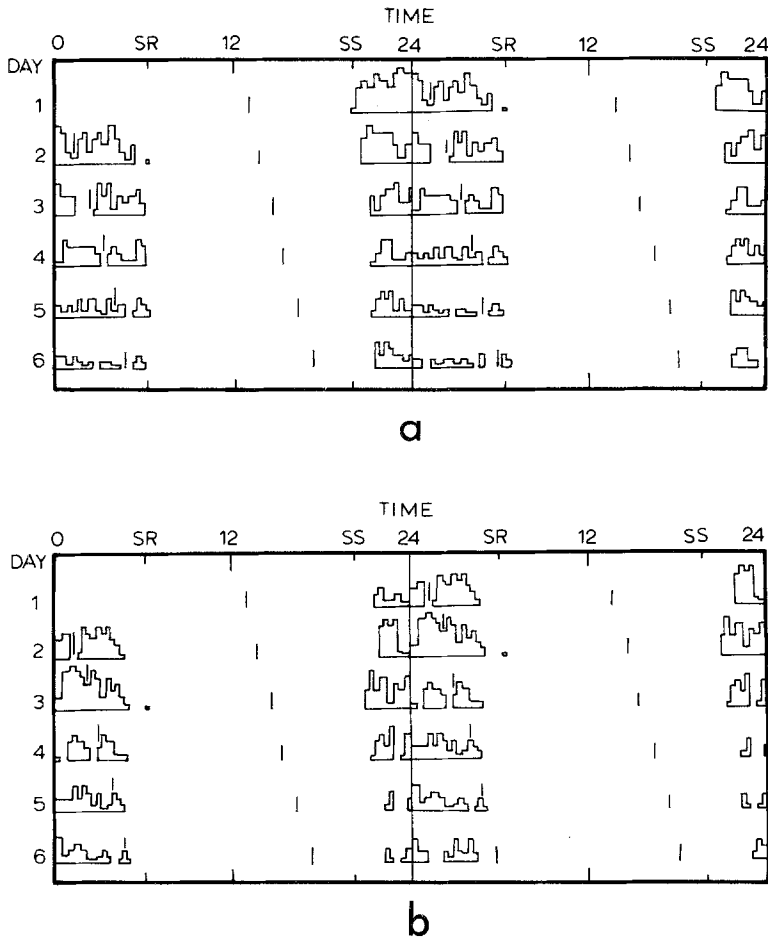
a

b

Fig. 6. Activity records of two individual sandhoppers recorded under natural light cycles for 6 days. The short vertical bars indicate the times of high tide on the home beach. Note the inhibition of activity at about the times of high tide. Both sandhoppers were on a sand substrate. The times of sunrise (*SR*) and sunset (*SS*) are indicated

a period of about 24 hrs for the first 7–11 days, and then show a marked increase in period to the stable value of 25 hrs. The days during which the major component of the rhythm shows an approximately 24 hr period corresponds to the time during which the influence of the circatidal clock is clearest. After 11 or so days, it is difficult to discover unequivocal evidence of inhibition in records for individuals, although a few populations still show predicted delay of activity onset at up to 20 days. This suggests that there may be some kind of interaction between the clocks, a possibility which is considered in more detail in the discussion.

The circadian clock is easily entrained and shows the conventional variation in light sensitivity according to phase which provides the mechanism for such entrainment. Several workers have put forward evidence that a few species
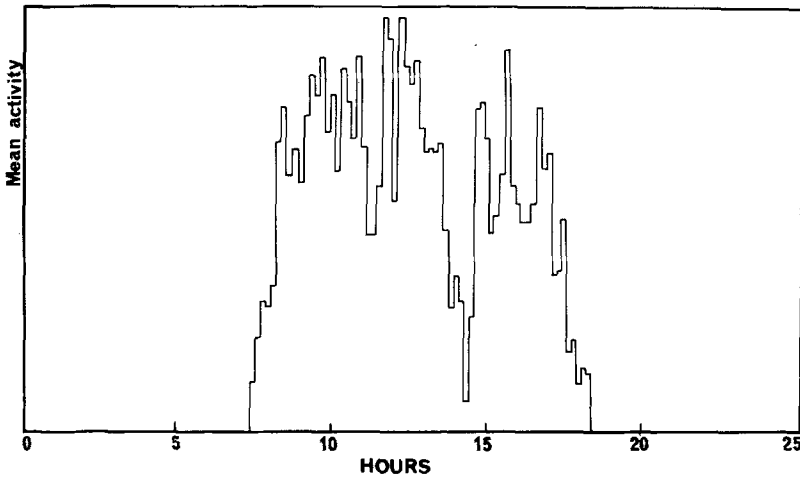
Fig. 7. Twenty-five hour form estimate of the six days of locomotor activity of a single sandhopper (Histogram Fig. 6a) under natural light cycles. The large dip in average activity is approximately in phase with the nocturnal high tides on the home beach, and is considered to provide evidence for tide-related inhibition of activity. Calculations were based on data collected at 10 min intervals. To obtain the form estimate, these data were arranged in a table consisting of 150 columns (25 hrs of the 10 min data) and the means of the activity counts of each column calculated and plotted on an arbitrary scale
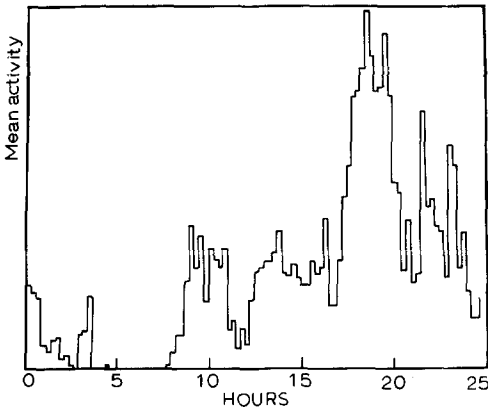

Fig. 8. Form estimate for 24.75 hrs of the locomotor activity of a population of sandhoppers in constant conditions (DD, 20 °C) for 8 days. The low mean activity at Hour 12 corresponds with the times of high tide at the home beach, and provides evidence for tide-related inhibition of activity. Calculations are based on data collected at 15 min intervals, and the form estimate was obtained in a similar way to that described for Fig. 7

have rhythms of circatidal period which are entrainable in the laboratory. For example, there is some evidence that wave-produced turbulence can entrain circatidal rhythms (Enright, 1965; Jones and Naylor, 1970; Klapow, 1972), and cycles of hydrostatic pressure change may also be effective (Morgan, 1965;

Fig. 9. Entrainment of the circatidal
clock of 2 populations of 15 sandhoppers
with vibration cycles under light entrainment
(LD 12:12). The phasing of the LD cycles
is indicated by the black and white bars below
the time scale. The short black bars show
the times at which vibration was applied,
and the vertical arrows the times of
inhibition of activity in the active phase
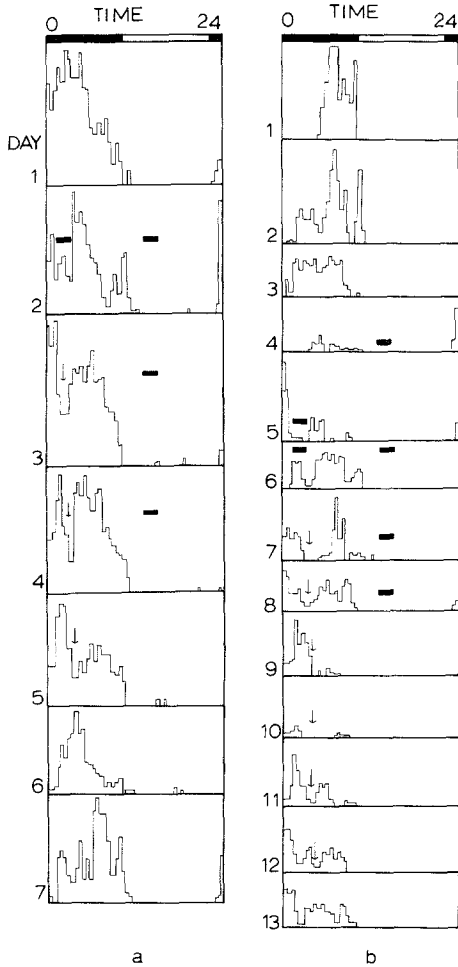
Gibson, 1971; Naylor *et al.*, 1971; Naylor and Atkinson, 1972). Since *Talorchestia* burrows anticipating onset of inundation, it seems unlikely that direct wave action is the Zeitgeber. Quilter (1971) found that substrate vibration may entrain the circatidal clock of the isopod *Scyphax* which has similar burrowing habits to *Talorchestia*.

Fig. 9 illustrates the result of application to two populations of *Talorchestia*, entrained to a LD 12:12 cycle, of 2 hr periods of vibration in an attempt to simulate the effects of wave vibration transmitted through the substrate. The sandhoppers had been in laboratory conditions for several days, and by Day 1 of the activity presented in Fig. 9a showed no inhibition of activity at the time of high tide. On Day 2 two pulses of vibration were applied. The first pulse occurred during the active phase so that the decrease in activity in direct response to the vibration can be observed. The pulses were then applied at 24.0 hr intervals for three days in the rest phase, with reduction of
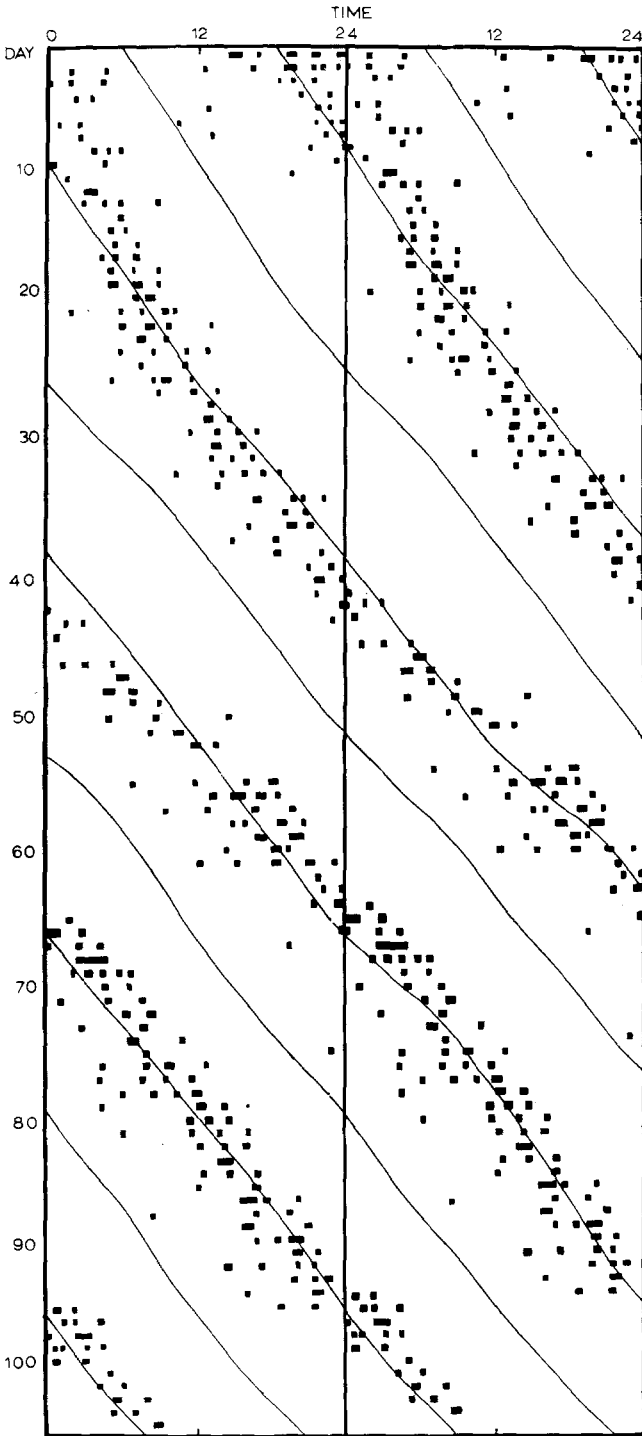
Fig. 10

activity at the points indicated by the arrows approximately 12.5 hrs after the pulse. When the entrainment cycle was discontinued, the series of entrained decreases in activity continued for a few days. A second population (Fig. 9 b) showed similar results, with entrainment lasting clearly for four days. A further population subjected to the same regime showed such unevenness of activity amplitude that no conclusion regarding entrainment could be drawn.

It can be tentatively concluded that a vibration-entrainable clock is active with a circatidal period. A bimodal circalunadian clock cannot satisfactorily account for the observed data.

In those parts of the world, such as New Zealand, where there are two high tides per day separated by an interval of about 12.4 hrs, the amplitudes of these tides undergo a periodic fortnightly change. Evidence has been accumulated from the isopod, *Excirolana* (Heusner and Enright, 1966; Enright, 1972) and the intertidal midge, *Clunio*, (Neumann, 1969) for the existence of rhythms of monthly (circalunar) or semi-monthly (circasemilunar) period. Two specimens of *Talorchestia* have been independently monitored for 105 days (Fig. 10). Neither record shows clear evidence of a circatidal component. That is, both appear to be predominantly circadian clock controlled with an average free-running period of 25.0 hrs. However, when three day averages of total activity counts (taken from noon to noon) are plotted on the same time scale as the fluctuation in amplitude of high tide, an interesting relationship between total activity and tide height emerges (Fig. 11). There is an inverse correlation between amplitude of activity and tide height. This is especially clear for the first 7 oscillations, and from that point the oscillations in activity appear to drift out of phase with the tidal periodicity, but a rhythm of circa-semilunar period remains unmistakable, particularly in the lower trace. There is a fairly distinct correspondence between activity and tide involving details of magnitude for the first few cycles. The larger the peak or trough in the tidal trace, the greater the magnitude of the corresponding inverse curve of the activity oscillation. For the major part of the record, however, the correlation is between peaks and troughs with no clear correspondence of magnitude. There are two unexplained anomalies; the split in peak 7 and the extra peak following peak 11 in the lower trace, but the overall pattern is clear. Possible control mechanisms are discussed below.

Discussion

Three strong lines of evidence support the hypothesis that there is a circatidal clock active in *Talorchestia* which inhibits activity at phase points separated by approximately 12.4 hrs. Firstly, activity is often inhibited whenever the pro-

Fig. 10. Activity of one sandhopper in DD for 105 days. The times of high tide on the home beach are indicated by the diagonal lines. The activity is considered to be controlled by a circadian clock with a free-running period of about 25 hrs. The constant phase relationship between activity and high tide is therefore coincidental. The other sandhopper which survived for the same length of time in DD showed a slightly longer free-running period, and hence no steady phase relationship with the tides. The substrate was damp filter paper on an expanded polysterene disc
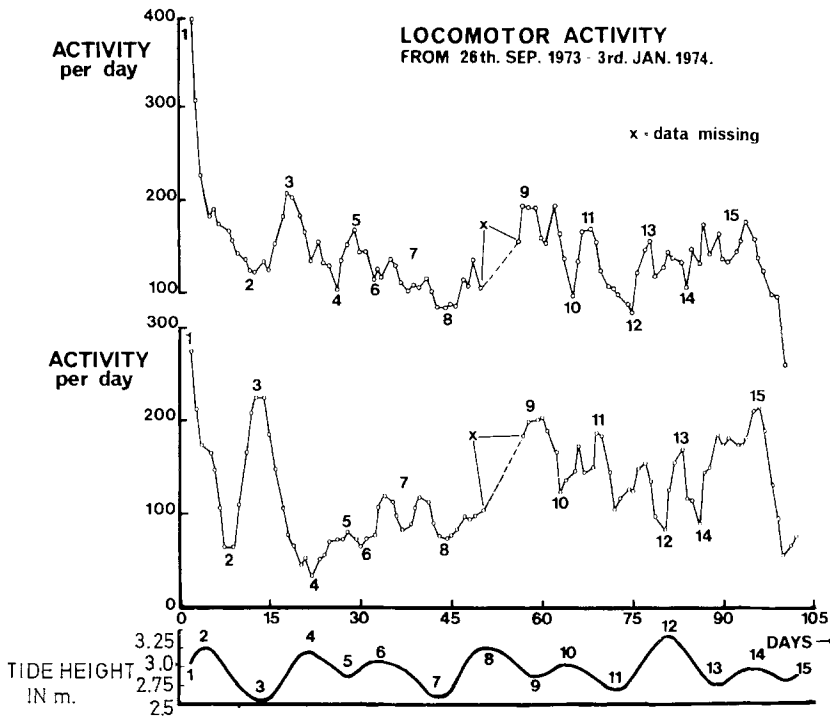
Fig. 11. The relationship between three day running averages of total daily activity of two sandhoppers in DD for 105 days and the high tide height on the home beach

jected time of high tide coincides with time of onset of activity predicted from the circadian component. Secondly, inhibition can be traced as the circatidal phase point scans the active phase of the circadian rhythm. Thirdly, there is evidence to suggest that pulses of vibration induce decreases in activity at multiples of 12.5 hrs later, i.e. may be entraining the circatidal clock.

In data from free-running individuals, those which show circatidal inhibition have a period of approximately 24.0 hrs, until the circatidal effect fades, at which point, usually after 7–11 days, the period increases to about 25.0 hrs, which is the free-running period of animals which show clear evidence of circadian control only. The change in period could simply be a spontaneous change such as has been observed in various free-running circadian rhythms (Eskin, 1971). However, possibly there is coupling between the two clocks, or both components are frequency transductions of a single master clock.

Such coupling would not be maladaptive because in natural conditions no large phase shifts occur in either system. On the contrary, if strong coupling is induced by entrainment of the circatidal clock, and if strong coupling results in a circadian clock period of 24.0 hrs in DD, then a delicate balance is achieved which would be especially adaptive for an organism which remains buried during the light phase of the day. The rhythm remains in phase with the day-night cycle by virtue of entrainment by mechanical vibration, reception of which

is enhanced by burrowing. Only when storm-induced tidal irregularities fail to cause normal entrainment of the circatidal clock would the animal be likely to emerge into the dangers of daylight, when the phase shifting effect of light on the circadian rhythm would advance or delay the rhythm to its proper phase relation with sunset.

These models must also be able to account for the presence of a modulation of total activity amplitude with an average period of about 15 days. The authors are hesitant to hypothesize yet another clock to account for this rhythm, although long period clocks have been suggested for intertidal crustacea (Enright, 1972). If the period of the circatidal clock in constant conditions increases to 12.9 hrs either by uncoupling from the circadian clock or increasing in period as a result of period increase in the circadian clock, it will scan the 25.0 hr circadian rhythm once every 15 days. By inducing greater diminution of activity, as it crosses the circadian activity peak, it will produce a rhythm in total activity of period 15 days. None of these values need be exact in constant conditions because the semi-monthly rhythm itself is quite imprecise.

However, it has been hypothesized on the basis of the evidence that the circatidal clock actually ceases to function (or at least is disconnected from the effector organs) after some days in constant conditions, and not that it remains active with increased period as is required in this hypothesis. Nevertheless, in natural conditions, the circatidal clock with period of 12.4 hrs would scan the entrained circadian cycle every 15 days. All of these parameters match the natural periodicities with which the various rhythmicities must be synchronised. How the 15 days rhythm comes to be in correct phase relation with the spring/neap tide cycle remains unexplained.

In conclusion, it appears from the data that the activity rhythm of *Talorchestia quoyana* is controlled by at least two clocks. A circadian clock with typical properties dominates control with a nocturnal active phase. Output of locomotor activity is modified by the action of a circatidal clock which inhibits activity at projected time of high tide. It is suggested that after 7–11 days in constant conditions the two clocks become uncoupled and free-run at longer periods. The inhibitory effect of the circatidal clock is no longer obvious after this time but may possibly manifest itself in combination with the circadian output as a 15 day rhythm in total amount of activity. It is further suggested that in natural conditions the circatidal clock is entrained by wave associated vibration and that coupling is strengthened between circatidal and circadian clocks so that the period of the former is about 12.4 hrs and the latter 24.0 hrs, and fluctuation from 24.0 hr period in the circadian clock is corrected by consequent exposure to light. The combined output of these two clocks may account for a semi-monthly rhythm of activity amplitude with maximum activity occurring on those days when the high tides are lowest. This combination of periodicities is an adaptation to the littoral environment where it is highly advantageous for *Talorchestia* to avoid predation during the daylight hours and to avoid exposure to wave action at high tide, and especially at spring tides.

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