

ACTIVITY RHYTHMS AND ORIENTATION IN SANDHOPPERS (CRUSTACEA, AMPHIPODA)

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1. ABSTRACT

The aim of the present review is to combine knowledge of locomotor activity rhythms with that of compass orientation in littoral arthropods. Talitrid amphipods (the sandhoppers) represent a good biological model in the fields of animal orientation and biological rhythms.

The paper examines the activity rhythms of different species of sandhoppers (mainly *Talitrus saltator*), as well as the chronometric mechanisms of compensation for the apparent motion of the sun and moon that these animals use in zonal recovery based on the two astronomical cues. The two chronometric mechanisms seem to be independent of each other and to operate throughout the 24-hour period. The speed of the chronometric mechanism of solar compensation appears to be related to the hours of light and is entrained by the same stimulus (light-dark alternation) that controls the circadian activity rhythm. Therefore, it is probable that in *T. saltator* the same mechanism regulates both the circadian locomotor activity and the solar compensation.

2. INTRODUCTION

The littoral environment is inhabited by many forms of arthropods, each characterized by particular eco-physiological requirements. Their spatial and temporal distribution has been investigated at the population level, although the studies are not very numerous. The littoral environment, especially a sandy beach, is largely characterized by a two-dimensional space, easily represented by two Cartesian axes: the X axis, corresponding to the shoreline, and the Y axis, corresponding to the sea-land direction. Numerous factors characterize the littoral environment and are often present in an intensity gradient, especially along the Y axis; they act differently, but constantly, on all the organisms. Indeed,

the sandy and rocky littoral environments are ecotones in which periodic and non-periodic factors of biotic and abiotic stress are particularly strong, so much so that they condition and modulate many of the physiological and behavioural activities of organisms (see 1-4). The distribution of these stress factors is certainly more intense along the Y axis: tidal alternation, waves, arrival of predators, and variations of temperature, salinity and oxygen are some of the main factors acting on littoral organisms. Some organisms tend to remain in the ecologically advantageous zone without moving horizontally, mainly using physiological compensation mechanisms. Others need to maintain a certain spatial position that preserves stable ecological characteristics. Thus they perform excursions along the Y axis of the shore in phase with the alternation of periodic stress factors or in relation to non-periodic factors.

Many studies concern the presence and function of locomotor activities with various types of periodicity (daily, circadian, tidal, lunar, circalunidian, etc.) which anticipate stressful environmental changes (for a review, see 5-8). Many others deal with the identification of orientation factors and the mechanisms used during homing or zonal recovery (see 9-12). Littoral arthropods, e.g. crabs of the genera *Carcinus* and *Uca* (5, 13), as well as various species of isopods and amphipods (6, 14-18), represent classic examples of these types of studies. Therefore, another review of this topic would seem to be of little interest. Instead, the relationship in littoral arthropods between the biological clock controlling the periodic cycle of locomotor activity and the mechanism of chronometric compensation for the astronomical orientation references has long been neglected. The purpose of this review is to briefly examine the current knowledge about rhythmic locomotor activity and then to devote more attention to the orientation ability and use of astronomical orientation

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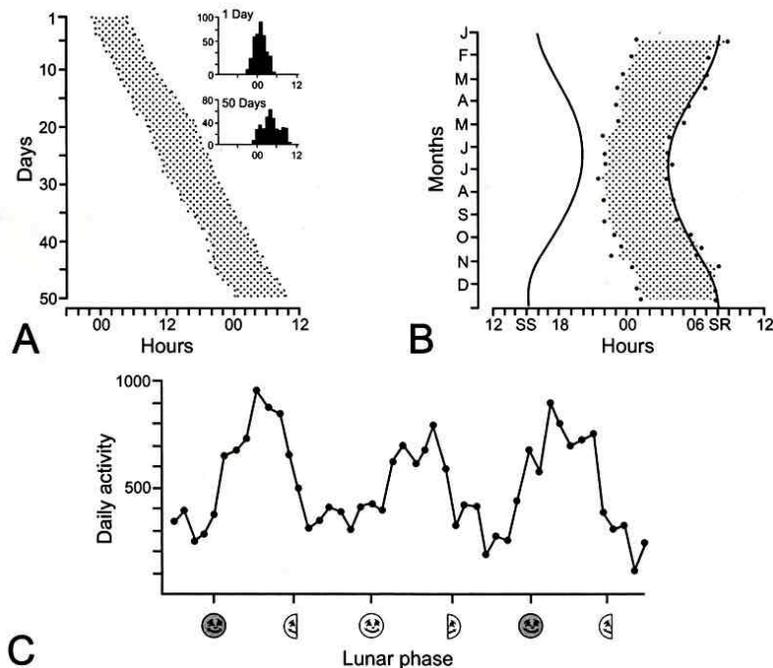


Figure 1. Locomotor activity rhythm of *T. saltator* adults. A, in constant conditions for 50 days (sample size = 50). Abscissa, hours of the day; ordinate, time in days. The gray area indicates the period of activity of the individuals in free running conditions (from 35 modified). The insets show the activity of sandhoppers the first and the last day of experiment. B, relationship between variation of the hour of dawn and dusk during the year and the phase-shift in locomotor activity. The limits of the gray area indicate the beginning and end of the activity. SR, sunrise, SS, sunset (from 37 modified). C, semilunar locomotor activity rhythm. On the abscissa, time expressed as lunar phases; on the ordinate, total daily locomotor activity (from 39 modified).

factors in a group of crustaceans, the talitrid amphipods; they are not only highly representative of the sandy littoral environment, but also constitute an excellent biological model for various types of studies.

3. LOCOMOTOR ACTIVITY RHYTHMS

It has been known for a long time that supralittoral amphipods are mainly nocturnal animals (19-22). However, the first quantitative (albeit preliminary) laboratory investigation of the locomotor activity cycle in *Talitrus saltator* and *Talorchestia deshayesii* was conducted by Ercolini (23). In field studies using fall-traps positioned at various distances from the shoreline, Geppetti and Tongiorgi (24) showed that *T. saltator* in Italy emerges at the surface of the wet band of sand near the water at sunset and performs landward migrations perpendicular to the shore to feed at night. The length of the migration (from a few tens of meters to more than 100) seems to depend mainly on the environmental humidity and temperature of the sand. This has largely been confirmed in recent studies (25-28). The return migration begins at the first light of dawn and ends in the early hours of the morning. Young individuals show a spatially reduced activity with respect to adults in *T. saltator*, and in *Orchestoidea tuberculata* also the phasing of activity differs in juveniles and adults (24, 29). Non-periodic factors, like rain or strong waves, can induce diurnal activity in sandhoppers. However, in regions with strong tides, *T. saltator* and other sandhoppers migrate

a few meters toward the sea to forage in the intertidal zone, although the migration is reduced or absent in conditions of high tide or abundant food in the wet zone (21, 30-32). The extensive study of sandhopper activity rhythms by Bregazzi and Naylor (30, also see 6) conducted in constant conditions clearly showed an endogenous circadian rhythm with nocturnal periodicity in *T. saltator*. It is regulated by the light-dark alternation and is independent of the tidal rhythm. Temperature variations within the natural range do not alter the function of the mechanism, although Pardi and Grassi (33) and Bregazzi (34) found that prolonged exposure to low temperatures (2- 6°C) could influence the chronometric mechanism of solar orientation and locomotor activity. More recently, Williams (35) showed that in constant conditions (free running activity rhythm) the activity period of *T. saltator* remains very stable for many days (up to 50, figure 1A) and that the photoperiod, particularly dawn (36), is the main factor of locomotor activity synchronization: in the laboratory, the phase of the rhythm only changes in relation to the shifting hour of dawn (figure 1B). The circadian rhythm of locomotor activity varies during the year; in fact, the peak of locomotor activity presents a constant phase-angle relationship with the hour of dawn (36, 37). Moreover, dawn is recognized as such by *T. saltator*, albeit with some individual variability, by an irradiance level of around 1-10 lux (36, also see 38) independently of the duration of twilight. Yet this probably cannot be generalized to all sandhoppers, since the entraining stimulus for *Talorchestia*

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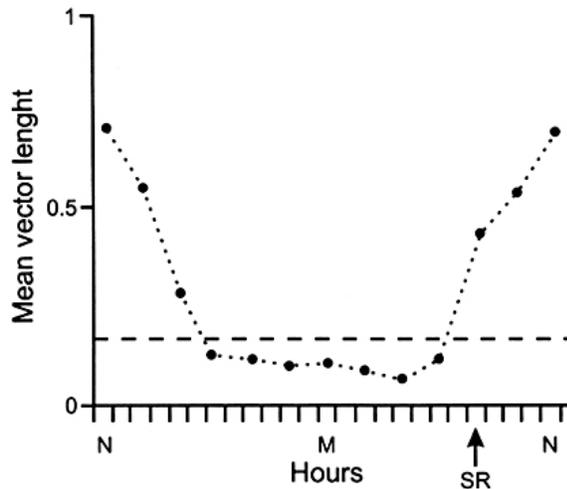


Figure 2. Circadian variation in the scototactic response of *T. saltator*. Releases in an arena equipped with a black screen occupying 60° of the horizon. On the abscissa, hours of the day; on the ordinates, length of the mean vector of each release. N, noon; M, midnight; SR, subjective sunrise. The dashed line indicates the limit of statistical significance ($P < 0.05$) for each release (from 43 modified).

deshayesii seems to be dusk (cf 37). Williams (39) also identified a variation in total daily locomotor activity of *T. saltator* with a circasemilunar rhythm (figure 1C) which, given the absence of a tidal rhythm, should be considered endogenous. In *Talorchestia quoiana* of New Zealand tested in constant conditions, a circatidal rhythm of locomotor activity seems to be present along with the circadian rhythm (40). Unlike in other crustaceans (see 8), there are no clear indications of circalunidian activity rhythms in amphipods.

There have been no studies of the location of the internal clock controlling the circadian rhythm. However, from experiments with selective cooling of different parts of the body conducted on the estuarine amphipod *Corophium volutator*, it seems that control of the circatidal rhythm of swimming activity is due to two different oscillators located in the supra- and subesophageal ganglia (41, 42).

4. CLOCK-CONTROLLED ORIENTATION

4.1. The sun compass

Various authors have demonstrated visual orientation responses (related to simple phototaxis and scototaxis) dependent on the circadian rhythm in *T. saltator* (figure 2) (43-45). However, many species of sandhoppers are certainly better known for their ability to use astronomical orientation references in zonal maintenance.

T. saltator was the first invertebrate species in which the ability to use the sun in zonal orientation was demonstrated, and one of the first animals in which the existence of the sun compass has been confirmed (46). This mechanism is composed of a reference direction, in littoral animals corresponding to the Y axis of the shore (toward

the sea if partially dehydrated, toward land if in water, figure 3A), and a chronometric part that allows variation of the angle of orientation with respect to the sun during the day, thus theoretically maintaining the ecologically effective direction (chronometric photomenotaxis). Figure 3B-E shows the results of releases in a confined environment (a transparent plexiglas bowl, see 48, for details). It is evident that individuals allowed vision only of the sky and sun maintained a virtually constant mean direction independently of the hour of the release (figure 3B, C). As shown by Papi (49) and Pardi and Grassi (33), if we subject the sandhoppers to a light:dark rhythm corresponding to the natural one in duration but clock-shifted by several hours (figure 3D), we will obtain a corresponding deflection of their orientation. Figure 3E illustrates that individuals subjected to a L:D = 12:12 rhythm of artificial illumination for 1 week, anticipated by 6 hrs with respect to the natural one, showed a deflection toward the theoretically expected direction of 114°, thus very near to the 90° expected on the basis of the number of hours of clock-shifting and the season in which the experiment was conducted. An experiment in which individuals of the same Italian population were released in Italy and in Argentina (49) showed that the ability to compensate for the apparent movement of the sun is not based on local references. It should be noted that the ability of solar orientation is maintained for months in adult individuals kept in captivity with artificial illumination (50) and is still present in adults kept in complete darkness for 17 days (51). The biological clock regulating the hourly compensation for the apparent motion of the sun is not affected by temperature variations, unless they exceed rather wide limits (33, 34) and it is active throughout the 24-hour period (52). Indeed, the mechanism of solar compensation is also active at night (as in bees and other arthropods, 53-55). As shown by Pardi (52) releasing sandhoppers clock-shifted by 12 hours during the day (and thus exposed to the sun during their subjective night), at night, sandhoppers compensate for the movement of the sun that passes not to the North (as in bees) but, after setting in the West, returns to the East by passing to the South. Therefore, compensation for the apparent solar motion is regulated by an endogenous circadian timing mechanism working throughout the 24-hour period.

4.2. Modality of compensation for the apparent solar motion

At any given latitude, the azimuthal speed of the sun's apparent motion varies during the year and at different times of the day. At equatorial latitudes in particular, these variations and the change in declination make it difficult to use the sun compass alone (18, 48, 56, 57). Use of the sun as a chronometric orientation reference is certainly simpler at intermediate latitudes, but the question of how the animals compensate for the hourly variation of the sun's azimuthal speed is still being investigated. Insects and birds with homing and sun compass abilities, which can move away from home even for great distances and for long periods of time, seem to possess the ability of differential compensation for the apparent solar motion. In other words, since the azimuthal speed of the sun is not constant during the day or during the

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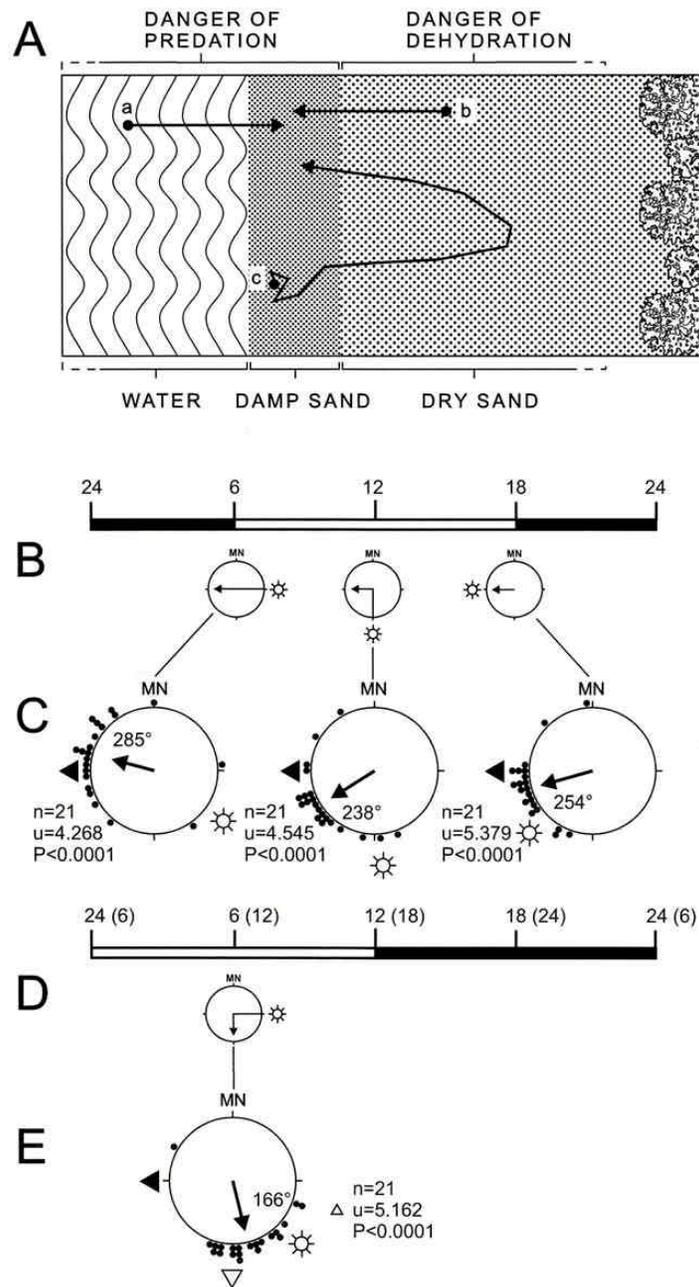


Figure 3. A, zonal orientation of *T. saltator*. Schematic representation of the trajectory followed by the animals when released in water (a), on dry sand (b), or disturbed in the zone of wet sand (c). From 95 modified. B-E, solar orientation of *T. saltator*. Releases in a confined environment. B, schematic representation of the angle the animals should assume with respect to the sun, at three hours of the day, after being kept in a L:D 12:12 cycle corresponding to the natural one; C, result of the releases (controls); D, schematic representation of the angle the animals should assume with respect to the sun, at three hours of the day, after being kept in a L:D 12:12 cycle anticipated by 6 hours with respect to the natural one (in brackets the subjective time); E, result of the releases (clock-shifted). Black triangle outside the distributions, home beach seaward direction; open triangle, expected direction for clock-shifting experiment. The arrow inside each distribution represents the mean vector (length varies between 0 and 1 = radius of the circle); black dots, sandhoppers' directions (each dot represents one individual). The symbol of the sun corresponds to the sun's azimuth at the moment of release. Sample size, n, V test value, u, with the probability level, P, are also given. The distributions were analysed with the procedures proposed by Batschelet (47).

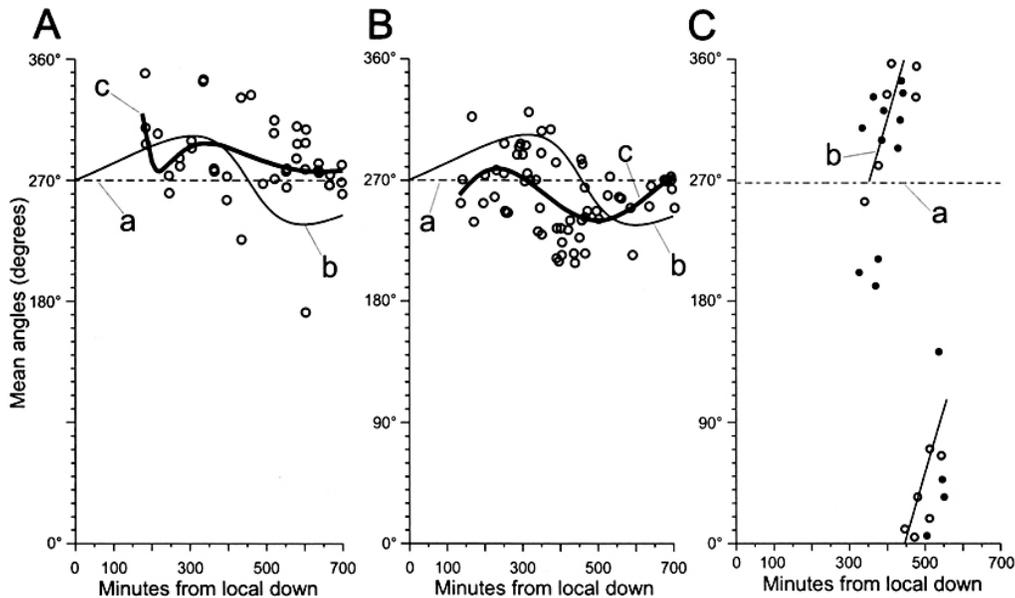


Figure 4. Solar orientation of *T. saltator*. Releases of individuals subjected to a L:D cycle corresponding to the natural one. A, individuals collected 1 day earlier, B, individuals collected about 20 days earlier; C, individuals collected about 20 days earlier but subjected to a L:D = 4:20 cycle. Each dot corresponds to the mean angle of each release. Empty dots, group releases; solid dots, releases of single individuals. Line a, expected direction for differential solar compensation; line b, expected direction for constant speed of solar compensation (see text and 66); line c, fractional polynomial interpolation. Circular correlation was used for the data in figure C (see text and 47 for further details).

year, they appear to have a type of ephemerids function that allows them to return home by taking account of the variations in the sun's azimuthal speed for that day and latitude, without committing errors that might derive from non-differential solar compensation (58-64).

In sandhoppers, experiments of solar orientation in freshly collected adult individuals performed in two different periods of the year showed an excellent correspondence between the theoretical curve for perfect compensation for the sun's azimuthal variation and the mean directions assumed by the animals(20). Therefore, it was hypothesized that the speed of this mechanism varies not only during the year but also during the day, and in this way theoretically perfect compensation for the sun's azimuthal variation (= differential compensation) can be achieved. Considering that sandhoppers move away from the wet band of sand only for a few minutes during the day (often only for a few seconds) and that they do not have homing mechanisms (the zonal orientation is unidirectional, see 65), I recently proposed an alternative model, simpler than that of differential compensation (66, 67). In this model, the chronometric mechanism of the sun compass compensates for azimuthal variation of the sun at a constant speed, according to the following formula:

$$EY_{L-S}(t) = K*t + AZs(t)$$

in which $EY_{L-S}(t)$ is the expected Y axis (landward or seaward) direction (expressed in degrees from North) that the sandhoppers must assume after t minutes from sunrise, $AZs(t)$ is the sun's azimuth at the time of the release. The angular speed of correction

K is

$$K = (AZsS - AZsR) / ML$$

where $AZsS$ and $AZsR$ are the azimuths of the sun at sunset and sunrise respectively, ML indicates the minutes of light from sunrise to sunset. If the sun's azimuthal speed is not constant during the day (e.g. around the equinox), EY will assume a curvilinear form (and not a horizontal line) because of the discrepancy between the speed of the compensation mechanism and the azimuthal speed of the sun.

Therefore, since the speed of compensation is determined on the basis of the relationship between the sun's azimuthal variation from dawn to sunset and the hours of light, the part of the ephemerids the sandhopper should know is restricted to the total amount of degrees the sun passes through in a certain day or period of the year, during the period of light. Information about the hours of light can very well be obtained by direct observation the day, or several days, before.

The model allows one to predict the amplitude and direction of oscillations of the theoretical directions around the Y axis of the home beach at various hours of the day. In the case of differential compensation, there should be no oscillation.

Experiments conducted far from the sea, in a confined environment, with adults collected about 20 days before (figure 4B) showed that the pattern of the mean angles during the day was sinusoidal, in good agreement

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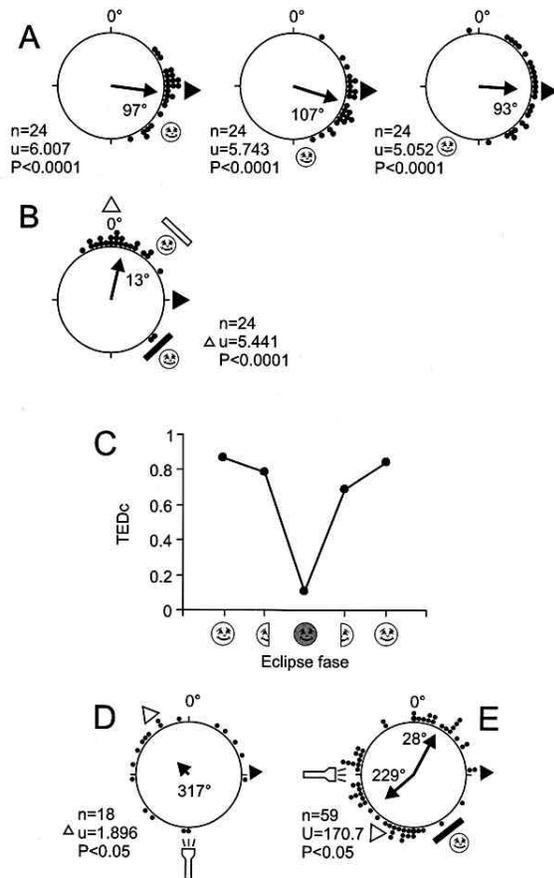


Figure 5. Lunar orientation of *T. saltator*. Experiments under the full moon. A, adults tested at different hours of the night. The symbol of the moon corresponds to the moon's azimuth at the moment of release B, mirror experiment: the lunar azimuth was deviated by 130° with a mirror, while vision of the true moon was blocked with a screen. C, eclipse releases: relationship between the portion of the moon that is visible (abscissa) and the goodness of orientation (=TEDc, see 47). D, releases performed in the new moon phase and E, full moon phase. The moon was screened and the beam of an electric torch was projected onto the sandhoppers in the bowl. All the releases were carried out without the horizontal component of the magnetic field. See figure 3 for further details.

with the theoretical pattern predicted by the model. Instead, the pattern of the mean angles deriving from releases of adults collected the day before the experiment was not sinusoidal but more rectilinear (figure 4A). That sandhoppers use the ratio between azimuthal variation of the sun and hours of light to establish the speed of solar compensation is supported by the results of the following experiment (66). Adult sandhoppers collected about 20 days before were subjected for 10 - 15 days to a cycle of artificial illumination L:D = 4:20, with the subjective noon in phase with the natural noon of the experimental site. In this way, the period of light was greatly reduced (from more than 15 hours to 4 hours). Therefore, according to the model, the chronometric mechanism should accelerate to

about 62°/hr (figure 4C, line b). In fact, the mean directions recorded in the experiments significantly agreed with the theoretical line ($R^2 = 0.485$, $n = 28$, $P < 0.001$, circular correlation). It should also be noted that the distribution of locomotor activity recorded during the L:D = 4:20 cycle agreed rather well with that of the imposed L:D rhythm.

4.3. The moon compass

The other astronomical orientation factor used by sandhoppers is the moon. However, the moon also presents some theoretical difficulties in its use as a chronometrically based orientation reference: it is not always visible during the synodic cycle, it is not always visible at the same hour and at the same azimuth, and it does not always present the same shape. These characteristics, together with the fact that among invertebrates a chronometrically compensated lunar orientation has been shown only in some species of sandhoppers (51, 68-72), has produced a great deal of scepticism towards the results achieved (see 73, 74).

Recently, experiments of lunar orientation have been resumed using different techniques. Releases in the plexiglas bowl occurred in the presence of sea water (about 1 cm) which motivates the sandhoppers to assume the ecologically effective direction. This overcomes the problem of the high degree of environmental humidity at night which decreases the motivation for orientation in sandhoppers previously dried for several minutes. Moreover, observation of the experimental subjects did not occur directly nor with the use of flash-illuminated photos. Instead, an infrared-sensitive videocamera situated beneath the bowl was used. Since some species of sandhoppers can use the natural magnetic field to assume a certain direction (see 18), the releases were carried out in an artificial magnetic field able to cancel the horizontal component of the natural magnetic field.

The experiments were performed at different hours of the night under a full moon, with adults collected during the previous new moon. They fully confirmed the existence of chronometrically compensated lunar orientation, independent of the possible use of the magnetic reference (figure 5A). The classic experiment of Santschi (75), in which the image of the moon (in our case) was reflected onto the animals from a randomly chosen azimuth while the true moon was screened, clearly showed that the lunar disk is used as an orientation factor (figure 5B). In fact, the mean direction of the sandhoppers was diverted by 84° with respect to that of the controls tested under the true moon.

That the shape of the moon is not a determinant factor for correct orientation was confirmed by experiments performed during the lunar eclipse of 1997 (76, figure 5C); it is obvious that the individuals were able to maintain a good orientation toward the ecologically effective direction despite the variations in the moon's shape. In addition, preliminary tests seem to indicate that the lunar clock works continuously throughout the synodic month. In fact (figure 5D), in the experiment during the new moon phase, in which the beam of an electric torch simulating the moon (otherwise invisible) was projected onto the animals from a

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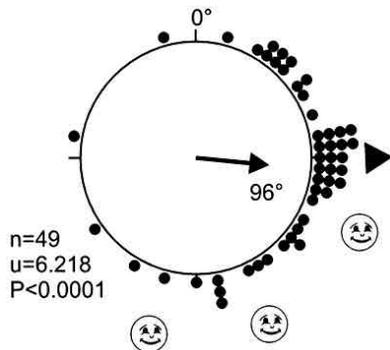


Figure 6. Lunar orientation of *T. saltator*. Inexpert young born in the laboratory. See figures 3 and 5 for further details.

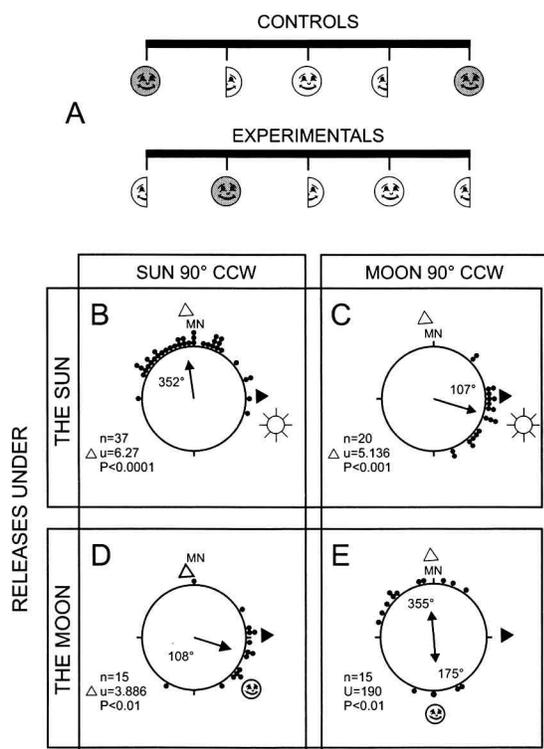


Figure 7. Lunar orientation of *T. saltator*. Moon clock-shifting. A, schematic representation of the phases of the natural synodic cycle and the cycle anticipated by 7 days (clock-shifted): the natural full moon phase in which the experiments were conducted corresponds to the subjective first quarter for the clock-shifted animals. B, D, (L:D = 12:12, anticipated by 6 hours = 90° CCW); C, E, tests with individuals clock-shifted for the moon. B, C, tests during daytime; D, E, tests at night. See figure 3 for further details.

randomly chosen azimuth, the sandhoppers assumed an angle of orientation with respect to the light stimulus comparable to that between the true moon and the ecologically effective direction, and thus in agreement with the theoretically expected direction for lunar orientation based on the true moon's azimuth at the time of the experiment. That the electric torch was used as an orientation reference (being confused with the moon) was

confirmed by the results of an experiment in which vision of the full moon was screened from the animals in the bowl and the torch beam was projected onto them from a randomly chosen azimuth. As seen in figure 5E, the mean direction of the individuals was diverted by a number of degrees (60°) comparable to that between the azimuth of the screened true moon and the azimuth of the false moon (62°).

It has been known for many years that the direction of the Y axis of the beach and the mechanism of compensation for the apparent solar motion are innate (77-79). Recent experiments with young *T. saltator* born in the laboratory and exposed to the natural sky only at the time of the experiments seem to show an innate ability of lunar orientation (figure 6).

4.4. Relationship between the sun and moon compasses

The relationship between the chronometric orientation mechanism regulating solar compensation and the one regulating lunar compensation was investigated by subjecting adult individuals of *T. saltator* to a L:D rhythm with a duration corresponding to that of the natural one but anticipated with respect to the latter so as to obtain an expected deflection of the orientation of 90°. Contemporaneously, another group of individuals was subjected to an artificial moon consisting of a small light bulb (diameter = 3 mm, irradiance = 0.1 micro watt cm⁻²) that was illuminated in relation to a synodic cycle anticipated by 7 days with respect to the natural one. In practice, when the sandhoppers were tested under the full moon, the lunar phase of the artificial cycle corresponded to the first quarter (figure 7A, also see 80). The controls were subjected to artificial L:D and lunar cycles with duration and phase corresponding to the natural ones. The experimentals and controls were released in the usual apparatus, both during the day (sun) and at night (full moon).

The results clearly showed that the experimentals with a clock-shifted L:D rhythm, tested under the sun, showed the deflection predicted on the basis of the clock-shifting (figure 7B) while other individuals of the same group tested at night were oriented in the same way as the controls (figure 7D). In contrast, the individuals with a clock-shifted lunar cycle showed a mean direction that agreed well with the expected one for the clock-shifting if tested under the moon (figure 7E), while they did not show any substantial deflection with respect to the controls if tested under the sun (figure 7C).

5. CONCLUSIONS AND PERSPECTIVES

Periods of locomotor activity with some relationship to the synodic cycle are very widespread in marine invertebrates. However, at least in littoral arthropods, most of the studies have dealt with the relationship between activity rhythms and a more macroscopic effect that the moon has on this environment: the tides (see 7, 8, 81); in addition to the periodicity of about 12.4 hours (tidal), a circalunidian activity rhythm (of about 24.8 hours) was also found fairly recently (see 82). A

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generalized presence of endogenous circatidal or circalunidian rhythms of locomotor activity has not been found in sandhoppers, despite the strong similarity of the environments inhabited by the various species and the (highly) probable similarity of the orientation mechanisms used in zonal recovery.

On the other hand, it is true that various types of rhythms with circatidal, semilunar, etc. periodicity are observed in many species of amphipods (e.g. see 83-87.). However, since the phylogenetic relationships within the amphipods and the Gammaroidea have not been completely clarified (88, 89) we can only hypothesize that in sandhoppers the chronometric mechanism controlling lunar orientation (which seems to be separate from the mechanism for solar compensation) is effectively controlled by a clock with circalunidian or circatidal periodicity of ancient origin and different function; on the basis of the ecological requirements of many sandhopper taxa (inhabitants of the supralittoral near the high tide limit), it could have been used by them to maximize the straightness of their movement along the Y axis of the sea-land ecotone.

Moreover, some preliminary studies (76, 90) indicate that, as for the sun compass (17), the ecologically effective direction and the mechanism of chronometric compensation of the lunar compass are innate in *T. saltator*.

The relationship between the chronometric mechanism controlling circadian locomotor activity and the one controlling solar compensation certainly deserves to be more thoroughly studied. Yet at present, it is sufficiently clear that the endogenous mechanism regulating solar orientation functions throughout the 24-hr period and is entrained by the same stimulus (light-dark alternation) that controls the circadian activity rhythm. Therefore, it is probable, as shown already in birds (see 73) and hypothesized by Bregazzi and Naylor (30) for sandhoppers, that in *T. saltator* the same mechanism regulates both the circadian locomotor activity and the solar compensation.

Regarding the lunar clock, there have been no studies relating the ability of compensation for lunar motion and the semilunar rhythm of locomotor activity in *T. saltator*. On the basis of preliminary studies, it seems that the chronometric mechanism of lunar compensation in adults functions throughout the synodic month (experiments in the new moon phase) and that vision of the moon could be one of the factors of entrainment (clock-shifting experiments). In young inexperienced sandhoppers, the zeitgeber regulating the innate ability to use the moon as a chronometrically compensated orientation factor is not known; however, in view of what has just been said, vision of the lunar disk could be an instantaneous entraining factor.

It is also interesting that the irradiance level at which *T. saltator* recognizes dawn as the important zeitgeber (about 1-10 lux) is greater than that of the full moon (0.3-0.8 lux). Therefore, moonlight is not a source of disturbance for the recognition of dawn by sandhoppers,

which however are able to use the moon as a nocturnal orientation factor.

Since the two chronometric mechanisms are separate and the clock-shifting of animals in conditions of free running activity rhythm is not corrected by an irradiance level of about 0.5 lux (comparable to the intensity of moonlight) (see 14, 39), it can be hypothesized that the chronometric mechanism regulating lunar orientation is independent of the one controlling locomotor activity, which instead is related to the one regulating solar compensation.

The experiments conducted under the sun during the subjective night and the releases performed both at night and during the day with natural and artificial illumination (77, 80, 91, 92) raise the interesting question of identification of the factors of astronomical orientation by sandhoppers. Given the relative lack of information, this subject cannot be included in the present review. However, it is appropriate to mention briefly that preliminary results seem to indicate an interrelationship between intrinsic characteristics of the orientation factor and the time of day (92).

During the day, it is of primary importance that the sandhoppers avoid stress from desiccation due to the high temperatures that can occur on the beach, combined with low levels of relative humidity (20). At night, however, these factors are rarely limiting. This is shown by the fact that at night the sandhoppers perform foraging excursions even some tens of meters away from their preferred zone. Therefore, the selective pressures that led to the lunar orientation (or at least some of them) are not the same as those operating during daytime. At night, use of the moon for zonal orientation is probably necessary to avoid biological stress factors. Although sandhoppers do not have specific predators, many invertebrates and vertebrates feed on them more or less occasionally (see 93-95) and it is known that sandhoppers play an important role in the energy flow of sandy littoral ecosystems (96).

Biological stress factors, along with the other non-biological stress factors already mentioned, may also have led to the refinement of the sun compass: escaping in a line perpendicular to the shoreline (the shortest route away from it) or returning to the preferred zone as quickly as possible would favour an individual's possibility of survival. However, to accomplish this, an optimisation of the ability of solar orientation, in terms of perfect compensation for the sun's motion, is not strictly necessary. For instance, in some species of *Cataglyphis* and *Polyergus* ants, it is certainly important to minimize the error of orientation deriving from a hypothetically imperfect solar compensation, since the consequence would be not being able to find the nest (punctiform destination), possibly with serious damage to the whole colony (97, 98). In sandhoppers, this problem does not exist since the destination is planar (the band of wet sand of the beach) and is easily reached when the animal is oriented in the right direction. Thus the error produced by non-differential compensation would be small, given the short movements

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occurring during the day and the potent backup systems of solar orientation (vision of the landscape, inclination of the substrate, natural magnetic field, see 18, 99, for reviews). Therefore, solar compensation in intertidal animals forced to move along the sea-land axis to remain in a zone with certain physico-chemical characteristics probably involves a simpler mechanism than the one required by central place forager arthropods.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. G. Branch & M. Branch. The living shores of Southern Africa. C. Struik Publ., Cape Town (1981)
2. A. McLachlan & T. Erasmus. Sandy beaches as ecosystems. Junk, The Hague (1983)
3. G. Chelazzi & M. Vannini Behavioral adaptation to intertidal life. NATO ASI Ser. A 151, 524 pp., Plenum Press, New York (1988)
4. D. Raffaelli & S. Hawkins. Intertidal ecology. Chapman & Hall, London (1996)
5. E. Naylor. Tidally rhythmic behaviour of marine animals. In: Physiological adaptations of marine animals. Ed: Laverack M.S. Symp. Soc. Exp. Biol. 39, 63-93. (1985).
6. E. Naylor. Clock – controlled behaviour in intertidal animals. In: Behavioral adaptation to intertidal life. Eds: Chelazzi, G. & Vannini, M. NATO ASI Ser. A 151. Plenum Press, New York (1988)
7. J. D. Palmer. Biological clocks in marine organisms. Wiley Interscience Publication, John Wiley & Sons, New York (1974).
8. J.D. Palmer. The biological rhythms and clocks of intertidal animals. Oxford University Press, New York (1995)
9. Rebach S. The use of multiple cues in short – range migrations of Crustacea. *Am. Midl. Nat.* 105, 168-180 (1981).
10. W.F. Herrnkind. Movement patterns and orientation. In: The biology of Crustacea. 7. Behaviour and Ecology. Eds: Vernberg, F.J. & Vernberg, W. Academic Press, New York (1983)
11. G. Chelazzi. Invertebrates (excluding Arthropods). In: Animal homing. Ed: Papi, F. Chapman & Hall, London (1992)
12. R. Wehner. Arthropods. In: Animal homing. Ed: Papi, F. Chapman & Hall, London (1992)
13. Palmer J.D. Contributions made to chronobiology by studies of fiddler crab rhythms. *Chronobiol. Int.* 8, 110-130 (1991)
14. Enright J.T. A virtuoso isopod: circa lunar rhythms and their tidal fine structure. *J. Comp. Physiol.* 77, 141-162. (1972)
15. Enright J.T. Orientation in time: endogenous clocks. *Marine Ecology* 2, 917-944 (1974).
16. J.T. Enright. Migration and homing of marine invertebrates: a poutpourri of strategies. In: Animal Migration, Navigation, and Homing. Eds: K. Schmidt – Koenig & Keeton W. Springer, Berlin (1978)
17. Pardi L. & A. Ercolini. Zonal recovery mechanism in talitrid crustaceans. *Boll. Zool.* 53, 139-160 (1986).
18. Ugolini A. The sun and the magnetic field in the orientation of equatorial sandhoppers. *Trends Comp. Biochem. Physiol.* IN PRESS (2002)
19. Verwey J. Einiges aus der Biologie von *Talitrus saltator* Mont. *10th Congr. Int. Zool.*, 1156-1162 (1929)
20. Pardi L. & F. Papi. Ricerche sull'orientamento di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). I. L'orientamento durante il giorno in una popolazione del litorale tirrenico. *Z. vergl. Physiol.* 35, 459-489 (1953)
21. Williamson D.I. Landward and seaward movements of the sand hopper *Talitrus saltator*. *Adv. Sci.* 11, 69-78 (1954)
22. Palluault M. Notes ecologiques sur le *Talitrus saltator*. *Arch. Zool. Exp. Gén.* 91, 105-129 (1954)
23. Ercolini A. Sul ciclo normale di attività in alcuni Talitridae litorali. *Boll. Ist. Mus. Zool. Univ. Torino* 6 (7), 165-170 (1958)
24. Geppetti L. & P. Tongiorgi. Ricerche ecologiche sugli artropodi di una spiaggia del litorale tirrenico. II. Le migrazioni di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *Redia* 50, 309-336 (1967)
25. Scapini F., L. Chelazzi, I. Colombini & M. Fallaci. Surface activity, zonation and migration of *Talitrus saltator* on a Mediterranean beach. *Mar. Biol.* 112, 573-581 (1992)
26. Scapini F., M. Audoglio, L. Chelazzi, I. Colombini & M. Fallaci. Astronomical, landscape and climatic factors influencing oriented movements of *Talitrus saltator* in nature. *Mar. Biol.* 128, 63-72 (1997).
27. Tsubakura T., S. Goshima & S. Nakao. Seasonal horizontal and vertical distribution patterns of the supralittoral amphipod *Trinorchestia trinitatis* in relation to environmental variables. *J. Crust. Biol.* 17 (4), 674-686 (1997)
28. Weslawski J.M., T. Kupidura & M. Zabicki. Sandhoppers, *Talitrus saltator* (Montagu, 1808) (Amphipoda, Gammaridea), at the Polish Baltic coast: season and spatial distribution patterns. *Crustaceana* 73, 961-969. (2000).
29. Kennedy F., E. Naylor & E. Jaramillo. Ontogenetic differences in the circadian locomotor activity rhythm of the talitrid amphipod crustacean *Orchestoidea tuberculata*. *Mar. Biol.* 137, 511-517 (2000)
30. Bregazzi P.K. & E. Naylor. The locomotor activity rhythm of *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *J. Exp. Biol.* 57, 375-391 (1972)
31. Craig P.C. Behaviour and distribution of the sand - beach amphipod *Orchestoidea corniculata*. *Mar. Biol.* 23, 101-109 (1973)
32. Pardi L., A. Ercolini & F. Ferrara. Ritmo di attività e migrazioni di un crostaceo anfipode (*Talorchestia martensii* Weber) sul litorale della Somalia. *Accad. Naz. Lincei, Rc. (Cl. SMFN)* 55, 609-623 (1974)
33. Pardi L. & M. Grassi. Experimental modifications of direction-finding in *Talitrus saltator* (Montagu) and *Talorchestia deshayesei* (Aud.) (Crustacea, Amphipoda). *Experientia* 11, 202-210 (1955)

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34. Bregazzi P.K. The effect of low temperature upon the locomotor activity rhythm of *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *J. Exp. Biol.* 57, 393-399 (1972)
35. Williams J.A. Environmental influence on the locomotor activity rhythm of *Talitrus saltator* (Crustacea, Amphipoda). *Mar. Biol.* 57, 7-16 (1980)
36. Williams J.A. The effect of dusk and dawn on the locomotor activity rhythm of *Talitrus saltator* (Crustacea, Amphipoda). *J. Exp. Mar. Biol. Ecol.* 42, 285-297 (1980)
37. Williams J.A. The light-response rhythm and seasonal entrainment of the endogenous circadian locomotor rhythm of *Talitrus saltator* (Crustacea, Amphipoda). *J. Mar. Biol. Ass. U.K.* 60, 773-785 (1980)
38. E. Bünning. Symptoms, problems and common features of circadian rhythms in plants and animals. In: Proc. Int. Symp. Circadian Rhythmicity Ed.: Bierhuizen, J.F. Veenman and Zonen, Wageningen (1971).
39. J.A. Williams. A semi-lunar rhythm of locomotor activity and moult synchrony in the sand-beach Amphipod *Talitrus saltator*. In: Cyclic phenomena in marine plants and animals. Eds: Naylor E. & Hartnoll, R.G. Proc. 13th European Mar. Biol. Symp., Pergamon Press, Oxford (1979)
40. Benson J.A. & R.D. Lewis. An analysis of the activity rhythm of the sand beach amphipod *Talorchestia quoyana*. *J. Comp. Physiol.* 105, 339-352 (1976)
41. Harris G.J. & E. Morgan.. The location of circa-tidal pacemakers in the estuarine amphipod *Corophium volutator* using a selective chilling technique. *J. Exp. Biol.* 110, 125-142 (1984)
42. E Morgan, G.J. Harris & F. Holmstroem. Circa-tidal pacemakers in the nervous system of an amphipod. In: Circadian rhythms in the central nervous system. Eds: Redfern et al. Mc Millan, Houndmills (1985)
43. Edwards J.M. & Naylor. Endogenous circadian changes in orientational behaviour of *Talitrus saltator*. *J. Mar. Biol. Ass. U.K.* 67, 17-26 (1987)
44. Mezzetti M.C., E. Naylor & F. Scapini. Rhythmic responsiveness to visual stimuli in different populations of talitrid amphipods from Atlantic and Mediterranean coasts: an ecological interpretation. *J. Exp. Mar. Biol. Ecol.* 181, 279-291 (1994)
45. Nardi M., L.E. Persson & F. Scapini. Diel variation of visual response in *Talitrus saltator* and *Talorchestia deshayesii* (Crustacea, Amphipoda) from high latitude beaches of low tidal amplitude. *Estuarine Coast. Shelf Sci.* 50, 333-340 (2000)
46. Pardi L. & F. Papi. Die sonne als kompass bei *Talitrus saltator*. *Naturwissenschaften* 39, 262-263 (1952)
47. E. Batschelet. Circular statistics in biology. London: Academic Press (1981)
48. Ugolini A. Relationship between compass systems of orientation in equatorial sandhoppers. *Anim. Behav.* 62, 193-199 (2001)
49. Papi F. Experiments on the sense of time in *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). *Experientia* 11, 201 (1955)
50. Ercolini A. Ricerche sull'orientamento solare degli anfipodi. La variazione dell'orientamento in cattività. *Arch. Zool. Ital.* 48, 147-179 (1964)
51. Papi F. & L. Pardi. Ricerche sull'orientamento di *Talitrus saltator*. II. Sui fattori che regolano la variazione dell'angolo di orientamento nel corso del giorno. L'orientamento di notte. L'orientamento diurno di altre popolazioni. *Z. vergl. Physiol.* 35, 490-518 (1953)
52. Pardi L. Esperienze sull'orientamento di *Talitrus saltator* (Montagu) (Crustacea, Amphipoda): l'orientamento al sole degli individui a ritmo nicotimerale invertito, durante la "loro notte". *Boll. Ist. Mus. Zool. Univ. Torino* 4, 127-134 (1954)
53. Lindauer M. Dahertänze im Bienenstock und ihre Beziehung zur Sonnenbahn. *Naturwissenschaften* 41, 506-507 (1954)
54. Lindauer M. Sonnenorientierung der Bienen unter der Äquatorsonne und zur Nachtzeit. *Naturwissenschaften* 44, 1-6 (1957)
55. Birukow G. Lichtkompaßorientierung beim Wasserläufer *Velia currens* F. (Heteroptera) am Tage und zur Nachtzeit. I. Herbst- und Winterversuche. *Z. Tierpsychol.* 13, 463-484 (1957)
56. Ugolini A. & L. Pardi. Equatorial sandhoppers do not have a good clock. *Naturwissenschaften* 79, 279-281 (1992)
57. Ugolini A. The orientation of equatorial sandhoppers during the zenithal culmination of the sun. *Ethol. Ecol. Evol.* IN PRESS (2002)
58. Whener R. & B. Lanfranconi. What do the ants know about the rotation of the sky? *Nature* 293, 731-733 (1981)
59. Neuss M. & H.G. Wallraff. Orientation of displaced homing pigeons with shifted circadian clocks: prediction vs observation. *Naturwissenschaften* 75, 363-365 (1988)
60. Schmidt-Koenig K., R. Ranvaud, J.U. Ganzhorn & O.C. Gasparotto. Retardation of homing pigeons' ephemerides? *Naturwissenschaften* 78, 330-333 (1991)
61. Wehner R. & M. Müller. How do ants acquire their celestial ephemerids function? *Naturwissenschaften* 80, 331-333 (1993)
62. Dyer F.C. & J.A. Dickinson. Development of sun compensation by honeybees: How partially experienced bees estimate the sun's course. *Proc. Natl. Acad. Sci. USA* 91, 4471-4474 (1994)
63. Towne W.F. & W.H. Kirchner. Honey bees fail to update their solar ephemerids after a displacement. *Naturwissenschaften* 85, 459-463 (1998)
64. Wiltschko R., M. Walker & W. Wiltschko. Sun-compass orientation in homing pigeons: compensation for different rates of change in azimuth?. *J. Exp. Biol.* 203, 889-894 (2000)
65. Jander R. Ecological aspects of spatial orientation. *Annu. Rev. Ecol. Syst.* 6, 171-188 (1975)
66. Ugolini A. & F. Frittelli. Photoperiod length and chronometric mechanism of the sun compass in Mediterranean sandhoppers. *J. Mar. Biol. Ass. UK* 78, 1155-1165 (1998)
67. Ugolini A., B. Tiribilli & V. Boddi. The sun compass of the sandhopper *Talitrus saltator*: the speed of the chronometric mechanism depends on the hours of light. *J. Exp. Biol.* 205, 3225-3230 (2002)
68. Papi F. & L. Pardi. Nuovi reperti sull'orientamento lunare di *Talitrus saltator* L. *Z. vergl. Physiol.* 41, 583-596 (1959)
69. Papi F. & L. Pardi. On the lunar orientation of sandhoppers (Amphipoda, Talitridae). *Biol. Bull.* 124, 97-105 (1963)

Activity rhythms and orientation in sandhoppers

70. Papi F. Orientation by the night: the moon. *Cold Spring Harb. Symp. Quant. Biol.* 25, 475-480 (1960)
71. Enright J. T. Lunar orientation in *Orchistoidea corniculata* Stout (Amphipoda). *Biol. Bull.* 120, 148-156 (1961).
72. J. T. Enright. When the beachopper looks at the moon: the moon-compass hypothesis. In *Animal orientation and navigation*. Eds: Galler S.R. et al. NASA SP-262, Washington D.C. (1972)
73. K. Hoffmann. Clock-mechanism in celestial orientation of animals. In: *Circadian clocks*. Ed: Aschoff J. North-Holland Publishing Co., Amsterdam (1965)
74. H.G. Wallraff. Clock-controlled orientation in space. In: *Handbook of behavioral neurobiology*. Ed: Aschoff, J. Plenum, New York (1981)
75. Santschi F. Observations and remarques critiques sur le mécanisme de l'orientation chez les fourmis. *Rev. Suisse Zool.* 19, 303-338 (1911)
76. Ugolini A., C. Melis & R. Innocenti. Moon orientation in adult and young sandhoppers. *J. Comp. Physiol. A* 184, 9-12 (1999)
77. Pardi L., A. Ercolini, V. Marchionni. & C. Nicola. Ricerche sull'orientamento degli anfipodi del litorale: il comportamento degli individui allevati in laboratorio sino dall'abbandono del marsupio. *Atti Accad. Sci.* Torino 92, 1-8 (1958)
78. Pardi, L. Innate components in the solar orientation of littoral amphipods. *Cold Spring Harb. Symp. Quant. Biol.* 25, 394-401 (1960)
79. Pardi L. & F. Scapini. Inheritance of solar direction finding in sandhoppers: mass - crossing experiments. *J. Comp. Physiol. A* 151, 435-440 (1983)
80. Ugolini A., C. Melis, R. Innocenti, B. Tiribilli & C. Castellini. Moon and sun compasses in sandhoppers rely on two separate chronometric mechanisms *Proc. R. Soc. Lond. B.* 266, 749-752 (1999)
81. D. Neumann. Tidal and lunar rhythms. In: *Handbook of behavioral neurobiology*. 4. Biological rhythms. Ed: Aschoff J. Plenum Press, New York (1981)
82. Palmer J.D. The clocks controlling the tide - associated rhythms of intertidal animals. *BioEssays* 22, 32-37 (2000)
83. Enright J.T. The tidal rhythm of activity of a sand - beach amphipod. *Z. vergl. Physiol.* 46, 276-313 (1963)
84. Morgan E. The activity rhythm of the amphipod *Corophium volutator* (Pallas) and its possible relationship to changes in hydrostatic pressure associated with tides. *J. Anim. Ecol.* 34, 731-746 (1965)
85. Fincham A.A. Rhythmic behaviour of the intertidal amphipod *Bathyporeia pelagica*. *J. Mar. Biol. Ass. U.K.* 50, 1057-1068 (1970)
86. Fincham A.A. Rhythmic swimming and rheotropism in the amphipod *Marinogammarus marinus* (leach). *J. Exp. Mar. Biol. Ecol.* 8, 19-26 (1972)
87. Forward R.B. Phototaxis of a sand - beach amphipod: physiology and tidal rhythms. *J. Comp. Physiol.* 135, 243-250 (1980)
88. Berge J., G. Boxshall & W. Vader. Phylogenetic analysis of the Amphipoda, with special emphasis on the origin of the Stegocephalidae. *Pol. Arch. Hydrobiol.* 47, 379-400 (2000)
89. Kim C.B. & W. Kim. Phylogenetic relationships among gammaridean families and amphipods suborders. *J. Nat. Hist.* 27, 933-946 (1993)
90. Ugolini A. The moon as an orientation factor. *Proc. R. Inst. Navigation Conference, Oxford*, 11 pp. (2001)
91. Ugolini A., B. Tiribilli & C. Castellini. Artificial light and sun compass orientation in the sandhopper *Talitrus saltator* (Crustacea-Amphipoda). *J. Comp. Physiol. A.* 182: 43-50 (1998)
92. Ugolini A., B. Tiribilli & C. Castellini. Sun and moon identification by sandhoppers (Amphipoda, Talitridae). *Abstr. Int. Conf. Invert. Vision, Backaskog Castle, Sweden*, p. 74 (2001)
93. P.R. Evans. Predation of intertidal fauna by shorebirds in relation to time of the day, tide and year. In: *Behavioral adaptation to intertidal life*. Eds: Chelazzi G. & Vannini M. NATO ASI Ser. A 151. Plenum Press, New York (1988)
94. Wildish D.J. Ecology and natural history of aquatic Talitroidea. *Can. J. Zool.* 66, 2340-2359 (1988)
95. Ugolini A. Jumping and sun compass in sandhoppers: an antipredator interpretation. *Ethol. Ecol. Evolution* 8, 97-106 (1996)
96. C.L. Griffiths, J.M.E. Stenton - Dozey & K. Koop. Kelp wrack and the flow of energy through a sandy beach ecosystem. In: *Sandy beaches as ecosystems*. Eds: McLachlan A. & Erasmus T. Junk Publ., The Hague (1983)
97. R. Whener. The ant's celestial compass system: spectral and polarization channels. In: *Orientation and communication in Arthropods*. Ed: Lehrer M. Birkhauser verlag, Basel (1997)
98. Ugolini A., F. Le Moli & A. Grasso. The homing of scouts and raiding columns of the slave - making ant *Polyergus rufescens*. *Proc. R. Inst. Navigation Conf., Oxford*, 10 pp. (1997)
99. A. Ugolini, F. Scapini, G. Beugnon & L. Pardi. Learning in zonal orientation of sandhoppers. In: *Behavioral adaptation to intertidal life*. Eds: Chelazzi G. & Vannini, M. NATO ASI Ser. A 151. Plenum Press, New York (1988).

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