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Environmental factors influencing the surface activity and zonation of *Tylos europaeus* (Crustacea: Oniscidea) on a Tyrrhenian sandy beach

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Abstract The present study considers a population of *Tylos europaeus* Arcangeli, 1938 living on a Tyrrhenian sandy beach (Burano, GR, Italy). Monthly surveys were carried out between April 1986 and March 1987 with directional pitfall traps so as to intercept the isopods moving in four directions on the beach surface. In addition a bimonthly study was performed from March 1991 to January 1992 using two methods of capture: pitfall traps joined by 10-cm high strips of fibreglas for surface-active individuals and sieving for those burrowed in the sand. Capture frequencies allowed analysis of annual abundance, daily activity and zonation of juveniles and male and female adults. Variations of these spatio-temporal data were correlated by means of multiple regression with many environmental parameters: temperature and relative humidity of the air and sand, evaporation, wind direction and speed, global radiation, atmospheric pressure, rainfall, the sand salinity and granulometric parameters. *T. europaeus* was found to be mainly active in summer and autumn and during the night, and was zoned along the eulittoral. The surface activity was influenced by almost all the environmental factors when they were limiting but especially by the relative humidity of the air. The mean zonation of active specimens, however, varied hourly according to the sand temperature. On the other hand, the zonation of the buried individuals depended on the mean grain size, which involves many other parameters, such as moisture and oxygen contents.

Introduction

Tylos europaeus Arcangeli, 1938 is one of the most common terrestrial isopods on the sandy beaches along the Mediterranean and Atlantic coasts of Europe. Despite the wide distribution and ease of capture of this species (usually referred to as *T. latreillei* Audouin, 1826), there have been few studies on its ecology. Only F. Mead (1968) and Tongiorgi (1969) have carried out field studies on its zonation and surface activity, while diurnal orientation was studied by Pardi (1955) and by Mead and Mead (1974). In the laboratory, the rhythm of locomotor activity was analysed by M. Mead (1968). Arcangeli (1953) and Giordani Soika (1954, 1972) provided some information about the ecology and behaviour of *T. europaeus*.

More extensive and detailed studies have been performed on other species of the genus. Hamner et al. (1968, 1969) analysed the orientation and population dynamics of *Tylos punctatus* Holmes & Gay, 1909, as well as its horizontal and vertical zonation, throughout a whole year. Holanov and Hendrickson (1980) studied the relationship of sand moisture to burrowing depth in this same species. Hayes (1977) studied its zonation parallel to the shoreline. Kensley (1974) investigated the vertical zonation of buried populations and hourly variation in *T. granulatus* Krauss, 1843 and *T. capensis* Krauss, 1843. Burrowing behaviour and cost have been studied in *T. granulatus* (Brown and Trueman 1996), and depth of burrowing has been related to sand moisture (De Villiers and Brown 1994). In *T. granuliferus* Budde-Lund, 1885 (referred to as *T. granulatus* Miers, 1877), Imafuku (1976) studied zonation in relation to grain size and the presence of beach debris. In addition, he compared diurnal and nocturnal activity throughout the year in relation to the substrate temperature and relative humidity of the air. Matthewson (1991, cited in Brown and Odendaal 1994) studied the relationship of various size classes of *T. capensis* subjected to a series of temperature/relative humidity combinations with rates of water lost. The results obtained confirmed that the regulation of water balance depends on behavioural rather than physiological mechanisms.

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Laboratory studies have been carried out on the genus *Tylos* (Imafuku 1976; Hayes 1977; Matthewson 1991, cited in Brown and Odendaal 1994), but mainly on other genera of isopods (Waloff 1941; Edney 1951, 1954; Bursell 1955; Cloudsley-Thompson 1956, 1977; Perttunen 1961; Warburg 1964, 1968, 1989), with regard to the effects of light, temperature, humidity, salinity and granulometric parameters. An extensive review on various aspects of the biology of the genus *Tylos* has been presented by Brown and Odendaal (1994).

In 1986/87, field research was carried out for the present paper at Burano, in which the principal aim was the understanding of how different arthropods exploit the beach/dune system using different spatial and temporal strategies. The behaviour and adaptations of some Coleoptera and Amphipoda living in this unstable environment have been the subject of previous papers (Colombini and Chelazzi 1991; Scapini et al. 1992; Colombini et al. 1994).

The present paper investigates the surface activity and zonation of *Tylos europaeus* during periods of activity and rest. For this study a different experimental design was employed in the field during 1991/92, in which new spatial and temporal intervals of sampling were adopted. Furthermore, environmental parameters were recorded in order to identify which of these might influence the spatial and temporal distribution of the isopods.

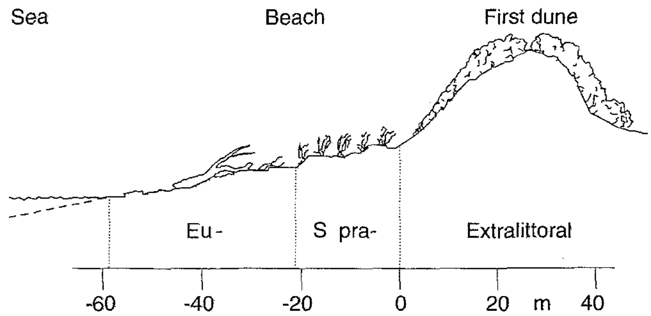

Fig. 1 Study area: cross-section of the beach/dune system

Materials and methods

Between the sea and the Burano Lagoon (Capalbio, GR, Italy, 42°23′30″N; 11°22′30″E), there is a sand bar (average width ca. 250 m) with two dunes covered with vegetation. A transect, ca. 100 m in length, running from the sea to the top of the first dune (Fig. 1) was analysed. Along this transect, three different zones were identified on the basis of morphology, exposure and vegetation cover. The eulittoral comprises the entire beach without vegetation and contains fresh debris washed up by the sea. Its width (ca. 35 m) and profile may vary according to wave action and sea currents. The supralittoral, with an average width of ca. 20 m, comprises the antedune where the first pioneer plants are found. Its vegetation cover depends on the particular pedological conditions and exposure to the winds. The extralittoral, which includes the two dune belts and extends to the lagoon behind, was studied only as far as the top of the first dune, the one closer to the sea. This part of the zone, namely the foredune and its summit, is covered by Mediterranean maquis.

Sampling took place in two different periods, always at the same point on the coast. From April 1986 to March 1987 sampling was carried out for 72 consecutive hours once every month. Tetradirectional pitfall traps were used (Scapini et al. 1992); one was placed in the eulittoral ca. 2 m from the sea, another one in the middle of the supralittoral and a third in the extralittoral at the summit of the first dune. The position of the first trap varied according to the width of the eulittoral and the roughness of the sea, whilst the other two traps were always placed at the same spots. During this period, the trapped arthropods were removed for analysis every 2 h. The directional pitfall traps were oriented in such a way as to intercept separately individuals moving on the sand in four directions: either from or to the sea, or from either the NW or the SE (i.e. parallel to the shoreline). However, only captures by the trap in the eulittoral, which accounted for virtually all individuals intercepted, were considered for the statistical analysis.

From March 1991 to January 1992 sampling was bimonthly, for 48 consecutive hours each time. A continuous transect from the sea to the summit of the dune was made, placing pitfall traps every 5 m and joining them by 10-cm high strips of fibreglas so as to intercept the isopods. Trap zero was situated next to a State Property boundary stone at the base of the first dune and, from this reference point, the other traps were numbered both towards the sea (–) and towards land (+). The length of the transect, and thus the number of seaward traps, varied according to the width of the eulittoral and the roughness of the sea. Every hour the individuals intercepted were counted and released.

During 1991/92, in order to sample the buried arthropods, a 1 m^2 patch of sand was sieved, during daylight hours, every 5 m along a transect similar to the previous one but ca. 40 m away from it. The sand was sieved to a depth of ca. 10 cm, but this varied according to the relative humidity of the sand, that is until the wet layer was reached and macroinvertebrates were no longer found. Also for this type of sampling, the reference patch was situated at the base of the dune and the length of the transect varied according to the width of the eulittoral and the roughness of the sea.

In this second period the experimental design permitted analysis of the activity patterns and zonation of *Tylos europaeus* on a smaller spatial (every 5 m) and temporal (each hour) scale but with bimonthly frequency to maximize climatic differences.

In addition, the temperature and relative humidity of the air and the temperature of the sand surface were recorded. In the period 1991/92, samples of sand were collected next to the patches sieved, to a depth of 6 cm from the surface, in order to measure the salinity, the humidity and granulometric parameters. These factors were determined according to standard methods (conductivity of 10 g of sand in 60 cm^3 of demineralized water, dry weight versus wet weight and sieving, respectively). The following granulometric parameters were considered: M_Z (mean size), σ_I (inclusive graphic standard deviation), Sk_I (inclusive graphic skewness) and K_G (graphic kurtosis) (Folk and Ward 1957). Data on the atmospheric pressure, the speed and direction of the wind, global radiation and rainfall were recorded at the meteorological station of Montalto di Castro (VT) located on the first dune belt ca. 6 km SE of the study area.

A program for circular statistics (Batschelet 1981) was used to analyse the daily components of surface activity. The Rayleigh test was used to analyse whether the population was significantly distributed around a particular month or hour of the day. The 95% confidence limits for the mean hour of activity were calculated by graphic interpolation (see Fig. 5.2.1 in Batschelet 1981). It should be noted that the graph for 200 specimens was used even when there was a larger number of captures. The *V*-test was used to determine whether the observed mean hour was significantly equal to the expected one (24:00 hrs for nocturnal individuals). The chi-square test (contigency tables) was used to compare the activity rhythms of the different groups of individuals (juveniles, i.e. specimens less than 6 mm in length, male and female adults).

Statgraphics Version 2.6 (STSC Inc.) was used to analyse the surface activity and zonation of *Tylos europaeus* in relation to the different environmental parameters, by means of multiple regression (Bliss 1970; Zar 1984) with the backward elimination method. For this analysis, wind direction was broken down into two components, along the sea–land and SE–NW axes: the polar co-ordinates of wind direction were transformed into Cartesian co-ordinates (sine and cosine of the angle) by making the x-axis point towards NW and the y-axis towards land.

To analyse the capture frequencies in terms of tidal hours, taking account of the alternation of high and low tide, the time between two successive high tides was divided into 12 periods. These time intervals were then summed and considered as time units which were independent of the real time periods between two high tides.

The orientation indices (R) were calculated, by the circular statistics, from the frequencies of captures in the four separate directions (sea, land, NW and SE). The V-test was used to determine whether the observed direction was significantly equal to the expected one.

Data from captures of five or more individuals considered for calculation of the barycentres and their confidence limits (95%) and for the circular statistic analysis. Probability levels were considered significant when < 0.05.

Results

Annual capture frequencies

All the results on surface activity in 1986/87 refer to the directional pitfall trap placed on the eulittoral. The other two traps on the supra- and extralittoral intercepted such a small number of isopods, respectively 29 and 6, that they were not considered sufficient for statistical analysis.

The capture frequencies determined monthly from April 1986 to March 1987 (Fig. 2a) reveal that, for the sexually mature specimens ($n = 3210$), the highest values occurred from June to October. No specimens were found to be active on the surface in August and January and only two in May. Immature forms ($n = 3545$) appeared abundantly in July and reached maximum numbers in October and November. After this, captures of juveniles decreased gradually, and, like the adults, they were absent in August and January. The mean month of surface activity for both adults and juveniles was calculated with circular statistic analysis. For adults, the mean activity period was the end of August (Rayleigh's test: $z = 633.513$, $p < 0.001$), whilst for the juveniles it was around the beginning of October ($z = 1400.161$, $p < 0.001$).

From March 1991 to January 1992, in spite of the bimonthly frequency of sampling, a total of 12 967 specimens were captured. July and September accounted for the maximum captures, whereas again in May a few specimens were caught. Generally the annual capture frequency obtained in 1986/87 was confirmed with this second trapping system.

Captures by sieving in 1991/92 show that *Tylos europaeus* (males, females and juveniles) were present, buried in the sand, throughout the whole year (Fig. 2b). Juveniles were more numerous than adults in July

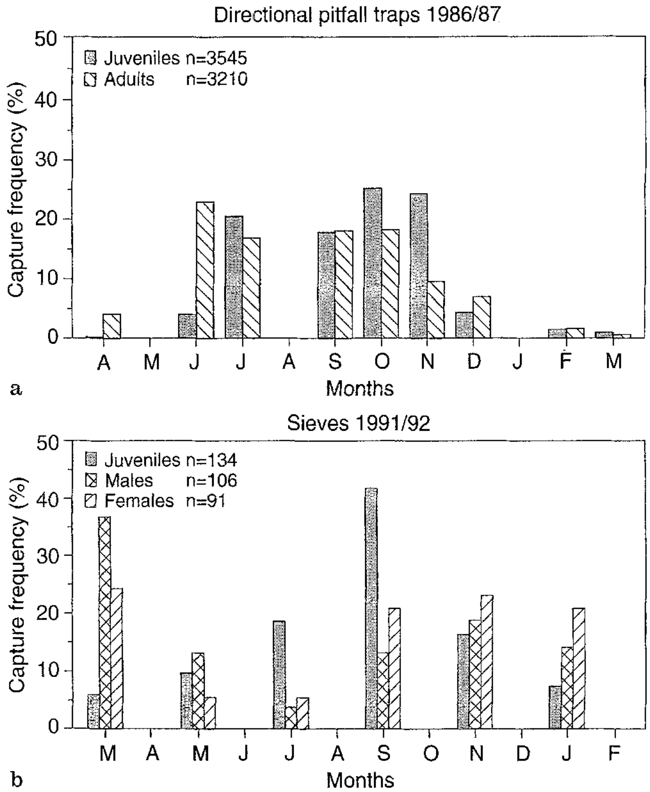

Fig. 2 *Tylos europaeus*. Annual capture frequency **a** with tetradirectional pitfall traps in 1986/87 and **b** with sieves in 1991/92

($n = 25$ and $n = 9$, respectively) and in September ($n = 56$ and $n = 33$, respectively), the month in which they reach maximum numbers only to decrease gradually in the subsequent months. The number of males was significantly greater than that of females in May $\chi^2 = 4.26316$, $p < 0.05$, $df = 1$), the month in which there was a maximum number of captures of both males ($n = 39$) and females ($n = 22$). However, the difference between captures of males and females throughout the whole year was not significant ($\chi^2 = 9.27618$, $p > 0.05$, $df = 5$), indicating that the sex-ratio was approximately equal to 1. May was the month with the smallest number of captures by sieving. The mean burrowing period of the adults was the middle of January (Rayleigh's test: $z = 14.067$, $p < 0.001$), whilst that of the juveniles was mid-September ($z = 29.682$, $p < 0.001$).

Daily rhythm of surface activity

From April 1986 to March 1987, a daily rhythm of surface activity was deduced for the months in which captures were abundant. Except in rare cases, the activity on the sand surface was concentrated in the hours of darkness and showed one or two peaks. For example, in June there was only one peak, at 22:00 hrs, both for adults and juveniles (Fig. 3a). In the following month, July, a second peak appeared (Fig. 3b), which by September (Fig. 3c) had a value similar to the first peak.

By March, the surface activity had shifted mainly to the second part of the night (Fig. 3d). The temporal distributions of capture frequencies were significantly different between adults and juveniles in June, July and September (respectively, $\chi^2 = 23.4232$, $p < 0.00005$, $df = 3$; $\chi^2 = 226.612$, $p < 0.00001$, $df = 6$; $\chi^2 = 56.6064$, $p < 0.00001$, $df = 5$), but not in March ($\chi^2 = 0.350243$, $p > 0.05$, $df = 2$).

However, when the mean hour of activity was compared between adults and juveniles (Fig. 4), the difference was significant only in June (mean hour: adults 23:48 hrs ± 20 min, juveniles 22:57 hrs ± 12 min) and September (adults 02:12 hrs ± 36 min, juveniles 00:46 hrs ± 44 min). A comparison of the different months shows that the mean hours of activity of adults and juveniles change according to the months, moving to the first or second part of the night.

Fig. 3 *Tylos europaeus.* **a–d** Capture frequency in four different months in 1986/87 with tetradirectional pitfall traps (*Continuous lines* adults; *dashed lines* juveniles; *shaded areas* periods of darkness)

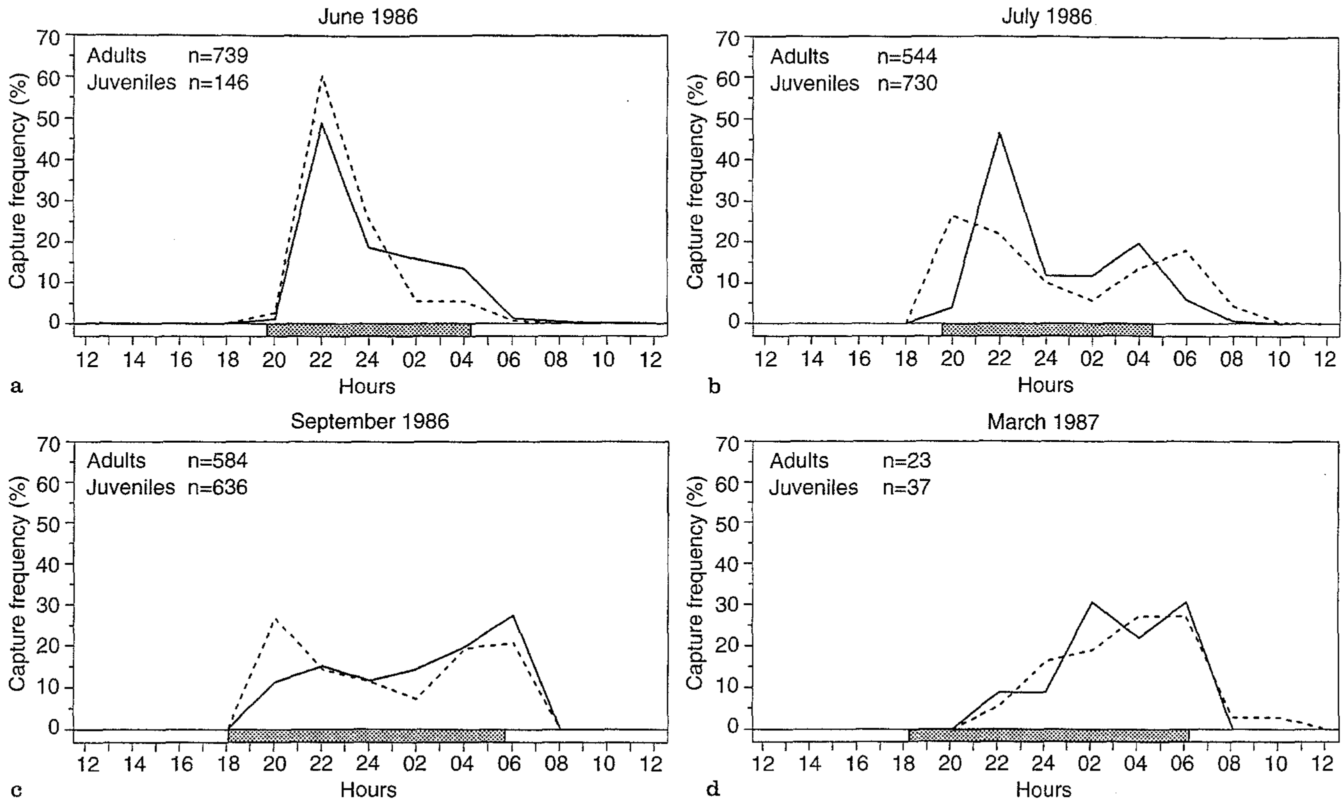

Fig. 4 *Tylos europaeus.* Mean daily activity in 1986/87. Confidence limits are not shown when inside the symbols (*Filled circles* adults; *open circles* juveniles; *shaded areas* periods of darkness)

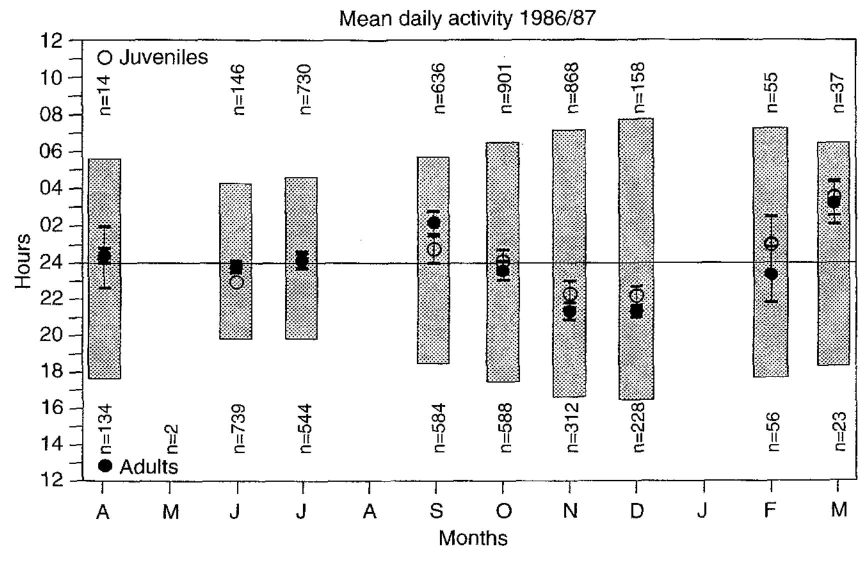

Table 1 *Tylos europaeus*. Multiple regression of surface activity in 1986/87 with environmental parameters (evaporation, air and sand temperatures, relative humidity, wind components along the sea–land axis and parallel to the shoreline (SE–NW), wind speed, global radiation, pressure, rainfall). Significant regression coefficients and their probabilities are shown. The constant (*Const.*) of the multiple regression equation and the coefficient of multiple determination (R^2) are also reported (– missing data; *N.S.* not significant)

	Const.	Evap. ($cm\,h^{-1}$)	Air temp. (°C)	Sand temp. (°C)	Rel. hum. (%)	Wind component		Wind speed ($m\,s^{-1}$)	Global rad. ($cal\,cm^{-2}\,min^{-1}$)	Pressure (mbar)	Rain (mm)	R^2
						s–l	SE–HW					
Annual	– 79.1992 $p < 0.0001$	N.S.	1.7248 $p = 0.0110$	N.S.	1.2226 $p < 0.0001$	21.7665 $p < 0.0001$	N.S.	N.S.	N.S.	N.S.	N.S.	0.1189
Apr	N.S.	N.S.	N.S.	N.S.	0.0266 $p = 0.0080$	–	–	–	–	–	–	0.2256
Jun	N.S.	N.S.	– 11.1941 $p = 0.0221$	N.S.	–	N.S.	N.S.	N.S.	N.S.	0.2347 $p = 0.0132$	N.S.	0.2437
Jul	15957.2 $p = 0.0249$	N.S.	N.S.	N.S.	2.5510 $p = 0.0023$	N.S.	N.S.	N.S.	N.S.	– 19.0164 $p = 0.0234$	N.S.	0.3788
Sep	18144.6 $p = 0.0071$	N.S.	N.S.	– 4.0715 $p = 0.0202$	N.S.	N.S.	N.S.	N.S.	N.S.	– 18.0578 $p = 0.0075$	N.S.	0.2670
Oct	N.S.	N.S.	N.S.	3.4540 $p = 0.0387$	3.3298 $p = 0.0001$	50.4211 $p = 0.0005$	N.S.	N.S.	N.S.	– 0.2819 $p = 0.0003$	N.S.	0.6724
Nov	N.S.	– 55.6838 $p = 0.0292$	N.S.	N.S.	0.6907 $p = 0.0002$	–	–	–	–	–	–	0.3935
Dec	2835.74 $p = 0.0062$	N.S.	N.S.	N.S.	N.S.	15.6789 $p = 0.0052$	N.S.	N.S.	N.S.	– 2.7866 $p = 0.0063$	N.S.	0.2184
Feb	31.4546 $p = 0.0022$	– 9.1093 $p = 0.0376$	N.S.	N.S.	– 0.3137 $p = 0.0061$	4.6019 $p = 0.0156$	N.S.	N.S.	N.S.	N.S.	N.S.	0.3550
Mar	N.S.	N.S.	0.4238 $p < 0.0001$	N.S.	N.S.	N.S.	N.S.	N.S.	– 0.0678 $p = 0.0038$	N.S.	N.S.	0.3796

For 1986/87, the surface activity of *Tylos europaeus* was analysed by multiple regression (Table 1). Surface activity was the dependent variable while the various environmental parameters recorded near the capture trap (first four parameters) and at the meteorological station of Montalto di Castro (all other parameters) were the independent variables. For the annual analysis, the months of April and November were excluded, because the relevant data from the station at Montalto di Castro were not recorded, and June was excluded owing to a lack of data for relative humidity. There was a significant positive correlation between the annual surface activity and different environmental parameters: air temperature, relative humidity and wind direction along the sea–land axis (i.e. towards land). However, it should be noted that the coefficient of multiple determination was very low ($R^2 = 0.1189$).

Multiple regression between the surface activity of *Tylos europaeus* and the same environmental parameters, but carried out only for the months with sufficient captures (Table 1), reveals that some parameters (evaporation, global radiation and pressure) are significantly correlated with surface activity even though they were not when the annual activity was considered. Surface activity was negatively correlated with evaporation only in November and February. Surface activity was negatively correlated with air temperature in June, with night-time temperatures between 16 and 20 °C, while there was a positive correlation in March. In that month, individuals did not show any surface activity on the first two nights, when the temperature was below 6 °C, whereas on the third night they were active with a temperature of around 13 °C. The results regarding the temperature of the sand's surface are similar; in September, which had a minimum night-time temperature of 20 °C, activity was negatively correlated with sand temperature, whilst in October with a minimum temperature of 13 °C, there was a positive correlation. In four of the months, the correlation between surface activity and relative humidity of the air was positive (as was the correlation with the annual activity). In fact, when relative humidity exceeded 70% during the night, the activity of *T. europaeus* increased. However, in February the correlation was negative; on the first two nights, the relative humidity was about 90% on account of rain, whereas on the third night it was about 70% and the individuals exhibited greater activity than on the previous two. There was a significant positive correlation between surface activity and wind direction along the sea–land axis in the autumn/winter months (that is, the number of captures increased when the wind came from the sea and vice-versa). In October and December, the wind came from the sea during the night and from land during the day. In October, the month with the largest coefficient of multiple determination, there was a significant positive correlation between the sea–land component and relative humidity ($r = 0.4202$, $p = 0.0107$). In February, there was virtually always wind from the mainland except on the third day. However, for this month the significance of the correlation between the activity and sea–land wind was less than in October and December. The surface activity was negatively correlated with global radiation only in March. Finally, the correlation between activity and atmospheric pressure was positive for June and negative for July, September, October and December. The range of pressure was lower in June (1007 to 1017 mbar) than in the other four months (1012 to 1024 mbar).

In general, the coefficient of multiple determination in the individual months always had a low value except in October, in which the probability model of the multiple regression included four of the environmental parameters considered.

The data recorded from March 1991 to January 1992 again showed that the surface activity of *Tylos europaeus* was almost exclusively nocturnal and that maximum peaks of activity changed from one month to the other. The distributions of the capture frequencies were principally unimodal especially for the summer months. Calculating the mean daily activity, this was around midnight for each month except for January.

The analysis of capture frequencies in the pitfall traps along the transect, reveal that, in March and November 1991, the months in which the activity of *Tylos europaeus* was distributed most widely throughout the eulittoral, the mean hour of activity varied significantly according to the distance from the sea. For example in March (Fig. 5), on average, individuals were active near the sea earlier in the night and towards land later. The same trend was found in November.

The data from hourly sampling in the period March 1991 to January 1992 were also analysed in tidal hours. The resulting distributions of capture frequencies are very variable among the different months. For example, a maximum peak of activity in July occurred immediately before low tide and in September at high tide.

Static and dynamic zonation

The results obtained from sieving sand at different distances from the base of the dune in 1991/92 revealed that during the day, when *Tylos europaeus* is not active on the surface, individuals were buried at different distances from the shoreline in different months (Fig. 6). The total mean static zonation of *T. europaeus*, i.e. juveniles, males and females combined, was situated significantly more towards land in March and November than in May, July and September and in November compared with January. In September, the total mean zonation was situated closer to the sea than in all the other months considered. Differences remain significant, except for November versus January, even when the distances of individuals from the shoreline (the position of which changes from month to month) are calculated. The annual mean zonation of adult females

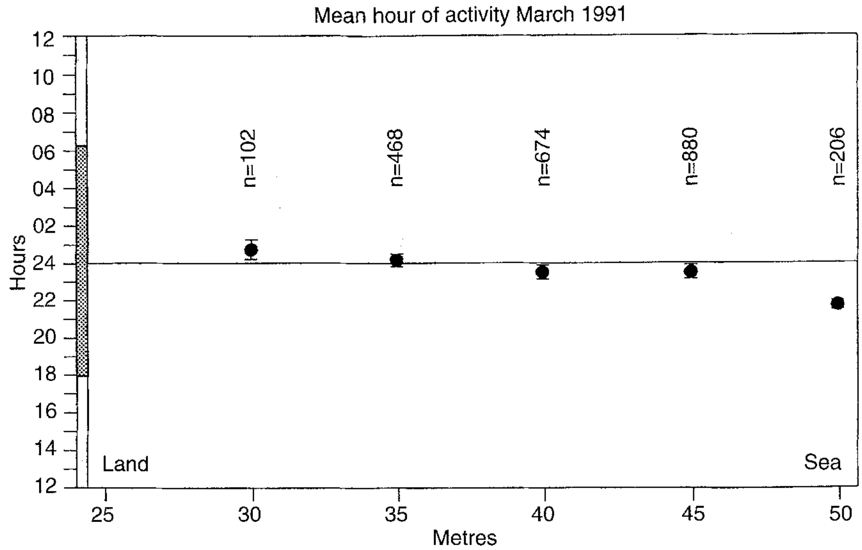

Fig. 5 *Tylos europaeus*. Mean hour of activity in the different zones of the eulittoral in March 1991 (*Filled circles* mean activity; *error bars* confidence limits; *shaded area* period of darkness)

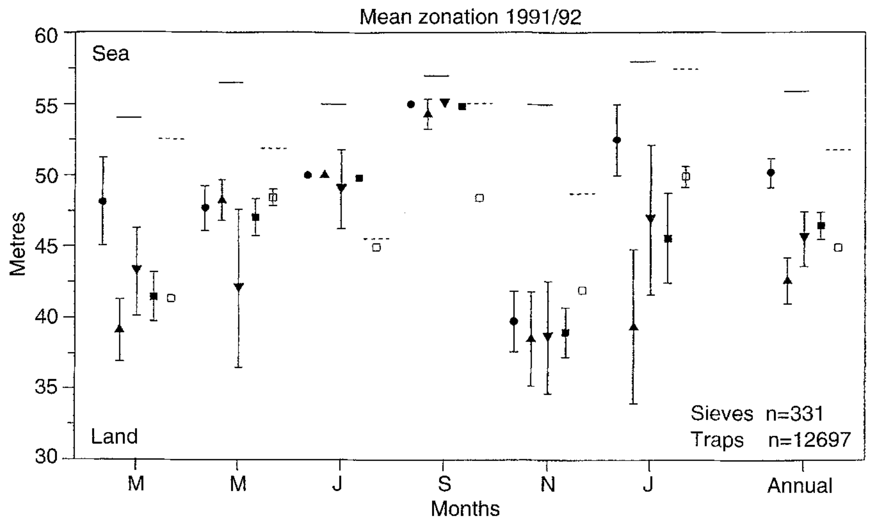

Fig. 6 *Tylos europaeus*. Monthly and annual mean zonation, both static and dynamic, in 1991/92. *Filled symbols* represent the captures with sieves of juveniles (●), males (▲) and females (▼). The total captures obtained with sieves are represented by *filled squares*, while *open squares* represent the total captures of surface-active individuals with the pitfall traps along the transect. Confidence limits are not shown when inside the symbols. *Horizontal continuous* and *dashed lines* represent the mean level of the shoreline corresponding to the sieve and pitfall trap transects, respectively

and males showed that they were zoned significantly more towards land than the juveniles. However, only in March and January were juveniles zoned significantly further towards the sea than the males, while the difference between juveniles and females was never significant. It should be noted that, in July and September, the captures of males, females and juveniles were more concentrated than in March and January and were located more towards the sea than in November, also when the variations of the beach's profile among the various months are considered.

Table 2 reports the results of the multiple regression of the static zonation of *Tylos europaeus* with the relative humidity and salinity of the sand and the granulometric parameters, M_Z, σ_I, Sk_I and K_G. There were significant positive correlations between the annual static zonation and these three parameters: the relative humidity and salinity of the sand and the parameter M_Z. A negative correlation was found with Sk_I. The coefficient of multiple determination was about 45%. A significant positive correlation with relative humidity was also found in March and September, while that with salinity occurred in May and January. Zonation was significantly correlated with granulometric parameters in May, July and November. Only in July, the mean grain size (M_Z) was negatively correlated with zonation, in agreement with the fact that the largest grains (smaller ϕ) are found close to the shoreline. In May and November, as in annual zonation, the correlation with M_Z was positive, whereas the correlation with Sk_I was negative. The values of these two parameters increase from the sea to the dune; in fact, the asymmetry of the sand grain sample passes from negative values (greater presence of larger fractions) to positive ones (greater presence of finer fractions). It must be kept in mind that the different granulometric

Table 2 *Tylos europaeus*. Multiple regression of specimens (captured by sieving in 1991/92) with the relative humidity and salinity of the sand and granulometric parameters (Folk and Ward 1957). For further explanations see Table 1

	Const.	Sand rel. hum. (%)	Sand sal. ($\mu S\,cm^{-1}$)	M_z (ϕ)	σ_I (ϕ)	Sk_I	K_G	R^2
Annual	– 71.6034 $p = 0.0074$	5.5067 $p = 0.0037$	0.0611 $p < 0.0001$	52.6275 $p = 0.0112$	N.S.	– 49.1588 $p = 0.0048$	N.S.	0.4485
Mar	N.S.	3.9493 $p = 0.0107$	N.S.	N.S.	N.S.	N.S.	N.S.	0.4947
May	– 36.5178 $p = 0.0063$	N.S.	0.0494 $p = 0.0001$	38.7462 $p = 0.0006$	19.2983 $p = 0.0105$	– 23.3736 $p = 0.0076$	– 12.5092 $p = 0.0026$	0.9926
Jul	N.S.	N.S.	N.S.	– 50.4683 $p = 0.0010$	186.0323 $p = 0.0007$	34.0513 $p = 0.0422$	N.S.	0.8050
Sep	– 72.5573 $p = 0.0001$	76.7750 $p < 0.0001$	N.S.	N.S.	N.S.	N.S.	N.S.	0.8137
Nov	N.S.	N.S.	N.S.	3.8235 $p = 0.0121$	N.S.	– 35.4338 $p = 0.0042$	N.S.	0.7233
Jan	N.S.	N.S.	0.1621 $p < 0.0001$	N.S.	N.S.	N.S.	N.S.	0.8582

parameters are highly correlated among themselves and this may produce changes in the sign of the regression coefficient. The coefficients of multiple determination in the individual months were always greater than 70%, except for March when it was about 50%.

From the capture frequencies of individuals active on the surface at different distances from the base of the dune (Fig. 6), it can be seen that, in March and November, *Tylos europaeus* was found more towards land than in the other months. However, the zonation of individuals was also significantly different between these two months. In May, September and January, individuals were found more towards the sea; further, the zonations recorded in September and in January were significantly different from each other, but not with that of May. In terms of mean distance from the sea, these isopods were ca. 0.5 m from the shoreline in July, while their distance from the sea in September, November and January did not differ significantly and had values of 6 to 8 m.

Comparing the zonation, measured from the shoreline, of buried individuals with that of individuals active on the surface, the latter were closer to the sea than the former in all months except for September, when the opposite occurred, and in March, when there was no difference. However, over the entire year, the zonation of *Tylos europaeus* with respect to the shoreline did not differ between the surface-active and buried individuals.

Mean hourly zonation

The mean zonation of *Tylos europaeus* at different hours of the night was calculated from the capture frequencies of the surface-active specimens recorded from March 1991 to January 1992. There were no significant differences in the mean hourly zonation during the night in May, July and January. However, in March, September and November, the zonation was significantly different at the various hours. For example, in March (Fig. 7) *T. europaeus* was active closer to the sea at 19:00, 20:00 and 21:00 hrs than in the following hours, with the exception of 06:00 hrs.

Table 3 reports the results of the multiple regression of mean hourly zonation with temperature of the air and sand and relative humidity of the air. For the whole year, only the correlation with sand temperature was significant. However, when the six months were analysed separately, that correlation was seen to be significant, with a coefficient of multiple determination of 0.9979, only in November. The data for sand temperature were incomplete for May and January. In those two months, there was a positive correlation with the air temperature. Again in May, the mean hourly zonation was positively correlated with relative humidity, whereas the correlation was negative in September. In March, no environmental parameter was significantly correlated with the mean hourly zonation. In July, since zonation was virtually constant at 45 m on account of the drying up of the whole high eulittoral, there were no significant correlations with environmental parameters. While the explained variability is consistently rather high in four of the months, it is zero in the other two, which may have caused the low value of R^2 for the entire year.

Orientation indices

From analysis of the capture frequencies obtained with the tetradirectional pitfall traps from April 1986 to March 1987, it can be seen that the direction of movement of the isopods varied throughout the night. In the different months, variations in the orientation index, calculated for movements along the sea–land axis, had a similar pattern throughout the night, and only two examples are here reported. In July (Fig. 8a),

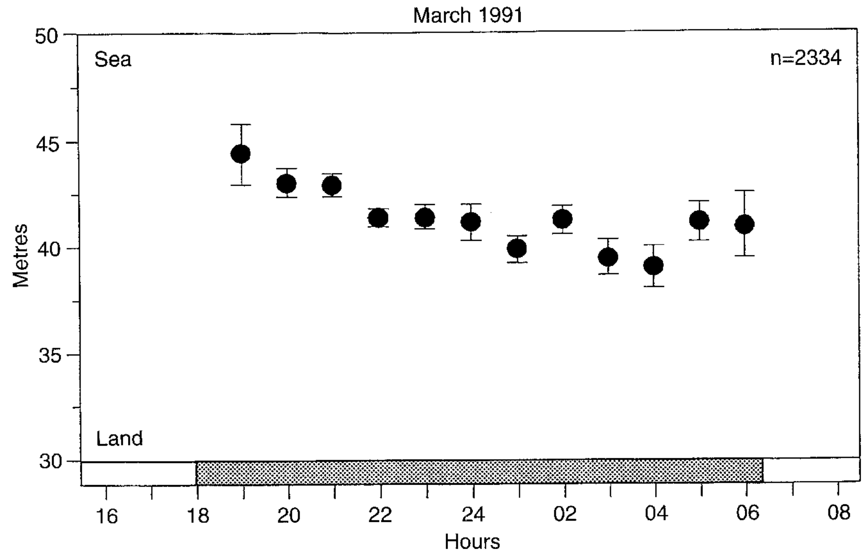

Fig. 7 *Tylos europaeus.* Mean zonation of surface-active specimens in the different hours of the night in March 1991 (*Filled circles* mean zonations; *error bars* confidence limits; *shaded area* period of darkness)

Table 3 *Tylos europaeus.* Multiple regression of mean hourly zonation of surface-active individuals in 1991/92 with environmental parameters. For further explanations see Table 1

	Const.	Air temp. (°C)	Sand temp. (°C)	Rel. hum. (%)	R^2
Annual	39.561 $p < 0.0001$	N.S.	0.3746 $p = 0.0043$	N.S.	0.2450
Mar	41.1575 $p < 0.0001$	N.S.	N.S.	N.S.	0.0000
May	38.6835 $p = 0.0001$	0.2870 $p = 0.0168$	–	0.0789 $p = 0.0157$	0.7661
Jul	44.9818 $p < 0.0001$	N.S.	N.S.	N.S.	0.0000
Sep	71.2345 $p < 0.0001$	N.S.	N.S.	– 0.2470 $p = 0.0005$	0.6178
Nov	N.S.	N.S.	3.2615 $p < 0.0001$	N.S.	0.9979
Jan	45.2644 $p < 0.0001$	1.3044 $p = 0.0029$	–	N.S.	0.9518

adults and juveniles had a similar pattern of movements, although the latter exhibited reversal of direction later than the adults, namely between 02:00 and 04:00 hrs rather than between 24:00 and 02:00 hrs. In October (Fig. 8b), the movements of the adults were oriented significantly towards land until 22:00 hrs and those of the juveniles until 02:00 hrs. At 24:00 and 02:00 hrs, the adults were significantly bimodal (respectively, $p < 0.05$ and $p < 0.005$) along the sea–land axis, whereas the juveniles were bimodal at 04:00 hrs ($p < 0.005$) along the NW–SE axis. At 06:00 hrs, both were significantly oriented towards the sea. The activity of the juveniles was oriented significantly towards the sea also during daylight hours. For both adults and juveniles, the inversion of the orientation index occurred between 02:00 and 04:00 hrs.

Discussion

The analysis of the capture frequencies obtained from the pitfall traps in the two periods studied show that the surface activity of *Tylos europaeus* depends not only on the environmental conditions of the different seasons, but also on those of that particular night of capture. A more realistic idea of the isopod abundance is obtained with sieving techniques. For example in winter months, when surface activity was depressed on account of the adverse climatic conditions, the sieves revealed that isopods were present in a good quantity, but were not moving at the surface. The total absence of surface activity recorded during August 1986 and 1987 can again be related to the high temperatures of the air and sand. The differences of the mean annual surface activity found between adults and juveniles in 1986/87 are not due to differences in their surface activity, but principally to where the activity took place on the eulittoral. As can be seen by the mean zonation, young individuals were located significantly more towards the sea than the adults and had a different speed of zonal recovery (see the orientation indices) and so a different motility. In fact, F. Mead (1968) stated that the locomotor behaviour of the juveniles is associated with the wetter zone of the beach. Thus the tetradirectional

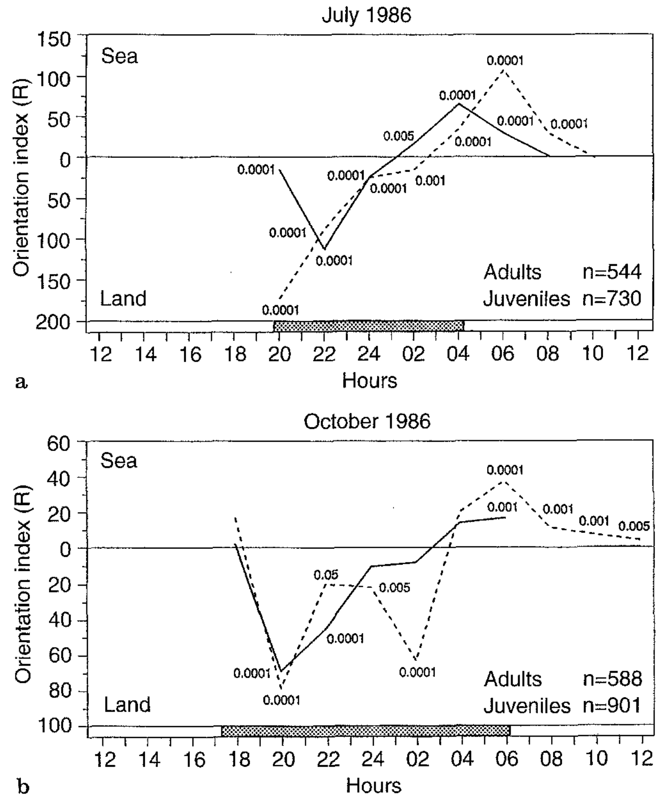

Fig. 8 *Tylos europaeus.* Orientation index (R), towards sea or land, obtained with tetradirectional pitfall traps and probability levels calculated with the *V*-test in **a** July and **b** October 1986. (*Continuous lines* adults; *dashed lines* juveniles; *shaded areas* periods of darkness)

pitfall trap could have caught the two age classes in different ways. Also for the mean annual burrowing period, obtained with sieves, differences were found between the adults and juveniles. In this case the trapping system shows a distribution in the capture frequencies that could reflect a more likely pattern in isopod abundances.

In general, the significant differences found between the two study years with regard to both the annual distribution and total number of captures are mainly due to the different climatic conditions and trapping systems employed.

Tylos europaeus was found to be mainly nocturnal with a prolongation of its surface activity into the early hours of the morning in some months. This extension of activity is due mainly to the juveniles which in July and October 1986 exhibited delayed zonal recovery compared with the adults. Furthermore, the juveniles also became active earlier than the adults in the months in which the dark phase is shorter; this may be due to the young taking more time to arrive at or return from the foraging area, or remaining there longer. Both the earlier initiation of activity by the juveniles and its subsequent prolongation, compared with the adults, was described by F. Mead (1968) again for the month of July. This author believed that the difference in activity was due to a different light threshold in the two age classes. We believe though that light per se is not a limiting factor, but rather that the microclimatic conditions during the day are critical. This same hypothesis was proposed for *T. granuliferus* by Imafuku (1976), who stated that the temperature and especially the relative humidity are very important for the activity of that species, since the rates of evaporation of water through the teguments and respiratory organs are dependent on these two parameters. However, Imafuku (1976) believed that *T. granuliferus* follows the light: dark cycle, since in nature it is the most stable environmental parameter and is generally associated with the cycle of humidity. In the case of *T. europaeus*, a difference in light threshold between the two age classes would be advantageous since it would permit the young to be active for a greater number of hours than the adults. Being zoned closer to the sea, the young experience temperature excursions to a lesser degree and thus can move first. Further, if the foraging area is the same as that of the adults, the distance to be covered is greater and for this reason they must move earlier.

In the two study years, the hourly distributions of capture frequencies were different from month to month owing to variation in environmental parameters. In the winter months, concentration of activity in one part of the night was due to the low temperature, which was a limiting factor in some hours. This indicates that surface activity is influenced by well-defined thresholds of the environmental parameters which cause the shift in time of the maximum activity. The presence of a temperature threshold has already been hypothesized in other isopods by Cloudsley-Thompson (1958b). However, the presence of two activity peaks, as seen in the summer months of 1986, can be accounted for by the fact that the tetradirectional pitfall trap intercepted only at one point in the eulittoral. Thus the trap captures individuals only at the beginning or end of the activity period and not during the central hours of the night. In summer, the isopods are concentrated close to the shoreline and thus, to reach the foraging area, they vary their zonation during the night more than in the other months. Using the same trapping system (cross traps), Tongiorgi (1969) also found both daily and monthly differences in the activity peaks of *Tylos europaeus* and, further, also reported a migration perpendicular to the shoreline. In 1991/92, when the second trapping system was used (a continuous transect), a unimodal distribution was obtained even during the summer months. This confirms the above statements that the directional pitfall trap caught the isopods when they were moving back and forth from the foraging area.

In some months of 1986/87, the mean hour of activity of adults and juveniles was shifted probably because of their different zonation. The differences between the various months were related to the environmental conditions which influenced the maxima of activity. It should be remembered that in 1991/92 the observed mean hour of activity being close to midnight was due to the system of trapping, which intercepted individuals along the whole eulittoral and thus was not affected by the hourly shifts in zonation.

For the entire period of study (1986/87), the variables correlating significantly with the surface activity were the temperature and relative humidity of the air and the sea–land direction of the wind. The importance of temperature and relative humidity for the locomotor activity of terrestrial isopods has been described by various authors (Edney 1951, 1954; Bursell 1955; Cloudsley-Thompson 1956; Perttunen 1961; Warburg 1964, 1968, 1989; Kensley 1974; Imafuku 1976). The very low value of the coefficient of multiple determination for the entire year indicates that perhaps other parameters might influence surface activity. However, the low value of R^2 might have occurred for other reasons: the use of climatic parameters from another locality (Montalto di Castro), a lack of data for some months, a large variability of the data among the different months and variation in the number of captures according to the life cycle of *Tylos europaeus*.

An analysis of the environmental parameters in the individual months reveals that air temperature is very important, in that it may become a limiting factor both in the cold months and in the hot ones. In fact, the correlation of surface activity with this parameter was positive in the autumn/winter months and negative in spring/summer. Without a doubt, there are lower and upper thresholds below and above which individuals will not be active. This was the case in January and March 1987, when the temperature was below 6 °C for a few hours or for the whole night. Also in other isopods, Cloudsley-Thompson (1958b) noted an inhibition of surface activity when the temperature fell below 5.5 °C, and further believed that the lower threshold of the nocturnal activity could vary seasonally (Cloudsley-Thompson 1974). The case of an upper threshold is exemplified by August 1986, during which the air temperature remained above 20 °C for the entire night and no surface activity was recorded. Warburg (1964) analysed the thermal preferences of certain species of terrestrial isopods and found that the specimens did not exhibit a preference within a gradient of 10 to 20 °C, whilst in the higher ranges the lower temperatures were clearly preferred.

There are also thresholds with regard to sand temperature and relative humidity of the air. In September and October 1986, the correlation of surface activity with sand temperature was respectively negative and positive, demonstrating that, also in this case, a preferred range of temperature between 13 and 20 °C exists. However, the correlation with relative humidity was always positive, with the exception of February 1987 during which activity was depressed when relative humidity was below 70% or exceeded 90%. In a study of the mechanisms involved in the reaction to humidity, Waloff (1941) demonstrated that terrestrial isopods avoid a relative humidity less than 68%. It is probable that *Tylos europaeus* regulates the onset of its nightly excursion on the basis of the temperature of the sand in the months in which this parameter is the limiting factor and once active is guided by other environmental variables.

Another factor that influences *Tylos europaeus* is the wind along the sea–land axis, which was always positively correlated with the surface activity. This means that the capture frequency increases when the wind blows from the sea, thus increasing the humidity on the eulittoral. Cloudsley-Thompson (1958a) described in terrestrial isopods an inverse relation between wind speed and the number of active individuals. Imafuku (1976) stated that a strong wind clearly suppresses the activity of *T. granuliferus*.

Another environmental parameter that is clearly important is atmospheric pressure. In general, when the trend of variation is towards high values, the surface activity tends to be depressed, whereas the opposite occurs when the trend is from low to intermediate values. The range of preference seems to be 1012 to 1017 mbar. Like sand temperature, pressure is a parameter that *Tylos europaeus* can sense even when it remains buried. This would allow individuals to evaluate whether the atmospheric conditions (relative humidity, wind) will be suitable for their surface activity in the subsequent hours.

Analysis of the static zonation of *Tylos europaeus* revealed variations in the resting places of the isopods related to the changes in the physical and chemical parameters of the beach. In summer, individuals were burrowed closer to the sea and were more concentrated, mainly due to the progressive drying out of the shore. In winter, microclimatic conditions assume a more uniform pattern allowing the isopods to exploit all of the eulittoral. Hamner et al. (1969) also recorded a change in the zonation of *T. punctatus* within the period of one year. In general, the juveniles, the females and the males were zoned on average in three successive bands from the sea towards land. As stated by F. Mead (1968), a probable cause of the different distributions of the three classes is their different size, with adult males being larger than females. This author believed that the adults could be found in a band further from the sea than the young on account of their greater ability to burrow.

The correlation of the static zonation of *Tylos europaeus* with various environmental parameters revealed that the isopods are influenced by the humidity and salinity of the sand and by granulometric parameters. The most important of the latter is the mean grain size. Coarser sand permits greater permeability which favours the exchange of oxygen and a greater moisture content. Hayes (1977) found that the importance of grain size for buried isopods depends on the physical properties related to it. The granulometric factors become less important when the humidity of the sand reaches critical values, as in September when zonation was concentrated close to the shoreline on account of the general drying out of the beach. A different situation occurred in January; there was a uniform and non-limiting humidity of the beach, and the isopods could have used the salinity of the sand to maintain their zonation near the shoreline.

Giordani Soika (1954) reported that *Tylos europaeus* could be found at depths up to around 20 cm. In *T. granulatus*, Kensley (1974) demonstrated an hourly variation of burrowing within a 40-cm deep layer, and De Villiers and Brown (1994) showed that this isopod prefers an environmental moisture range from 3.4 to 13%. Holanov and Hendrickson (1980), in *T. punctatus*, showed that the depth of burrowing is largely determined by the moisture content of the substratum and 1.5 to 2% moisture content of sand was preferred. In the present study, we investigated a layer only about 10 cm deep. In the Mediterranean area, large tidal excursions do not occur, and the conditions remain rather constant with time. In this case, the wet layer remains rather superficial, obstructing the circulation of oxygen and consequently also limiting the presence of the isopods. Therefore, the layer that we investigated was probably sufficient to evaluate the abundance and zonation of *T. europaeus*.

Analysis of the dynamic zonation of *Tylos europaeus*, based on distance from the sea, revealed that surface-active individuals varied their mean point of activity in different months of the year. These displacements were probably related to monthly variation of the suitable foraging area. When dynamic and static zonations are compared, it can be seen that, in various months, the isopods were active in a band that was closer to the sea than the mean point of burrowing. In September, the opposite situation occurred since the static zonation was closely correlated with the humidity of the sand. However, comparison of the annual static and dynamic zonations shows that there was no significant difference between the mean point of surface activity and that of burrowing. This is due to the fact that the annual zonation is an average of the various months. Another explanation might be that, since *T. europaeus* does not cover great distances during the night, the location of its resting site depends to a great degree on where the isopods had been active.

With regard to the mean hourly zonation of the surface-active individuals in 1991/92, there were significant displacements on the eulittoral during the night in some months. In fact, there was a change in zonation first towards land and then, in the early hours of the morning, towards the sea. In other months, instead, the changes in position were not significant, either because individuals were already in the foraging area or because the number of captures was low, therefore increasing the width of the confidence limits. Confirmation of these displacements comes from the captures obtained with the tetradirectional pitfall traps in 1986/87. The orientation indices show that *Tylos europaeus* migrated towards land in the first part of the night and then turned towards the sea in the morning. The juveniles returned towards the sea later than the adults both on account of their slower motility and the fact they had to cover greater distances. Further, young individuals might require a longer foraging period. F. Mead (1968) also noticed a different duration of the activity period between adults and juveniles. In fact, the latter start their activity before the adults and continue it even after the adults have burrowed. Tongiorgi (1969) also demonstrated that, on the eulittoral, *T. europaeus* perform an outward migration towards land in the first part of the night and a seaward one in the second part of the night. Hamner et al. (1969), in *T. punctatus*, and Chelazzi and Ferrara (1978), in *Littorophiloscia tropicalis* Taiti & Ferrara, 1986 [referred to as *L. compar* (Budde-Lund, 1893)], described the opposite migratory behaviour, namely first towards the sea and then towards land. However, in those cases, in addition to there being large tidal excursions, kelp did not accumulate along the high tide mark but in a zone that was closer to the sea. In the Mediterranean area, where large tidal excursions do not occur, *T. europaeus* burrows in a band of the eulittoral close to the sea and goes to feed during the night in an area of the eulittoral situated more towards land, where the deposition fronts of beach debris are located. In fact, this isopod, not having to deal with changing positions of the trophic zone and the burrowing point in relation to the tides, does not exhibit a tidal rhythm.

Finally, the correlation of mean hourly zonation of surface-active individuals with environmental parameters revealed that mainly the sand temperature influences the dynamic zonation of *Tylos europaeus*. When the sand temperature dropped, the isopods moved more towards land. However, the isopods might also have responded to other parameters (such as the relative humidity of the sand), at least in the months in which none of the three parameters analysed was significant. In September, dynamic zonation was negatively correlated with the relative humidity of the air; in other words, the distance from the base of the dune decreased with an increase in the relative humidity.

In conclusion, *Tylos europaeus* regulates its daily rhythm of surface activity according to the relative humidity of the air. On the other hand, its mean hourly zonation depends on sand temperature; that is, once active on the surface, the isopod is influenced principally by this last parameter.

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