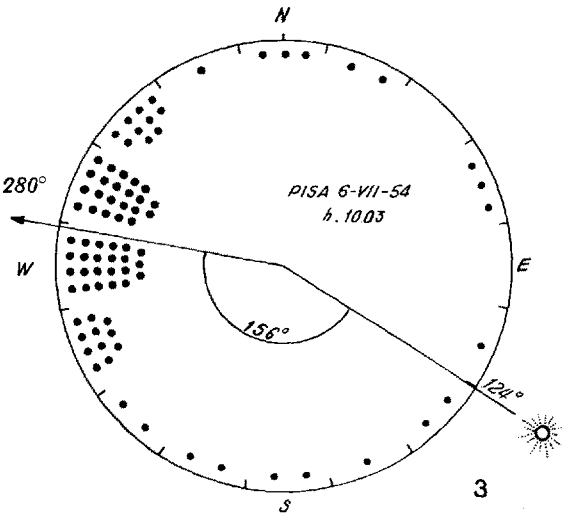
with a glass[1] and placed where the sight of the sky was not obstructed by objects of the landscape. The animals, as always, crowded up in determined sectors trying to escape. Their distribution was recorded by nine photographs taken between 10·38 and 11·05 h (Argentina mean solar time). Figure 1 represents the total distribution of the 117 positions observed, the resulting direction of escape, and the position of the sun at 10·49 h[2] (average time in which the photographs were taken). Consequently the animals on the average keep the sun on their left side by 38° 30′.



3

As soon as the results of this experiment came to my knowledge[3], I performed a controlling test in Pisa with the same number of specimens at the Italian local time (14·38–15·05 h), corresponding to the time of the above-quoted experiment in Argentina. The result is shown in Figure 2. The azimuth of the sun at the average time was approximately 254°, the resulting direction amongst the 117 positions of the animals observed was 291°. Therefore the animals keep the sun on their left side by 37°. The statistical analysis of the results of this experiment and the one performed in Argentina show that the differences between the distribution in relation to the sun have no significance ($P > 0·9$). I must acknowledge that the results agree more exactly than was to be expected, considering the variability of the orientation angle even at a determined hour of the day.

Another experiment was made to establish which orientation angle the animals would assume if they had compensated for difference of longitude. Nine more photographs were taken in Pisa of a group of animals placed in a basin at approximately 10·03 h (average time), i.e. the Italian time at which the experiment was made in Argentina reduced by the difference of the time between Pisa and Rosario (approximately 4·46 h). The resulting direction between the 85 positions observed was 280°, the angle with the sun 156° (Figure 3). The difference between this distribution and the one given by the Rosario experiment appears to be statistically significant ($P < 0·001$).

[1] For the experimental methods see L. PARDI and F. PAPI 1953, *l.c.* (pp. 461–463).

[2] I wish to express my gratitude to Com. ENRICO LAJ of the Accademia Navale of Leghorn for assistance with the astronomical calculation.

[3] N. ARRIGHINI, *in lit.* June 26, 1954.

The animals behaved therefore at 10·49 h in Rosario in the same manner as they would have behaved in Pisa at 14·49 h. Consequently it is proved that the *Talitrus*, transferred to a different longitude, adjust their orientation angle with the sun according to the time of the place of origin and not according to local time. This phenomenon demonstrates the existence of an internal sense of time[1].

F. PAPI

Institute of Zoology and Comparative Anatomy, University of Pisa, October 28, 1954.

Riassunto

Il Crostaceo Anfipode *Talitrus saltator* (Montagu) possiede un meccanismo di orientamento solare che gli permette, mediante una variazione regolare e continua dell'angolo di orientamento nel corso del giorno, di mantenere una direzione di fuga praticamente costante. Esemplari del litorale tirrenico, trasportati in Argentina, hanno assunto un angolo di orientamento col sole conforme all'ora italiana nel momento della esperienza. Ciò dimostra che la regolazione dell'angolo di orientamento è dovuta ad un fattore endogeno e cioè che esiste in *Talitrus* un senso del tempo.

[1] L. PARDI and M. GRASSI (cfr. this same number) comes to the same conclusion, following different method.

Experimental Modification of Direction-Finding in *Talitrus saltator* (Montagu) and *Talorchestia deshayesei* (Aud.) (Crustacea-Amphipoda).

By influencing the time-keeping mechanism ("innere Uhr") of starlings, K. HOFFMANN[1] succeeded in modifying a given direction-finding that these birds had learnt in connection with the position of the sun.

After training two starlings to find food in a given direction, HOFFMANN placed the birds in a dark room and subjected them to an illumination 6 h behind-hand in respect to normal conditions, i.e. the animals were kept in artificial light from 6 h after local sunrise to 6 h after local sunset. The temperature also was adjusted to conform with the new light conditions. After 12–18 days of this treatment, their steering by the sun for the direction they had learnt was modified exactly as expected. For example, the bird trained to find food to the west now sought it towards north, which can easily be accounted for, on recollecting that the "internal watch" of the animal was 6 h late. HOFFMANN, moreover, proved that when the animals are again subjected to normal conditions of light (in the open air), they return to the direction learnt in their early training within a period of not more than 12 days.

It was interesting to apply this beautiful experiment of HOFFMANN to *Talitrus*, for this Amphipod can steer its course on the shore in reference to the azimuthal position of the sun (mirror experiment) (PARDI and PAPI; AURICH in VON BUDDENBROCK; PAPI and PARDI[2]).

Specimens removed from the moist sand return to it at any time of day, always following a more or less

[1] K. HOFFMANN, Naturwissenschaften *40*, 608 (1953).

[2] L. PARDI and F. PAPI, Naturwissenschaften *39*, 162 (1952); Z. vgl. Physiol. *35*, 459 (1953). – AURICH in W. VON BUDDENBROCK, Sinnesphysiologie, Verlag Birkhäuser (1952). – F. PAPI and L. PARDI, Z. vgl. Physiol. *35*, 490 (1953).

straight track, whose angle with the sun varies continually during the course of day. The fact that here the sun is the pre-eminent factor of orientation, as it is in bees (v. FRISCH[1]) and in birds (KRAMER, MATTHEWS[2]), seems to imply, even in the case of *Talitrus*, a capacity for taking the time of day into account (PARDI and PAPI, PAPI and PARDI[3]). In these earlier papers, the authors advanced the opinion that the daily adjustment of the angle of orientation depended on an endogenous factor based on processes following a daily rhythm ("Zeitsinn"), and at the same time they excluded the influence of various external factors on this adjustment.

HOFFMANN's experiment was most appropriate for testing the validity of such a hypothesis for *Talitrus*.

Having ascertained in some recent unpublished experiments that *Talorchestia deshayesei* (Audouin) also possesses this same mechanism of orientation, we extended our experiments to this species. The population of *Talitrus saltator* of San Rossore (Pisa), where the coast line follows approximately a N.-S. direction and the sea lies to the west, has an average daily line of escape towards 263° (in May and June). The specimens of *Talorchestia deshayesei* were gathered in Lavagna (East Riviera), where the coast line runs in a N.W.-S.E. direction. This population has an average line of movement towards 216°, i.e. roughly towards S.W., as was ascertained in a limited number of tests carried out this spring.

At Turin the following experiments were performed:

(1) *Talitrus saltator* (March-June). Experimental batch: from 12 to 24 h, artificial light and temperature 24°C; from 24 to 12 h, darkness and temperature varying somewhere between 15°C and 19°C. Controls: a batch in normal conditions of light and temperature.

(2) *Talitrus saltator* (April-June). Experimental batch: from 12 to 24 h, artificial light; from 24 to 12 h, darkness; temperature constant throughout, 24° ± 0·5°C. Controls: one single batch in normal conditions of light and temperature.

(3) *Talitrus saltator* (May-June). Experimental batch: from 12 to 24 h, artificial light; from 24 to 12 h darkness; variable room-temperature. Controls: as in the preceding experiment.

(4) *Talorchestia deshayesei* (June-July). Experimental batch: artificial light from 6 h after local sunrise to 6 h after local sunset (Turin). Variable room-temperature. Controls: artificial light from local sunrise to local sunset and variable room-temperature.

(5) *Talorchestia deshayesei* (June-July). Artificial light from 12 h after local sunrise to 12 h after local sunset. Controls: as in the preceding experiment.

The day following the commencement of the experiment, and later at more or less regular intervals in connection with meteorological conditions, 5 specimens of each batch (both experimental batches and control) were exposed to the sunlight and their orientation was registered by means of repeated counts. The orientation tests were all made between 15·00 and 16·00 for experiments 1–4; between 16h30′ and 17h30′ for experiment 5.

The essential conclusions are the following:

(1) Shifting the period of illumination, as in experiments 1–4, the line of movements is shifted constantly, though to a varying extent, in the expected direction, i.e. towards north. In figure 1 the average line of escape for *Talitrus saltator* (268° between 15 and 16 h in the spring months[1]) considered equal to 0°, while all the resultant directions observed from the fourth to the 26th day after the beginning of the experiment are shown as deviations in degrees from the average line of escape, with positive values when the deviation is towards

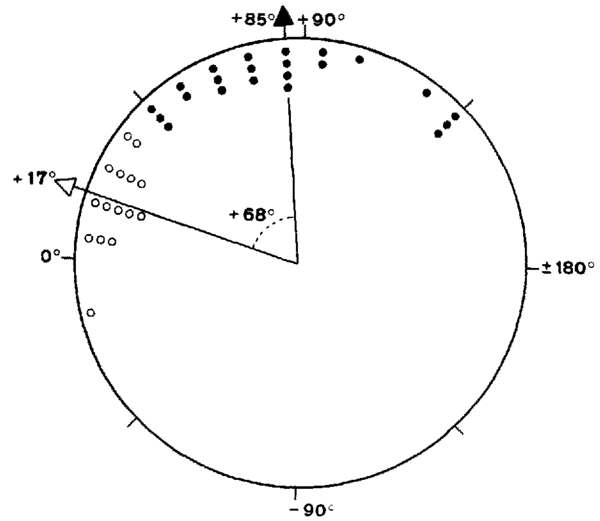


Fig. 1.–*Talitrus saltator* (Montagu). Experiment 1–3 (artificial light from 12 h to 24 h): observed resultant directions of the control (white circles) and experimental individuals (black circles) are shown as deviation in degrees from the average line of escape considered equal to 0°. Other explanation in text.

north, with negative values when towards south. The deviations obtained vary for the specimens under experiment from a minimum of + 45° to a maximum of + 146° with an average of + 85°; for the controls, which also show a slight shifting northwards in their line of movement (+ 17°), the minimum and maximum values found respectively are − 16° (southward deviation) and + 38°. The difference (+68°) between the two distributions is statistically significant ($P < 0{\cdot}01$).

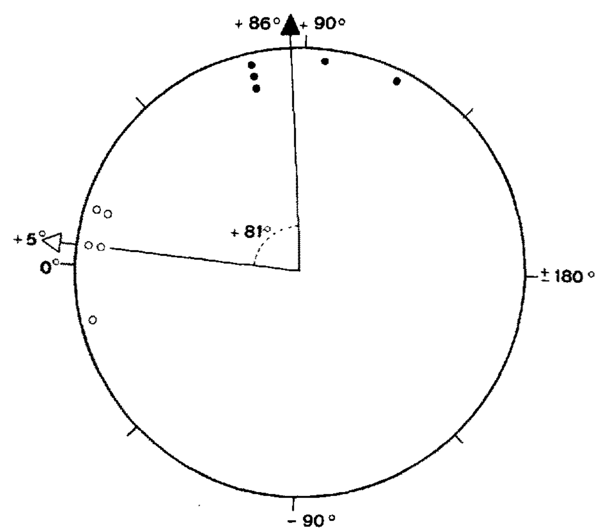


Fig. 2.–*Talorchestia deshayesei* (Audouin). Experiment 4 (artificial light from 6 hours after sunrise to 6 hours after sunset). Explanation as in Figure 1.

Figure 2 shows the results obtained with *Talorchestia*, which correspond exactly to those obtained with *Tali-*

[1] K. VON FRISCH, Exper. *5*, 142 (1949).

[2] G. KRAMER, Exper. *94*, 265 (1952). – B. V. T. MATTHEWS, J. Exp. Biol. *30*, 243 (1953).

[3] See footnote 2, p. 202, loc. cit.

[1] L. PARDI and F. PAPI, Z. vgl. Physiol. *35*, 470 (1953).

trus. While controls present a small deviation in their line of movement (+ 5° in average) the specimens under experiment have a marked northward deviation (+ 86° in average). (Difference between controls and experimental specimens = + 81°, $P < 0{\cdot}01$).

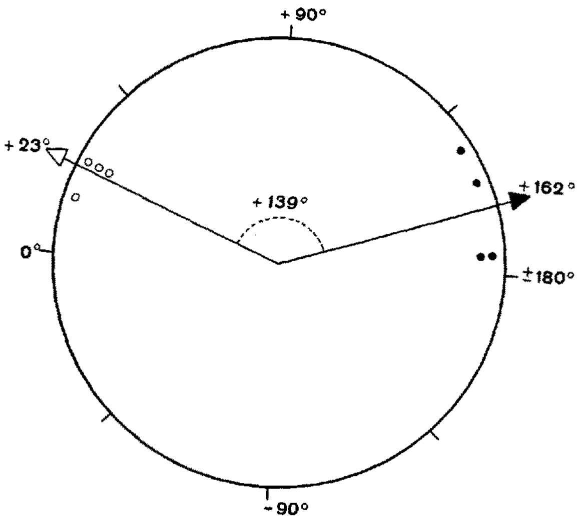


Fig. 3.–*Talorchestia deshayesei* (Audouin). Experiment 5 (artificial light from 12 h after sunrise to 12 h after sunset). Explanation as in Figure 1.

Though the causes of the considerable variability which was noted are as yet to be investigated, it is definitely ascertained that *changes in the period of illumination*, as described above, *cause a deviation of flight very near, in average value, to what was expected* (+ 90°). In other words, the animals under experiment, exposed to the afternoon sun, behave as if it were morning.

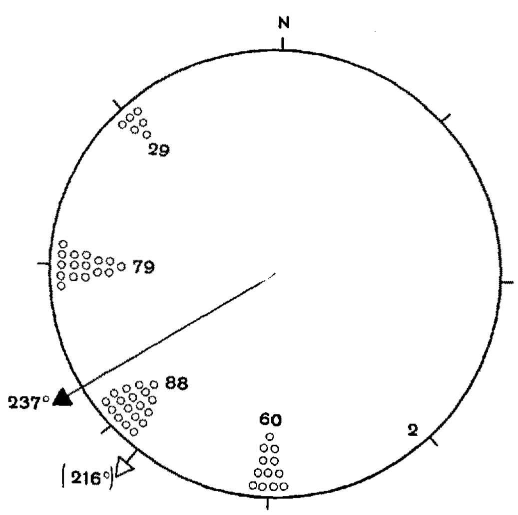


Fig. 4*a*.–*Talorchestia deshayesei* (Audouin). Experiment 5 (artificial light from 12 h after sunrise to 12 h after sunset). Observed positions of the *control* individuals between 17 h 07′ and 17 h 12′ on 19th of July, 9 days after the beginning of the experiment. Each circle: 5 observed positions of the animals which show a very little deviation from the average line of escape (216°).

(2). The deviation in the line of escape takes place by degrees and, within the first four days of treatment, reaches its maximum, which is subsequently maintained with more or less considerable fluctuations.

(3) Also with a constant temperature (experiment 2), results were instructive: a considerable shift of line of escape having been obtained in the expected direction (average of 100°); this proves that the normal daily variations of temperature have no perceptible influence on the processes by which the angle of direction is regulated.

(4) When the individuals used in these experiments were returned to normal conditions, i.e. to natural light or even to artificial light synchronized with local daylight-time (i.e. from dawn to sunset) they all returned gradually, within the space of 4 days, to their normal line of escape.

(5) When light is given in periods, which are 12 h behind normal daylight time (experiment 5 with *Talorchestia*), the change of the line of movement in the foreseen direction is still more marked (average of + 162°) (Fig. 3 and 4); the animals exposed to sunlight late in the afternoon, behave as if it were early morning[1].

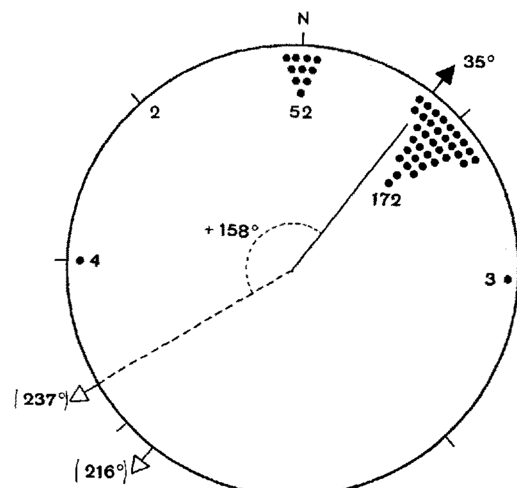


Fig. 4*b*.–*Talorchestia deshayesei* (Audouin). Experiment 5 (artificial light from 12 h after sunrise to 12 h after sunset). Observed positions of the *experimental* individuals between 16 h 52′ and 17 h 00 on 19th of July, 9 days after the beginning of the experiment. Each circle: 5 observed positions of the individuals which show a very great deviation from the average line of escape (216°). The line of escape of the controls (237°) and the deviation from the controls is also given

(6) Together with orientation, rhythm of surface activity also changes: In experiment 1, repeated counts of the number of animals on the surface showed that the curve of activity was retarded by about 5 h as compared with that of controls kept in natural light.

These are the results of the "HOFFMANN experiments". In the present experiment, an attempt has also been made to modify the line of movement by raising and lowering temperature. During 18 h preceding the experiment in sunlight, the animals (*Talitrus saltator* and *Talorchestia deshayesei*) were subjected to temperatures of + 35° to 37° or of + 4° to 6°C. The exposure to low temperature gave no valuable results; but the high temperatures, in both the experiments carried out, caused a limited but significant change in the line of escape: *when exposed to sunlight, the individuals which were subjected to high temperatures move with a sharper angle than the controls*. For *Talitrus saltator* and *Talorchestia deshayesei*, a difference of 31° and of 46° respectively was ascertained between controls and

[1] Corresponding results have been obtained this summer with a different population of *Talitrus saltator* (line of escape = 201° ca.).

specimens subjected to high temperatures. The inference may be drawn that the high temperature accelerates, to a slight degree, the internal process by which the angle of orientation is determined: the internal time-measuring apparatus of the animals subjected to high temperatures is thus in advance when compared with that of the controls.

Further experiments are being carried out. At present the results obtained appear to prove the following points:

(a) As had been assumed, and in accordance with what HOFFMANN has established for Birds, the adjustment of the angle of orientation in *Talitrus* and *Talorchestia* is based on an endogenous factor, i.e. on physiological processes having a daily rhythm ("innere Uhr", "Zeitsinn")[1].

(b) It is possible, also in the case of *Talitrus* and *Talorchestia*, to influence these processes by altering the period of illumination; and this alteration takes place even if temperature conditions remain unchanged.

[1] F. PAPI, following a different method, comes to the same conclusion (*vide* this same volume).

(c) It is possible to determine a perceptible change in the line of escape by exposing the animals to comparatively higher temperatures before investigating the direction in which they escape.

L. PARDI and M. GRASSI

Zoological Institute of the University of Turin (Italy), October 29, 1954.

Riassunto

Talitrus saltator (Montagu) e *Talorchestia deshayesei* (Aud.) (Crustacea-Amphipoda), allontanati dalla sabbia umida della spiaggia, sono in grado di ritornarvi, mantenendo una direzione di fuga praticamente costante in base alla posizione azimutale del Sole. La regolazione dell'angolo di orientamento col Sole nel corso del giorno è basata sul senso del tempo, come dimostrano esperienze simili a quelle recentemente eseguite da HOFFMANN per gli Storni. È possibile ottenere una significativa deviazione della direzione di fuga, sottoponendo gli animali a temperature relativamente alte prima di saggiare il loro orientamento.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Über die Genauigkeit der Brenztraubensäure-Bestimmung als 2,4-Dinitrophenylhydrazon

Vergleich der direkten kolorimetrischen mit der quantitativ-chromatographischen Methode

Von S. MARKEES[1], Basel

Die Wichtigkeit zuverlässiger Nachweismethoden für die Brenztraubensäure (BTS) ist bei Betrachtung der in den letzten Jahren gewaltig angewachsenen Zahl von Arbeiten über dieses Stoffwechselprodukt unverkennbar. Dabei muss daran erinnert werden, dass für die früher unter die Gruppe der «bisulfitbindenden Substanzen» gerechnete und als solche quantitativ bestimmte Säure erst 1939 von LU[2] ein praktisch brauchbares Bestimmungsverfahren für das Blut entwickelt worden ist, das nach dem Prinzip der Umsetzung mit dem 2,4-Dinitrophenylhydrazin arbeitet. Durch FRIEDEMANN und HAUGEN[3], nach deren Angaben die BTS heute ziemlich allgemein bestimmt wird, ist dieses Verfahren nur hinsichtlich des Extraktionsmittels – Toluol statt Essigester – abgeändert worden. Das mit der BTS gebildete 2,4-Dinitrophenylhydrazon ist in alkalischer Lösung rot gefärbt und gut kolorimetrierbar. Diese Reaktion ist naturgemäss unspezifisch, da alle α-Ketosäuren und teilweise auch einige β-Ketosäuren sich mit dem Reagens umsetzen. Über die Ausschaltung der wesentlichsten Fehlerquelle, der Azetessigsäure, durch kurzfristiges Erhitzen des Blutfiltrates vor dem Zusatz des 2,4-Dinitrophenylhydrazins haben wir früher berichtet[1]. Den mit dem Aufkommen der Papierchromatographie in der Literatur[2] auftauchenden Zweifeln an der Genauigkeit der kolorimetrischen Bestimmung der BTS als 2,4-Dinitrophenylhydrazon sind wir dann gemeinsam mit GEY[3] in einer Arbeit entgegengetreten, in der das direkte kolorimetrische Verfahren mit dem quantitativ-chromatographischen verglichen worden ist. Es hat sich damals eine ausreichende Übereinstimmung der gefundenen Absolutwerte ergeben. Nachdem aber neuerdings in der Literatur[4] darüber berichtet worden ist, dass bei Verwendung einer fermentchemischen Methode mit Milchsäure-Dehydrogenase, die als spezifisch angesprochen wird, niedrigere Werte für die BTS gefunden werden als mit der 2,4-Dinitrophenylhydrazin-Methode, haben wir es für erforderlich gehalten, dieses Verfahren noch einmal sehr eingehend auf Genauigkeit, bzw. seine Spezifität zu überprüfen. Dabei hat sich vor allem die Notwendigkeit herausgestellt, die papierchromatographische Methode grundlegend neu zu bearbeiten und sie

[1] Aus den Medizinischen Laboratorien der F. Hoffmann-La Roche & Co. AG., Basel.

[2] G. D. LU, Biochem. J. *33*, 249 (1939).

[3] TH. E. FRIEDEMANN und G. E. HAUGEN, J. Biol. Chem. *147*, 415 (1943).

[1] S. MARKEES, Exper. *7*, 314 (1951).

[2] D. CAVALLINI, N. FRONTALI und G. TOSCHI, Nature *163*, 568 (1949).

[3] S. MARKEES und F. GEY, Helv. physiol. Acta *11*, 49 (1953).

[4] K. JOHANNSMEIER, H. REDETZKI und G. PFLEIDERER, Klin. Wschr. *32*, 560 (1954).