

LOCOMOTOR ACTIVITY RHYTHMS IN *Tryphosella kergueleni* (Miers)
AND *Cheirimedon femoratus* (Pfeffer) (CRUSTACEA, AMPHIPODA)

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ABSTRACT. Trap samples, trawling and visual observations show that the swimming and feeding activity of *T. kergueleni* and *C. femoratus* occurs during the hours of darkness. In summer, *C. femoratus* is the more active of the two, but in winter it displays much reduced activity.

The activity of both species is partly under the control of an internal physiological clock, which maintains the rhythmic pattern of behaviour in constant conditions in the laboratory. The clocks are synchronized by cyclic changes in light intensity. Also, darkness generally promotes activity, whereas a change from darkness to light causes the animals to seek shelter.

During winter, the activity rhythm of *C. femoratus* in constant conditions is ill-defined, but that of *T. kergueleni* is relatively persistent.

The adaptive significance of the internal timing mechanism is considered to be a measure for conserving energy.

Some information is given concerning the rhythmic swimming activity of other Antarctic amphipod species.

INTERNAL or endogenous timing mechanisms which control a variety of physiological processes have been described in many different species of animal and plant. Mammals and crustaceans dominate these studies, and of the latter, amphipods have received considerable attention (e.g. Pardi, 1960; Enright, 1963; Morgan, 1965; Fincham, 1970; Wildish, 1970).

Changes in light intensity have been shown to be an important, and probably the main, environmental synchronizer for rhythms of circadian frequency, and polar regions, with their widely divergent seasonal light regimes, are areas of obvious interest in this respect. Almost all studies of this sort have been undertaken in Arctic regions on account of their relative accessibility. Swade and Pittendrigh (1967) have reported endogenous circadian locomotor rhythms in rodents at Point Barrow, Alaska (lat. 71°19'N.). A period of complete darkness is not necessary to effect entrainment of these rhythms and the crucial factor is the ratio between maximum and minimum intensities of light. If the ratio is too small, "free-running" of the rhythms occurs and synchronization is lost. These authors found that the nocturnal species of Arctic rodent showed apparent difficulty in maintaining the proper phase of the rhythm during the summer solstice. In a study of wolf spiders at lat. 69°N., Papi and Syrjamaki (1963) found that fluctuations in light intensity were sufficient to entrain the rhythm of sun orientation in these animals. Nymphs of *Baetis vernus* Curtis, which are active during darkness under normal light/dark conditions, do not show a rhythmic activity cycle in the permanent light of polar regions (Muller, 1965).

Extensive studies on the effect of different day lengths over the year upon the activity of several organisms, especially insects, have been undertaken recently in high northern latitudes (*in Phänomene der Tages—und Jahresperiodik in hohen nordischen Breiten. Oikos*, supplement 13, 1970). The daily activity patterns vary considerably between different species and between different seasons. Studies of this nature, under conditions of natural light and darkness do not necessarily imply the possession of an internal physiological timing mechanism, although it would be surprising if this were not true for most cases, on account of the selective advantages of such timing mechanisms (Aschoff, 1964).

Hamner and others (1962) have demonstrated the maintenance of some physiological rhythms of hamsters, *Drosophila* and bean plants at the South Pole, but the only studies to date on the endogenous timing mechanism of an Antarctic organism appear to be those of Emlen and Penney (1964) and Penney and Emlen (1967) on the solar navigation ability of the Adélie penguin.

Signy Island (lat. 60°43'S.), in the South Orkney Islands group, does not experience continuous daylight at mid-summer or continuous darkness at mid-winter. There is considerable variation in day length over the year, there being 19·2 hr. from sunrise to sunset at mid-summer and 5·5 hr. at mid-winter. Twilight periods are particularly long during summer. As far as

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marine organisms are concerned, the effect of long days in the summer is to some extent offset by the presence of dense phytoplankton and other suspended particles in the water at this time, which reduces light penetration considerably, even in 5 or 10 m. of water (Fig. 1).

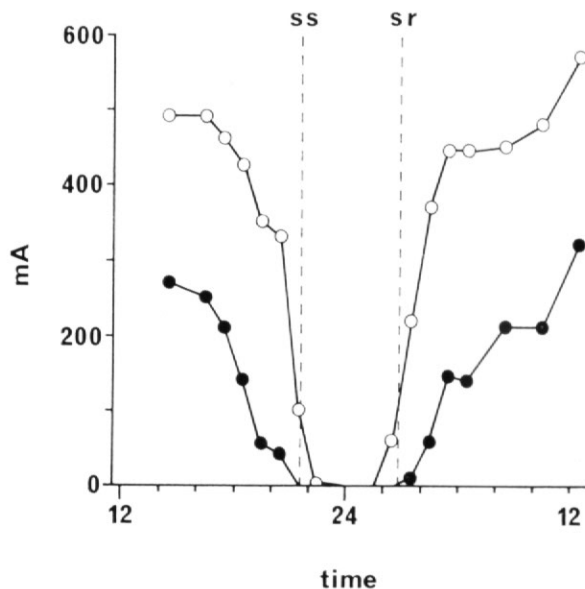


Fig. 1. Incident light, measured by a photocell (in mA), at the surface (open circles) and 6 m. below the surface (closed circles). Factory Cove, Signy Island; 7-8 January 1969. ss: sunset; sr: sunrise.

The present study was undertaken at Signy Island to examine the rhythmic locomotor behaviour of two species of Antarctic amphipod, and to determine whether an endogenous timing mechanism was involved in the control of this activity. Field observations were complemented by laboratory studies.

MATERIAL AND METHODS

The amphipods *Cheirimedon femoratus* (Pfeffer) and *Tryphosella kergueleni* (Miers) are common in shallow inshore sands in certain parts of the Antarctic. They are night-active, emerging from the sand around sunset, and are taken in baited traps at all times of the year, especially at night. Ovigerous females of *C. femoratus* and small juveniles of both species are not taken in traps, but ovigerous females of *T. kergueleni* bearing early-stage broods are regularly attracted to bait (Bregazzi, 1972a).

Trap samples were obtained at hourly intervals for 24 hr. periods upon five different occasions, during autumn, winter (twice), spring and summer, using 30 cm. by 15 cm. funnel traps baited with half heads of fish (*Notothenia* spp.), similar pieces of fresh bait being used for each sample. Two traps were set on the half-hour near site A, Factory Cove (Bregazzi, 1972a), either from a boat or through a hole cut in the sea ice, and left in position for 10 min. One trap lay on the bottom and the other was suspended by means of a buoy 1.5 m. vertically above it. All amphipods taken in the traps were preserved in 5 per cent neutral formalin.

During the summer, some trawls were made by night with a 0.46 m. diameter plankton net, and visual observations were made at different times of the year by aqualung divers.

Between 5 and 7 February 1969, the partial failure of the pump which supplied sea-water to aquaria in the station laboratory provided an opportunity for regular mid-water sampling. Swimming amphipods and other organisms were sucked into the intake pipe, the mouth of which was about 1 m. above the sandy bottom of Factory Cove, and delivered, unharmed upon this occasion, at the outlet in the laboratory where they were retained in a sieve. Sampling

took place on two consecutive nights from 18.00 to 04.00 hr. The sieve was removed and the contents examined at hourly intervals, on the half-hour.

Investigations into spontaneous locomotor activity were conducted in a constant-temperature room at $0 \pm 1^\circ \text{C}$, and under conditions of light, provided by two 40W strip lights about 1 m. vertically above the experimental tanks (about 100 lux), or with dim red background illumination of immeasurably low intensity. The Perspex tanks were 17 cm. by 17 cm. by 22 cm. and were filled with fresh sea-water to about 15 cm. A 60 cm. length of stainless steel wire with a 10 cm. loop at one end was suspended in the water close to one side of each experimental tank so that when an animal swam against it, it was displaced slightly and made contact with a copper plate a few centimetres above the water surface. This completed an electrical circuit and resulted in a mark being made on the moving chart paper of a Rustrak event recorder (Fig. 2).

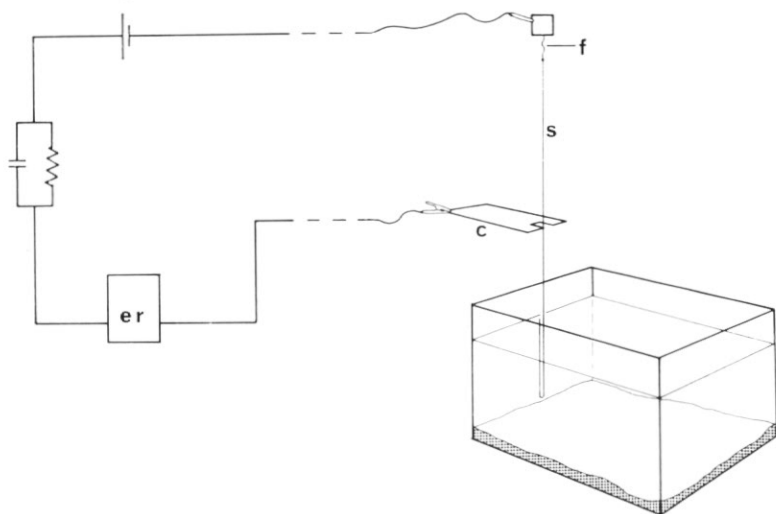


Fig. 2. Apparatus for recording swimming activity of *T. kergueleni* and *C. femoratus*. c: copper contact plate; er: event recorder; f: thin fuse wire, allowing wire pendulum to swing; s: stainless steel wire with loop.

Power for the circuit was provided by a mains-operated transformer which produced a rectified current at 12 V. Recordings were also made with another type of circuit in which contact between the stainless steel wire and the copper plate caused the closing of a reed-switch relay, completing a circuit into which a solenoid pen-marker and Nife battery were connected. The pen-marker moved against a smoked drum revolving once every 24 hr. at about 1.27 cm./hr. Both types of recording circuit contained a condenser and resistance of appropriate value in parallel, so that prolonged making of the contacts by an animal which had become wedged between the wire and the side of the tank was still recorded as a single mark. This was particularly important with the solenoid pen-marker, with which continuous current caused overheating and a rapid draining of the battery. Records were made and observed in a laboratory several metres away from the constant-temperature room.

This apparatus was suited to the exploratory swimming habits of these amphipods, but occasionally the wire pendulum was avoided. For some experiments, cardboard covers were provided for the tanks, in which case a very small amount of light entered the tanks through a 2 mm. by 2 mm. hole through which the wire pendulum passed.

For experiments in constant conditions, satisfactory results were obtained by introducing about 1 cm. of fine pebbles into the tanks, amongst which the animals could remain when inactive. On other occasions about 1 cm. of fine or coarse sand was also used into which the animals could burrow.

The largest animals available were used for experiments as these developed greater momentum for operating the wire pendulum and were also less liable to adhere to the surface film. The individuals used, therefore, were non-ovigerous females of *C. femoratus* and early-stage

ovigerous females of *T. kergueleni*, both collected in flesh-baited traps and thus recently fed. Additional experiments with males of both species, ovigerous females of *C. femoratus* and non-ovigerous females of *T. kergueleni* revealed no differences in behaviour patterns. Unless otherwise stated, ten freshly collected animals were used in each tank.

Results are expressed as number of events recorded per hour. Mid-activity points have been calculated for the activity in a 24 hr. period by using those hourly values which are greater than the mean for that period, and differences between successive mid-activity points provide a measure of the period of the locomotor rhythm.

All times given are local time.

Trap samples

FIELD OBSERVATIONS

The limitations of sampling with baited traps have been outlined by Bregazzi (1972a). The results of the five 24 hr. hourly samples obtained from the trap on the sea bed are given separately for each species in Fig. 3.

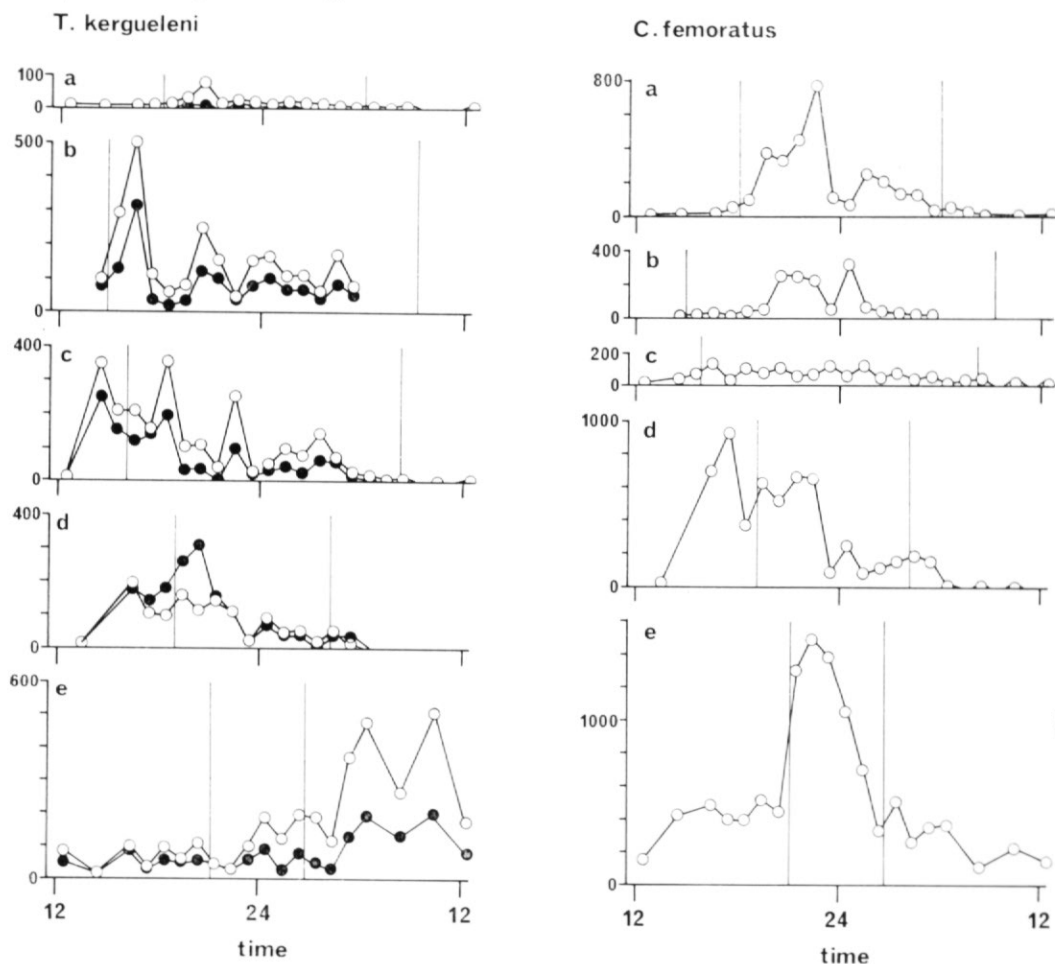


Fig. 3. Numbers of *T. kergueleni* and *C. femoratus* taken in five 24 hr. hourly samples, with fish-baited trap on the sea bed. Factory Cove, 6 m.

a: autumn (25-26 March 1968). b: winter (1-2 July 1968). c: winter (31 July-1 August 1968). d: spring (18-19 October 1968). e: summer (7-8 January 1969). Vertical lines denote times of sunset and sunrise. Sampling stopped at 05.30 hr. on 2 July 1968 due to weather conditions. Open circles, non-ovigerous animals; closed circles, ovigerous animals.

T. kergueleni. Small numbers of this species were taken during the autumn sample, with a peak during the early part of the night (Fig. 3a). Both winter samples show an overall increase in numbers (Fig. 3b and c), which is in accord with the suggested influx of this species into the sampling area during winter (Bregazzi, 1972a). The greatest numbers taken again coincide with the beginning of the night. The first peak of the second winter sample occurs before sunset, and this is probably accounted for by the dense cloud cover which appeared between 12.30 and 14.30 hr. on this occasion. The fluctuations in numbers of both samples over the 24 hr. period suggests that this species may possess some degree of gregariousness when active. The spring sample (Fig. 3d) produced somewhat fewer numbers, with a peak during the early part of the night. The large increase in numbers between 13.30 and 16.30 hr. is again likely to be due to the change from bright sun to cloudy conditions which occurred during this time. The summer sample showed the anticipated low numbers to begin with, and also throughout the night, but the appearance of large numbers soon after sunrise was quite unexpected. It may be supposed that a "swarm" of this species settled in the sand around the sampling point following the previous night's activity. Core samples taken by day in the sampling area have shown that lower numbers of *T. kergueleni* occur in Factory Cove during the summer (Bregazzi, 1972a).

C. femoratus. The numbers of this species caught with the bottom trap follow the hours of darkness reasonably closely, but many more were taken during spring and summer than during winter. Bottom core samples taken during day-time, however, indicate that there is no great reduction in numbers of *C. femoratus* inhabiting a given area of bottom sand in Factory Cove during winter, and also that growth does not occur at this time of year (Bregazzi, 1972a). It is suggested, therefore that the activity and perhaps metabolism of this species are at a relatively low level during winter.

Orchomene plebs (Hurley). This species was taken, in small numbers, during the night-time of the autumn and winter samples only (Table I). This supports the data discussed by Bregazzi (1972a) which have led to the suggestion that *O. plebs* is a migratory species, moving into shallow inshore waters during winter.

TABLE I. NUMBERS OF *Orchomene plebs* TAKEN DURING FIVE 24 hr. HOURLY TRAP SAMPLES

	18.30	19.30	20.30	21.30	22.30	23.30	00.30	01.30	02.30	03.30	04.30	05.30	06.30
25 March 1968	—	—	—	—	1	—	—	4	—	1	1	3	3
1 July 1968	4	1	2	2	—	12	24	3	2	1	6	4	No sample
31 July 1968	—	—	—	—	—	2	—	2	—	3	—	—	—
18 October 1968	—	—	—	—	—	—	—	—	—	—	—	—	—
7 January 1969	—	—	—	—	—	—	—	—	—	—	—	—	—

Other species. Small numbers of *Orchomene nodimanus* (Walker), *O. rotundifrons* (Barnard) and *Lepidepcreum cingulatum* Barnard were taken in the bottom trap at most times of the year, and all during the hours of darkness. Total numbers for each 24 hr. sample are given in Table II.

Top trap samples. Amphipods taken in the trap 1.5 m. above the sea bed were much fewer in number than those from the trap on the bottom and included *C. femoratus* and *T. kergueleni* only. Total numbers for each 24 hr. sample are given in Table III. Very few *T. kergueleni* were taken throughout the year. Most *C. femoratus* were obtained during summer and autumn, in which cases the hourly numbers followed the pattern of the bottom trap closely. Very few *C. femoratus* were taken in winter.

TABLE II. TOTAL NUMBERS OF THREE SPECIES OF AMPHIPOD TAKEN IN A TRAP ON THE SEA BED IN EACH OF FIVE 24 hr. HOURLY SAMPLES

	<i>Orchomene nodimanus</i>	<i>O. rotundifrons</i>	<i>Lepidepcreum cingulatum</i>
25 March 1968	36	1	4
1 July 1968	3	—	10
31 July 1968	—	—	9
18 October 1968	2	1	10
7 January 1969	1	—	5

TABLE III. TOTAL NUMBERS OF AMPHIPODS TAKEN IN A TRAP 1.5 m. ABOVE THE SEA BED IN EACH OF FIVE 24 hr. HOURLY SAMPLES

	<i>T. kergueleni</i>	<i>C. femoratus</i>
25 March 1968	4	183
1 July 1968	6	8
31 July 1968	17	12
18 October 1968	18	46
7 January 1969	13	60

Sieve collections

The results of the two periods of sampling by sieve at the aquarium pump outlet during summer are given in Fig. 4. Many more specimens of *C. femoratus* (Fig. 4a) were taken than *T. kergueleni* (Fig. 4b), supporting the probability that *C. femoratus* is an active vertical swimmer and that *T. kergueleni* tends to remain near the bottom. Although sampling took place only from 18.30 to 03.30 hr., it seems clear that the amphipod species taken swim away from the bottom only during the hours of darkness. Specimens of *T. kergueleni* appear in the samples chiefly during the early part of the night, whereas the other species were taken throughout the hours of darkness, with a peak around midnight.

Visual observations

Most diving was conducted during the hours of daylight when amphipods were rarely seen, either in open water or on the sand surface. However, some dives were undertaken during dusk and darkness at different times of the year, and the observations, all from within Factory Cove in water 5–10 m. deep, may be summarized as follows.

Around dusk, amphipods emerge from the sand. At first, only a few heads are seen, but gradually, more and more animals appear on the bottom surface. At this time they were often seen to form clumps composed of many species, feeding upon damaged amphipods or other animal remains. Following relative inactivity on the sand surface, bursts of swimming occur, which take the animals a few centimetres away from the bottom. During winter, this appeared to be the extent of the activity of most individuals. For example, at 21.00 hr. on 14 August 1968 (about 5 hr. after sunset), many amphipods, mostly *C. femoratus*, *Pontogeneiella brevicornis* (Chevreux) and *T. kergueleni*, were observed on the sand surface, largely inactive, with a few making short vertical excursions. Several were noted with only the anterior parts visible,

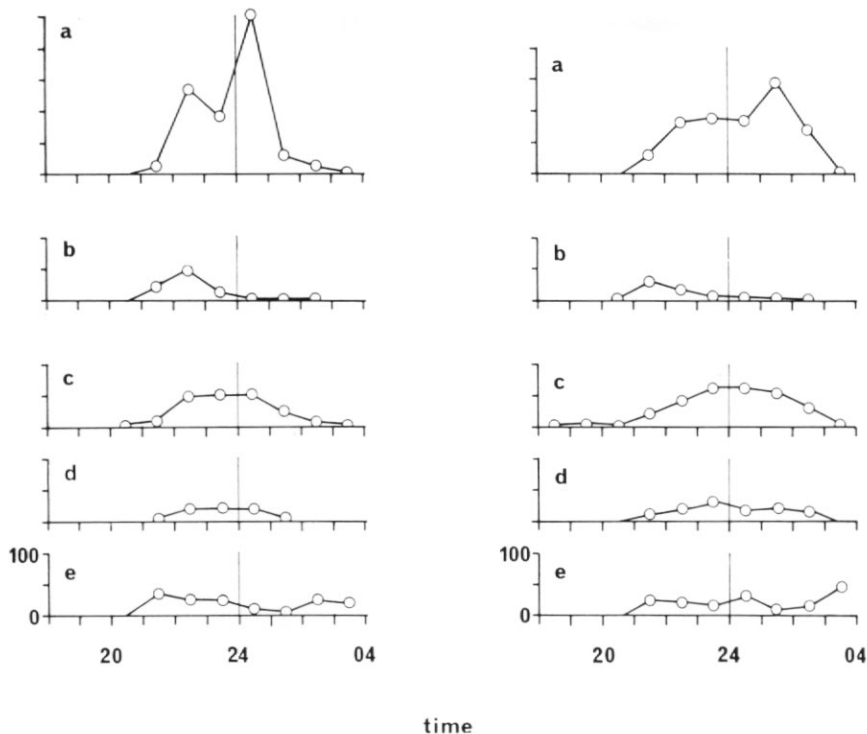


Fig. 4. Numbers of amphipods taken by sieve at hourly intervals during two successive nights (5-6 and 6-7 February 1969). Sunset 20.20 hr., sunrise 04.05 hr. a: *C. femoratus*. b: *T. kergueleni*. c: *Pontogeneia antarctica*. d: *Pontogeneiella brevicornis*. e: *Lepidepecreum cingulatum*.

partly buried in the sand. By disturbing the bottom sand by hand, it was clear that many more amphipods were completely buried.

Observations during summer nights revealed a different pattern of activity. There were much greater numbers of all species on the sand surface and swimming near to it, but also, very large numbers of *C. femoratus* were observed and captured swimming in open water. These, including ovigerous females all bearing hatched broods, were found to be feeding upon the dense planktonic organisms (Bregazzi, 1972a). The greatest densities of this species were noted at the edge of Factory Cove, but "swarms" were also encountered in mid-Cove, from near the bottom up to the water surface. On very calm nights, several *C. femoratus* were seen trapped in the surface film. The appearance of increasing numbers of this species in the open water as night fell was observed on several occasions from land and from boats.

A few plankton-net trawls, each of 10 min. duration, were made at hourly intervals during certain summer nights from a slowly moving boat, such that the net was kept 1-2 m. above the sandy bottom near the edge of Factory Cove. These also produced increasing numbers of amphipods with the onset of darkness, mostly *Pontogeneia antarctica* Chevreux and *C. femoratus*, but also a few *Pontogeneiella brevicornis*, *T. kergueleni* and *Lepidepecreum cingulatum*. Two other species of sand-dwelling amphipod, *Parharpinia rotundifrons* Barnard and *Monoculodes scabriculosus* Barnard, common in the sand of Factory Cove, were never taken in net trawls, and they appear not to swim extensively under normal circumstances.

LABORATORY INVESTIGATIONS

Both *T. kergueleni* and *C. femoratus* displayed rhythmic swimming activity in conditions of constant light and temperature.

Continuous dim red background illumination

The total activity of four groups of ten early-stage ovigerous females of *T. kergueleni* over 4 days from 3 January is given in Fig. 5a. There is a clear circadian locomotory rhythm with maximum activity around midnight to begin with, but at a successively later time during the following days. Male and non-ovigerous female animals were found to be similarly rhythmic.

The mean period length for eight experiments, each carried out for 4 days during 31 August–7 January was 25.25 hr. (s.d. ± 0.76 hr.).

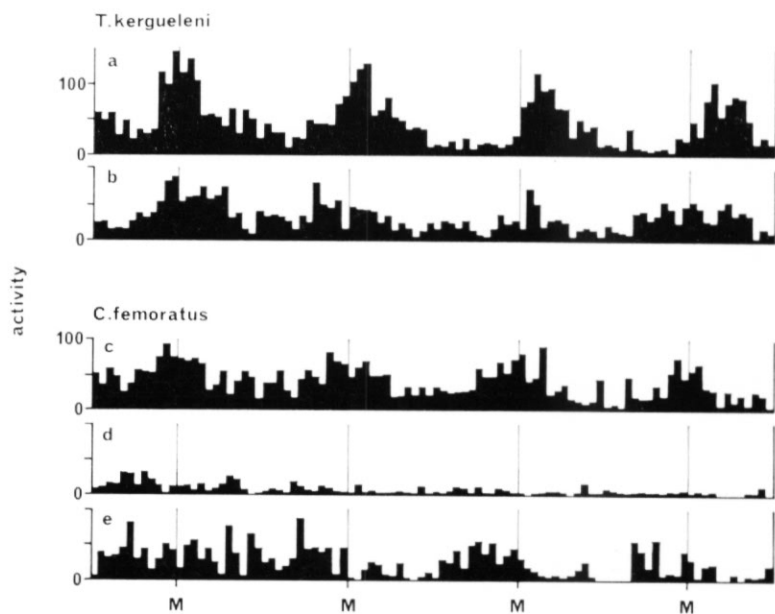


Fig. 5. Total spontaneous swimming activity recorded in continuous dim red background illumination for 4 days. *T. kergueleni*. a: 3–7 January. b: 19 June–10 July. *C. femoratus*. c: 29 December–2 January. d: 19 June–10 July. e: 19–23 June. a, b, c and d: each, four groups of ten animals; e: two groups of ten animals; M: midnight.

The total activity of four groups of ten *C. femoratus* over 4 days from 29 December is given in Fig. 5c. A clear circadian rhythm is again apparent, with maximum activity around midnight on successive days. Similar rhythms were obtained using males and ovigerous females, the latter collected by dredging on account of their non-carrier feeding habits.

The results of nine experiments carried out at times between 17 August and 6 March, each for 4 days, gave a mean period length of 24.37 hr. (s.d. ± 0.65 hr.).

Around mid-winter, the extent of spontaneous swimming activity of both species was reduced. The total activity of four groups of *T. kergueleni* recorded at times between 19 June and 10 July is given in Fig. 5b. A circadian rhythm is still apparent but it is not so clearly defined as that obtained during summer. The swimming activity of *C. femoratus* recorded at mid-winter was in most cases arrhythmic, as indicated in Fig. 5d, which plots total activity of four groups of this species recorded at times between 19 June and 10 July. Apparent rhythmicity was recorded around mid-winter in only two groups of animals, between 19 and 23 June, and the combined records of these are shown in Fig. 5e.

Continuous light

Swimming rhythms of circadian frequency were recorded under continuous light in a few experiments with both species. In these, the rhythms were not so clear-cut as those recorded under continuous dim red background illumination, and the extent of activity was reduced.

The period of the rhythm appears to be similar to that obtained under dim red illumination in the case of *C. femoratus* and somewhat shorter in the case of *T. kergueleni*.

Synchronization of the locomotor rhythm

Correct synchronization of an endogenously timed rhythm is achieved by response to regular environmental changes, and, to test the role of light, freshly collected groups of *T. kergueleni* in spring were subjected to artificial cycles of light and darkness of 12 hr. each. With two groups, onset of darkness was 6 hr. in advance of natural conditions and in another two groups it was delayed by 6 hr. These conditions were maintained for two 24 hr. cycles and during this time the animals were active only during their respective times of darkness. After this, all groups were allowed to "free-run" in dim red background light. Activity for the 3 days in constant conditions following the artificial light/dark cycles, summed over 24 hr. is given in Fig. 6a. It can be seen that both groups of animals, under changed light/dark conditions, have re-synchronized their swimming rhythms so that activity occurs during the time of subjective "night". There is, however, a secondary peak, in both cases around midnight, which indicates that re-synchronization may not be complete.

Another experiment tested the ability of *T. kergueleni* to re-synchronize to artificial cycles of 12 hr. light and 12 hr. dark when onset of darkness was advanced 9 hr. from natural sunset during early December. Four experimental tanks were given one, two, three and four light/dark

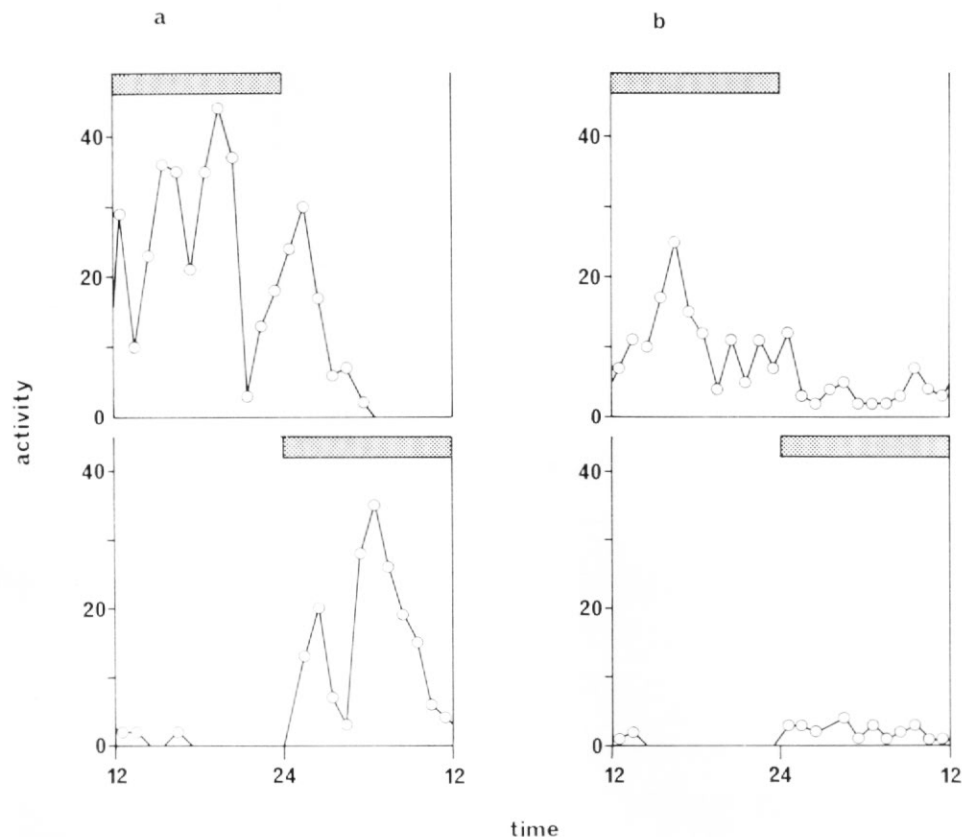


Fig. 6. a. Total activity of *T. kergueleni*, summed over 24 hr., for 3 days in continuous dim red background illumination following artificial light/dark cycles for 2 days.

b. Total activity of *C. femoratus*, summed over 24 hr., for 3 days in continuous dim red background illumination following light/dark cycles for 4 days. Shaded area, time of "expected night".

cycles respectively before being allowed to free-run in constant darkness (cardboard covers). Actual records for the four tanks are given in Fig. 7. These show that re-synchronization occurs progressively, and that it is not complete, under these conditions, until after at least 4 days of the experimental cycles.

In a further experiment, some *T. kergueleni* were kept in continuous dim red light for 12 days during September, and then 18 animals were placed in each of two experimental tanks. There was a low level of activity for 2 days and no apparent rhythm (Fig. 8). Then both tanks were given 6 hr. of artificial light from 18.00 to 24.00 hr., and one tank was given a second period of 6 hr. of light and at the same time next day. In both tanks activity proceeded at a higher level following the light shocks and in a rhythmic manner at circadian frequency. It seems that a single 6 hr. period of light at the intensity here used is sufficient to induce a rhythm of locomotory activity that was previously absent or obscured.

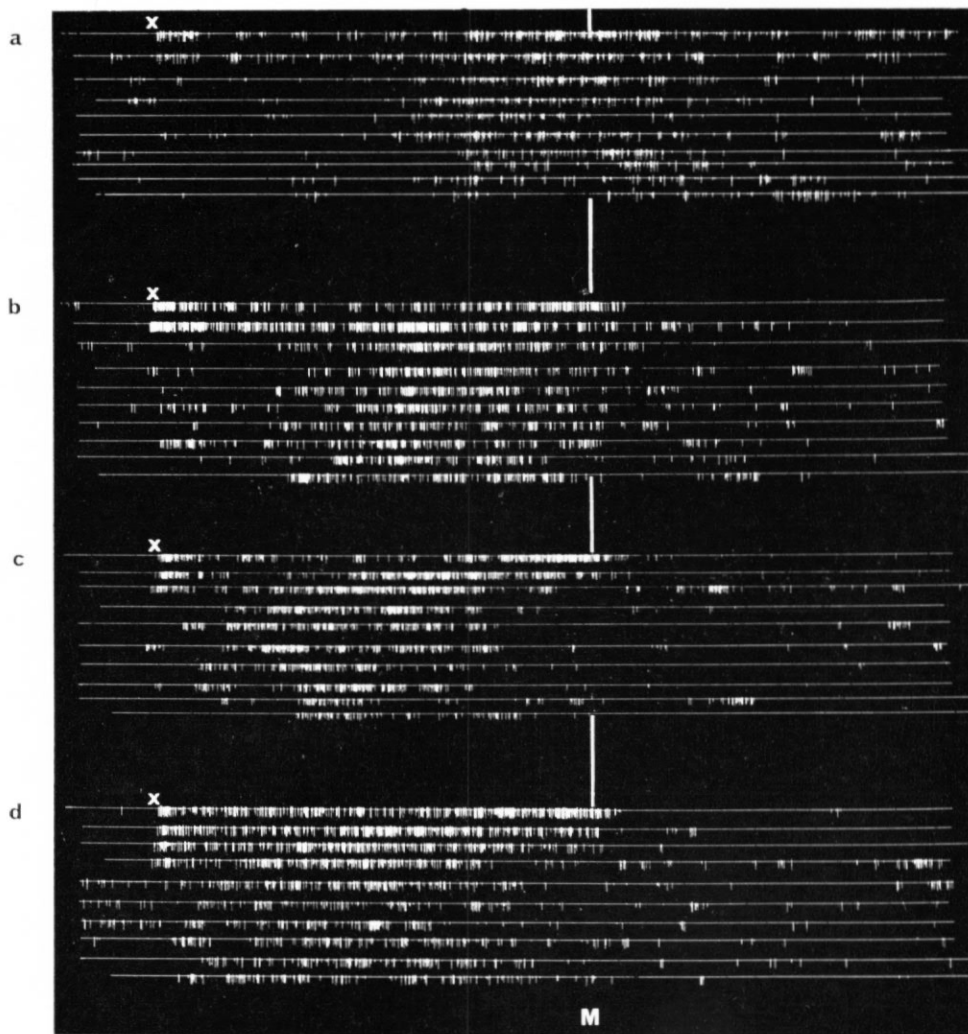


Fig. 7. a, b, c and d: groups of ten *T. kergueleni*, given 1, 2, 3 and 4 artificial cycles respectively of 12 hr. darkness and 12 hr. light, with darkness beginning at 12.00 hr. (X) followed by continuous darkness. Each line represents activity for 1 day. Each group recorded, on smoked paper, for 10 days. M: natural midnight.

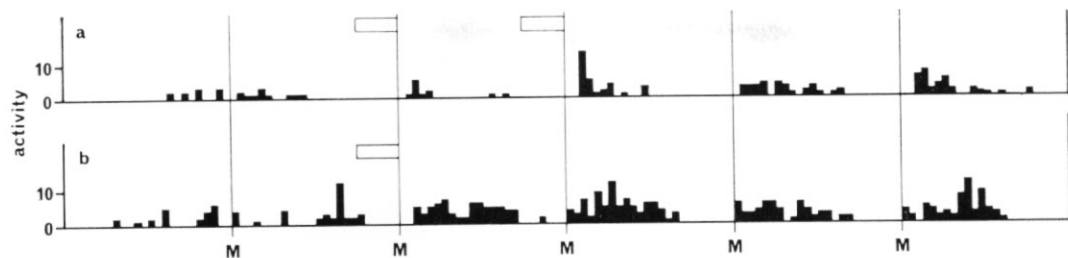


Fig. 8. Two groups (a and b) of 18 *T. kergueleni* in continuous dim red background illumination. Unshaded rectangles: periods of artificial light; M: natural midnight.

Although attempts to re-synchronize apparently arrhythmic *C. femoratus* were unsuccessful, the timing of the activity patterns of rhythmic animals could be re-phased by artificial light/dark cycles, although the rate of re-phasing was not so rapid as that of *T. kergueleni*. Also, after a few days in captivity under light regimes which were different from normal, the swimming activity of *C. femoratus* was usually reduced to a low level. Fig. 6b shows the total hourly activity for ten *C. femoratus* over 24 hr., from records of 3 days' activity in constant dim red light following exposure to light/dark cycles of 12 hr. each for 4 days. The two artificial cycles represented an advance, and delay, in the onset of "night" of 6 hr. from natural conditions.

Effect of different substrates

Swimming activity was recorded at various times in constant dim red background illumination and constant light using tanks with small pebbles, coarse sand, fine sand and no substrate. Circadian rhythms were apparent in both species in all instances, except for *T. kergueleni* when fine sand was used in continuous light, in which case swimming activity was entirely suppressed. Activity was usually at a low level in both species when fine sand was used under constant dim red illumination.

Effect of long and short nights

The effects of the natural long and short nights of different times of the year upon the timing of the spontaneous swimming rhythm of both species under conditions of dim red illumination are given in Fig. 9. The mean hourly activities for different numbers of experiments of 3 or 4 days' duration are averaged over 24 hr. For *T. kergueleni*, the mid-activity points of the 24 hr. periods for autumn (a), winter (b), spring (c) and summer (d) are 00·60, 01·13, 22·68 and 02·37 hr., respectively, and the corresponding times for *C. femoratus* are 00·11, 18·54, 20·63 and 23·77 hr. For *C. femoratus* the timing of the rhythm is such that the mid-point of activity occurs at around midnight when the nights are short and towards the beginning of the night when they are long. In *T. kergueleni* the same pattern is not apparent and mid-activity is recorded around midnight throughout the year. However, this may be due to lack of appropriate data, because when summer and winter activity recordings for 4 days are compared side by side (Fig. 5a and b), the timing of the winter rhythm appears to be generally in advance of that of the summer rhythm.

In both species, total activity is greater, and the peaks of activity more pronounced, during summer than during winter.

Several experiments were carried out between September and March in which animals were subjected to long (18 hr.) and short (6 hr.) artificial nights by maintaining the tanks under continuous light and employing individual covers as appropriate. After from 2 to 9 days of artificial light/dark cycles, swimming activity was recorded under continuous dim red background illumination for 3 or 4 days.

For short "nights", the swimming activity of the animals was always re-timed in both species to coincide with darkness, whether the onset of darkness was advanced by 6 or 12 hr. or delayed by 6 hr. Re-timing of the rhythm was achieved in from 2 to 5 days and was maintained in the constant conditions that followed the artificial cycles.

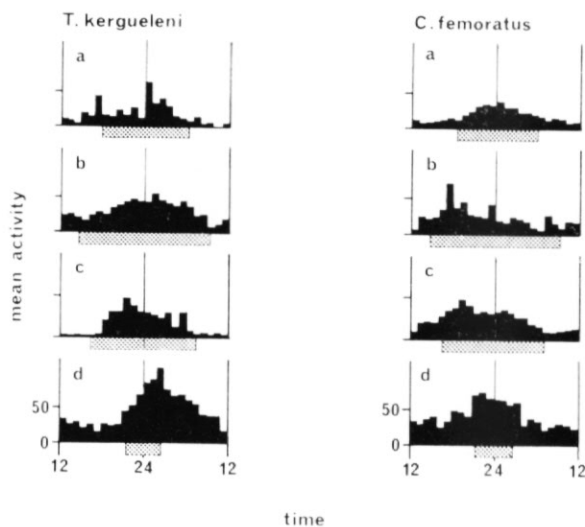


Fig. 9. Mean hourly activity, over 24 hr., for *T. kergueleni* and *C. femoratus* under continuous dim red background illumination for 3 or 4 days, at different times of the year.

Dates, with number of experiments, each with ten animals:

	<i>T. kergueleni</i>	<i>C. femoratus</i>
a: autumn	7 March–12 April (3)	6–21 March (7)
b: winter	19 June–19 July (4)	19–22 June (2)
c: spring	3 August–4 September (2)	13 August–4 September (5)
d: summer	29 December–6 January (4)	29 December–1 January (3)

Shaded area, "expected" darkness.

When long "nights" of 18 hr. were employed, the extent and direction of the re-timing of the activity rhythm varied, but insufficient results were obtained to enable firm generalization to be made. With *C. femoratus*, the peak of activity either advanced slightly towards the beginning of the dark period or else remained around the middle of the dark period. With *T. kergueleni*, the activity peak usually drifted towards the end of the dark period, whereupon its drift was arrested, but on one occasion the activity peak advanced to the beginning of the dark period. Fig. 10 shows the results of experiments carried out during September (Fig. 10a) and March (Fig. 10b), at which times sunrise and sunset occurred at very nearly the same hours (about 06.00 and 18.00 hr.). The onset of experimental darkness was therefore about 3 hr. in advance of sunset and the artificial "midnight" was the same as natural midnight. The relative positions of the activity peaks of the two experiments were maintained in the subsequent 4 days of continuous dim red illumination.

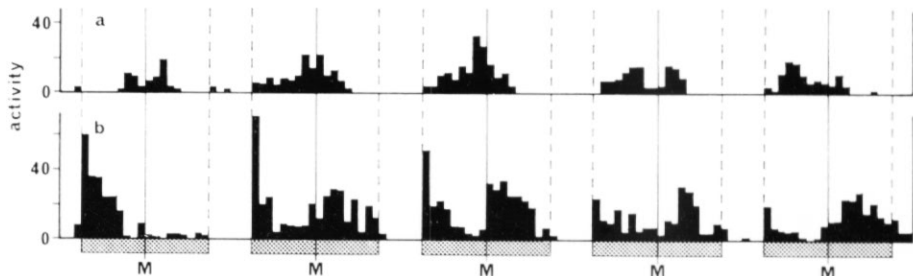


Fig. 10. Activity of two groups of ten *T. kergueleni* under the same artificial cycles of 18 hr. darkness and 6 hr. light during September (a) and March (b). Shaded area, artificial darkness; M: natural midnight.

Fig. 11 shows the activity of three groups of *T. kergueleni* during March in constant dim red illumination following exposure to five 24 hr. cycles, which included 6, 12 and 18 hr. of darkness, respectively. Although onset of darkness occurred at the same time in all cases (15.00 hr.), the response of the different groups clearly varies according to the duration of the darkness.

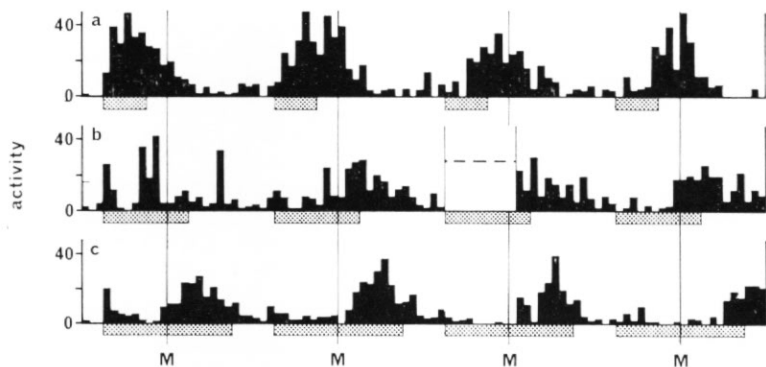


Fig. 11. Activity of three groups of ten *T. kergueleni* under constant dim red illumination, following different artificial light/dark cycles for 5 days, recorded simultaneously. Shaded area, "expected" darkness; M: natural midnight.

Effect of light to dark and dark to light changes

In several experiments using artificial light/dark cycles, it was noted that the immediate responses of the two species to light changes differed from each other, in spite of the fact that both species are dark-active. With *C. femoratus*, a change from dark to light usually caused increased swimming activity, and a change from light to dark usually caused decreased activity. With *T. kergueleni*, opposite effects were observed.

The effect of light/dark changes at different times of the activity cycle, and also with different substrates, was tested by exposing separate groups of both species to cycles of 3 hr. light and 3 hr. dim red illumination. Fig. 12 shows the results of one such experiment in which the artificial cycles lasted for 3 days. Hourly activity values are averaged over a 24 hr. period for each group

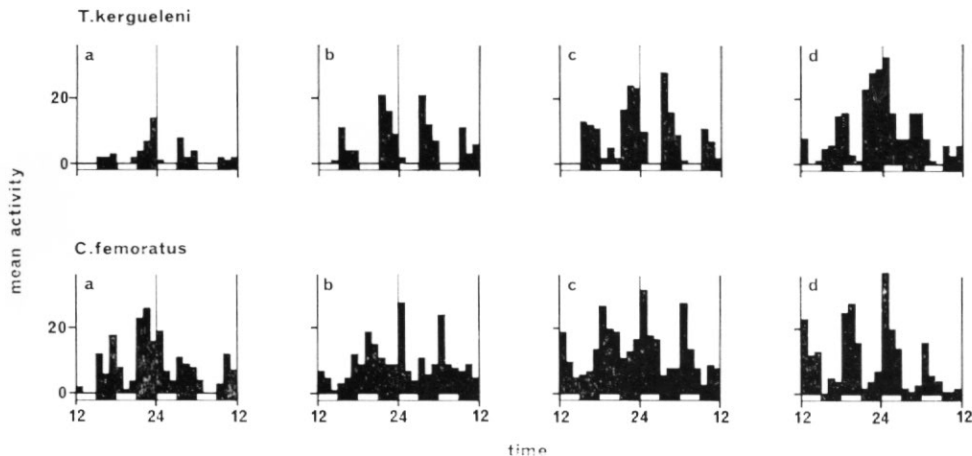


Fig. 12. Mean hourly activity, over 24 hr., for groups of ten *T. kergueleni* and *C. femoratus* under cycles of 3 hr. darkness and 3 hr. light for 3 days, with different substrates.

a: fine sand; b: coarse sand; c: small pebbles; d: no substrate.

Shaded and open areas on abscissae represent times of darkness and light, respectively.

of animals. The substrates provided were fine sand, coarse sand, small pebbles and no substrate (Fig. 12a, b, c and d, respectively).

With *T. kergueleni*, the bulk of swimming activity occurs during the darkness. The change from light to darkness causes an immediate increase in swimming activity in all cases, and the change from darkness to light causes a reduction in swimming activity, except in some cases in the tank with no substrate.

With *C. femoratus*, clear increases in swimming activity at the onset of and during darkness are only apparent in the tank with fine sand. In the tanks with coarse sand, pebbles and no substrate, there are clear increases at the onset of and during light, with a reduction in activity during darkness.

It is possible to account for these differences in response to light changes by considering the substrate preferences of the two species. When *C. femoratus* is disturbed by an increase in light intensity, it seeks shelter, and having a clear preference for finer grades of sand (Bregazzi, 1972b), swimming activity soon ceases in those tanks provided with it. When other substrates or no substrate are available, the shelter-seeking response is extended and appears to be greatest in those tanks having the least favourable substrate. This response ceases only after the onset of darkness.

T. kergueleni possesses a much wider tolerance for different substrates (Bregazzi, 1972b) and consequently, the shelter-seeking response is rapidly satisfied. The only sign of an increase in activity following an increase in light intensity is in the tank with no substrate, but even so, this response is much less than that for *C. femoratus*.

In all tanks, the underlying circadian activity rhythm is apparent, with maximum activity around midnight and minimum around noon. The timing of this rhythm was maintained in all cases in the continuous dim red illumination which followed the 3 hr. +3 hr. light cycles.

Similar experiments were carried out on four other occasions, with similar results.

DISCUSSION

Visual observations, trapping samples and other methods have shown that the swimming activity of *C. femoratus* and *T. kergueleni* occurs during the hours of darkness and not normally during the day-time. Similar patterns also appear to occur with five other species of Antarctic amphipod.

In the cases of *C. femoratus* and *T. kergueleni*, the activity appears to be controlled in part by an internal physiological timing mechanism, because rhythmic patterns of behaviour were recorded in the laboratory under conditions of constant light intensity and temperature.

Although both species lie buried in the bottom sand during the day-time, the presence of food odours at this time will cause them to emerge and swim towards the odour source. Thus, presence of animals in a trap does not necessarily mean that they are normally active at the time that they are taken, and it is necessary to compare numbers taken at different times of the day and night in order to gain an idea of the activity state of the animals.

The reduction in light intensity in the field at sunset undoubtedly is the direct cause of animals emerging from the sand. The increase in numbers trapped during the day-time following a reduction in light intensity by cloud cover suggests that the animals are also sensitive to light/dark changes at times other than sunset. Various laboratory experiments, especially those under cycles of 3 hr. light and 3 hr. dark, show that the reaction to decreased light intensity can occur at all times of the 24 hr. cycle. It seems that this response, as well as the internal "clock", must contribute to the control of the daily activity pattern in the field. The response also probably helps to account for more animals of both species being taken in traps in the first half of the night than in the second half.

The spontaneous activity rhythms recorded in constant conditions in the laboratory show that an increase in light intensity is not necessary to terminate an activity period, but also, laboratory experiments with light and dark cycles indicate that in the field, an increase in light intensity at dawn is likely to be responsible, at least in part, for causing the animals to seek a suitable substrate for shelter.

It appears that the endogenous timing mechanism concerned in the control of swimming activity is itself synchronized by light and dark changes in the field. The laboratory experiments

suggest that the manner in which synchronization is achieved is complex and may vary seasonally. Although the timing of the rhythm of both species probably advances relative to midnight during winter, synchronization is unlikely to occur through a simple response to the light to dark signal alone.

The shelter-seeking activity which occurs at the end of the main period of locomotory activity, does not appear to be under control of a separate timing mechanism. The response to an increase in light intensity is greatest at the time of maximum locomotor activity, not at dawn, and is least at the time of minimum locomotor activity (Fig. 12).

It is not surprising that no tidal component was evident in the locomotor rhythm. In the area of study, the maximum extent of the tides is only about 2 m. and they vary over the lunar cycle, being obviously twice daily at some times and virtually once daily at other times.

In view of the clear responses to changes from light to dark and from dark to light which are able to occur at all times of the 24 hr. cycle, the adaptive significance of the internal physiological clock is best viewed as a measure for conserving energy. It enables the animal to be at full physiological readiness when the cyclically varying environmental conditions become most favourable for activity. Thus the need to maintain metabolism at a high energy level throughout the 24 hr. cycle is avoided. The adaptive significance of a physiological clock in burrowing animals has been further discussed by Bregazzi and Naylor (1972).

Mature females of *C. femoratus* produce a single brood in spring or summer and growth ceases during winter. *T. kergueleni*, however, produces both winter and summer broods, and growth may proceed throughout the year (Bregazzi, 1972a). The seasonal differences in swimming activity in *C. femoratus* discerned by visual observations and by trapping samples likewise point to the reduced general activity of this species during winter. The generally higher numbers of *T. kergueleni* taken in traps in winter, however, are accounted for by the seasonal movement of this species into the sampling area and also indicate the maintenance of a higher level of activity than in *C. femoratus* during winter. Furthermore, the endogenous locomotor rhythm seems to be more persistent in *T. kergueleni* than in *C. femoratus* during winter. Support is thus gained for the assertion that *T. kergueleni* is relatively free from the seasonal environmental fluctuations, whereas *C. femoratus* is very dependent upon them (Bregazzi, 1972a).

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APPENDIX

Rhythmic locomotor activity was displayed under laboratory conditions of constant light intensity and temperature by one other species of amphipod. A few experiments were carried out during the winter of 1968 with specimens of *Orchomene nodimanus* (Walker) obtained from fish-baited traps. Although the extent of swimming activity and the degree of rhythmicity were variable, some good results were obtained of which the following are examples.

Fig. 13 shows the swimming activity of two groups of four non-ovigerous females of *O.*

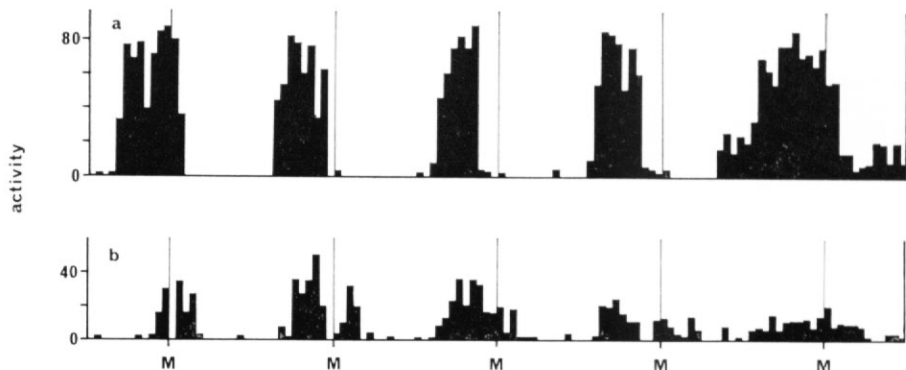


Fig. 13. Spontaneous swimming activity of two groups of four non-ovigerous *Orchomene nodimanus* recorded simultaneously for 5 days from 17 to 24 June 1968.

a: group in continuous darkness; b: group in continuous light (about 100 lux); M: midnight.

nodimanus, recorded in the manner described above, under conditions of continuous darkness (Fig. 13a) and continuous light at about 100 lux (Fig. 13b). This species is not a sand-dweller, and the experimental tanks were provided with about 1 cm. of small pebbles. The animals in darkness show more activity than those in light. In both cases, the bulk of activity is well in advance of midnight. These results are consistent with trapping data described above which indicate that *O. nodimanus* is a night-active species, and also that it possesses a conspicuous internal timing mechanism.