ACTIVITY RHYTHMS IN THE LARVAE OF MYRMELEON OBSCURUS RAMB. (NEUROPTERA, MYRMELEONTIDAE)

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1. INTRODUCTION

There is very little information available on the general biology of myrmeleontid larvae, and nothing is known of their activity rhythms. It is known that most ant-lion larvae construct small conical pits in dry sand, apart from which they show very little activity. They do occasionally crawl around on the surface of the sand but this generally indicates that they are about to moult or pupate. In this investigation the daily (circadian) and lunar activity rhythms of a local ant-lion, Myrmeleon obscurus Ramb., were studied. Pit building was used as the criterion of activity throughout. Because pit building in these animals was the basis of the whole study a thorough investigation into factors affecting pit construction was undertaken before studying any activity rhythms.

Although nothing is known of ant-lion activity rhythms there is a wealth of literature on this subject in other insects. It is well known that most animals restrict their activity to certain limited periods of the day (Enright, 1965), and that these activity periods occur at regular intervals related to the daily 24 hour cycle. These circadian activity rhythms have been extensively studied and there is a number of important reviews on this subject. One of the earliest reviews was that of Welsh (1938), who discussed diurnal changes, not only in the general activity of various animals, vertebrate and invertebrate, but also diurnal fluctuations in light production, eye pigments, and many other processes. This review was followed by that of Bruce and Pittendrigh (1957), which is largely a list of the rhythms recorded in various animal species. It was not until the review by Harker (1958), that some indication was obtained of the physiological processes involved in the control of activity rhythms. There is also a large number of recent reviews, such as those of Cloudsley-Thompson (1961), Harker (1961), Aschoff (1963), Bünning (1964), Aschoff (1965), and Sollberger (1965), which give a very comprehensive coverage of the field of activity rhythms.

In general it is agreed that circadian activity rhythms have a basic periodicity of about 24 hours. This basic or endogenous periodicity becomes apparent when the animal is maintained under constant conditions, and is known as the free-running period of the rhythm. The free-running rhythms seldom have a period of exactly 24 hours and under natural conditions, the rhythm is synchronized to precisely 24 hours by external environmental factors, such as light or temperature changes. Light is generally the most important synchronizing factor, and reversal of the light-dark cycle will produce a *reversed activity rhythm. With this information as a background the circadian and lunar activity rhythms of ant-lions were investigated.*

2. MATERIAL AND METHODS

All the ant-lion larvae used in this investigation were collected in the Grahamstown district. Large numbers of larvae of various sizes were collected, and from this population random samples were withdrawn for use in the experiments. The larvae were extremely difficult to identify to species, and where possible, all the larvae used in the experiments were reared to the adult stage to facilitate identification. About 95% of the ant-lions used were identified as Myrmeleon obscurus Ramb. by Tjeder (1966). The remaining 5% included Myrmeleon medialis Banks, and a few species which remained unidentified. This small percentage of alien larvae did not appear to affect the results obtained. The population was thus regarded as being uniform, and no allowance was made for the occasional alien larvae.

In the laboratory, the animals were kept in plastic dishes with a square top (sides 10.5 cm.) tapering to a base with sides of 8.5 cm. The depth of the dishes was 8 cm. These containers were filled with sand from the natural habitat which had been sifted to remove debris. All the ant-lions were fed on custodian ants, Plagiolepis custodiens Santschi, and under these conditions successfully completed their development.

A major problem encountered in this study was to find a suitable way of measuring the daily pit building activity of the *ant-lions. For obvious reasons, conventional mechanical actographs were unsuitable for such small sand dwelling insects. Attention was thus turned to recent developments in electronic transducers in an attempt to find a circuit which could be adapted for the study of ant-lion activity. A number of electronic transducers have been developed by various workers in an effort to find a sufficiently stable, sensitive instrument for recording the activity of very small insects. Powell, Esch and Craig (1966), for example, recorded the activity of mosquitoes using a series of parallel wires connected to the terminals of a battery, such that the circuit was completed when the animal walked on the wires. A circuit of this design was obviously not suitable for an ant-lion moving in a dish of sand. More sensitive electronic transducers with a wider application have been devised using changes in capacitance to detect insect movements. Instruments operating on this principle have been used by Lofqvist and Stenram (1965), to record the activity of various insects, while Schechter, Dutky and Sullivan (1963), have recorded the activity of small insects by detecting frequency changes in capacitance, with an FM detector. Both these electronic actographs, however, are expensive, tend to be unstable, and are not very sensitive. Based on the ideas of Schechter, Dutky, and Sullivan (1963), a circuit was designed for recording ant-lion activity (Grobbelaar, Morrison, Baart and Moran, 1967). This recorder was extremely versatile and was used not only with ant-lions, but with minor modifications* *was also used to monitor the activity of hamsters, aquatic insects, and very small insects such as Drosophila and thrips. The apparatus was extremely sensitive, relatively inexpensive, with negligible drift, and used batteries as a power source.*

2.1 Circuit Description (Fig. 1)

The instrument comprised a sensor in which the insect was confined, an oscillator, a frequency detector, amplifier and recorder. The basic principle was that the oscillator frequency was changed by a variation in the capacitance between two electrodes when the insect moved in the sensor chamber. This variation in frequency was converted into a voltage variation which was amplified and used to drive a 1 ma D.C. recorder. A small change in the impedance between point A and earth changed the frequency of the oscillator (TR 1). This impedance was that of the series resonant circuit consisting of the coil L 1, the isolating capacitor C 3 and the capacitance between the sensor electrodes, one of which was connected to the metal chassis (earth). The variation in impedance was produced by a change in the capacitance of the sensor caused by movements of the insect. The coil L 1 was tuned so that the resonant frequency of the series circuit was close to the oscillator frequency. In this way a large frequency variation was obtained for a small capacitance variation. The employment of a resonant circuit, instead of a capacitor alone, gave an increase in sensitivity of about five times.

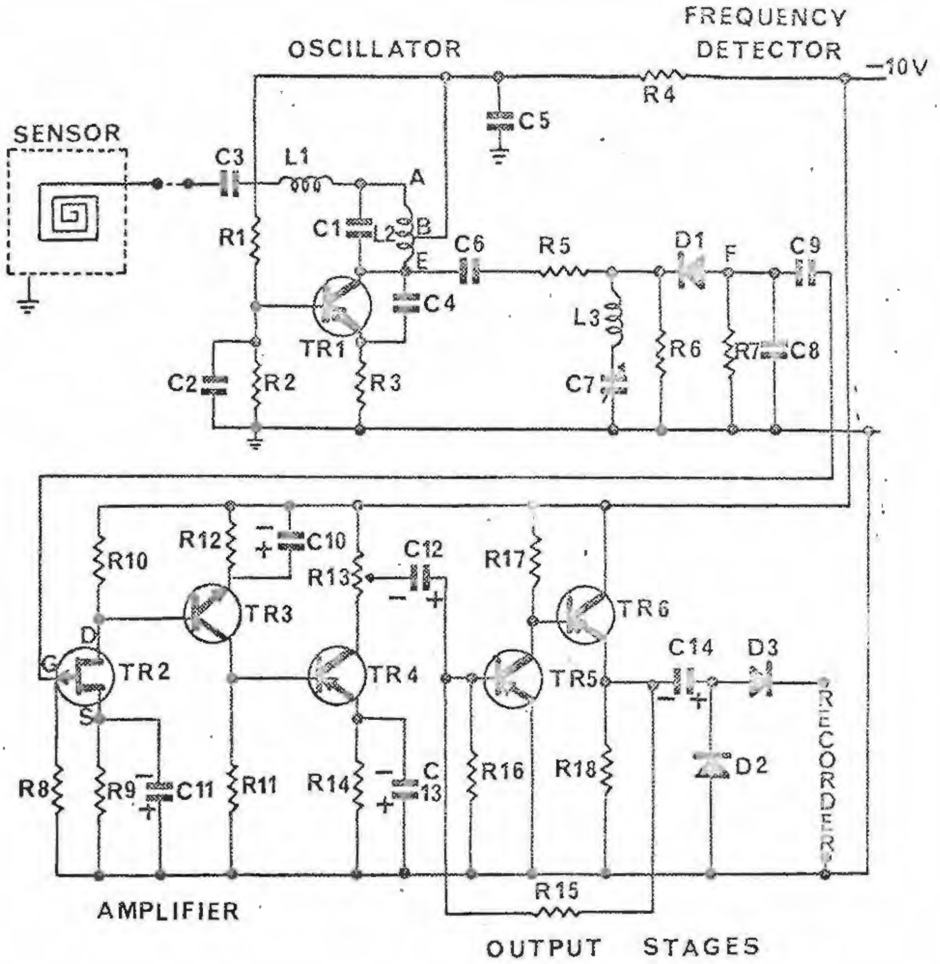


Fig. 1. Circuit diagram for capacitance sensitive insect activity recorder. For explanation and list of components see text.

The frequency detector operated as follows. The output of the oscillator from point E was fed to a further resonant circuit (L 3, C 7), the impedance of which varied sharply with the change in frequency. The resistance R 5 and this impedance formed a potential divider. The change in frequency was thus converted to a change in voltage amplitude across the resonant circuit. This voltage was detected by the diode D 1 using a time constant of 25 milliseconds and the output was taken through a capacitor (C 9) to the gate G of a field-effect-transistor (F.E.T.) which was the first stage of the amplifier. The amplifier consisted of a low noise input stage (TR 2) followed by two complementary stages (TR 3 and TR 4). (With small alterations in the biasing of the transistor a low noise transistor could be used in place of the F.E.T.). Although these three stages were directly coupled, no serious drift was encountered since each individual stage was well stabilized.

The output stage was an emitter follower (TR 6) which was driven by a common emitter (TR 5) the input amplitude to which was controlled by a potentiometer (R 13). This determined the size of the recorder deflection. The recorder was fed with unidirectional signals obtained by rectifying the output of TR 6 using the diodes D2 and D3. The rectifier was fed through the capacitor C 14 so that drift in the amplifier had no effect on the recorder.

The circuit was shielded by means of a metal cover, and the leads to the battery and recorder were screened to prevent oscillation. A single accumulator was used as the power source with the current drain being 10 milliamps. If dry batteries are used it would be advisable to use a separate battery for the output stages. This eliminates the risk of oscillation when the batteries begin to run down. A well regulated D.C. supply, powered by the mains, can also be used.

2.11 Components

(i) Capacitors: C 1 - 10 pf, C 2 - 0.1 mfd, C 3 - 250 pf, C 4 - 20 pf, C 5 - 0.1 mfd, C 6 - 56 pf, C 7 - 0 to 10 pf trimmer, C 8 - 0.25 mfd, C 9 - 3 mfd 50 V paper, C 10 - 500 mfd used for low leakage, C 13 - 500 mfd 30 V, C 14 - 1000 mfd 30 V.

(ii) Resistors: R 1 - 3.9 KΩ, R 2 - 1.8 KΩ, R 3 - 1.5 KΩ, R 4 - 1 KΩ, R 5 - 27 KΩ, R 6 - 150 KΩ R 7 - 100 KΩ, R 8 - 470 KΩ, R 9 - 6.8 KΩ, R 10 - 10 KΩ, R 11 - 5.6 KΩ, R 12 - 3.9 KΩ, R 13 - 5 KΩ potentiometer, R 14 - 4.7 KΩ, R 15 - 100 KΩ, R 16 - 22 KΩ R 17 - 2.2 KΩ, R 18 - 1 KΩ.

(iii) Diodes: D 1, D 2, and D 3 - all 0A85.

(iv) Transistors: TR 1 - 0C44, TR 2 - 2N3820, TR 3 - 0C140, TR 4 - 0C44, TR 5 - 0C71, TR 6 - 0C71.

(v) Coils: L 1 - 24 turns of 24 gauge enamel-covered wire on a $\frac{1}{2}$ inch former. A piece of ferrite three inches long cut from the antenna of a portable radio receiver was used as the core inside the former. L 2 - 55 turns of 36 gauge enamel-covered wire on a $\frac{1}{2}$ inch former tapped 15 turns from one end, the 15 turns being between B and E. A powdered iron core fitting the standard $\frac{1}{2}$ inch former was used for tuning. L 3 - 31 turns of 24 gauge enamel-covered wire on a $\frac{1}{2}$ inch former. A short piece of ferrite rod as was used for L 1, was inserted for tuning.

2.12 Sensors

To record the activity of insects, using this circuit, their movements first had to be converted into variations of the capacitance between two electrodes. These variations were then converted by the frequency detector into voltage variations and it was therefore important that a large percentage variation of capacitance was obtained for any insect movements in the sensor. A pair of parallel plates, as has been used by other workers, will not give a large variation in capacitance and for maximum sensitivity the electric field between the two electrodes must be made as non-uniform as possible. The sensor used to record the activity of ant-lions consisted of a flat wire spiral as shown in Fig. 1. This was connected to the sensor terminal of the circuit. The dish containing the ant-lion larva was placed between this sensor and the chassis. A fairly large container could thus be used in which the animal could easily build its pits. In these experiments a 60 cc. non-conducting plastic container was used.

2.13 Tuning procedure

The oscillator frequency was adjusted to approximately 4.5 Mc/s by moving the core in the coil L 2. This frequency can be checked with a radio receiver if no other equipment is available. The sensor was connected to the circuit and tuned for resonance by adjusting the inductance of coil L 1. To do this the probe of a sensitive oscilloscope was placed close to the sensor, but not in contact with it, and the ferrite core of coil L 1 was moved in and out until the maximum signal was obtained on the oscilloscope. The coil L 1 was then slightly detuned so that the signal detected by the oscilloscope was about seven-eighths of the maximum signal. The resonant circuit containing the coil L 3 was then tuned to a frequency differing slightly from the oscillator frequency. This was done by connecting a D.C. millivoltmeter across the detector output, between earth and point F (Fig. 1). The trimmer capacitor C 7 was then tuned to the oscillator frequency which was indicated by a minimum reading on the millivoltmeter. The trimmer was then fractionally detuned so that the millivoltmeter reading increased slightly. The position of the trimmer giving maximum sensitivity for the circuit was determined by trial and error.

3. FACTORS AFFECTING PIT CONSTRUCTION

A number of different factors were found to affect the size of the pits constructed by myrmeleontid larvae. These factors were studied before the investigation into activity rhythms was undertaken. This was especially relevant in monitoring lunar rhythms as pit volume was the only criterion used, and the conditions under which the larvae built pits might also be expected to affect the daily activity rhythm.

Factors affecting pit construction include:

(i) the size of the ant-lion larvae
(ii) crowding of the larvae
(iii) the amount of food obtained
(iv) sand grain size
(v) temperature
(vi) disturbance of the pits.

Experiments were performed to determine the effect of each of these factors on pit construction.

3.1 THE SIZE OF THE ANT-LION LARVAE

One of the most obvious factors which would be expected to affect pit construction, especially the size of the pits, is animal size. It would be expected that the larger ant-lions would construct bigger pits. The size of the sand pits constructed by individual larvae, however, varied considerably from day to day and large animals often constructed very small pits before moulting or pupating.

The pits of 50 myrmeleontid larvae were measured in the field. The animals were then sifted out of the sand and their head-widths measured. The width of the head capsule was used as an indication of animal size. Body length was not used, since the bodies of these larvae are very extensible, and the measurement obtained would have depended on how much the animals were stretched during measurement.

Pit volume and animal size have been correlated in Fig. 2. This figure shows that there was some correlation between animal size and pit size, although this correlation was not as close as might be expected. This was due to the fact that a few very large animals built very small pits. When Spearman's Rank correlation test was applied to these data a correlation coefficient of 0.71 was obtained. This indicated that there was a fairly high degree of correlation between animal size and pit size. This is, in fact, the result one would expect, and bears out the results of Haub (1942). Working on <u>Myrmeleon</u> <u>immaculatus</u> De Geer, Haub found that the size of the pit varied directly with the size of the larva. Haub, however, used body weight as his criterion of size rather than head width, and his results might have been affected by the amount of food obtained by the larva before weighing.

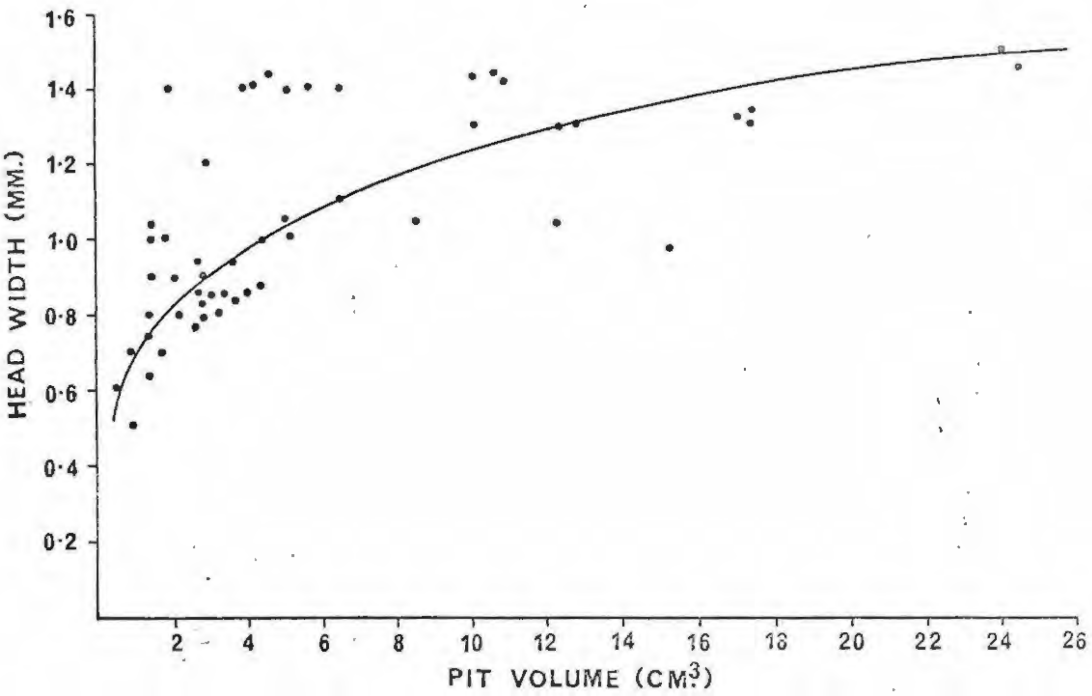


Fig. 2. *The correlation between animal size and pit size in a group of 50* *__Myrmeleon__* *__obscurus__* *larvae. Animal size is expressed as width of the head capsule.*

The size of the larvae thus had a marked effect on pit construction, and to eliminate the effects of animal size in the experiments on activity rhythms, random samples were used. These random samples were withdrawn from a large population in which the head widths ranged from 0.5 mm. to 1.4 mm.

3.2 CROWDING OF THE LARVAE

The size of the pits constructed by Myrmeleon obscurus larvae was found to depend, not only on the size of each individual, but also on the number of other ant-lions in the immediate vicinity. The more crowded the animals were the smaller, and closer together, their pits tended to become, until a point was reached where competition between the larvae resulted in mortality.

The effects of crowding were clearly demonstrated in two different experiments. In the first experiment, groups of 10 Myrmeleon obscurus larvae were placed in the following areas of sand: 2,576 cm.^{2}, 1,288 cm.^{2}, 644 cm.^{2}, 322 cm.^{2}, and 161 cm.^{2} (400, 200, 100, 50, 25 square inches). Each area was half the preceding one. In the second experiment, varying numbers of animals were placed in each of six identical containers. The groups consisted of 5, 10, 20, 30, 40 and 50 ant-lions respectively, in an area of about 858 cm.^{2} (132.7 square inches). In the first experiment the number of animals *remained the same while the area available was decreased, while in the second experiment the area remained constant while the number of animals increased. The pits constructed by the* _Myrmeleon obscurus_ *larvae in each group were measured daily and the mean pit volume calculated. The average distance between adjacent pits was also determined for each group. These distances were measured from the edges of the pits concerned, and not from the centres. Both experiments were continued for a period of nine weeks. Comparison of the two experiments was based on the area of sand available per individual ant-lion.*

The mean pit volume for each group is shown in Fig. 3, and from this figure it can be seen that when the ant-lions were crowded they constructed smaller pits. It can be seen that the animals in experiment two consistently built smaller pits than those in experiment one. This difference was due to differences in temperature at the time of the experiments. The first experiment was conducted during January when the temperature of the laboratory was about 18° C, while the second experiment was conducted in August when the temperature was only about 16° C. The differences between the two experiments therefore had nothing to do with crowding. The important point is that both sets show that the mean pit volume decreased with increased crowding.

With increased crowding one would also expect the pits to be closer together. This is in fact the case as shown

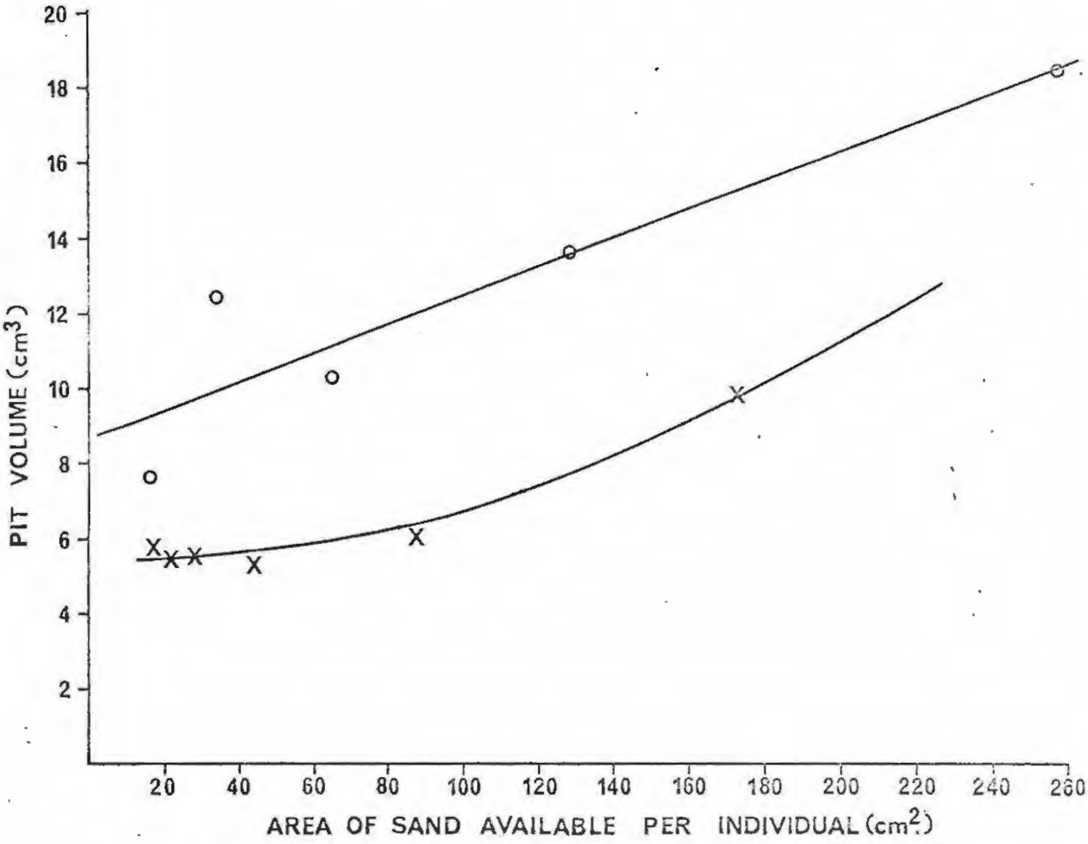


Fig. 3. *The mean pit volume over a period of nine weeks for various groups of* *Myrmeleon obscurus* *larvae under crowded conditions. The degree of crowding is expressed as the area of sand available per individual. The results of two different experiments are shown.*

o———o experiment 1 in which groups of 10 ant-lions were placed in decreasing areas of sand.

x———x experiment 2 in which groups containing different numbers of ant-lions were placed in the same area of sand.

For further details see text.

Fig. 4. It is, however, interesting to note that the number of deaths in the population also increased with increasing density. This is illustrated in Fig. 5, which shows the percentage mortality after nine weeks in the various experimental groups. From this figure it can be seen that the percentage mortality increased very rapidly as the area of sand available per individual was decreased. Calculation of the percentage mortality in these experiments was not affected by pupation, since animals which pupated were counted as being alive at the end of the experimental period. The high death rate in the crowded groups was due to competition between the larvae. It was observed that when a large number of larvae were crowded into one dish, and their pits were close together, they often fell into each others pits. When this occurred the larvae attacked and killed each other.

The above experiments show that crowding the larvae of Myrmeleon obscurus had a marked effect on the size of the pits constructed by these larvae. In all the experiments on activity rhythms, therefore, each larva was placed separately in a dish and thus the effect of crowding on pit size could be disregarded. Maintaining the larvae in separate dishes also eliminated competition. The dishes used for most of the activity experiments had a surface area of 1,102.5 $cm.^2$, and did not restrict the size of the pits constructed. Smaller dishes with an area of about 19.6 $cm.^2$ were

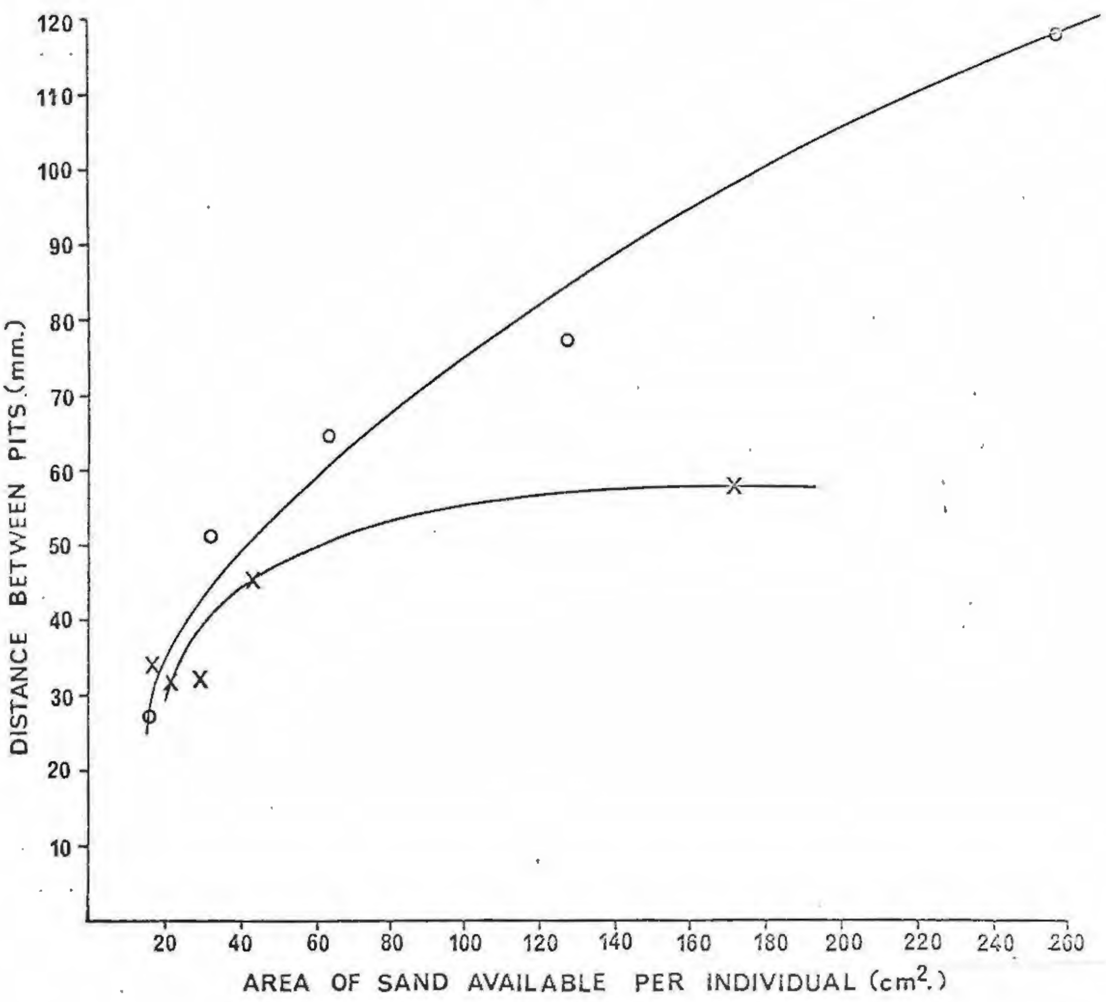


Fig. 4. *The average distance between adjacent pits in groups of* *__Myrmeleon obscurus__* *larvae under crowded conditions over a nine week period. The degree of crowding is expressed as the area of sand available per individual. The results of two different experiments are shown.*

o———o *experiment 1 in which groups of 10 ant-lions were placed in decreasing areas of sand.*

x———x *experiment 2 in which groups containing different numbers of ant-lions were placed in the same area of sand.*

For further details see text.

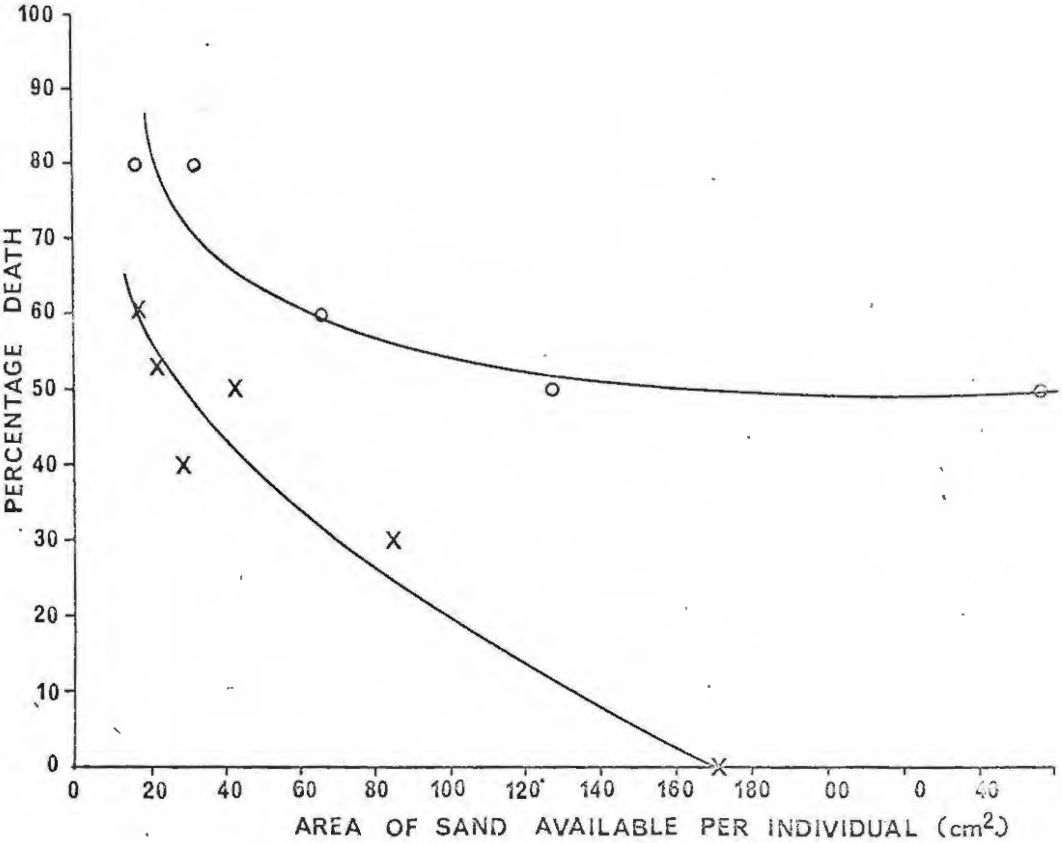


Fig. 5. The percentage mortality after nine weeks in groups of <u>Myrmeleon</u> <u>obscurus</u> larvae under crowded conditions. The degree of crowding is expressed as the area of sand available per individual.

The results of two different experiments are shown.

o——o *experiment 1 in which groups of 10 ant-lions were placed in decreasing areas of sand.*

X——X *experiment 2 in which groups containing a different number of ant-lions were placed in the same area of sand.*

For further details see text.

used in the electronic activity recorder. These dishes may have restricted the size of the pits constructed but, in these particular experiments, the time at which the activity took place was important and not the size of the pits.

3.3 THE AMOUNT OF FOOD OBTAINED

It has been shown that the size of the pits constructed by Myrmeleon obscurus larvae was dependent on the size of the larvae. An increased food supply would increase the rate of development of the larvae and hence the amount of food obtained might also be expected to affect the size of the pits.

In the experiments to investigate the effect of feeding frequency on pit size, six groups of 12 ant-lions were maintained at a constant temperature of 25.5 ± 1°C. They were allowed seven days to acclimate to laboratory conditions, during which time each individual was fed one ant a day. Thereafter the feeding frequency for each group was altered as follows: group A, 2 ants a day; group B, 1 ant a day; group C, 1 ant every 3 days; group D, 1 ant every 7 days; group E, 1 ant every 14 days; group F, continued starvation. Daily readings of the pit size were taken for 50 days and the mean pit volume calculated for each group. These results are shown in Fig. 6.

From Fig. 6 it is clear that in groups A, B and C, where the interval between feeds was three days or less,

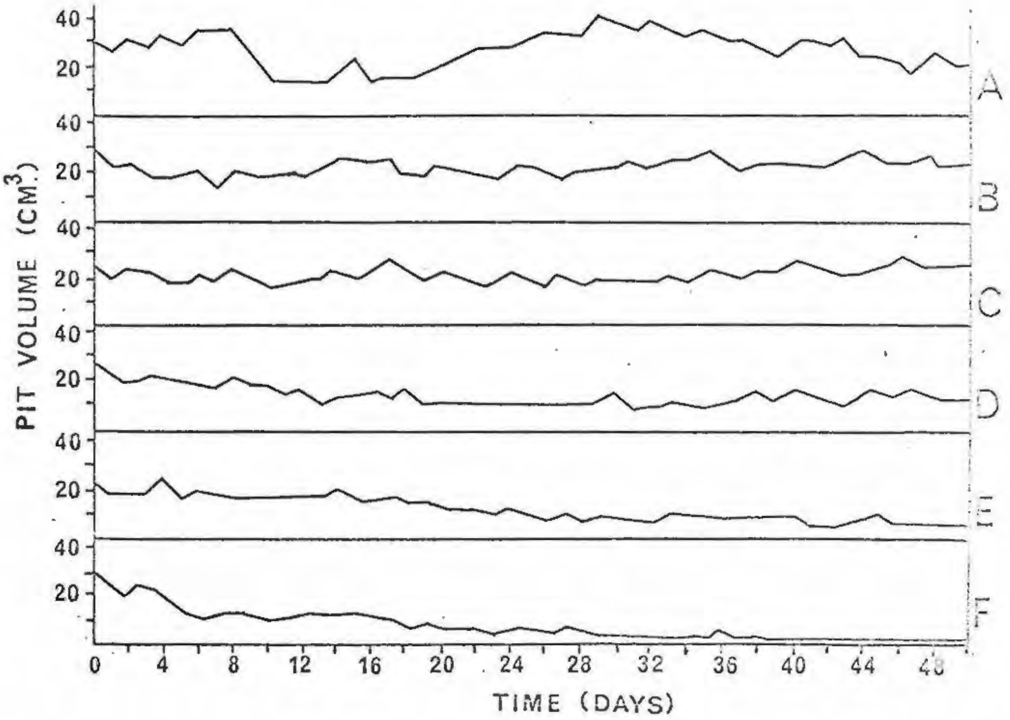


Fig. 6. The effect of feeding frequency on the volume of the pits constructed by different groups of <u>Myrmeleon obscurus</u> larvae at a constant temperature of 25.5 ± 1°C. Group A, 2 ants a day; group B, 1 ant a day; group C, 1 ant every 3 days; group D, 1 ant every 7 days; group E, 1 ant every 14 days, group F, continued starvation.

there was a slight overall increase in the pit volume with the passage of time. In groups D, E, and F, on the other hand, where the feeding interval was more than three days, there was a general decrease in pit volume.

There were three possible sources of error in these experiments, pupation, mortality, and the increasing size of the larvae. In group A, for example, seven of the 12 animals pupated. Pupation results in the removal of the largest larvae from the population. It has already been shown (section 3.1) that the larger the larvae the larger the pits, therefore pupation could cause a drop in the mean pit volume. In group A, however, the mean pit volume showed a slight increase during the experimental period and this seemed to indicate that pupation was a negligible source of error in this group. In the remaining groups, pupation showed a progressive decrease as the feeding frequency was reduced. Finally in group F only one animal pupated.

The second possible source of error was mortality. In group F, which was starved completely, there was 50% mortality within 50 days. If starvation killed mainly the larger larvae a drop in the mean pit volume would result. The animals which died, however, were of various sizes and it appeared that no particular size group was any more or less resistant to starvation than the others. Since death appeared to be independent of size, this factor would not affect the general shape of the graph. In the remaining groups no mortality was recorded.

Finally, another complicating factor was the increase in the size of the ant-lions during the experimental period. It was noted that in the groups which were fed frequently many animals pupated, while in the groups which were starved few animals pupated. This indicated, as could be expected, that a high feeding frequency increased the rate of development of these animals. Since it has been shown that the pit size is dependent on the size of the ant-lions, the increase in pit size at high feeding frequencies could be due to the general increase in animal size. At low feeding frequencies, however, the pit volume decreased, although the ant-lions did not shrink in size.

In general these experiments showed that there was an increase in pit volume when the feeding frequency was high, and a decrease in pit volume when the feeding frequency was low. This relationship is shown in Fig. 7 where the mean pit volume for each group is plotted against the feeding interval. (Fig. 7 is based on the same data as Fig. 6). Fig. 7 shows that, as the interval between successive feeds was increased from 0.5 to 7 days, the pit volume dropped rapidly. At feeding intervals of more than 7 days the decrease was far less rapid, and the pits appeared to be reaching a minimum volume below which they were not reduced.

Taking this into account, in all further experiments on activity rhythms the ant-lions were fed one ant a day. This

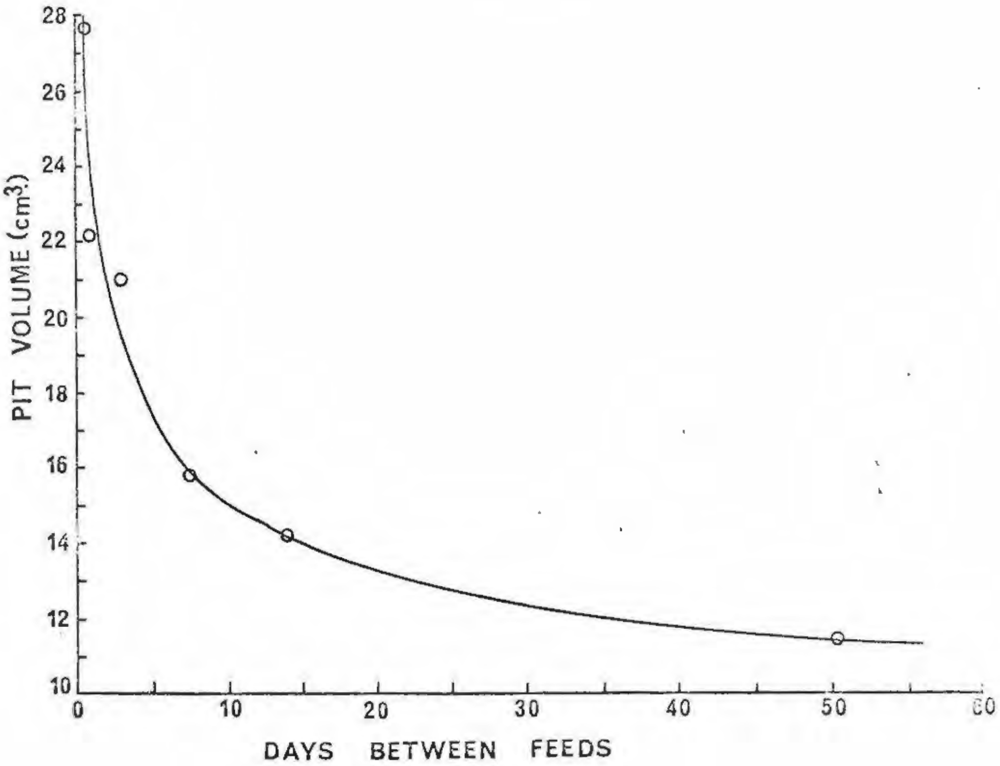


Fig. 7. *The mean pit volume, over a period of 50 days, for groups of* <u>*Myrmeleon obscurus*</u> *larvae at various feeding frequencies. The larvae were kept at a constant temperature of 25.5 ± 1°C. For details of feeding frequencies see text.*

value was chosen since it allowed growth and development of the larvae without a marked increase in the rate of pupation. Animals which were fed more than one ant a day pupated very rapidly and could therefore not be used in long term experiments. If the animals were fed less than one ant a day the size of the pits was reduced.

3.4 SAND GRAIN SIZE

In these experiments sand collected from the animals natural habitat was sifted through a mechanical sifter to obtain the following grain sizes: greater than 599 μ, 500 μ, 353 μ, 295 μ, 211 μ, 152 μ, and less than 152 μ. 12 ant-lion larvae were placed in each grade of sand, and the number and size of the pits constructed by each group was recorded over a period of six days.

In order to determine the percentage composition of sand from the natural habitat a known weight of sand was sifted, the resulting fractions weighed, and expressed as a percentage of the total weight. The results of this experiment are expressed in Table I.

Table I

Sand grain size in µ .	Composition of sand (% of total weight).	% of animals building pits in each grain size.	Mean pit Volume (cm^3.)
> 599	5.63	62.33	11.2
500	3.57	18.16	14.5
353	19.78	88.41	29.6
295	14.14	88.41	38.8
211	26.60	100.00	35.0
152	10.16	72.09	14.0
< 152	20.12	72.09	23.7

From this table it can be seen that in sand grain sizes of more than 599µ, and 500µ, few pits were constructed by the ant-lion larvae, and the pits were of a small size (11.2 cm^3, 14.5 cm^3). It would seem that such coarse sand is not suitable as a habitat and ant-lions are certainly never found in sands as coarse as this under natural conditions. Firstly, it was noted that larvae below 8 mm. length made no pits at all in this coarse sand, probably because of some physical limitation imposed by the large size of the sand grains. Secondly, ants also escaped far more readily from pits constructed in this coarse sand than they did from pits constructed in normal sand. It took P. custodiens ants 21-29 secs.

(mean 25 secs.) to escape from a pit with a diameter of 17 mm. and a depth of 45 mm. constructed in sand with a grain size of greater than 599 μ, while it took them 53-64 secs. (mean 60 secs.) to escape from a pit of the same size constructed in sand with a grain size of 211 μ. In each case the times quoted were based on ten trials.

In the experiments on activity rhythms sand from the natural habitat was used. It is significant that 211 μ was the most abundant grain size in sand from the natural habitat and that 100% of the animals constructed large pits in this sand (Table I). A quantity of sand was collected from the habitat at the start of this investigation and the same sand was used throughout. This ensured that the composition remained constant and did not affect the circadian or lunar rhythms in these insects.

3.5 TEMPERATURE

Temperature also had a very marked effect on the size of the pits constructed by Myrmeleon obscurus larvae. Two groups of 12 animals were kept at 25.5 ± 1°C for seven days, and thereafter the temperature was raised or lowered by 1-2°C/day until pit construction ceased. Fig. 8 shows that pit construction ceased below 11°C and above 42°C. This is a considerably greater temperature range than that found by Haub (1942) for Myrmeleon immaculatus. Haub reported that the larvae of this species only constructed pits between

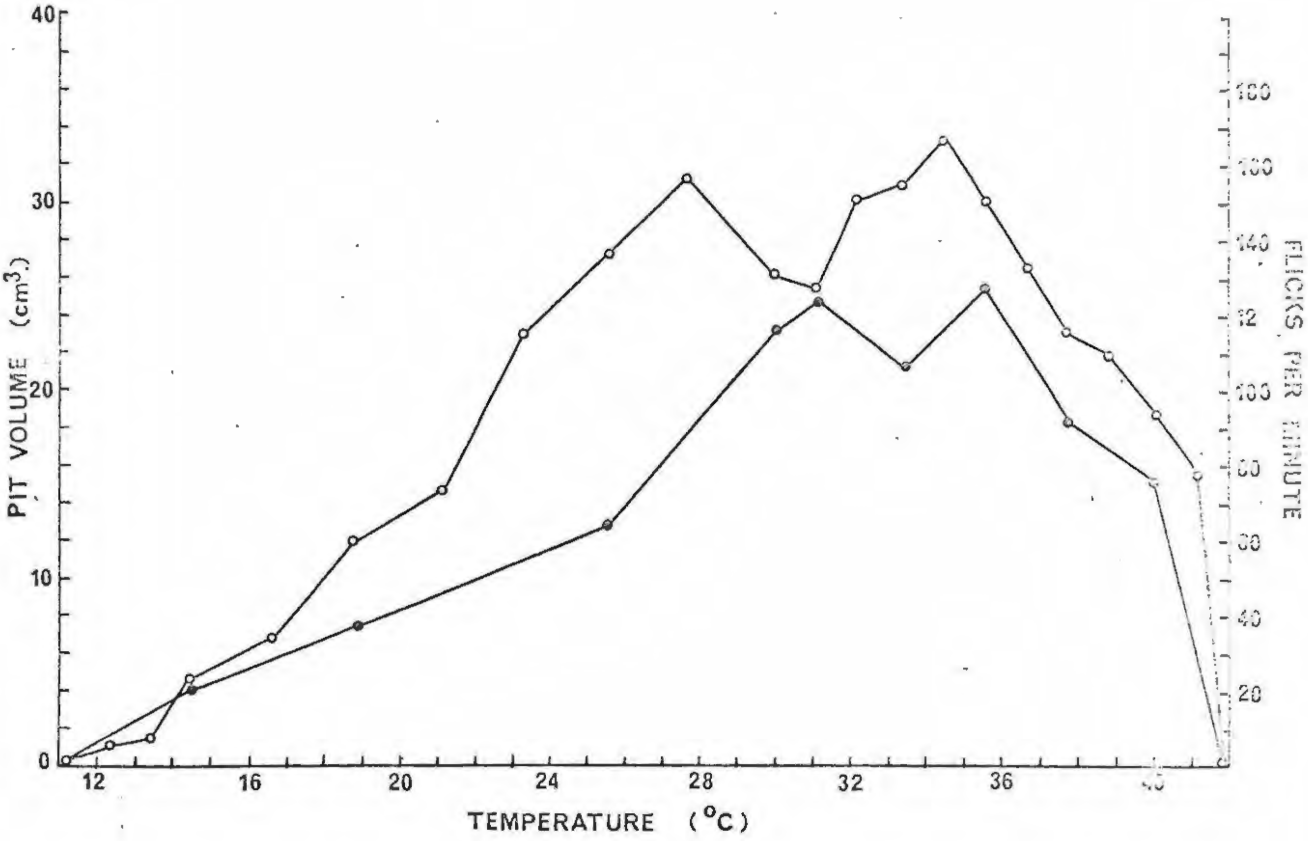


Fig. 8. The mean pit volume of groups of 12 Myrmeleon obscurus larvae at various temperatures (open circles). Number of flicks per minute observed during pit construction (closed circles) at various temperatures. For further details see text.

20°C and 28°C. Below this temperature range he found that the pits were small or nonexistent, while above this temperature migration or death occurred.

The temperature relations of Myrmeleon immaculatus have also been studied by Green (1955). He observed the activity of the ant-lions in the field at various temperatures, which were measured 1 mm. below the base of the pit. Green states that the ant-lions became active at about 2°C, and their optimal range was between 16°C and 34°C. Activity finally ceased at about 48°C. The results obtained by Green thus differ considerably from those obtained by Haub (1942). This difference is due to the different criteria used by the two workers to assess the activity of the ant-lions. Haub studied pit building, while Green considered the ant-lion to be active if "its mandibles were exposed at the base of the pit," or if it reacted to sand grains and prey falling into the pit.

In the experiments on Myrmeleon obscurus pit building was the criterion used to study activity rhythms and therefore only the critical temperatures for pit construction were determined. It was, however, noted that the upper and lower critical temperatures for pit construction did not cause the death of any animals even after two days exposure. When the temperature was returned to 25.5 ± 1°C, after exposure to these temperature extremes, all the animals constructed normal pits.

Fig. 8 also shows that the optimal temperature for pit construction was in the region of 35°C. The exact value is uncertain due to the dip in the graph between 25°C and 35°C. This dip in the graph is thought to be related to the lunar cycle which will be dealt with in a later section of this work.

Ant-lion larvae construct their pits by flicking away the sand with their heads and, as might be expected, temperature also had an effect on the rate at which sand was flicked out of the pits during construction. The number of flicks per minute was recorded for five animals in each group at the various temperatures and the mean rate of flicking was closely correlated with pit size, as shown in Fig. 8.

In the experiments shown in Fig. 8 the temperature for each particular day was plotted, but it could also be expected that the previous thermal history would be important.

The pits of a field population of ant-lions, consisting of about 50 animals, were measured on the day of every first and last lunar quarter over a period of a year. Only pit measurements on the first and last lunar quarter were used since, as will be shown later, the moon is important in determining the size of the pits constructed by Myrmeleon obscurus larvae and at first and last lunar quarters the pits are intermediate in volume. By using only these results the influence of the moon could be discounted in this experiment. Temperature summations for each of the 1 to 14 days preceding the first and last quarters were then calculated, the temperature being expressed as hour degrees centigrade above 10°C. (The base line of 10°C was chosen since below this temperature no pits were constructed).

The pit volume for each first and last lunar quarter was plotted against the temperature summation for the preceding day and the results are shown in Fig. 9. From this figure it can be seen that there was no correlation between the pit volume and the temperature summation. A similar lack of correlation was found when the temperature summations for the preceding two days were used. Thereafter the correlation gradually increased until, with temperature summations for seven days before first and last quarters, a very close correlation with pit volume was achieved, as shown in Fig. 10. The correlation showed a progressive decrease with temperature summations for longer periods of time.

Fig. 10 shows that, in a field population of ant-lions, the pit volume increased as the temperature summation for the preceding seven days increased. This increase in volume tended to tail off at very high, and very low, temperatures.

These field results have been confirmed in the laboratory. A group of 24 ant-lions was maintained at a constant temperature of 14 ± 1°C for seven days. At the end of this time the mean pit volume for the group was recorded and the temperature summation (hour degrees above 10°C) for the

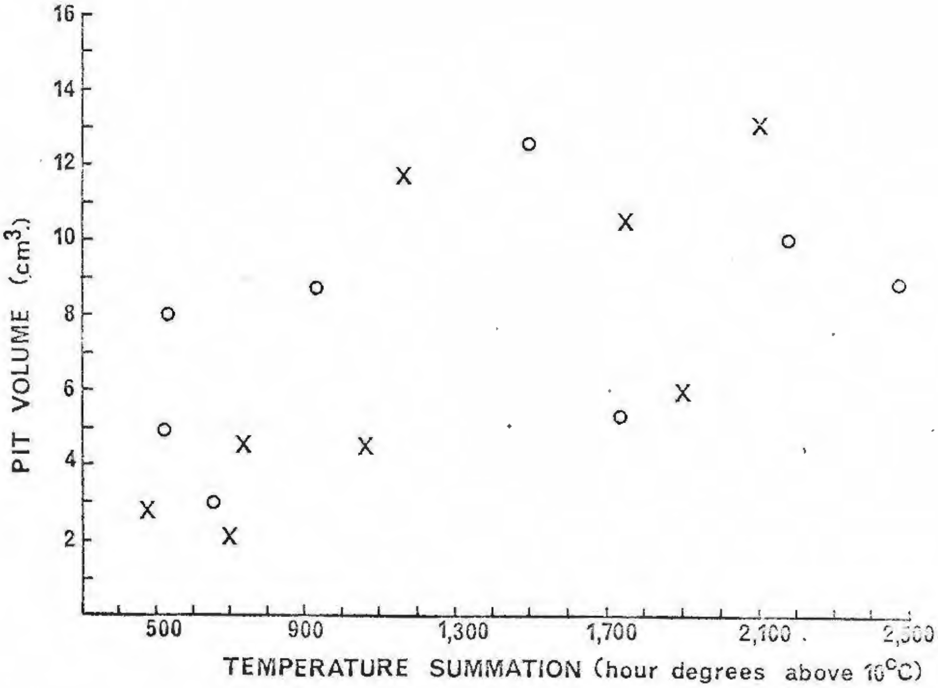


Fig. 9. The mean pit volume of a field population of 50 Myrmeleon obscurus larvae plotted against the temperature summations in degrees centigrade for the preceding day. The temperature is expressed as hour degrees above 10°C. The readings of pit volume were taken on the days of first and last lunar quarters. x----x first lunar quarter, o-----o last lunar quarter. For further details see text.

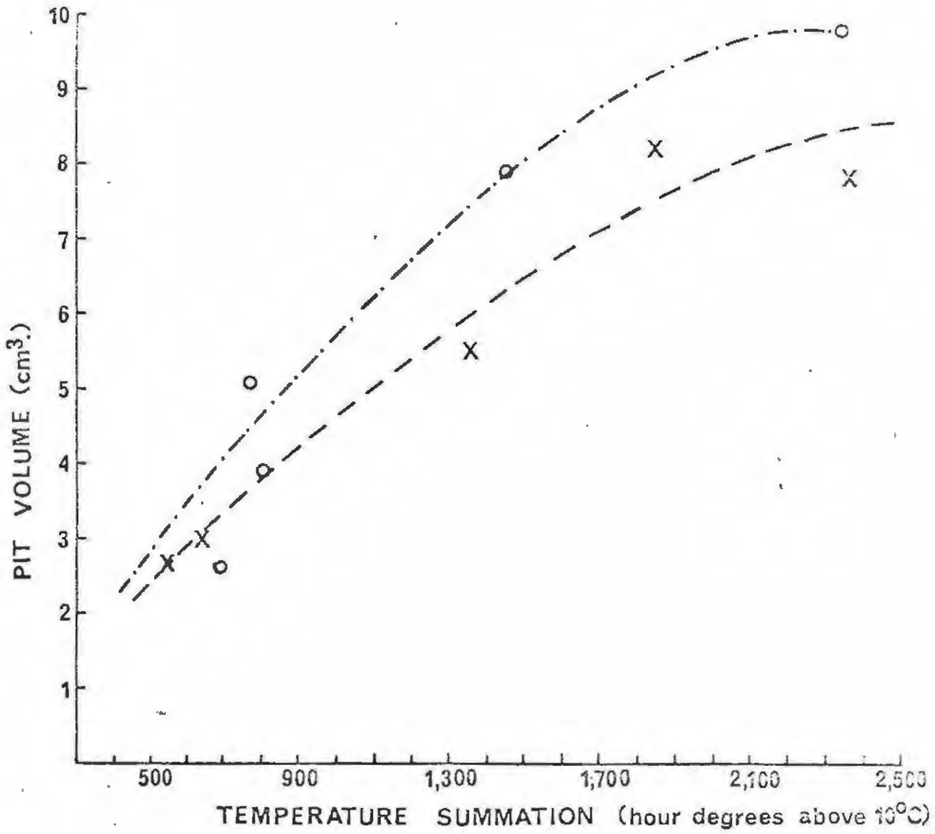


Fig. 10. The mean pit volume of a field population of 50 *Myrmeleon obscurus* larvae correlated with the temperature summation in degrees centigrade for the preceding seven days expressed as hour degrees above 10°C. The graphs are those for the first lunar quarter X———X, and the last lunar quarter o———o. For further details see text.

seven days calculated. The animals were then returned to the laboratory, where the temperature was 18 ± 3°C for eight days. This procedure was repeated with constant temperatures of 22°C, 19°C, 17°C, and 13°C all ± 1°C. After each exposure in the constant temperature room the experimental ant-lions were returned to the laboratory for eight days to eliminate any effects of the previous thermal treatment. The mean pit volume at the various temperatures was plotted against the temperature summations for the previous seven days, and the results are shown in Fig. 11. No readings were taken over full or new moon and these results can therefore be compared with the field results which were illustrated in Fig. 10. Fig. 11 shows that the pit volume increased most rapidly between 500 and 1,500 hour degrees, while above and below this range the pit volume increased very little with rising temperature.

Both the field and laboratory experiments on the effects of temperature on pit construction showed that the pits increased in size with increasing temperature. The ant-lions were also affected by the temperatures experienced during the preceding seven days. In view of these results all the ant-lions used in the experiments on activity rhythms were kept in the laboratory for seven days before being used to standardize the effects of their previous thermal history. The laboratory was also maintained at a temperature sufficiently high to allow the construction of large pits.

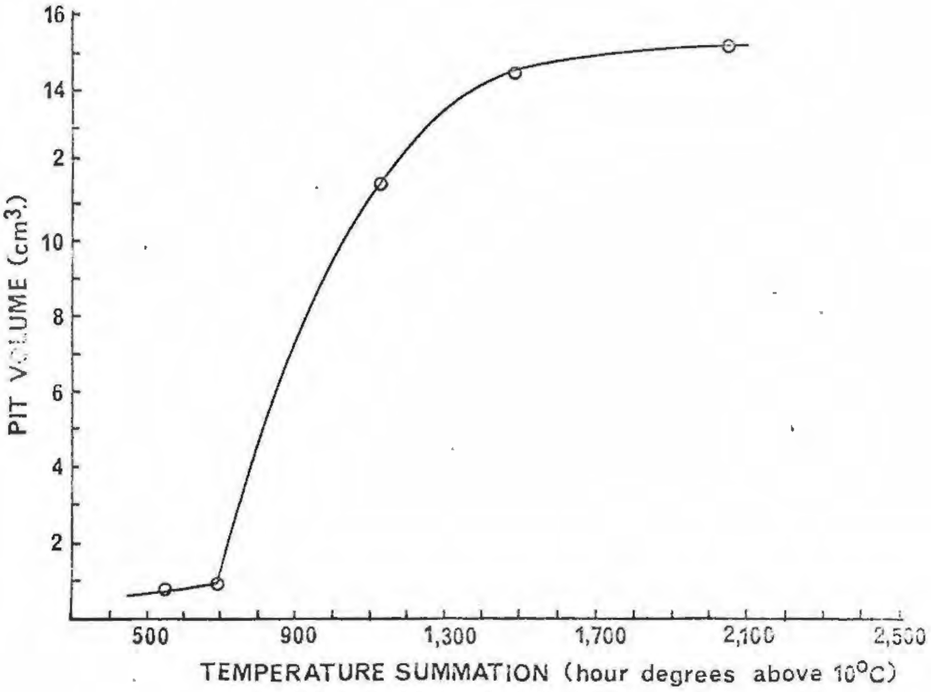


Fig. 11. The mean pit volume of a laboratory population of 12 Myrmeleon obscurus larvae correlated with the temperature summations for the preceding seven days expressed as hour degrees centigrade above 10° C.

3.6 DISTURBANCE OF THE PITS

In the experiments on activity rhythms the pits of the ant-lion larvae had to be destroyed before daily pit construction could be observed. Pits which were not disturbed tended to collapse and become irregular in shape, thus giving rise to inaccurate readings. In the electronic circuit recordings the pits also had to be destroyed each day in order to clean away sand from the sensor. It was thus essential to investigate the effects of pit destruction on pit size.

In an initial experiment a group of 96 Myrmeleon obscurus larvae was kept in the laboratory under normal daylight conditions and a temperature of 18 ± 3°C. These animals were each fed one ant a day at times which were randomly determined. For the first 70 days of the experiment the pits were left completely undisturbed. The pits were then disturbed each day after feeding for the following 22 days. The results of this experiment are shown in Fig. 12 and from this figure it can be seen that the destruction of the pits on day 70 caused an immediate drop in pit volume. The pit volume of the group remained at this low level until the end of the experiment.

The effect of pit destruction on pit size was further clarified in an experiment in which two groups of 12 ant-lions were used. In the control group the pits were left undisturbed so that the larvae did not need to construct a new pit each day, while in the test group the pits were destroyed each day after feeding. Measurements of the pit sizes in

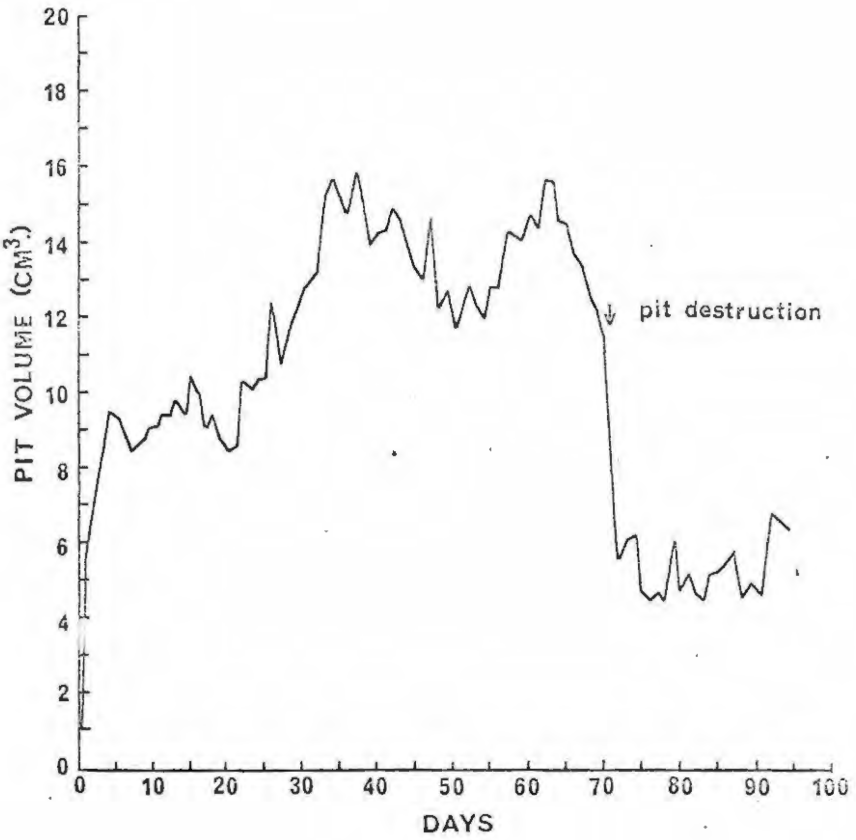


Fig. 12. *The effect of pit destruction on the mean pit volume of a group of 96 <u>Myrmeleon</u> <u>obscurus</u> larvae. The pits were left undisturbed until day 70. Thereafter the pits were disturbed each day after feeding. The ant-lions were kept in the laboratory at a temperature of 18 ± 3°C and each fed one ant a day.*

both groups were taken over a period of eight days. The pit sizes in these two groups of ant-lions varied from 0.5 cm^3. to 82 cm^3. and this range was divided into 5 cm^3 class intervals. The number of pits in each class interval was determined for both the control (undisturbed) and the test (disturbed) groups, and these numbers were expressed as a percentage of the total number of pits constructed by the respective groups. For example, in the control group it was found that there were 23 pits in the 21-25 cm^3. class interval, and this was 17.25% of the pits constructed by the group during the eight days. The results obtained from this experiment are shown in Fig. 13. From this figure it is clear that in the disturbed group there was a far higher percentage of pits in the smaller size ranges than was the case in the undisturbed control group. In the disturbed group 36% of the pits were in the 1-5 cm^3. class interval, while in the undisturbed group only 2.6% of the pits were as small as this.

These experiments established that daily destruction of the pits greatly reduced the pit size. In the experiments on activity rhythms, however, it was necessary to destroy the pits each day. Destruction of the pits in all experiments was thus carried out immediately after feeding each day (feeding times were randomly determined) and the amount of disturbance in all experiments was therefore the same.

At this point it seems necessary to summarize briefly the

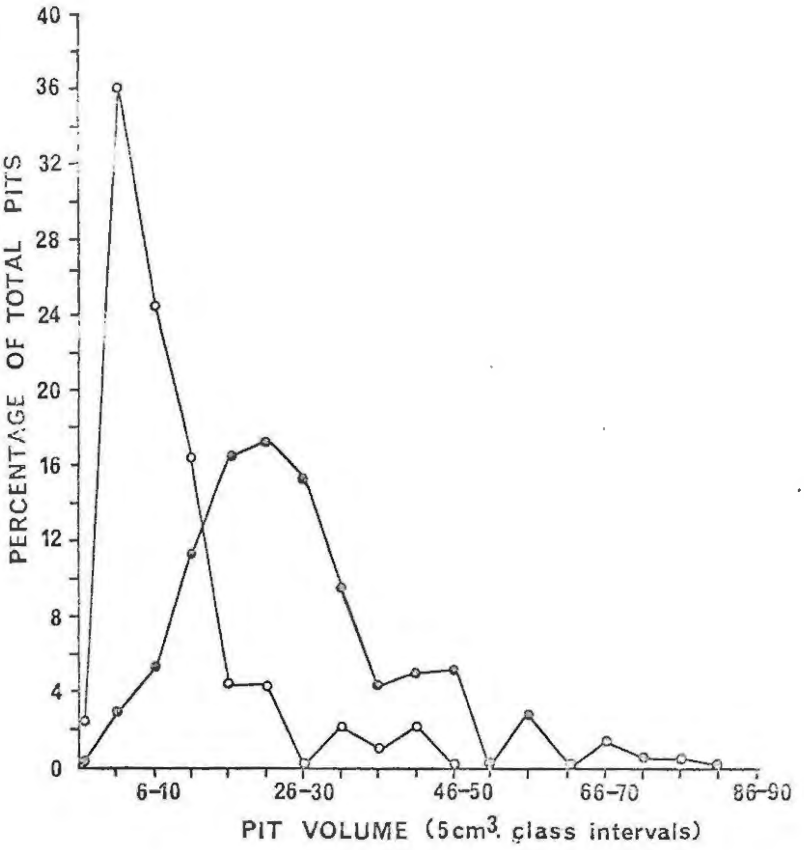


Fig. 13. The frequency of various pit sizes in two groups of 12 Myrmeleon obscurus larvae. The pits of the control group were left undisturbed, ●———● while the pits of the test group ○———○ were disturbed each day after feeding for eight days. The range of pit volumes recorded in both groups was divided into 5 cm^3 class intervals. For both the control and the test groups the number of pits in each 5 cm^3 class interval was expressed as a percentage of the total number of pits constructed during the eight day period.

effects of various factors on pit construction and the influence that this had on the design of the activity rhythm experiments.

(i) *It was shown that pit size was dependent on the size of the Myrmeleon obscurus larvae and random samples of ant-lions were used in the activity experiments.*

(ii) *Crowding the ant-lion larvae led to a decrease in pit size and an increase in mortality due to competition. The effects of crowding were avoided by keeping all the larvae in separate dishes.*

(iii) *Each ant-lion larva was fed one ant a day since this allowed growth and development without markedly increasing the rate of pupation. Feeding times were randomly determined.*

(iv) *Sand grain size had a marked effect on pit construction and in all the activity experiments the animals were kept in sand from the natural habitat. The composition of this sand was known and it proved to be a suitable habitat since all the ant-lions constructed large pits.*

(v) *The ant-lions in the activity experiments were kept at a constant temperature since it had been shown that the pit volume was greatly affected by temperature. No pits were constructed at all when the* temperature was very high or very low.

(vi) Pit destruction reduced the size of the pits but this effect was standardized by destroying all the pits each day immediately after feeding.

With these considerations in mind the circadian and lunar pit building activity rhythms in *Myrmeleon obscurus* may be discussed.

4. DAILY ACTIVITY RHYTHMS

4.1 THE EXISTENCE OF DAILY RHYTHMS

Preliminary observations indicated that the larvae of Myrmeleon obscurus tended to build their pits only in the late afternoon and evening, and a number of experiments were performed to test this. A population of ant-lions was maintained in the laboratory, where they were subjected to normal daylight fluctuations. The temperature of the laboratory was kept at 16 ± 3°C. An animal from this laboratory population was placed in the capacity sensing apparatus, and its activity recorded for two months, after which time it pupated. The ant-lion was fed one ant each day at a time which was determined randomly, and after feeding the pit was destroyed. The first 30 days of this activity record are shown diagrammatically in Fig. 14. The remainder of the activity record has been omitted since the activity continued as before during this time. In Fig. 14 the records for succeeding days are placed one below the other, activity being represented by the dark blocks. The height of the blocks is proportional to the height of the deflections on the original trace.

From this activity record (Fig. 14) it is clear that the ant-lion was mostly active at about 7 p.m. The exact time of activity varied slightly from day to day, as did the amount

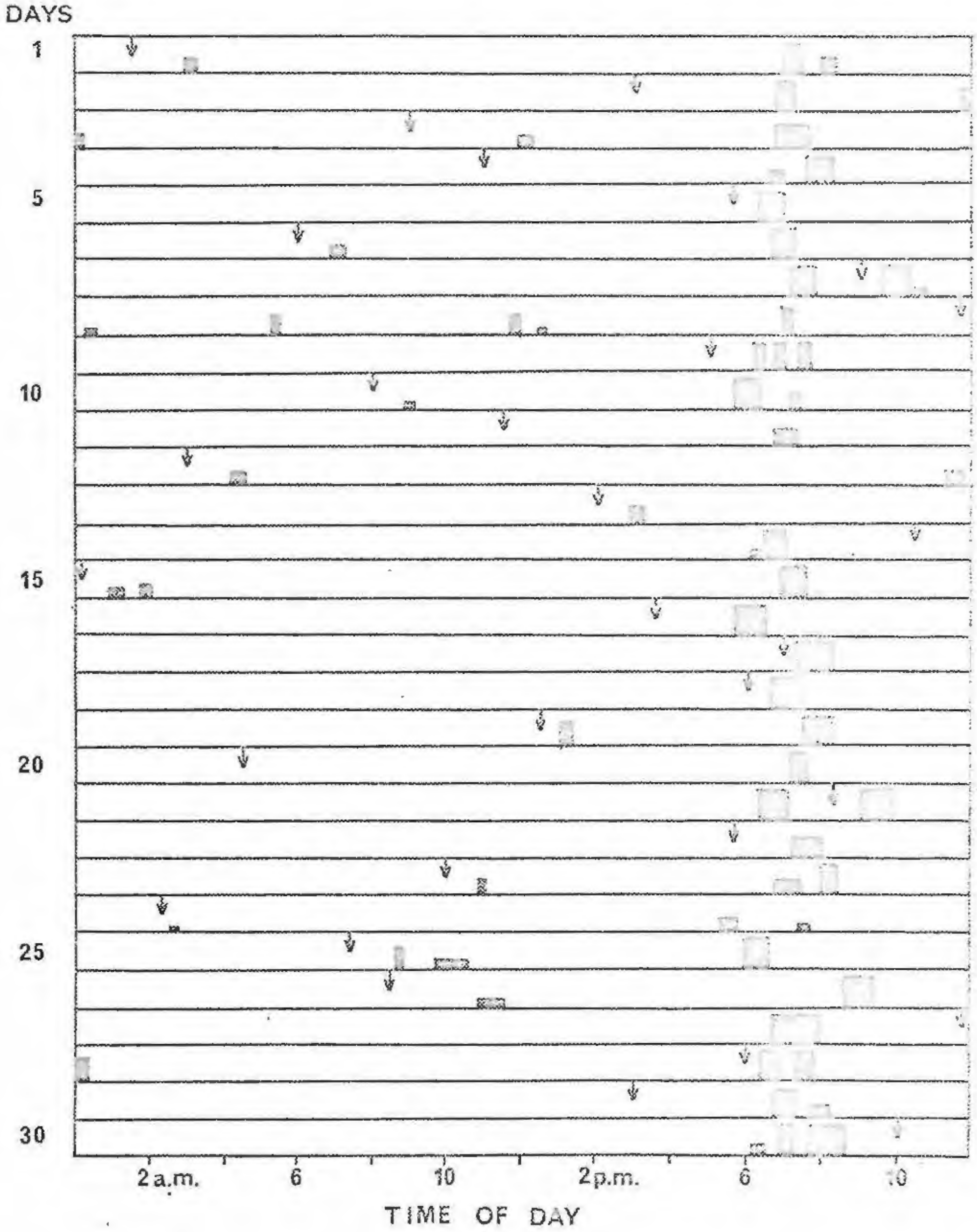


Fig. 14. *The activity record of a* *Myrmeleon obscurus* *larva under natural daylight conditions. The records for each day are placed one below the other and activity is represented by the dark blocks. The height of each block is proportional to the deflection recorded on the original trace. The times of feeding and pit destruction are indicated by arrows.*

of activity observed. Apart from the main activity peak at dusk smaller peaks were sometimes recorded within an hour or two of feeding. Activity at these times was generally low and did not involve the construction of a complete pit, such construction only occurring at the normal time (7 p.m:). Exceptions to this were noted on days 7 and 21 when feeding took place at 9 p.m. and 8.15 p.m. respectively, that is shortly after the normal activity time. In these instances two separate pits were constructed by the larva, one at the normal time and a second one after feeding.

This experiment was repeated with five different individuals, and in all cases each individual was found to show definite activity peaks at dusk. The time of activity for each individual varied slightly, and this variation was shown in an experiment in which a different Myrmeleon obscurus larva was placed in the activity recorder each day. 12 different individuals were used and these activity records are shown in Fig. 15. From this figure it is clear that all the animals showed an activity peak between 5 p.m. and 8 p.m. As in the previous experiment some slight activity was often observed shortly after feeding, although the main activity peak occurred at dusk.

The daily pit building activity rhythm was also demonstrated by direct observation. A group of 96 Myrmeleon obscurus larvae was kept in the laboratory under natural daylight conditions, and the pit building activity of these animals observed every 15

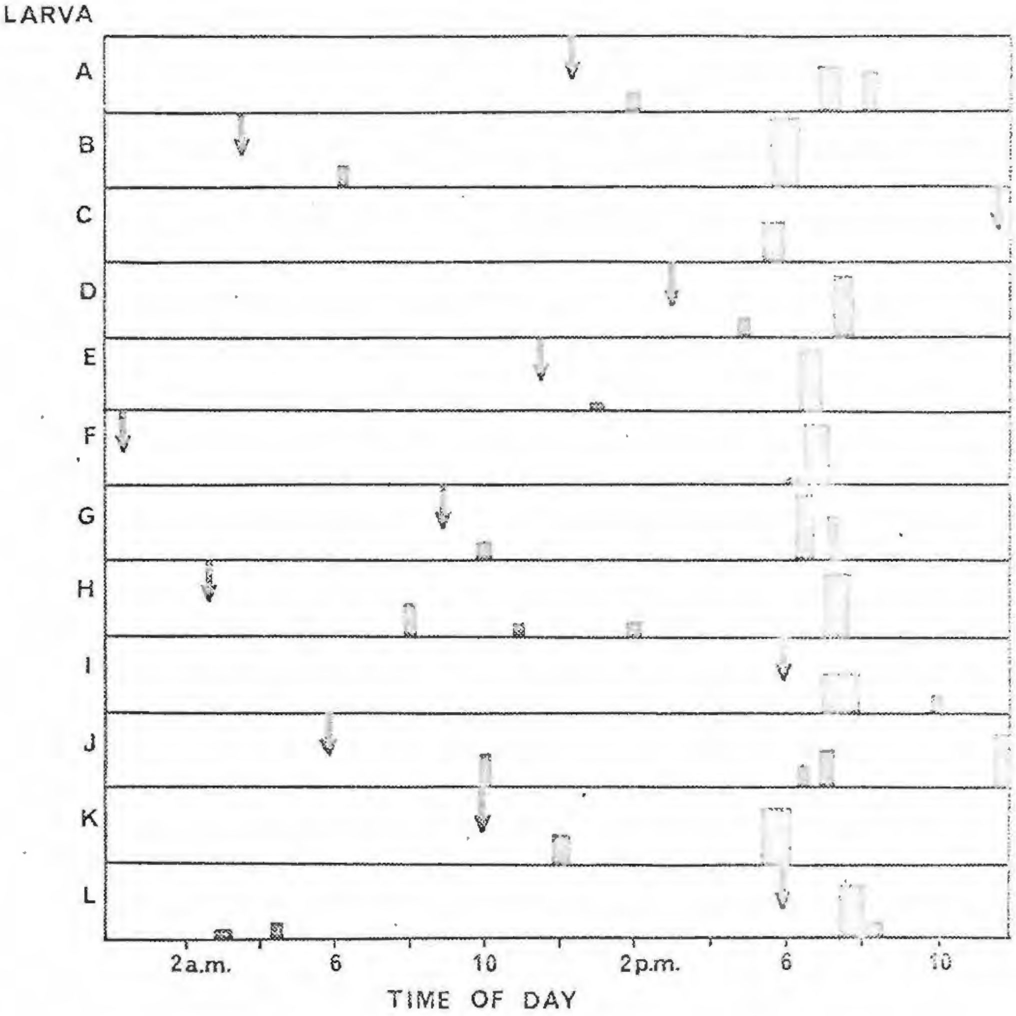
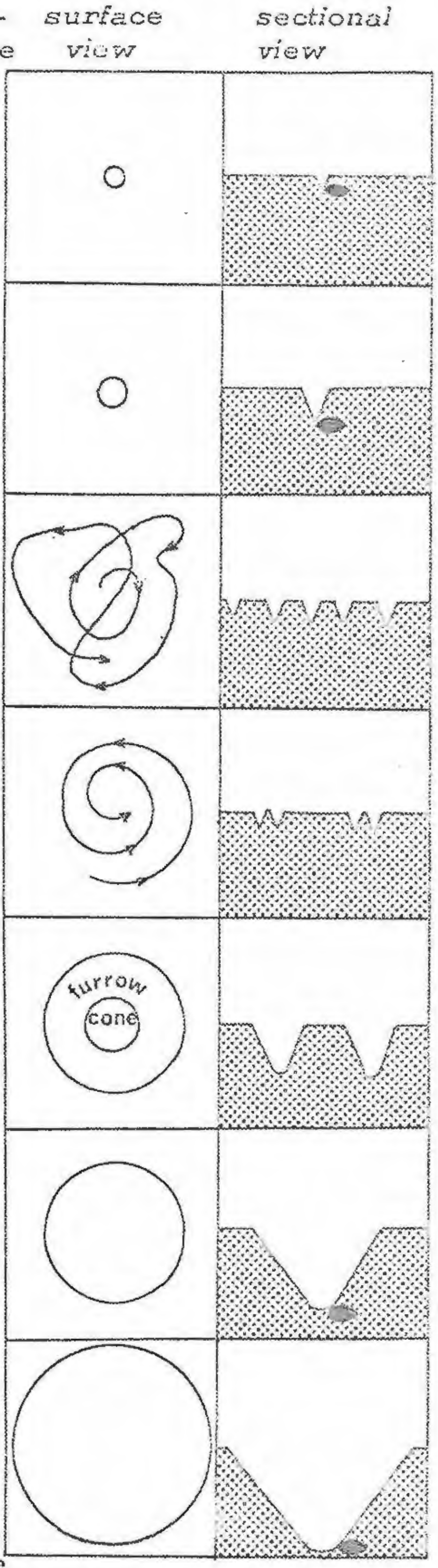


Fig. 15. *The daily activity of 12 individual* Myrmeleon obscurus *larvae, A to L, under natural daylight conditions. The activity is represented by the dark blocks the height of which is proportional to the deflections on the original trace. The times of feeding are indicated by arrows.*

minutes for 24 hours. In order to assess the activity of these ant-lions various stages in the pit building activity had to be recognised. A number of workers such as Haub (1942), and Wheeler (1930) have described the pit building activity of myrmeleontid larvae, but have not divided this activity into its various stages. These stages in <u>Myrmeleon</u> <u>obscurus</u> are as follows.

First the animal comes to the surface of the sand (Fig. 16(1)), where it often excavates a very small pit by remaining in one place and flicking the sand away from itself with its head (Fig. 16(2)). These two stages together are generally completed within 4-5 minutes, but the ant-lion may remain in either of these stages for a number of hours. If the pit is destroyed in the early morning the animal generally remains in one of these two stages until dusk when actual pit construction takes place. If the pit is destroyed in the late afternoon the ant-lion may proceed directly to stage 3. Fig. 16(3) shows the third activity stage in which the ant-lion crawls haphazardly round the dish flicking the sand away as it goes, thus leaving a shallow furrow to mark its progress. Having crawled around in this manner for some time (from a few minutes to several hours), the animal begins to move in a circle spiralling inwards, as shown in Fig. 16(4). This indicates the beginning of actual pit construction. The ant-lion then spirals inwards, increasing the depth of the furrow as it goes and, as the furrow increases in depth, a central cone



Approximate time taken	
1 min.	<u>Stage 1</u> The larva is visible at the surface of the sand but has not made a pit.
3 mins.	<u>Stage 2</u> A very small pit has been excavated by the animal which remained stationary and flicked away the sand with its head.
5 mins. to 3 hours	<u>Stage 3</u> The larva crawls haphazardly round the container leaving small furrows. The direction of movement is indicated by arrows.
3-10 mins.	<u>Stage 4</u> The larva begins to move in a circle which has a diameter slightly smaller than the diameter of the complete pit.
5-10 mins.	<u>Stage 5</u> The pit is half completed and consists of a small central cone of sand surrounded by a deep furrow.
5-10 mins.	<u>Stage 6</u> The completed pit.
bursts of activity, each lasting a few secs., may continue for many hours	<u>Stage 7</u> Pit enlargement by the larva remaining at the bottom of the pit and continuing to flick out sand.

Fig. 16. The various stages in the construction of a pit by a <u>Myrmeleon obscurus</u> larva. The approximate times taken for each stage are indicated. The times vary considerably depending on the size of the pit being constructed. For further details see text.

of sand is left which is gradually undermined and flicked out of the pit (Fig. 16 (5)). At this stage the pit is half complete. Finally the last of the central cone of sand is removed and the pit is complete (Fig. 16 (6)). The time taken for the completion of stages 4, 5 and 6 depends largely on the size of the pit being constructed. Occasionally the pit may be further enlarged, as in Fig. 16 (7), by the animal remaining at the bottom of the pit and continuing to flick out sand. These were the various stages observed in pit construction by <u>Myrmeleon</u> <u>obscurus</u> larvae.

In observing the activity of the group of 96 ant-lions, each change from one stage of pit construction to another was scored as one point. Often the ant-lions passed through several stages during the 15 minute observations, and they were scored one point for each level passed. For example, if at 8.30 a.m. there were 5 animals in stage 1 (score 5), and 1 animal in stage 2 (score 2), the total activity for the group was recorded as 7. At 8.45 a.m. the score might be as follows: 2 animals in stage 1 (score 2), 5 animals in stage 3 (score 15), total 17. The difference between these two totals gives a measure of the activity which has taken place during the 15 minutes between 8.30 a.m. and 8.45 a.m.

During the hours of darkness some light was obviously necessary in order for the activity to be observed and a very dim red light was used for this purpose. The ant-lions appeared to be insensitive to this red light since, while switching on a normal light temporarily stopped pit construction, switching on the red light had no such disturbing effect. In fact, the animals did not appear to react in any way to the red light.

This experiment was repeated with four groups of 96 animals and the average activity for each hour of the day is shown in Fig. 17. This figure shows that maximum activity took place between 6 p.m. and 8 p.m. Thereafter the level of activity remained fairly high until 1 a.m., after which it decreased to its lowest point from about 8 a.m. to 11 a.m. As in the actograph experiments it should be noted that even though the pits were destroyed early in the morning very little pit construction took place until dusk. Most of the activity observed during the day was confined to the animals coming to the surface (stage 1 of pit construction). These experiments therefore, confirmed the results obtained from the capacity sensitive circuit, and showed that ant-lions had a daily rhythm of pit building activity.

In addition it was shown that ant-lions were more likely to accept prey during their period of maximum pit building activity. A group of 24 ant-lions was maintained at a constant temperature of 25.5 ± 1°C. These animals were fed as often as they would accept food, or at least once every hour between 9 a.m. and 11 p.m. This was continued for 48 days and the average number of ants eaten by the group during each hour from 9 a.m. to 11 p.m. was calculated. The results are shown

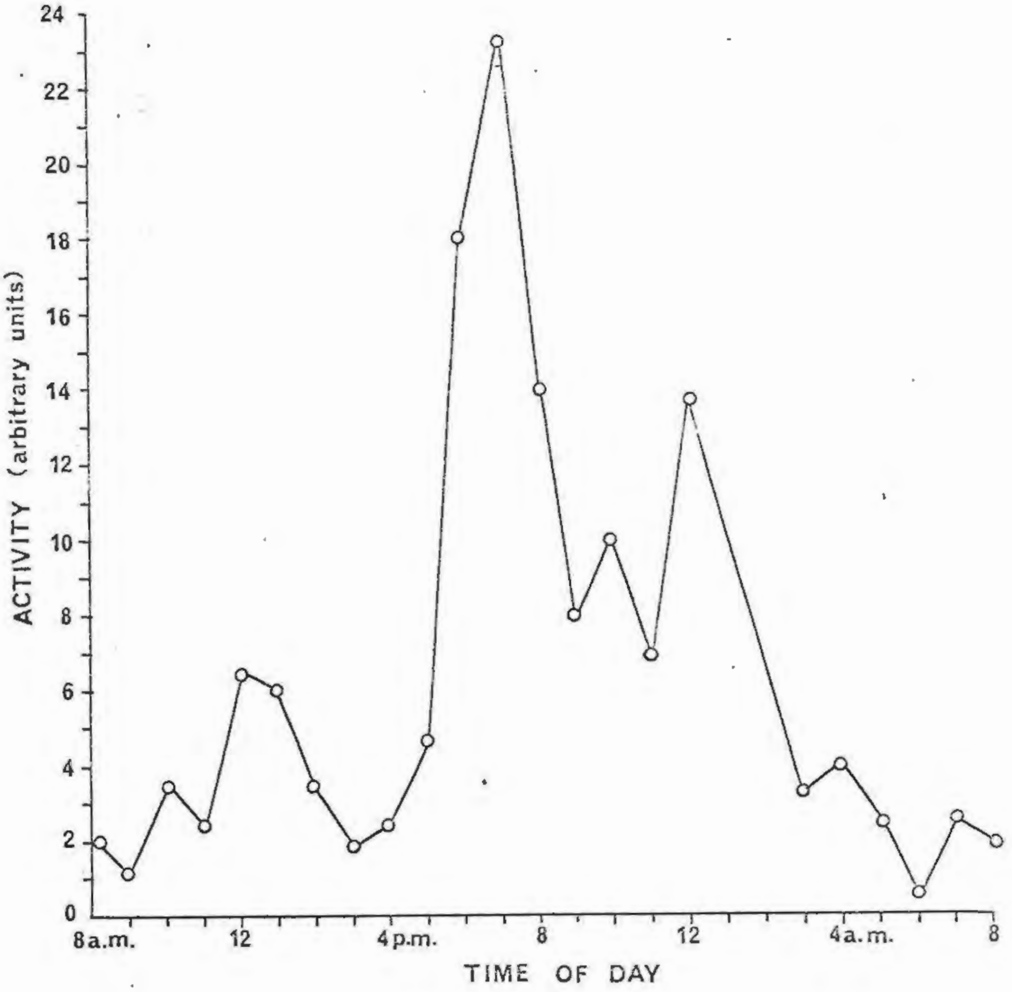


Fig. 17. *The average activity of four groups of 96 Myrmeleon obscurus larvae observed over a period of 24 hours. The larvae were kept in the laboratory under natural day-night conditions and a temperature of 18 ± 3 °C. The activity was estimated as described in the text.*

in Fig. 18. From this figure it is obvious that the maximal feeding activity occurred during the period 7 p.m. to 11 p.m. Thus the time of maximal feeding was correlated with the increase in general pit building activity. At this time of day the ant-lions were also observed to destroy their own pits. They did this by spiralling upwards round the sides of the pit causing the sand to slide into the pit. Having destroyed the pits they then reconstructed them. This behaviour was not observed at any other time of the day.

It has thus been shown that at dusk ant-lion larvae showed a general increase in activity which involved an increase in the construction and destruction of pits as well as an increase in feeding activity. While the experiments described above have shown the existence of an activity rhythm, they have not given any indication as to whether it is purely a direct response to some environmental factor, such as the light-dark transition, or whether it is an endogenous rhythm, such as has been described in a number of other insects. In order to assess whether the rhythm was endogenous or due to some environmental factor, a study of the effect of constant conditions on daily rhythms was necessary. It is generally agreed in the literature (Roberts, 1960; Sollberger, 1965) that the persistence of daily rhythms under constant laboratory conditions is evidence for their endogenous nature.

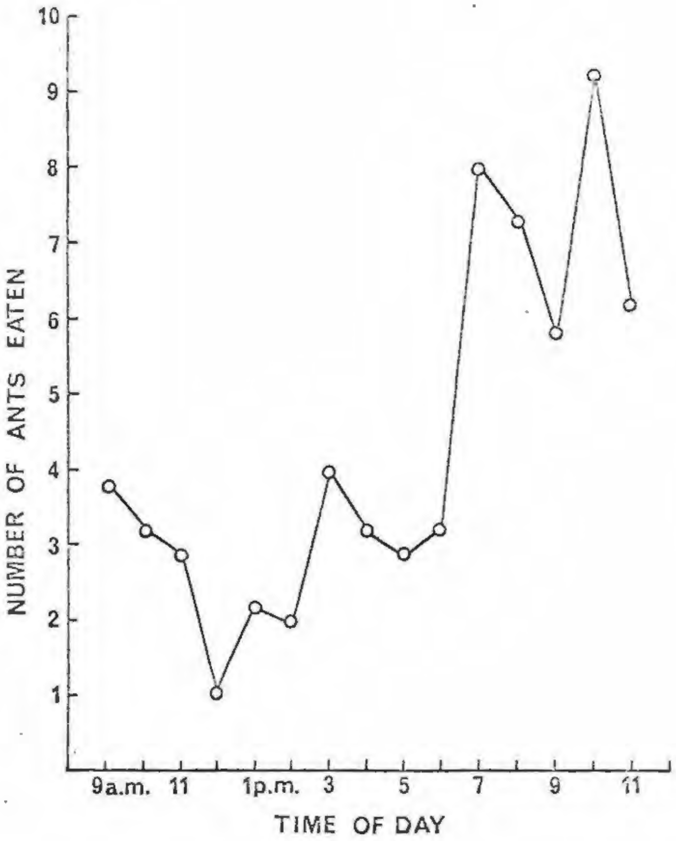


Fig. 18. The average number of ants eaten by a group of 24 Myrmeleon obscurus larvae at each hour of the day between 9 a.m. and 11 p.m. The ant-lions were maintained at a constant temperature of 25.5 ± 1°C and fed at least once every hour.

4.2 THE DAILY ACTIVITY RHYTHM UNDER CONSTANT CONDITIONS.

Both constant light and constant dark conditions were used. The temperature in the laboratory was maintained at 16 ± 3 °C. The departmental constant temperature rooms could not be used since the actograph circuit proved to be sensitive to the vibrations and switching of the compressors. In all these experiments the normal activity rhythms of the Myrmeleon obscurus larvae were determined under fluctuating conditions for a few days before the animals were placed in constant conditions. It should be emphasised that only 1-3 replicates of each experiment were possible since each experiment lasted for at least 30 days and only one electronic circuit was available.

4.21 Constant dark

The first experiment was conducted under conditions of constant dark. These conditions were achieved by placing the circuit in a light proof box. This box, containing a piece of unexposed photographic film, was tested for light leaks by exposure to the sun for 30 minutes. After this time the photographic film was removed in the dark and developed. It showed no evidence of any exposure to light, and the box was presumed to be completely light proof. This box was only opened during the hours of darkness when the animals were fed. Some light was necessary for this operation so a very dim red light was used.

Three different individuals were tested in the constant dark. The activity record obtained from one of these animals is shown in Fig. 19, where the activity traces for each day have been placed one below the other. The record shows that the activity was slightly reduced for the first few days after the onset of darkness, although it still occurred at the normal time. Thereafter there was a tendency for the activity to occur later each day until day 37, after which time the rhythm was lost, small amounts of activity occurring at all hours of the day. In this individual the daily activity rhythm continued for 35 days in constant dark, and under these conditions had a period of slightly more than 24 hours. During the 35 days in darkness the rhythm shifted by about three hours and this represents an average shift of almost five minutes a day. The free-running period in constant dark would thus appear to be about 24 hours 5 minutes.

The remaining two individuals which were tested in constant dark both pupated within four weeks. During the time they were in darkness their rhythms showed no tendency to fade and, as in the first individual, it seemed likely that their rhythms would continue for at least 35 days in constant dark. The free-running period was calculated for both individuals on the data available. The free-running rhythms had period of 24 hours 17 minutes and 24 hours 2 minutes respectively. In the ant-lions tested in constant dark therefore the activity occurred later and later each day, but the exact length of the free-running

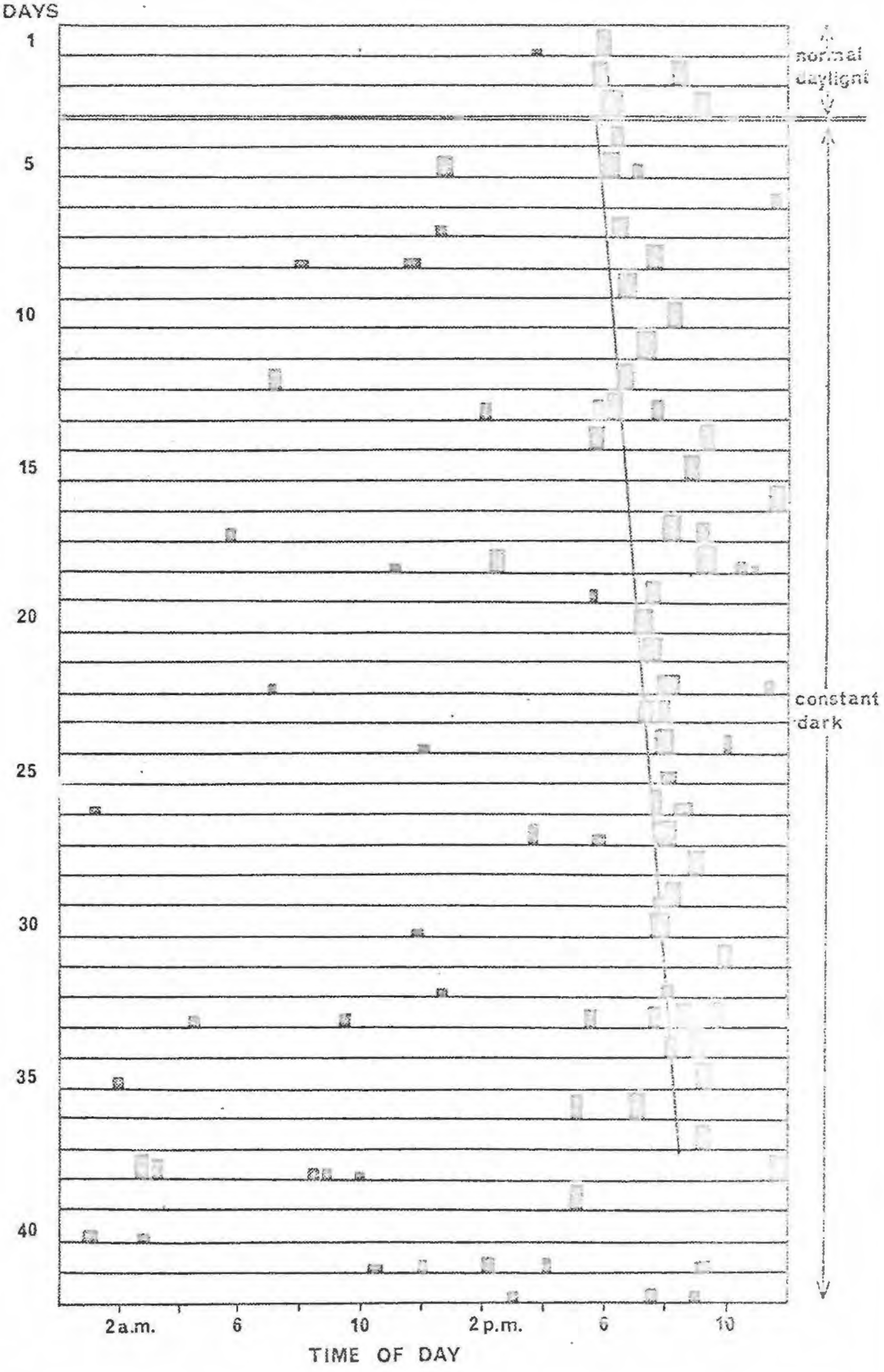


Fig. 19. The daily activity of a Myrmeleon obscurus larva under normal daylight conditions and in constant dark. During the first three days of the experiment the animal was under normal daylight conditions, thereafter it was in constant dark. The ant-lion was fed one ant a day at times which were randomly determined.

period varied from individual to individual.

4.22 Constant light

Experiments were also conducted under conditions of constant light (approximately 1,500 lux). Two different individuals were tested. One of these pupated within a week and the results were thus discarded. The activity record obtained from the second Myrmeleon obscurus larva is shown in Fig. 20. For the first four days under constant light the activity peak still occurred during the early evening but the rhythm was not nearly as precise as normal. After this time the rhythm showed a general tendency to occur earlier each day. This trend was still obvious when the experiment was concluded on day 30 (due to the pupation of the ant-lion). After 25 days in constant light the rhythm occurred about seven hours earlier than normal, and this represents a daily shift of about 16 minutes. The free-running rhythm under constant light of 1,500 lux would thus appear to be about 23 hours 44 minutes.

4.23 Synchronization of the rhythm

These experiments have shown that Myrmeleon obscurus larvae have a daily rhythm of pit building activity, the free-running period of which increases in constant dark and decreases in constant light. Since this rhythm deviates from 24 hours under constant conditions there must be some factor which, under normal fluctuating conditions, sets the rhythm to

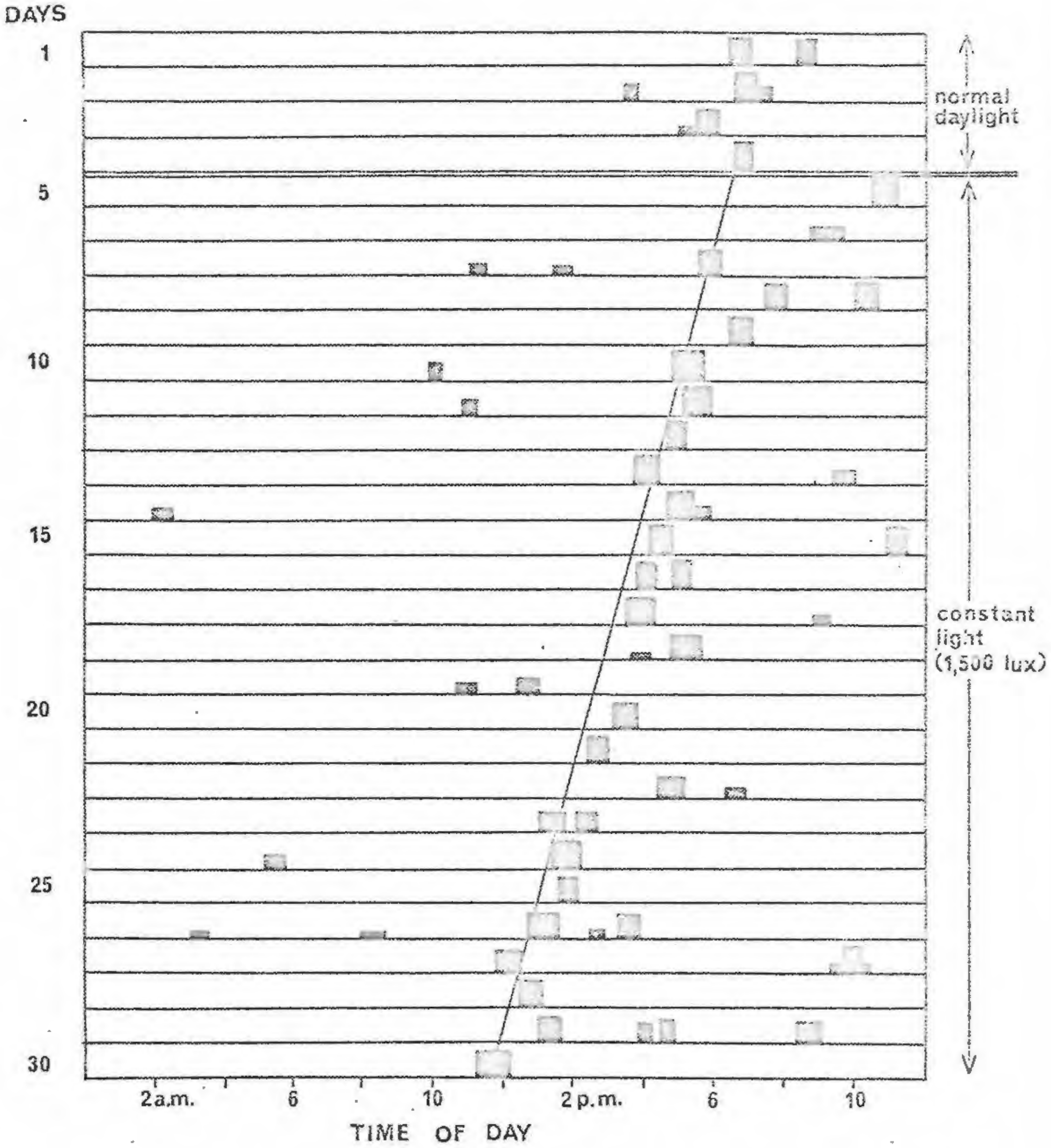


Fig. 20. The daily activity of a Myrmeleon obscurus larva under normal daylight conditions and in constant light of approximately 1,500 lux. During the first four days of the experiment the ant-lion was under normal daylight conditions. The ant-lion was fed one ant a day at times which were randomly determined.

exactly 24 hours. Light-dark changes are generally regarded as being the most universal phase setting factors, or Zeitgebers, and it is likely that this applies to rhythms in Myrmeleon obscurus.

If the activity rhythm is being set by light then reversal of the light-dark cycle should reverse the activity rhythm. In order to test this, an animal was placed in a reversed light-dark cycle in which the onset of darkness began at 10 a.m. and the onset of light at 8 p.m. After eight days under this reversed light regime the ant-lion was placed in constant darkness and its activity recorded for a further eight days. The results of this experiment are shown in Fig. 21. During the first four days of the experiment the ant-lion was subjected to normal fluctuating light conditions, in which the onset of darkness was at about 6 p.m. Under these conditions the larva was always active just after the onset of darkness. On day five the light regime was altered so that darkness began at about 10 a.m. when the daily activity rhythm shifted, so that activity still occurred shortly after the onset of the dark period. The rhythm shifted immediately and no transients were obvious. When the animal was placed in constant dark, having been under a reversed light cycle for eight days, the rhythm was lost completely, activity occurring at all hours of the day.

From this experiment it was concluded that light changes were capable of rapidly resetting the phase of the daily rhythm,

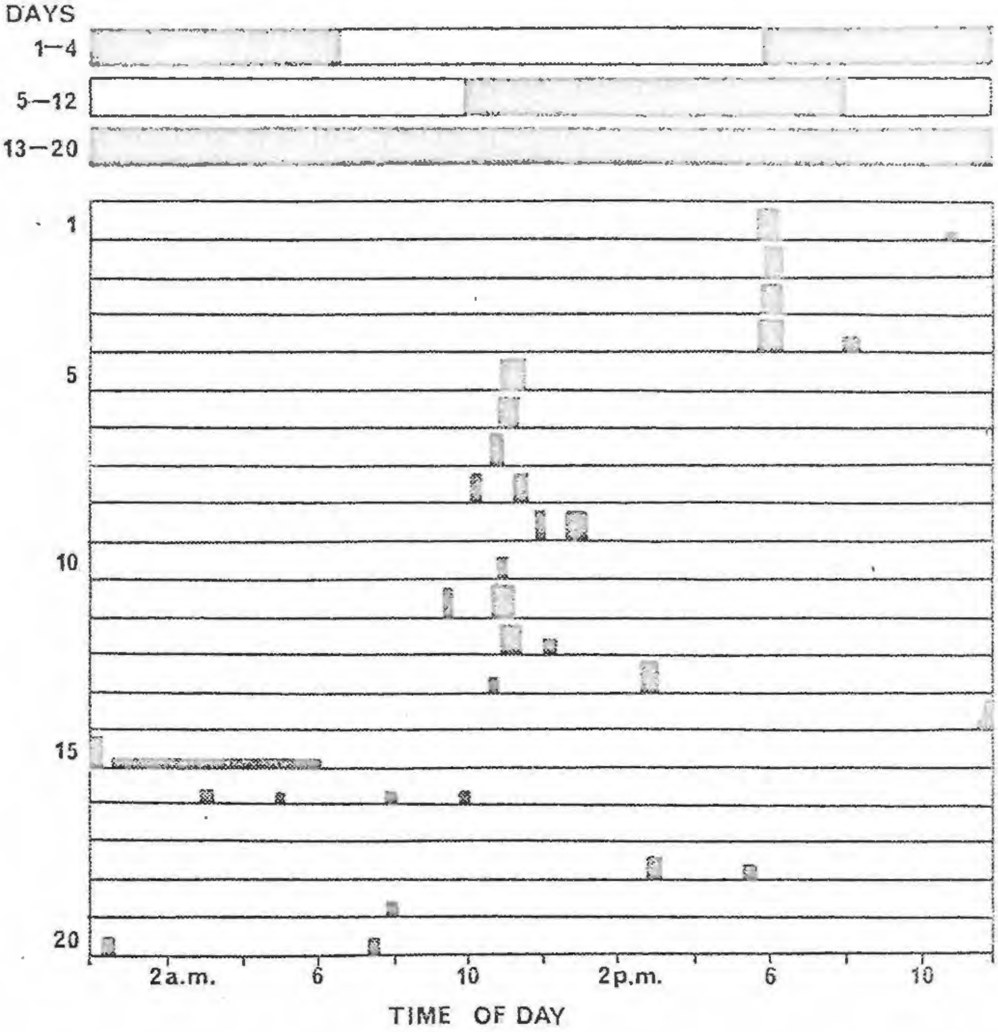


Fig. 21. The daily activity of a Myrmeleon obscurus larva under normal and reversed light regimes followed by a period in constant dark. The position of the light and dark fractions are indicated at the top of the figure where the white bands denote light of approximately 1,500 lux while the black areas denote darkness. The antlion was fed one ant a day at times which were randomly determined.

but this new rhythm was not maintained under constant conditions. It might, however, be maintained if the reversed light cycle was continued for a longer period of time.

In the absence of light changes it has been found that other factors, such as regular times of feeding and pit destruction, may act as synchronizing factors. This was illustrated in an experiment in which a Myrmeleon obscurus larva was placed in constant light of 1,500 lux but, instead of being fed at randomly determined times as in all previous experiments it was always fed at exactly the same time of day. During the first 15 days of the experimental period the animal was always fed at exactly 7.45 p.m. as indicated by the arrow at the top of Fig. 22. Although the ant-lion was in constant light it did not show a free-running rhythm as was the case in other experiments in which the feeding time was randomised. Instead the rhythm maintained a 24 hour periodicity with the activity peak occurring shortly after feeding time each day. When the feeding time was altered to 11 a.m. on day 16 (indicated by the arrow in Fig. 22) the activity peak immediately shifted. The activity now occurred shortly after the animal had been fed. As in the experiments in which the light cycle was reversed, no transients were observed.

The change in activity time, which took place when the feeding time was altered, might have represented a direct response to feeding and pit destruction rather than actual

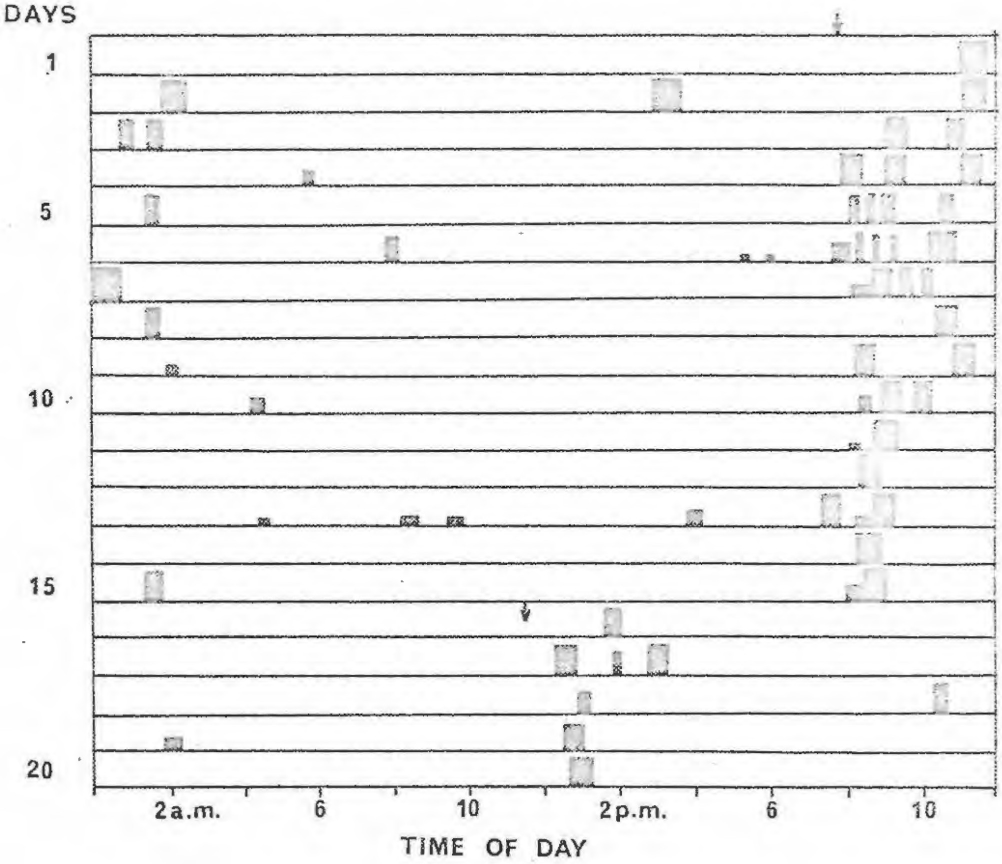


Fig. 22. The daily activity of a Myrmeleon obscurus larva in constant light of about 1,500 lux when fed at specific times of the day. From day 1-15 the animal was always fed at 7.45 p.m. as indicated by the arrow at the top of the figure. On day 16 the feeding time was switched to 11.30 a.m. as shown by the arrow.

resetting of the endogenous rhythm. This, however, seemed unlikely since in earlier experiments (section 4.1) it was shown that, when the pits of ant-lions under normal fluctuating conditions were destroyed in the morning, new pits were not constructed until the usual activity time at dusk. In order to clarify this point two further experiments were performed.

In the first experiment a <u>Myrmeleon</u> <u>obscurus</u> larva was placed in the capacity sensitive circuit and its activity recorded for five days under normal fluctuating conditions. The animal was then placed in constant light of 1,500 lux and its activity recorded for a further 25 days. The ant-lion was fed at exactly 12.15 p.m. each day, until day 25, after which the feeding times were randomly determined. Fig. 23 shows the results of this experiment. During the first four days under fluctuating light conditions the ant-lion always showed its main activity peak at about 6 p.m. although it was fed at 12.15 p.m. Some slight activity was recorded after feeding but this did not involve the construction of a complete pit. When placed in constant light, however, considerably more activity took place after feeding, and included the construction of complete pits. Large peaks of activity were also observed at other times of the day but no relationship could be found between these peaks and the experimental conditions. From day 26 onwards the feeding time was randomly determined. If the previously constant feeding time had reset the endogenous

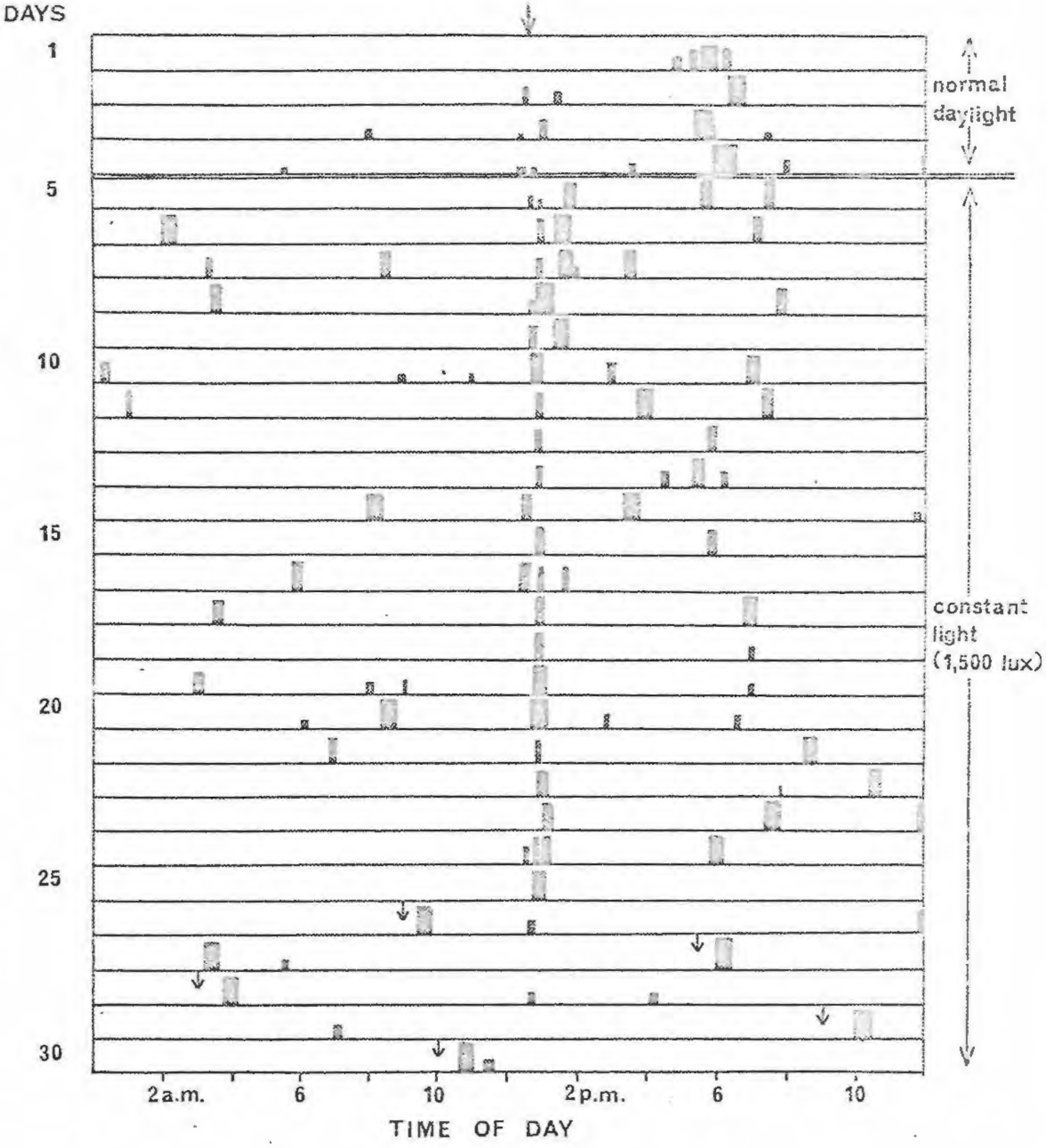


Fig. 23. *The daily activity of a Myrmeleon obscurus larva under fluctuating and constant light conditions when fed at 12.15 p.m. each day. The animal was subjected to normal daylight conditions for the first four days and thereafter to constant light of approximately 1,500 lux. The time of feeding is indicated by the arrow at the top of the figure. After day 25 the feeding time was randomly determined as indicated by the arrows.*

rhythm it might be expected that this rhythm would continue when the feeding time was randomized. This, however, did not occur. Instead the main activity peak occurred immediately after feeding each day. This experiment indicated that a set time of feeding cannot reset the endogenous rhythm in the absence of light changes.

In the second experiment the ant-lion was fed every 15 hours instead of being fed at the same time each day. The activity of this animal was recorded for four days in normal fluctuating light conditions and then for a further 22 days in constant light of about 1,500 lux. The results illustrated in Fig. 24 show that for the first 19 days of the experiment the main activity peak always occurred in the early evening, regardless of the time at which the ant-lion was fed. The endogenous rhythm thus continued under these conditions although some slight activity was observed after feeding time each day. After day 20 the endogenous rhythm appeared to be lost and the ant-lion became active immediately after feeding. Thus by feeding the ant-lion every 15 hours it was induced to show a 15 hour periodicity. This activity rhythm, however, was purely a direct response to feeding, and only became apparent when the normal endogenous 24 hour rhythm was lost.

The evidence presented thus far indicates that Myrmeleon obscurus larvae have an endogenous daily rhythm of pit building activity. The daily rhythm continued for 35 days in constant dark and showed a free-running period of between 24 hours

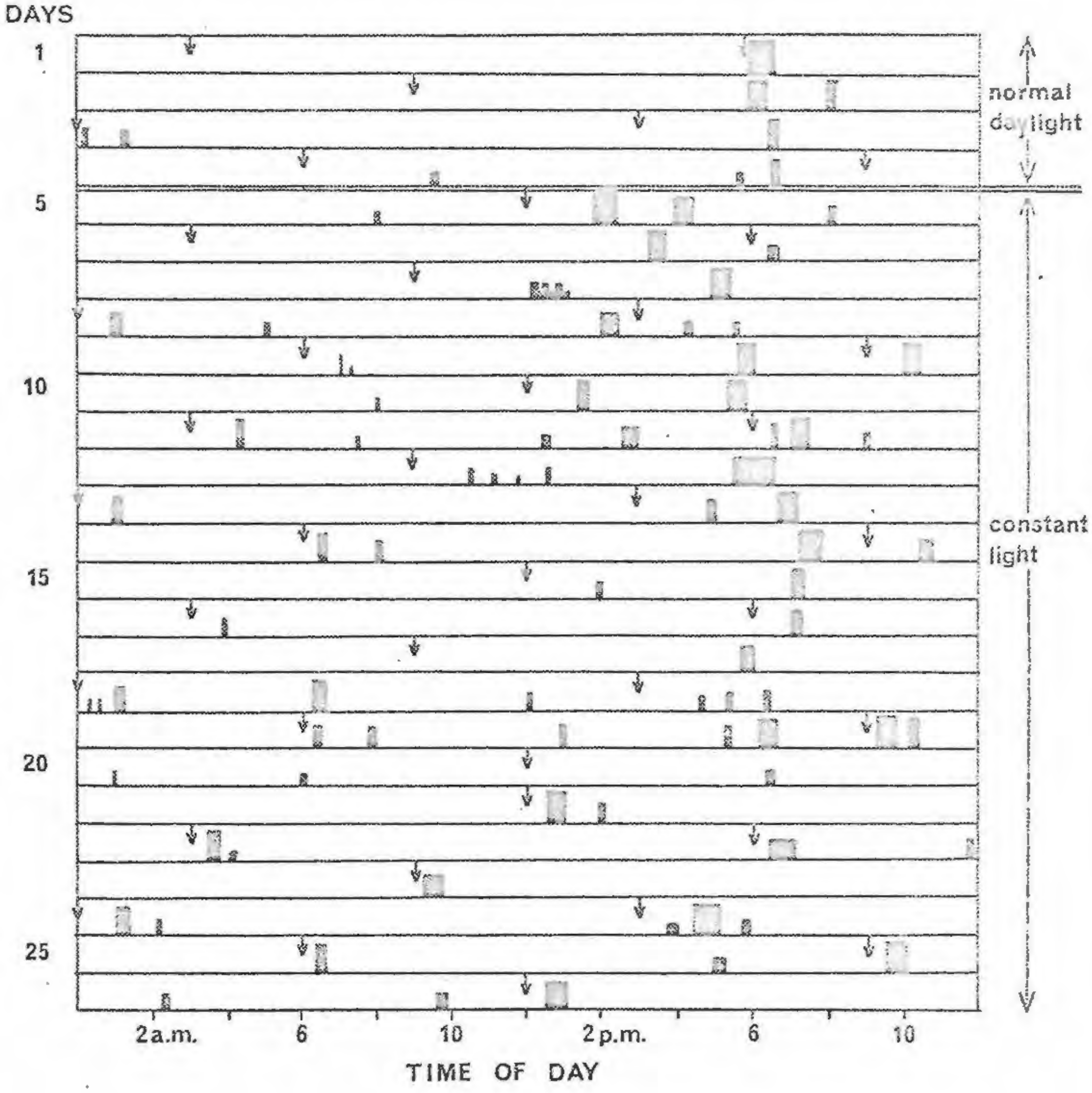


Fig. 24. The daily activity of a Myrmeleon obscurus larva under fluctuating and constant light conditions when fed every 15 hours. The animal was subjected to normal daylight conditions for the first four days, and constant light was begun at feeding time on day four. The light was approximately 1,500 lux. The times of feeding are indicated by arrows.

2 minutes and 24 hours 17 minutes. In constant light of about 1,500 lux the rhythm continued for at least 25 days with a free-running period of 23 hours 44 minutes. This endogenous rhythm was rapidly altered by reversal of the light cycle, but it was not reset by changes in the time of feeding.

4.3 EXPERIMENTS TO DETERMINE THE LOCATION OF THE "CLOCK" CONTROLLING THE DAILY ACTIVITY RHYTHMS

The design of experiments to locate the "clock" controlling the endogenous daily rhythms of Myrmeleon obscurus larvae must rest heavily on the findings of other workers in different insects. Much of our knowledge of the timing devices or "clocks" controlling insect diurnal rhythms is due to the work of Harker (1956, 1960, 1961). Working on the cockroach Periplaneta americana L. she established that the control of the diurnal rhythm was hormonal. She demonstrated this by using the technique of parabiosis. The site of the hormone was found to be the sub-oesophageal ganglion and this was borne out by experiments in which ganglia were transplanted from one cockroach to another. In addition to this technique of transplanting ganglia, Harker also demonstrated the existance of the "clock" by chilling the sub-oesophageal ganglion in situ. Provided that the ganglion was transplanted to an arrhythmic animal immediately after chilling, the clock was stopped. If, on the other hand, the ganglion was left in position chilling had no effect on the rhythm. This suggested that, in the intact animal, something else was maintaining the rhythm during the chilling period. Harker concluded that there were, in fact, not one but two "clocks" controlling the daily rhythm of P. americana. The second "clock" was found to be located in the corpora cardiaca and was reset by chilling the cockroach in the dark.

More recent work (Fingerman, Lago and Lowe, 1958; Rensing, 1964; Roberts, 1966) has tended to cast doubt on Harker's results and to implicate instead the pars intercerebralis of the brain as the major controlling centre or "clock" for daily activity rhythms. Roberts (1966) working on P. americana, and using the same technique as Harker, was unable to demonstrate the importance of the sub-oesophageal ganglion or the corpora cardiaca in the control of daily rhythms. By cutting out various parts of the brain Roberts was able to show quite clearly the importance of the pars intercerebralis of the brain. Brady (1967 a, b, and c) also worked on P. americana. He was unable to confirm either Harker's or Roberts' results and suggested that the divergent results obtained by the various workers could only be explained if the controlling "clock" in the brain was assumed to be electrical rather than hormonal. This electrical centre in the brain would control neuro-endocrine systems in the ventral nerve cord. There is also some evidence in other insects which implicates the brain, rather than the sub-oesophageal ganglion, in the control of activity rhythms. Rensing (1964), for example, has shown that there are circadian changes in the neurosecretory activity of the brain of Drosophila which are related to the activity cycle.

From the literature it can be seen that four main techniques have been used to investigate the "clocks" controlling activity rhythms, parabiosis, chilling, histological techniques, and ganglion transplants or other surgical techniques. Because pit building was used as the criterion of activity, chilling techniques were obviously most suitable for the study of "clocks" in Myrmeleon obscurus larvae.

As much of the recent literature points to the importance of the head region, particularly the brain, it was decided to chill this region first. The brain in Myrmeleon obscurus larvae lies very close to the dorsal surface of the head which is very much flattened to act as a shovel for digging. The brain is thus easily chilled from the dorsal surface. Whole ant-lions were also chilled in an attempt to stop any neurosecretory "clocks" located in other parts of the body.

Chilling of ant-lion heads was carried out using a short, thin piece of copper wire, one end of which was passed into a container of solid carbon dioxide. The free end of the wire was placed on the centre of the ant-lion's head with the aid of a micromanipulator. The animal itself was not anaesthetized but was firmly held with plasticine. The exact temperature at the surface of the head was not determined but it was noted that the copper wire remained frosted with ice crystals throughout the chilling period.

It has been shown that the larvae of <u>Myrmeleon</u> <u>obscurus</u> were normally active at approximately 6 p.m. Chilling was thus performed between 5 p.m. and 6 p.m. as it was assumed that at this time chilling would have its greatest effect on the activity rhythm. As a control an animal was held down with plasticine for the same length of time without being chilled. Both ant-lions were then placed in actographs in the constant dark and their activity recorded for a further five days. Each animal was fed one ant a day and the laboratory was maintained at a temperature of 18 ± 3°C. This experiment was repeated with a second individual and the results obtained confirmed those from the first individual.

The results of this chilling experiment are shown in Fig. 25 where the activity records for both control and chilled animals are given. Chilling the brain initially inhibited all activity for a few days, but thereafter the rhythm continued as before. There was no visible shift in the rhythm at all. Two alternative explanations are possible. Firstly, the brain might have nothing to do with the control of activity rhythms in ant-lion larvae. Secondly, the daily rhythm might have been maintained by another "clock" located in some other part of the body. Such a situation would be similar to the one reported by Harker (1956) for <u>P</u>. <u>americana</u>. Harker reported that a second "clock", located in the corpora cardiaca, could be reset by placing the animal in darkness during the

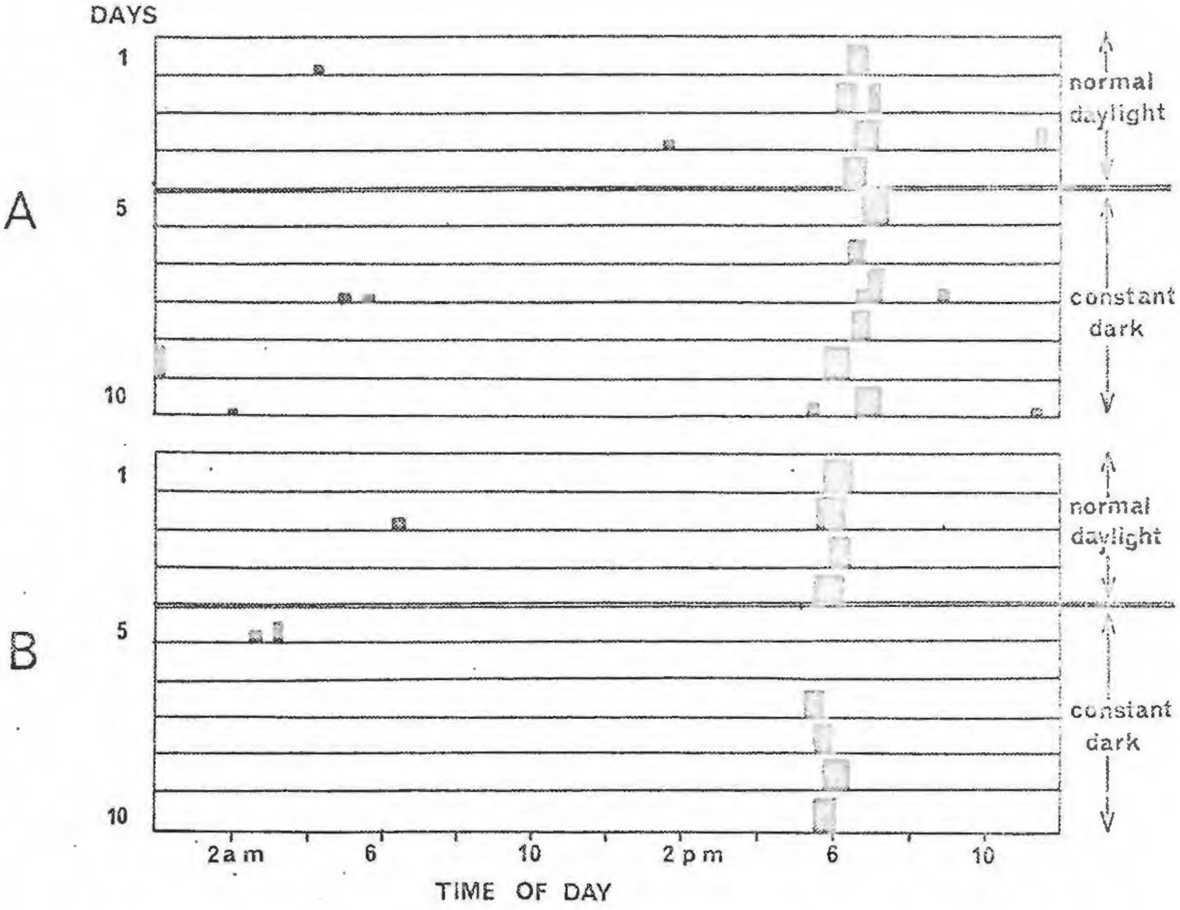


Fig. 25. *The daily activity of two* *__Myrmeleon obscurus__* *larvae. Animal A was the control which was kept under normal daylight conditions for four days. On day five it was held down with plasticine between the hours of 5 p.m. and 6 p.m. It was then replaced in the actograph and its activity recorded, in the constant dark, for a further five days.*

Animal B was kept in normal daylight conditions for four days. On day five its head was chilled between the hours of 5 p.m. and 6 p.m. The activity of this chilled ant-lion was then recorded for a further five days.

chilling period. An experiment was thus performed in which the brain of a Myrmeleon obscurus larva was chilled in the dark. As in the earlier experiments the chilling was performed between 5 p.m. and 6 p.m. and the activity of the antlion was recorded in constant dark.

The results of this experiment are shown in Fig. 26. From this figure it can be seen that chilling initially inhibited all activity but thereafter the rhythm showed two activity peaks. The first of these activity peaks tended to occur earlier each day between day 8 and day 12. The second activity peak generally occurred at the normal time, that is, at the same time as in the unchilled animal. On some days this peak was up to 30 minutes later than usual but this variation was no greater than that found in a normal unchilled animal. The significance of the two distinct peaks noted in this experiment is doubtful. It may indicate that there were two "clocks", one of which was put out of action by being chilled in the dark, while the second "clock" continued unchanged. This explanation, however, seems unlikely since chilling would be expected to delay the clock rather than speed it up. Apart from the two main activity peaks some slight activity was also evident at other times of the day but this was no more marked than in a normal animal.

The results obtained in these experiments were indecisive and it was decided to turn to group experiments. A group of 96 Myrmeleon obscurus larvae was placed in a refrigerator

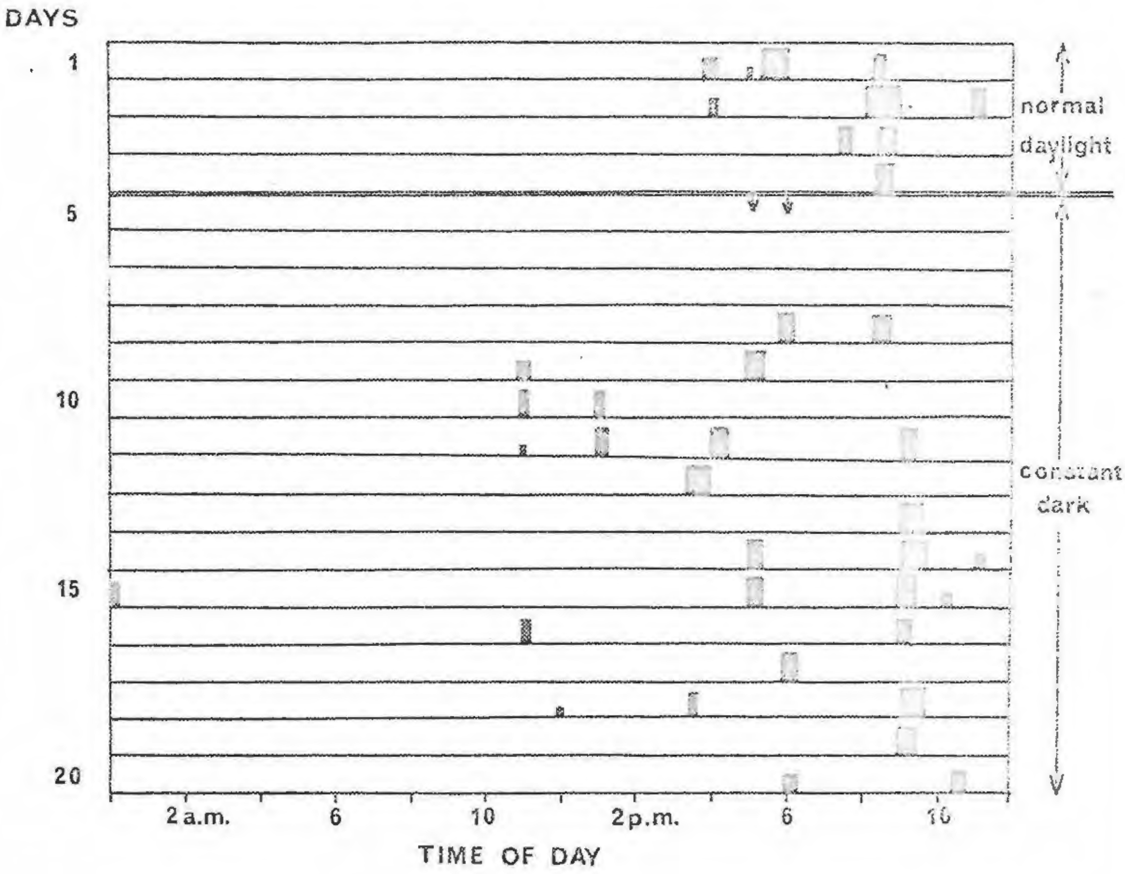


Fig. 26. The daily activity of a Myrmeleon obscurus larva before and after chilling the brain. During the first four days of the experiment the animal was kept in normal daylight conditions. On day five its head was chilled between the hours of 5 p.m. and 6 p.m. During this chilling period the ant-lion was kept in the dark. The activity was then recorded in the constant dark for a further 16 days. The temperature of the laboratory was 18°C ± 3°C and the ant-lion was fed one ant a day.

at a temperature of 4.4°C. The temperature of 4.4°C was chosen since it has been shown that no pit building activity takes place below 11°C. The internal temperature of the larvae was not recorded during the chilling period but, since the larvae were completely exposed without any insulating sand, it was assumed that their internal body temperature would drop to a figure very close to the chilling temperature. The group of ant-lions was chilled between 3 p.m. and 6 p.m. After chilling the larvae were replaced in their dishes of sand and returned to the laboratory where they were kept under natural daylight conditions and a temperature of 18 ± 3°C. By the following morning the entire group had constructed pits. These pits were destroyed and the activity of the ant-lions was then observed every 15 minutes for the following 24 hours. A control group of 96 unchilled ant-lions was observed at the same time. The activity of both groups was estimated as described in section 4.1 and the results are shown in Fig. 27.

From Fig. 27 it can be seen that there was very little difference between the chilled and unchilled groups except at dawn when the chilled group showed an unusually high level of activity. It may be that the normal peak of activity at dusk was controlled by a "clock" which was insensitive to low temperatures, while the dawn peak of activity could have been due to a second "clock" which was delayed by the period of chilling. This suggestion, however, seems unlikely since the delay in the second "clock" was longer than the original

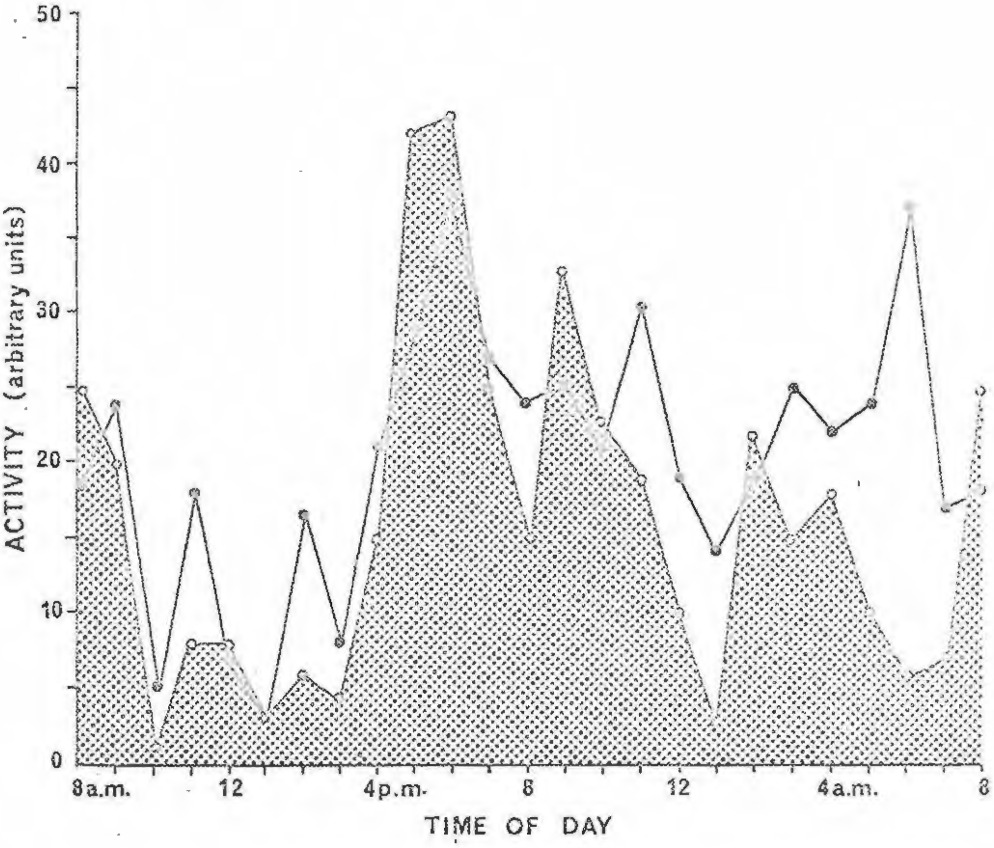


Fig. 27. The activity of two groups of 96 Myrmeleon obscurus larvae observed over a 24 hour period. The activity was estimated as described in the text (section 4.1). The shaded area represents the control group which was kept in the laboratory under normal daylight conditions and a temperature of 18 ± 3°C. The unshaded graph represents the test group, which was chilled to 4.4°C in a refrigerator between the hours of 3 p.m. and 6 p.m. on the day preceding the activity observations. After chilling these ant-lions were returned to the laboratory and kept under normal daylight conditions and a temperature of 18 ± 3°C.

period of chilling. These results also conflicted with results of earlier experiments in which the second "clock" appeared to be speeded up by the chilling of the brain.

Further experiments were thus performed in which different groups of ant-lions were chilled for varying lengths of time. Four groups of 48 ant-lions were used. One of these groups (group A) was the control which remained unchilled. The remaining three groups (groups B, C and D) were chilled in the refrigerator at 4.4°C on the day preceding the activity observations. The groups were chilled for the following lengths of time: group B, 2 hours (4 p.m. to 6 p.m.); group C, 4 hours (2 p.m. to 6 p.m.); group D, 6 hours (12 noon to 6 p.m.). All groups were then returned to the laboratory where they were maintained under natural daylight conditions and a temperature of 18 ± 3°C. The activity observations were begun at 8 a.m. the following morning and continued for a period of 24 hours. The activity of the various groups was estimated as described in section 4.1.

The results of this experiment are shown in Fig. 28. From this it can be seen that chilling reduced the general level of activity. Group D, which was chilled for the longest period of time, showed the lowest activity. In all groups, except group D, there was a marked peak of activity in the early evening. This peak occurred from one to two hours later in the chilled groups than it did in the control.

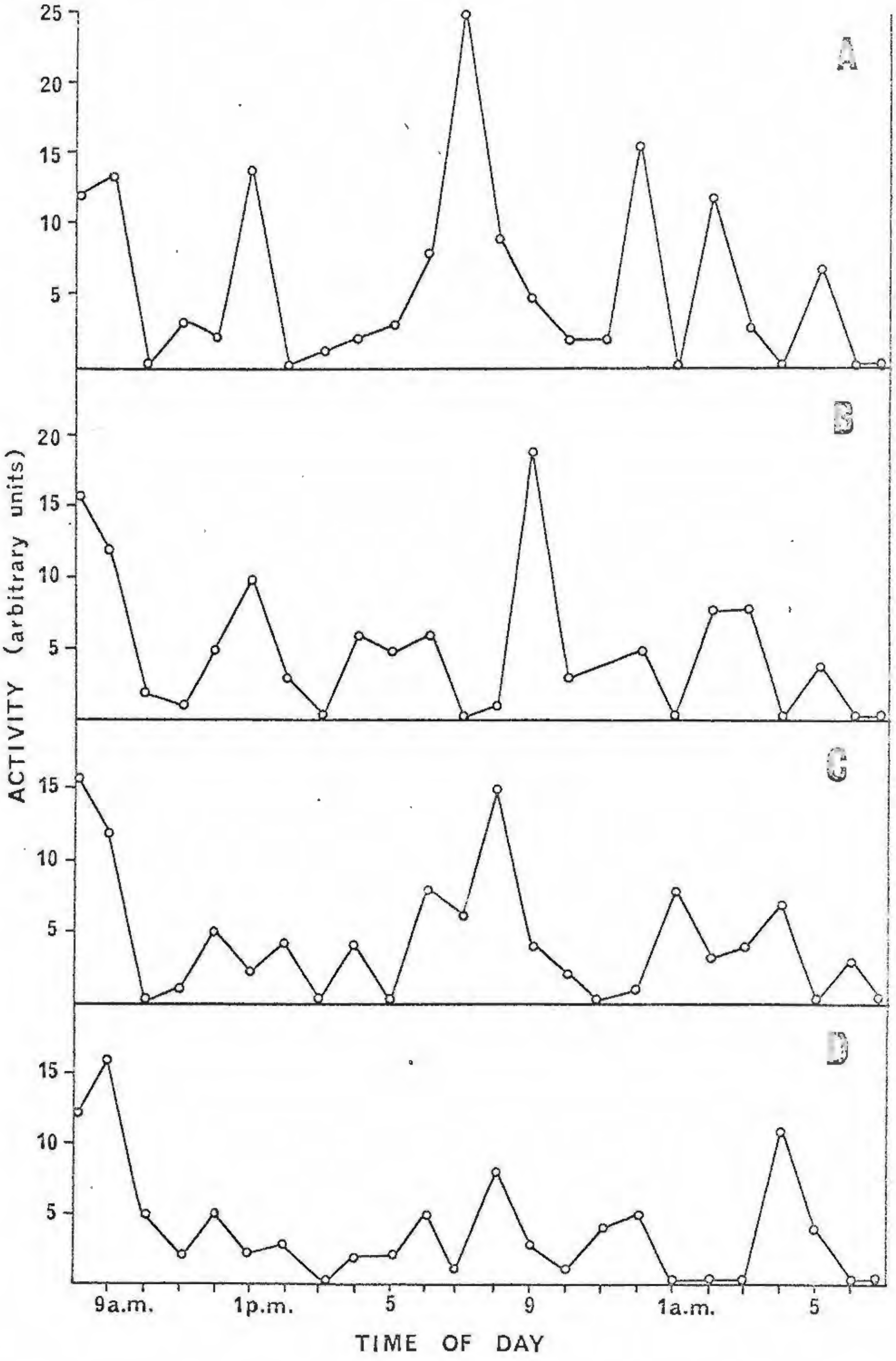


Fig. 28. The activity of four groups of 96 Myrmeleon obscurus larvae chilled for various lengths of times. The activity of each group was observed over a 24 hour period as described in section 4.1. Group A, control kept in laboratory at 18 ± 3°C; group B chilled in the refrigerator for 2 hours; group C, chilled for 4 hours; group D, chilled for 6 hours. All groups were chilled to 4.4°C. For further details see text.

This delay in the activity was not related to the length of time for which the groups had been chilled. All the groups also showed several peaks of activity during the night but again no relationship was evident between these peaks and the length of the chilling period.

The results obtained from this series of chilling experiments were thus inconclusive. In some experiments there appeared to be evidence for a "clock" controlling the activity rhythm which was upset by chilling to low temperatures. In other experiments, however, chilling did not appear to have any effect at all, apart from lowering the general level of activity. No definite conclusions could thus be drawn about the location of a "clock" controlling activity in Myrmeleon obscurus.

5. THE LUNAR RHYTHM OF PIT BUILDING ACTIVITY

5.1 THE EXISTENCE OF A LUNAR RHYTHM IN FIELD AND LABORATORY POPULATIONS

In many of the preliminary experiments on the effects of feeding frequency, temperature, and crowding, it was found that there were peaks in the pit building activity which could not be explained by the experimental conditions. These peaks, which occurred in addition to the normal diurnal activity rhythm, were found to be correlated with the phases of the moon. The lunar rhythms were expressed as variations in pit volume and were therefore investigated by taking daily measurements of the pit sizes of many individual animals. The depth, and diameter, of each conical pit was measured, and these readings were then converted to volumes using the normal formula for a cone. The times of moonrise and moonset, together with other lunar information, was obtained from the Astronomical Ephemeris (Southern Latitudes -35° plus 10 minutes).

Two examples of lunar activity peaks are shown in Fig. 29. The first of these graphs (Fig. 29A) was obtained from experiments on feeding frequency (section 3.3), while the second graph (Fig. 29B) was obtained from crowding experiments (section 3.2). In both cases the pit volume reached its maximum at the time of full moon.

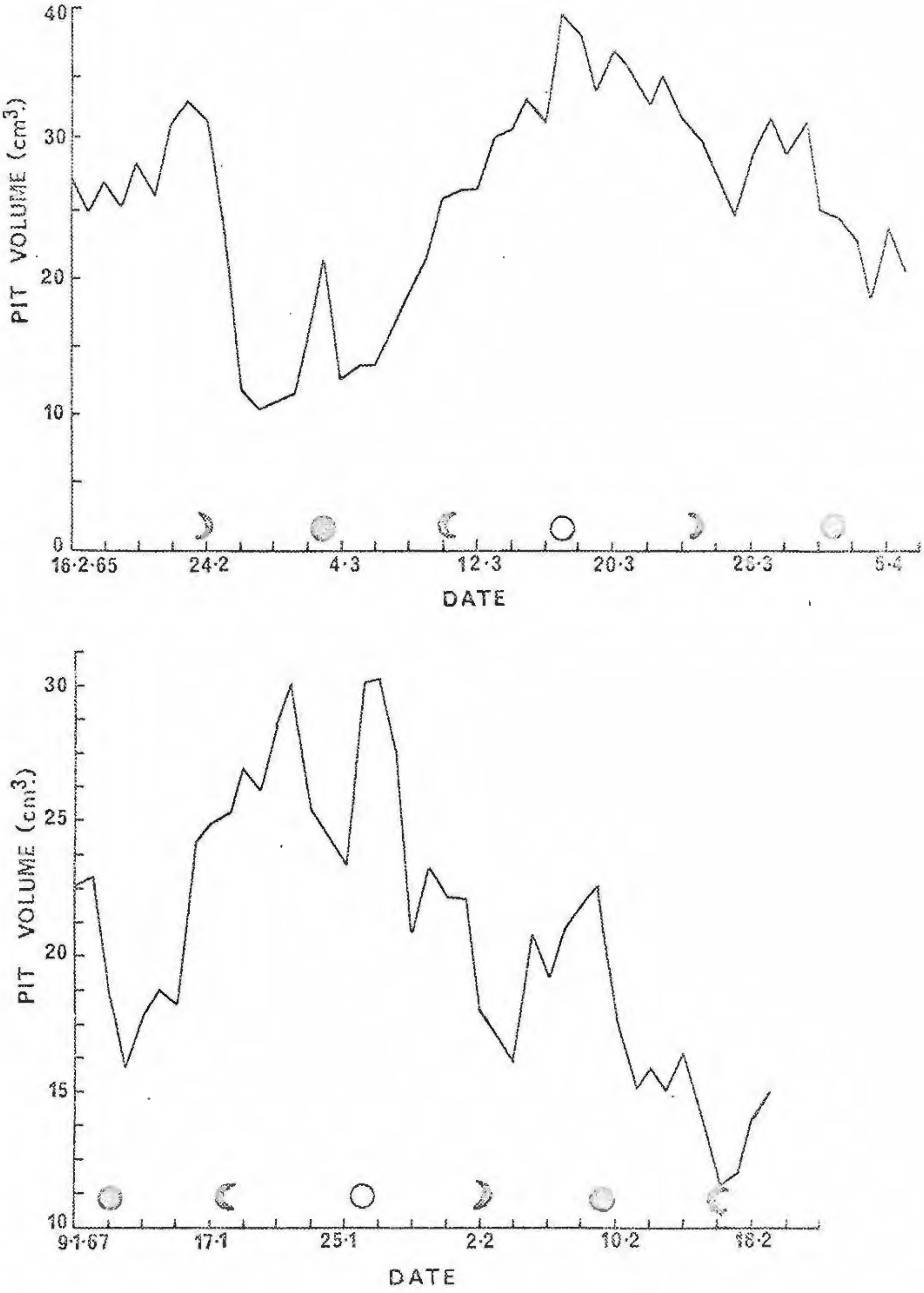


Fig. 29. Fluctuations in the mean daily pit volume of two groups of Myrmeleon obscurus larvae and the correlation of these fluctuations with the lunar cycle. ○ full moon, ☽ last quarter, ● new moon, ☾ first quarter.

A. Group A from the experiments on feeding frequency (section 3.3). This group of 12 ant-lions was kept under normal daylight conditions and each individual was fed two ants a day.

B. Results from an experiment on the effects of crowding on pit size (section 3.2). This group consisted of 10 ant-lions. The animals were subjected to normal daylight conditions and each individual was fed one ant a day.

The effect of a lunar pit building rhythm was also noted in the experiments which were performed to investigate the effect of pit disturbance on pit size (section 3.6). In both the undisturbed and the disturbed groups the pits increased in volume before full moon. After full moon the pits in the disturbed group dropped rapidly in size. The pits of the undisturbed group, on the other hand, were not actively destroyed but showed a gradual collapse due to disturbance by prey etc. This led to pits of irregular shape. These observations suggested that myrmeleontid larvae were more active in constructing pits at full moon than at other stages of the lunar cycle.

In order to investigate the lunar pit building rhythm, the pits of a field population consisting of about 50 Myrmeleon obscurus larvae were measured every day for a year. The number of pits constructed by this population fluctuated slightly from day to day but, since there was such a large population, this did not affect the mean pit volume significantly. There were a number of reasons for the fluctuations in the numbers of pits. Larvae which were moulting did not construct pits, some ant-lions also pupated during the experimental period while new larvae hatched. After disturbance of the pits by wind or rain some individuals reconstructed their pits faster than others and this also led to fluctuations in the number of pits present each day. During periods of rain or strong wind no pits were present and thus the record is incomplete.

The results obtained from this experiment are shown in Fig. 30, where the mean daily pit volume for the group is plotted and the times of full moon indicated. This figure shows that in the field the pits reached their maximum volume at about the time of full moon. These lunar peaks of pit building activity were most marked during the summer months from October to February. During the winter months the peaks decreased in amplitude and were hardly visible at all during August and September. It has been shown (section 3.5) that low temperatures decreased the size of the pits constructed by ant-lions and it seemed likely that low temperatures were inhibiting the expression of the lunar cycle.

Suppression of the lunar activity peak by low temperatures has, in fact, been demonstrated in laboratory experiments. In these experiments four groups of 12 ant-lions were used. All the groups were kept at 25.5 ± 1 °C for seven days to eliminate any effects of their previous thermal history. The various groups were then subjected to the following temperatures for the next 14 days:

group A - 25.5 ± 1°C
group B - 27 ± 1°C for five days and 29 ± 1°C for nine days
group C - 18 ± 1°C
group D - 21 ± 1°C

Thereafter all the groups were returned to 25.5 ± 1°C for a further seven days.

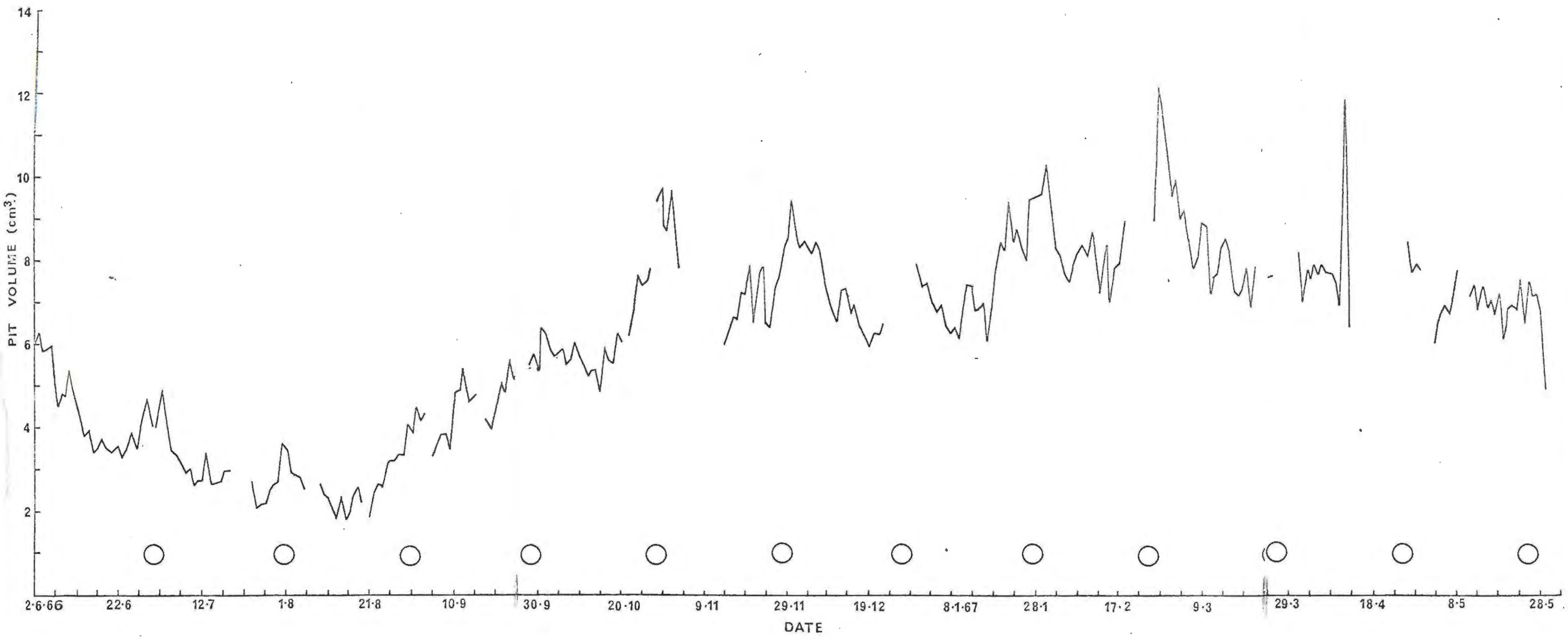


Fig. 30. The mean daily pit volume of a field population of about 50 Myrmeleon obscurus larvae. Gaps in the record reflect periods when, due to rain or wind, no pits were present. The times of full moon are indicated by circles.

The results obtained from groups A, B, C and D are illustrated in Figs. 31 A and B. The times of full moon are also illustrated. It can be seen that the control group A, which was kept at a constant temperature throughout, showed a very clear lunar peak. It was thought, however, that this peak might be due to the large number of pupations which took place towards the end of the experimental period. Pupation results in the removal of the largest ant-lions from the population and could thus have caused a drop in the mean pit volume. The mean pit volume was therefore recalculated, excluding any readings from animals which had pupated during the experimental period. From Fig. 31 (A) it can be seen that, although this correction decreased the level of the graph slightly, it did not alter the shape significantly. Also, since graphs of individual animals showed similar trends, the peak could not have been due to the procedure used to obtain mean values. The peak shown in group A thus represented a true lunar cycle of pit building activity.

In group B the temperature was raised to 27-29°C, and these results did not differ significantly from those of the control group. In groups C, and D, the lunar cycle was clearly depressed by the decrease in temperature. This depression was most marked in group C where the temperature was lower than it was in group D. The pit volume of both groups returned to normal once the temperature was raised.

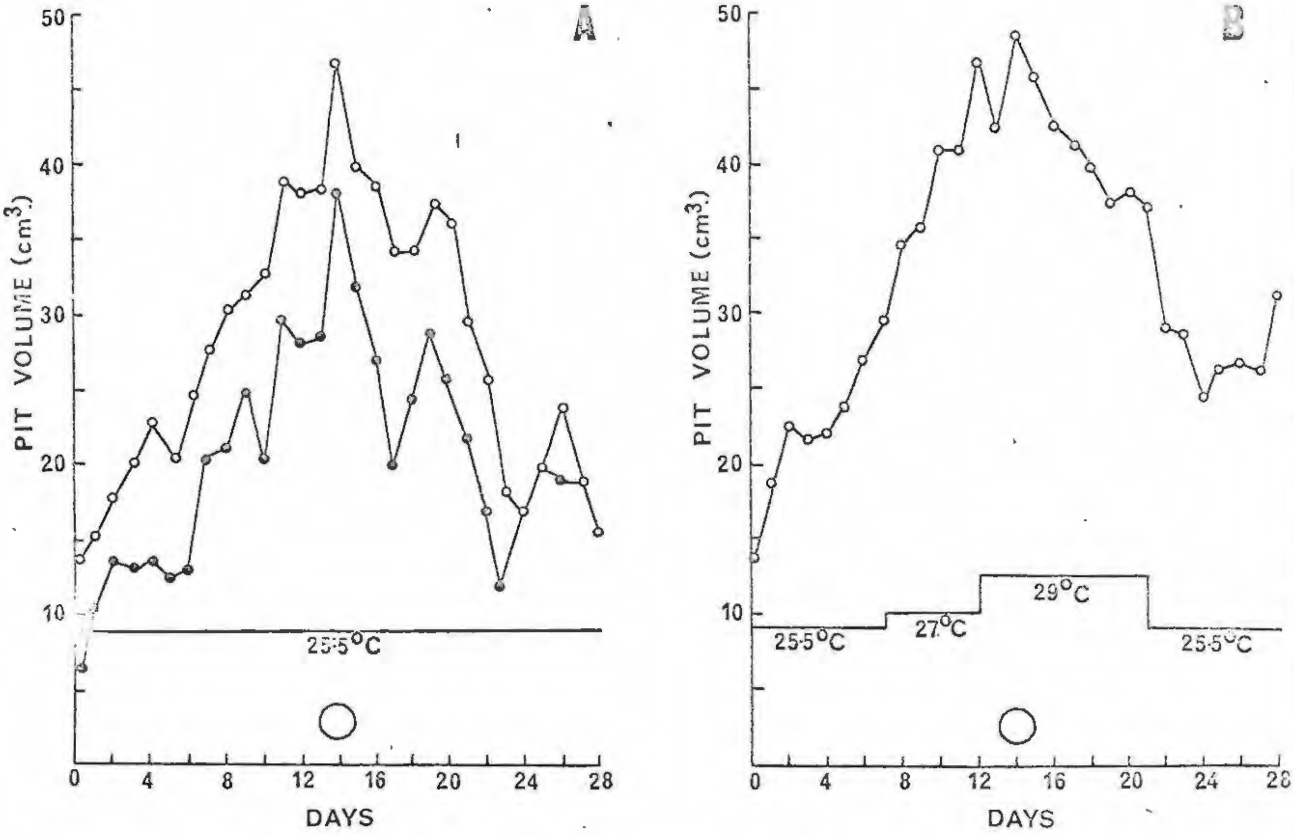


Fig. 31 A. The mean pit volume of different groups of Myrmeleon obscurus larvae subjected to changes in temperature. The larvae were each fed one ant a day.

A. Control group kept at a constant temperature of 25.5 ± 1°C

The corrected values shown in the lower graph (o———o) include only those animals which did not pupate during the course of the experiment.

B. Group in which the temperature was raised to 27-29°C (both ± 1°C).

The times of full moon are indicated by the circles at the bottom of each graph.

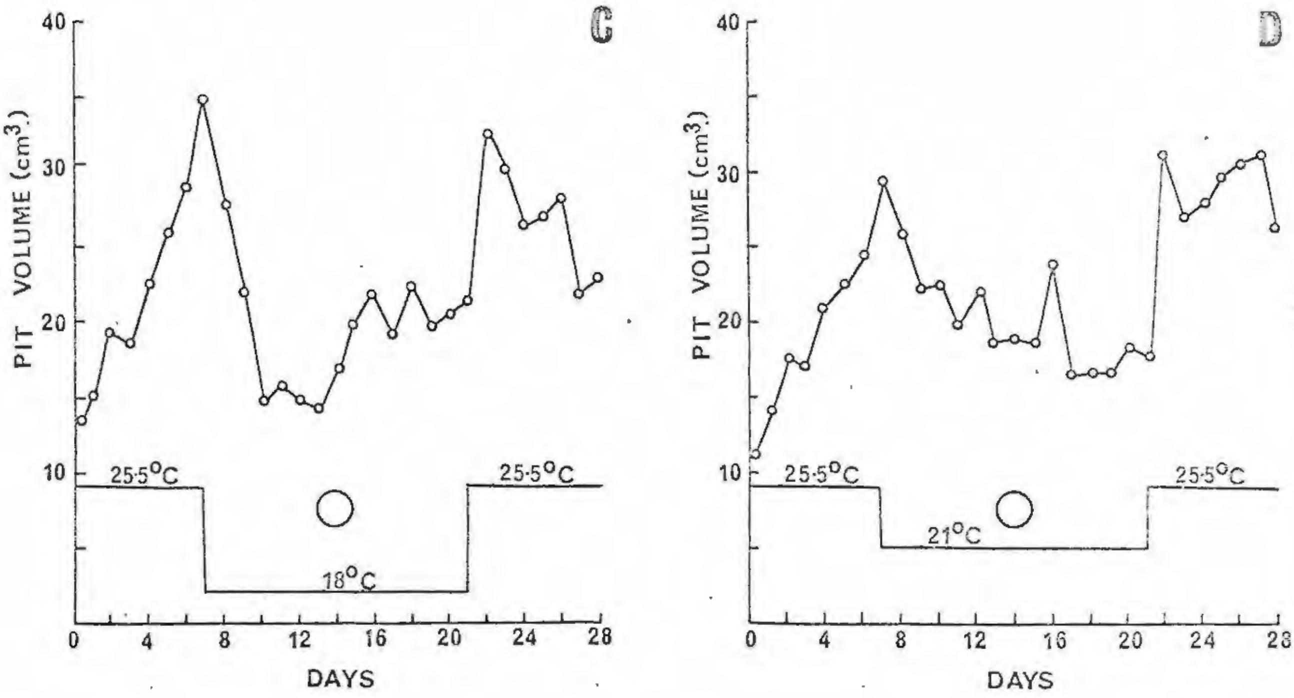


Fig. 31 B. The mean pit volume of different groups of *Myrmeleon obscurus* larvae subjected to changes in temperature. The larvae were each fed one ant a day.

C. Group in which the temperature was decreased to 18 ± 1°C

D. Group in which the temperature was decreased to 21 ± 1°C

The times of full moon are indicated by the circles at the bottom of each graph.

This experiment indicated that the absence of some lunar peaks in the field experiment could have been due to a temperature effect. In working on lunar rhythms in the laboratory therefore, it is essential to keep the temperature constant.

The effect of temperature on the lunar cycle was more clearly demonstrated in the experiments involving temperature summations (section 3.5). In these experiments pit volumes at various stages of the lunar cycle were correlated with the temperature summation for the preceding seven days. (The temperature summations were expressed as hour degrees centigrade above 10°C). The results obtained for full moon, new moon, first and last quarters are shown in Fig. 32. This figure shows that at low temperatures the pit volume was small regardless of the stage of the lunar cycle. At higher temperatures, however, the pit volume was much greater at full moon than it was at new moon, while intermediate values were obtained at first and last quarters. For example, at a temperature summation of 1,000 hour degrees centigrade per week the pit volume at both new and full moon was about 3.2 $cm.^3$ At 2,000 hour degrees centigrade, on the other hand, the full moon pit volume was about 10.0 cm^3., while the new moon value was only 7.2 cm^3. Thus, although a rise in temperature always caused an increase in the pit volume, this increase was much greater at full moon than it was at new moon. This experiment also confirmed that low temperatures could override the lunar cycle, since at low

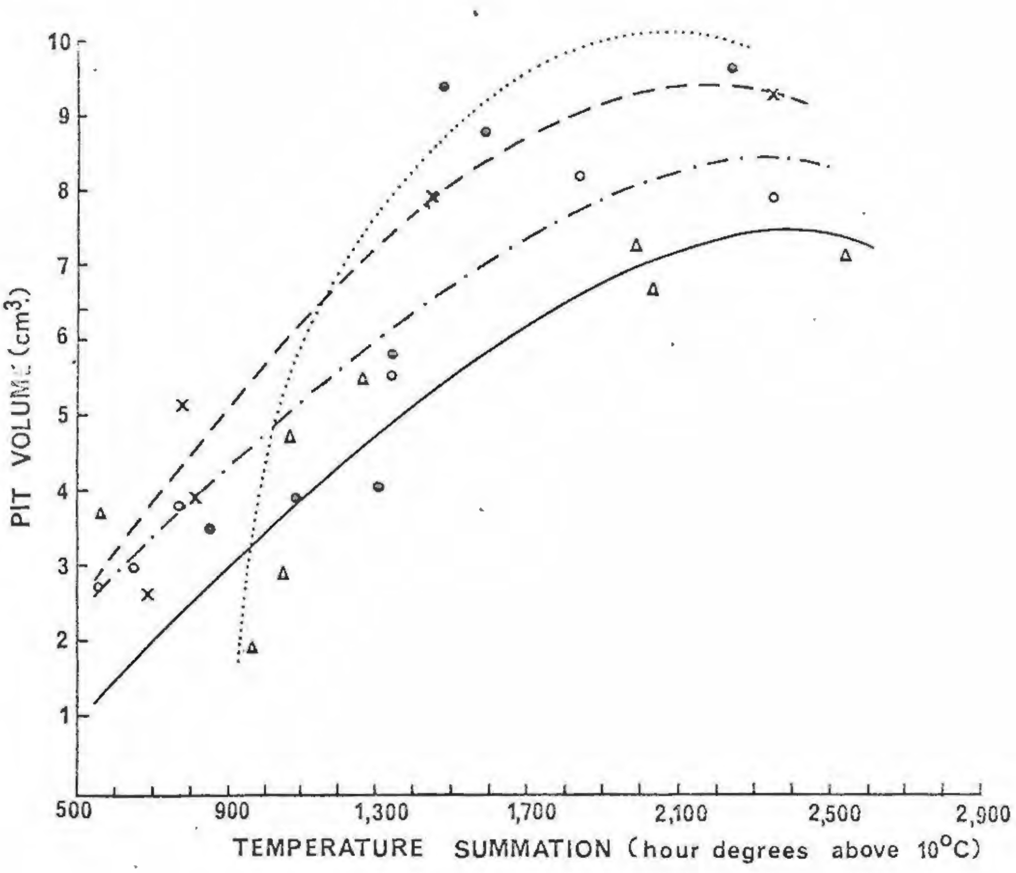


Fig. 32. The pit volume of a field population of about 50 Myrmeleon obscurus larvae correlated with the temperature summations for the preceding seven days. The temperature summations are expressed as hour degrees above 10°C. The pit volumes were recorded at new moon △———△ , full moon ●··········● , first quarter x-----x , and last quarter o-·-·-o .

temperatures there was no difference between the new and full moon values.

This work established that Myrmeleon obscurus larvae had a lunar cycle of pit building activity, with the peak occurring at the time of full moon. As with the daily activity rhythms, it was important to determine whether this lunar rhythm was purely a direct response to external environmental factors, such as the light of the moon, or whether it was endogenous. The criterion of an endogenous rhythm is that it will continue for a time under constant conditions, and the lunar rhythm was thus studied both in constant light and in constant dark.

5.2 THE LUNAR PIT BUILDING RHYTHM UNDER CONSTANT CONDITIONS

There is very little information on lunar rhythms in insects and most of this work appears to indicate that these rhythms are purely direct responses to the light of the moon. For example, Vanderplank (1941) showed that the biting activity of Glossina pallidipes had a lunar component. He states, however, that these insects were more active on moonlight nights than on dark nights, and correspondingly less active during the day after full moon. Their lunar rhythm was thus a direct response to the light of the moon. The remaining work on lunar rhythms in insects is largely restricted to reports of insect catches at light and suction traps (Williams and Singh, 1951; Williams, Singh and El

Ziady, (1956), and to lunar swarming of the so-called "lake flies" (including mayflies, chironomids and chaoborids) by Corbet (1958), Fryer (1959), Macdonald (1956), and Hora (1927). Finally Kerfoot (1967) has discussed the lunar periodicity in the foraging behaviour of a nocturnal bee, Sphecodogaster. In none of these cases is there any evidence that the activity was due to endogenous lunar rhythms, such as are found in a large number of marine animals (Enright, 1963). The only evidence for an endogenous lunar activity rhythm in insects is that of Hartland-Rowe (1958) who worked on the mayfly Povilla adusta at Lake Victoria. These mayflies regularly emerge in their greatest numbers on the second night after full moon. This rhythm was shown by Hartland-Rowe to be maintained after the larvae had been kept in darkness for ten days, and in the case of two individuals after six weeks. No experimental details are given, however, and the existence of endogenous lunar rhythms in insects remains in doubt.

Preliminary observations had indicated that the lunar pit building rhythm of Myrmeleon obscurus larvae might be endogenous and hence a number of experiments were performed under constant conditions. A group of 12 ant-lion larvae was kept at a constant temperature of 29 ± 1°C in continuous darkness. The temperature of 29°C was used since it was known that at this temperature the lunar cycle would be clearly expressed (see section 5.1). The ant-lions were each fed one ant a day, at times which were randomly determined. Some light was obviously necessary during feeding and pit measurement and a very dim red light, to which these animals are insensitive, was used. The size of the pits was measured each day for a period of 92 days, after which time the experiment had to be discontinued due to the pupation of a large number of larvae. The results illustrated in Fig. 33 show that the ant-lions in constant dark exhibited a very clear lunar rhythm for two months, after which time the rhythm was lost.

The above experiment indicated that the lunar activity rhythm was endogenous since it continued unchanged for two months in constant dark, after which time the rhythm broke down. This evidence is, however, by no means conclusive, and an attempt was made to clarify the position by experiments conducted in constant light.

As in the previous experiment a group of 12 ant-lions was maintained at a constant temperature of 29 ± 1°C. Instead of being in constant darkness these animals were exposed to light of approximately 1,500 lux, which was obtained from two 75 W light bulbs. The lights were placed at least three feet away from the experimental animals so that they did not affect the temperature significantly. Readings of pit size were continued for 52 days, after which time the pits were extremely small and difficult to measure. A large number of larvae had also pupated. The results which are

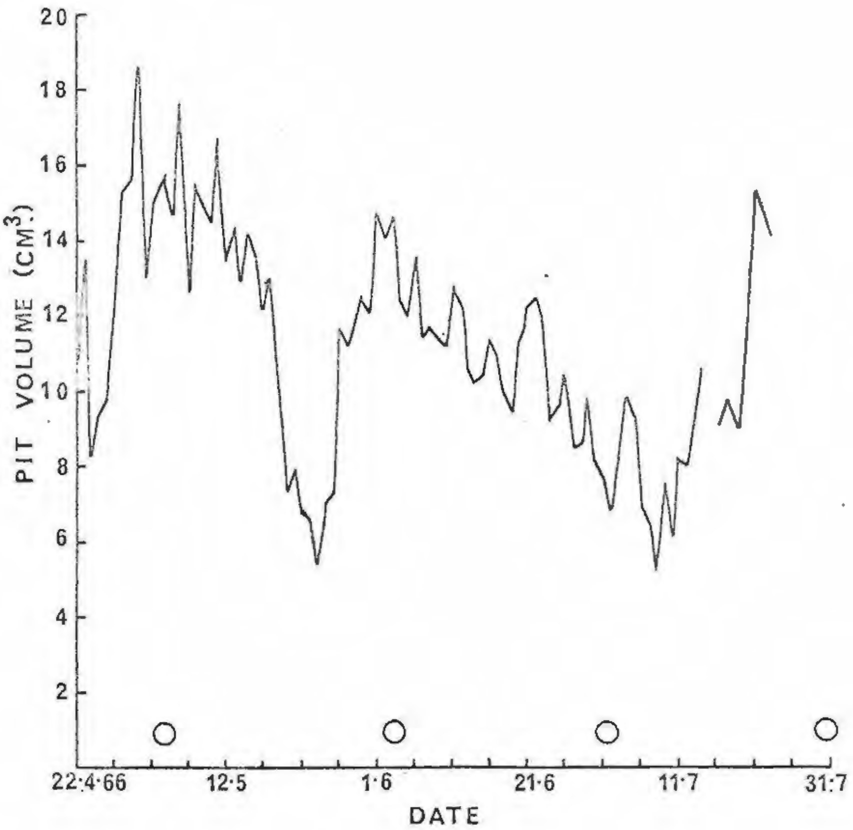


Fig. 33. *The mean daily pit volume of a group of 12 Myrmeleon obscurus larvae in continuous darkness at a constant temperature of 29°C. The times of full moon are indicated by the circles at the bottom of the figure.*

expressed in Fig. 34 show that the lunar cycle occurred as normal during the first month. Thereafter the pit building activity appeared to be inhibited by the constant light, as the pit size dropped steadily until the end of the experiment.

The above experiments indicated that the ant-lion larvae had an endogenous lunar cycle of pit building activity. The largest pits were constructed at the time of full moon and this rhythm continued for at least two months in constant dark and one month in constant light. After a month in constant light not only was the lunar rhythm lost but all pit building activity was inhibited. Such rapid damping of a rhythm by light is well known in circadian rhythms, and a number of examples are quoted by Harker (1958). In addition to damping the rhythms, continuous light is also reported by Harker to completely inhibit activity in a variety of different animals, including Ephestia and Drosophila. The fact that the lunar rhythm of ant-lions did not continue for more than a month in constant light need thus not be regarded as evidence against its endogenous nature.

5.3 LUNAR RHYTHMS IN VERY YOUNG LARVAE

The lunar pit building rhythm observed in field and laboratory populations of Myrmeleon obscurus larvae was thought to be endogenous and it therefore seemed likely that it would also be innate. If the rhythm was innate it should be present in the ant-lion larvae at the time of hatching, although the light

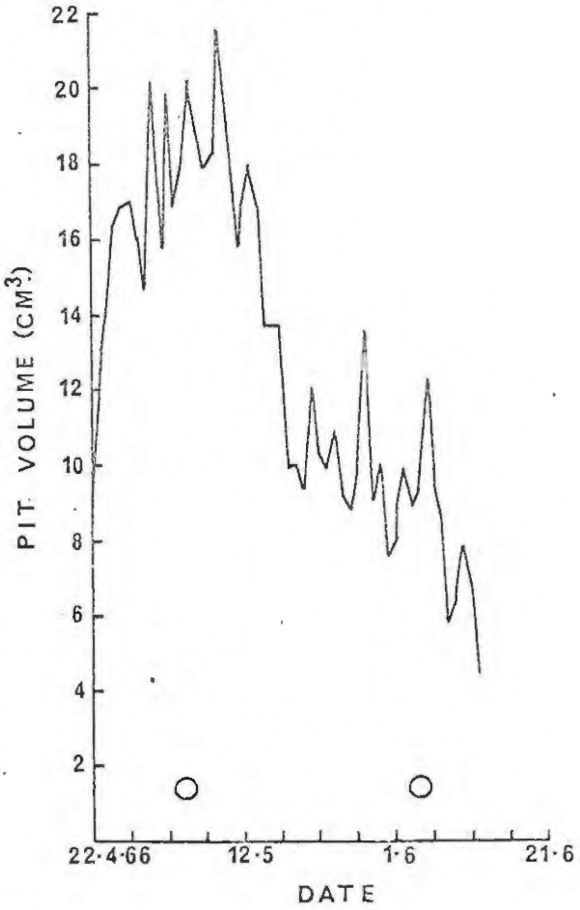


Fig. 34. *The mean daily pit volume of a group of 12* *__Myrmeleon obscurus__* *larvae in continuous light of approximately 1,500 lux. The temperature was kept at 29 ± 1°C. The times of full moon are indicated by the circles at the bottom of the figure.*

of the moon might be required to set the rhythm accurately. In order to determine whether or not the lunar rhythm was innate the rhythms of very young Myrmeleon obscurus larvae were studied. Originally it was hoped that newly hatched larvae, obtained from the breeding of adult insects, could be used. During the three years of this research, however, it was not possible to get the adults to breed at all in captivity, although they survived for up to two weeks. Had breeding been successful the lunar and daily rhythms could also have been followed through several generations bred under different conditions.

As newly hatched larvae were not available, a large number of ant-lions were collected in the field and, using body length as a measure of age, was sorted into the following groups: less than 5 mm. (group A), 5-7 mm. (group B), 7-8 mm. (group C), 8-9 mm. (group D), and 9-11 mm. (group E) body length. The width of the head capsule was not used as a measure of age in these experiments due to the difficulty of measuring very small animals while they are still alive. Ant-lions with a body length of less than 5mm. were very young larvae and the smallest that could be obtained in the field. Pit building in these very young larvae was compared with that in the older larvae to establish whether the lunar rhythm was innate.

Each group contained 12 Myrmeleon obscurus larvae which were maintained in the laboratory under normal daylight conditions. The ant-lions were, however, shielded from the light of the moon. The mean daily pit volume for each group was recorded over a period of 18 weeks and the results are shown in Fig. 35, where the times of full moon are indicated by the vertical broken lines.

Fig. 35 shows that in groups B, D and E, which contained large larvae, an obvious lunar pit building rhythm was present. Group C also contained large larvae but this group showed a very indistinct lunar rhythm. Why the rhythm should have been so weak in this particular group is not clear. Group A, containing very small larvae with a body length of less than 5 mm., at first showed very indistinct lunar cycles. Measurements taken during this early stage of the experiment were, however, very inaccurate, due to the difficulty of measuring such small pits. The fact that the results were plotted in cubic centimeters also tended to eliminate small differences in pit size, since a difference of a few millimeters was not obvious when the results were converted to centimeters. As the ant-lions in group A increased in size, however, the lunar rhythm became more evident, until by the fourth full moon period the amplitude was equal to that of the larger groups. By this time the larvae had grown considerably in size and were, in fact in the 5-7 mm. size range.

From this experiment it was concluded that the very young larvae did possess a lunar pit building rhythm, the amplitude of which increased as the animals grew in size. More detailed

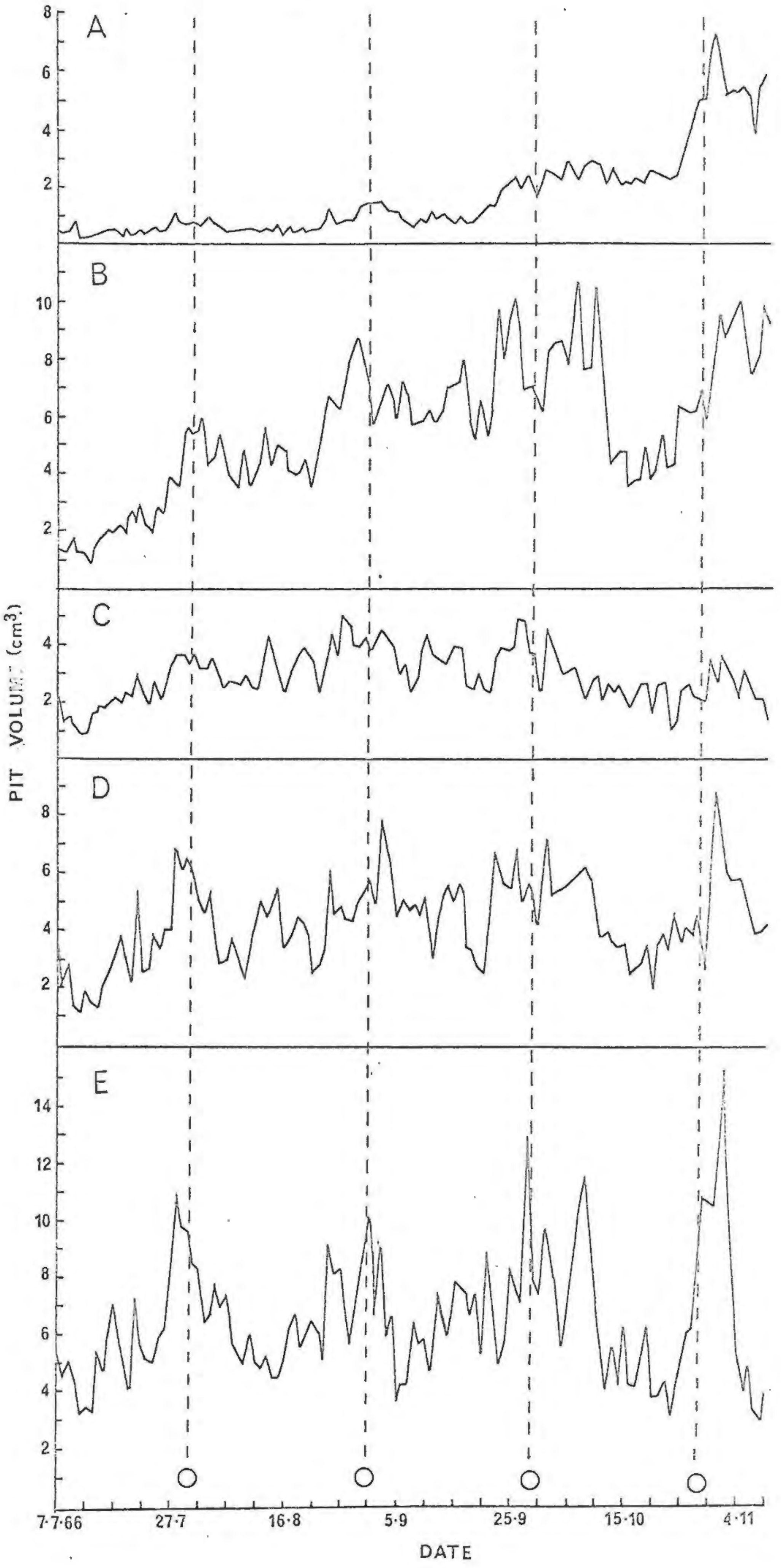


Fig. 35. *The mean daily pit volume for groups of* *__Myrmeleon obscurus__* *of various ages. The length of the body, from the base of the mandibles to the tip of the abdomen, was used as a measure of age. There were 12 ant-lions in each group which consisted of the following sizes: group A, less than 5 mm.; group B, 5-7 mm.; group C, 7-8 mm.; group D, 8-9 mm.; group E, 9-11 mm.*

The times of full moon are indicated by the circles at the bottom of the figure and the vertical broken lines.

experiments were, however, performed on newly hatched larvae. These larvae were obtained from eggs which had been extruded from the abdomen of an adult Myrmeleon obscurus female, after it had been half eaten by a larger ant-lion species. The eggs hatched successfully, and within a few hours the larvae had constructed their small pits. These newly hatched larvae were kept in the laboratory where they were subjected to normal fluctuating daylight conditions, but where they were shielded from the light of the moon.

A micromanipulator was adapted for measuring the pits and the pit volume was expressed in mm^3. instead of cm^3. A far more accurate measurement of the very small pits was thus obtained. The results are shown in Fig. 36, and from this figure it can be seen that the most obvious trend was one of rapidly increasing pit size. Although no definite lunar rhythm was evident there was a number of peaks in the pit volume. Some of these peaks occurred over the time of full moon, as in a normal lunar rhythm, while other peaks occurred at the time of new moon. Peaks in pit volume at the time of new moon have also been found occasionally in other experiments, although they were generally much smaller than the full moon peaks. Examples of new moon increases in pit volume are shown in Fig. 37.

Fig. 37 shows that, in addition to the normal full moon peaks, there were often secondary peaks in the pit volume over the new moon period. It is interesting to note that these

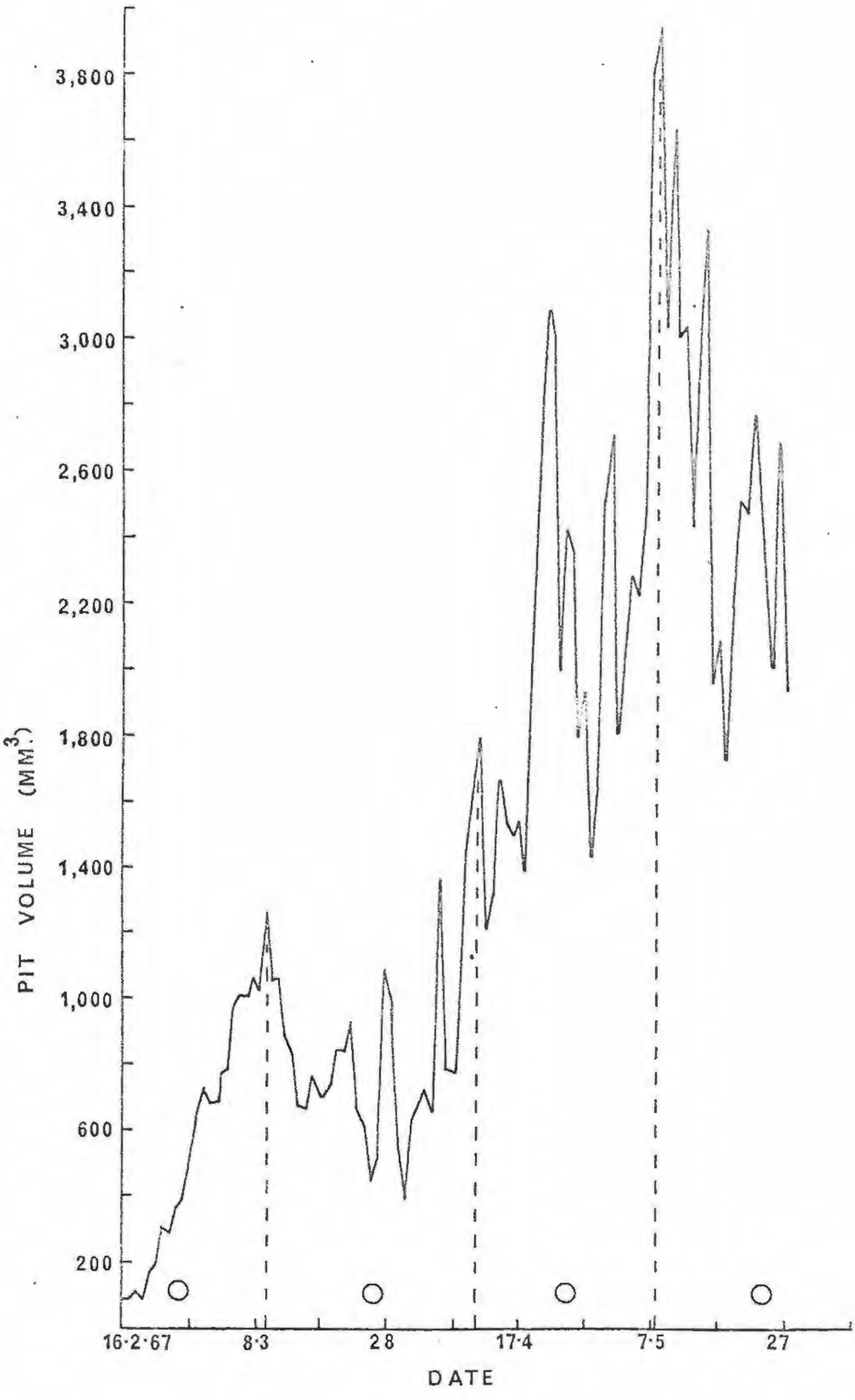


Fig. 36. The mean daily pit volume of a group of 12 newly hatched Myrmeleon obscurus larvae. The times of full moon are indicated by the circles at the bottom of the figure while the times of new moon are indicated by the vertical broken lines.

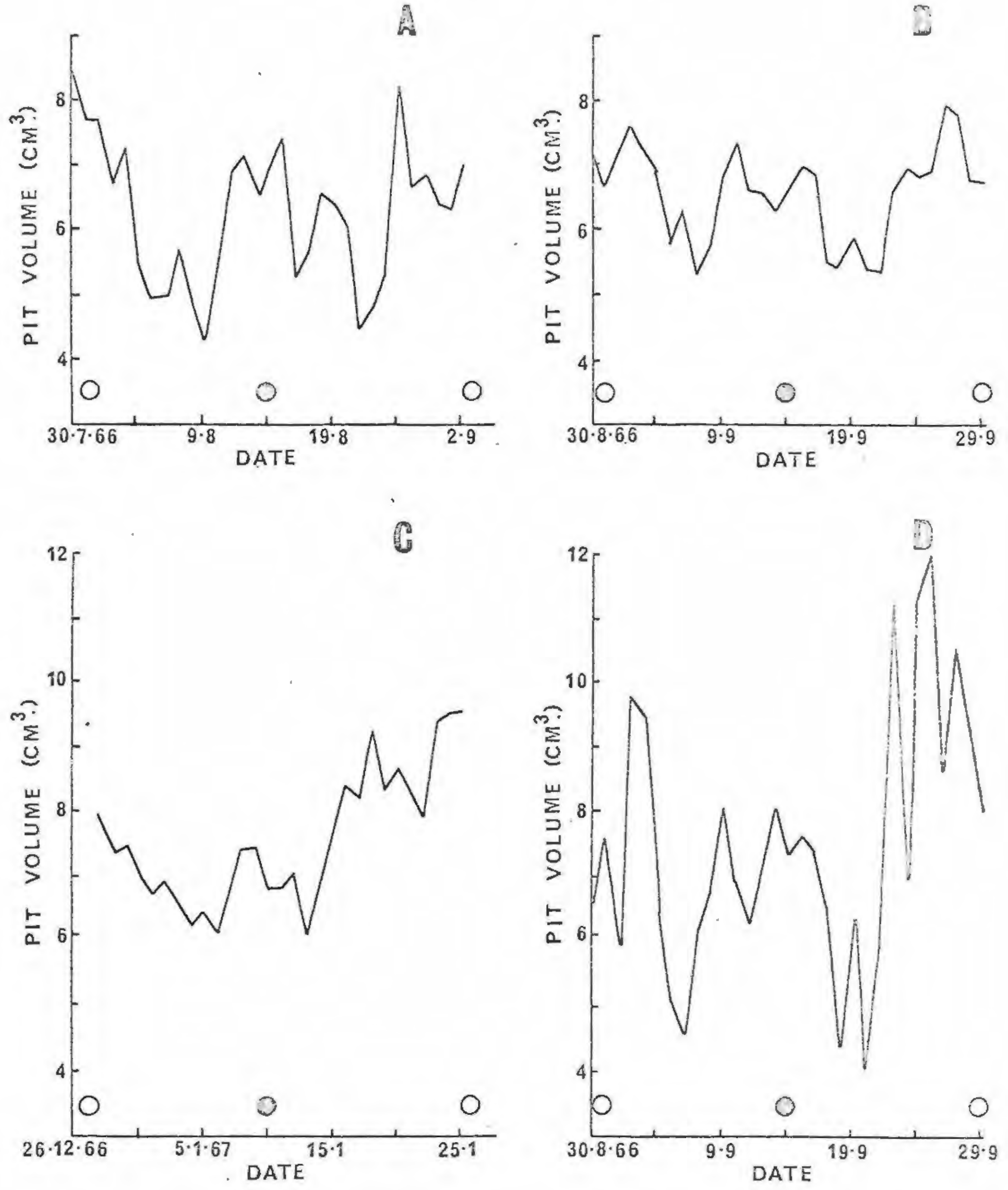


Fig. 37. *The pit volume of different groups of Myrmeleon obscurus larvae showing new moon peaks. A. Group of animals with body length 8-9 mm. from experiments on ant-lions of different ages. B. Group of animals with body length 7-8 mm. from experiments on ant-lions of different ages. C. Results from observations on the lunar cycle of a field population. D. Group of 5 animals in 858 sq. cm. of sand from experiments on the effects of crowding on pit size.*

○ *full moon,* ● *new moon.*

new moon peaks were more marked in the laboratory (Fig. 37 A, B and D) than they were in the field (Fig. 37 C). It is suggested that this was due to the fact that in the laboratory the animals were shielded from the light of the moon. The normal full moon peak was thus not reinforced each month by the action of moonlight and the new moon peak became more evident.

From these experiments on very young larvae it was concluded that young larvae obtained from the field had a lunar pit building rhythm while larvae which hatched in the laboratory did not have a rhythm. It therefore seems likely that moonlight is necessary to set the lunar rhythm. It is interesting to note that the larvae in the laboratory hatched shortly before full moon and it is probable that they would have shown a rhythm had they been exposed to the light of the moon at this time. It would be interesting to attempt to set the phase of the lunar rhythm in newly hatched larvae by exposing them to light of low intensity at times other than the normal full moon period. No further eggs could be obtained, however, in order to carry out these experiments.

5.4 EXPLANATION FOR THE LUNAR PIT BUILDING CYCLE

An endogenous lunar rhythm has been shown to exist in field and laboratory populations of Myrmeleon obscurus larvae. The obvious question arising from this is why such a rhythm should exist. A number of marine animals, such as crabs

(Naylor, 1958; Barnwell, 1963) and annelids (Market, Market and Vertrees, 1961) have been shown to possess lunar rhythmicities. These rhythms are in all cases related to the tides and to spawning. In insects a lunar rhythm has been reported in the inter-tidal midges of the genus Clunio by Neumann (1966) and here again the rhythm is related to the tides and reproduction. The remaining lunar rhythms which have been recorded in insects are largely restricted to aquatic, although not marine forms. In these insects, such as mayflies (Corbet, 1958), chaoborids (Macdonald, 1956), and chironomids (Fryer, 1959), the adults are very short lived, and the lunar rhythm ensures that all the adults will emerge at the same time, thus increasing their chances of finding a mate. In contrast to the above Myrmeleon obscurus larvae are completely terrestrial and the lunar rhythm is thus obviously not related to tidal conditions. A lunar rhythm was recorded in the larvae of ant-lions, not in the adults, and the rhythm is therefore probably not related to the synchronization of reproduction. The functional significance of the lunar rhythm in Myrmeleon obscurus if thus difficult to understand. Three different possibilities are suggested below.

(1) prey capture

(2) distribution of ant-lions in their habitat

(3) the combination of solar day and lunar day rhythms.

Each of these possibilities was investigated experimentally.

5.41 Prey capture

The first suggestion for the functional significance of the lunar rhythm in Myrmeleon obscurus larvae is that it is dependent on the numbers of prey present at various times of the month. The efficiency of ant-lions as predators depends to a large extent on the size of their pits, since it is more difficult for prey to escape from a large pit. For example, it took an ant 25-33 secs. (mean 30 secs. from 10 readings) to escape from a pit 77 mm. in diameter and 26 mm. deep, while it took only 5 - 16 secs. (mean 10 secs. from 10 readings) to escape from a pit 30 mm. in diameter and 15 mm. deep. The large pits constructed at the time of full moon should, therefore, be more efficient in trapping prey. It is possible that the lunar cycle might in some way be connected with the numbers of prey present at various stages of the lunar cycle. This explanation for the lunar cycle has the disadvantage that it involves postulating a lunar rhythm in the prey species, in addition to that found in the ant-lions themselves. The basis of this suggestion was, however, tested experimentally.

The first step in these experiments was to determine what the larvae of Myrmeleon obscurus would in fact eat. It has been reported by Turner (1915) that ant-lions eat a large variety of small invertebrates, but in order to test the food preferences of Myrmeleon obscurus larvae, numerous small animals were caught in the field and fed to ant-lions in the laboratory. In addition to ants of the genus Pheidole, which were the most abundant type of prey in the field, it was found that the larvae would eat almost any small invertebrate in the right size range, except for some very hard beetles. The list of animals that the ant-lions accepted is a long one and includes spiders, isopods, small millipedes, centipedes, large collembolans, flies, small earthworms, and some soft bodied beetles. Fast moving animals, such as spiders, were not captured as easily as the slower moving ones but, once caught, were readily eaten. By examining the remains of the prey it could easily be seen whether or not they had been eaten, since when ant-lions feed they suck all the body contents out of the prey and flick away the dried remains.

The abundance of the various prey species in the field at different times of the month was investigated using a series of traps. These traps were set in the midst of ant-lion colonies. Each trap consisted of a bottle buried level with the ground into the neck of which was inserted a funnel of about the same diameter as an ant-lion pit. This funnel led into a small collecting bottle containing a dilute solution of teepol and water. (This was found to be the most satisfactory solution since it evaporated very slowly, and did not give off any odour which might repel insects from the lip of the funnel). The ground was carefully smoothed around the lip of the funnel. This method proved to be very satisfactory in trapping small invertebrates.

In these experiments three traps were used and the contents were collected morning and evening for five months.

The totals for all morning and evening catches and the grand totals for each day were plotted. The results are shown in Fig. 38 and Fig. 39. These figures show that, while there was a considerable variation in the number of animals caught at different times, these variations did not follow any regular pattern. The numbers were probably determined by the prevailing weather conditions rather than the lunar cycle. The total number of animals caught showed a general increase with the approach of summer and the peaks on 18/11/66, 1/12/66, and 9/12/66 coincided with warm still days. Fewer animals were caught during cold and wet weather conditions (4/8/66, 5/9/66).

From these experiments it was concluded that the number of prey available was not determined by the lunar cycle. A further calculation was, however, performed to verify this conclusion. All the results obtained during the first four months were taken and the total catches for all full moon days summed. This process was repeated with one day after full moon, two days after full moon etc. From the results, which are shown in Fig. 40 it can be seen that the number of animals caught in the traps did, in fact, vary at different stages of the lunar cycle. The numbers increased shortly before new moon and decreased again thereafter. It is suggested, however, that these results are not of any great significance for two reasons. Firstly, for fluctuations in the number of prey to give rise to the very definite lunar rhythms

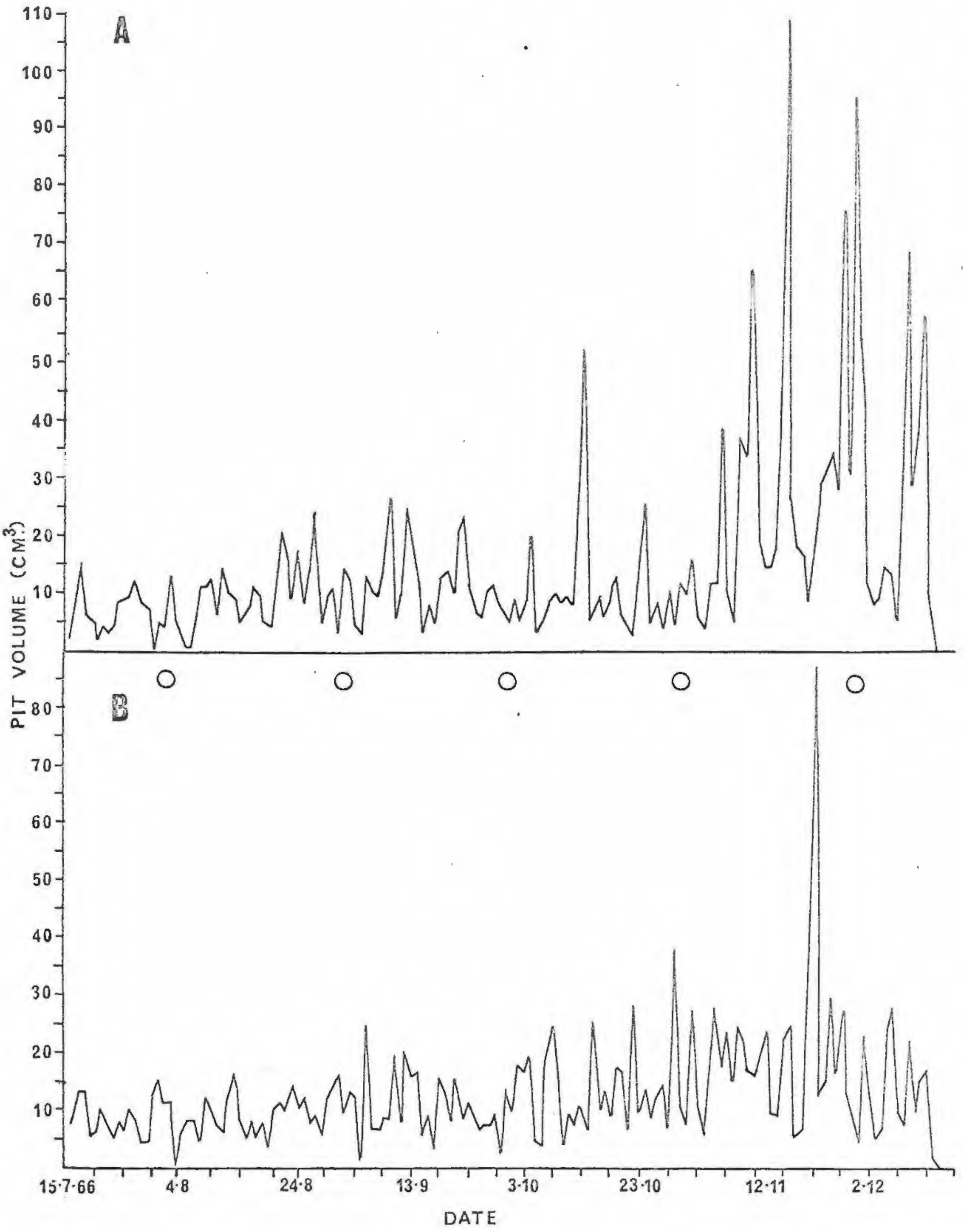


Fig. 38. The number of prey animals caught in three traps, set in the middle of ant-lion colonies, over a period of five months. Graph A represents the morning collection while graph B represents the evening collection. The times of full moon are indicated by the circles in the centre of the figure.

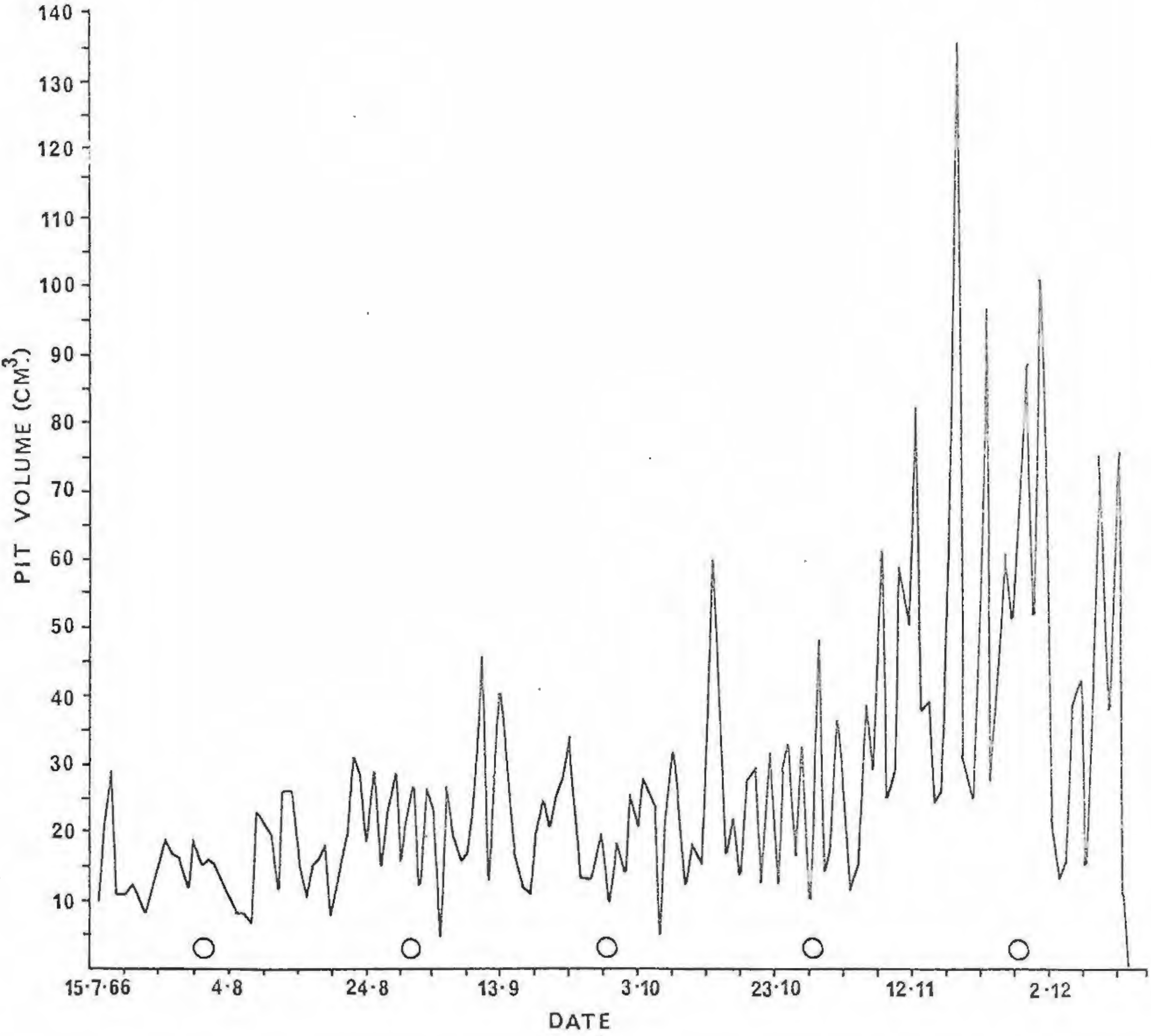


Fig. 39. The total daily catch of prey animals over a period of five months. The animals were caught in three traps set in the middle of ant-lion colonies. The times of full moon are indicated by circles at the bottom of the figure.

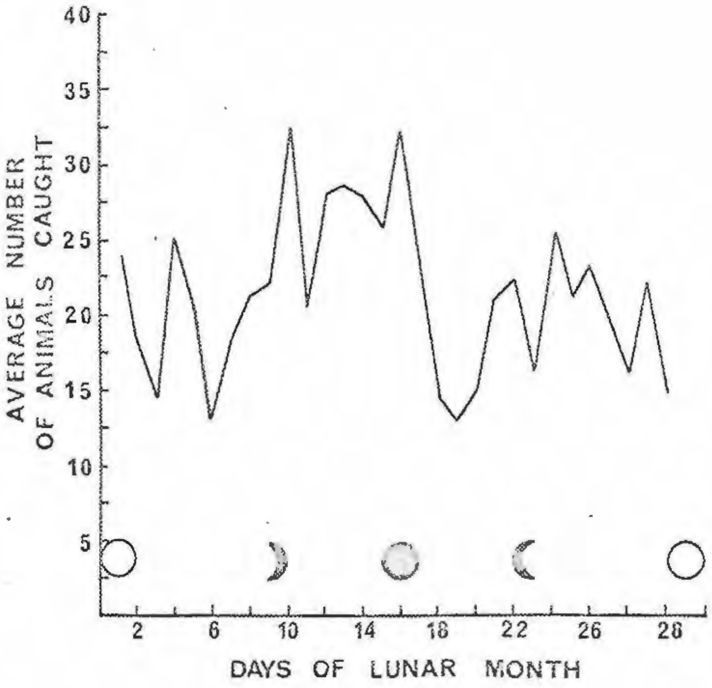


Fig. 40. The average number of prey animals caught in traps at various stages of the lunar cycle. ○ full moon, ☽ last quarter, ● new moon, ☾ first quarter. The number of animals caught in traps over a four month period was taken and all the catches for the days of full moon summed, one day after full moon etc. The average values for each day of the lunar cycle were then calculated. For further details see text.

which were observed in ant-lions, the fluctuations should be obvious each month in the field. Secondly, Enright (1963, 1965) has shown that the method used in this calculation is not always reliable since, while it may show up a true lunar rhythm, it may also show a rhythm which does not rise above the background variation of the experiment. It was therefore concluded that the lunar pit building rhythm was not determined by the number of prey available at different times of the month.

5.42 Distribution of ant-lions in their habitat

The larvae of Myrmeleon obscurus normally live in limited areas of sand, as under hedges or rocky overhangs. Within these habitats some areas are more suitable than others in terms of temperature, sand grain size, abundance of prey etc. The larvae thus tend to congregate in the more favourable areas, and this may be seen in the field. There are, however, disadvantages in the larvae being too close together, since individuals in the centre of the colony would not get sufficient food. The prey would be more likely to be trapped in the peripheral pits. It is therefore necessary for the ant-lions to space themselves out in the habitat, and it is suggested that the lunar pit building rhythm is involved in controlling the distribution of the larvae.

It has been observed that when Myrmeleon obscurus larvae are crowded in the laboratory, sand flicking by one member of the group often stimulates the others to start flicking as well.

This sand flicking is thought to space the animals in the habitat. When the pits are close together, much sand will be flicked from one pit to another during normal pit construction. Pits which are continually having sand flicked into them will tend to collapse and therefore be less efficient in trapping prey. Some larvae might thus be stimulated to move away to other parts of the habitat, and, in the field, tracks indicating the migration of larvae from one part of the habitat to another have often been observed. This migration of larvae would tend to space the pits more evenly.

When ant-lion larvae construct big pits they flick out large quantities of sand, and the larger the pits the more effective the spacing mechanism would be. It may be, however, that the larvae would not always construct pits of the maximum size and it is possible that increases in pit size, and hence sand flicking, only take place during certain limited periods. These increases in pit size could correspond to the increases in pit volume observed at the time of full moon. The lunar pit building rhythm may therefore be related to the sand flicking which spaces the Myrmeleon obscurus larvae in the habitat. If this is the case, the lunar pit building rhythm would be analagous to the epidietic displays described by Wynne-Edwards (1962) for a large number of different animals. These epidietic, or competitive displays, are involved in spacing of animals in their habitats and occur at regular intervals.

If the lunar pit building rhythm is involved in spacing

Myrmeleon obscurus larvae in the habitat, it could be expected that the lunar rhythm would be more pronounced when the animals were crowded. In uncrowded conditions the need for spacing would be reduced and the lunar rhythm might be less obvious. Experiments were therefore performed to determine the effect of crowding on the lunar rhythm. Crowding may also exaggerate the lunar rhythm in another way. In the laboratory migration of overcrowded larvae is not possible, and it therefore seems likely that some animals may be prevented from building pits altogether. The larger larvae construct bigger pits and flick out more sand than the smaller larvae. The small larvae may thus be prevented from building pits. If this occurs then the mean pit volume for the group would increase and the lunar rhythm would be exaggerated still further. In the crowding experiments therefore, the number of pits constructed each day was also noted, in order to determine whether or not some animals were being prevented from building pits.

In these crowding experiments three groups of Myrmeleon obscurus larvae, containing 5, 40 and 50 animals respectively, were placed in dishes with an area of about 858 cm^2. These ant-lions were kept in the laboratory under normal fluctuating conditions, and the mean pit volume of each group was recorded daily for a period of nine weeks. Each day the number of ant-lions constructing pits was also noted.

Fig. 41 shows the mean daily pit volume for the three

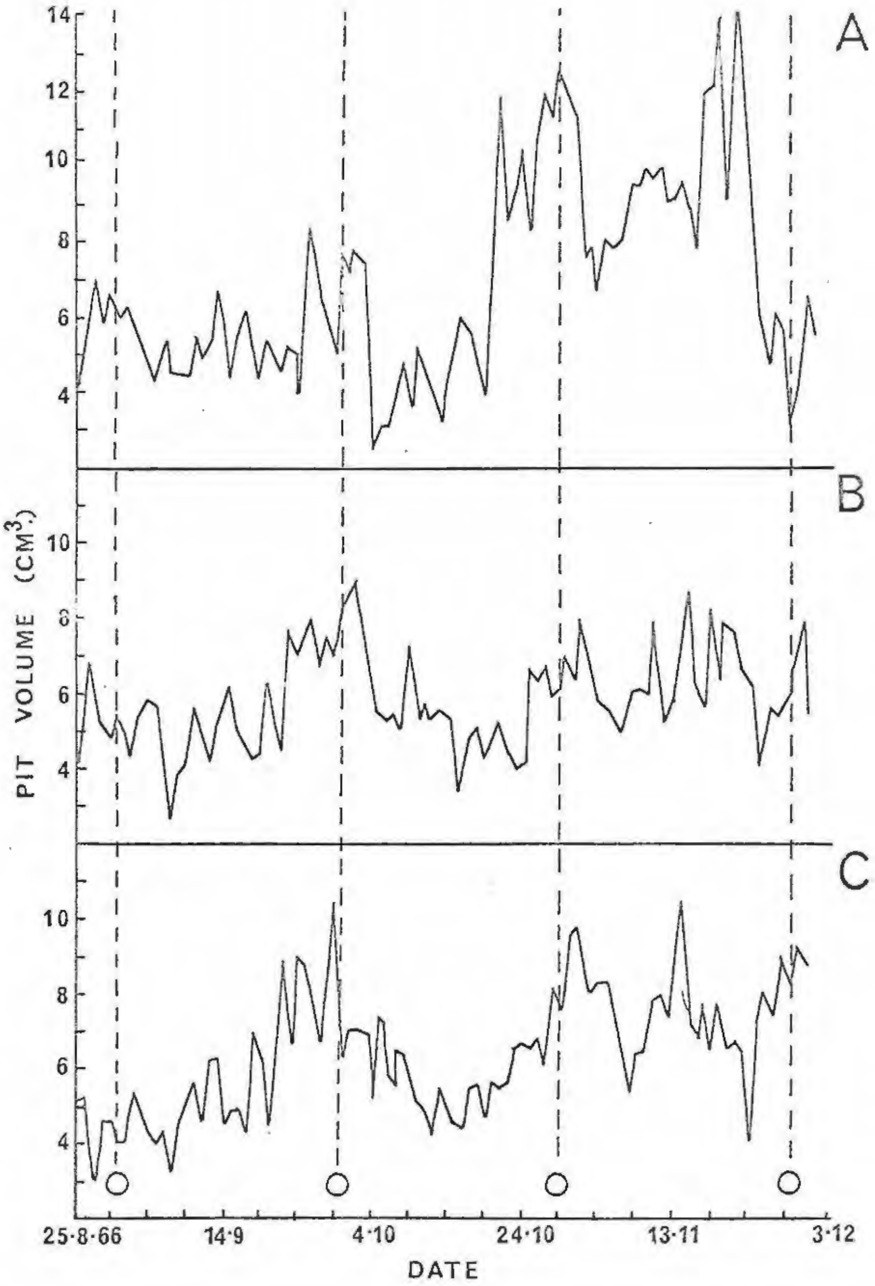


Fig. 41. *The mean daily pit volume of various groups containing different numbers of Myrmeleon obscurus larvae. A, 5 ant-lions; B, 40 ant-lions; C, 50 ant-lions. The times of full moon are indicated by the circles at the bottom of the figure and the vertical broken lines.*

groups. All the groups showed clear lunar activity cycles. The lunar rhythm in the crowded groups, containing 40 and 50 ant-lions, was no more marked than in the uncrowded groups containing only five animals. Crowding the animals thus had no effect on the lunar pit building cycle.

The number of pits constructed each day by the various groups is shown in Fig. 42. In all groups the number of pits constructed each day fluctuated considerably. In neither of the crowded groups was there any evidence that fewer pits were regularly constructed at the time of full moon. It is, however, interesting to note that a far smaller proportion of larvae constructed pits in the crowded groups containing 40 and 50 animals than in the uncrowded group containing five animals. In the group containing 50 Myrmeleon obscurus larvae the maximum number of pits constructed was 19, while in the group containing five larvae all five often constructed pits. This decrease in the percentage of pits constructed by the crowded groups is due to the increased mortality which takes place when the larvae are crowded (section 3.2).

The above experiments showed that crowding the larvae of Myrmeleon obscurus did not exaggerate the lunar rhythm and it was therefore concluded that the lunar rhythm was not involved in spacing the larvae. The number of pits constructed by the crowded groups of ant-lions did not decrease at full moon, and it was concluded that increased sand flicking at this time was not preventing the small larvae from constructing pits. In

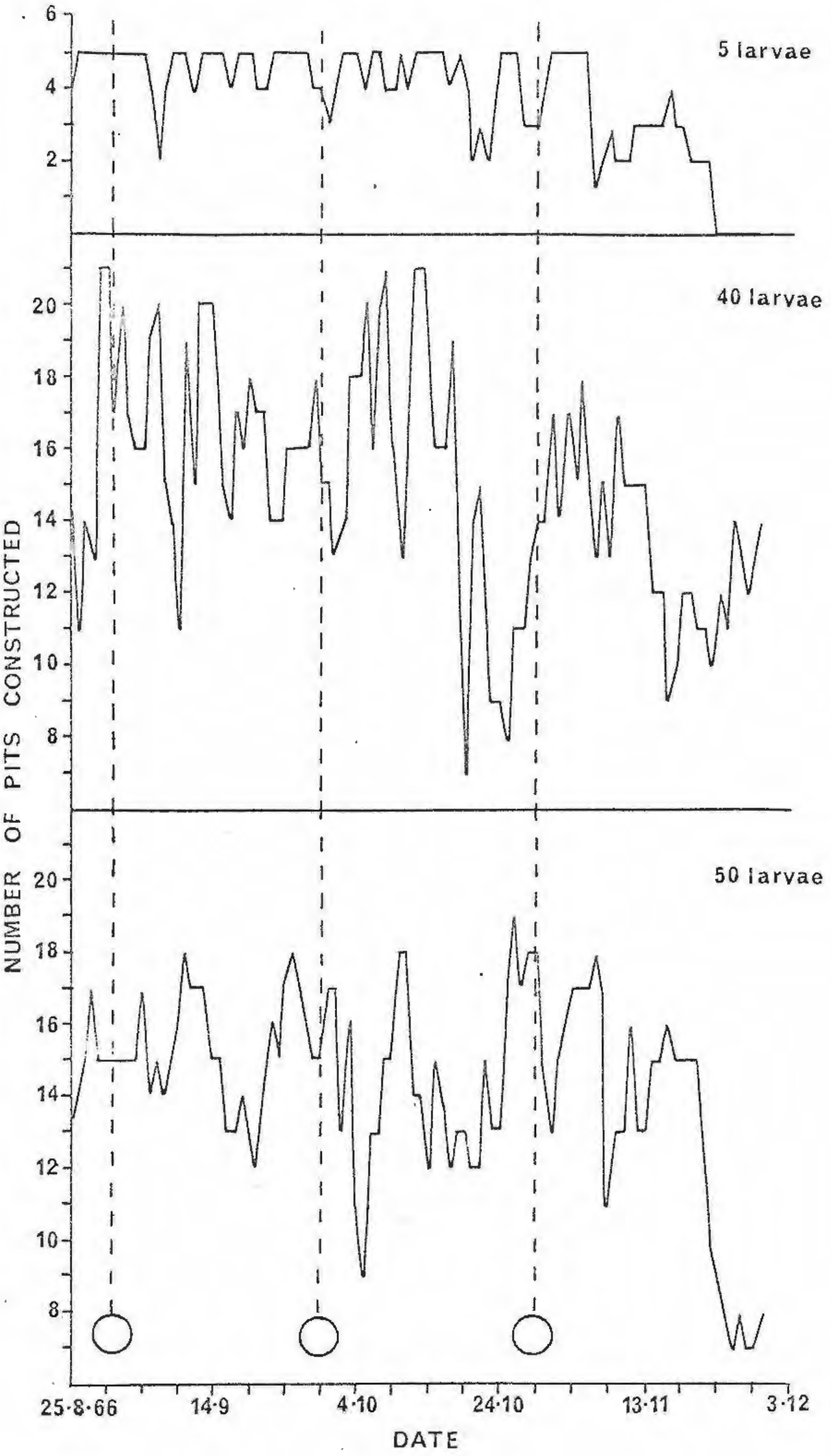


Fig. 42. *The number of pits constructed each day by three different groups of* *<u>Myrmeleon</u>* *<u>obscurus</u>* *larvae. The groups consisted of 5, 40 and 50 ant-lions respectively in an area of about 858 cm^2. The ant-lions were subjected to normal daylight conditions but were shielded from the light of the moon. The times of full moon are indicated by the circles at the bottom of the figure and by the vertical broken lines.*

general it was concluded that, although sand flicking was probably the mechanism spacing the ant-lions in the habitat, this flicking behaviour was not related to the lunar cycle.

5.43 The combination of solar day and lunar day rhythms

The third possible explanation for the lunar pit building rhythm in Myrmeleon obscurus larvae is that it may have no functional significance in itself, but may arise by the combination of daily solar activity rhythms occurring once every 24 hours, and daily lunar activity rhythms occurring once every 24.8 hours. These two activity rhythms would come into phase once during every lunar cycle, giving rise to a large peak of activity. The combination of a solar day and a lunar day activity rhythm has been observed in a number of different marine animals. In these animals the lunar day rhythm is generally bimodal with peaks corresponding to definite phases of the tidal cycle. Such a lunar tidal rhythm in combination with the solar rhythm gives rise to peaks of activity every 15 days. Rhythms with a 15 day periodicity have been demonstrated in Uca (Brown et al, 1953) and Carcinus (Naylor, 1958). A number of other examples have been quoted by Korringa (1957) and Cloudsley-Thompson (1961), while an extensive study has been undertaken on midges of the genus Clunio by Neumann (1966). Clunio has a rhythm of emergence with a period of about 15 days, corresponding to the semi-lunar cycle of spring and neap tides. Under certain conditions, however, some cultures showed a 30 day rhythm. These insects may thus have either a semi-lunar or a lunar rhythm and, since emergence always takes place at the same time of day, they also have a solar day rhythm.

The above examples all refer to marine or inter-tidal animals, and the only evidence for lunar day rhythms in terrestrial insects is that of Campbell (1964) who worked on mealworm larvae. These larvae have a lunar day rhythm of oxygen consumption, with maxima at the times of moonrise and moonset. The combination of this lunar day rhythm with the normal solar day rhythm gives rise to peaks of oxygen consumption at the time of third lunar quarter. It is thus possible that the lunar pit building rhythm observed in <u>Myrmeleon</u> <u>obscurus</u> larvae might have been due to the combination of lunar day and solar day rhythms. Should either of these daily rhythms be bimodal it could give rise to a bimodal lunar rhythm, which would account for the new moon peaks observed in some experiments.

A daily rhythm of pit building activity has already been demonstrated in <u>Myrmeleon</u> <u>obscurus</u> larvae. The important question which must be answered is whether or not ant-lion larvae possess a lunar day activity rhythm in addition to the solar day activity rhythm. To determine whether such a lunar day rhythm existed, the daily activity traces of a single individual, covering the period 5th April, 1966 to 19th May, 1966, were analysed. This was done by counting the number of deflections of the pen on the activity trace of the period in question. Only deflections greater than 5 mm. were counted so as to exclude all background noise. The times of moonrise and moonset for each day were found from the Astronomical Ephemeris for 1966 and the activity for all the hours of moonrise was summed. This procedure was repeated with the hours of moonset, one hour after moonrise/moonset etc. Considerable difficulty was experienced, since the time between moonrise and moonset varies greatly depending on the time of the lunar month. In an attempt to compensate for this, the average number of pen deflections was calculated for each hour of the lunar day. These results have been plotted in Fig. 43.

Fig. 43 shows that there were a number of activity peaks during the lunar day, while there was a very low level of activity shortly before moonset. The fluctuations were, however, too great to allow any definite conclusions to be drawn. In order to clarify the position, further experiments were performed.

The daily activity of two groups of 96 <u>Myrmeleon</u> <u>obscurus</u> larvae was observed over a 24 hour period, as described in section 4.1. The activity of the first of these groups was observed on 23-24th February, 1966, while the activity of the second group was observed on 30-31st March, 1966. The times of moonrise and moonset were found for these dates and the activity summed as in the previous experiment. The results are shown in Fig. 44. From this it can be seen that there was a very definite activity peak about four hours after moonrise.

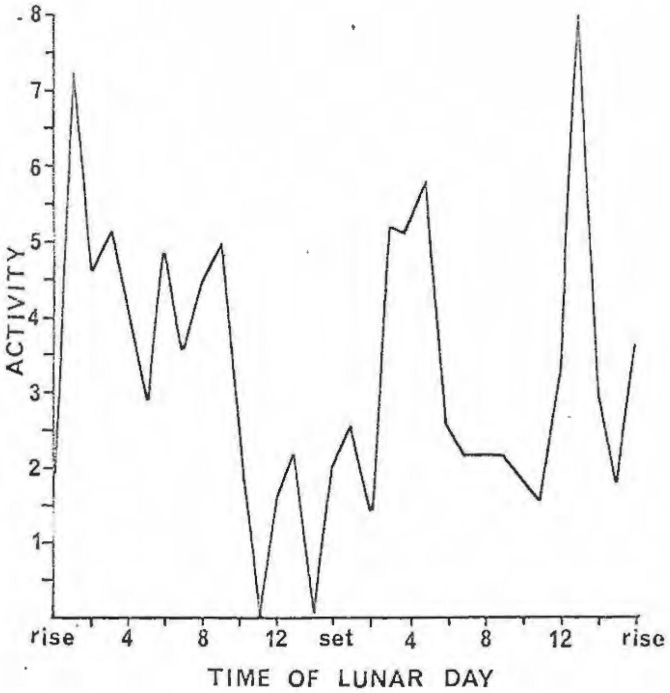


Fig. 43. The activity of an individual Myrmeleon obscurus larva at various times of the lunar day. The results were obtained from the daily activity record covering the period 5th April to 19th May, 1966. The number of deflections of the pen, hence activity, was determined for each hour of this period. The activity for all the hours of moonrise was summed, one hour after moonrise etc. During the activity recording the ant-lion was kept in normal daylight conditions but was shielded from the light of the moon.

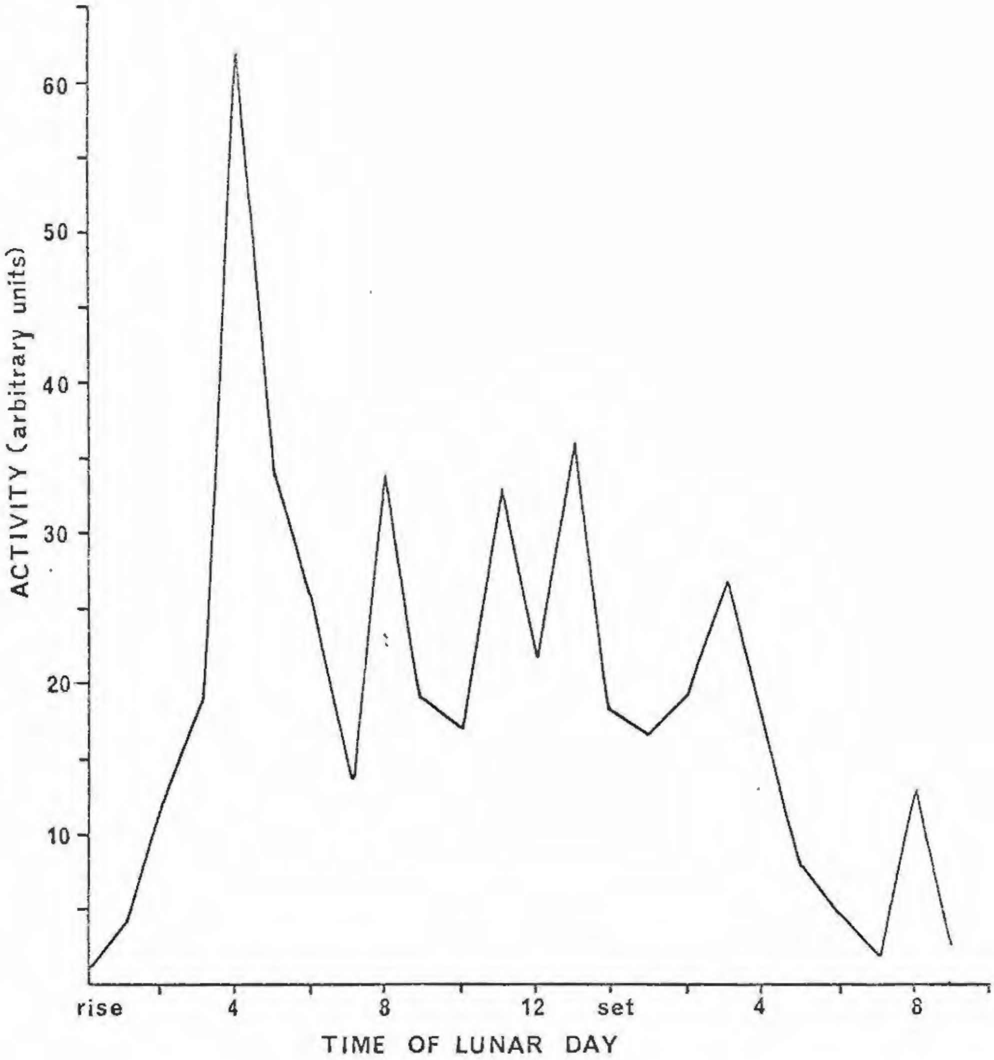


Fig. 44. The activity of two groups of 96 Myrmeleon obscurus larvae at various times of the lunar day. The activity of the two groups was observed for 24 hours on 23rd - 24th February, 1966 and 30th - 31st March, 1966, respectively. On 23rd February the moon rose at 8.25 a.m. and set at 8.43 p.m. In March the moon rose at 2.25 p.m. on 30th and set at 0.09 a.m. on 31st. The activity of these two groups of larvae was estimated as described in section 4.1. All the activity for the hours of moonrise was summed, one hour after moonrise etc.

A single lunar day activity peak (as shown in Fig. 44) in combination with the normal solar day activity rhythm, could give rise to the observed monthly lunar pit building rhythm. If the lunar day activity peak occurs shortly after moonrise, then this peak would coincide with the solar day activity peak (at dusk) when the moon rises shortly before sunset. It is, in fact, at the time of full moon that the moon rises shortly before sunset. The lunar day and solar day activity rhythms would thus coincide at the time of full moon.

In winter the moon rises earlier than it does in summer (for example, in February, 1967 it rose at 7.38 p.m., while in July, 1967 it rose at 4.47 p.m.), and the lunar day peak would thus occur earlier. For the solar day and lunar day rhythms to remain in phase it is therefore also necessary for the daily activity peak to occur earlier in winter. In order to determine whether or not this did occur, the daily activity traces of individual <u>Myrmeleon</u> <u>obscurus</u> larvae, obtained at different times of the year, were studied. The results of this study are shown in Fig. 45, which indicates that the daily peak of activity did, in fact, occur earlier in winter than it did in summer. No results were available for the midsummer months, November to January, and it was thus not possible to state exactly how much earlier the activity peak was in winter than it was in summer. The difference, however, appeared to be of the order of four to five hours.

The above results may be criticized since each trace represented only a single ant-lion. It was shown in section 3.1 that there was considerable individual variation in the time at which activity took place. The differences shown in Fig. 45, however, are considerably greater than that normally found between individuals measured at the same time of year. Further proof that the seasonal shift in activity was not an artifact, was given by experiments in which the activity of groups of ant-lions was observed directly for 24 hours.

Two groups of 96 <u>Myrmeleon</u> <u>obscurus</u> larvae were used in these experiments. Both groups were kept in the laboratory under normal daylight conditions and a temperature of 18 ± 3°C. Their activity was estimated by direct observation as described in section 3.1.

The activity of one group was observed during March (summer), while the activity of the second group was observed during July (winter). The results illustrated in Fig. 46 show that there was a considerable difference in the time at which maximum activity took place in these two groups. During March the activity peak occurred between 6 p.m. and 8 p.m., while in July the peak was between 4 p.m. and 7 p.m. This experiment thus confirmed that the daily peak of activity occurred earlier in winter than it did in summer.

<u>Myrmeleon</u> <u>obscurus</u> larvae have therefore been shown to possess both solar day and lunar day activity rhythms, both of which occur earlier in winter than in summer. If the building of bigger pits at the time of full moon is due to the

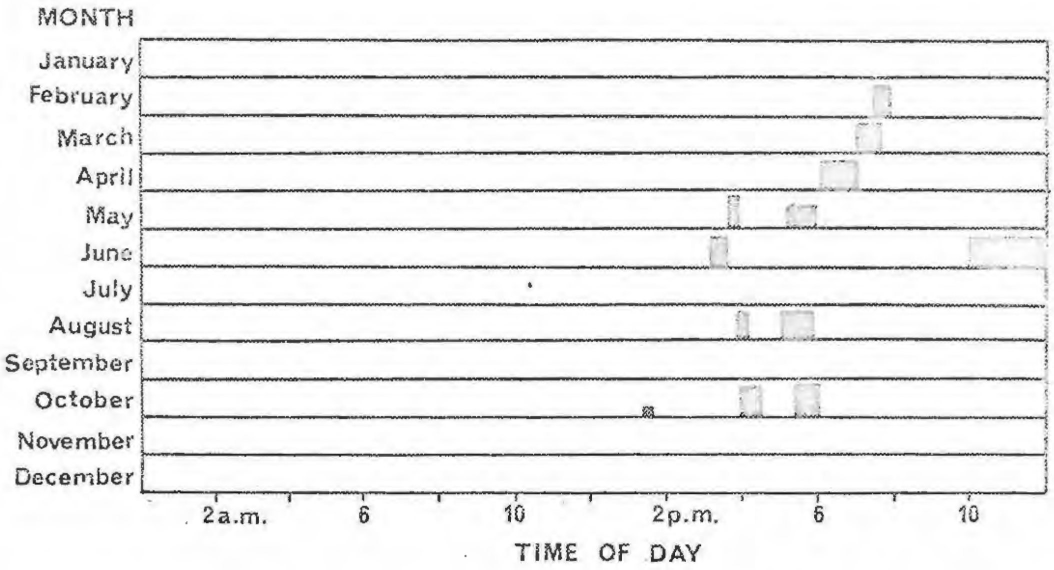


Fig. 45. *The daily activity of <u>Myrmeleon</u> <u>obscurus</u> larvae at different times of the year. The activity of seven different individuals is shown. The animals were kept in natural daylight conditions.*

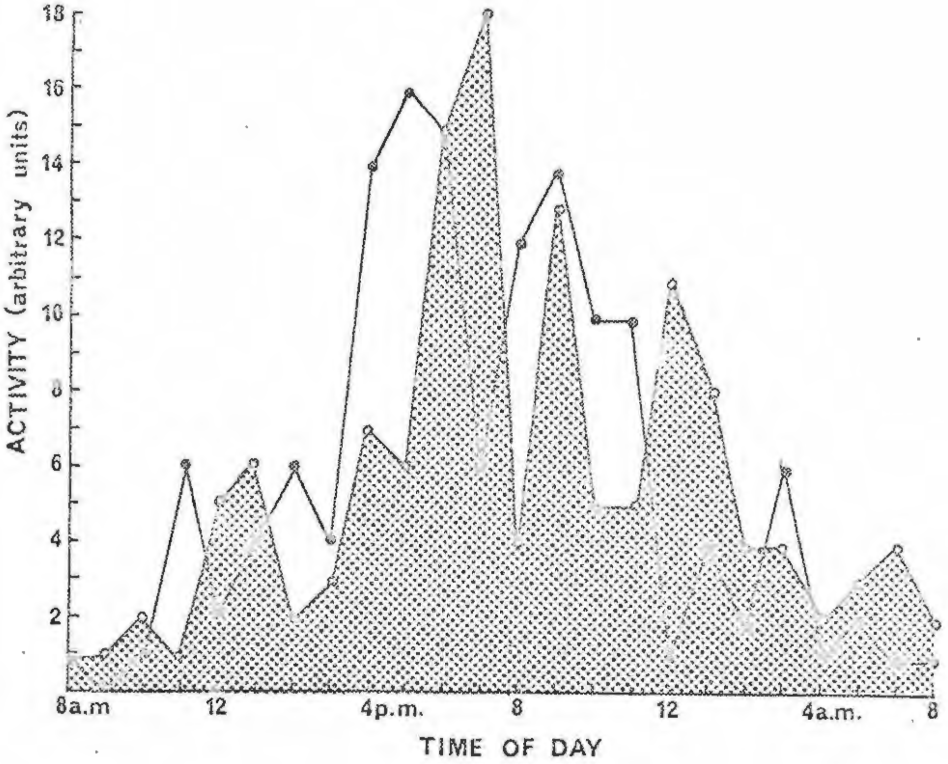


Fig. 46. The daily activity of two groups of 96 Myrmeleon obscurus observed at different times of the year. Group A observed during March, 1966 (unshaded graph). Group B observed during July, 1966 (shaded graph). The activity of both groups was observed over a period of 24 hours as described in section 4.1.

combination of these two rhythms, then changes in either of them should produce changes in the lunar pit building cycle. An experiment was therefore performed in which the solar day activity rhythm was altered by a reversed light-dark cycle, and the effect of this reversal on the lunar cycle noted. If the lunar day activity peak occurred at moonrise, then the lunar monthly peak of pit building activity should occur when the time of moonrise coincided with the time of the new (reversed) solar day activity peak. In Fig. 44, however, it was shown that the lunar day activity peak in Myrmeleon obscurus larvae actually occurred some four hours after moonrise. The lunar monthly peak of activity should thus be obvious when the activity peak, four hours after moonrise, coincides with the reversed solar day activity peak.

A group of 12 Myrmeleon obscurus larvae was placed in darkness from 9.45 a.m. to 7.45 p.m. and in light, of about 1,500 lux, from 7.45 p.m. to 9.45 a.m. Such a reversed light cycle has already been shown (section 4.23) to reverse the solar day activity rhythm, so that the animals become active at about 10 a.m. instead of at dusk as in normal larvae. Under the conditions of this particular experiment it was therefore predicted that the monthly lunar activity peak would occur when the moon rose four hours before the solar day activity peak. If the moon rose at 6.00 a.m. the lunar day activity peak, occurring four hours after moonrise, would coincide with the solar day activity peak at 10 a.m. This prediction was, in fact, borne out by the results of the experiment which are shown in Fig. 47. From this figure it can be seen that there was no peak in the pit building activity at the time of full moon (20th August, 1967). A peak of activity did, however, occur on 3rd September, 1967, on which day the moon rose at 6.06 a.m. It should be emphasised that this was the date on which the solar day activity rhythm, with the peak at 10 a.m., coincided with the lunar day activity rhythm, with the peak four hours after moonrise.

From these experiments it was concluded that the monthly lunar rhythm of pit building activity was due to the combination of a solar day activity rhythm, with the peak at dusk, and a lunar day activity rhythm, with the peak four hours after moonrise. Both the peaks occurred earlier in winter than in summer and therefore remained in phase throughtout the year.

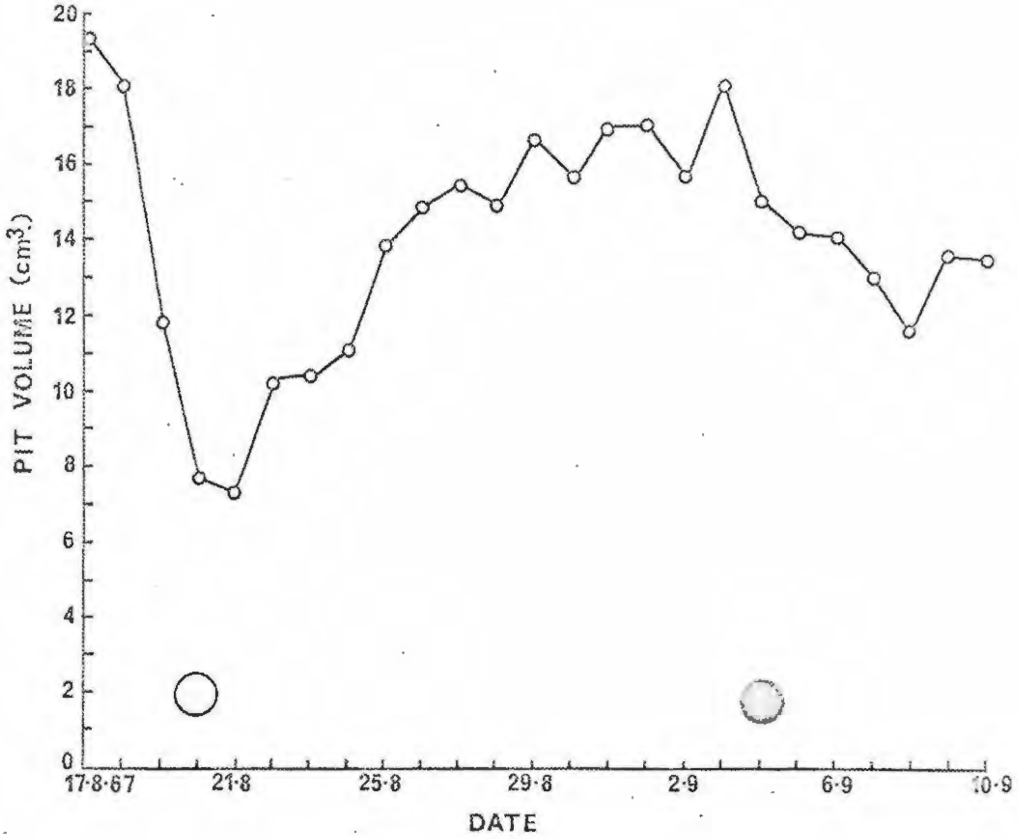


Fig. 47. The mean daily pit volume of a group of 12 Myrmeleon obscurus larvae under reversed light conditions. The animals were subjected to light of approximately 1,500 lux from 7.45 p.m. to 9.45 a.m. Each ant-lion was fed one ant a day. The times of full moon and new moon are indicated by the circles at the bottom of the figure.

6. DISCUSSION AND CONCLUSIONS

This work has shown that Myrmeleon obscurus larvae possessed two main activity rhythms, the solar day rhythm of pit construction and feeding which occurred at dusk, and the lunar day activity rhythm which occurred four hours after moonrise. These two rhythms combined to give rise to a peak in the pit building activity at the time of full moon. These peaks at the time of full moon were expressed as an increase in the volume of the pits constructed.

There are a number of comments which can be made about the daily activity rhythm. In general this rhythm appeared to be similar to other insect circadian rhythms, such as those which have been described for the cockroach by Harker (1956) and Roberts (1959), grasshoppers (Fingerman, Lago and Lowe, 1958), Drosophila (Roberts, 1956), fruit moths (Chiba, 1966 a and b), and many others. Like the rhythms described in other insects, that found in ant-lions would appear to be endogenous, since it continued for at least a month both in constant light and in constant dark. In contrast to the other rhythms, however, the daily rhythm in Myrmeleon obscurus larvae did not conform to Aschoff's Rule (Aschoff, 1960; Harker, 1964; Hoffman, 1965; Sollberger, 1965) which states that the period of the activity rhythm should be shorter than normal in constant dark for dark active animals. The period should also be longer than normal for such animals in constant light, while the opposite applies to animals which are active during the day. Ant-lions are active after dark and one would thus expect their period of their activity rhythm to decrease in constant dark and increase in constant light. In actual experiments, however, the opposite was found to apply. In the literature there are a number of other examples of insects which do not obey Aschoff's Rule. For example, Drosophila melanogaster is said by Pittendrigh (1960) to violate the Rule, while Roberts (1960) has shown that some cockroaches in constant dark have an activity period which is longer than 24 hours. In constant light, however, all the cockroaches showed an increased period in contrast to ant-lions where the period decreased. In their responses to constant light and constant dark, Myrmeleon obscurus larvae seemed to behave more like diurnal animals than nocturnal ones.

The free-running period lengths calculated for Myrmeleon obscurus larvae in the constant dark varied from 24 hours 2 minutes to 24 hours 17 minutes. This variation is not surprising since Lohmann (1967) has shown that each species has a whole range of free-running periods. In the cockraoch Leucophaea, for example, the range of free-running periods is from about 23 hours 3 minutes to 24 hours 39 minutes in the constant dark. The free-running period of 23 hours 44 minutes obtained for Myrmeleon obscurus in constant light was only obtained from a single individual. A very large number of experiments need to be conducted before the average free-running period in either constant light or constant dark can be determined for the species as a whole.

Another unusual feature of the ant-lions daily activity rhythm was the lack of transients in the experiments with reversed light dark cycles (see section 4.23). Normally when any animal is placed in an altered light-dark cycle it takes several days for the daily activity rhythm to adjust to the new conditions. During this period of adjustment the rhythm shifts by a small amount each day. These small shifts are known as the transients. Although the number of transients found in various animals differ considerably under different conditions, normally at least one or two days are required for complete adjustment to new conditions. For example, Warnecke (1966) found that it took beetles of the genus Geotrupes from one to eight days to adjust to a reversed light cycle, depending on the intensity of the light used. It took the beetles two days to adjust to the new light cycle when the light intensity was 400 lux, and five to seven days when the light intensity was 50 lux. The higher the light intensity the more rapidly the adjustment takes place. The lack of transients in Myrmeleon obscurus rhythms was perhaps due to the very high light intensity used (1,500 lux).

It seems that the solar day activity rhythm may be of great survival value, especially in protecting the ant-lion larvae from extremes of temperature. It is well known (Buxton, 1923, 1924) that the surface layers of the sand may get very hot and very cold, while a few centimeters below the surface the temperature is more moderate. Schaefer (1967) has, in fact, shown that the temperature of the surface sand in Myrmeleon obscurus habitats are well above the lethal limit during the heat of the day. The larvae can therefore not construct their pits during the day, since in the early stages of pit construction they are exposed on the surface of the sand. Pit construction must thus take place when it gets cool. The earlier such construction can take place, however, the more chance the ant-lion has of capturing prey. If construction of the pits only took place at dawn no prey could be captured during the night. Pits are therefore built shortly after dark, and are available through the night for prey capture. This pit construction after dark is controlled by the solar day activity rhythm.

During the heat of the day Schaefer (1967) has shown that Myrmeleon obscurus larvae migrate away from the base of the pits to cooler sand and it is suggested that this migration is also controlled by the solar day activity rhythm. This activity rhythm is controlled by light changes. The larvae thus probably migrate away from the pit base a set number of hours after sunrise, and return to the surface a set number of hours later.

The functional significance of the solar day activity rhythm thus seems fairly obvious, but no obvious function can be ascribed to the lunar day activity rhythm. Why the Myrmeleon obscurus larvae should be active four hours after moonrise is not clear, but this rhythm may possibly be related to "subtle geophysical factors" (Brown, 1960). These so-called subtle factors include cosmic rays, barometric pressure changes, and the earth's magnetism. A number of different animals have been shown to respond to these factors by Brown (1960), Brown, Webb and Macey (1957), Brown, Brett and Webb (1960), and Brown and Park (1965). Recently Picton (1966) has also shown a statistically significant response in Drosophila to weak magnetic and electrostatic fields. It is therefore possible that the lunar day activity might be related to such subtle factors. These subtle factors would have been present even under the so-called constant conditions described in this work. Should a relationship be established between these geophysical factors and the lunar day rhythm, however, this would not explain the significance of the activity rhythm in the lives of the animals. Attempting to establish a relationship between geophysical factors and the lunar day activity rhythm does not appear to be a profitable line of study. The significance of the lunar day rhythm could only be determined by a thorough investigation of the biology of Myrmeleon obscurus larvae. At present no suggestions for the functional significance of the lunar day activity rhythm can be made. It is possible that this rhythm is due to the combination of other rhythms as was shown to be the case for the monthly lunar rhythm.

The monthly lunar activity rhythm which was demonstrated in Myrmeleon obscurus larvae, was shown to arise by the combination of the solar day activity rhythm and the lunar day activity rhythm. The monthly rhythm did not appear to have any functional significance in itself.

7. SUMMARY

1) An investigation was undertaken into the various activity rhythms found in the larvae of Myrmeleon obscurus (Neuroptera, Myrmeleontidae). Pit construction was used as the criterion of activity.

2) The capacity sensitive electronic circuit used to monitor the daily pit building activity is described.

3) Various factors affecting the size of the pits constructed by ant-lion larvae were investigated. Pit size was found to be correlated with the size of the larvae, larger larvae constructing bigger pits. Population size was also shown to have an effect, since the more crowded the animals were the smaller and closer together their pits became. The death rate due to competition between the larvae also increased as the ant-lions became more crowded.

4) Feeding frequency also affected pit size. Ant-lions which were starved constructed very small pits.

5) Pit size was affected by the texture of the sand, maximal pit size being attained in sand with a grain size of between 353 μ and 211 μ . This was shown to be the most abundant grain size in sand from the natural habitat. In sand coarser or finer than this, pit size was reduced.

6) *Generally smaller pits were constructed at lower temperatures, but this response depended largely on the lunar cycle. The upper and lower critical limits for pit construction were found to be 11°C and 42°C. Below and above these temperatures no pits were constructed, although the ant-lions remained alive.*

7) *Daily destruction of the pits decreased the pit size.*

8) *The existence of the daily pit building activity rhythm, which occurs at dusk, was demonstrated in individuals and in populations of ant-lion larvae. This rhythm was studied using the capacity sensitive circuit, and also by direct observation of groups of animals over a 24 hour period.*

9) *The daily activity rhythm appeared to be endogenous, since it continued for at least a month both in constant light and in constant dark. The rhythm was altered by reversals in the daily light-dark cycle, and it was concluded that light was the main phase setting factor.*

10) *An attempt was made to determine the location of the "clock" or "clocks" controlling the daily activity rhythm. This was investigated by chilling the heads of individual ant-lions with a fine copper wire, and also by chilling large groups of animals in the refrigerator. From these experiments no definite conclusions could be drawn about*

the location of the "clock".

(11) The existence of a lunar pit building cycle was demonstrated in both field and laboratory populations of antlions. Maximal pit size was attained at full moon. Very low temperatures were found to suppress these lunar peaks.

(12) The lunar pit building rhythm was shown to be endogenous, since it continued for at least two months in constant dark and one month in constant light.

(13) The lunar pit building rhythm of very young larvae was investigated, and it was shown that while larvae which hatched in the field appeared to possess a lunar rhythm, larvae which were hatched in the laboratory did not have a rhythm. It was therefore concluded that the light of the moon was necessary to establish the lunar rhythm.

(14) Some possible explanations for the lunar cycle were investigated experimentally. It was concluded that the lunar rhythm was not related to the number of prey present at various times of the lunar cycle or to the spacing of the animals in their habitat. The lunar rhythm was shown to be due to the combination of a lunar day activity rhythm and a solar day activity rhythm.

(15) *Some aspects of the daily rhythm are discussed, and this rhythm is compared with those found in other insects. Probable functional reasons for the rhythm are presented.*

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