The Influence of the Natural Light-Dark Cycle on the Activity Rhythm of Pond Bats (*Myotis dasycneme* Boie, 1825) during Summer

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Summary. 1. The activity cycle in a colony of about 150 pond bats is studied throughout the summer season in Berlikum (Netherlands). The natural diurnal roosts of the bats are sited between rafters of a church loft where a nocturnal darkness reigns day and night. The onset of activity generally takes place in two phases: I—descending into and waiting in a narrow exit chamber from where daylight can be seen, II—flying out to the feeding grounds. The investigation includes electronic recording of passages of bats, and of light intensity during morning and evening twilight.

2. Phase I is regulated by an endogenous circadian timing system which is synchronised to the seasonal variation in daylength. The main entraining agent is daylight which the bats can only have observed, either when waiting in the exit chamber at dusk on the preceding day, or when flying home at dawn. The large time lapse occurring between the moments of arrival of first and last descender in the exit chamber (2 hrs), and between the return of the first and last homeflyer (1 hr) on a given day, is ascribed to lack of precision in timing of the right moment. This lack is thought to originate from two facts. First, the almost dayly variation of cloudiness of the sky at the time of departure causes the rate of decrease of daylight to fluctuate irregularly. Second, at the times of departure and return most of the bats are confronted with very low light intensities only being poor entraining agents. In addition, there is some evidence that each individual has an oscillatory entrained activity cycle causing it to be among the first descenders (homeflyers, resp.) at one day, among the last descenders (homeflyers, resp.) at another day. Some relevant literature data on response-curves concerning other nocturnal mammals are discussed.

3. The seasonal phase relationship between the daylight cycle and the activity rhythm observed in pond bats accords with the Aschoff-Wever model, but only as far as the light-dark ratio is concerned. Twilight duration does not seem to be an important factor in this respect. The value of light intensity at the moment of departure of the first outflyer decreases as the season proceeds.

4. Phase II (the flying out) is regulated *grosso modo* by the absolute value of light intensity at the moment of departure. It is made plausible that the releasing stimulus for departure of the first outflyer, on clear evenings, is the surpassing of a threshold value of the rate of relative decrease of light intensity.

5. Literature data on activity cycles of other bat species are discussed.

1. Introduction

The factors regulating the timing of nocturnal activity in microchiropterid bats are of endogenous as well as exogenous origin. An endogenous circadian timing system is now wellknown to form the basis of 24-hr activity rhythms throughout the animal kingdom. Under natural circumstances, this rhythm is
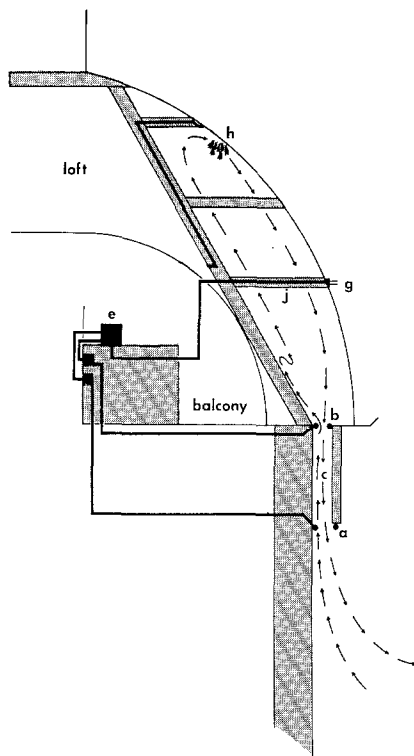
Fig. 1. The route of the bats (arrows) from a roosting site (*h*) to the exit chamber (*c*) and back. Position of the instruments which score the interruptions of two infrared light beams (*a* and *b*, placed parallel to the church wall), and the light intensity outside the church (*g* selenium photocell). They are all connected with the same recorder (*e*)

synchronized to the earth's rotation. In higher vertebrates, the main entraining agent or "Zeitgeber" is the natural light-dark cycle (Aschoff, 1960, 1964, 1969). Endogenous rhythms in bats were first described by Griffin and Welsh (1938). Their synchronization by light-dark cycles was shown by Decoursey and Decoursey (1964).

A number of other variables may affect bat activity patterns. Reproductive and other physiological conditions can eventually play a role. Meteorological parameters such as precipitation, temperature and wind speed may influence times of onset and end as well as intensity of nocturnal hunting activity.

Yet light is presumably the most important variable involved in the regulation of activity times. In the course of the season, the natural light-dark cycle undergoes considerable variation in two parameters: daylength and twilight duration. The main objective of the present study has been to assess the start and the end of activity in a colony of vespertilionid bats throughout the summer season, and to evaluate the effect of several environmental parameters, especially light.

The investigation, extensively reported upon in a thesis by Voûte (1972), involved electronic recording of passages of bats through the exit holes of the colony, as well as of light intensity during morning and evening twilight.
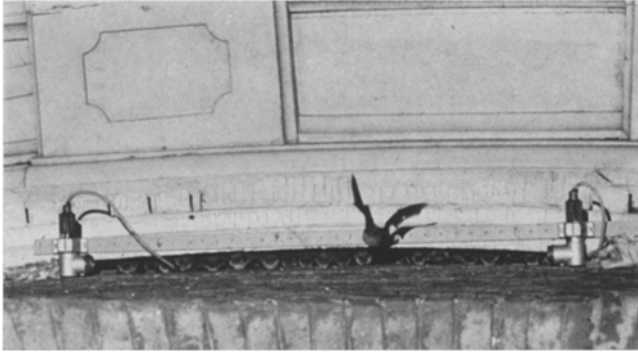
Fig. 2. Exit hole seen from below at the moment when the first outflyer is passing the outside infrared beam between lamp (right) and photocell (left) which are mounted on a perforated copper strip. Note the waiting bats on the background in the exit chamber. Photo: Stam (S.F.W.)

2. Situation of the Colony

The object of our study has been a nursing colony of pond bats (*Myotis dasycneme*) numbering about 150 adult females roosting on a church loft in the frisian village of Berlikum (lat. 53° 15′N, long. 5° 40′E). This loft is dome-shaped, has no window and contains a "jungle" of rafters providing the bats with many protected sites to hide during daytime. Exit holes are found between a wooden ornament just beneath the eaves gutter and the stone wall of the church. In Fig. 1, the route from a roosting site to an exit hole and vice versa is drawn schematically. Each of these holes is shaped as a narrow chamber (3 cm wide) having a height of circa 100 cm and a breadth varying from 25 to 80 cm.

Bats entering an exit chamber at its upper end (*cf.* Fig. 1, b) will be referred to as "descenders"; those who leave this chamber at the bottom will be called "outflyers". Bats moving in the opposite direction will be called "homeflyers" and "ascenders", respectively.

3. Methods

In order to record the movements to and from the exit chamber, two photocell detectors with infrared beams were placed: one above, and one below the exit chamber (Figs. 1 and 2). Interruptions of these beams were written down on a Philips potentiometric recorder by means of two event-markers. An example of this recording is shown in Fig. 3. At the same time, this recorder registered the signal originating from a selenium photocell (diam. 67 mm) placed on the roof of the church and informing about outside light conditions at appropriate times of each day and night. In order to simulate as much as possible the spectral sensitivity of the vertebrate eye, a commercial rhodopsine filter was placed on the entrance of the photocell. The recorder was placed on a balcony inside the church (Fig. 1), so that the observers could read it without disturbing the bats on the loft.

An example of the recording of daylight intensity can also be seen in Fig. 3. The distance (*z*) from the mark of the first outflyer (*b*) to the corresponding point on the light curve (*A*) is a measure for the intensity of the daylight (*E*) at the moment when it flies out. The tangent (*d*) enables us to calculate the slope of the light curve at that moment, from which we may calculate the rate of relative decrease of the light intensity: $S = \dfrac{1}{E}\dfrac{dE}{dt}$. Additional light measurements on the church loft during daytime were taken with a Vitatron galvanometer.
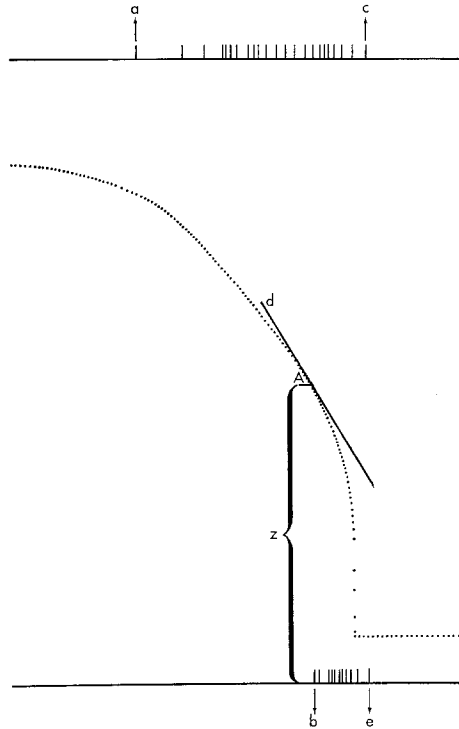
Fig. 3. Example of the recording of infrared beam interruptions above (*a—c*) and at the bottom (*b—e*) of the exit chamber, and of the decrease in light intensity outside the church. Vertical: light intensity (*E*). Horizontal: time; *d* the tangent to the light intensity curve at the moment (*A*) when the first bat flies out (*cf. b*)

One of the main disadvantages of field observations on a rather large colony is the impossibility to recognize individual bats. In order to be able to discriminate a small group of them from the rest of the colony, we have captured 14 adults and banded them with rings covered with strongly reflecting white Scotch lite tape. These white rings could be recognized easily on flying bats, even during the darkest hours of the night, when watched through infrared field glasses.

4. Results

4.1. *Survey of the Nocturnal Activity of the Whole Colony*

Fig. 4 presents a summary of all activity records made. The gaps in this figure are due either to technical failures of the recorder or to bad weather conditions. The bats may already begin to descend into the exit chamber (Fig. 1) some time before sunset and go on descending until at least one hour after sunset; among the outflyers, starting always more than 30 min after sunset, a smaller variation is observed.

The time of the first homeflyer can be generalized less easily. In some cases it is followed by the second homeflyer after more than one hour. In that case, the time of the first homeflyer is not at all characteristic for the onset of the
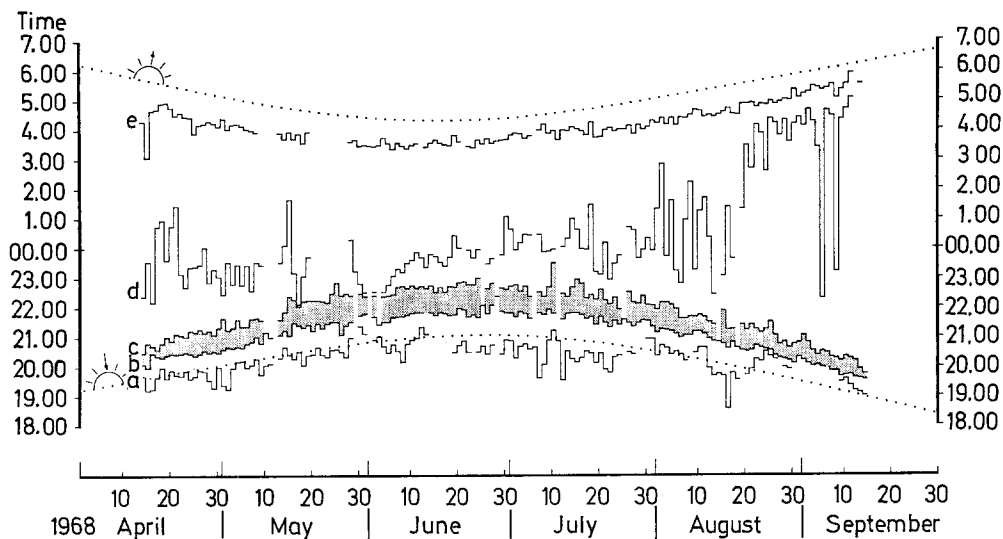
Fig. 4. Survey of the nocturnal activity of the colony during the 1968 season (April—Sept.). From bottom to top: sunset (dotted line), first descender (*a*), first outflyer (*b*), last outflyer = last descender (*c*), first homeflyer (*d*), last homeflyer = last ascender (*e*), and sunrise (dotted line)

homeflight of the colony as a whole. In order to meet this bias as far as possible, we have arbitrary decided to consider the first homeflyer as a "regular one" only if it was followed by a second one within 30 min, and if no time lapses of more than 45 min occurred in the subsequent flow of homeflyers.

Nevertheless, the moment of the first homeflyer fluctuates considerably (*cf.* Fig. 4). After this moment the bats continue to fly in and out during many hours of the night, especially during the breeding season (from June till the middle of August). These extra in- and outflights are not recorded in Fig. 4. They are most probably related to the feeding of young during the night, but will be dealt with in a separate paper.

Summarizing the features which are relevant to the present study, we come to the following conclusions. The nocturnal outdoor activities of the bats last throughout the night, as long as it is sufficiently dark outside. Their onset is marked by descending of bats from their hiding places on the loft into the exit chamber, but those who descend too early have to interrupt the chain of events for some time. They wait behind the wooden ornament (Fig. 2). As will be shown in the next pages, it is this phenomenon which enables us to analyse separately the regulating influence of the endogenous timing system and of the natural light-dark cycle on the nocturnal activity cycle of the bats of this colony as described above.

Three main questions to be answered are:

a) What makes the bats start their nocturnal activity by descending into the exit chamber, and end it by ascending into the loft finally ? (*cf.* part 4.2.).

b) How is the relationship between the natural daylight cycle and the endogenous activity cycle of the bats? (*cf.* part 4.3.).

c) What is the releasing stimulus for the bats waiting in the exit chamber to fly out towards their hunting grounds? (*cf.* part 4.4.).

4.2. The Endogenous Timing System

The experimental way to study the endogenous circadian cycle is to expose an animal to complete and permanent darkness for several days (for bats: *cf.* Decoursey and Decoursey, 1964).

On the Berlikum church loft a nocturnal darkness most probably reigns day and night at the favourite roosting sites of the bats, as an extremely sensitive Vitatron-galvanometer did not react at all when placed at these sites on the sunny afternoon of March 28, 1972. At another place, opposite and quite near to a little hole in the roof, a light intensity of max. $100 \ \mu W/m^2$ could be measured at the same day. Therefore, it seems very unlikely that the bats being on this loft can observe dusk. This means that the onset of their nocturnal activity can hardly be initiated by the daylight situation on the same day. As a crucial experiment we have covered the main small holes and crevices occurring in the old wooden church roof with lightproof material, making the darkness on the loft almost complete during 1 week permanently. This did not have any influence on the usual behaviour of the bats.

In Fig. 4 it is shown that the last home-flyers arrive at the church loft approximately 40 min before sunrise, when the first daylight is manifest already. But, as we will show in a separate paper on the whereabouts of these bats during the night, many of them must have taken the decision to fly home much earlier, as they were far out in the field several kilometers from the church. So, the end of nocturnal activity is not likely to be initiated by the daylight situation of the same date either.

Anticipation of both dusk and dawn are additional arguments to the likeliness of an endogenous rhythm controling primarily both onset and end of activity. The endogenous rhythm is likely to be adjusted secondarily by daylight features occurring around the moments of sunset and sunrise observed by the bats when waiting in the exit chamber in the foregoing night, and (or) when coming home in the foregoing morning.

4.3. Synchronisation of the Activity Rhythm to the Natural Daylight Cycle

The moments of flying out and coming home of bats are roughly controlled by the light-dark cycle. As this cycle undergoes considerable variation in the course of a season, these moments must be characterized by the time intervals between sunset and the flying out, and between the coming home and sunrise. Our main difficulty of measuring the relevant values of the light intensity (E) has been that frequently the first outflyer departs at values of E which are too low (less than $100 \ \mu W/m^2$) to be measured with our instruments, if the selenium photocell is placed on the S.E. side of the church, where the main exit hole happens to be. Another exit hole existed on the S.W. side, and light measurements

Table 1. Number of minutes after sunset when first outflyers came out of two different exit holes during the 1967 season

Date		Exit hole S.E.	Exit hole S.W.	Time diff. in minutes	"Cloudiness"
May	31	36	36	0	8
June	12	43	35	+8	1
	13	40	40	0	3
	14	40	39	+1	7
	15	44	44	0	4
	29	42	39	+3	8
July	13	44	43	+1	2
	24	46	50	−4	2
	25	53	50	+3	3
	26	52	55	−3	2
	27	53	55	−2	3
	28	39	42	−3	8
August	17	37	34	+3	3
	31	33	34	−1	8

at that place proved to be more satisfactory. Unfortunately, we had to close this western exit in the winter of 1968 (like all other exit holes but one), as it gave no opportunity to place an infrared beam at its top. So in the 1968 season, light was measured on the S.W. side, while the bats' activities were observed at the main exit hole at the S.E. side of the church.

This circumstance does not constitute a serious bias, as one might think. When studying the effect of decreasing daylight on the flying out, only the light situation shortly before the moment when the first outflyer comes out is relevant. Following outflyers may be influenced by daylight too, but the moment of their departure may be influenced in addition by the starting of their precursors. During the 1967 season, when both exit holes were still used simultaneously, we observed that the first outflyers from the two exits depart at approximately the same time each night. In Table 1, the small time differences recorded between them are shown to have neither a positive nor a negative trend, and not to depend on the cloudiness of the sky. Cloudiness has been recorded in this table as degrees of covering of the sky increasing from 1 to 8, as published by the meteorological station of the airport at nearby Leeuwarden.

On clear days, one might expect the values of E (which we were not yet able to measure in 1967) to differ significantly on different sides of the church, but this does not seem to prevent the first outflyers to come out at approximately the same time.

4.3.1. Influence of Twilight Duration and Cloudiness on Flying out and Coming Home

According to the usual definition twilight duration at dawn is the time interval between sunset and the moment when the sun stands 6° below the horizon. Its
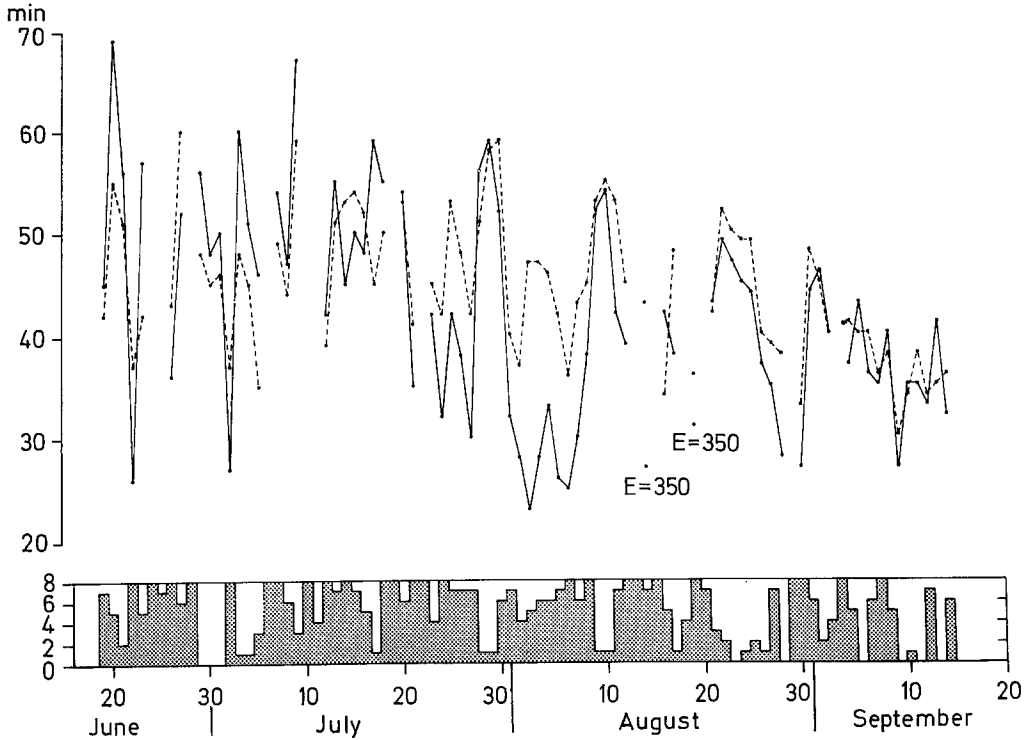
Fig. 5. Time intervals in minutes between sunset and the moment when light intensity $E = 350 \, \mu\text{W/m}^2$ (line), and between sunset and the moment of the first outflyer (interrupted line), both plotted against the 1968 season. In the lower part of the figure cloudiness is recorded as degrees of sky covering from *1–8*

influence on bats can be evaluated by recording the time interval between sunset and the reaching of a certain value of E. Arbitrary, we have chosen $E = 350 \, \mu\text{W/m}^2$, being a quantity of light that still can be measured quite reliably with the aid of our instruments.

Unfortunately, we could start the recording of E not earlier than June 19 of the year of these observations (1968). This recording was continued with slight interruptions (see p. 224) till September 14 of the same year.

In Fig. 5 it is shown that the $E = 350$ interval decreases in the course of the season, as could be expected. At the same time, the interval between sunset and the first outflyer is decreasing in the same sense. It must be noticed, however, that both intervals also vary considerably from one day to another mounting to differences of 15 min within 1 week.

As is shown in Fig. 5, these differences are correlated to differences in cloudiness of the sky during twilight on the relevant nights.

We conclude that the moment of the first outflyer roughly correlates with the duration of the sunset-$E = 350$ interval, but that this duration on a given night
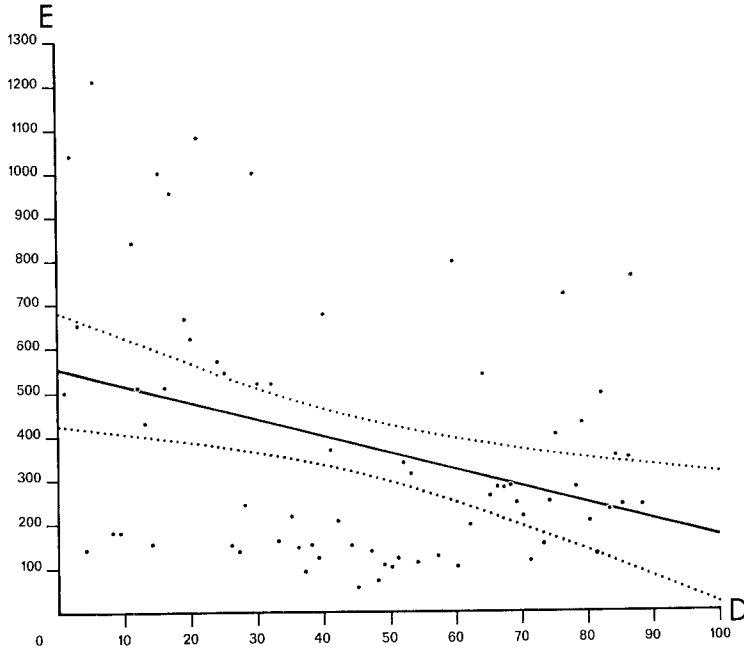
Fig. 6. Daylight intensity (E in $\mu W/m^2$) at the moment of the first outflyer plotted against days (D) during the period from June 19 till September 14, 1968. Dotted lines mark the 0.95-confidence limits of single points of the regression line

depends much more on the incidental degree of covering of the sky than on the seasonal variation of twilight duration. We are not able to appreciate the influence of twilight duration and cloudiness at dawn on the coming home of pond bats, as the last homeflyer reaches the church loft always before sunrise at a moment when daylight intensity is still too low to be registered by our instruments. The only thing we can say about it is that many of these bats start in the evening when there is still more daylight than there is the next morning when they come home again.

4.3.2. Influence of Light Intensity and Light-Dark Ratio on the First Outflyer

The daylight intensities at which the first outflyers depart each night during the period from June 19 till September 14, 1968, are recorded in Fig. 6. Although these values of E vary considerably, a regression line can be calculated as drawn in the figure. According to this line we conclude that the light intensity at which the colony starts flying out decreases *grosso modo* from June to September. Laufens (1972) came to the same result for *Myotis nattereri*. An explanation of this phenomenon could be that in June, when the nights are short, the bats will tend to use the dark-time more completely for their activities than in September, when the nights are much longer.
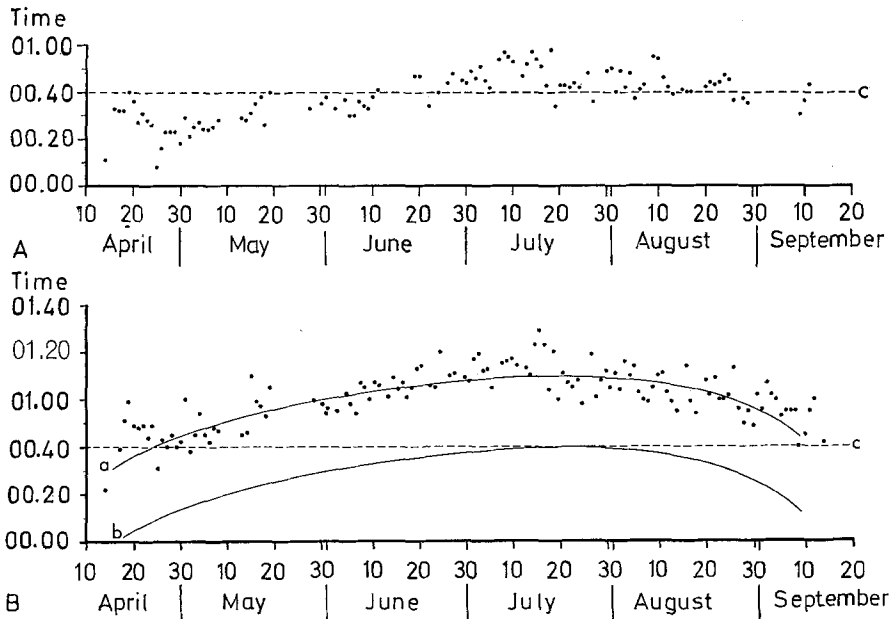
Fig. 7 A and B. Shift of the midpoint of bat-activity in relation to the midpoint of dark-time from April to September (line *c*), 1968. (A) The midpoint between the times of the first outflyer and the last homeflyer. (B) The midpoint between last outflyer and last homeflyer (line *a*); line *b* represents the likely midpoint of average bat activity

4.3.3. The Seasonal Phase Relationship between the Light-Dark Cycle and the Activity Rhythm

Phase differences between the cycles involved here are—in other organisms— known to shift systematically with the season (Aschoff, 1964). As we have no experience with a cyclic night activity of pond bats during winter, we can only regard the oscillation of their activity during spring and summer. According to Aschoff (1964), the best way to measure the appropriate phase relation in dark-active animals is to compare the midpoint of activity with the midpoint of darktime.

If we regard the colony as a whole, the activity midpoint can be calculated by regarding the times of the first outflyer and the last homeflyer; the midpoint of dark-time by taking the midpoint between the times of sunset and sunrise. In Fig. 7 A the activity midpoint has been plotted against the season.

If we try to determine the activity midpoint for one individual, the difficulty arises that we are dealing with 150 bats at least, none of which can be recognized individually. The only reliable checking points we have got are the moments of the first and the last outflyer, and that of the last homeflyer (*cf.* part 4.1.). According to Engländer and Laufens (1968) who watched a much smaller colony of Natterers bat (*Myotis nattereri*) the first and the last outflyer tend to be the last and the first homeflyer, respectively. Our experience with 14 white ringed bats (*cf.* part 3.) gave no positive evidence for similar tendencies in *Myotis*
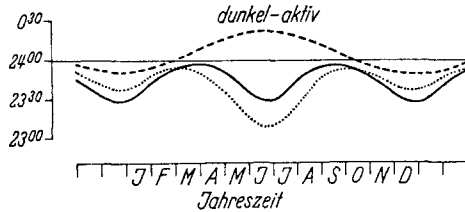
Fig. 8. Predicted seasonal shift of the phase difference concerning nocturnal animals:
----- calculated with regard to the duration of daylight only (constant duration of twilight 1 h);
...... calculated with regard to the duration of twilight only (constant duration of daylight
12 hrs); ———— calculated with regard to the duration of both daylight and twilight (after
Wever, 1967, p. 274)

dasycneme. The white rings were spread over the large flow of unbanded outflyers
many nights in a different way. This problem will be further discussed in part 5
of this paper.

In order to solve our problem in an arbitrary way, we assume that the duration
of nocturnal activity is almost the same for all individuals of the colony. Thus
the last outflyer is likely to be the last homeflyer too, on a given day. Calculating
the midpoint of activity for these individuals and comparing this with the mid-
point between sunset and sunrise we get a graph as shown in Fig. 7 B. The curve (a)
fitted to the points relates to the last outflyer-homeflyer, whereas we are aiming
at the average one. In order to estimate the level of the average curve, we may
use the fact that the median outflyer has been observed to depart, on an average,
approximately half an hour before the last one. This results in a curve (b) for
the likely middle of bat activity. From both Fig. 7A and B we conclude that the
phase difference between the biological cycle and the natural light-dark cycle
decreases as the nights grow shorter and increase as they grow longer. If we take
midnight (00.00 hr) as a reference, the former cycle is always in advance in rela-
tion to the latter one. This conclusion accords with the rule of Aschoff (1964)
which he claimed to count for many other nocturnal animals, and with the results
which Laufens (1972) obtained in some other bat species.

As to the factors causing this type of seasonal shift, some controversies have
risen between Aschoff (1964) and Wever (1967), the former claiming daylight
intensity and light-dark ratio to be the main factors, and the latter adding dura-
tion of twilight as one of the main ones. These resulted in the Aschoff-Wever
model, which has been accepted now by both authors (Aschoff, 1969). In our
Fig. 8 we have cited a figure dealing with the prediction of seasonal variation
in phase-angle differences in nocturnal animals as originating from this model
(Wever, 1967). In part 4.3.1., we came already to the conclusion that the start of
nocturnal activity of pond bats roughly correlates with twilight duration. Our
results on the seasonal shift of phase-angle differences (Fig. 7) do, however, not
indicate the role of the seasonal variation of this duration to be as dominating in
this respect as it is supposed to be in the Aschoff-Wever model (*cf.* Fig. 8). They
are more in accordance with the original seasonal rule of Aschoff, 1964 (compare
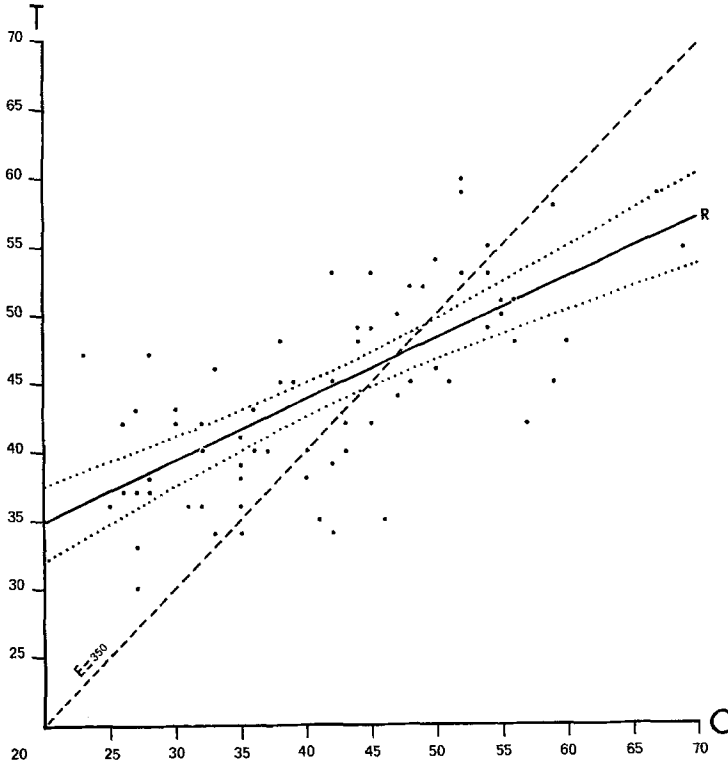Fig. 7 with the interrupted line in Fig. 8).

Fig. 9. Time interval in minutes between sunset and the moment of the first outflyer (T) plotted against time interval between sunset and the moment when light intensity $E = 350\ \mu W/m^2$ (C), during the period from June 19 till September 14, 1968. R regression line; dotted lines = 0.95-confidence limits of single points of R; points laying on the interrupted line indicate cases when the first outflyer moves at the moment when $E = 350$ is reached

4.4. The Releasing Effect of Decreasing Daylight on Flying out

So far, we have paid much attention to descenders and ascenders, outflyers and homeflyers, as they mark the onset and the end of nocturnal activity. We have seen that early descenders have to wait in the exit chamber till the light situation outside has become suitable for flying out (*cf.* p. 225).

On the next pages, we will deal with two most appropriate features of this light situation. They are: the total radiance energy (E) of the daylight and its rate of relative decrease (S).

4.4.1. The Light Intensity at the Moment of the First Outflyer

From Fig. 6 it can be concluded that the majority (61%) of the first outflyers waited till a light intensity below 350 μ W/m^2 was reached. The rest flew out at higher E-values, mounting up to $1210\mu W/m^2$. It seems reasonable to assume that E-values above $1250\mu\ W/m^2$ prevent pond bats to fly out, whereas values below 350 are always acceptable. E-values between 350 and 1250 may occasionally be accepted.
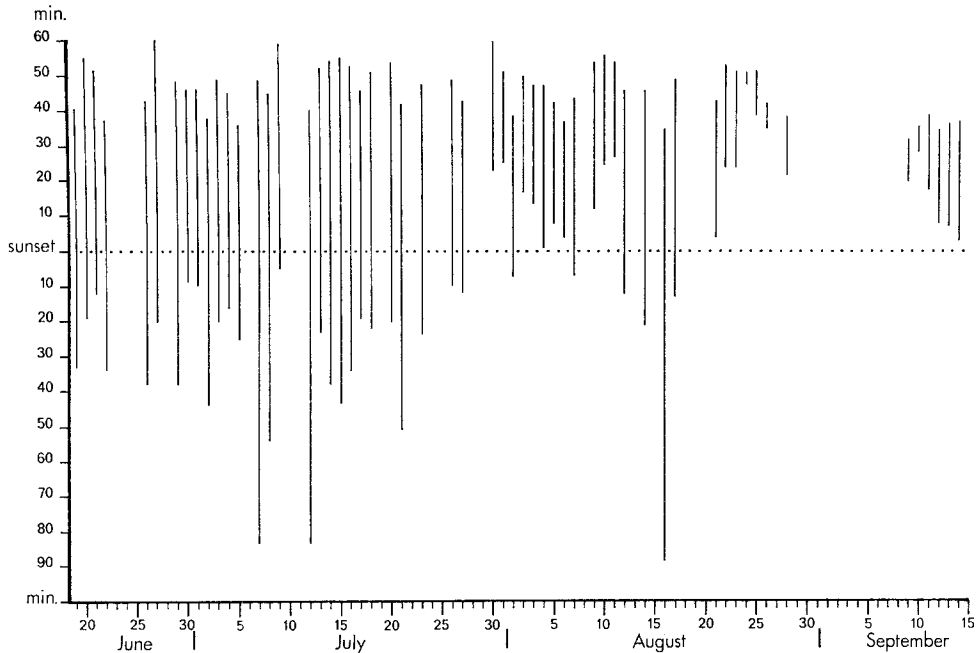
Fig. 10. Time intervals in minutes between the moments of the first descender and the first outflyer during the period from June 19 to September 14, 1968 (cf. Fig. 4)

A first simple hypothesis would be that the first outflyer waits until some threshold of absolute light intensity is surpassed. Were this the case, one would expect this bat to come out earlier as the sky is more clouded. And indeed, in Fig. 5 a positive correlation between time lapses from sunset to the first outflyer and to $E = 350 \mu W/m^2$ is shown. Yet the slope of this relationship certainly deviates from 1:1 (Fig. 9). This means that on cloudless days the first bat leaves the church at light intensities fairly higher than on heavily clouded and overcast days.

The amount of variation independent of cloudiness has to be attributed to additional factors. There may be individual differences in physiological conditions. Another factor we can think of in this respect is the time during which the first outflyer has been waiting in the exit chamber. This latter factor can be approximated by the time interval between first descender and first outflyer. Needless to say that we have no definite check that indeed the same individual is involved. These time intervals are depicted in Fig. 10. The duration of these time intervals appears to fluctuate from 3 to 132 min. At the moment of descending, this fluctuation may be due to inaccuracy in synchronising the endogenous timing system to the natural light-dark cycle (cf. part 5). So, at least the onset of the waiting time is independent of the cloudiness on the evening involved, as the effect of cloudiness on the daylight intensity cannot be seen on the loft (cf. part 4.2.). The moment of flying out (=end of the waiting time) must, however, be directly correlated indeed to the light conditions outside. The
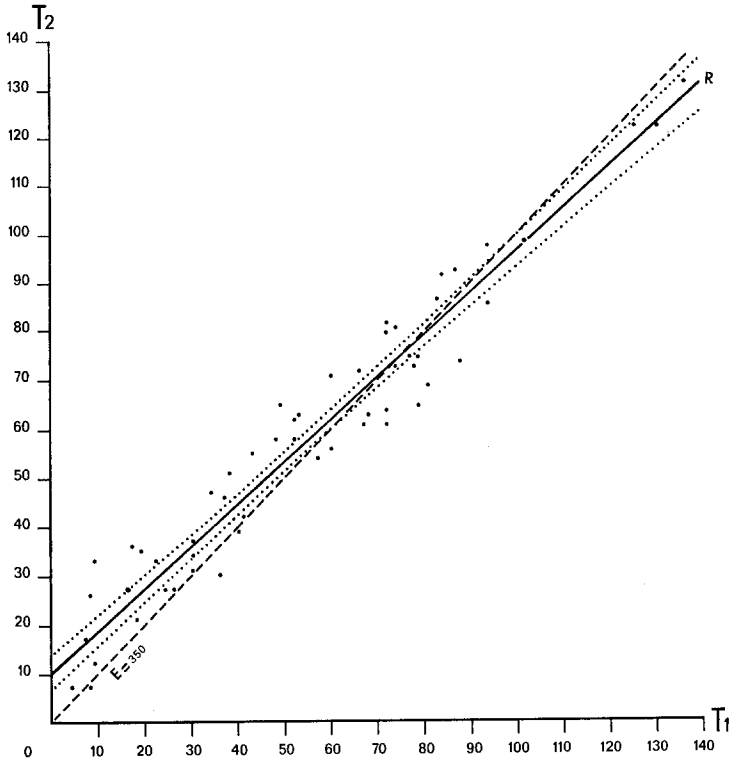
Fig. 11. Time interval in minutes between the first descender and the first outflyer (T_2) plotted against the interval between the first descender and the moment when $E = 350\ \mu W/m^2$ (T_1), during the period from June 19 till September 14, 1968. R regression line. For dotted and interrupted lines see legend of Fig. 9

suggestion made above, that the duration of waiting in the exit chamber is also in some way related to the time of departure of the first outflyer, can be judged to some extent by regarding Fig. 11. According to these data, practically all individuals which have waited in this chamber for less than 50 min have not flown out until E-values below 350 were reached. A part of those which have probably waited longer did the same, but about 50% of them left at higher values of E.

4.4.2. The Rate of the Relative Decrease of Daylight Intensity

The observation of Ringelberg (1964) that a certain value of the rate of the relative decrease of light intensity acts as a releasing stimulus on the phototactic behaviour of the Cladoceran *Daphnia magna* Straus drew our attention to this rate in connection with the behaviour of the first outflyer. The values of this rate at the moments when the first bat flies out (S) during the period from June 19 till September 14, 1968, are plotted against the season in Fig. 12, in the same way as the corresponding E-values are recorded in Fig. 6.
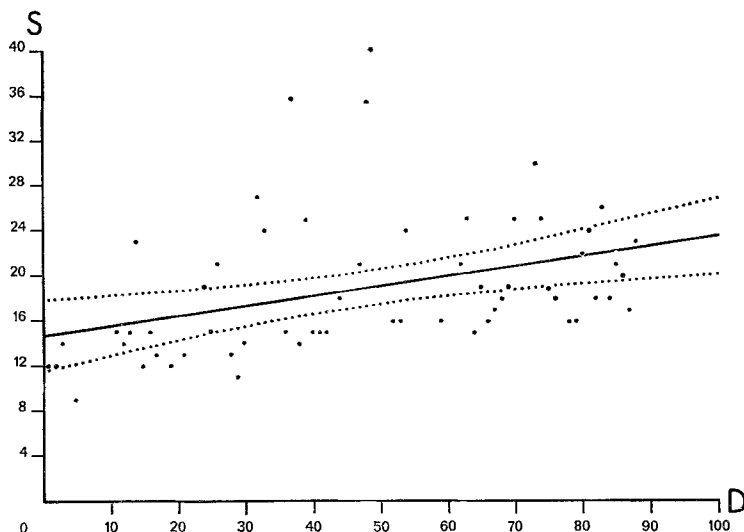
Fig. 12. The rate of the relative decrease of daylight intensity per second at the moment of the first outflyer, multiplied by 10^4 (S), plotted against days (D) during the period from June 19 till September 14, 1968. For dotted lines see legend of Fig. 6

According to Fig. 12 these S-values range from 9 to 40, and increase as the season proceeds. This increase could be expected, as the corresponding E values are decreasing (Fig. 6).

In order to analyse the interrelation between these daylight features more closely, the S-values are plotted against the E values concerned here in Fig. 13. According to this figure, practically all first outflyers wait till $S = 11$ is surpassed. For those flying out at $E < 350$, the actual value of $S > 11$ does not seem to matter a great deal, but those flying out at higher E-values seem to depend on S-values between 11 and 20.

4.4.3. Conclusions

It is clear that the decision of the first outflyer to depart depends primarily on the absolute value of the total radiance energy of the daylight outside (E). On the other hand, a rather wide range of E-values appears to be acceptable (cf. Fig. 6). Thus, the actual E-value at the moment of decision can hardly be the releasing stimulus to fly out in every case.

Physiologically, the absolute value of E is not likely to be a releasing stimulus as it is decreasing which is not easy to evaluate by the eye generally. The rate of relative decrease of the light intensity (S) is, on the contrary, a rapidly increasing factor during that part of the evening when the first outflyer uses to depart. Therefore, S is more appropriate to be a releasing stimulus (cf. Ringelberg, 1964).

According to the data recorded in part 4.4. we come to the following hypothesis. If the first outflyer waits till $E = 350\,\mu\,W/m^2$ is surpassed, no special releasing stimulus is needed to make her fly out. If the first outflyer leaves at $E > 350\,\mu\,W/m^2$, values of $S > 11$ are needed as a releasing stimulus. A duration
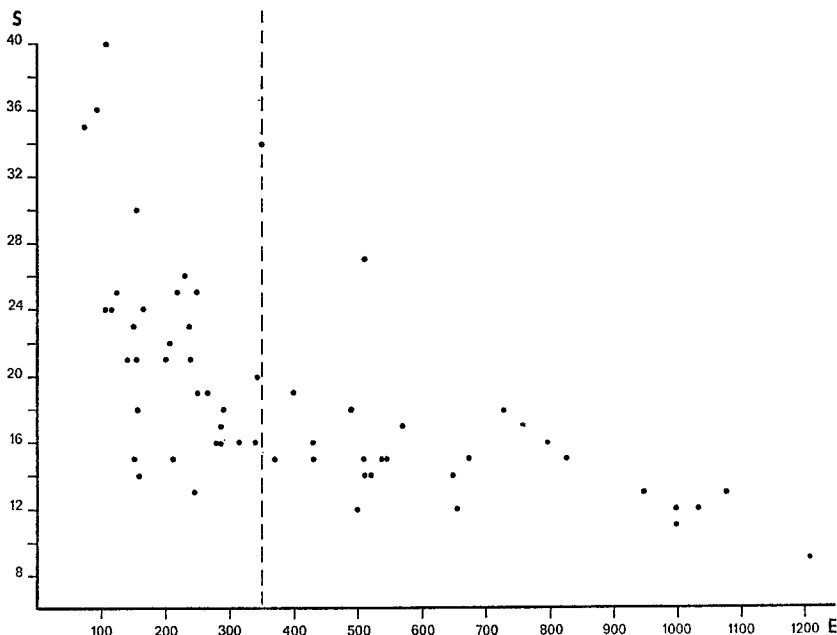
Fig. 13. The rate of the relative decrease of daylight intensity per sec, multiplied by 10^4 (S), plotted against the total radiance energy of daylight (E in $\mu W/m^2$), during the period from June 19 till September 14, 1968. Each dot relates to one first outflyer

of more than 50 min of waiting in the exit chamber promotes the decision to fly out in spite of these higher E-values. The consequences of this hypothesis will be discussed in part 5 of this paper.

5. Discussion

The reader must be aware of the fact that our conclusions originate from observations on pond bats having their diurnal roosting places in the almost absolute darkness of the Berlikum church loft. In other situations and in other bat species things may be essentially different. Bat roosts are not always that much deprived of daylight as in our case, and various bat species have a different sensitivity to daylight as an inhibiting factor. For instance: *Pipistrellus pipistrellus*, *Nyctalus noctula*, and *Eptesicus serotinus* at our latitude fly out earlier in the evening than pond bats do, and the first outflyer may occasionally come out even before sunset. In Finland, Nyholm (1965) observed *Myotis mystacinus* to fly in bright daylight during May and October when the nights are too cold to feed. Hence, our conclusions may not be generalised over all kinds of bats and over all latitudes. On the other hand, results similar to ours have been reported in other bats species living in other situations.

5.1. Onset and End of Activity in Myotid Bats

Dispersion among outflyers and homeflyers has been reported by many authors in many bat species. It is known to vary with the season. In Table 2, average

Table 2. Average times of departure and return in minutes after sunset (a.s.) and before sunrise (b.s.), respectively

Species Country Author	Start first outflyer	Start last outflyer	Return first homeflyer	Return last homeflyer	Duration outdoor activity
Myotis dasycneme Netherlands Voûte (1972)	45 a.s.	105 a.s.	100 b.s.	40 b.s.	45 a.s. till 40 b.s.
M. nattereri E. Germany Böhme and Natuschke (1967)	45 a.s.	120 a.s.	120 b.s.	40 b.s.	45 a.s. till 40 b.s.
M. mystacinus Finland Nyholm (1965)	45 a.s.	100 a.s.	120 b.s.	60 b.s.	45 a.s. till 60 b.s.
M. myotis E. Germany Böhme and Natuschke (1967)	35 a.s.	120 a.s.	150 b.s.	55 b.s.	35 a.s. till 55 b.s.
M. nattereri W. Germany Laufens (1972)	35 a.s.	70 a.s.	100 b.s.	30 b.s.	35 a.s. till 30 b.s.
M. Bechsteini W. Germany Laufens (1972)	30 a.s.	—	—	50 b.s.	30 a.s. till 50 b.s.

times of departure and return during summer have been estimated from literature data as far as they originate from recordings with the aid of a photocell detector which is comparable with our apparatus (*cf*. part 3).

From *Myotis daubentoni* only average times of all outflyers and all homeflyers are published (Nyholm, 1965), being 45 min a.s. and 55 min b.s., respectively. According to these data the time lapses observed in european myotid bats between first and last outflyer, first and last homeflyer, and the duration of outdoor activity of the whole colony do not differ very much from those in *M. dasycneme* in the Berlikum situation.

Unfortunately, times of the onset of indoor activity could be recorded only in Berlikum. In our opinion, the times recorded for descenders demonstrate the biological significance of having an endogenous 24 hr activity rhythm in a most convincing way (*cf*. part 4.2.).

5.2. The Activity Rhythm in Pond Bats

In this part we will recapitulate and discuss the main events in the chronological sequence as observed each night again in the Berlikum pond bat colony. The onset of the nocturnal activities of the colony takes place in two phases. In phase I the bats descend into the exit chamber, and most of them wait there for some time. In phase II they fly out through the exit hole of this chamber. The beginning of phase I depends on an endogenous 24-hr activity rhythm which is roughly synchronised with the light-dark cycle in such a way that this rhythm
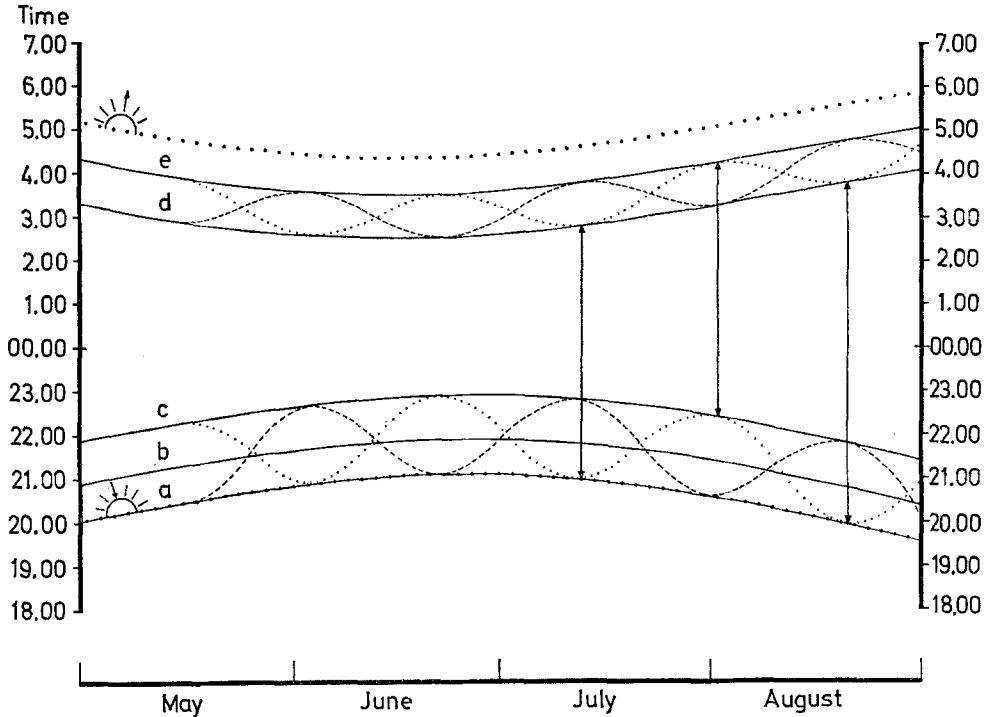
Fig. 14. Simplified survey of the nocturnal activity of the bat colony (*cf.* legend of Fig. 4). Arrows indicate duration of nocturnal activity of one individual, on the assumption that the first descender is the first homeflyer, and the last descender is the last homeflyer

becomes adapted to the seasonal variation of the light-dark ratio (*cf.* Fig. 4). The onset of phase II (the flying out) is correlated with daylight intensity in such a way that the bats can cope with the irregular fluctuations of this intensity caused by differences in cloudiness of the sky which may occur from one day to the next (*cf.* Fig. 5).

5.2.1. Thesis on the Synchronisation of the Activity Rhythm to the Natural Daylight Cycle

To begin with, we will regard the synchronisation of the endogenous cycle mentioned above. In Fig. 14 the survey of the nocturnal activity of the colony (*cf.* Fig. 4) has been schematically simplified for convenience sake. The first of the descenders (line a) has been assumed to coincide with sunset, on an average, and the maximal variation among descenders to be constantly two hours (*cf.* line c); the last of the ascenders to arrive 40 min before sunrise (line e) and the maximal variation among ascenders to be constantly 1 hr (*cf.* line d). As a matter of fact Fig. 14 gives limits counting for a colony of 150 bats as a whole, but no information on the behaviour of one individual. Laufens (1972) observed the first outflyer to be in most cases the last homeflyer, the second outflyer to be

the last homeflyer but one, etc., etc., ending with the last outflyer to be identical with the first homeflyer in a colony of eight Natterers bats (*Myotis nattereri*).

If this were true for the pond bats in the Berlikum situation, late descenders (being identical with late outflyers) would descend and ascend again at moments when there is practically no daylight. As there cannot penetrate any appreciable amount of daylight on the church loft (*cf.* part 4.2.), late descenders would live in a constant and almost complete darkness. They would miss any opportunity to synchronise their endogenous cycle to the seasonal variation of the light-dark ratio. This would mean that many of the bats in this colony would depend mainly on some kind of social "Zeitgeber" in order to be able to descend on the right moment. It seems much more probable that every bat of the Berlikum colony uses to see some daylight at least once a day: either in the evening after descending early to the exit chamber, or early in the morning when staying out sufficiently long to be confronted with dawn. As we recorded no individual activity data, we have no proof for the existence of two oscillators in bats. But if so, their behaviour would fit fairly well in the recent model of Pittendrigh postulating a "night" and a "morning-oscillator" to be involved in the control of the activity rhythm in higher vertebrates (cited from Daan, 1973, p. 258). According to this hypothesis the moment of descending on a given night would be coupled with the night-oscillator of the preceding night for one part of the Berlikum colony, and with the morning-oscillator of the preceding morning for another part of this colony.

Another question is wether or not each individual has such a rigid activity pattern that it always belongs either to the former, or to the latter part of the colony mentioned above. If this were true, the time differences between the moments of first and last descender, and of first and last ascender, as recorded in Fig. 14, would be only consequences of individual differences, *e.g.* in sensitivity to daylight intensity. In addition, such a rigid activity pattern demands a rather high precision of entrainment. There are good reasons, however, to doubt the adjustment to the synchronising daylight to be so accurate really. First, cloudiness of the sky may be totally different on subsequent days (*cf.* Fig. 5), causing the light intensity at dawn and dusk to fluctuate irregularly. Second, intensity of daylight (the entraining agent) is mostly very low at the moments when the bats can see it, especially at dawn (see p. 229). Therefore, we expect the individual activity pattern to lack so much precision of entrainment that the same individual depends on the night-oscillator on one day and on the morning-oscillator another day. This may cause the moments of descending, and those of ascending, of each individual to oscillate between two extremes (lines a and c, lines d and e) as indicated in Fig. 14. The interrupted and dotted lines in this figure illustrate this schematically for two of them being in opposite phases. The frequency suggested in Fig. 14 is completely fictive, as we know nothing about it.

Summarising we suggest that the usual dispersion in time among all descenders and ascenders of the colony on a given day is caused by individually different phases of an oscillatory entrained activity cycle. This means also that the first descender on a given day may become the last one on another day; the same counts for the ascenders. Interindividual phase differences could be initiated by the fact that many bats of this colony arrive on different days at Berlikum when returning from their hibernation quarters during April (Sluiter *et al.*, 1971).

A first attempt has been made to sustain the hypothesis mentioned above by observing individuals marked with white rings (see p. 224), but further experiments are needed to prove it. For the time being, we can only refer to some results which other authors obtained from experiments with other mammals, but which may be relevant to our problem with bats.

Early descenders which arrive in the exit chamber before sunset (*cf.* Fig. 10) are confronted with rather high daylight intensities. These may be comparable with experimental lightshocks which were given by Decoursey (1961, 1964) to nocturnal rodent species being kept in permanent darkness in order to study response-curves. Such a lightshock given during 10 min just before the time of the onset of activity had a retarding effect on this onset and on the end of activity. Laufens (1972, p. 19) showed a small colony of Natterers bats (*Myotis nattereri*) to react in the same way when floodlight was given near their natural roost just before the usual time of flying out. If similar response-curves would exist in bats as were found by Decoursey (1961, 1964) in rodents, it seems to be a reasonable suggestion that early descenders in Berlikum will delay their activity period as a reaction on a "light-shock". They will descend a little later the next evening and ascend a little later the next morning. Going on in this direction they will join the descenders after sunset which are supposed to see very little daylight at all. Lack of precision in the timing of their activity period which is expected to occur then may confront them some day with another "lightshock" at dawn and make them move their activity period backwards again (*cf.* Fig. 14).

5.2.2. Thesis on the Releasing Effect of Decreasing Daylight on the Flying out

The next point to discuss is the regulation of the time of flying out of the exit chamber, being phase II of the onset of nocturnal activity of the pond bats. Fig. 15 illustrates the consequences of our hypothesis on the daylight features which are suitable (o) resp. unsuitable (•) for a pond bat to fly out (*cf.* part 4.4.3.). As examples, we have recorded in this figure the relevant situations on three nights: August 6 (a), June 19 (b) and 20 (c), 1968. On night (a) the sky was heavily clouded, and the first outflyer came 36 min after sunset. The light intensity at this moment was as low as $E = 125 \mu$ W/m² (o), meaning that nightfall was almost completed; the rate of relative decrease of E was accordingly high then: $S = 40$ (o). According to the time of the first descender of night (a) (3 min after sunset), the first outflyer of this night can have waited no longer than 33 min in the exit chamber before these very favourable values of E and S were reached.

On the nights (b) and (c), however, the sky was much more clear than on night (a). The first outflyer came at 42 (b) and 55 min (c) after sunset at values of $E = 500$ (•) and $E = 1035 \mu$W/m² (•), respectively, meaning that in neither of both cases a favourable light intensity to fly out ($E < 350$, *cf.* part 4.4.1.) had been reached. On the other hand, the assumed critical threshold value $S = 11$ (o) (*cf.* part 4.4.2.) had just been surpassed at the moments when both first outflyers started. First descenders on the nights (b) and (c) came 74 and 75 min before the corresponding first outflyers. So it is quite possible that these first outflyers have waited more than 50 min (*cf.* 4.4.1.) till $S = 11$ (o) was surpassed. Unfortunately, this cannot be ascertained, as we are not able to prove the first descender to be the same individual as the first outflyer generally.
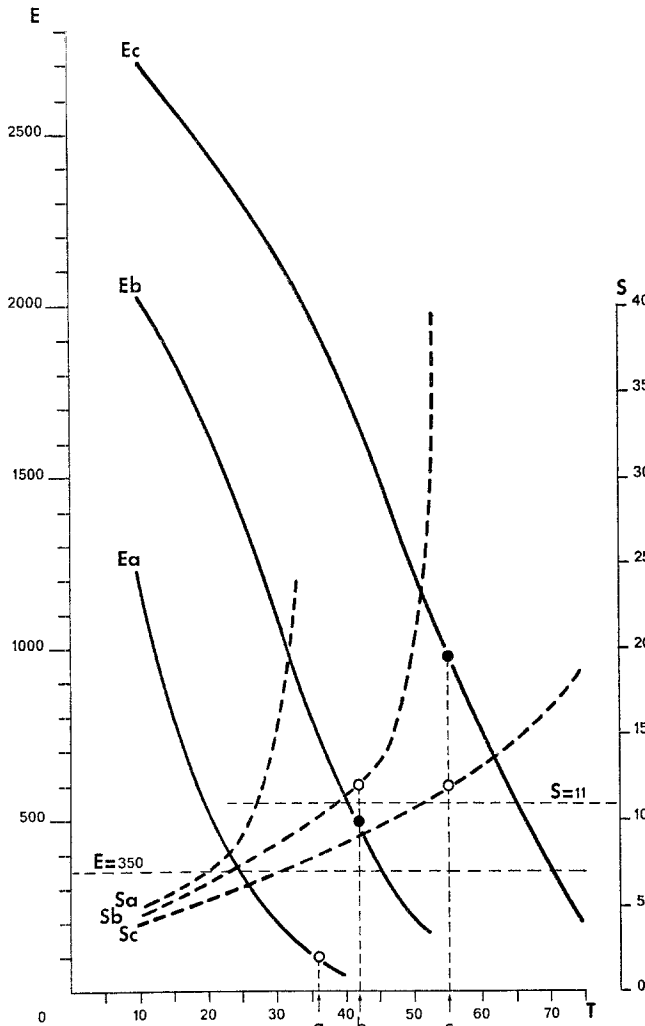
Fig. 15. Daylight changes as regards E and S on three nights (a, b, and c) with different degrees of cloudiness plotted against time after sunset in minutes (T). Arrows indicate moments when the first outlfyer came. For dots and rings see text on p. 240

With regard to the hypothesis mentioned above and according to the data recorded in Fig. 15, the first outflyer of night (a) could have departed already at 28 min after sunset, as $S = 11$ ($\circ$) had been surpassed then. Night (a) represents a case of extremely heavy cloudiness of the sky and, therefore, the time of 28 min after sunset may be generalised as a minimum value for all first outflyers during the period from April till September, 1968. In fact, no first outflyer has been recorded earlier than 30 min after sunset. As night (c) was a rather clear one in June, the time of 52 min after sunset should be near the maximum value for all first outflyers during this part of the season. In fact, no first outflyer has been ever recorded to depart later than 60 min after sunset.

Finally, it must be asked what might be the survival value of having a certain value of the rate of relative decrease of daylight (S) as a releasing threshold for flying out. It must be noted in this connection that each value of S generally "predicts" how long it will last before a certain value of daylight intensity will be reached. In case of the example used in Fig. 15, on night (b), the value of $S = 12$ garantees a favourable light intensity ($E < 350$) to be reached in less than 5 min. Reasoning along the same line, it can be concluded that the first outflyer on night (c) could expect this favourable light intensity to be reached in 15 min after her departure. So, by sticking to a releasing threshold of $S = 11$, the first outflyers on clear nights (when they accept rather high E-values) can be sure that the safety of darkness will come soon.

5.3. Light Sampling Behaviour in Myotid Bats

We consider the waiting of the pond bats in the exit chamber of the Berlikum church as a sampling behaviour, in order to judge the light situation outside. Other authors describe sampling flights of myotid bats e.g. to a window on a loft (Decoursey and Decoursey, 1964), to an exit of a cavern (Twente, 1955) which do not result in their actual departure immediately. Nyholm (1965) observed a colony of Myotis mystacinus to become active in its roost shortly before flying out and to sample the daylight by flying or crawling to the exit.

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