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ACTIVITY DURING NATURAL HIBERNATION IN THREE SPECIES OF VESPERTILIONID BATS

by

SERGE DAAN*

(Laboratory of Animal Physiology, University of Amsterdam, The Netherlands)

CONTENTS

1. INTRODUCTION

Hibernation is an eco-physiological adaptation developed by several mammalian taxonomic groups, and serving to cope with yearly re-turning periods of food shortage in the temperate zones (reviews in EISENTRAUT, 1956; KAYSER, 1961; FISHER *et al.*, 1967). Its dominant

* Present address: Max Planck Institut für Verhaltensphysiologie, Seewiesen und Erling-Andechs, West Germany.

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physiological characteristic is the greatly reduced body temperature: Instead of being regulated at the normal homeothermic level, body temperature during hibernation is allowed to drop to the ambient temperature, or is regulated at a level only slightly exceeding the latter. The metabolic rate is reduced to a fraction of its value in the homeothermic state, when involved in maintaining such minimal temperature differences with the environment (e.g., HENSHAW, 1968). It has long been realized that it is this reduction in energy expenditure, that endows survival value on the whole complicated set of physiological and behavioural mechanisms now known to be involved in hibernation. Indeed, highly specialized adaptations contributing to the success of hibernation range from peculiarities such as MERZBACHER's (1904) wing-spread reflex in torpid bats loosened from their foothold to such physiological accomplishments as accurate timing based partly upon endogenous circannual oscillators (PENGELLEY, 1967, 1968) and navigation of bats in long-distance migration to their winter quarters (STRELKOV, 1969; GRIFFIN, 1970).

The vast amount of research into hibernation accumulated in the last decades (MAYER, 1964), has concentrated largely on physiology, but neglected ecological topics. Few data are available on population biology in hibernators. These suggest that rates of mortality attain maxima around the end of hibernation. This has at least been shown in several species of bats (*Myotis myotis:* EISENTRAUT, 1947; *Rhinolophus ferrumequinum:* RANSOME, 1968). STEBBINGS (1969) reported that 90% of his findings of dead bats away from roosts were made in April and May. To the extent that starvation during this yearly returning minimum body weight period may be held responsible for a peak in mortality, we may hypothesize that the rate of body weight loss during the winter is of vital interest for the species. BEER & RICHARDS (1956) calculated from the average rate of body weight loss in hibernating *Eptesicus fuscus* in Minnesota that the amount of body fat stored in the autumn by this species would allow hibernation for about 195 days, which matches the average period with frosty days in that area: A slightly longer winter would put a severe toll on the population.

Hibernation is not continuous. DUBOIS (1896) already observed the frequent spontaneous interruptions of the lethargic state in the Alpine marmot *(Marmota marmota)*. In recent years this phenomenon has been found to occur in a more or less distinctly periodic pattern in many hibernators (e.g., PENGELLEY & FISHER, 1961; KRISTOFFERSON & SOIVIO, 1964; SAINT GIRONS, 1965). Due to the enormous differences in metabolic rate between the torpid and homeotherm states, and to the extra energy involved in warming up the body in the arousal process itself, such interruptions utilise large amounts of body reserves.

KAYSER (1952) was the first to realize that these interruptions together account for the major part of the energy expenditure during the winter. He made the paradoxical statement: "...il est évident que le coût de l'hibernation n'est pas dû à la léthargie mais aux réveils pendant lesquels les hibernants se comportent en homéothermes". This conclusion was based on actographic recording of hibernating ground squirrels *(Citellus citellus)* during a complete hibernating season. Multiplication of the total time spent awake with the active (summer) metabolic rate and of the total time of torpidity with lethargic metabolic rate gave a figure of 90% of the total energy expenditure to be accounted for by the interruptions, which made up only 7.5 % of the total hibernation time. This 'Paradox of Kayser' can today be confirmed for a number of species that have been studied under laboratory conditions (SOUTH & HOUSE, 1967), though very little is known about arousal frequencies in natural circumstances.

If it is true that also in nature these arousals have such a predominant share in the cost of hibernation, we must logically presume that activity during hibernation is one of the weakest links in the hibernator's life cycle, and that natural selection would act with according strength on all genetic variation affecting the amount of such activity. From such considerations, and the general occurrence of periodic arousal, one is inclined to postulate somepositive biological function of winter activity, which outbalances the selection pressure against more rapid utilisation of body food reserves. Of course, arousal is as inherent to hibernation as is waking up to sleep. But the large amount of interspecific, seasonal and individual variation justifies the question as to the functional differences of these patterns. The existence of interscapular brown fat—first described as a "hibernation gland" (BARKOW, 1843), but since the detection of its role in nonshivering thermogenesis during arousal from hibernation (HAYWARD & LYMAN, 1967) better regarded as an "arousal organ"—adds to the probability of a functional aspect.

Many other authors have investigated the regularity of spontaneous arousals (*e.g.*, TWENTE & TWENTE 1965, 1967, 1968; FISHER & MANERY 1967; FISHER & MROSOVSKY 1970; PENGELLEY *et al.*, 1971). This interest has been directed to the causes rather than to the possible function of arousals. The research presented here is directed towards the question of function. It was designed as an attempt to formulate as complete a picture as possible of the amount and nature of the activity during hibernation in three species of cave roosting bats.

Scanning the relevant part of the vast literature on hibernation, it amazed me what little information is available on natural hibernation

in the field. The only hibernators that have frequently been studied in their natural environment are cave-dwelling microchiropterans. This is obviously related to the accessibility of their winter quarters where they can be observed without difficulty, often in large numbers together. None of the many burrowing rodent hibernators present this opportunity. I think it is this ease of observation which gave rise to what now impresses one as a long tradition of anecdotical papers, often filled with notes from the waste-basket of large-scale bat banders. VAN NIEUWENHOVEN (1956) has summarized most of the older papers, but the tradition has unattenuatedly continued since that time (MUMFORD, 1958; MUIR & POLDER, 1960; TINKLE & MILSTEAD, 1960; TWENTE, 1960; PEARSON, 1962). Most of the serious studies on bat hibernation behaviour involved large-scale banding (KRZANOWSKI, 1959, 1961; BEER & RICHARDS, 1956; TINKLE & PATTERSON, 1965), and since the disturbance effect of banding and handling is now known to be considerable (STEBBINGS, 1966, 1969; SLUITER et al., 1971) we can give little weight to their rough estimates of, e.g., population activity and turnover.

Careful long-term investigations on the behaviour of hibernating bats have seldom been made (VAN NIEUWENHOVEN, 1956; NEVRLÝ, 1963; RANSOME, 1968). Still, however accurate some of this work may be, it does not, except for RANSOME's (1971) recent data, allow the calculation of hibernation periods and other parameters of activity. Therefore a series of field investigations in bat hibernacula was set up aimed at a quantative evaluation of winter activity. These studies have been carried out partly in the limestone quarry area in South Limburg (The Netherlands) and partly in an old abandoned ice cellar in northwestern Holland.

Bats are variable in many aspects of their behaviour. There is, for instance, large individual variation in the time of arrival in the winter quarter, and in their selection of particular environments. Some of their behaviour is strongly affected by individual tradition. Bats may do anything between flying spontaneously from one cave to another in severe winter conditions and staying for six months in one little hole hundreds of meters away from daylight. Any description of the behaviour is therefore necessarily a matter of relative frequencies, if not a mere qualitative account of incidental observations.

On the other hand, there is of course interspecific variation. Some of the patterns found will turn out to be valid for more species, others only for those under study, still more perhaps may be restricted to the particular latitude or type of hibernaculum. The limitations of general validity can only in few instances be inferred from literature.

In the South Limburg limestone caves 14 bat species have been found to hibernate, out of 20 species occurring in northwestern Europe. Three of these *(Myotis mystacinus, M. daubentoni* and *M. dasycneme)* together make up an estimated 90% of the hibernating populations. The behaviour of these species differs in details but is comparable in most general patterns. In many ways (DAAN & WICHERS, 1968) the three form an intermediate group between two groups of the scarcer species: On one side the hardier kinds (such as *Plecotus auritus, Eptesicus serotinus, Myotis nattereri, Pipistrellus pipitrellus)*, which seldom visit the caves, and then only the front part, being content with less protected abodes; on the other hand those southern species usually found in the warm and stable rear of the caves, and nowadays either declining *(Myotis emarginatus, M. myotis, Rhinolophus hipposideros)* or nearly extinct in the Netherlands *(Rhinolophus ferrumequinum)*. Observations on single specimens of these other species may, in a few instances, give some idea of how far reasonable extrapolations may be made.

2. METHODS

2.1. Frequency of movements

Several methods have been used by students of natural winter activity in hibernating bats. First, there are qualitative approaches, such as: assigning to the animals observed an arbitrary index of physiological state, ranging from torpidity to full activity (DWYER, 1964), using fresh faecal pellets as information about previous hunting activity (RANSOME, 1968), and netting bats outside the hibernacula (O'FARRELL *et al.*, 1967).

Quantitative attempts to assess duration of hibernation periods ('bouts') in the field have been made by DE WILDE & VAN NIEUWENHOVEN (1954), NEVRLÝ (1963), GAISLER (1966) and recently by RANSOME (1971). Such estimates are always based on series of standardized searches in the hibernacula, during which the exact location of every bat found is noted. Disappearing from a location is then considered to be the result of spontaneous arousal (1), whereas finding a bat at the same location in two subsequent searches is assumed to prove that the animal has been continuously torpid during the interval (2).

The former of these assumptions is supported by measures taken by the authors to avoid any disturbance of the bats. No bands are used, or coloured bands (GAISLER & NEVRLÝ, 1961) which can be read from a distance. In my investigations plastic head stikcers (DAAN, 1969) were used for identification purposes. With these markings, even the fixing can often been done without the bats waking up. The drawback

of the method is that the markings are lost during the summer (and in very few instances also during the winter): they are useless for identification during more than one winter season. The standard search procedure used in this study involved avoidance of any noise or touch. Electric torches were the only light source used. Generally, a bat was observed for no longer than a few seconds needed to identify species, headmark and location number. All locations where a bat was found were exactly marked by a number on the white cave wall. Notations were made on position, dew cover, and postural changes since the previous search.

The second assumption is not always justified: Animals may wake up between two searches, and either enter torpidity again without having left their location, or return to it after a flight through the cave. The bias caused by assuming continuous torpidity results in an underestimation of activity. The stronger the tendency per individual to return to a particular location after arousal, the stronger the bias. This means that especially the longest hibernation bouts found (e.g., the maxima computed by NEVRLÝ, 1963 and GAISLER, 1966) will be strongly affected by it. Therefore I prefer to compute the frequency of movements in the population (DAAN, 1969) rather than duration of individual non-moving. By this treatment the bias is spread out over the population, and an over-all estimate of the error introduced can be computed (see section 4.5).

The number of movements detected is of course inversely related to the duration of the intervals between two searches. It will be argued (section 4.5) that long and irregular intervals as used by NEVRLÝ (1963), GAISLER (1966), DAAN & WICHERS (1968) are essentially inadequate to estimate the real amount of activity of the bats. The numbers and frequencies of searches in the present investigation are shown in Table I. Searches were made usually in daytime (900 h–1800 h) and occasionally at night (1800 h–2400 h).

2.2. Recording of flights through passages

Activity recordings of bats in their summer colonies have been made by various authors, making use of either mechanical (KOLB, 1959) or photocell detectors (NYHOLM, 1957, 1965; BÖHME & NATUSCHKE, 1967; ENGLÄNDER & LAUFENS, 1968; LAUFENS, 1969; VOÛTE, 1972). No such studies have been made in winter quarters so far. In the investigations presently described a sensitive photocell device was used (for details see DAAN, 1970), with a long light beam reflecting concertina-wise between two parallel mirrors mounted in an iron rrame of 75 × 100 cm. It was designed to cope with the difficulty of the large flight passages present in the quarries rather than the small holes often giving access

TABLE I

Methods used in the Barakkengroeve (see Kuipers & Daan,1970),the Koelebosgroeve (I, see Daan, 1969; II see Daan, 1970) and in Middenduin (see De Rue&Daan, 1972).

	Locality			
Methods	Barakken-groeve 1967/68	Koelebos-groeve I, 1967/68	Koelebos-groeve II, 1968/69	Middenduin 1969/70
Number of Searches	13	111	31	159
Intervals (days)	10–16	2	7	1–2
Approximate duration	3 days	3–5 hrs	3–5 hrs	30 min
Recording	—	—	camera	camera double detector

to summer colonies. Recording was by means of an automatic camera, triggered by a photocell-amplifier and viewing the 'window' (Pl. I). The advantage of photographic above event-pen recording is obviously the possibility to identify species and sometimes even individuals.

The photographic bat recorder has been in use in the cave, 'Koelebosgroeve', in 1968/69 and in the ice-cellar, 'Middenduin', in 1969/70. In the latter locality (see Fig. 7) it was used in combination with another photocell-eventmarker system. The latter, similar to the double device used by Böhme & Natuschke (1967), discriminated direction and recorded inward and outward movements through the narrow entrance passage separately.

2.3. Meteorological measurements

In order to analyse the influence of environmental parameters on bat activity, it was necessary to have a sound knowledge of the climatic structure of the hibernacula. Use has been made of the following techniques:

(a) Continuous recording of temperatures on a potentiometric twelve-channel recorder (Philips PR3210U): ambient temperatures at twelve stations were sensed by Pt-resistances, and recorded on a scale either from 0 to 10°C ($\pm$ 0.1) or from -5 to $+15$°C ($\pm$ 0.2). The recorder has been used in the 'Koelebosgroeve' in 1967/68 and 1968/69.

(b) Air temperature readings at a larger number of stations in the hibernacula were made at regular intervals (usually two weeks). For this purpose a YSI-telethermometer was used, equipped with six NTC-thermistors. Readings could be made with a precision of 0.2°C.

At every station temperature was measured in the upper ($\pm$ 10 cm from the ceiling) and in the lower air layer ($\pm$ 10 cm above the floor). Such measurements were made in all parts of the investigation.

(c) Determinations of relative humidity were made at irregular intervals with an Assmann aspiration psychrometer.

(d) The direction of circulation in upper and lower air layers was qualitatively assessed by using tobacco smoke. Measurements of wind velocity were sometimes made with a Luga-anemometer.

(e) Mapping of the hibernacula was done with help of compass, candles, measuring tape, and a garden hose with water for assessing the height differences.

(f) For outside conditions use has been made of the weather reports from the KNMI (= Royal Netherlands' Meteorological Institute) Stations Beek (1967/69) and Schiphol Airport (1969/70).

3. LOCALITIES

3.1. Koelebosgroeve

Of the series of subterranean limestone quarries in South Limburg, the Koelebosgroeve (nr. 35 in the lists of BELS, 1952 and VAN WIJN-GAARDEN, 1967) is a medium-sized example (corridor-surface 8300 m²; greatest distance from entrance 350 m). It is situated near the village of Bemelen in the eastern slope of the Meuse valley (Fig. 23). The quarry is nearly horizontal (Fig. 1b) and consists of regular corridors ($\varnothing$ roughly 3 × 3 m) with relatively few crevices. There is one entrance, closed with a wooden gate and lock, so that nobody except myself can have entered the quarry during the two seasons of investigation. Two ventilation shafts are also present: one small (6 m high) half-way in the quarry, and a large (35 m high) one at the end (Fig. 1a).

The small shaft appeared to have hardly any influence on circulation and climate in the quarry. The larger one is more important. In the winter of 1967/68, this shaft was completely filled with litter from a farmhouse on the plateau, preventing any circulation through it. During August, 1968 it was emptied. As a consequence, in 1967/68 the Koelebosgroeve belonged to the so called 'static horizontal caves' (VAN NIEUWENHOVEN, 1956), but acted as a 'dynamic cave' in 1968/69. The difference in circulation is illustrated in Fig. 2. In the static situation exchange of air with outside takes place through the main entrance only. Differences between outside and cave temperature establish a convection current with the top air layer moving down the temperature gradient (*i.e.*, outward on cold days and inward on warm days),
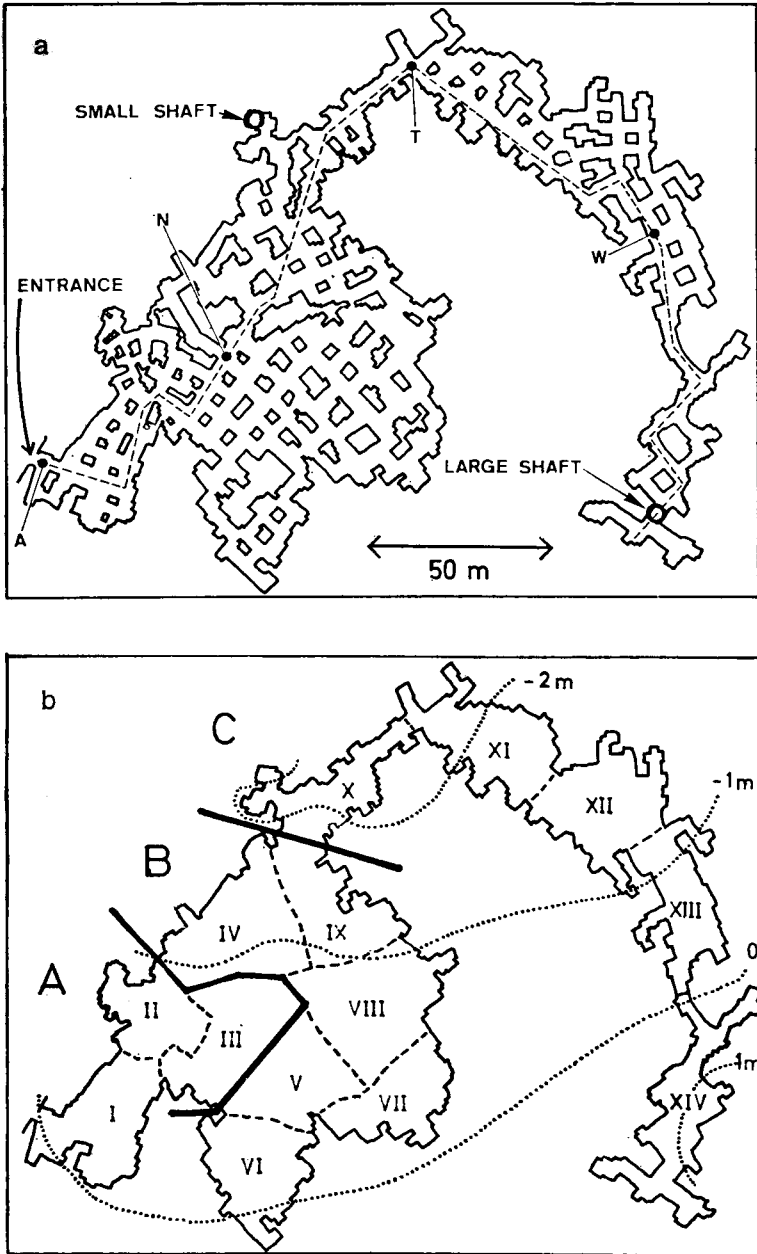
Fig. 1. Koelebosgroeve: topography. a. plan; A, N, T, W indicate temperature stations along the main route. b. Sections (I–XIV) and areas (A, B, C) distinguished. The level of the cave floor relative to the entrance level is indicated by dotted lines.

and the bottom air layer moving in opposite direction. The two com-
pensate each other. The two ways of circulation in both dynamic and
static type will be called 'summer circulation' and 'winter circulation'
henceforth, though both can occasionally occur at suitable temper-
atures in the other season. In the dynamic situation the same currents
flow through the entrance, but now the cold lower current is much
stronger. It is compensated by a fast one-way current through the
ventilation shaft, running down in summer and upwards in winter.

The over-all result is a much stronger circulation in the dynamic
condition, which leads to increased influence of outside weather in the
cave. The effect on air temperature in the cave is illustrated in Fig. 3:
The ambient temperature in the rear of the quarry is extremely stable
in the stationary condition (range of variation at point W over four
months: 9.0–9.1°C), but shows damped outside fluctuations in the
dynamic state. There is no appreciable overall-difference in outside
temperatures during the two winters (Fig. 14) to account for these
differences.
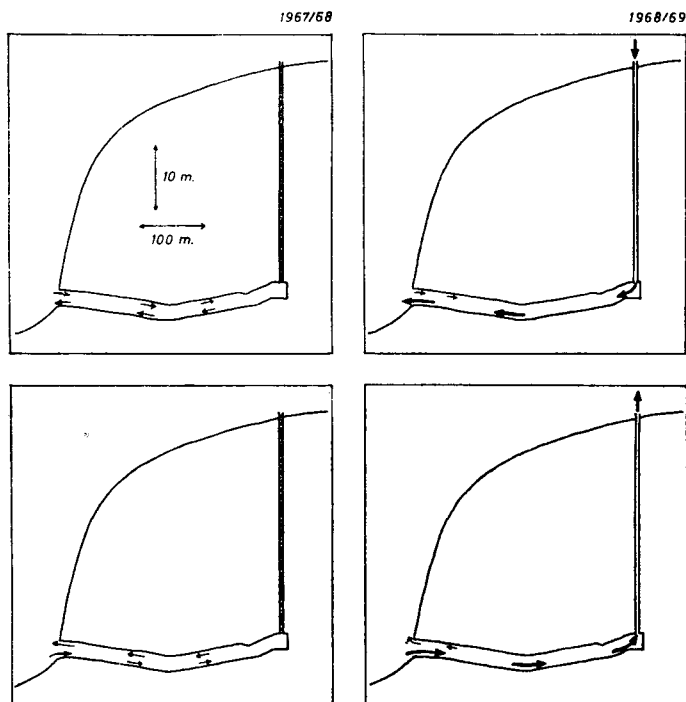
Fig. 2. Koelebosgroeve: Patterns of air circulation. Left: Static condition, right:
dynamic condition. Top: 'Summer circulation', bottom: 'Winter circulation'.
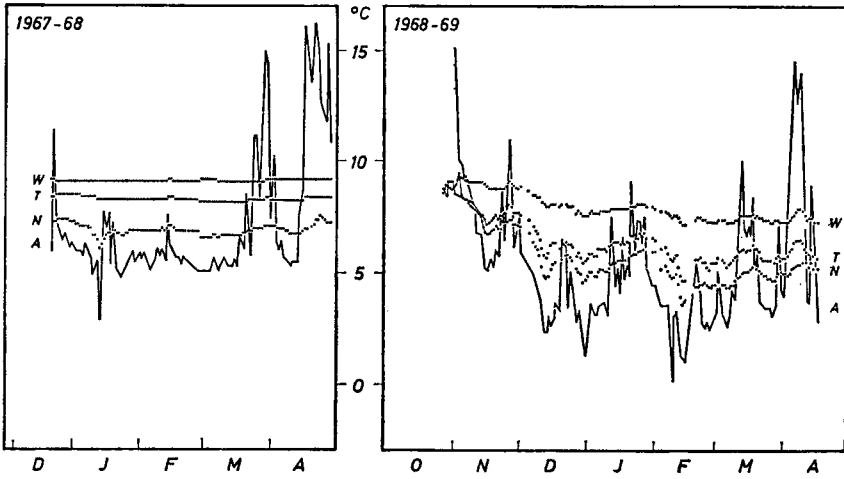
Fig. 3. Seasonal fluctuations of air temperature near the ceiling at the four stations shown in Fig. 1.
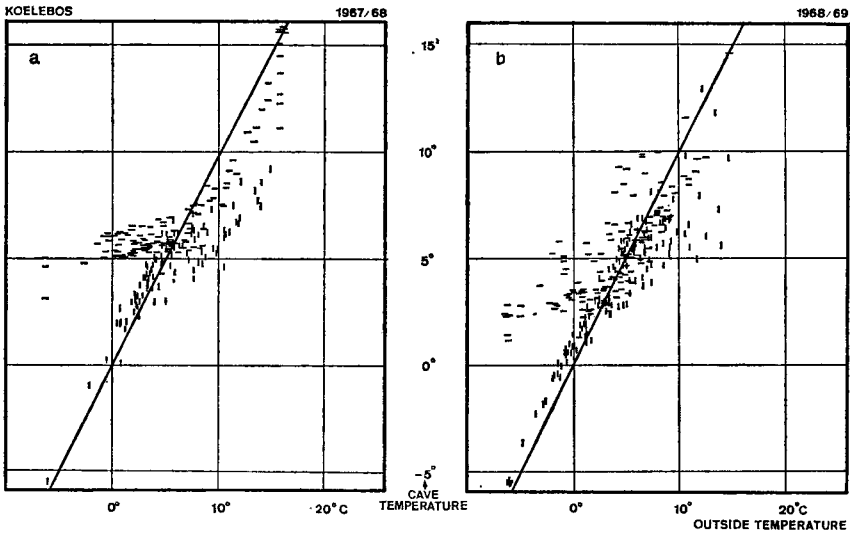
Fig. 4. Dependence of cave air temperature in the entrance (station A) on outside temperature. | : Measured 10 cm from floor, –: measured 10 cm from ceiling. (Daily temperature chart readings at 12.00 hrs.).

Fluctuations of ambient temperature in the front part of the quarry are likewise different in both conditions. With 'static circulation' temperature in the upper air layer at point A rarely drops below 5°C, whereas such drops were normal in 1968/69 in the dynamic situation. The dependence of air temperature at point A on outside temperature (Fig. 4) is indicative of differences in the circulation patterns. Static circulation causes roof ambient temperature in the entrance to be largely independent from outside temperatures as long as the latter remain below 5°C (Fig. 4a). Floor temperature then equals outside temperature. On warmer days, the bottom air layer remains more stable. The switch point, *i.e.*, the outside temperature at which circulation in the cave entrance reverses, remains more or less fixed between 5 and 6°C. In the dynamic condition the switch point underwent more change in the course of the season. This caused an increased dependence of entrance air temperature on outside conditions (Fig. 4b).

From fortnightly readings at 30 stations in the quarry, isotherm maps were constructed, a selection of which is shown in Fig. 5. The top row of these figures reveals that in summer and autumn the lowest part of the cave (see Fig. 1b) is also the coldest. As winter advances the entrance parts become cooler. This holds for both the static and the dynamic situation, though the differences are greater in the latter.

Taking into account all meteorological data available, the cave was divided into fourteen sections, which were again grouped together in three areas (Fig. 1b).

Relative humidity is constantly 99–100% in the greater part of the cave. Lower humidities were measured only in the entrance section (I), usually in the lower air current, which is the colder one of the convection currents—rising in temperature and therefore decreasing in relative humidity—both in summer and winter circulation. Static and dynamic condition are not different in this respect. The lower air layer is of only minor importance to us, as the majority of the bats hibernate in the upper half of the corridors. In the upper air layer of the entrance section, varying humidities below 99% occur only in summer circulation with dry outside conditions. With moist outside air in summer, and with winter circulation, the upper air layer in a large part of the quarry gets oversatured and condensation takes place. This results in the formation of mistbanks and a dew-cover on various objects, among which the fur of bats hibernating in exposed positions. From the relative frequency of those exposed locations where dew formation was recorded at least once in 1967/68, a general impression can be derived of the extension of condensation in the quarry (Fig. 6). This frequency decreases from entrance to rear and is obviously positively correlated with the average difference between top and
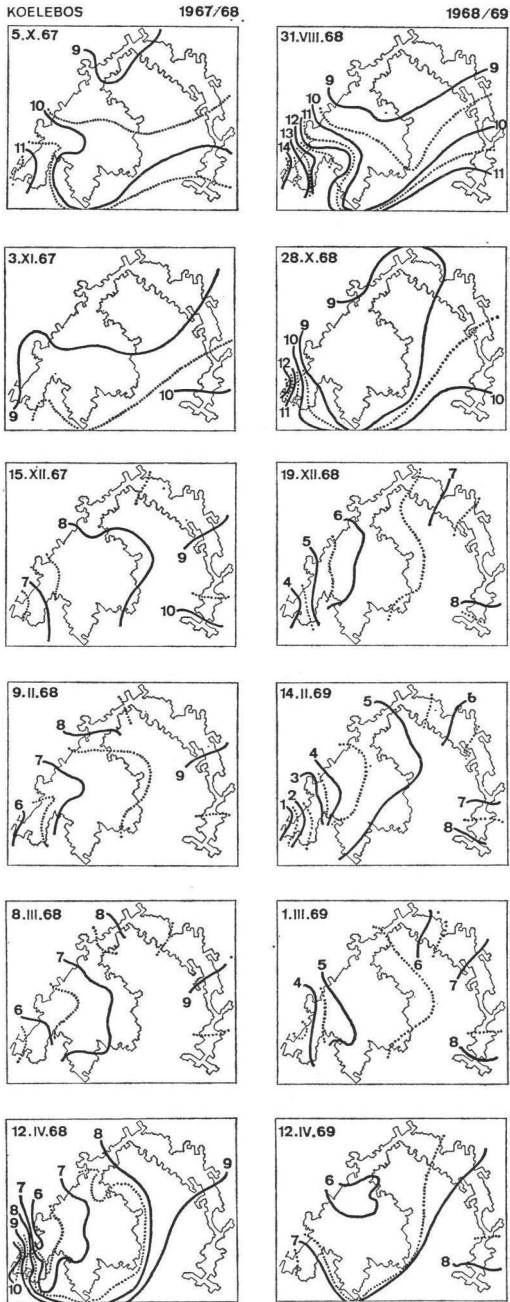
Fig. 5. Isotherms showing the dynamics of air temperature near the ceiling in the course of the season. Isotherms in centigrades. Half-centigrades: dotted lines.

bottom ambient temperature: Oversaturation is partly caused by cooling down of the upper air layer through radiation and turbulences with the lower. The rate of caloric exchange—and therefore of condensation—is proportional to the temperature difference between the two.

In conclusion, the Koelebosgroeve is a meteorologically simple representative of the static—in 1968/69 dynamic—horizontal caves. Most of the South Limburg limestone quarries—*e.g.*, Apostelhoeve-cave (VAN NIEUWENHOVEN, 1956), Sibbergroeve (DORGELO & PUNT, 1969), Geulhemergroeve (DAAN & WICHERS, 1968), Barakkengroeve (KUIPERS & DAAN, 1970)—are complications of this climatic type.

3.2. Barakkengroeve

Parallel to the investigation in the Koelebosgroeve, a similar though less extensive program of searches was run in the quarry Barakken-groeve (BELS, 1952 nr. 83). This quarry is situated at a distance of two
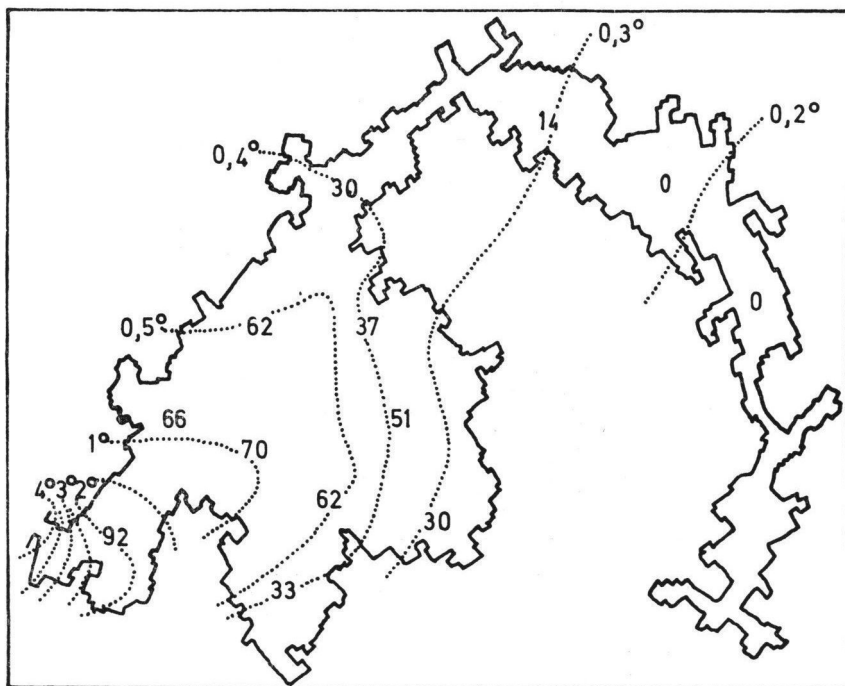
Fig. 6. Condensation and vertical temperature gradient. Dotted lines connect points with equal average temperature difference (°C) between top and bottom air layer. Numbers inside the cave outline are percentages of exposed bat locations where dew formation was noted at least once.

kilometers from the Koelebosgroeve in the southern slope of the Geul-valley (see Fig. 23). It is about four times as large as the Koelebosgroeve (corridor-surface 35.000 m²), and more complicated in structure and climate.

In this large excavation, the investigation had to be limited to 13 searches and meteorological samples in one winter season. It was considered worthwhile to gather results here also, first to judge the general validity of activity patterns found in the Koelebosgroeve, and second to determine what, if any, impact the climatic differences between the two had on these patterns. At present, the Barakkengroeve is probably the most populous bat hibernaculum in the whole area (maximum count on one day in 1967/68: 185 bats).

In a previous paper (KUIPERS & DAAN, 1970) the structure and microclimate of this cave were briefly described. The major characteristic is that the quarry is dug out in four superimposed marl layers. The middle one of the three (!) systems formed has a steep downward entrance behind which cold air is trapped in winter. Thereby, the small area behind remains a few degrees colder than the rest of the quarry throughout the year. In the other parts similar conditions to those described for the Koelebosgroeve prevail, but two ventilation shafts complicate the climatic picture. The results obtained in the Barakkengroeve are less complete than those from the Koelebosgroeve. Only in a few instances they will be referred to in the following chapters.

3.3. Ice cellar Middenduin

The third hibernaculum where investigations were carried out is of a quite different nature. It is a so called 'ice cellar', of a type not infrequently built during the nineteenth century on large estates in the Netherlands and other European countries. Designed as a store for natural ice, cut from canals and ponds in winter, and to be used for household purposes during the summer, such cellars have the property of being fairly uninfluenced by meteorological fluctuations. The thermostability in winter, usually combined with high relative humidity, makes them suitable roosts for wintering bats. Many of the still extant ice cellars have been found to lodge several species of bats in winter (VAN WIJNGAARDEN & BRAAKSMA, 1969).

Middenduin is such an estate with a well-preserved ice-cellar. It is situated in the municipality of Overveen, behind the dunes bordering the North-Sea coast at the latitude of Amsterdam. It consists of a circular vault, 9 meters high and 5 m in diameter, which is accessible through a narrow corridor (Fig. 7). Climatological isolation of the cellar has been achieved by a thick layer of earth on top of it, and by
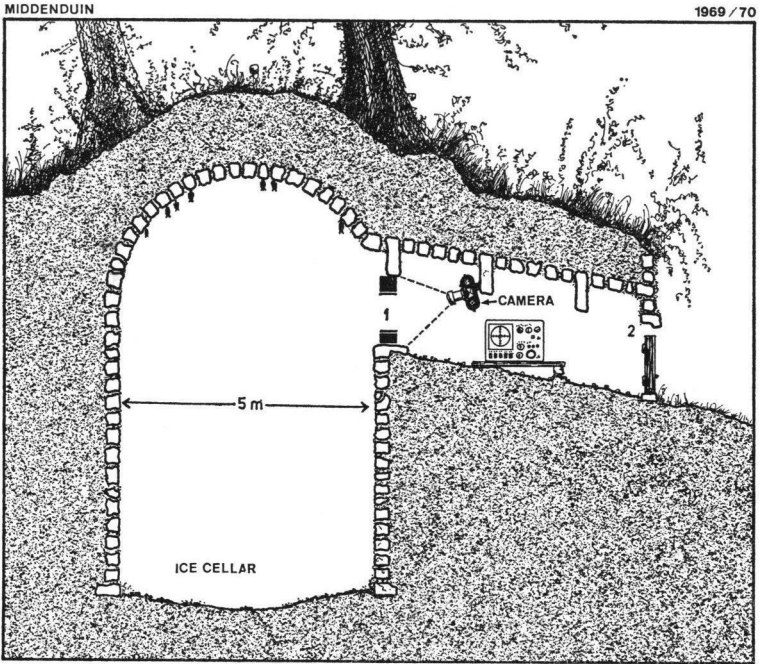
Fig. 7. Ice cellar Middenduin: vertical transsection. 1. window with photocell and mirrors; 2. double photocell detector.

double walling. That is, we are not sure if there is a layer of air around the whole cellar, but such an inter-wall space exists at least near the connection of cellar and corridor.

Up to twenty bats are normally found solitarily hibernating in the vault or in wall fissures in the corridor. Roughly 90% of them belong to the species *Myotis daubentoni*. Incidental visitors of other species are: *M. nattereri*, *M. mystacinus* and *Plecotus* spec. The seasonal changes in population strength have been described earlier (DE RUE & DAAN, 1972). The authors found by marking-recapture estimates, and by counting the number of inward and outward flights recorded in the entrance (section 4.7) that the bats found in cellar and corridor are only a sample from a population two to three times larger than that. It was presumed that the non-visible bats hibernate probably in the inter-wall space around the cellar, which is accessible through fissures.

Recordings of bat activity were made in 1969/70 at two places in the hibernaculum (fig. 8). The photographic recorder (DAAN, 1970) was mounted in the passage between vault and corridor. In a flyway above the entrance door the other, direction discriminating detector

(see section 2.2) was mounted. The latter operated only from November 3, 1969 onwards; the photographic recordings cover nearly the whole hibernation season, from October 14, 1969 to April 30, 1970. Event recorder and amplifiers were on the corridor floor. A 150 m cable connected the experimental set-up with the nearest mains in a forestry office.

Air temperature fluctuations in the ice-cellar have been described by DE RUE & DAAN (1972). Here, it may suffice to mention that the range of temperatures measured from October to April was 5.9–11.8°C in the corridor and 6.6–12.0°C in the cellar itself. During January through March these ranges were even as narrow as 5.9–6.9°C and 6.6–8.1°C, respectively. Daily fluctuations were not recorded, but are doubtlessly of an average amplitude far below 1°C. No circulation of any measurable strength is permitted by the door in the entrance. Measurements of relative humidity invariably indicated 100%. Water usually condenses into drops on the walls. Light penetrates through the flyway but its intensity in the vault will not exceed the order of magnitude of .01 Lux.

4. RESULTS

4.1. Population estimates

In the Koelebosgroeve eight species of bats have been found during the investigation: Plecotus cf. auritus (Linnaeus, 1775)*, Myotis mystacinus (Leisler, 1819)*, Myotis daubentoni (Leisler, 1819), Myotis dasycneme (Boie, 1825), Myotis nattereri (Kuhl, 1819), Myotis myotis (Borkhausen, 1797), Myotis emarginatus (Geoffroy, 1806), Eptesicus serotinus (Schreber, 1775). For the first four species the numbers counted in each visit are shown in Fig. 8. The other species were never represented by more than one animal at a time, and are therefore of no importance in the present study. The Barakkengroeve contains larger numbers of the same species except for Myotis emarginatus and Eptesicus serotinus,

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* Both Plecotus auritus and Myotis mystacinus are currently considered (BAUER, 1960; HANÀK, 1970) to embrace actually two twin species, on the basis of minor but consistent morphological differences. The South Limburg populations of Plecotus include both P. auritus and P. austriacus (Fischer, 1829) (VAN BREE & DULIČ, 1963). However, I am not quite convinced that my identification of these species in the field, from some distance, and without handling, is correct. I therefore refer to the Large-eared bats found as Plecotus cf. auritus. Whether M. mystacinus in the area actually belongs partly or completely to Myotis brandti (Eversmann, 1845) is unknown. There are no field characters so far to distinguish the species. Therefore, these bats are traditionally referred to as M. mystacinus.
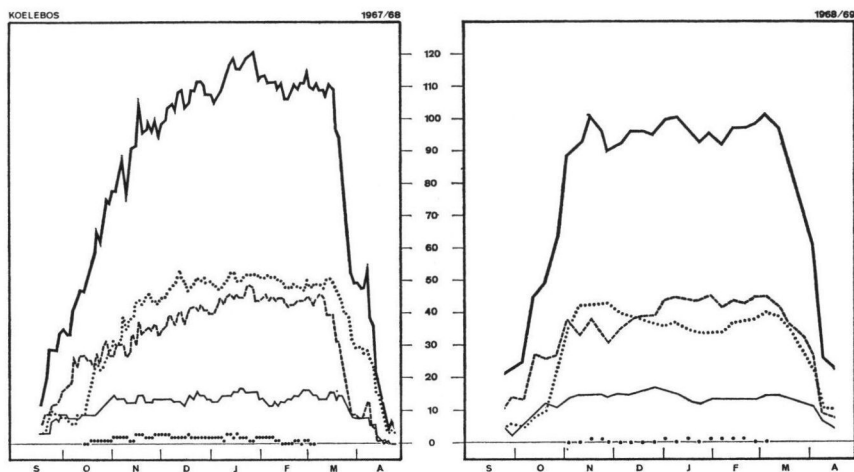
Fig. 8. Numbers of bats counted in the Koelebosgroeve during two-daily searches in 1967/68 (left) and weekly searches in 1968/69 (right). ... *M. mystacinus*, - - - *M. daubentoni*, — *M. dasycneme*, ●●● *Plecotus* cf. *auritus*. Upper curve: species added.

and has in addition a few specimens of *Rhinolophus hipposideros* (Bechstein, 1800).

The numbers counted in the cave should be treated as samples from the hibernating populations present, since an unknown number of bats escapes observation. In trying to estimate the total population size from the proportion of marked bats in the sample, one is confronted with several problems. The popular Lincoln index,—which divides the actual number of animals marked by the relative frequency of marked ones in a sample to estimate population size—as well as its extension to series of samples (HAYNE, 1949), are based on the assumption that sampling is random. This means that the probability of occurring in the sample should be equal for marked and non-marked individuals, and in the case of sample series that subsequent samples should be independent. The latter condition is of course not met in the bat searches. Each sample is highly dependent on the previous one as the majority of bats simply stay where they are. The former condition is doubtful. It will be shown (section 4.3) that there is some tendency to return to previously occupied locations in the cave. This must have the effect of a greater probability for a marked than for a non-marked bat to return in the sample. Any estimation based on 'recapture' rate of marked specimens will be biased by this effect. It would lead to underestimation of the population size in the same way as 'trap-proneness' of some individuals does in population studies of rodents (CROWCROFT & JEFFERS, 1961).

Other methods have been developed (*e.g.*, MARTEN, 1970) which do not require the assumption of equal catchability of the marked and non-marked animals. These however put other, more severe limitations, such as independence of subsequent samples over a period of time during which population size should remain constant. None of these conditions is met in the cave bat searches.

Despite the inherent bias in population estimation it was useful to calculate for the marked animals the average proportion that appeared in the samples. For each species a monthly figure was obtained. For istance those bats which had been tagged before January 1 and were refound at least once after January 31, were considered to constitute the tagged population in January. The average fraction of these bats that was present in the samples is shown in Table II as 'relative sample size'. Although no quantitative evidence can be given, it is felt that these values, obtained with marked animals are closely approaching those for the whole population; the intuitive arguments are that returning to previous locations is not the dominant behaviour (section 4.3), and that the marked bats must themselves in fact be close to the whole population, at least in the Koelebosgroeve: Nearly every bat found without head-mark was immediately given one. The overall fraction of non-identified

TABLE II

Relative sample sizes and population estimation.

Species:		M. mystacinus			M. daubentoni			M. dasycneme			M. myotis	Plecotus spec.
Cave*:	K1	K2	B	K1	K2	B	K1	K2	B	B	B
Relative sample size, individuals tagged												
Oct	99	75	—	80	68	—	91	83	—	—	—
Nov	92	92	—	77	76	—	84	80	—	—	—
Dec	95	85	—	84	77	—	75	87	—	—	—
Jan	93	81	—	85	90	—	72	87	—	—	—
Feb	95	81	—	86	92	—	65	87	—	—	—
Mar	98	90	—	90	95	—	69	95	—	—	—%
Yearly r.s.s.	95	85	82	84	84	65	74	87	78	97	60%
Maximum monthly mean sample size	50	39	81	45	44	59	15	16	20	9	8
Estimated max. population	53	46	99	54	52	91	20	18	26	9	13
Number of indiduals tagged	67	57	77	66	58	71	2	16	20	9	8

* K1 = Koelebosgroeve 1967/68; K2 = Koelebosgroeve 1968/69; B = Barakken-groeve 1967/68.

individuals was thereby kept as small as 1.9% in 1967/68 and 3.7% in 1968/69.

Apart from slightly overestimating the real relative sample sizes, the monthly figures in Table II are pertinent only to this investigation in this particular cave. The range of variation is relatively small. The relative sample sizes calculated for both the Barakkengroeve and for NEVRLÝ's (1963) investigation in a disused water canal in the Czecho-slovakian Iser Mountains tend to be below this range. BELS's (1952) figure of 10% bats overlooked is in apparent agreement with the Koelebosgroeve results, but his was a fully intuitive estimate and his sample sizes have doubtlessly been much smaller than 90%. Smaller relative sample sizes (68%, 55–80%) can also be concluded from direct evidence obtained by PUNT & VAN NIEUWENHOVEN (1957) with radio-active tagging of bats. Still much smaller sample sizes must have been involved in most other studies reported, such as that by KRZANOWSKI (1959), who found on the average two bats at a time, out of a popu-lation that allowed him to band 666 individuals in the course of five seasons. The abundance of crevices as well as the climatic conditions forcing the bats to hide in them varies of course from eave to cave.

Division of the actual monthly sample size by the relative sample size of marked individuals would yield a kind of integrated Lincoln index. This calculation is made in Table II to estimate the maximum population size. For the reasons set out before, these estimates are no more than rough approximations. Comparison of these figures with the total number of marked individuals shows that, in the Koelebos-groeve the latter figure has usually been larger: not in the Barakken-groeve where tagging was restricted to the period before December 1st, 1967. Apart from the bias mentioned, this difference may partly be caused by occasional tag shedding, and by movements to other hiber-nacula. By the first effect, a few individuals may have been tagged more than once. A few female Daubenton's bats were found with heavily worn tags as a result of neckbiting by the males in copulation. Mating in the hibernaculum seems to be most frequent in this species (section 4.4). Movements between hibernacula must be regarded as rare phenomena (section 4.6).

The relative sample sizes in the ice cellar 'Middenduin' were in the order of 30–40% (DE RUE & DAAN, 1972), a value far lower than in the marl caves. This is caused by the special structure of the ice cellar, where about two-thirds of the population probably hibernates be-tween the two walls (see section 3.3).

4.2. Population development and turnover

The most rapid increase in population strength of bats in the Koelebos-

groeve took place from the middle of September to the middle of November (Fig. 8). The numbers of *M. daubentoni* continued to increase, though at a slower rate, until the middle of January. Median dates of arrival and departure can be roughly estimated by the times at which the sample size crossed half its maximum value. These dates were for *M. mystacinus* in 1967/68: 1.XI and 13.IV (1968/69: 27.X and
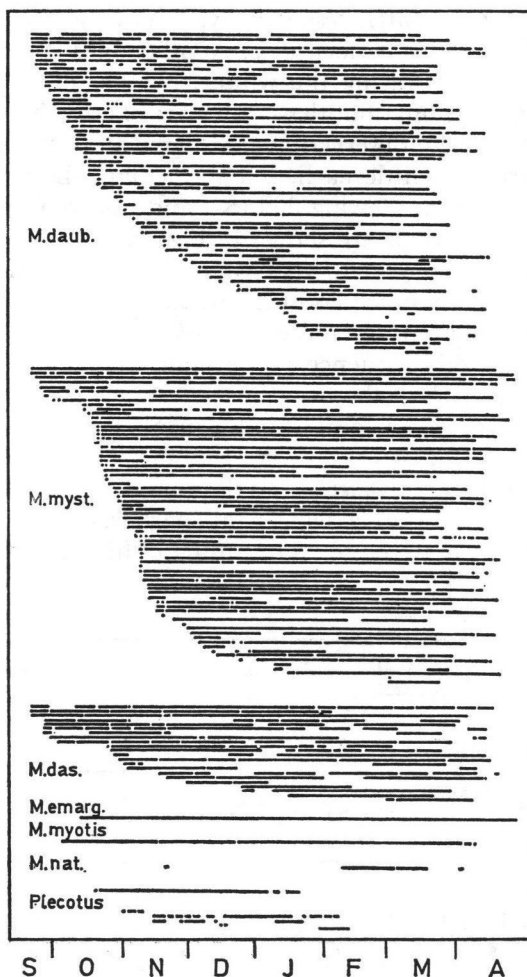

Fig. 9. Summary of the observations on individually marked bats in the Koelebosgroeve 1967/68. Each horizontal bar represents one individual bat. The smallest interruptions of the bars indicate movement to a new location, longer interruptions temporary absence from the sample.

5.IV); *M. daubentoni:* 10.X and 24.III (12.X and 6.IV); *M. dasycneme:* 26.IX and 31.III (12.X and 8.IV). The corresponding estimates of total mean hibernation duration would be: *M. mystacinus:* 1967/68 164 days (1968/69 160 days), *M. daubentoni* 165 days (175), *M. dasycneme* 187 days (177).

A more detailed picture can be derived from the observations of individually marked bats, summarized in Fig. 9. Of course individual dates of first and last observation in the cave need not exactly be the arrival and departure dates, respectively. There may be differences by temporary stay at invisible locations. Another bias comes from a few cases of tag shedding during the winter. Neither of these errors can be estimated exactly, but doubtlessly they are of only limited influence. Both tend to make the observed average arrival a little later and the observed departure a little earlier. The median—being less affected than the mean by such skewing factors—and quartile distributions of first and last observations are shown in Fig. 10.

Both individual and year-to-year variation in dates of first and last observation in the cave are considerable. Specific differences in this respect are such that *M. dasycneme, M. daubentoni* and *M. mystacinus* tend to arrive in this sequence in autumn, In spring, *M. dasycneme* stays a little longer than the others.

The sexes travel separately: females in all three species arrive in the quarry on the average before the males (median to-median distance 14 days in *M. mystacinus,* 36 days in *M. daubentoni,* 14 days in *M. dasycneme*). In spring, they leave again earlier, except *M. mystacinus.* Similar differences between sexes in timing of hibernation were found
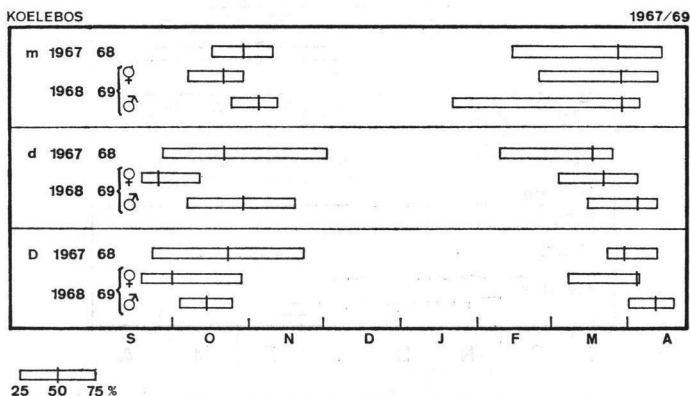
Fig. 10. Quartile distributions of dates of first and last observations of marked individuals. m = *M. mystacinus,* d = *M. daubentoni,* D = *M. dasycneme.* In 1967/68, animals were not handled when being marked, and thus, sexes not identified.
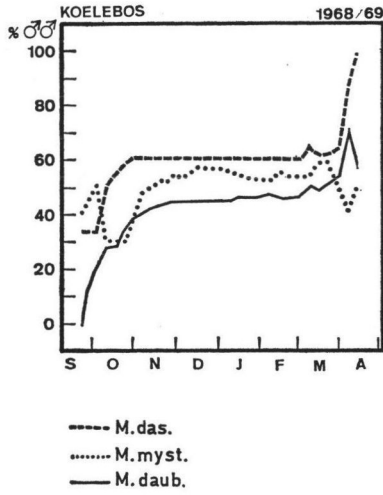
Fig. 11. Sex ratio in the course of the season.
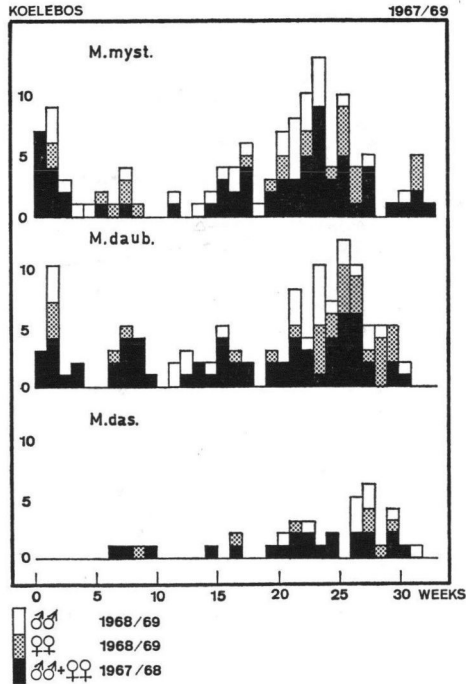
Fig. 12. Frequency distributions of time elapsed between first and last observation of marked individuals in the hibernaculum.

by NEVRLÝ (1963) in *M. daubentoni* and *M. myotis*. The sex ratio (Fig. 11) thereby undergoes considerable change in the course of the season. Some of the disproportionate sex ratios often reported in hibernating bats (*e.g.*, DAVIS, 1959; BEZEM *et al.*, 1960) may be related to different timing of hibernation. However, other behavioural differences between the sexes (habitat selection, clustering behaviour; see DAVIS & HITCHCOCK, 1964; RYSGAARD, 1942) can play a role here as well.

In Fig. 12 the frequency distributions of time distance between first and last observation of marked bats are shown. The histograms are significantly skewed to the left by the combined incidences of tag shedding and movements to both invisible locations and other hibernacula. The mode of the distributions is thought not to be affected thereby.

The modal duration of hibernation in the Koelebosgroeve was: *M. mystacinus* 23 weeks (males 21; females 25), *M. daubentoni* 25 weeks (males 23, females 25), *M. dasycneme* 27 weeks (males 26, females 27). These estimates come very close to those based on half-maximum
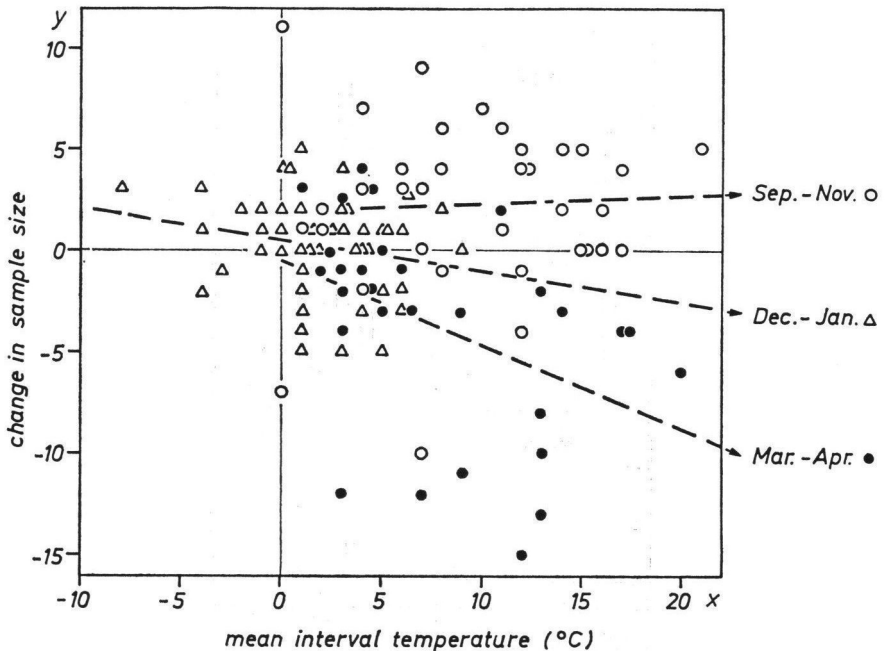
Fig. 13. Change in sample size (species combined) per two-day interval as a function of average temperature. Linear regressions: Sep–Nov: $y = 1{,}9 + 0{,}04 \cdot x$ $(r = 0{,}047)$; Dec–Feb: $y = 0{,}5 - 0{,}14 \cdot x$ $(r = -0{,}195)$; Mar–Apr: $y = -0{,}4 - 0{,}42 \cdot x$ $(r = -0{,}430)$.

sample sizes (see above). *Plecotus* cf. *auritus* and *M. nattereri* were never present for longer than 15 weeks. The single specimen of *M. myotis* stayed 27 weeks in 1967/68 and 28 in 1968/69, and for *M. emarginatus* these figures are 27 and 29. In all, we find very nearly the same specific order as found by DAAN & WICHERS (1968, Table 10) on basis of earliest and latest observations of the species in the South Limburg quarries.

In Fig. 13, the dependence of arrival and departure on the temperature is investigated. There is no significant relation between change in sample size and outside temperature per interval during autumn and winter. In spring, however, more bats leave in the warmer intervals (coefficient of correlation: -0.430; $p < 0.05$). In spring 1968, there were two sharply marked warm periods, one from March 20 to 30, and one from April 13 to 23 (Fig. 14). About half of the total populations left the quarry during the first warm period, and the other half during the second. In the first ten days of April, which were cold, the populations remained constant at about 50 bats, as is visible in the right half of Fig. 8a.

If last observations of marked individuals tell us something about the departure from the cave—and it was shown above that they do—such data must relate in some way to what has been called population turnover (MUMFORD, 1958). During the three winter months December to February, the sample sizes remain relatively stable (Fig. 8). However, some new animals appear, and others are not refound (Fig. 9). The fraction of marked bats found during a month that was not refound later indicates the size of such changes (Table III).

These apparent turnover figures overestimate real turnover, again as both tag shedding and movements to invisible locations are included. Yet, the values attained are very low—usually below 10%—indicating that winter population turnover was extremely small. (See also section 4.6.) This is in sharp contrast to the common notion in bat literature, where much larger values of population turnover in hibernating bat populations are reported (*e.g.*, MUMFORD, 1958; DE WILDE & VAN NIEUWENHOVEN, 1954; KRZANOWSKI, 1959; TINKLE & PATTERSON, 1965). Although there may be considerable interspecific variation in this respect, it can be argued that the frequent disturbance inherent to the banding method used by nearly all authors who studied bat hibernation behaviour in the field has led to overestimations of population turnover. Speculations such as the frequently cited statement by ANCIAUX (1948, p. 21), that a hibernating bat population in Belgian caves would renew itself at least every eight days, say less about the activity of the bats involved than of the investigators.
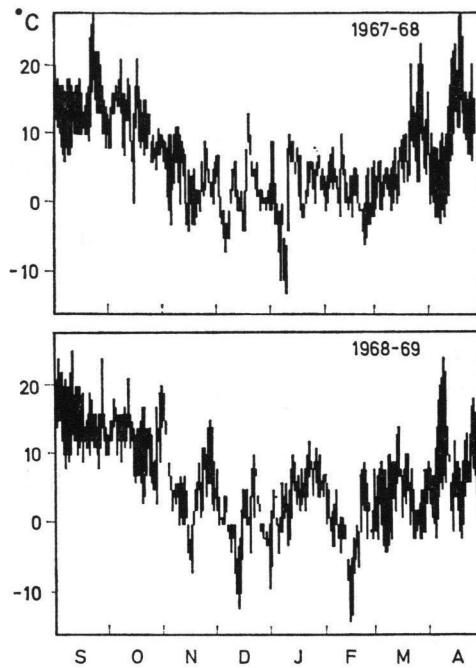
Fig. 14. Daily range of temperature measured at KNMI wheather station Beek during
the two seasons of investigation in South Limburg.

STEBBINGS (1966, 1969) has clearly reported the adverse effect his
banding intensity had on the bat population in Horringer caves
(Suffolk, England). The maximum number of bats found each year
was negatively correlated with the number of searches made. In five
seasons of visits to the Koelebosgroeve I found a positive rather than
a negative correlation. ((The maximum number of bats was in 1966/
67: 63 (4 searches), 67/68: 121 (111), 68/69: 101 (31), 69/70: 96 (2),

TABLE III

Percentages of bats lost from the hibernaculum during the winter months.

| | Koelebosgroeve 67/68 | | | Koelebosgroeve 68/69 | | | Iser Mts. 57/58* | | |
	D	J	F	D	J	F	D	J	F
M. mystacinus	4	0	6	6	9	10	0	0	0
M. daubentoni	2	8	8	2	6	4	4	6	12
M. dasycneme	0	0	10	7	0	0			
M. myotis							0	0	0

*Calculated from data by NEVRLÝ (1963).

70/71: 83 (2)). Certainly, several variables, among which differential search effort, are involved here. Yet, I think the results of STEBBINGS and myself demonstrate the difference between investigations with and without handling of the bats.

4.3. Microhabitat selection

Bats were found in 737 locations in the Koelebosgroeve in 1967/68 and in 577 in 1968/69. Of each location three characteristics were recorded: the topographical position in the quarry, the degree of exposure (to air currents *etc.*) and, about once every fortnight, the ambient temperature near the bats. Several aspects of habitat selection described previously (DAAN & WICHERS, 1968) can now be analysed with greater accuracy.

Intracave distribution.—The percentage distribution of the bats in the three cave areas distinguished (Fig. 15) shows once more the phenomenon of "internal migration" (DAAN & WICHERS, 1968; DORGELO & PUNT, 1969; KUIPERS & DAAN, 1970): The relative number of bats in the front part (area A as defined in Fig. 1c) of the cave increases during the season, while the fraction in the rear decreases. The intermediate cave area contained about half of the population throughout the year. This was found in both seasons equally. The difference between the two seems too small to attach any speculations on the role of altered climatic conditions. All species studied followed the same pattern (Fig. 16).

In spring, with the departure of bats from the cave the situation is
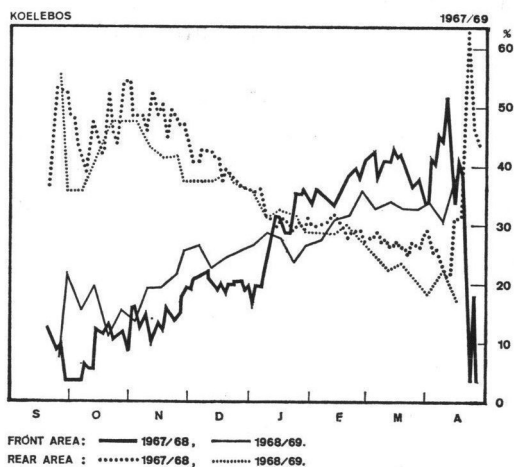
Fig. 15. Percentage of bats (species combined) in rear and front areas of the Koelebosgroeve.
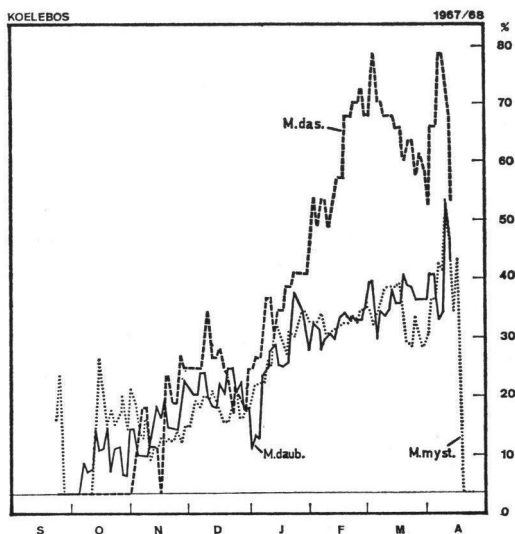
Fig. 16. Seasonal change in percentage of bats in front area of the Koelebosgroeve.

rapidly reversed: The front area fraction dropped from about 40% to 30% in the first warm period of 1968 (late March). In the following cold first week of April, it rose again to over 50%, and then fell to zero in the warm second half of April. The percentage increase in the rear in such warm intervals in spring is caused mainly passively by bats from the front area leaving the quarry.

Also during the winter months (December to February) the shift of the distribution towards the entrance area was negatively correlated

TABLE IV

Effect of temperature on direction of intracave movements. Koelebosgroeve; period: December 1, 1967 to March 1, 1968.

Average outside temperature per interval (°C)	Number of intervals	Mean increase in front cave area as percent of total sample	Mean distance (in sections) of movements in marked individuals (− = towards rear)
<0	12	1.3	1.9
0–2	11	0.5	0.4
2–4	12	0.4	0.6
4–6	7	−0.3	−0.6
>6	3	−1.1	−2.2

with outside temperature (see Table IV). Low outside temperatures evidently favour intracave migration towards the entrance. With temperatures above 4 to 6°C, this tendency is reversed. This temperature is about the so-called switch-point (see section 3.1). When rising above the switch-point, outside temperature causes the thermocirculation in the cave to reverse, and bats tend to move away from the entrance area. As long as winter circulation prevails, they tend to prefer this area.

Ambient temperature.—Air temperature near hibernating bats were measured at regular intervals in both seasons in the Koelebosgroeve. The resulting averages are plotted as a function of season in Fig. 17, together with the standard deviations in one species *(M. daubentoni)*. Standard deviations in the other species were roughly the same, and are omitted in the figure for the sake of simplicity. The range of roof air temperatures available in the quarry (shaded area in Fig. 17) is derived from the fortnightly measurements at 30 stations in the quarry (see section 2.3).

The average temperatures selected by the three species studied are very close to each other. The differences are statistically insignificant. All averages tend to lie in the lower part of the range of temperatures available in the quarry. This is the result of (active) "internal migration" (see above): the bats select the cold rear of the quarry in autumn, and the entrance area when this is the coldest part later in winter. On the other hand the decrease in air temperatures near the hibernating bats is caused (passively) by gradual cooling of large parts of the cave. Obviously, the latter effect has been stronger in the dynamic (1968/69) than in the static situation (1967/68).

Exposure.—BEZEM *et al.* (1964) have shown that there exist large specific differences in the tendency for bats to select either exposed positions or to hide in crevices *etc.* This behaviour is further complicated by a stronger preference for protected positions in the entrance area of a cave (DAAN & WICHERS, 1968). This aspect of habitat selection was again studied by assessing the relative frequency of "highly protected positions" (as defined by BEZEM *et al.*, loc. cit. p. 345). This figure was computed per species per cave area per month, but as seasonal variation appeared to be insignificant, the yearly averages are given in Table V. The tendency to hide away in the entrance area is confirmed by the data. In this area roughly twice as many of the bats were found in crevices as in the rest of the quarry. It can be inferred from this observation that the relative number of unseen animals will be highest in the entrance area.
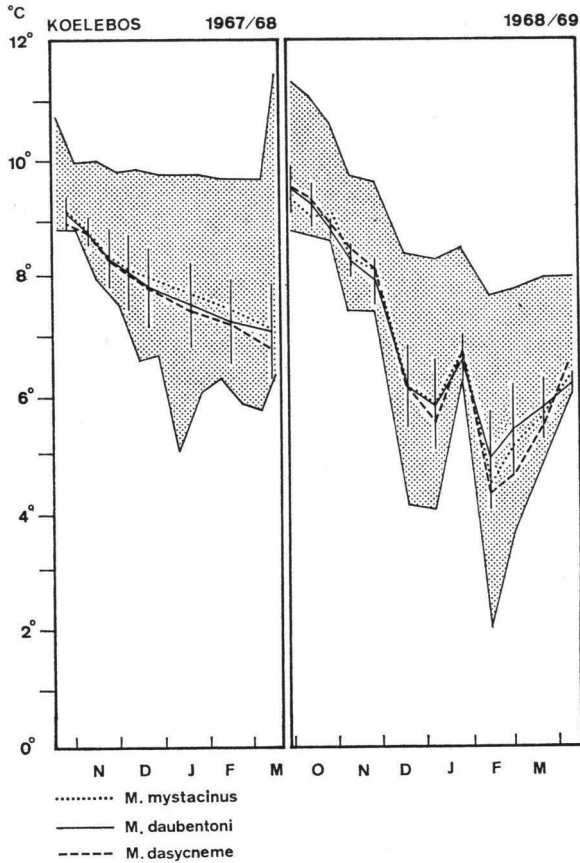
Fig. 17. Seasonal change in mean ambient temperature near hibernating bats. Vertical lines indicate standard deviation for *M. daubentoni*. Shaded area: range of roof air temperatures available in the cave.

The figures obtained in 1967/68 and 1968/69 are strikingly similar. It may be concluded that the climatological differences between the two seasons (see section 3.1) had no detectable effect on hiding behaviour. Variations between two caves are considerable, however, and the specific sequence in hiding behaviour appears to be less consistent than was suggested earlier (DAAN & WICHERS, 1968).

Social factors.—One assumption made so far has been that the individual bats behave independently in habitat selection as well as in activity. This is supported by the general solitary habits of the hibernating bat species studied. In gregarious bats, the social parameters of behaviour

Relative frequencies of "highly protected positions" (in %).

Cave	Season	M. mystacinus	M. daubentoni	M. dasycneme
Koelebosgroeve:				
Entrance area	67/68	46	47	95
Entrance area	68/69	44	44	94
Rest of cave	67/68	18	21	48
Rest of cave	68/69	13	24	53
Whole cave	67/68	25	26	63
Whole cave	68/69	20	28	62
Geulhemergroeve:				
Whole cave	63/64	41	58	42

seem to outweigh the effects of autecological factors, and even make the latter difficult to reveal. A movement of one cluster of, say, a hundred bats from one cave part to another, would of course be far less significant than if a hundred solitary bats simultaneously and independently decided to perform this activity. Much of the work in the South Limburg limestone caves was based upon this advantage, offered by a relatively small number of solitary bats as compared with gregarious colonies found in many other countries.

An attempt was made to check the degree to which the bats studied were really solitary, and what remains of their gregarious summer habits in hibernation. Therefore, the relative frequency of locations occupied by two or more individuals—both simultaneously and at different times in the season—was computed (Table VI).

TABLE VI

Relative frequencies of locations shared by more individuals. Koelebosgroeve.

Species	Season	Number of locations	Percentage of locations shared		
---------	--------	---------------------	with other bats	with conspecific bats	simultaneously with conspecific bats
M. mystacinus	67/68	322	17.7	6.5	2.5
	68/69	236	13.1	5.9	1.3
M. daubentoni	67/68	309	24.6	11.9	2.5
	68/69	249	15.2	8.8	3.2
M. dasycneme	67/68	75	34.7	10.7	10.7
	68/69	65	27.7	12.3	7.7

Indeed, few of the locations appear to have been occupied by more than one bat, and still less if only conspecific bats are considered. In the majority of such instances in *M. mystacinus* and *M. daubentoni*, the bats did not occupy the location simultaneously. It is felt, therefore, that incidental gatherings of two individuals in these species need not be explained as social behaviour, but would occur also by chance in similar frequency. This is supported by the fact that nearly all locations shared are in crevices and holes *etc.*, where lack of space helps the animals to select one particular spot.

In *M. dasycneme* most locations shared were occupied at least once by two specimens simultaneously. Sometimes three or four bats were found in one hole together which is the more significant as *M. dasycneme* is smaller in numbers than the other two species. Thus in this species some social tendency in the selection of locations is present, but most roost solitarily.

Tradition.—It is quite common to find one bat occupying one location several times during the season. Some individuals make use of two or three locations in the cave alternately. Such "traditional" behaviour would partly tend to obscure *e.g.*, ecological preferences expressed in habitat selection. In an attempt to quantify this component of behaviour the frequencies of returns to previously occupied locations were calculated: absolute (Table VII, e) and relative (f) to the total number of periods the bats spent on any location (b+e), except for each first period (a). Again, the bias in this estimation is that in some instances bats have returned to a location immediately after an active period. Such interruptions and returns escape observation. The values in Table VII, f therefore underestimate the real frequency of returns. As was stated earlier (section 2.1), this bias is stronger as the search intervals increase. It is not astonishing therefore to find the values obtained in 1968/69 (with 7-day intervals) in the three species to be lower than in 1967/68 (2-day intervals).

If the tendency to return to the last location occupied would be about the same as to return to other previous locations, the overall returning frequency can be calculated to be underestimated by about 40%. The real relative frequencies of returns would than be in the order of 20% *(M. mystacinus)*, 45% *((M. daubentoni)*, 40% *(M. dasycneme)*.

'Tradition' also appeared to survive the long summer absence from the hibernaculum. This can best be illustrated by the movements of a specimen of *M. myotis*. During both seasons of investigation, one bat of this species was present in the Koelebosgroeve. As the headmarks used all become lost during the summer, it was uncertain if these two

TABLE VII

Frequency of returns of tagged bats to previous locations. Koelebosgroeve.

| Species: | M. mystacinus | | M. daubentoni | | M. dasycneme | |
Season:	67/68	68/69	67/68	68/69	67/68	68/69
a. Number of bats tagged	67	57	66	58	22	16
b. Number of visible locations occupied by tagged bats	328	240	342	272	94	70
c. Mean number of visible locations per bat per year (c = b/a)	4.9	4.2	5.2	4.7	4.3	4.4
d. Percent of locations used twice or more by one animal	11.6	7.9	17.8	17.6	29.8	9.4
e. Number of returns to a former location	44	23	110	74	22	6
f. Percent of times a previous location is selected (f = 100.e/(b + a − a))	14.4	11.2	28.5	25.7	26.2	10.0
g. Mean number of periods in visible locations per bat (g = (b + e)/a)	5.6	5.0	6.8	6.0	4.8	4.7

were actually the same animal. The specimen present in 1967/68, marked AV, occupied the locations **56–303–455–715**–722 in this sequence. The mouse-eared bat in 1968/69 (JO) was found in seven locations: **56**–1015–**303**–**455**–1242–1273–1242–**715**. The two series have four locations in common (heavy type). The only conclusion from this correlation can be that AV and JO have most probably been the same animal. The same conclusion could be drawn for a single specimen of *M. emarginatus*.

To see in how far similar statements about the more abundant species would be justified, a little more rough statistical calculation is needed. Using the average of 5 visible locations per year (Table VIIc), 600 locations in the second year, of which 250 had also been used in the first, it can be computed that two or three locations in an individual's series matching by chance any of about 150 series in the first year, would be in the order of .075 and .0004, respectively. Actually, 25 out of 137 marked bats in the second season occupied such series of locations: 11 with 2, 11 with 3, 2 with 4 and 1 with 5 locations combined also in the first season. In all these cases the bats were each year of the same species. Those with three or more corresponding places

3

can with high probability be assumed to be the same animal, those with two in common are guessed to be so. An example of the locations and movements of one Pond bat in two subsequent seasons is shown in Fig. 18.

Movements between two locations made in both seasons by assumed identical bats were in the same direction in 41 out of 52 cases (79%, sign-test: p < 0.01.) This holds for both forward intracave movements (16 out of 19 copied in the same direction: p < 0.01) as in the backward and within-section movements (25 out of 33: p < 0.01). Accordingly, there is a significant positive correlation between dates of first observations at the same location of assumed identical bats in both seasons (Fig. 19). Again, this correlation is also positive when cave areas A, B and C are considered separately (A: r = .83; p < .05, B: r = .44; p < .01, C: r = .47; p < .05. Whole cave: r = .51; p < .001).

The phenomenon of internal migration would lead us to expect bats in subsequent years on their way from rear to front to make use of identical locations in the same order, as the overall meteorological changes repeat themselves. This explantation however does account neither for the fact that also backward- and within-section movements tend to be repeated in the same direction, nor for the fact that, say, a front area location incidentally selected in November in 1967/68 tended to be selected by the same bat at the same time of the next season. Tradition does not only determine which cave (often at many kilometres distance from the summer quarters) is selected for hibernation, and then influences what particular spots of a few square centimetres become occupied in the several hectares of quarry surface,
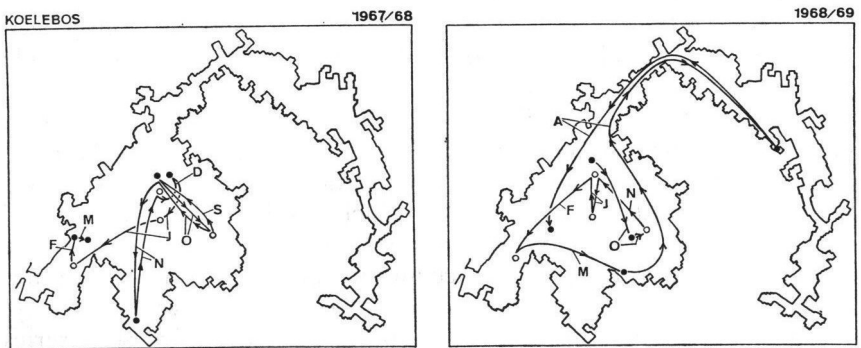
Fig. 18. Movements of *M. dasycneme* (tagged BF) in 1967/68 and of *M. dasycneme* (tagged KG) in 1968/69. ○ = locations shared in both cases, ● = locations occupied in one season only. Capitals indicate months in which movements took place.
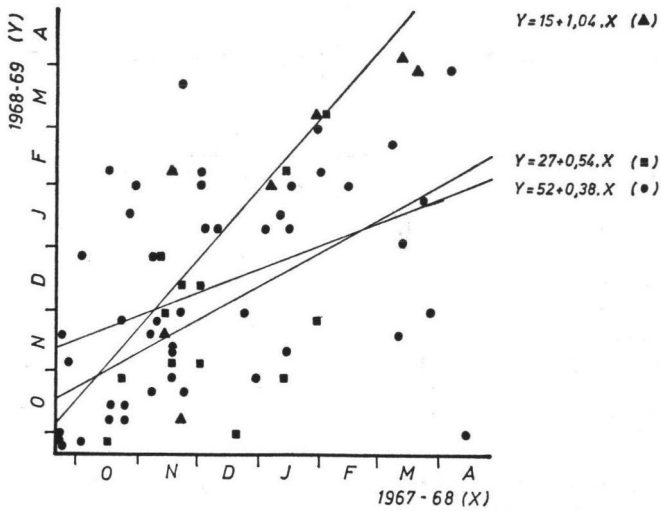
Fig. 19. Correlation between dates of first observation of presumably identical bats in two subsequent seasons. Each symbol represents one location in the Koelebosgroeve. (▲ = location in entrance area, ● in middle part, ■ in rear). Linear regressions are expressed in number of days after September 26.

but also affects the sequence in which more locations are revisited one after another!

4.4. Copulations

During the investigations a total number of fifteen bat copulations was observed. This is too few to speculate much about the frequency of copulation in winter. As little is known, however, about times of copulation in nature, it was thought useful to include a table with the observations (Table VIII). The overall distribution suggests a concentration in autumn, especially if one takes into account that the bat populations are not yet at their full strength at this time of the year. The scarcity of copulations in *M. mystacinus* as compared to the other species is another conspicuous feature. The daily distribution of observed copulations is of course largely determined by the distribution of searches, which were concentrated between 900 h and 1800 h.

4.5. Intracave movements

Frequency of movements as an estimate of arousal frequency.—The frequency with which bats change sites within the hibernation quarter bears some relation to the frequency of arousal, but obviously the two are not the same. In this paragraph we will see in how far systematic differences can be traced and estimated.

TABLE VIII

Observations of copulation in the Koelebosgroeve 1867/69 and Barakkengroeve 1967/68.

Month	Species		
	M. mystacinus	*M. daubentoni*	*M. dasycneme*
September	—	—	—
October	—	—	2
November	—	4	2
December	—	2	—
January	—	4	—
February	1	—	—
March	—	—	—
April	—	—	—

(a) In a previous paper (DAAN, 1969) the relative frequency of disappearings from a location was used as an estimate of the individual frequency of arousal (RFD):

$$RFD_i = D_i/N_i,$$

where N_i is the number of bats observed before the i-th interval, and D_i the number among these having left their place during the interval. Or, if more intervals and cave sections are pooled:

$$RFD = \Sigma D_i/\Sigma N_i$$

Apart from the sources of error described below under (b) and (c), this figure estimates correctly the instantaneous arousal frequency in the population in any particular interval or group of intervals. It can be used for instance to trace the direct effect of climatological stimuli on activity. However, to estimate the mean individual frequency over longer periods of time, RFD is a correct parameter only when N_i is relatively constant. In a period when many bats have just entered the cave or cave area, the RFD values will be too small, whereas mass disappearing from the cave would give rise to too high values to describe the long-run frequency of spontaneous arousal. The effect of variation in N_i can be corrected for by modifying RFD to a 'Relative frequency of movements' (RFM):

$$RFM = \Sigma (D_i + A_i)/\Sigma (N_i + N_{i+1}),$$

A_i being the number of bats appearing in new locations during the interval i. As long as N is constant, D_i equals A_i and RFM equals RFD. If N decreases, D_i is larger than A_i and RFM is smaller than RFD RFM is larger than RFD when N increases.

(b) Both RFD and RFM are of course strongly dependent on the duration of the interval between successive searches: The longer the interval, the more movements will escape observation. The nature of

this relationship was theoretically supposed to be exponential by
DAAN & WICHERS (1968)—who had to calculate a cumbersome cor-
rection to compare results from different interval lengths—but could
not be empirically established at that time. The results collected in
the Koelebosgroeve in 1967/68 with two-day intervals gave the op-
portunity of checking this relation. Each search can be compared
with one after two, four, six days, *etc.*, and the resulting RFM-values
divided by the interval duration. This was done for the pooled results
for the period December to February in each of the three species
studied (Fig. 20).

In *M. daubentoni* and *M. mystacinus* the effect of interval duration on
estimation of RFM is close to linear. Linearity in *M. dasycneme* is less
evident, but for this species fewer results are available. Differences in
slope of the regressions can partly be attributed to differences in
tendency to return to former locations.

If the curves are extrapolated to zero interval duration, the real
RFM-values are approached. We can conclude from Fig. 20 that
underestimation of arousal frequency due to search interval duration
would be in the order of:

<div align="center">

0.5– 2% for 1-day intervals
1– 3% for 2-day intervals
6–12% for 7-day intervals
20–32% for 16-day intervals
36–53% for 28-day intervals

</div>

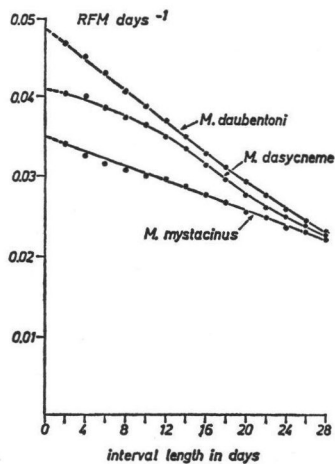(c) Another source of underestimation of arousal frequency is due

Fig. 20. Effect of interval duration on the estimation of frequency of movements.
Koelebosgroeve 1967/68.

to a certain fraction of the bats that arouse, but stay at their location, without any difference in postural adjustment to reveal the last arousal. We cannot hope for more than to get a very general impression of the frequency of such undetected arousals:

Among a total of 8874 observations of marked bats in the Koelebosgroeve in 1967/68, there were 75 instances of a bat either found awake at its location or absent from the sample during one search only. Among these 75, there were 20 cases in which the particular individual had resumed exactly its original position when the next search was made two days later. In 55 the animal moved to another site. This would point to a general underestimation of arousal frequency by RFM in two-day intervals of 27%.

Taken together, the figures of the latter two sources of error indicate that the relative frequency of individual arousal is estimated by RFM to be about one-third too low with intervals of up to one week, and down to half the real frequency when longer intervals are used. Application of these figures to other species or to studies with varying interval lengths seems doubtful.

(d) RFM is a point estimate of the fraction of bats moving around in an interval. It is based on a total number of movements and a number of nonmoving animals. Thereby it is not feasible to calculate the dispersion of RFM-values. This excludes also statistical comparison. Use of the χ^2-test as usual with relative frequencies is of no help as the basic data are not independent.

The above considerations show that the figures for frequency of displacements given by DAAN & WICHERS (1968, Table V), tell little about the frequency of arousal, although they may reflect roughly the same seasonal and specific variation. Also the results of others (VAN NIEUWENHOVEN, 1956; NEVRLÝ, 1963; GAISLER, 1966) probably highly overestimate the duration of individual hibernation periods. In a recent paper RANSOME (1971), employing the same technique, claims the accuracy of his observations of arousal frequency in *Rhinolophus ferrumequinum*; the argument being that even a minute change in foot-position in this species is the result of complete arousal. His daily observations in a colony of up to 20 bats constitute the most accurate data currently available. However, also RANSOME's results to some unknown extent underestimate arousal frequency (or, overestimate hibernation duration): The problem is not that some bats are incorrectly noted as having aroused when changing foot-position, but that some eventually aroused, without changing position, the discontinuity thus escaping observation.

Seasonal variation.—Relative frequencies of movements were calculated in the three species studied for every month in both seasons of investigation in the Koelebosgroeve. All curves (Fig. 21) agree to one pattern of systematic seasonal variation: The frequency of movements generally drops from September to February by about two-thirds of its initial value, and rapidly increases again in March and April. Slight specific differences in frequency of movements seem to persist throughout both seasons, to the extent that greatest activity is found in *M. daubentoni*, and lowest in *M. dasycneme*. The curves for the second year generally fell below those of the first. This is at least partly due to the longer interval duration (7 days) used in 1968/69.

DAAN & WICHERS (1968, p. 277) found no indication of seasonal variation in activity in the thermo-constant back part of the cave, and formed the hypothesis that seasonal variation in ambient temperature in the other parts causes the regular change in activity. With the data from the Koelebosgroeve, the existence of such differences can not be checked. This is done in Fig. 22 for the year 1967/68. During that winter the rear part of the cave was constant in temperature: the range of air temperature variation in this area was as small as $0.3°C$ at point T and $0.1°C$ at point W (see Fig. 3). Nevertheless, both *M. mystacinus* and *M. daubentoni* exhibit essentially the same pattern of seasonal variation in the rear as in the other cave parts. A difference occurs only in *M. dasycneme*, where activity in the rear is more constant than in animals hibernating in the other cave parts. However, this curve is based on a small number of observations, though, and little significance can be attached to it.

Influence of temperature.—In the above, temperature variation was already mentioned as a factor possibly contributing to variation in
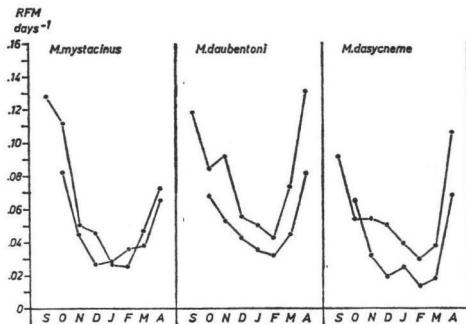
Fig. 21. Relative frequency of movements (RFM): Seasonal variation. Koelebosgroeve.
●- - -● 1967/68, ○- - -○ 1968/69.
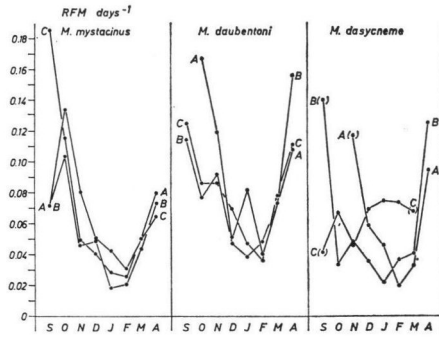
Fig. 22. Frequency of movements: Seasonal variation in different cave parts. A = entrance area; B = intermediate; C = rear. Koelebosgroeve 1967/68. (·) indicates values based on less than 25 observations.

activity. There are two ways in which temperature may be involved in the frequencies of arousal and intracave movements of hibernating bats: The frequency of spontaneous arousal may be affected by the mean level of ambient temperature, and changes in temperature may directly or indirectly (*i.e.*, via other meteorological factors) act as stimuli to instantaneous arousals. Below I will try to trace to what degree such effects play a role in activity in the natural environment. Two parameters are considered: outside temperature and ambient cave temperature.

The effect of outside temperature on activity was measured by comparing displacement frequencies in cold and warm intervals. Mean temperatures were calculated from 3-hr weather reports of the KNMI Meteorological station Beek, for the 46 2-day intervals in the winter (December to February) of 1967/68. Restriction to the winter months largely excluded the effect of seasonal variation. The intervals were pooled in three mean temperature classes (< 0, 0–4, and $> 4°C$), and the corresponding RFD-values calculated for front area and rest of the cave.

Table IX shows that in the three species, activity is positively correlated with outside temperature in the front area, not in the rest of the cave. This effect is obscured to some extent in similar calculations of RFM: the tendency of bats to move towards the front area in cold intervals (Table IV) adds to the RFM-values in this cave part. The frequency of displacements, being the better estimate of instantaneous arousal frequencies (see above) is therefore given in Table IX. That outside temperature affects the rate of activity only in the front area is obviously connected with the limited penetration of temperature fluctuations into the cave.

TABLE IX

Relative frequencies of displacements (RFD) in dependence of outside temperature (expressed in days^{-1}).

Mean interval temperature (°C)	Number of intervals	M. mystacinus		M. daubentoni		M. dasycneme	
		front area	rest cave	front area	rest cave	front area	rest cave
<0	12	.023	.025	.040	.044	.000	.024
0–4	24	.036	.036	.043	.050	.031	.053
>4	10	.038	.035	.074	.035	.057	.038

In order to analyse correlations of activity with ambient temperature, an 'average' top air layer temperature was assigned to each section for every interval: from the fortnightly 30-point readings and the 12-point continuous temperature record, it was possible to estimate the dominant temperature within limits of .5°C. Cave sections and intervals were then pooled in three classes: 3–5, 5–7 and 7–9°C. Calculation was restricted to the periods December 20, 1967 to February 20, 1968 (limited by the temperature record available) and December 1, 1968 to March 1, 1969. In the first season temperatures ranged from 5–10°C, in the second from 2–9°C. Observations below 3°C and above 9°C, were however too few to be included.

In Table X the RFM values computed for the three *Myotis* species in both seasons are given. In five out of six cases the correlation of RFM with ambient temperature is positive. The exception is *M. daubentoni* in 1967/68 with a lower frequency in 7–9°C than in the 5–7°C class.

TABLE X

Relative frequency of movements in winter in three classes of ambient temperature (days^{-1}). In parentheses the corresponding corrected estimates of mean duration of hibernation bouts (days). Koelebosgroeve.

Species		Ambient temperature (°C)		
		3–5	5–7	7–9
M. mystacinus	1967/68	—	.023 (23)	.036 (20)
	1968/69	.026 (25)	.029 (23)	.035 (19)
M. daubentoni	1967/68	—	.052 (14)	.046 (16)
	1968/69	.031 (22)	.037 (18)	0.46 (14)
M. dasycneme	1967/68	—	0.39 (19)	.051 (14)
	1968/69	.018 (37)	.019 (35)	.023 (29)

The values of 1967/68 are generally higher than those of 1968/69 in the same temperature classes: This is partly due to different interval length. Using the correction factors derived before, estimates of duration of hibernation bouts were computed. The variation due to relatively small differences in temperature available in the cave can become quite considerable. In 3–5°C, the estimated duration of hibernation periods is 1/2 to 1/4 longer than in 7–9°C.

Direction of movements.—The division of the quarry in fourteen sections, numbered 1–14 from the entrance to the far rear (see Fig. 1b) provides an opportunity to quantify the directional aspect of movement. To every individual intracave movement a natural number between −13 and +13 can be assigned, denoting the number of sections the bat moved towards the entrance (positive) or towards the rear (negative). Many movements (55% in *M. mystacinus*, 44% in *M. daubentoni*, 32% in *M. dasycneme*) were made within sections. This is obviously related to traditional aspects in habitat selection (see section 4.3. *Tradition*). For the remaining movements over one or more section boundaries, the mean direction and distance travelled was calculated in five classes of outside temperature. These figures, shown in Table IV, demonstrate the effect that low outside temperature generally causes awake bats to move outward in the cave, high temperatures favouring inward movements. They agree with the procentual increase in the front area during cold intervals.

4.6. Extracave movements

The results obtained on activity outside the cave are twofold: they consist of incidental observations of exchange of marked individuals with other caves, and of electronic recordings of flights through the entrances.

Outside flights can occur throughout the winter. However, it could be demonstrated that they are rare in the populations studied (DAAN, 1970). The—overestimated—mean fraction of *Myotis*-bats flying out of the Koelebosgroeve from December to February was in the order of 0.5% per day. In the same period, the frequency of intracave movements was at least ten times higher. In *Plecotus* (probably *P. auritus*) exit flights occur with a frequency two orders of magnitude larger. The record from the entrance of the ice-cellar Middenduin is more complete than the one from the Koelebosgroeve and offers a possibility to verify the figures obtained. Allowance has to be made, though, for the method differences: In the Koelebosgroeve outward and inward movements were recorded by means of photographic recording (DAAN, 1970). In Middenduin a direction discriminating photocell

system was used that did not allow the identification of individual or genus in the records. As a speculative estimate of the relative contributions of both genera *(Plecotus* and *Myotis)*, their fractions in the photographs of intracave flights in the same period were used. However accurate and complete the records themselves are, the occurrence of one or two highly active *Plecotus*—specimens in the ice-cellar in winter, renders some of the calculations for *Myotis* less reliable.

Moreover, many 'outward flights', especially those in daytime were immediately followed by returns. Such records are considered to represent 'light sampling behaviour' and are not entered in the exit flight calculations of Table XI. Finally, the differences in specific composition between Koelebosgroeve and ice cellar, to mention only one of many variables, would not let us expect the same values in both hibernation quarters.

Nevertheless, the orders of magnitude of outside flight frequencies agree reasonably well, and the same seasonal tendency is evident. In addition, Table XI shows that the few outside flights occurring in winter tend to be concentrated during mild nights ($> 4°$).

The next question to be asked in this connection concerns the direction of extracave movements. Does spontaneous intercave winter migration exist? Many examples of intercave migration can be found in the literature. In nearly all cases, however, the data concern bats that were banded and thus disturbed before migrating. In two years of more or less regular visits to two dozen caves in South Limburg, in which together 586 hibernating bats were marked with head stickers,

TABLE XI

Extracave flights recorded in Middenduin 1969/70.

Movements	Nov	Dec	Jan	Feb	March	April
			Month			
Inward: Total	18	11	5	7	12	18
Outward: Total	9	2	3	10	50	55
Per day	.37	.05	.12	.36	2.08	2.99
Per day <0° C	—		.09		—	—
0–4° C	.33		.17		1.59	3.33
>4° C	.56		.38		2.57	1.56
Estimated per bat (Myotis) per day*	.006	.001	.002	.008	.037	.22
	(.012)	(.006)	(.007)	(.007)	(.020)	(1.15)

* Numbers in parentheses are data from Koelebosgroeve 1968/69 (DAAN, 1970).

we came across 13 instances of spontaneous intercave migration (not stimulated by our research methods). These are summarized in Fig. 23.

Though of little quantitative value, these results contain two patterns worth mentioning. Firstly, the intercave movements seem to be concentrated in autumn, and occur far less frequently in winter and spring.
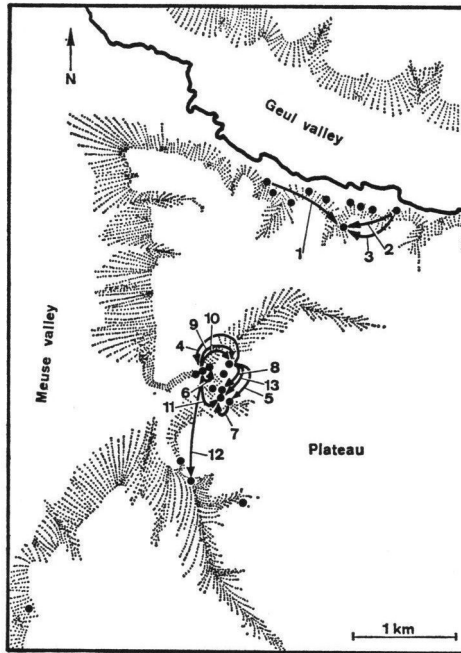
Fig. 23. Entrances of caves regularly visited during the investigations, and spontaneous winter movements registered.
Movements: (cave numbers corresponding to BELS 1952)
Barakkengroeve area:
   1. *M. mystacinus* tagged XS Dec 67 (cave 164 → 83)
   2. *M. daubentoni*   ,,   XC Nov 67 (82 → 83)
   3. *M. dasycneme*   ,,   XB Nov 67 (82 → 83)
Koelebosgroeve area:
   4. *M. daubentoni* tagged DH Nov 67 (35 → 33)
   5. *M. mystacinus*   ,,   CZ Nov 67 (35 → 117)
   6. *M. mystacinus*   ,,   IK Nov 67 (33 → 34)
   7. *M. mystacinus*   ,,   CZ Jan 68 (117 → 38)
   8. *M. daubentoni*   ,,   AR Mar 68 (35 → 37)
   9. *M. dasycneme*   ,,   gh Nov 68 (33 → 35)
  10. *M. nattereri*   ,,   ga Nov 68 (33 → 35)
  11. *M. dasycneme*   ,,   gi Dec 68 (33 → 38)
  12. *M. mystacinus*   ,,   gv Dec 68 (33 → 42)
  13. *M. mystacinus*   ,,   LT Dec 68 (35 → 38)

Secondly, the movements are of limited geographical distance: They connect closeby situated caves in the valley around the Koelebosgroeve, and similarly in the slopes around the Barakkengroeve, but apparently do not bridge the two kilometres over the plateau between the two regions. Also, no exchange between the Koelebosgroeve and the 'Keerderberg' caves—a third rich area, another two kilometres to the southwest—could be established.

In all of the more frequent bat species there are examples of such small-distance intercave migration. Nevertheless, we must consider this a very rare phenomenon. Dealing with estimates of population turnover we arrived at the same conclusion (section 4.2). Both in the larger and in the small caves the wintering populations seem to be very stable during midwinter, both in total number and in hibernating individuals.

4.7. Circadian rhythm in activity

In several parts of the investigation we collected information concerning the natural 24-hour distribution of bat activity in winter. A first rough approach is the calculation of RFD-values obtained in short-term series of short-interval searches. Such results were collected on three occasions during student excursions to the quarries. Also, the data reported by DE WILDE & VAN NIEUWENHOVEN (1954) are appropriate for evaluation of this aspect.

Except for the Apostelgroeve data, all figures in Table XII agree in showing lower displacement frequencies in daytime (800 h–1600 h) than during the night (1600 h–800 h). The overall figures per day show remarkably little variation, except for the Koelebosgroeve results which were obtained in March, and thus would be expected to be higher than those taken in January (see section 4.5).

For a closer look at the daily distribution of activity we have to turn to the automatic recordings. The results obtained with the photographic recorder in the Koelebosgroeve in 1968/69 (DAAN, 1970) deal only with flights to and from the hibernaculum. They revealed outward flights exclusively between sunset and sunrise. This certainly does not reflect activity distribution inside the winter quarter. In Middenduin, the next winter, the same apparatus recorded flights through a passage inside the ice-cellar (Fig. 7). Moreover, daily checks of the recorder made its operation nearly continuous throughout the winter of 1969/70. Fig. 24 summarizes the 24-h distributions of all recorded bat flights per month. The histograms show that in October pronounced nocturnality characterizes the activity pattern. As the season proceeds, however, activity of the population becomes less rhythmic. Nocturnality returns with the increase in activity between

TABLE XII

Relative frequencies of displacements per 8 hr period for mixed-species populations.

Period of observation	Apostel-groeve* Jan 52/54	Koelebos-groeve March 1968	Sibber-groeve Jan 1969	Barakken-groeve Jan 1970
		Cave		
0– 8 hr	.026	.067	.026	.020
8–16	.022	.018	.008	.008
16–24	.016	.042	.026	.022
0–24	.064	.127	.060	.050

* From data in DE WILDE & VAN NIEUWENHOVEN, 1954.

February and April, as can also be noticed from Table XIII, columns 2 and 3.

Another feature deserving attention here is that the peak of activity falls late during the night in December and January and is earlier in autumn and spring (Fig. 24; Table XIV). Since this points to some interesting hypotheses with regard to the circadian system involved, it is necessary to see if this difference is statistically consistent. Statistical analysis of 24-h distributions commonly involves use of the Rayleightest for departure from randomness in circular samples (BATSCHELET, 1965). This, and other statistics are valid only for samples composed of independent observations. The passages of bats through the window are certainly not independent events. For each individual bat they tend to be grouped together in one or a few hours just after arousal from hibernation. Therefore, we can not rely upon the high 'significances' indicated in the first two columns of Table XIV.

TABLE XIII

Summary of the activity of bats *(Myotis daubentoni)* in the ice cellar Middenduin 1969/70.

Month	Estimated mean population	Recorded flights/hour Day	Night	Total	Flights/ bat/ day	RFM/ day	Flights/ active bat
October	?	.11	.93	.56		.32	
November	66	.41	.72	.61	.221	.21	1.05
December	75	.16	.39	.31	.100	.14	.71
January	81	.11	.17	.15	.045	.072	.62
February	83	.16	.23	.21	.058	.078	.77
March	68	.36	.66	.50	.177	.197	.90
April	17	.10	.30	.19	.264	.18	1.46
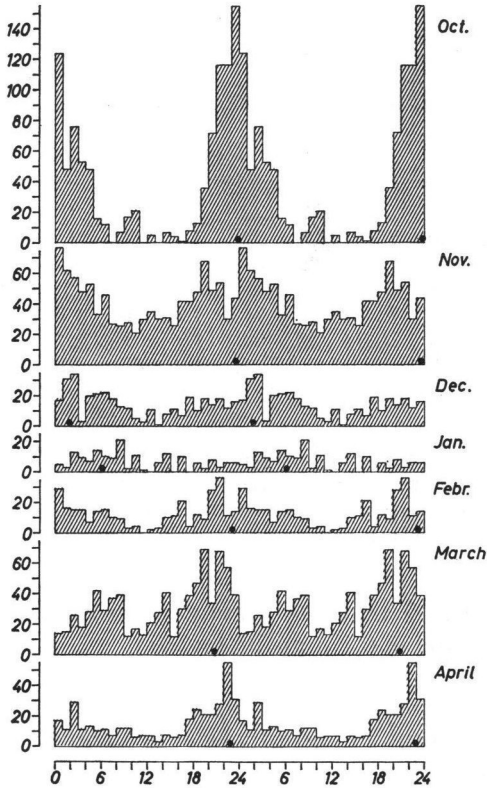
Fig. 24. Twentyfour-hour distributions of total number of recorded intracave flights. Middenduin 1969/70. Distributions are shown two times. Black dots indicate circular means per month.

To test the difference in activity time between winter and autumn/ spring, it was preferred therefore to take the daily circular means as independent sample points, and to compute the desired statistics over these values (Table XIV, column 3). Use of these daily mean vectors is a crude reduction of the original data. The insignificant Z-values in column 3 therefore do not exclude a diel rhythm in activity, as can be rapidly seen in Fig. 24, November and March, for instance. But the daily means can with certainty be considered independent. Thus, the hypothesis is tested that the distribution of daily mean vectors in winter (December-February) is the same as in autumn and spring. A parametric test described by BATSCHELET (1965, p. 33) is used yielding $F_{1, 133} = 5.07$, which is significant at the .025 level. So we have to conclude that with decrease in activity towards midwinter, the amplitude of the circadian rhythm decreases, and its phase shifts backward.

TABLE XIV

Statistics of 24 hr distributions of recorded intracave flights. Middenduin 1969/70.

	All bats				Identified Myotis only				Daily mean vectors of all bats			
	m	s	n	z	m	s	n	z	m	s	n	z
October	23.8	3.1	955	**431**	22.1	3.7	37	**10**	00.4	2.3	9	**5.9**
November	23.5	4.9	1007	**34**	22.5	4.8	327	**15**	00.3	4.2	28	4.1
December	01.8	4.7	344	**22**	00.2	4.6	135	**12**	03.0	4.8	21	0.9
January	06.1	4.7	164	**9.6**	03.3	5.0	68	1.3	04.3	4.4	14	1.6
February	23.1	4.4	298	**35**	21.0	4.6	110	**8.2**	01.7	4.4	20	2.3
March	20.8	4.9	775	**25**	00.1	4.9	204	**7.1**	23.0	4.8	30	1.5
April	22.8	4.3 hrs	374	**53**	21.4	4.0 hrs	142	**28**	23.1	3.0 hrs	13	**6.3**

m = circular mean (clock hours); s = circular dispersion; n = number of observations. Rayleigh-statistic $z = R^2/n$, where R is the length of the mean vector. Z-values appearing in heavy-type indicate significant difference from random distribution at the .01 level (however, see text).

Table XIV indicates that the gravity point of activity in all bats together gets as late as 6.1 a.m. in January.

As stated above, winter bat flights from the Koelebosgroeve took place exclusively between sunset and sunrise. The arrangement of a double-beam photocell device in the entrance of Middenduin surprised us with another aspect of rhythmicity in the behaviour of the bats. The records of extracave movements are far from dense. Table XI indicates a maximum frequency of 0.12 per hour in April. Nevertheless, it occurred fairly regularly that an outward flight was almost imme-diately—within one minute—followed by an inward interruption of the photocell beams. We assumed such instances represented trial flights, and established their 24-hr distribution separately from other inward and outward flights. This interpretation of these recordings is not necessarily correct in all cases. However, the very nature of the overall results supports my feeling that it is correct in the majority of instances.

Fig. 25 contains all results from passages through the entrance of Middenduin. Outward flights appear to be concentrated mostly in the beginning of the night (circular mean at 21.2 p.m.). Subsequent outward and inward flights occur distinctly more often in daytime than at night. They peak in the late afternoon (circular mean 16.1 p.m.). Evidently such recordings represent "light sampling behaviour" (TWENTE, 1955) where trial flights of bats result in their return to the
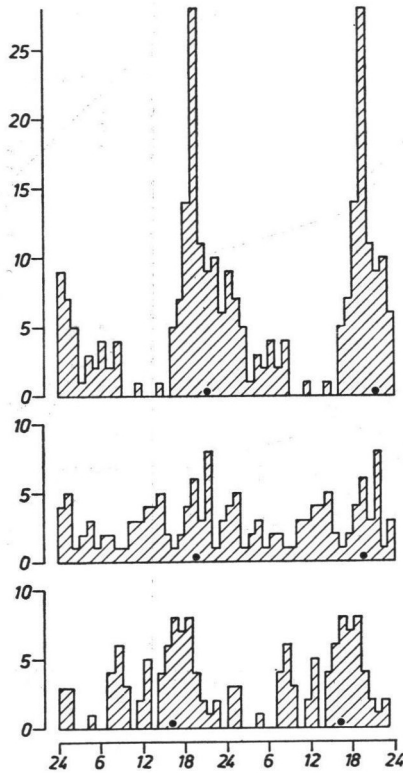
Fig. 25. Twentyfour-hour distributions of flights recorded through the entrance. Middenduin 1969/70. The histograms are shown two times. Black dots indicate circular mean vectors of the sample. Top: Single outward flights, Middle: single inward flights, Bottom: outward flights followed immediately by inward flights.

hibernaculum by daylight. Strangely, single inward flights are made far less exclusively at night than single outward flights.

4.8. Weight loss and activity

In the Koelebosgroeve during 1968/69 and in the ice cellar in 1969/70 all bats were weighed and sexed at the time of marking. Those bats still hibernating in the cave on April 20, 1969 were weighed once more. Furthermore, a few large samples of bats were weighed in 1968 and 1969 in the Barakkengroeve and in the Keerderberg cave. Monthly means and standard deviations of all weights are plotted in Fig. 26, together with the linear regressions calculated over the periods September 26–December 31 and December 1 to April 20 (see also Table XV).

4
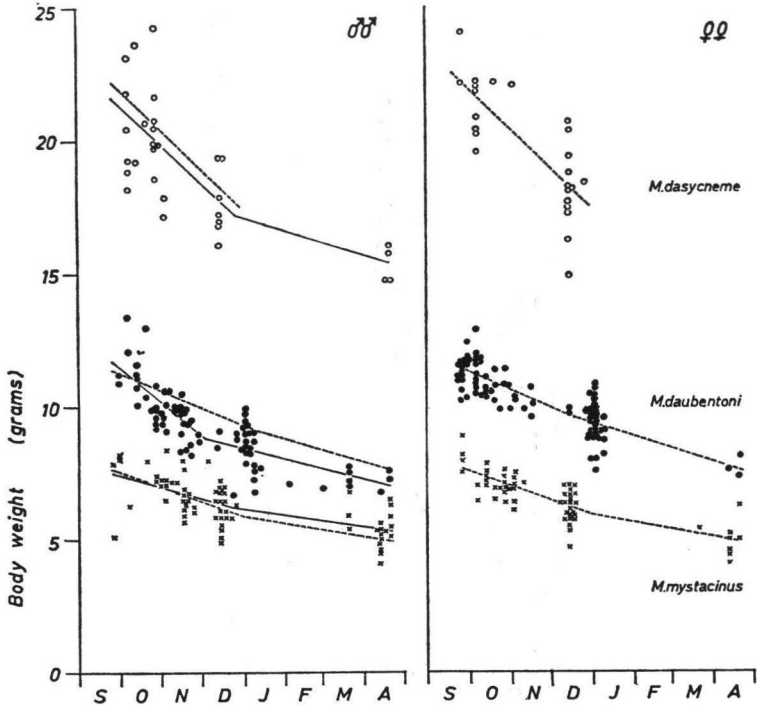
Fig. 26. Variation of body weight in the course of the hibernation season. Regression lines calculated from values of different caves and years in the periods September–December and December–April. Regression lines of females (dotted) are repeated in the left picture for comparison with males.

TABLE XV

Estimated mean weight losses in hibernating bats under natural circumstances. Koelebosgroeve 1968/69.

Species	Sex	Weight at arrival[1]	Weight at departure[1]	Loss	Daily loss[2]	
					Sept-Dec	Dec-April
M. mystacinus	m	6.9 (7.XI)	5.5 (31.III)	1.4 (20)	15	8
	f	7.1 (24.X)	5.1 (31.III)	2.0 (28)	18	10
M. daubentoni	m	10.2 (1.XI)	7.1 (7.IV)	3.1 (30)	42	14
	f	11.3 (28.IX)	8.2 (24.III)	3.1 (27)	22	16
M. dasycneme	m	20.6 (17.X)	15.5 (14.IV)	5.1 (25)	48	19
	f	21.9 (3.X)	— (7.IV)	— —	50	—
		g	g	g %	mg	mg

[1] Values of the regression lines in fig. 27 at the dates (in parentheses) of median arrivals and departures in fig. 10.
[2] Slope of the linear regressions in fig. 26.

A consistent sexual difference is observed only in *Myotis daubentoni*, females of this species being about half a gram heavier than males. All species conform in showing steeper slopes in body weights in autumn than in winter and spring.

It would be of interest to know if the rate of decrease in body weight is related to the amount of individual activity performed during the winter. The animals for which individual weight losses are known are too few, to permit this calculation. However, unpublished results from two experiments, one in seminatural, the other under laboratory conditions, that are pertinent to this question.

In the first experiment, two corridors in the Barakkengroeve, one in a relatively cold and one in a warm part of the cave, were closed by wire netting in January 1969. Ten female specimens of *Myotis daubentoni* were placed in each corridor. At the beginning and end of the experiment, the body weights were taken. Frequencies of movements in both groups were assessed weekly during a period of 63 days.

According to the data in Table XVI the daily body weight loss in female *M. daubentoni* at 10.5°C is twice as high as at 5°C (difference significant by t-test at the 0.001 level). The frequencies of movements differ by about the same factor. Body weight loss at 5°C is at the same level as in natural circumstances (Table XV) in the second half of the hibernation season, and so is activity. Indeed, corridor I is in the entrance area of the Barakkengroeve where most bats are found hibernating by January-March, and corridor II is in the thermoconstant rear which is nearly depopulated by this time of the year (KUIPERS & DAAN, 1970).

In the second experiment weight loss and frequency of arousal of *Myotis daubentoni* were tested in climatized rooms under different conditions of temperature and relative humidity. The bats were kept for two to four weeks in continuous darkness in light spring-suspended cages, a modification of the type used by RAWSON (1960). They had continuous access to water, but no food was given. Movements of the cages were recorded on an Esterline Angus event recorder. In

TABLE XVI

Weight loss and activity in female *Myotis daubentoni* in seminatural conditions. Barakkengroeve January–March 1969.

Corridor	Ambient temperature	Number	Weight loss (mg/day)	RFM/day
I	4–6° C	10	17.7 ± 5.7	0.030
II	10.5 ± 0.1° C	10	37.6 ± 6.2	0.063

Fig. 27 all data concerning weight loss and activity of individuals at 100% relative humidity and ambient temperature of either 10°C or 5°C are plotted.

There is a significant (p < 0.05) positive correlation between decrease in body weight and frequency of arousal from hibernation. The frequencies measured are an order of magnitude larger than frequencies of movements found in nature. Also, the weight losses are considerably higher. The experiments were stopped prematurely as most animals lost weight too rapidly, and apparently did not show normal hibernation behaviour in their state of confinement. Therefore, firm conclusions can not be based on these results. Nevertheless, Fig. 27 indicates a similar tendency to the other experiment, *viz.*, that high temperatures stimulate both frequency of arousal and rate of weight loss. Few results were obtained with low relative humidities (Table XVII) due to increased mortality in these groups. They suggest that dry air leads to large mean daily weight losses without clearly affecting rate of arousal.

The positive correlation between frequency of arousal and weight loss of the hibernating bats, indicated by both experiments, is most probably involved in the change in rate of weight loss during the season.
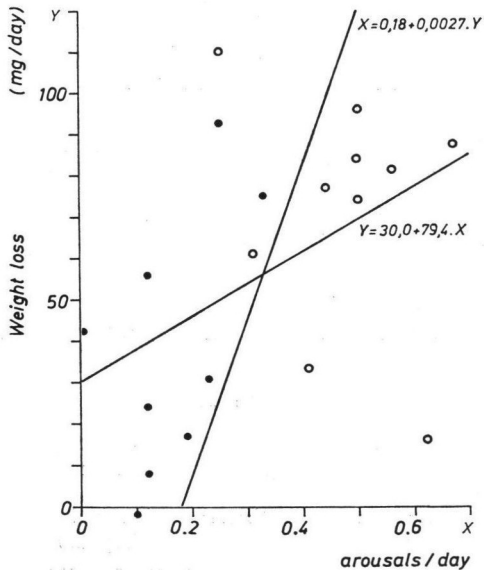

Fig. 27. Correlation between body weight loss and frequency of arousals in *Myotis daubentoni*, kept in constant conditions at 100% relative humidity. ● 5° C, ○ 10° C. Coefficient of correlation = 0.460.

5. DISCUSSION

5.1. The functions of activity

The basic adaptive function of hibernation is to enable microchiropterans from temperate zones to survive winter by conservation of energy over a period of food scarcity. It is especially the share of activity in the overwintering budget that will be discussed here. Before dealing with the cost of activity I shall first discuss the biological functions served by winter activity, and our present knowledge about the factors causing interruptions of hibernation.

Reproductive activity is one of the functions performed at least partly during the hibernation season, for which the aroused state is a prerequisite at least in males. It is well-known from histological studies that most hibernating bats in the temperate zone must copulate mainly in September–October, the spermatozoa being stored for several months in the female reproductive tract (*e.g.*, GAISLER, 1966b; WIMSATT, 1969). STRELKOV (1962) states that with northern vespertilionids copulations were at least as frequent in winter and spring as in autumn. My observations of copulations (Table IX) indicate a slight concentration in autumn when species are considered together, though they definitely occur during the winter too. The copulatory behaviour of *Myotis daubentoni* has recently been described by ROER & EGSBAEK (1969). These authors mention a number of copulations observed in limestone quarries in Denmark. Such observations were either made in October–November or in March–April. This is not highly significant, as the search effort was not the same throughout the season.

Habitat selection is a second function supported by interruptions of torpidity. Habitat selection changes in the course of the season occur with changes in cave microclimate. This has the general pattern: In many caves in South Limburg bats of the more abundant species tend to select the rear of the cave in autumn and the front parts later in

TABLE XVII

Weight loss and activity in *Myotis daubentoni*; experimental conditions.

Experimental group	Temp	RH	N	Weight loss (mg/day)	Arousals/day
1	5° C	100%	9	38.2 ± 31.6	0.16 ± 0.10
2	5° C	80%	2	88.2 ± 36.4	0.37 ± 0.18
3	10° C	100%	10	72.0 ± 28.4	0.48 ± 0.13
4	10° C	80%	2	112.2 ± 4.2	0.39 ± 0.12

the season (VAN NIEUWENHOVEN, 1956; DAAN & WICHERS, 1968; DORGELO & PUNT, 1969). This phenomenon of "internal migration" turns out to be subject to some variation revealing its true nature: Usually, in horizontal caves the entrance part is relatively warm in autumn. In the Barakkengroeve, so structured that the entrance part is coldest throughout the year, this area is preferred instead of avoided by bats entering in autumn (KUIPERS & DAAN, 1970). Furthermore, the direction of intracave movements turns out to be determined by outside temperature (Table V): Low temperatures promote the selection of locations in the cold entrance area, high outside temperatures urge the animals backward. It appears to be a continuous trial and error process. By the selection of low temperatures it may help to limit the energy expenditure both by minimizing the metabolism in hypothermia and by reducing the frequency of spontaneous arousal from hibernation. Low temperatures can in winter usually be found near the cave entrances, and are thereby positively correlated with relatively large temperature fluctuations giving rise to additional arousals. The bats avoid the effects of temperature fluctuation to some extent by withdrawing in holes and fissures in the entrance area (Table V), but can probably not avoid them completely. It is a changing balance between energetically favourable and adverse microclimatic factors that causes the population to shift through the cave. Slightly below-saturation humidities may play a part in the avoidance of the entrance area in autumn. But later in winter, when the whole top-air layer is continuously saturated with water, humidity plays no role in changing habitat selection.

The picture of habitat selection as governed by microclimatic factors is complicated by individual tradition. Signs of tradition were found at all levels (section 4.3): Bats tend to return not only to the same cave (BELS, 1952), not only to the same section, but even to exactly the same spot in a large subterranean labyrinth. Quite often, they may alternate between two or more spots. If bats hibernate for two or more years in the same cave they may use in part exactly the same locations and tend to do so in a constant time-sequence. The latter observation suggests the existence of an endogenous annual program clock, to which the selection of locations in the cave may sometimes by linked. Circannual rhythms are now well-known to regulate yearly behavioural cycles, *e.g.*, in hibernating mammals (PENGELLEY, 1968) and in migrant birds (GWINNER, 1971). The bats' sequences of locations may contain the first indication known in animals of a rhythm producing annual replications of individual-specific behavioural patterns. As such, it would be comparable to the endogenous circadian program clock driving mice (*Peromyscus* spec.) to repeat night after night individual time sequences

of eastward and westward wheel-running in experiments by KAVANAU & RISCHER (1968).

Both tradition and the response to microclimatic factors involved in habitat selection seem to favour energy economy during hibernation. Certainly, the effect is toward minimizing rather than maximizing winter activity.

The majority of movements is made within the hibernation quarters. Only very few bats venture outside. Even in November, the month during which most inter-cave migrations were recorded (Fig. 23), the relative frequency of outward movements hardly exceeds 1% per day (Table XI). The relative frequency of arousal (Table XVIII) exceeds the frequency of outside movements by a factor between 5 and 10 throughout the year, except in March and April when mass migration from the cave occurs.

Both the Koelebosgroeve and the ice cellar Middenduin sheltered very few insects in winter—less than ten dormant Lepidoptera and a a few localised dipterans have been seen. The possibility that feeding of any importance took place during intracave activity must be ex-cluded. Neither remnants of prey, such as moth wings, nor bat drop-pings were ever found. So we can conclude for 80–90% of all activity periods in *Myotis*-bats that no food has been taken. Whether the remaining few incidental outward flights did yield any feeding success is uncertain.

In the literature, one finds with about equal frequency the opinion, that hibernating bats feed during arousals (BURBANK & YOUNG, 1934; SWANSON & EVANS, 1936; GRUET & DUFOUR, 1949; RANSOME, 1968; ROER, 1969) and that they do not (HAHN, 1908; GUTHRIE, 1933; FOLK, 1940; TWENTE, 1955; SAINT GIRONS et al., 1969; DAAN, 1970). The truth being probably that winter feeding depends both on species (*Plecotus* tend to feed while *Myotis* don't: ROER, 1969; DAAN, 1970) and on climatic factors subject to variation by geographical location, by time of year and by year-to-year differences. Such climatic variation, affect-ing the availability of insect food, may be hold responsible for the apparent contradiction in the findings of GRUET & DUFOUR (1949), SAINT GIRONS et al. (1969) and RANSOME (1968) concerning winter feeding of the Greater Horseshoe Bat *(Rhinolophus ferrumequinum)*.

Successful feeding on mild winter days would give a positive value to a variable frequency of arousal, in terms of the hibernation energy budget. RANSOME (1968) has presented some evidence that Greater Horseshoe bats actively adapt their selection of ambient temperatures to previous weather conditions, thereby affecting their frequency of spontaneous arousal.

The difference between *R. ferrumequinum* and *Myotis* winter activity

is once more illustrated by its circadian organization. RANSOME (1968, p. 101) supposes that waking up in the Greater horseshoe bat is synchronized with dusk. In our investigations this was evident only in October. Nocturnality disappeared with the decline of activity towards January and reappeared again slowly towards the end of the season. It was curious to find that in midwinter the peak of activity occurred early in the morning rather than late in the evening. One hypothetical explanation of this phenomenon is that the circadian rhythm giving rise to spontaneous arousal (STRUMWASSER et al, 1967; DAAN, in press) is not synchronized during the hibernation bouts, and has, generally speaking, a free-running period longer than 24 hrs. It is interesting to note that in Myotis lucifugus a seasonal variation in free-running period has been found (MENAKER, 1961) the latter being longer than 24 hrs in winter and shorter than 24 hrs in summer. Free-running circadian rhythms in natural conditions have hitherto only been found in the arctic summer (e.g., in Field mice: ERKINARO, 1969). The absence of synchronization with the light-dark cycle outside also suggests that the ecological significance of winter activity in the three Myotis species is different from that of summer activity.

    After waking up the bats fly around and may eventually try to leave the hibernaculum. When active in daytime they are likely to be compelled to return by the light outside. Such "light sampling behaviour" is now well known from a number of species to occur both in winter (TWENTE, 1955; GAISLER, 1963; RANSOME, 1968) and in summer colonies (DE COURSEY & DE COURSEY, 1964; light sampling behaviour in a dramatic form was recently described in Myotis dasycneme by VOÛTE, 1972).

The opportunity to take up water is another aspect of winter activity that has been proposed to have functional significance (FISHER & MANERY, 1967; DORGELO & PUNT, 1969). Indeed, dehydration is one of the more important hazards to which a hibernating bat is subject (KALLEN, 1964). Bats lose water by continuous evaporation, dependent on the vapor pressure deficit of the air, and by incidental urine ex-cretions as invariably accompany arousals. In the limestone quarries in South Limburg, conditions and habitat selection are such that the hazard of evaporation is minimized: Relative humidity of the upper air layers is 100% throughout, except in the entrance area on warm days in autumn, i.e., the time of year when this cave part is avoided by the bats. Oversaturation in large parts of the cave is usual during most of the winter. In circumstances where dew often forms on the bats' fur, evaporation must certainly be neglegible. Dew on some of the bats and on isolated wooden and iron objects is the only liquid water

available in the caves studied. I have observed several times, as have other authors (see FISHER & MANERY, 1967) that an awake bat may lick its fur and thereby ingest water. However, this behaviour was equally observed in bats waking up with dry fur. There is no evidence that drinking always—or even usually—accompanies arousal, as FISHER & MANERY (1967) suggest to be a special characteristic of bat hibernation. KALLEN (1964) reports that the little brown bat *(M. luci-fugus)* kept in a cold room would survive hibernation when either high humidity or drinking water was present. This suggests that the intake of water may be obligatory in non-saturated hibernacula whereas it remains a facultative feature of winter activity in biotopes with relative humidities of 100% FISHER & MANERY (1967) also made the appealing suggestion that clustering might be a behavioural means of bats to reduce evaporation. It is interesting to note in this connection that only vestiges of clustering behaviour are found in the water-saturated conditions in the South Limburg limestone quarries (section 4.3).

5.2. *The causes of arousal*

In section 4.5 a positive correlation was found between outside temperature and the relative frequency of displacements of bats in the entrance part of the cave. From this observation it is inferred that elevated temperatures in winter have a direct effect. The stimulus leading to arousal may either be the rise in temperature itself, or reversed thermocirculation (section 3.1) in the entrance area. It has been proposed (VAN NIEUWENHOVEN, 1956) that rising atmospheric pressure would lead to arousing stimuli by total replacement of the air in the entrance part of a quarry. It can be calculated however, that even a dramatic change in atmospheric pressure (say, 50 millibars in 3 days) would, in a 30.000 m³ cave with an entrance diameter of 5 m², lead to no more than a 1 mm/sec air movement through the entrance. This is neglegible compared to normal entrance thermocirculation, where wind speeds of 10–40 cm/sec are fairly common.

However, it is clear that meteorological fluctuations can exert some limited influence in the entrance area, which adds to the total number of arousals. The close agreement between RFM-values in different cave parts (Fig. 22) suggests that the majority of arousals is spontaneously determined, and is organized on a clear endogenous seasonal basis, with high frequencies in autumn and spring and infrequent arousals in midwinter.

The physiological stimuli leading to spontaneous arousal in hibernators have attracted considerable attention in the past decade. The fact that all hibernators seem to conform in invariably urinating shortly after arousal has largely influenced the hypotheses made. One

of the earlier suppositions, according to which distention of the slowly filling bladder were the arousing stimulus, dates at least as far back as HORVATH (1879). This hypothesis has been rejected in the Ground Squirrel (PENGELLEY & FISHER, 1961) on the basis of independence of bladder content from the time elapsed since entrance into hibernation. It could not be completely ruled out for the bat *Myotis lucifugus*, in which KALLEN & KANTHOR (1967) measured urine flow by urethric cathetering: they found urine flow sufficient to be of significance in bladder distention at temperatures down to 6.6°C. PENGELLEY & FISHER (1961) postulated metabolic end products—in the homeothermic state cleared by kidney function—to accumulate in the blood and perhaps thereby triggering arousal. The Q_{10} of arousal frequency in *Citellus lateralis* being close to 2.0 (TWENTE & TWENTE, 1965) suggests a metabolic process. Yet, neither non-protein nitrogen concentration in the blood, nor such electrolytes as Na, K, Ca and Mg (PENGELLEY *et al.*, 1971) nor d-glucose (TWENTE & TWENTE, 1967) could be found to increase between one arousal and the next. Sensitivity to injections of saline solution and of epinephrine increases in the course of the ground squirrel's hibernation bout (TWENTE & TWENTE, 1968a and b), and arousal response to potassium ions is stronger than to sodium (FISCHER & MROSOVSKY, 1970). But these facts remain only indications as long as no accumulation of such materials can be shown.

One more important aspect in the physiology of arousals is that they tend to occur in a circadian fashion, at least in bats (MENAKER, 1959), ground squirrels (STRUMWASSER *et al.*, 1967) and dormice (DAAN, in press). Yet, despite intensive research, there is at present no general and comprehensive view about the nature of the physiological stimulus triggering arousal from hibernation. Any theory shall have to account for distinct patterns of variation in the frequency of spontaneous arousal with season, taxonomic group, and environment factors such as temperature and humidity.

5.3. The cost of winter activity

A number of parameters of hibernation behaviour have been assessed in the foregoing chapters. It is realized that the variation around the mean is large in most cases. Yet, I think it useful to construct a 'standard' *Myotis mystacinus*, a standard *M. daubentoni* and a standard *M. dasycneme*, and see what can be inferred about their activity and use of energy during the winter. The important parameters are summarized in Table XVIII.

In this Table no distinction is made between the sexes. It should be realized, however, that there are sexual differences: Females tend

TABLE XVIII

Estimated mean parameters of hibernation in three species of bats. Koelebosgroeve; means over 1967/68 and 1968/69.

		Species	
	M. mystacinus	M. daubentoni	M. dasycneme
Date of arrival in cave[1]	29.X	11.X	4.X
Date of departure from cave[1]	9.IV	31.III	5.IV
Duration of hibernation[2]	162	171	183 days
Relative frequency of arousals[3] O–N	.084	.105	.059 days^{-1}
D–F	.044	.061	.042 ,,
M–A	.068	.091	.053 ,,
Number of arousals[4]	9.4	13.3	9.0
Weight at arrival[5]	7.0	11.0	21.5 g
Weight at departure[5]	5.2	7.5	15.7 g
Total weight loss[6]	26	32	27 %
Daily weight loss[7] O–D	17	32	49 mg/day
D–A	9	15	19 mg/day

[1] Mean dates at which populations were at half maximal strength.
[2] Days between mean arrival and mean departure.
[3] Mean RFM, corrected according to section 4.5.
[4] Calculated from 1 and 3.
[5] Mean of male and female regression values (Fig. 26) at arrival and departure dates.
[6] In % of weight at arrival.
[7] Mean slopes of male and female regressions (Fig. 26).

to arrive in the quarters two to four weeks before the males, and to leave in spring again one to two weeks earlier (section 4.2), thereby having a slightly longer overall hibernation period. It is interesting to note that a similar sexual difference in date of onset and in duration of hibernation was recently demonstrated in the ground squirrel, *Citellus lateralis* (PENGELLEY & ASMUNDSON, 1971). Female bats are furthermore slightly heavier (section 4.8) than males, both at arrival and departure, and their rates of weight loss are approximately equal. No indications have been found of sexual differences in activity or in habitat selection.

At present, no direct measurements of energy expenditure are available in the three bat species studied here. Extensive metabolic data (HOCK, 1951; HENSHAW, 1968, 1970) have accumulated for the North American Little Brown Bat, *Myotis lucifugus*. This bat is considered (*e.g.*, FINDLEY, 1970) to be taxonomically closely related to *M. daubentoni*. It hibernates in caves, under circumstances similar to

those met in the South Limburg limestone quarries, though its tendency to form large clusters is highly dissimilar from our species. In size (5–12 gram) the Little Brown Bat compares well with *M. mystacinus* and *M. daubentoni*. Metabolic data from the Little Brown Bat seem to be the most appropriate to use in estimating the cost of hibernation in the species here studied.

Basic metabolic rates of hibernating Little Brown Bats at 5°, 7° and 9°C can be estimated from the data presented by HOCK (1951, fig. 2), to amount to 0.04, 0.05 and 0.06 ml O_2/g/hr respectively. Prevailing R.Q.'s of 0.7 in hibernating mammals (KAYSER, 1961) indicate that fat is the prime source of metabolic energy. Accounting for a caloric content of fat of 9.4 kcal/gram, and neglecting eventual loss of water content, we can transpose HOCK's metabolic figures into relative rate of weight loss by multiplication with a factor of 1.2 (= 4.7 × 24/94) to get % weight loss per day. Thus, during continuous hypothermia at 5, 7 and 9°C, a *Myotis lucifugus* would lose .048, .060 and .072 % of its body weight daily, if its water content is assumed to remain the same. Actually, the picture is complicated by the data recorded by HENSHAW (1970) which show that in autumn the basal metabolic rate of *M. lucifugus* at 5°C is higher than later on in the hibernating season: The body-to-ambient thermal difference is kept at a few degrees higher level in November than in March. The possible, though not necessary, existence of similar seasonal fluctuations in basal metabolism and regulation of body temperature in our species renders the following calculations even more speculative than they would be otherwise. For the sake of simplicity we have to assume here that basal metabolic rate at one temperature would remain stable throughout the season (as it actually does in *Myotis sodalis*; HENSHAW, 1970). Taking into account the actual environmental temperatures selected in different parts of the season (Fig. 18), we may then estimate what part of total weight loss would have to be attributed to hypothermia alone. The calculations made are shown in Table XIX.

It turns out that the actual weight losses are about a factor of 2–3 larger than those calculated from metabolic rates in all three species. This difference is obviously caused by the extra energy expenditure involved in phases of arousal and activity. From the difference between calculated and observed weight losses the amount of weight loss per arousal can be estimated (bottom line of Table XIX). The specific percentages thus obtained are in good agreement with each other. Weight loss per arousal in autumn is about two times as high as later on in the winter. This points to prolonged and/or more intense activity periods in autumn. It is interesting to note in this connection that in this part of the season both copulations (section 4.4) and inter-

TABLE XIX

Estimates of energy expenditure during hibernation.

	M. mystacinus			M. daubentoni			M. dasycneme		
	O–N	D–J	F–A	O–N	D–J	F–A	O–N	D–J	F–A
1. days	32	62	68	50	62	59	57	62	64
2. temperature (°C; 68/69)	9	6	5	9	6	5	9	6	5
3. BMR ml O_2/g/hr	.06	.045	.04	.06	.045	.04	.06	.045	.04
% wt/day	.072	.054	.048	.072	.054	.048	.072	.054	.048
4. Initial weight g	7.0			11.0			21.5		
5. Wt loss in hypoth. mg/day	5.0	3.7	3.2	7.9	5.7	4.9	15.5	11.1	9.6
mg	161	229	216	396	355	290	883	690	612
total mg		610			1040				2180
6. Actual wt loss mg/day	17		9	32		15	49		19
total mg			1700			3400			5200
7. Extra wt loss mg/day	12		5.5	24		10	34		9
ar/day		.084	.056		.105	.076		.059	.047
mg/ar	143		98	224		132	576		192
%/ar	2.1		1.4	2.1		1.2	2.7		0.9

cave migrations (section 4.6) are concentrated. Estimating how long an average period of activity could be supported by the calculated rates of weight loss per arousal, would lead us too far away from the original data. However, from GRIFFIN's (1958, p. 41) estimate of bat metabolism during flight (200 cal/g/hr), we may derive an impression of the order of magnitude of flight activity that might be performed after arousal. Assuming fat to be the main source of energy again, 200 cal/g/hr can be converted to 2.1% body weight/hr. This figure would tell us that bats might perform little less than one hour of flight in autumn and less than half an hour when awaking in winter. In laboratory experiments (unpublished) we found the mean duration of 118 activity periods to be 4.5 hrs. MENAKER (1964) found an average of 3.16 hrs over 67 arousals in *M. lucifugus*. This is not contradictory to the above figures, as only a fraction of the activity periods is spent in flights, and metabolism in the rest of the activity period must be significantly lower.

5.4. Comparison with other hibernators

FISHER & MANERY (1967) have discussed, in a comparative way, the cost of hibernation in animals of different weight. This discussion is based on the assumption that metabolic rate in hibernation is a function of body surface rather than of body weight. Higher rates of basal metabolism per unit body weight in smaller animals would urge them to store more fat in order to endure the same total hibernation period as heavier animals. However, KAYSER (1964 a, b), comparing hypothermic metabolic rates in a large number of hibernators, found that these are—contrary to summer basal metabolic rates—strictly proportional to body weight. They can be described by the equation $Q = 3.20 \times P^{1.03}$;, where Q is kcal/24 hrs and P = body weight in kgr. As KAYSER (1964 a) has pointed out, this deviation from the surface exponent (0.67) is at least partly due to differences in body temperature. Larger hibernators maintain a higher body temperature than smaller species, having the same metabolic rates per gram body weight. Probably the body-to-ambient thermal difference in hibernating bats is further reduced than in other hibernators (SOUTH & HOUSE, 1967), which may be viewed as one way to reduce energy expenses. Still, during arousal from hibernation, in the homeothermic state, the surface law is followed again. This means that, theoretically, arousals cost more in smaller animals. In as far as interruptions from hibernation have the largest share in the total energy expenditure, the reasoning by FISHER & MANERY remains correct in its consequences, therefore: Higher aroused metabolism in smaller animals would thus require more fat storage to endure the same total hibernation period. However, the maximum potential fat reserve seems to be a limited fraction of body weight (MORRISON, 1960). FISHER & MANERY estimate that animals smaller than 165 grams would have to store more than the maximum amount of ± 50% fat in order to hibernate without food for 292 days (being the maximum artificial hibernation period in *Citellus lateralis*). They collected evidence from the literature that indeed hibernating rodent species of more than 200 grams do not eat during the winter, while three species smaller than 120 gram body weight (Dormice, Hamster, Chipmunk) do take food during arousals. Naturally, such herbivores have the opportunity to store complementary food reserved outside their body, in special provision rooms inside the burrows. Bats—at the very lower end of the body weight sequence—are as insectivores deprived of this possibility. FISHER & MANERY (1967, p. 247) generalize from literature data that bats "probably do not eat, but equally probably do drink during the whole hibernation period." They suggested that one way to cut down on hibernation energy expenses in order to endure equally long winters

as larger animals were to reduce the number of arousals. No data on the pattern of arousals in bats existed at that time, only misleading incidental observations of midwinter activity (*e.g.*, ANCIAUX, 1948; VERSCHUEREN, 1949), FISHER & MANERY postulated that bats may have significantly fewer arousals than do larger animals. The data obtained by RANSOME (1971) for the Greater Horseshoe Bat in Southern England, and in the present study in three *Myotis* species now allow an evaluation of this hypothesis.

In Table XX a survey is given of arousal frequency and weight loss as far as these data could be derived from the literature, *i.e.*, in four non-bat hibernators and four bat species. Male and female bats are again lumped together in this Table. Most of the variables are rough estimates, and are based on highly variable original data, especially in the bat investigations in natural conditions.

Nevertheless a few tendencies are conspicuous: The total weight loss, expressed as percent of initial body weight is somewhat less in bats (roughly 1/4–1/3) than in larger hibernators ($\pm$ 1/3–1/2). These figures are in good agreement with data published in other species

TABLE XX

Weight loss and activity in a number of hibernators.

Species	A	B	C	D	E	F	G	H
Erinaceus europaeus								
(finnish)	830	49	200	35	.17	1.4	4.2° C	KRISTOFFERSON & SOIVIO, 1964
(german)	830	35	241	31	.13	1.1	4.2°	KRISTOFFERSON & SOIVIO, 1964
Citellus lateralis	270	30	192	24	.12	1.2		FISHER, 1964, FISHER & MANERY, 1967
Citellus citellus	215	42	189	22	.12	1.9	5°	KAYSER, 1952
Eliomys quercinus	131	54	151	23	.15	2.3	semi-natural	GABE et al., 1963
Rhinolophus ferrumequinum f	28.5	35	169	33	.19	0.9	natural	RANSOME, 1968, 1971
m	23.5	25						
Myotis dasycneme	21.5	27	183	9	.049	3.0	natural	this study
Myotis daubentoni	11.0	32	171	13	.076	2.4	natural	this study
Myotis mystacinus	7.0	26	162	9	.058	2.8	natural	this study
	gr	%	days		days^{-1}	%		

A = Maximum autumnal weight; B = Weight loss during winter, in % of A; C = Duration of hibernation; D = Number of arousals; E = Frequency of arousals; F = Weight loss per arousal + hibernation bout; G = Conditions; H = Calculated from data in

(GRUET & DUFOUR, 1949; BEER & RICHARDS, 1956). This indicates a smaller relative fat storage in bats, perhaps related to their flight requirements.

The overall frequency of arousals as compared with non-bat hibernators is high in *R. ferrumequinum* and low in the *Myotis* species. Accordingly the weight losses calculated per arousal turn out to be generally larger than in non-bat hibernators in *Myotis* and smaller in *Rhinolophus*.

The latter difference is supported by the evidence RANSOME (1968) has given for winter feeding in the Greater Horseshoe Bat, and by the evidence for fasting (section 5.1) in the three *Myotis* species.

In conclusion bats are faced with the problem of winter energy conservation more dramatically than other hibernators. First, by the higher basal metabolism due to their small body size, though in hypothermia this is largely compensated for by a lower body-to-ambient temperature difference (SOUTH & HOUSE, 1967). Secondly, by their naturally limited opportunities of energy storage: no external food reserves, and limited autumn fattening, perhaps in relation with flight restrictions.

Apparently, there are at least two solutions to the problem. Either increased activity, which must, however, be supported by regular food intake. *Rhinolophus ferrumequinum* appears to do so, at least during mild spells in the relatively warm climate of SW. England (RANSOME, 1968). Also, the high winter activity in *Plecotus* cf *auritus* (see DAAN, 1970) is probably sustained by regular feeding on diapausing moths (ROER, 1969). On the other hand, a strong reduction of the frequency of arousal in mid-winter is possible both by endogenous seasonal regulation and behaviourally by habitat selection. This is shown by all three *Myotis* species, and is guessed to be similar in *M. myotis* and *M. emarginatus*. It is clear that bats are going further in this reduction than any other hibernator, the longest period of uninterrupted sleep recorded being over 85 days (MENAKER, 1964).

No ecological reasons for the remnants of activity in mid-winter could be detected. I think that in general activity is of positive value in the very beginning of the hibernation season, when copulation and habitat selection are their strongest requirements. At this time of the year, as well as in spring, activity may incidentally be supported by some feeding on insects flying during mild nights. The proximate hypothesis would be, then, that the physiological processes leading to arousal have become modified on an endogenous seasonal basis to produce high arousal frequencies in autumn and spring, and low frequencies in mid-winter, the complete blocking of these processes being physiologically incompatible with maximizing them in other parts of the year.

PLATE I
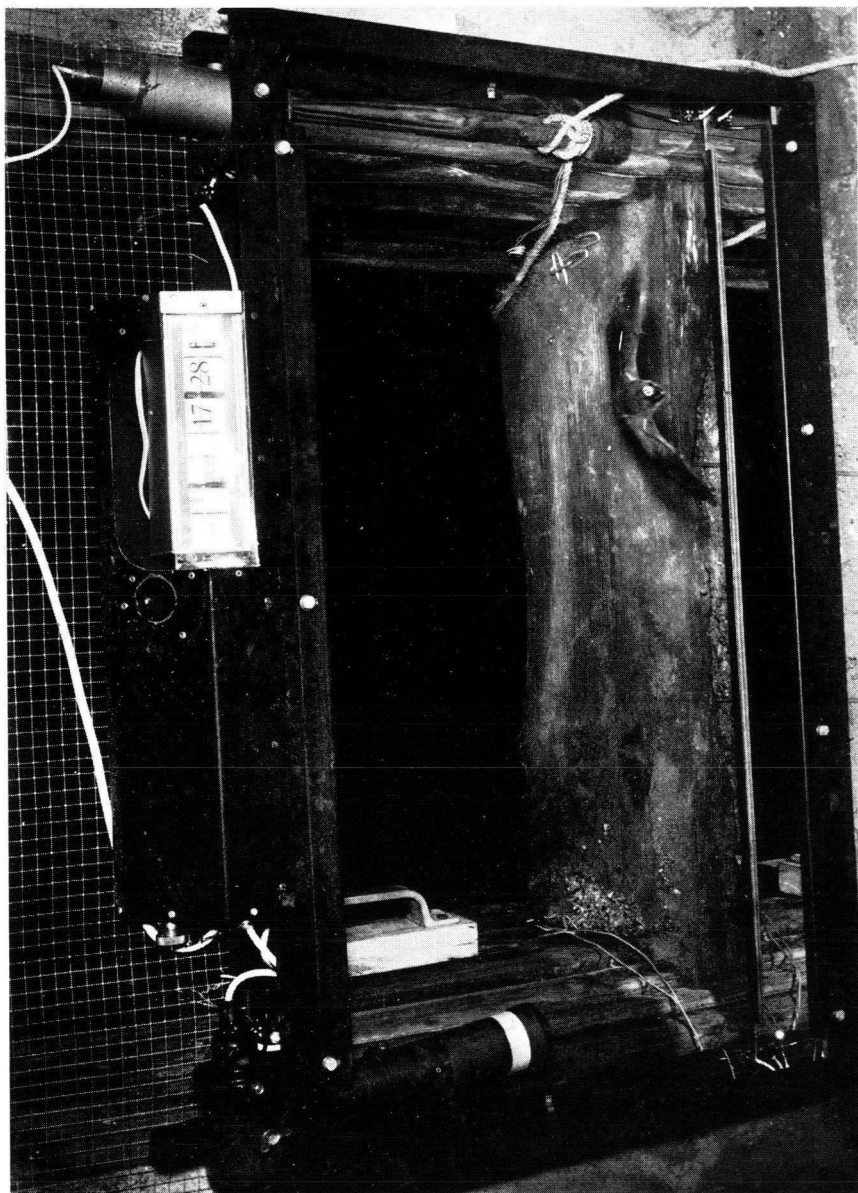
Automatically taken photograph of a tagged bat flying through the photocell-window in the ice-cellar Middenduin. Note lamp and photocell-house in the upper left and right corners of the window, the datum-clock and the lower one of the two mirrors at the bottom of the window.

6. SUMMARY

1. Patterns of winter-activity in three species of bats *(Myotis mysta-cinus, M. daubentoni, M. dasycneme)* were studied in three hibernation quarters in The Netherlands. The methods of investigation involved: individual marking, automatic recording of intracave and extra-cave flights and assessment of the frequency of movements between hibernation sites by short-interval searches.
2. Although differing in details, the species studied show close agreement in most aspects of hibernation behaviour. Major differences probably exist with other species in other climates.
3. Arrivals in the cave takes place gradually from September to December. Most bats leave the cave between the middle of March and the middle of April. There are consistent specific and sexual differences in the timing of hibernation. Females tend to arrive and to leave earlier than the males. Departure from the cave in spring is correlated with the prevailing temperature, while the arrival in autumn is not.
4. Population turnover during the winter is small. No more than an estimated 10% of the bats venture outside the cave upon waking. Recorded extracave migrations took place mainly in autumn and over distances not exceeding 1 km.
5. Microhabitat selection is such that the bats throughout the winter tend to concentrate in the coldest parts of the cave. Low outside temperatures cause cooling of the cave near the entrance during the winter season, and the bats respond by shifting their positions from the rear to the front of the cave, whereas on occasional warm days they tend to move to the rear. In the front part of the cave where stronger air circulation and temperature fluctuations prevail, bats tend to hide in fissures, while in the thermoconstant rear they prefer exposed locations.
6. *Myotis mystacinus* and *M. daubentoni* are solitary hibernating bats. In *M. dasycneme* 10% of the locations were shared simultaneously by two or more individuals. Observed matings were concentrated in autumn.
7. Some bats return to exactly the same locations in consecutive winters. A positive correlation between sequences of locations occupied by (presumably) the same individuals in different years leads to the hypothesis that the selection of locations, which were occupied in former years, is connected to an endogenous annual program clock.
8. The frequency of intracave movements shows large seasonal variation, being relatively high in autumn and spring, and low in mid-winter. The occurrence of the same patterns in the rear part of the

cave under almost constant conditions suggests the endogenous nature of a seasonal variation in arousal frequency.

9. The frequency of movements from one resting site to another was positively correlated with ambient temperature. High outside temperatures (directly or indirectly, by reversed thermocirculation) had a positive effect on the frequency of movements from locations in the front part of the cave.

10. The daily distribution of recorded flights shows that the activity of the hibernating bats is almost exclusively nocturnal in October. With a decrease in activity towards mid-winter, flights become more uniformly distributed over the 24 hrs and their mean shifts significantly to a later part of the night. The increase in activity in spring is accompanied by the return of nocturnalism.

11. During the hibernation extracave flights take place mainly between sunset and sunrise. Occasional outward flights in daytime are usually immediately followed by returns into the hibernaculum.

12. Weight loss of the bats is rapid in the first half of the hibernation season and occurs at a slower rate in the second half. It is concluded that feeding does not occur during the major part of the winter.

13. Energetical implications of winter activity in bats are discussed, and compared with other hibernators. The arousals from hibernation constitute a major proportion of bat energy expenditure during the winter. This expenditure of energy with the possibility of starvation in spring is among the greatest hazards to which temperate zone bats are exposed. The hypothesis is advanced that the energetic balance between gain due to successful feeding activity on mild autumn and spring days and loss due to arousal and movements gives positive survival value to an endogenous seasonal variation of arousal frequency.

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