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**ANALYSIS OF THE CIRCADIAN RHYTHM OF A SNOWSHOE HARE (*LEPUS AMERICANUS*, LAGOMORPHA) FROM TELEMETRY DATA**

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**Abstract:** The pattern of rest and activity of an adult male snowshoe hare was monitored over 26 months to illustrate seasonal changes in the circadian pattern. Data were collected in east-central Minnesota, U. S. A. using an automatic radio tracking system. Average daily percentage activity is used to define onset and end of activity in each 24 hour period. Phase-angle differences of onset, end and midpoint of activity and activity time show a strong similarity in successive years suggesting that telemetry data and our method of determining of onset and end are consistent and suitable for studies of rhythms in free-ranging animals. In general, onset followed sunset much more closely than end followed sunrise or midpoint followed darkness midpoint. Highest values of activity time occurred in winter and lowest values in summer. In terms of biological requirements, the large positive phase-angle difference in summer, with a short dark period, may indicate that the hare had to begin activity several hours before sunset to have sufficient time for feeding, grooming and other activities. During long winter nights the hare had ample time to complete required activities. Use of telemetry on free-ranging animals has the advantage of eliminating effects of cages and disturbances caused by human activities. Furthermore, telemetry allows measurement of all locomotor movements, not just activity measured by running wheels, at food or nest boxes or on perches. In addition, telemetry provides an opportunity to determine how factors such as temperature, precipitation, food, cover and reproduction interact with photoperiod in determining the circadian pattern.

**INTRODUCTION**

Seasonal changes in circadian activity have been studied extensively in caged animals (Aschoff 1969, Daan and Aschoff 1975). Development of radio telemetry techniques has made it possible to investigate the pattern of activity of free-ranging animals living under natural conditions. Examples of such studies include Girelli (1969) on mink (*Mustela vison*), Maxson (1977) on ruffed grouse (*Bonasa umbellus*), Mech et al. (1969) on cottontail rabbits (*Sylvilagus floridanus*) and snowshoe hares (*Lepus americanus*), Rongstad and Tester (1971) on snowshoe hares and Tester (1978) on grey squirrels (*Sciurus carolinensis*).

Comparison of data indicates that activity patterns of free-ranging animals are less precise in terms of the time of onset and end of activity than are patterns of caged animals. As a result, determinations of typical circadian characteristics such as period, phase relationships and  $\alpha : \rho$  ratios (see Aschoff 1965 for definitions) are more difficult from telemetry data.

This paper describes the type of information on activity that can be obtained by telemetry and presents a quantitative technique for determining time of onset and end, key circadian characteristics. This technique is applied to observations of a snowshoe hare over a 26 month interval to illustrate seasonal changes

in the circadian pattern. This animal was selected because it had been monitored continuously for more than 2 years and data on seasonal changes in activity could be compared from year to year.

#### METHODS

Field data were collected at the Cedar Creek Natural History Area in east-central Minnesota (93° 12' W., 45° 24' N.) from March 1966 to April 1968. The hare, an adult male, was captured on 16 March 1966 in a National live trap and was fitted with a radio transmitter in the 53 MHz range. The transmitter was designed as a collar with the broadcasting antenna forming the collar loop. Weight of the transmitter was 35 grams, life was 180 days and range was about 1.6 km. Every six months the hare was recaptured using a drive net and the battery in the radio transmitter was replaced.

Radio signals from the hare monitored by the Cedar Creek automatic radio tracking system (Cochran et al. 1965) and the data were used to determine location and activity. Signals were recorded on microfilm every 45 seconds.

When the hare was motionless the radio signal appeared as a continuous black bar on the microfilm records, whereas when the hare was moving the signal appeared as a broken or interrupted bar. These breaks were caused by modulation of the radio signal due to a change in capacitance of the circuit as the collar changed position on the neck of the mammal. The appearance of the radio signal on the microfilm record provided a means of determining, to the nearest minute, when the hare was active or resting. Specific types of activity, such as running or feeding, could not be differentiated from the radio signal. In our analysis, data were summarized by five minute intervals. The hare was considered "active" if two or more of the signals exhibited the broken pattern. At times, no signal was received due primarily to failure of equipment.

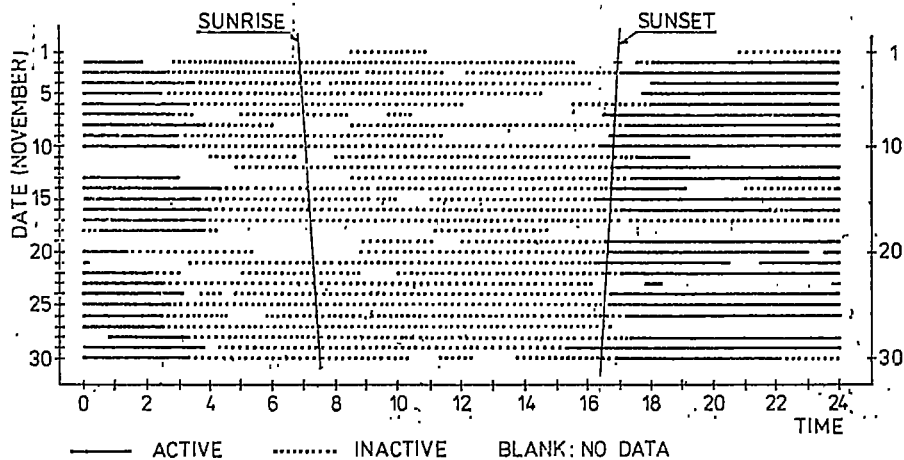


Fig. 1. Daily activity pattern for an adult male snowshoe hare for November 1967 in east-central Minnesota, U. S. A.

Data from the film record were recorded on tabulation sheets and then on computer punch cards for further analysis on a Control Data Cyber 74 system. The analysis program was designed to produce one output showing the daily pattern of rest and activity for an individual animal by months (Fig. 1). These data correspond to the strip chart records of captive animals in running wheels or spring-suspended cages.

The second computer output summarizes data on activity and rest for each month (Fig. 2). These data are presented as a composite 24 hr graph showing the percent of days of the month the hare was active for each 5 minute interval throughout the day.

For every 24 hour monthly composite, we calculated the average daily percentage activity. This is the total minutes active for the month, i. e., the summation of all 5 minute intervals, divided by the total minutes known times 100. This average activity, shown as a horizontal line in Figure 2, is used for defining onset and end of activity. Periods when percent activity is above this average line are defined as activity time, or " $\alpha$ ". Periods below the average line are defined as resting, " $\rho$ ". In the evening, for a night active species, the time when the percent activity rises above the average is defined as onset of activity. The time in the morning when the percent activity falls below the average is defined as end of activity. These onset and end of activity times along with the average sunset and sunrise times for each month are used to calculate  $\Psi$  onset and  $\Psi$  end.

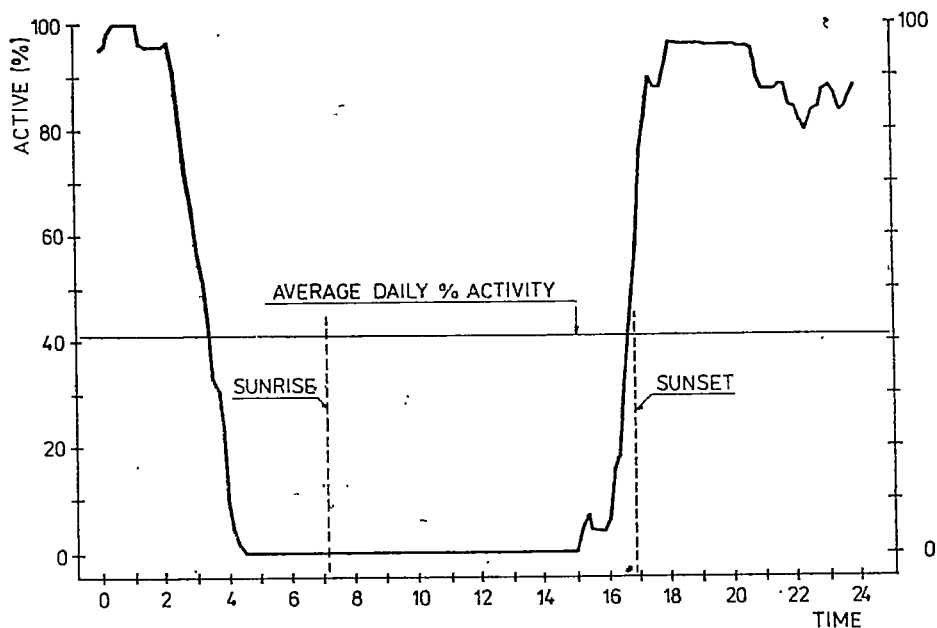


Fig. 2. Activity pattern for an adult male snowshoe hare for November 1967 showing percent of days the hare was active for each 5 minute interval during the 24 hours.

Actual onset and end of activity are probably somewhat different from these values. However, because the definitions are consistent for all months studied, the relative values should be valid in revealing general trends of the circadian pattern.

Weather data were recorded at the U. S. Weather Bureau Station at the Cedar Creek Natural History Area. Sunrise and sunset times were determined from U. S. Naval Observatory Chart No. 1155 for Minneapolis.

## RESULTS

### A. Phase angle

Phase angle,  $\Psi$ , is defined as the time difference between 2 corresponding phase of synchronized oscillations (A s c h o f f 1965). Usually 3 values of  $\Psi$  are determined. In night-active animals the phase-angle difference of onset,  $\Psi_{\text{onset}}$ , is the time between the beginning of activity and sunset. The phase-angle difference of end,  $\Psi_{\text{end}}$ , is the time between cessation of activity and sunrise. The phase-angle difference of midpoint,  $\Psi_{\text{midpoint}}$ , is the time between the midpoint of activity and the midpoint of the night.

Monthly average of times of activity onset, end and midpoint for March 1966 through March 1968 are given in Figure 3. Darkness midpoint is the

calculated midpoint between sunset and sunrise. In comparing the 3 sets of data, it is apparent that onset followed sunset much more closely than end followed sunrise or midpoint followed darkness midpoint. Similar patterns were observed for each year.

Figure 4 illustrates  $\Psi_{\text{onset}}$  as a function of time of year. From October—March, onset of activity occurred near sunset ( $\Psi_{\text{onset}} = 0$ ). The largest values (+2.3) occurred in the summer. This annual pattern, which is consistent for the entire period of study, has been shown to exist for caged night-active animals (D a a n

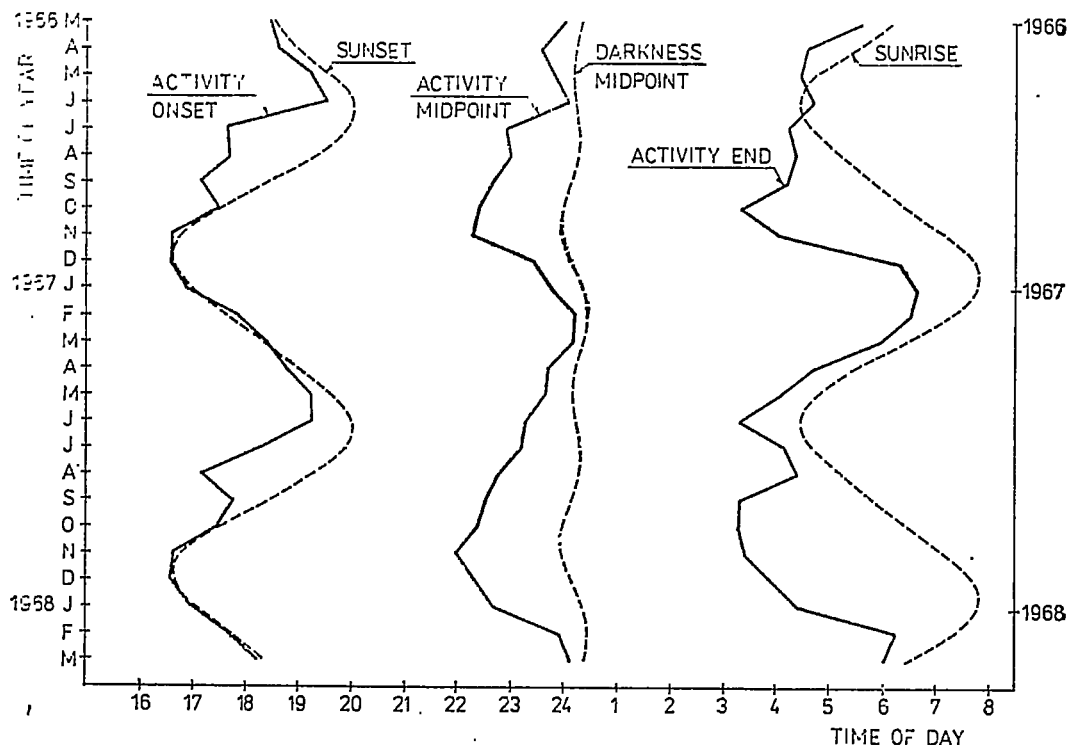


Fig. 3. Comparison of the monthly average of activity onset with sunset, midpoint with darkness midpoint, and end with sunrise for an adult male snowshoe hare from March 1966 through March 1968.

and Aschoff, 1975).  $\Psi_{\text{onset}}$  for golden hamsters (*Mesocricetus auratus*) ranged from approximately  $-1.5$  to  $+1.0$  in southern Germany ( $48^\circ$  N. latitude), and from approximately  $-3.0$  to  $+3.0$  at the Arctic Circle ( $66^\circ$  N. latitude).

As noted previously, onset of activity occurred near sunset for the months of October through March. The positive values of  $\Psi_{\text{onset}}$  occurred from May through September which is the time in which there are leaves on the trees and sunset occurs later in the evening. If onset is related to light intensity, leaves on the trees will make the forest darker earlier compared to the actual sunset. Thus the onset of activity would be earlier than sunset, giving positive values of  $\Psi_{\text{onset}}$ .

$\Psi_{\text{end}}$  as a function of the time of year is shown in Figure 5. Lowest values occurred in the summer (activity end close to sunrise) and the largest values in winter. This pattern has also been described for night-active caged mammals

by Daan and Aschoff (1975).  $\Psi_{end}$  for golden hamsters ranged from approximately 0.0 to +2.5 in southern Germany and from -0.5 to +6.0 at the Arctic Circle.  $\Psi_{end}$  for flying squirrels (*Glaucomys volans*) ranged from approximately +0.5 to +1.3 in southern Germany and from -2.0 to +6.0 at the Arctic Circle. Since the length of darkness is greatest during the winter, the snowshoe hare does not have to be active during the entire night, thus creating large positive values for  $\Psi_{end}$ .

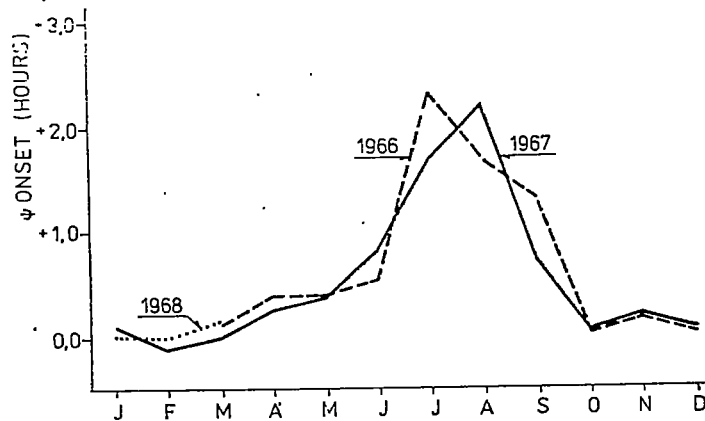


Fig. 4. Phase angle differences of activity onset of a snowshoe hare and sunset as a function of time of year.

Upon further examination of Figure 5, large differences between years can be seen in the values for January, June, and December. Low values of  $\Psi_{end}$  occurred for December 1966 and January 1967. There were some problems in defining the end of activity for these two months because of oscillations of as to the end of activity. For June 1967, the percent active line dropped below the average line in the morning giving uncertainty to the average line and stayed near, but below, the average line for about an

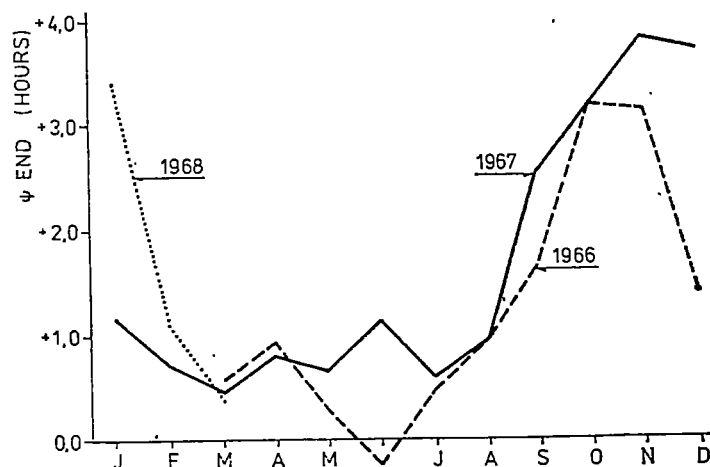


Fig. 5. Phase angle differences of activity end of a snowshoe hare and sunrise as a function of time of year.

hour before dropping to the 0% active value. June 1967 contained a large amount of unknown time (52%) which may have affected these percent active and average active lines.

Aschoff et al. (1970) reported that, for golden hamsters at the Arctic Circle, the end of activity changes its position relative to sunrise more than did onset of activity to sunset (Rule No. 5a; Aschoff and Wever 1962). For the snowshoe hare, maximum  $\Psi_{\text{onset}}$  equals approximately 2.3 and maximum  $\Psi_{\text{end}}$  equals approximately 4.0, thus supporting this rule.

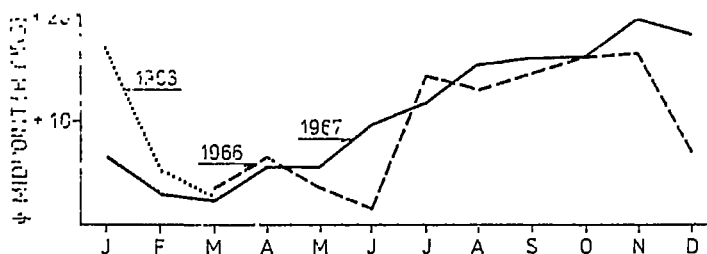


Fig. 6. Phase angle differences of activity midpoint of a snowshoe hare and darkness midpoint as a function of time of year.

Figure 6 illustrates  $\Psi_{\text{midpoint}}$  as a function of time of year. The range of values for  $\Psi_{\text{midpoint}}$  (approximately from 0.0 to +2.0) is less than the range for  $\Psi_{\text{onset}}$  or  $\Psi_{\text{end}}$  which makes it somewhat more difficult to detect seasonal trends.  $\Psi_{\text{midpoint}}$  was always positive and had its lowest values from February — June and its highest values from July — January. Aschoff et al. (1970) reported  $\Psi_{\text{midpoints}}$  always being positive for golden hamsters at the Arctic Circle.  $\Psi_{\text{midpoint}}$  reached its largest positive values in the midwinter and minimal positive values at the equinoxes (not found for the snowshoe hare).  $\Psi_{\text{midpoint}}$  for golden hamsters ranged from approximately 0 to 3.0.  $\Psi_{\text{midpoints}}$  for golden hamsters reported by Daan and Aschoff (1975) ranged from approximately -0.3 to +1.0 in southern Germany and from approximately +0.5 to +2.5 at the Arctic Circle.  $\Psi_{\text{midpoint}}$  for flying squirrels ranged from approximately +0.1 to +0.5 in southern Germany and from approximately -1.0 to +1.5 at the Arctic Circle. As far as discovering general trends for  $\Psi_{\text{midpoint}}$ , Daan and Aschoff (1975) described their findings by stating, "The seasonal changes in activity time result from roughly mirror-like changes in the times of onset and end of activity relative to sunset and sunrise, cancelling each other out. Therefore the midpoint of activity stays relatively stable; remaining minor changes in the midpoint do not produce a general seasonal pattern".

#### B. Activity and rest

Activity time,  $\alpha$ , is defined as the time interval in minutes between onset and end of activity. Rest time is 24 hours minus  $\alpha$ . A strong correlation normally exists between  $\alpha$  and the length of the night, with the exact nature of the relationship being determined by the length and light intensity of the twilight periods.

Activity time as a function of the time of year is shown in Figure 7. Highest values of  $\alpha$  occurred in the winter with the lowest values in the summer. Activity time generally followed the dark-light cycle for the year but was

a sharp increase of  $\alpha$  from June — August and then a decrease from August — October which deviates from the general trend. This phenomenon occurred for both 1966 and 1967 which indicates that some strongly influencing, but unknown, factors are affecting the activity of the snowshoe hare during the time of the year.

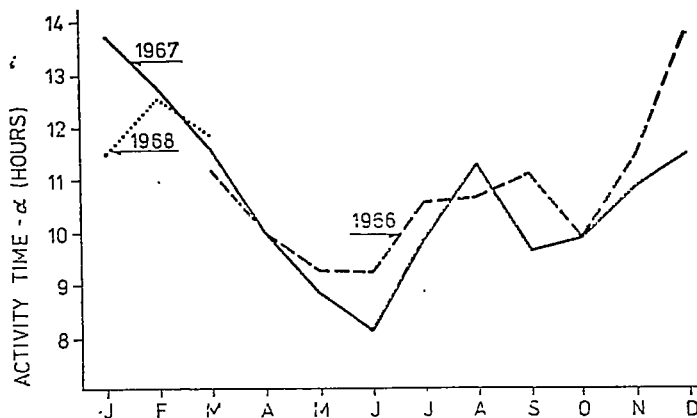


Fig. 7. Activity time ( $\alpha$ ) of a snowshoe hare as a function of time of year.

Figure 8 shows how  $\alpha$ , as a function of darkness, progressed for each month throughout the year. From February — June of each, the points follow quite closely along the theoretical value of  $\alpha/\text{darkness} = 1$ . From July — December the values deviate from this theoretical value producing a more horizontal

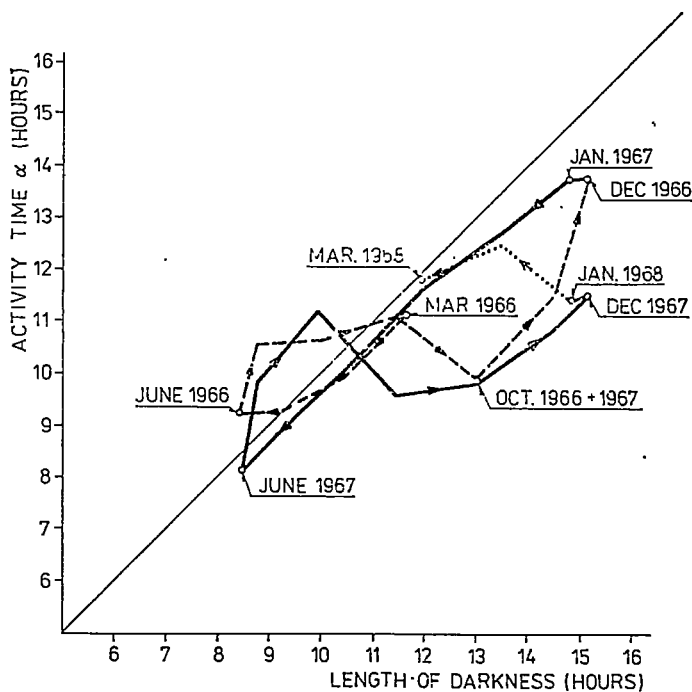


Fig. 8. Activity time ( $\alpha$ ) of a snowshoe hare as a function of length of darkness. Data points are monthly averages.

line. This trend was fairly consistent for the 25 month period studied. In fall,  $\alpha$  was shorter than in spring for a given length of darkness. This phenomenon has also been shown for caged day-active male starlings (*Sturnus vulgaris*) primarily due to changes in the physiological state relating to the reproductive cycle (Gwinner and Turek 1971).

For golden hamsters at the Arctic Circle, Figure 4 of Aschoff et al. (1970) resembles Figure 8 quite closely but does not indicate the data points as separate months which are needed to compare changes in  $\alpha$  between spring and fall for golden hamsters.

Activity data for a woodmouse (*Apodemus flavicollis*) (Erkinaro 1970) resemble Figure 8 quite closely. Spring activity time was also larger than fall activity time, but activity time, as a whole, was less than shown for the snowshoe hare.  $\alpha$  was never greater than length of darkness for the woodmouse.

Using a slightly different approach Daan and Aschoff (1975) compared  $\alpha$  and duration of sunlight. Their curves are also generally similar to Figure 8. However, only for golden hamsters in southern Germany was  $\alpha$  in spring greater than  $\alpha$  in fall. This did not appear to be true for flying squirrels in southern Germany or at the Arctic Circle or for golden hamsters at the Arctic Circle.

#### DISCUSSION

Threshold and niveau are the 2 important determinents of onset and end of activity (Aschoff and Wever 1962). When the periodic function crosses the threshold, activity begins or ends. Calculating the threshold as the average daily percent activity, as we have done for the night active snowshoe hare, has the advantage of reducing the effect of activity during daylight, which occurs mainly in summer. This method appears to be especially appropriate for animals which are not clearly day-active or night active, such as waterfowl (Ball et al. 1975), *Microtus* in spring and fall during the phase change (Erkinaro 1961) and *Odocoileus virginiana* (Kammerneyer, K. E. and R. L. Marchinton 1977). Use of the average daily percent activity as the threshold for such species would permit determination of the phase of activity throughout the year.

All of the calculated circadian characteristics of the snowshoe hare, phase-angle differences of onset, end and midpoint of activity and activity time, show a strong similarity in successive years. This suggests that the telemetry data and our analyses are consistent and suitable for studies of circadian rhythms in free-ranging animals. In addition, the yearly similarities in circadian characteristics suggest that exogenous conditions which might affect rhythms must have been generally the same in each year of the investigation. Pohl and West (1976), on the other hand, found that circadian rhythms of redpolls (*Acanthis flammea*) were different during 2 consecutive winters with similar ambient temperatures. They reported that the differences might be due to cage effects or to the transfer of birds from one locality to another prior to the study.

Use of telemetry on free-ranging animals has the advantage of eliminating effects of cages and disturbances caused by human activities such as feeding and maintenance. Furthermore, telemetry allows measurement of all locomotor movements, not just activity measured by running wheels, at food or nest boxes or on perches.



It is important to realize, however, that animals living in the wild are subject to the entire range of biotic and abiotic environmental forces. Many of these might influence the activity rhythm by coupling with photoperiod, the strongest Zeitgeber (Hoffmann 1969). For example, food supply, breeding behavior and weather factors such as temperature and precipitation would likely have strong effects on the physiology and behavior of an organism.

Most aspects of the circadian rhythm of the snowshoe hare appear to be related to its biological needs, i. e., seasonal changes in rhythms can be interpreted in terms of physiological, ecological and behavioral requirements. However, we are not able to explain the reason for the marked decrease in activity time in September 1966 and October 1967 (Fig. 7). Leaves begin dropping from deciduous trees at this time causing an increase in light intensity and length of photoperiod which may affect activity time. This may be especially important in the dense shrub cover typically used by snowshoe hares. The reproductive season ends in late summer or early fall and may affect activity through changes in hormone levels. Gwinner and Turek (1971) observed differences in activity of birds in spring and fall under the same photoperiod and attributed the lower fall level to a reduction in testes size.

The actual difference in the dates when the decrease occurred each year may be somewhat obscured by using monthly averages. However, weather conditions were different with 1967 being cooler during night, warmer during day and much drier than 1966. Frost occurred earlier in 1967. These weather conditions might affect rhythms directly, but more likely they affected such aspects as leaf fall, reproduction and food supply and these, in turn, may have affected activity time.

Examination of phase-angle differences reveals a similarity in  $\Psi_{\text{onset}}$ ,  $\Psi_{\text{end}}$  and  $\Psi_{\text{midpoint}}$  between the snowshoe hare living at 45° latitude and several species of night-active mammals in southern Germany at 48° latitude (Aschoff 1969, Daan and Aschoff 1975). This shows that determining onset and end of activity using the 50% level produces results comparable to those obtained by other methods.

It is important to note, however, that the actual values of  $\Psi_{\text{onset}}$  and  $\Psi_{\text{end}}$  are several hours longer in Minnesota than in southern Germany. With reference to  $\Psi_{\text{midpoint}}$ , Aschoff (1969) stated that the summer increase in photoperiod should result in a lower value for  $\Psi_{\text{midpoint}}$  in night-active animals. In the first half of the year the snowshoe hare follows this rule, but the expected fall minimum value does not occur.

In terms of biological requirements, the large value of  $\Psi_{\text{onset}}$  in summer, with a short dark period, may indicate that the hare had to begin activity several hours before sunset to have sufficient time for feeding, grooming and other activities. Winter nights are longer, and  $\Psi_{\text{onset}}$  was near 0 and  $\Psi_{\text{end}}$  was 3—4 hours suggesting that the hare had ample time to complete required activities.

In general, activity of the snowshoe hare appears to follow circadian rules. This indicates that radio-telemetry methods could be very helpful in determining activity patterns of other free-ranging animals. An advantage of using this method is elimination of cage effects as described by Lehmann (1976) for *Microtus agrestis*. In addition, telemetry provides an opportunity to determine how factors such as temperature, precipitation, food, cover and reproduction interact with photoperiod in determining the circadian pattern.

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