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Reviewed work(s):
Source: Copeia, Vol. 1999, No. 1 (Feb. 5, 1999), pp. 146-152
Published by: American Society of Ichthyologists and Herpetologists (ASIH)
Stable URL: http://www.jstor.org/stable/1447395
Accessed: 01/10/2012 08:49

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Circannual Rhythms of Appetite and Ecdysis in the Elapid Snake, *Hemachatus haemachatus*, Appear to Be Endogenous

GRAHAM J. ALEXANDER AND ROBERT BROOKS

We demonstrate that appetite and the probability of ecdysis vary seasonally in the temperate African elapid *Hemachatus haemachatus*. The periods of lowest food acceptance, and lowest probability of shedding, coincided with the late autumn and winter months of May through July, and we interpret these patterns as adaptations to avoid the costs of arrested digestion and of ecdysis during the coldest part of the year. The seasonal patterns we document have persisted in our animals without measurable attenuation for seven years to date, despite the snakes being kept at constant temperature (25°C) and photoperiod (12:12 h) for the duration of the study. Because these are the most likely cues for exogenous control of the seasonal patterns observed, we argue that we have provided good evidence that these rhythms are, at least partly, under endogenous control.

Many vertebrate species, including several snakes and lizards, show well-defined seasonal or circannual patterns in growth (Andrews, 1982; Hendriks et al., 1997), behavior (Gregory, 1982; Moore 1986), appetite (Gibbons and Semlitsch, 1987; Mushinsky, 1987), reproduction (Licht, 1973; Ferguson and Snell, 1986; Seigel and Ford, 1987), and activity (Gibbons and Semlitsch, 1987; Ford et al., 1991). Environmental factors such as photoperiod and variation in temperature, rainfall, and food supply are often correlated with these cycles (Gregory, 1982; Moore 1986). However, a correlation does not necessarily mean that there is a causal link between these extrinsic factors and such rhythmic phenomena.

In the 1950s and 1960s, a vigorous debate was waged between those proposing that control of persistent biological rhythms is only via environmental cues and those arguing for a truly endogenous "biological clock" that may or may not be entrained to environmental periodicities (Edmunds, 1976; Gwinner 1981). The latter case seems to have been widely accepted, and much research effort has been focused on the cellular and biochemical mechanisms that underpin "the clock." However, despite reports of circannual patterns as diverse as oxygen consumption rates in bean-sprouts and food uptake in woodchucks (Brown, 1976), far more research effort has been focused on circadian rhythmicity (1988 for overviews, see Palmer, 1976). Although true circannual clocks might underlie circannual biological rhythms (Pengelly and Asmundson, 1974), it is also possible that these rhythms are the result of an interaction between circadian clocks (which are relatively well understood) and day length or other extrinsic factors (Pittendrigh, 1981; Palmer, 1988; but see Gwinner, 1981). It follows from this argument that circannual rhythmicity would be expected to attenuate in the absence of the extrinsic cues. To test this prediction, we examined seasonal patterns of appetite and ecdysis in snakes kept under constant photoperiod and temperature for seven years.

The endogenous nature of any rhythm is notoriously difficult to verify because demonstrating the persistence of the rhythm after controlling a few potential extrinsic cues does not rule out the possibility of other potential cues, no matter how obscure. Conversely, endogenous control is an eminently testable hypothesis, with each extrinsic factor that is controlled being a critical test. If the pattern persists, the hypothesis has not been falsified. In this study, we kept snakes in a laboratory facility under constant temperature (25°C) and photoperiod conditions (12:12 h) for seven years, to test whether these factors are the basis for the rhythmicity of appetite and ecdysis.

The Rinkhals, *Hemachatus haemachatus*, is a cobralike elapid that is restricted to the cool, southern and eastern parts of southern Africa and appears to be a cold-adapted species (Alexander, 1996). Adaptation to a temperate environment should result in strong seasonal rhythmicity in appetite. Environmentally imposed low body temperature constrains digestion rates and could select for endogenously controlled rhythms that ensure a reduced appetite, even during warm bouts in winter. It may be too risky to eat in winter because of the nature of the weather, which may rapidly and unpredictably become unfavorable for digestion, causing undigested food to putrefy in the intestine. Also, in natural habitats, the availability of...
food, the digestive capabilities of the animal, or appetite may limit food consumption. In the laboratory, one can remove the confounding influence of food availability and temperature-related changes in digestive capability.

There are good reasons to expect rates of ecdysis to be seasonally modulated, especially in temperate-zone species (Gwinner, 1986), such as *H. haemachatus*. Not only is the frequency of ecdysis related to growth rate in snakes, but high temperatures are important for the physiological processes involved in epidermal cell growth (Gibson et al., 1989). As far as we are aware, ours is the first study to report on the seasonality of ecdysis under controlled laboratory conditions.

We present evidence that, in *H. haemachatus*, circannual rhythms in appetite and ecdysis persist unattenuated for at least seven years after the two most likely extrinsic cues for these rhythms have been removed. This observation provides two critical tests of the hypothesis that these traits are under endogenous control. Moreover, we show that rhythmic variation in these two traits is statistically verifiable.

**Materials and Methods**

Twenty-eight *H. haemachatus* (15 males, 13 females) were collected from their natural habitat close to Johannesburg, South Africa (26°S 28°E, altitude > 1600 m). The mean mass of individuals at the start of the measurement period was 400 g (SD = 288 g; range 6-1168 g). Individuals were housed in wooden cages in a windowless, temperature-controlled room. The ambient temperature was kept at a constant 25°C, and fluorescent lighting on an automatic timer provided a 12:12 h, light:dark cycle, with local dawn at about 0600 throughout the duration of our investigation.

Individuals were fed dead mice, with the exception of four individuals that refused to eat mice and were fed frogs (*Xenopus laevis*; approximately 10% and 20% of their own body mass at each feeding, of mice or frogs, respectively). Prey were placed headfirst into the entrance of each individual’s retreat so that the individual could consume the meal without exposing itself, thereby reducing predator-risk effects on food acceptance. The numbers of prey offered, accepted, and refused were recorded. The prey type and the prey mass offered to each individual remained constant year-round. The number of food items presented to each snake at each feeding did not vary markedly (numbers of prey increased to make up the required proportion of body mass, as snakes grew) and was kept constant during the last 18 months of measurement.

Food acceptance was calculated as the proportion of offered prey that was accepted by each individual at each feeding. These proportions were averaged for all individuals to produce a food acceptance measure for the test group of *H. haemachatus* for each month. Because food acceptance measures were in proportions, we arcsine transformed these data (Zar, 1996), tested for normality (Lilliefors test, $P > n$s), and homogeneity of variance (Bartlett’s test, $X^2 = 11.1, n$s). Twice in 1991 and once in 1993, snakes could not be fed within one week of the midmonth scheduled feeding time (due to logistical difficulties); thus, data from these years could not be included in the repeated measures ANOVA that we used to test for circannual trends in appetite. One-way ANOVA using the data from these years yields very similar results to the repeated measured ANOVA.

All ecdysis events over a seven-year period were recorded. Ecdysis rates were calculated by dividing the number of ecdysis events by the number of individuals for each month. These measures were arcsine transformed, tested for normality (Lilliefors test, $P > n$s), and homogeneity of variance (Bartlett’s test, $X^2 = 20.7, P = 0.037$). Even though these data showed some degree of heteroscedasticity, we used a repeated measures one-way ANOVA to examine monthly variation in ecdysis rate over a seven-year period (April 1990 to March 1997). We believe that we are justified in using ANOVA in this analysis because Zar (1996) notes that analysis of variance is robust, operating even with considerable heterogeneity of variances when all $n$s are equal or nearly equal, as was the case in our analysis. For both food acceptance and ecdysis rates, Tukey pairwise comparisons were used to establish which months differed significantly from one another in food acceptance or rates of ecdysis. In the case of food acceptance, Tukey posthoc tests were performed on the results of the one-way ANOVA because this incorporated data from more years.

Regression analysis was used to test the relationship between measures for the last 12 months of measurement, and the monthly means of the previous five years (for food acceptance) and six years (for ecdysis rates). These regressions test for the attenuation of rhythm over the measurement period (an attenuating rhythm would result in a regression slope of less than 1, whereas an intensified pattern would result in a higher slope value than 1).

We tested the relationship between body mass
Fig. 1. Monthly mean (and standard error) food acceptance by captive individuals. Data are from January 1991 and March 1997.

Fig. 2. Monthly mean (and standard error) proportion of test-group shedding. Data are from April 1990 to March 1997.

and the rate of ecdysis independent of season, to assess whether any of the residual variation in ecdysis rates could be explained by body mass. To do this, we regressed the interval between subsequent sheds against body mass for each individual, after the effect of season had been factored out. We did this by using the following formula, which provides a measure of shedding frequency in units of ‘per year’:

\[
12 \times \sum_{t=1}^{12} \left( P_{\text{shed},t} \times \text{days} \right) / P_{\text{shed[year]}},
\]

where \( z \) is the transformed number of days between subsequent sheds; \( P_{\text{shed},t} \) is the probability of any given individual shedding in month \( t \); days, is the untransformed number of days in month \( t \) between sheds; and \( P_{\text{shed[year]}} \) is the average frequency of shedding per year measured for the test group as a whole.

**RESULTS**

**Appetite.**—Repeated-measures ANOVA revealed that there were significant differences in measures of food acceptance among months \((F_{11,1} = 665.3, P < 0.001, \text{Fig. 1})\) but not among years \((F_{3,35} = 0.672, P = 0.575)\). Because this analysis excluded the data from two early years, we performed a one-way ANOVA that yielded similar results (between months: \( F_{11,58} = 7.3, P < 0.001 \)). Differences in food acceptance therefore were consistent throughout the six years in which data were collected. Tukey pairwise comparison revealed seven (of 66) month-on-month comparisons that were significantly different. The low acceptance rates in the midwinter months were significantly different from acceptance rates during the summer months.

Regression analysis of food acceptance measures from the last 12 months against the first five years \((R^2 = 0.703, n = 12, P = 0.001)\) showed a coefficient of 1.396 (SE = 0.287), the 95% confidence limits of which comfortably include a line of unity slope. This indicates that the rhythmic pattern in food acceptance persisted and that our snakes became neither more nor less likely to conform to the pattern during the seven-year measurement period.

**Ecdysis.**—Individuals shed a mean of 2.1 times per year (SD = 0.2; no significant differences among years, \( F_{6,66} = 0.530, P = 0.784 \)). We found significant differences in rates of ecdysis between months \((F_{11,1} = 44.922, P < 0.001, \text{Fig. 2})\), indicating that differences in ecdysis rates were consistent throughout the seven years in which data were collected. Tukey pairwise comparison revealed 11 of a possible 66 month-on-month comparisons that were significantly different. Rates of ecdysis peaked in March and were significantly higher during this month than from April to July, which coincides with the Austral winter. Ecdysis increased significantly during August to December, which is spring and summertime in southern Africa, and then declined during January and February, before peaking again in March. The regression of monthly ecdysis rates for the last year against mean rates for the first six years of the study \((R^2 = 0.588, n = 12, P = 0.004)\) yielded a slope \((1.128, \text{SE} = 0.298)\), which is not significantly different from 1. Like the pattern in food-acceptance measures, individuals became neither more nor less likely to follow the pattern in shedding over the period of our study.

Regression analysis did not reveal a significant relationship between ecdysis rate and body mass in our test group of *H. haemachatus* \((R^2 = 0.08; n = 28; \text{ns})\). The lack of a significant relationship indicates that body mass, per se, and thus probably age, had little influence on shed-
discussed frequencies in *H. haemachatus* under the lab conditions of our study.

**DISCUSSION**

*Hemachatus haemachatus* exhibited strong seasonal trends of appetite and ecdysis throughout the seven-year period of our study, despite being kept under a constant photoperiod and thermal regime. Thus, although our snakes were unable to perceive seasonal change by two of the most obvious seasonal environmental variables, they maintained rhythmicity in two important physiological attributes, without any significant change over seven years. The maintenance of these seasonal trends is strong evidence that appetite and ecdysis are regulated by endogenously controlled rhythms, in that the endogenous control hypothesis has survived the two most obvious critical tests that could be executed. Other environmental variables (e.g., humidity or barometric pressure) were not controlled explicitly in our study. However, many of these variables, such as humidity, were modified by the heating and cooling systems in the environmental control unit used to control the temperature of the room housing the snakes. Modification of an environmental attribute in this way is likely to render it unreliable, at least, as a rhythmic seasonal cue.

Although our results do not rule out the possibility that environmental variables other than temperature and photoperiod influence the observed trends, we agree with Alcock (1989) that temperature and photoperiod are the most likely candidates for exogenous control of seasonal rhythms. Such rhythms are likely to be adaptations for optimizing the well-documented relationships between temperature and growth, digestion (Alexander, 1996) and other aspects of reptile physiology. Two studies in lizards have shown that temperature is responsible for the induction of seasonal reproductive behavior patterns (Licht, 1973; Moore, 1986). Photoperiod is perhaps a less ambiguous cue (Gwinner, 1986), because it provides an “unfaltering and noise-free indication of past or coming season” (Rismiller and Heldmaier, 1991) and is not influenced by short-term climatic variance.

Many biological rhythms have an endogenous component that is longer than the rhythm cycle and are entrained by external stimuli such as pacemaker potentials (Eckert et al., 1988) and changes in lighting conditions (Engelmann, 1981; Pittendrigh, 1981), respectively. When the entraining stimulus is absent, the rhythm moves out of synchrony because of the slightly longer period of the endogenous component (Cowgell and Underwood, 1979; Gwinner 1981). We have not addressed the role of environmental variables in modifying (i.e., entraining as zeitgebers) the rhythms we observed (Edmunds, 1976), a situation that would require manipulative experimentation. If the seasonal cycles we report are entrained to external stimuli however, they would be expected to have shifted out of synchrony, which would have resulted in apparent attenuation of our reported pattern.

**Appetite.**—Generally, snakes in the wild tend to eat less in the winter than during the summer months (for review, see Gibbons and Semlitsch, 1987; Mushinsky, 1987). This is thought to be related to variation in ambient temperature, prey availability, activity levels, habitat, and stage of ecdysis cycle (Andrén, 1982; Shine and Lambeck, 1985; Reinert, 1993). However, because we offered our *H. haemachatus* constant amounts of food throughout the year, changes in consumption cannot be ascribed to prey availability. Furthermore, because prey were placed into the entrance of each individual’s retreat, rates of prey encounter are unlikely to be dependent on activity level as described by Madsen and Osterkamp (1982) for *Lycodonomorphus bicolor*. Thus, our results challenge the conventional wisdom that snake appetite is (proximally) modulated by ambient temperature and prey availability and encounter rates. Rather, it supports the position that decreases in appetite are a consequence of a real tendency not to eat as much in the winter months.

In reality, seasonal trends in appetite may be even more marked than is evident in our study, because of some characteristics of our experimental design, as well as some natural correlates of appetite in free-ranging snakes. We restricted food intake by imposing an artificial maximum, because captive individuals of *H. haemachatus* soon become overweight if fed ad libitum (Alexander, 1996). This restriction limited our snakes’ food intake, especially in summer when they were eating most of what was offered. Also, appetite is usually reduced during the opaque stage leading up to ecdysis (Broadley, 1983; Reinert, 1993). Because our *H. haemachatus* shed more frequently in summer, this effect is likely to dampen peaks in appetite during the spring and summer and lessen the difference in food consumption between seasons. Refusal of food may also result from sickness, which is not necessarily seasonal. However, sickness was very rare among individuals in our study (with no deaths in the last two years; seven deaths in seven years) and is unlikely to have had any major influence on the seasonal appe-
tite trends we report. Last, although various studies have shown that snakes rely partially on visual cues to locate prey (Czaplicki and Porter, 1974; Drummond, 1983; Teather, 1991), Broadley (1983) notes that H. haemachatus has relatively poor vision and is able to clearly distinguish only moving objects. Because prey were killed before being fed to our snakes, it is likely that some prey were not eaten because they were stationary (dead) and were thus not discovered by the snakes. Thus even at times of high food acceptance, prey items could be missed.

**Ecdysis rates.**—Rates of ecdysis peaked in March and were significantly and consistently higher during this month than in April through July. High rates of ecdysis in March may be the result of a "prewintertime shed." Because high temperatures are important for the physiological processes involved in epidermal cell growth (Gibson et al., 1989), it is advantageous for snakes to shed while warm temperatures permit the selection of higher body temperatures. The vision of snakes is impaired during the opaque stage (Reinert, 1993), resulting in reduced activity, apparently as a result of increased vulnerability to predation (Gibson et al., 1989). Thus, snakes tend to spend a greater portion of time sequestered in retreats during the opaque period and may have too few thermoregulatory opportunities in winter to attain the body temperatures required for the growth of new cells.

Our prediction that ecdysis would be highest in summer and lowest in winter because of the positive relationship between growth and ecdysis was supported by our results. However, the observation that ecdysis peaked in March, and to a lesser extent in August through October, as opposed to the midsummer months of November through February was somewhat perplexing. This result could be explained if H. haemachatus is adapted to avoid shedding in the winter months, because the higher temperatures of summer are likely to shorten the shedding process (Tu and Hutchison, 1995). Some snake species shed more frequently during the summer, at higher temperatures or after increases in temperature (Cliburn, 1976; Semlitsch, 1979; Maderson, 1984). However, it is difficult to ascertain whether higher shedding frequencies recorded during summer are purely the result of higher temperatures or also include other causal components (Peterson et al., 1993). Individuals avoiding winter molts would shed before winter, even if they had not quite undergone enough growth to normally warrant shedding, which would explain the peak in March. Likewise, they would “hold out” for the end of winter rather than shedding during winter, explaining the peak in the spring months. We predict that, if this is the case, skins shed in March will be significantly thinner and thus less heavy and skins shed in the spring will be significantly thicker and thus heavier than those shed at other times of the year.

We did not find a significant relationship between body mass and measures of shedding frequency for individuals. The lack of significant covariation between these two variables goes against the argument that small, young snakes grow faster and shed more frequently than larger, older individuals (Smith, 1976). However, Alexander (1996) has shown that growth rates of H. haemachatus are independent of body size or age, once the effect of food intake has been factored out. Rather then, it is a case of small, young snakes eating more, more often (if given the opportunity), which results in increased rates of growth, which in turn results in increased ecdysis rates. Food intake for small and large snakes was similar and controlled in our study; thus, the relationship between size and food intake had no opportunity to affect our measured rate of ecdysis.

**ACKNOWLEDGMENTS**

We thank M. Sledge and C. Marshall for commenting on a draft of this manuscript. The Animal Ethics Screening Committee of the University of the Witwatersrand cleared the procedures (clearance numbers 90/173/1, 92/103/3, 94/56/3, and 96/132/3). We thank the Transvaal Provincial Administration for granting permits for capture, transport, and maintenance of H. haemachatus in captivity. The study was funded by the University of the Witwatersrand and the Foundation for Research Development.

**LITERATURE CITED**


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