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DIURNAL AND SEASONAL LOCOMOTORY ACTIVITY IN THE GILA MONSTER, HELODERMA SUSPECTUM COPE

by

Peter James Lardner

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF BIOLOGICAL SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN ZOOLOGY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Peter James Lardner entitled Diurnal and Seasonal Locomotory Activity in the Gila Monster, Heloderma suspectum Cope be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

CH Lowe	May 14, 1969
Dissertation Director	Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

Wm J McConnell	14 May 1969
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[illegible]	15 May 69

*This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.

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SIGNED: Peter J. Lawler

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ABSTRACT

Field and laboratory locomotory activity of the Gila Monster, *Heloderma suspectum suspectum*, was studied using collection and observation records from the vicinity of Tucson, Pima County, Arizona, and by analysis of wheel-turning activity in the laboratory. Data for six years, 1959-1964, were utilized.

Field Locomotory Activity

It was shown that *Heloderma suspectum* has a seasonal activity period that extends from March to November ($\bar{x}$ = 228 days). During this period there are two obvious peaks in activity. The first peak occurs in April-May and the second in August.

Field locomotory activity is subject to modification by environmental factors such as temperature, precipitation, and light. Increasing daily thermal maxima tend to force natural activity into a crepuscular orientation. Very few *Heloderma* have been observed to be active before dawn civil twilight or following dusk civil twilight. It would appear that civil twilight is as important for *Heloderma* as it has been shown to be for other vertebrate species, as in birds. The effect of thermal inhibition is somewhat lessened following the onset of the summer rains. At this time, *Heloderma* may be found active during or following rain storms.

Laboratory Locomotory Activity

Activity orientation and natural period relationships were studied in the laboratory using large running wheels. The length of the natural period was found to vary between 22.5 and 25.3 hours. There is no apparent difference in the length of the natural period due to age or sex, but an indication of a seasonal difference does exist. In general, the seasonal changes in natural period length and activity orientation can be summarized in the following manner.

1. Emergence from hibernation
   a. The first days after emergence in February or March are often characterized by arhythmicity, followed by activity which exhibits a natural period length of greater than 24-hours.
2. Activity during spring
   a. The length of the natural period is approaching or less than 24-hours.
   b. Activity orientation becomes predominantly crepuscular in April-May.
3. Activity during summer
   a. The length of the natural period is less than or equal to 24-hours.
   b. Activity orientation is predominantly crepuscular.
4. Activity during fall
   a. The length of the natural period is $\approx$ 24-hours.

b. Activity orientation tends from crepuscularity toward diurnality.

5. Entrance into winter estivation

a. The length of the natural period is $\approx$ 24-hours.

b. Activity is completely diurnal.

Locomotory activity in the laboratory can be modified by several factors. Among these, temperature, light, and food deprivation were studied. Modifications are reflected in the phase relationship of the natural period and in the duration of activity.

The relationship of the electroencephalogram to locomotory activity was studied to determine if change in EEG amplitude might be a more sensitive measure of timekeeping ability.

INTRODUCTION

Man has long recognized the existence of rhythmicity in the overt activity and the more covert physiologic processes of plants and animals. This rhythmic activity is assumed to be under the control of a biological clock which provides information of a temporal nature to the organism and thus permits the plant or animal to orient certain activities within the span of a solar day and to repeat those activities at approximately the same time on succeeding days. Since these rhythms have a length of about one day (circa diem) they have been variously termed diurnal rhythms, 24-hour rhythms, or, more recently, circadian rhythms (Halberg, 1959).

Other rhythmic activities with much shorter periods (mitotic division) or longer ones (annual reproductive cycles) also receive time information from a biological clock. Whether these time-sensing mechanisms are similar or identical to the clock which controls circadian activity is not certain, but they are obviously very closely related. The site and the structure of the biological clock are unknown, but it is most commonly assumed to be a subcellular, temperature-compensated, biochemical or biophysical oscillating system that is under rather strict genetic control (see Pittendrigh, 1960; Aschoff, 1965).

An important question, still unanswered, is whether the clock consists of an endogenous, self-sustaining oscillation (Pittendrigh;

1954, 1960) or an exogenous, phase labile oscillator which receives temporal cues from environmental sources (Brown; 1958, 1960). Here it is assumed that the clock in reptiles and, most likely, in all ectothermal animals, is endogenous or self-sustaining, but that the overt responses mediated by the clock (e.g., locomotion) are modified by behavioral and physiologic homeostatic mechanisms which vary with species. Bartholomew (1959) states, for instance, that the effective day length in reptiles may be determined more by thermoperiod than by photoperiod. That thermoperiod can modify natural activity has been well demonstrated in various studies concerned with behavioral thermoregulation in reptiles (see, for example, Saint-Girons and Saint-Girons, 1956; Heckrotte, 1960; Heath, 1965).

The measure of circadian rhythmicity most commonly utilized in animal and plant studies is the natural period or the free-running period (Aschoff, 1960). This is simply a measure of the time from one point in a cycle to the same point in the next cycle, or the period of the rhythm. For locomotory activity the period of the rhythm from the time of initiation of activity on Day 1 to the time of initiation on Day 2 is approximately 24-hours. Within a population this value will tend to vary, but a mean period length of about 24-hours is usually observed (Lowe et al., 1967).

Assume, for example, the following two values for the length of the natural period: (1) 23 hours, 40 minutes and (2) 24 hours, 20 minutes. In the former, the initiation of activity is advanced 20 minutes on each succeeding day; in the latter, the initiation of activity is retarded 20 minutes on each succeeding day. An organism with a natural period of 24 hours, 00 minutes would initiate activity at the same time on succeeding days. By plotting the time of initiation on time (as days) it is then possible to calculate by linear regression a value for the slope of the line which is equal to the deviation in minutes from 24-hours.

In mammals such as *Peromyscus* (Kavanau, 1963) and *Dipodomys* (Justice, 1959; Lowe *et al*., 1967) the correlation of the time of initiation of activity on time (as days) is quite strong. In reptiles, on the other hand, this correlation is not so strong and is often very weak (Hoffmann, 1957b; Lowe *et al*., 1967). This phenomenon is the direct result of the labile homeostatic mechanisms exhibited by the ectothermal animals and the response of these animals to changes, often subtle, in macro- and microenvironmental factors of several kinds and intensities.

An essential criterion of circadian rhythms is that the natural period relationships of the function in question be maintained under conditions in which photoperiod, thermoperiod, or some other periodic environmental variable is varied or eliminated. Light is the variable most often manipulated and, as a result, organisms are exposed to artificial photoperiods which may differ significantly from environmental photoperiod. For example, an animal collected from a locality at which the environmental photoperiod is 14 hours of sunlight and 10 hours of darkness (L:D, 14:10) may be placed into an artificial photoperiod of 8 hours of light and 16 hours of dark

(L:D, 8:16). The animal might also be placed into constant light (L:L) or into constant dark (D:D). Different light regimes may then alter the phase relationships of the rhythm (*i.e.*, its orientation within a 24 hour period) or, to some extent, the length of the natural period, but the essential nature of rhythmicity is not lost.

The problem of an apparently labile time-keeping mechanism in reptiles is an interesting one, particularly in the light of statements concerning the ubiquitousness of the clock mechanism in the plant and animal kingdoms and the reliability of the time sense (Pittendrigh, 1960) and the temperature-independence of the biological clock and the resultant rhythm (Bruce and Pittendrigh, 1956; Hastings and Sweeney, 1957; Sweeney and Hastings, 1960). How then does a reptile, or any other ectothermal animal, remain oriented within the limits of a solar day?

Studies dealing with circadian rhythmicity and analyses of seasonal locomotory activity in reptiles are not abundant in the literature and many are anecdotal or non-quantitative in nature. The more critical of these studies are the following: Barden, 1942 (*Cnemidophorus*); Bartholomew, 1959 (review); Fischer, 1960 (*Lacerta*); Fox and Dessauer, 1958 (*Anolis*); Heath, 1962 and 1965 (*Phrynosoma*); Heckrotte, 1960 (*Thamnophis*); Hoffmann, 1955, 1957a, 1957b, 1959, 1960, and 1963 (*Lacerta*); Klauber, 1956 (*Crotalus*); Marx and Kayser, 1949 (*Lacerta*); Park, 1938 and Park, Barden and Williams, 1940 (*Geomyda*, *Thecadactylus*, *Sphaerodactylus*, *Anolis*, *Basiliscus*); Rahn and Rosendale, 1941 (*Anolis*); Saint-Girons and Saint-Girons, 1956.

The study reported herein is an attempt to analyze the seasonal and circadian locomotory activity of the Gila Monster, Heloderma suspectum suspectum as a population within an eco-physiological framework. Pertinent literature on Heloderma is not extensive. Because Heloderma is the only known venomous lizard, much of the literature is of a popular and sometimes sensational nature. This has led, unfortunately, to many erroneous concepts and general misconceptions regarding this rather pleasant and interesting lizard. The literature to 1956 has been reviewed by Bogert and Del Campo (1956), and the literature following that monograph to date has dealt primarily with aspects of the bite and of the venom.

MATERIALS AND METHODS

Specimens of the subspecies _Heloderma suspectum suspectum_ Cope were collected from the Santa Cruz Valley and associated bajadas (Santa Catalina, Tucson, Rincon, Tanque Verde, and Santa Rita Mountains) in the vicinity of Tucson, Pima County, Arizona. Individuals of this population are most commonly collected from rocky bajadas supporting a saguaro (_Cereus_)-paloverde (_Cercidium_) association. Less frequently, specimens were collected from desert-grassland and from the more xeric creosotebush (_Larrea_)--bur-sage (_Franseria_) associations.

The collection of _Heloderma_ has been aided by residents of the study area who have notified the Department of Zoology or some other organization when a Gila Monster has been observed or collected. Because of the potential danger of the bite to humans (particularly to children) and to domestic animals; the lizards were quite often removed from the collection locality to the laboratory. Some animals were marked and released in open desert areas near the site of collection.

Field Locomotory Activity

Records have been maintained by C. H. Lowe for all specimens of _Heloderma_ brought to the University laboratory since 1950. Records for the years 1959-1964 are used here. Included in the record for each individual is the locality of collection, the time of collection, the date of collection, estimated age, length and weight measurements, and sex. Many of these data were recorded on Specimen Record cards which remained with the animal at all times while in the laboratory, and data for all animals were also recorded in a permanent Heloderma catalog. These records have provided much of the necessary data for the analysis of field locomotory activity.

During the six year period reported, 277 Heloderma were observed and/or collected. Fourteen individuals lacked sufficient data to definitely assign them to the study area. Of the remaining 263 individuals, 56 were accompanied by insufficient collection data and were not included in all aspects of the analysis. The most common deficiency was an accurate time of collection on first observation.

Climatic data has been obtained from Local Climatological Data and Local Climatological Data, Supplement issued for Tucson International Airport (Tucson Municipal Airport until March, 1963). This station is at an elevation of 2584 feet and is situated at the approximate geographic center of the study area.

Indicators of environmental photoperiod, such as values for the time of sunrise and sunset, were obtained from the Tables of Sunrise, Sunset, and Twilight and corrected, when necessary, to 32.2° Lat. N and 110° Long. W.

In order to facilitate analysis, all months of the year have been divided into three periods. The first and second periods

(=tri-monthly intervals) are always 10 days in length. The third interval may be 8 or 9 days in length (February), or 10 (April, June, September, November), or 11 (January, March, May, July, August, October, December) days in length. Thus there are 36 tri-monthly intervals for any one or combination of years; these are coded 1, 2, 3..., 36 in all figures and tables (see, for example, Figure 1).

Values for field locomotory activity were assigned to the appropriate tri-monthly intervals. In addition, for each interval, frequency distributions were calculated for 1) daily thermal minima, 2) daily thermal maxima, and 3) precipitation.

Certain of the field activity parameters were analyzed by regression methods. Appropriate tests of significance were made when necessary.

Laboratory Locomotory Activity

Heloderma which were to be used for laboratory analysis of rhythmicity were typically introduced to the experimental situation no longer than 24 hours following the time of their original collection in the field. Between collection and experimentation the animals were fed one or two chicken eggs, which they usually ate, and allowed to drink water *ad libidum*.

Early experience demonstrated that an accurate activity record for *Heloderma* could not be obtained by use of a stabiliometer, as the activity of the animal often becomes oriented unidirectionally, resulting in the lack of a discernible record. It was found, however, that running wheels would permit reliable recording of locomotory activity rhythms.

Galvanized steel running wheels with a radius of 19 cm. and a width of 9 cm. were utilized in this study. These wheels are identical to those described and illustrated in Sage et al. (1962). The animals were maintained individually within a wheel for the course of each experiment. Rotation of the wheel activated either a microswitch or a magnetic proximity switch which momentarily completed a circuit with the recorder.

An Esterline-Angus event recorder equipped with 20 channels was used to record the wheel turning activity of each animal. Each rotation of the wheel caused a 0.25 inch deflection of a stylus on a continuously moving (0.75 inch/hour) chart. At the end of a 24 hour period the chart paper was divided into its 20 component records and the resultant 18 inch strips were affixed with an adequate adhesive to a sheet of poster board. In this way, a composite of the daily activity record of an individual could be compiled and easily analyzed (see, for example, Figure 8).

To house the running wheel and to provide light, temperature, and relative humidity control, three enclosures were used interchangeably throughout the course of the study. A single exception occurred when locomotory activity was recorded under environmental conditions.

The first of these enclosures, a series of constant temperature cabinets (Precision Constant Temperature Incubators), had an effective thermal range of 5^{o} to $50^{o}C$ with temperature control to $\pm 0.3^{o}C$. Relative humidity within the cabinets normally varied between 40% and 60% during tests. Only a single running wheel could be housed within this type of enclosure.

The other two enclosures were so instrumented as to permit control of both temperature and relative humidity. Of these, one was a light-tight room fitted with controls which permitted regulation of ambient temperature to $\pm 1^{o}C$ and regulation of relative humidity to $\pm 5\%$. This area was large enough to house 15 of the running wheels.

The third enclosure, an American Instrument Company Aminco-Aire Test Chamber in which temperature could be regulated to $\pm 0.1^{o}C$ and relative humidity to $\pm 5.0\%$ provided the most rigorous environmental control of all three areas. The test chamber could be made light-proof and was large enough to house a maximum of 6 running wheels.

Within the constant temperature cabinets and the Aminco-Aire Test Chamber the running wheels were not housed separately. In the constant temperature room, however, each wheel was isolated in an insulated, light-proof wooden box supplied by a ventilation system with temperature and relative humidity conditioned air from the room. Each box was provided with a 15 watt incandescent bulb which was wired to a master control panel. The control panel was so designed as to permit the maintenance of four separate, but simultaneous, light regimes.

Temperature was monitored by battery operated telethermometers and thermistor probes. In instances when a continuous record of temperature (e.g., body temperature) was required, a Yellow Springs Model 80 recorder, which samples temperature at a rate of once every two seconds, was employed.

Relative humidity within the experimental enclosures was monitored by an electrically driven wet bulb-dry bulb psychrometer and by an American Instrument Company hygrometer with lithium chloride hydroscopic elements.

Electroencephalography

The cortical electroencephalogram (EEG) was recorded with the aid of a three channel Physiograph (E & M Instrument Company). This system incorporates adequate amplification components with sufficient frequency range to permit recording and reasonable quantification of gross EEG potentials.

As a result of the high resistance of the *Heloderma* integument ($>$ 100,000 ohms), it was necessary to surgically implant EEG electrodes. Stainless steel electrodes, 0.5-0.7 mm. in diameter and lacquered to within 0.5 mm. of the flat tip, were inserted through the frontal and parietal bones to a point on the dura mater of the right and left cerebral hemispheres. The electrodes were maintained in position with an acrylic plastic which hardened to form a tough protective base.

During all operative procedures the animals were hypothermic. Accurate positioning of the pathways through the integument and cranium was accomplished with a stereotaxic device fitted with a Rusch 3/0 or 4/0 dental drill.

Three electrodes were implanted in all experiments. Two of these were positioned in the manner described above, while the third was placed in the premaxilla and used as a reference electrode. Short leads which extended to the region of the pectoral girdle were soldered to a three conductor female receptacle which was maintained in place by a strip of tape around the neck.

Gross cerebral potentials were recorded while the animal was maintained in a running wheel of the type described above. This permitted simultaneous recording of overt locomotory activity and central nervous system activity, and the subsequent correlation of the initiation of locomotory activity with the level of central nervous system excitation.

RESULTS AND DISCUSSION

General Field Locomotory Activity

Field activity records for 263 *Heloderma suspectum* collected from the Santa Cruz Valley in the vicinity of Tucson, Pima County, Arizona over a period of six years (1959-1964) indicate that the population is normally active from mid-March to early November. The length in days of the active period is variable (Table 1) and is dependent, in some part, upon macroenvironmental temperature in March and November.

For instance, in 1964 (activity period = 218 days) there was a March snow at the Tucson International Airport and November was characterized by sub-normal temperatures early in the month. In 1962 (activity period = 241 days), although March was the second coolest since 1924, November was the warmest since 1954. Other factors which are known to cause *Heloderma* to leave a refuge and which might, therefore, influence collection records in March and November are high precipitation and runoff, and the activity of man (*e.g.*, mining, excavation, road construction).

The six year mean field activity for 263 individuals is shown in Figure 1. It is evident that there exists a distinct bimodality in seasonal locomotory activity, with a primary activity peak occurring in May and a secondary peak occurring in August. The

Table 1. Length of the Seasonal Activity Period and Total Number of Heloderma Collected

YEAR	LENGTH OF ACTIVE PERIOD (DAYS)	NUMBER OF HELODERMA COLLECTED
1959	228	42
1960	227	28
1961	233	36
1962	241	34
1963	221	39
1964	218	84
1959-1964	$\bar{X} = 228$	$\Sigma = 263$

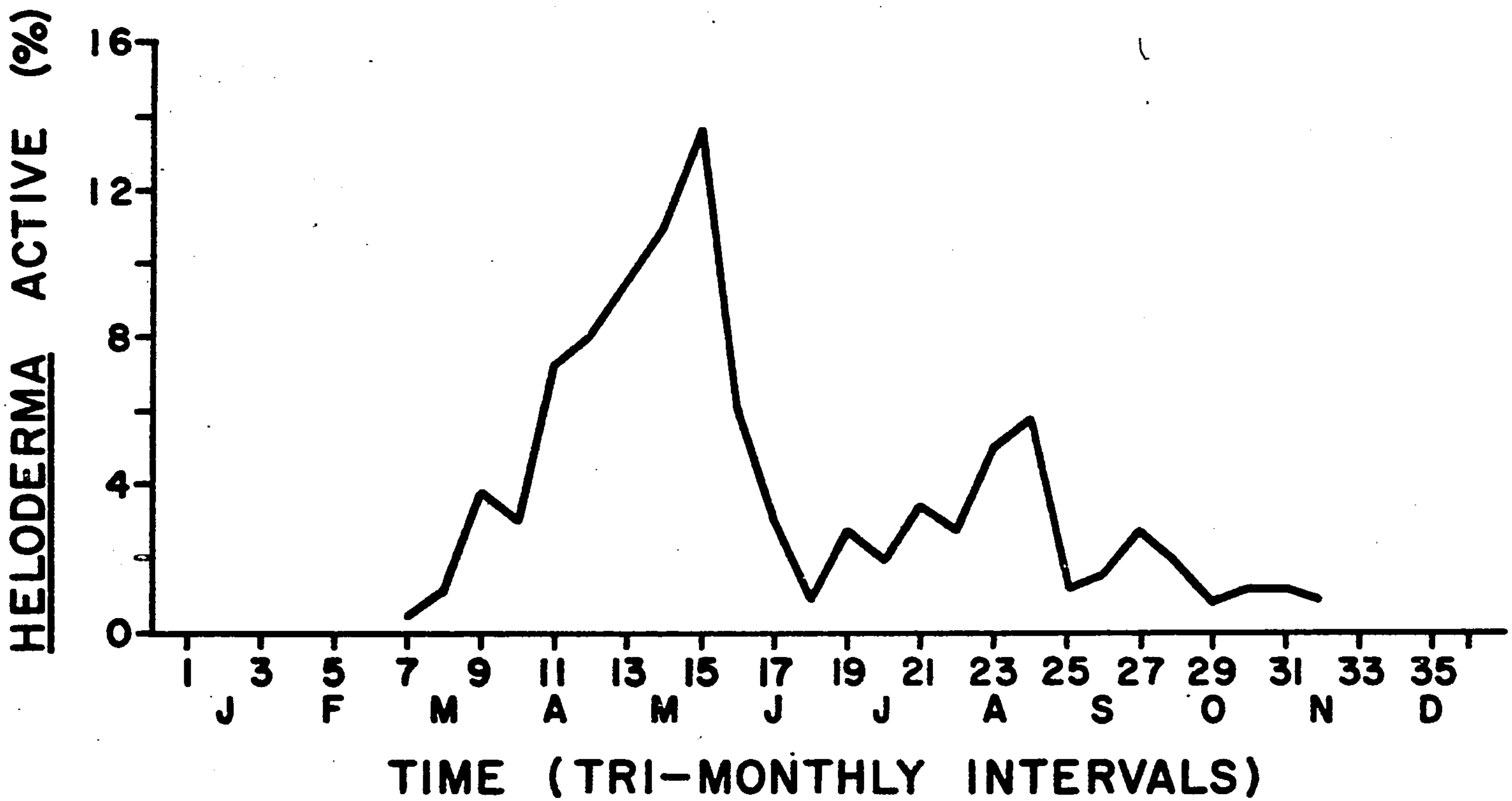

Figure 1. Six Year (1959-1964) <u>Heloderma</u> Field Activity Expressed as Per Cent of Collection Against Tri-Monthly Intervals

activity records for individuals years (see Appendix A) demonstrates that this bimodality is not an artifact of summation, but, in fact, does occur to some extent each year.

It is not inconceivable that the analysis discussed above might be biased by the fact that while Heloderma is predominantly diurnal during the period March-May, the population assumes a crepuscular activity orientation for the greater part of the remaining seasonal activity period, resuming a diurnal orientation during the period October-November. Relevant to the probability that this transition from diurnality to crepuscularity is not the result of a decrease in the total number or kind of observations, is the fact that during this period the great majority of the collecting efforts, including nocturnal collecting, are being conducted by herpetologically oriented individuals. Very few Heloderma have been collected on or off roads following the dusk photic transition. Furthermore, the human animal tends to respond to the increased temperatures of the midday in much the same manner as Heloderma and presumably, as a result of this behavior, the probability of observation would remain fairly constant. A bias, if it exists at all, is therefore not considered to be of sufficient magnitude to alter significantly the activity relationships of the study area population as presented here.

Field activity normally commences in March at a point when both the macroenvironmental daily thermal maxima and minima rise

Table 2. Six Year (1959-1964) Mean <u>Heloderma</u> Field Activity and Macro-Climatic Correlates by Tri-Monthly Intervals

TRI-MONTHLY INTERVAL	NUMBER OF <u>HELODERMA</u> COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (^{o}F)	DAILY THERMAL MINIMUM (^{o}F)
Jan 1	0	0.0	62.5 ± 0.96	36.4 ± 0.87
2	0	0.0	60.1 ± 1.31	34.2 ± 1.00
3	0	0.0	65.3 ± 0.83	38.7 ± 0.74
Feb 4	0	0.0	68.0 ± 1.72	38.8 ± 0.93
5	0	0.0	64.8 ± 0.97	39.0 ± 0.92
6	0	0.0	64.3 ± 1.17	36.3 ± 0.81
Mar 7	1	0.4	69.1 ± 1.17	40.0 ± 0.77
8	3	1.1	71.3 ± 0.95	40.7 ± 0.79
9	10	3.8	75.3 ± 0.98	46.1 ± 0.67
Apr 10	8	3.0	80.4 ± 1.09	49.7 ± 0.80
11	19	7.2	84.4 ± 0.95	52.1 ± 0.73
12	21	8.0	81.2 ± 0.92	50.6 ± 0.85
May 13	25	9.5	86.7 ± 1.01	54.6 ± 0.92
14	29	11.0	91.4 ± 0.84	58.0 ± 0.72
15	36	13.7	90.8 ± 0.60	57.7 ± 0.77
Jun 16	16	6.1	94.2 ± 0.47	62.2 ± 0.54
17	8	3.0	99.4 ± 0.65	67.6 ± 0.86
18	2	0.4	101.3 ± 0.50	71.7 ± 0.61
Jul 19	7	2.7	98.7 ± 0.60	74.3 ± 0.52
20	5	1.9	100.6 ± 0.43	74.5 ± 0.57
21	9	3.5	96.4 ± 0.60	73.0 ± 0.32
Aug 22	7	2.7	95.1 ± 0.69	72.3 ± 0.34
23	13	4.9	94.7 ± 0.71	72.3 ± 0.41
24	15	5.7	93.7 ± 0.62	70.6 ± 0.37
Sep 25	3	1.1	94.0 ± 0.75	70.2 ± 0.47
26	4	1.5	92.1 ± 0.71	68.1 ± 0.69
27	7	2.7	90.4 ± 0.69	63.5 ± 0.70

Table 2, <u>Continued</u>

TRI-MONTHLY INTERVAL		NUMBER OF *HELODERMA* COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM ($^{\circ}$F)	DAILY THERMAL MINIMUM ($^{\circ}$F)
Oct	28	5	1.9	87.3 ± 0.91	59.4 ± 0.71
	29	2	0.8	82.9 ± 0.94	56.7 ± 0.73
	30	3	1.1	81.5 ± 0.97	53.9 ± 0.67
Nov	31	3	1.1	74.7 ± 0.93	48.7 ± 0.65
	32	2	0.8	68.4 ± 1.18	43.9 ± 0.93
	33	0	0.0	71.7 ± 0.72	42.1 ± 0.70
Dec	34	0	0.0	65.0 ± 1.08	40.7 ± 0.95
	35	0	0.0	62.6 ± 0.95	38.4 ± 0.77
	36	0	0.0	64.4 ± 0.81	38.9 ± 0.60

significantly above winter levels (Figure 2). This increase is undoubtedly reflected in a rise of the microenvironmental temperature within the winter refugia (rock crevices, rodent burrows, mines) of Heloderma. Locomotory activity at this time appears to be sporadic. Within a period of only several weeks, however, a rather high activity level is attained which leads, in turn, to the primary activity peak in May.

The mean springtime increase in both daily thermal maxima and minima for the period 1959-1964 are listed in Table 2. The same data for individual years are listed in Appendix B. In each instance it can be seen that the initiation of field activity is related to environmental temperature increase.

There is no indication that an abnormally cold winter, in the sense of especially low extremes, has any effect on the time of spring emergence, assuming an optimal environmental temperature for March. An extended winter or a spring cold snap, however, can delay emergence.

A warm winter preceeding a warm March, on the other hand, seems to result in early emergence. For instance, the winter of 1958-1959 was atypical in the following respect. A snowfall of 6.4 inches was recorded on November 16, the first significant snowfall in November since 1895. The 24-hour total snowfall on this date was the greatest recorded to that time for any month. The temperature during the following tri-monthly interval (November 20-30) was above normal.

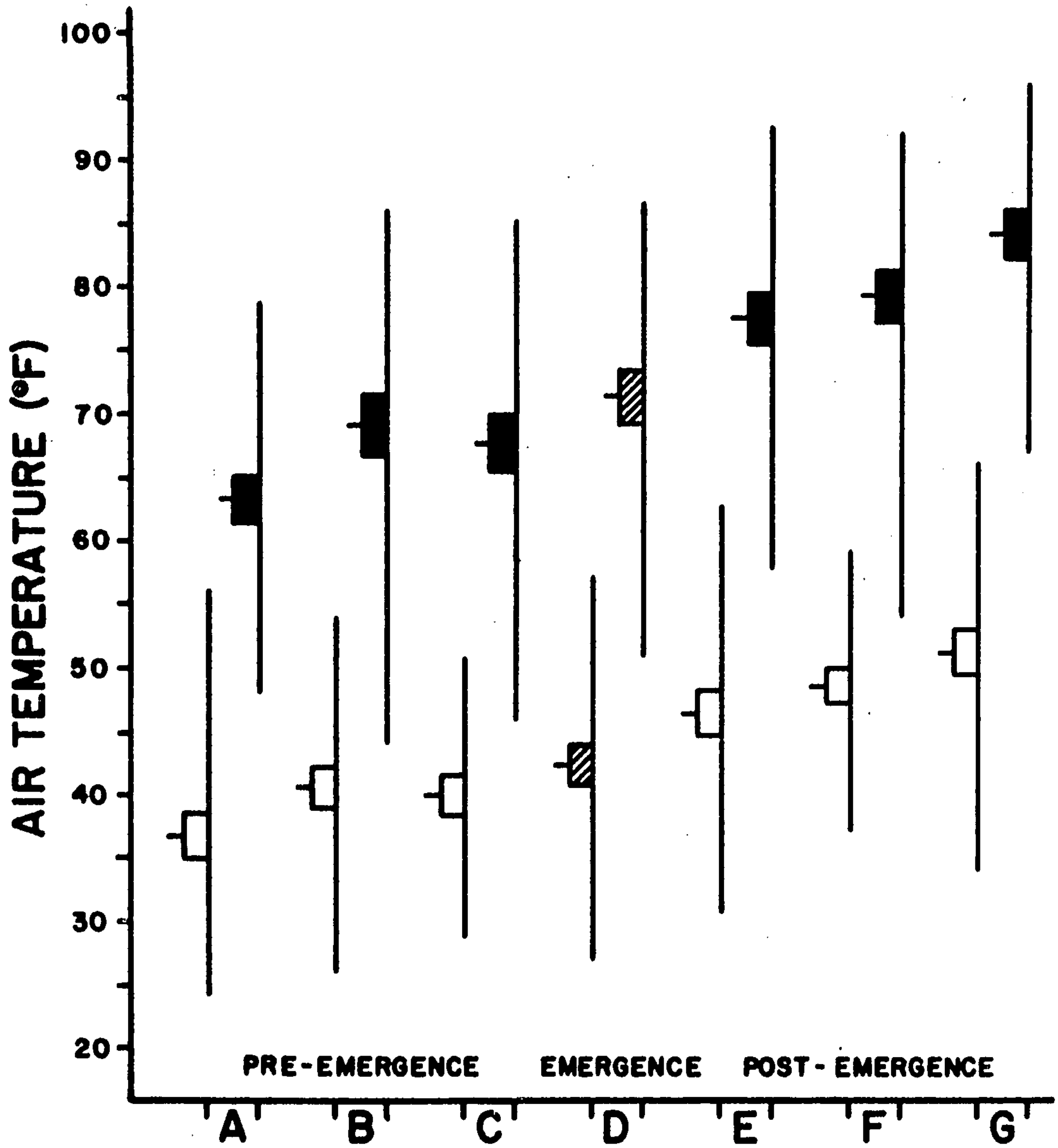

Figure 2. Mean Macro-Environmental Thermal Maxima (Closed) and Minima (Open) Preceding Emergence, At Emergence and Following Emergence for the Period 1959-1964

The month of December was dry, the first rainless December since 1917; and warm, the average monthly temperature was the highest since 1950. January, 1959, was also warm and dry with daily mean temperatures above normal on 25 days. The mean temperature for February, 1959, was only slightly below normal. The first three days of March averaged 5°F above normal and the first Heloderma was observed on March 4.

The remainder of March, although slightly above normal temperature, was the coolest March in seven years and no further Heloderma were observed until March 31. The increase in the daily thermal minima during March, 1959, was not as dramatic as in other years and this fact may have accounted, in part, for the lack of Heloderma observations.

It is important to recognize here that neither emergence nor immergence is wholly dependent upon temperature as an indicator of time, or the zeitgeber of Aschoff (1960,1965). There undoubtedly exists an endogenous bio-chronological cuing mechanism which stimulates the physiological mechanisms leading to initiation and/or cessation of seasonal activity.

The role of environmental temperature, while perhaps not a dominant factor in the operation of the timing mechanism, does certainly influence the initiation, cessation, and duration of overt locomotory activity. This is adequately demonstrated by information of the period discussed above for the March, 1959 collection data.

Table 3. Summation of Thermal Maxima and Minima of those Tri-Monthly Intervals for which Emergence and Immergence (D) are Established and the Tri-Monthly Intervals Immediately Preceding (A, B, C) and Following (E, F, G)

EMERGENCE

TRI-MONTHLY INTERVAL SUMMATION	N (DAYS)	THERMAL MAXIMUM (^{o}F)	THERMAL MINIMUM (^{o}F)
A	57	63.4 ± 0.87	36.8 ± 0.93
B	55	69.2 ± 1.24	40.6 ± 0.84
C	58	67.7 ± 1.12	38.9 ± 0.80
D	62	71.3 ± 1.02	42.4 ± 0.85
E	63	77.5 ± 1.04	46.3 ± 0.88
F	61	79.3 ± 1.03	48.5 ± 0.70
G	60	84.1 ± 1.06	51.1 ± 0.88

IMMERGENCE

TRI-MONTHLY INTERVAL SUMMATION	N (DAYS)	THERMAL MAXIMUM (^{o}F)	THERMAL MINIMUM (^{o}F)
A	60	88.1 ± 0.83	62.9 ± 0.91
B	62	83.6 ± 1.19	56.5 ± 0.86
C	61	81.2 ± 1.17	54.0 ± 0.85
D	61	77.6 ± 1.33	51.9 ± 1.14
E	62	75.7 ± 1.14	47.9 ± 0.93
F	60	71.4 ± 0.96	45.3 ± 0.74
G	60	61.9 ± 1.22	41.5 ± 1.10

The primary activity peak in May can most likely be attributed in part to reproductive activity. Copulatory behavior, however, seems to occur, for the most part, in July following the onset of the summer rains as reported by Ortenburger and Ortenburger (1926), Kauffeld (1943), and observed by Arizona residents. Reproductive activity, therefore, is most closely associated with the secondary activity peak (Figure 1) and it is at this time that the sexually active males and females will tend to aggregate.

In the present study, 152 Heloderma, or 57.8% of the total sample of 263, were collected or observed during the three months of March, April, and May. Throughout the period June-November (= six months) 42.2%, or 111 Heloderma were recorded.

It is obvious that any animal which has been inactive for a period of three to four months will require certain nutrients, particularly if it is to be physiologically sound during the breeding period. Thus it is probable that during the period March-May Heloderma is out and actively seeking food. Bogert and Del Campo (1956) summarize the literature concerning the diet of Heloderma suspectum and the Mexican Beaded Lizard, Heloderma horridum and they indicate that the diet of H. suspectum consists mainly of juvenile rodents and lagomorphs, the eggs of ground-nesting birds, and the eggs of reptiles. Of these, the juvenile mammals and the birds' eggs are available in sufficient quantity during the spring and summer months.

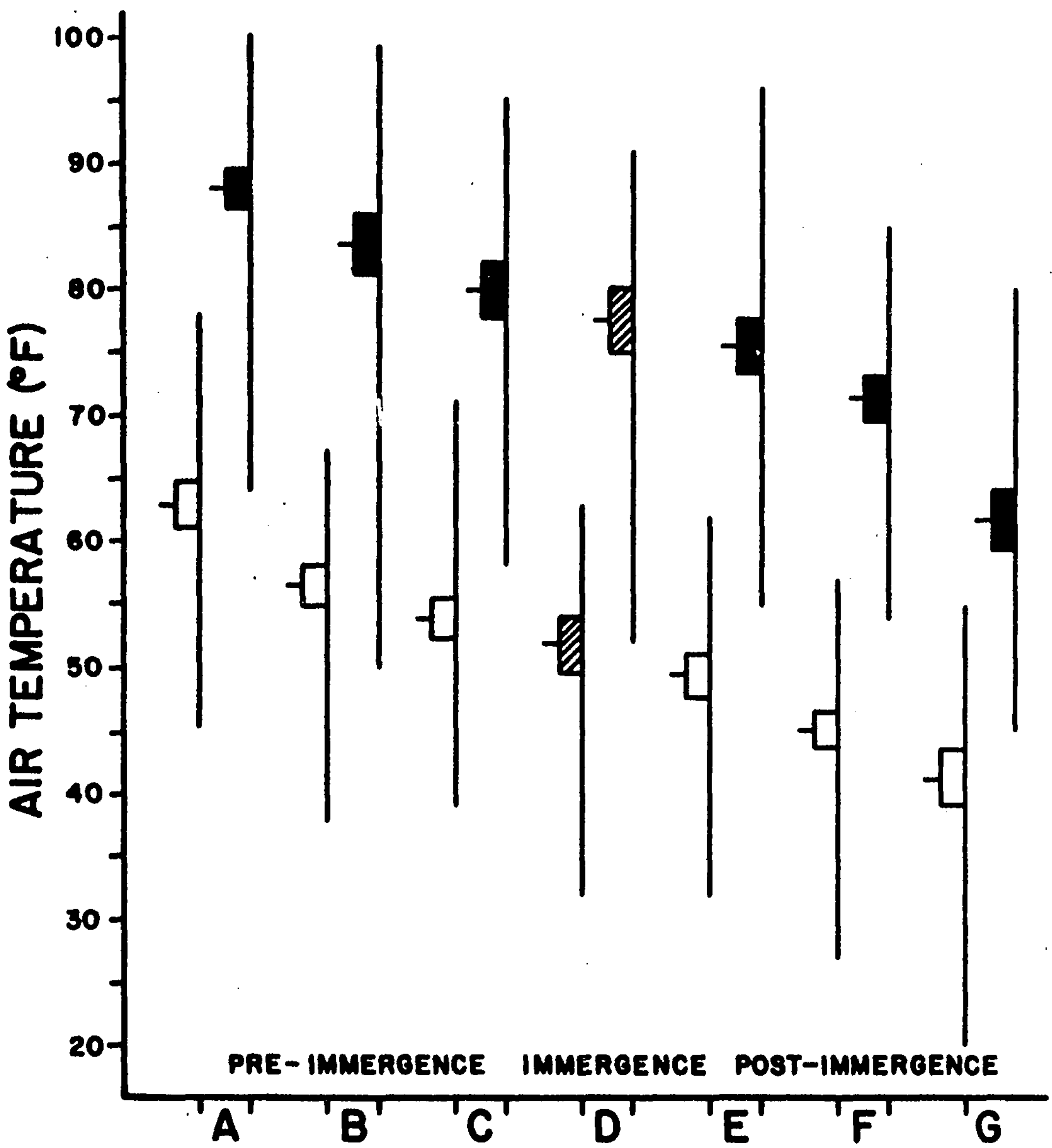

Figure 3. Mean Macro-Environmental Thermal Maxima (Closed) and Minima (Open) Preceding Immergence, At Immergence and Following Immergence for the Period 1959-1964

Basking behavior may be exhibited to a large extent in March and April, and it is possible that the animals are more prone to capture while they are lying exposed to incident solar radiation. Once again, collection in this instance is a function of the probability of observation and it is assumed here that this does not differ to a large extent between spring, summer, and fall.

Precipitation plays an important role in the activity of Heloderma during the period April-June. It is during this interval that the study area experiences an annual pre-summer drouth. This is discussed more fully below, but examination of Figure 5 will demonstrate that during the period of maximum field activity, there has been only a small amount of precipitation recorded for the study area. The lack of available water becomes severely limiting and Heloderma withdraws until the onset of the summer rains.

At a point which is indicated by the onset of the summer rains (late June-early July) Heloderma activity is reinitiated, although it never approaches the activity level demonstrated in May. The secondary activity peak in August is due to three factors: (1) food-seeking in response to nutritional deficits generated by the June estivation, (2) activity in response to precipitation, and (3) the breeding behavior discussed above.

In late October and November the period of seasonal activity comes to an end. Like emergence, the actual time of immergence appears to be dictated by daily thermal minima (Figure 3). The relationship of the time of immergence to thermal minima for individual years, 1959-1964, can be seen in Appendix B.

Modification of Field Locomotory Activity by Climatic Variables

Temperature

The locomotory activity of *Heloderma* in the study area is severely modified by environmental temperature regimes. The records discussed above indicate that there is a gradual transition in the diel relationships of the activity pattern from diurnality to crepuscularity. It is important to recognize that among reptiles, particularly desert reptiles, a survival value may be placed upon this transition, as it serves to remove the species naturally from daily thermal maxima and minima that may exceed physiologically critical levels.

Field and laboratory studies have shown that the preferred body temperature of *Heloderma suspectum* is approximately 28°C, a low value for a desert lizard, and that the maximum voluntarily tolerated temperature is 32° to 34°C; a critical thermal level (CTM) is reached at 41° to 43°C (Lowe, unpublished data). The range in environmental temperature to which *Heloderma* would normally be exposed is shown in Table 2. From this table it can be seen that in order to survive *Heloderma* must modify activity patterns in response to environmental thermal stimuli. The major response to annual change in temperature is a seasonal change in the orientation of the activity period.

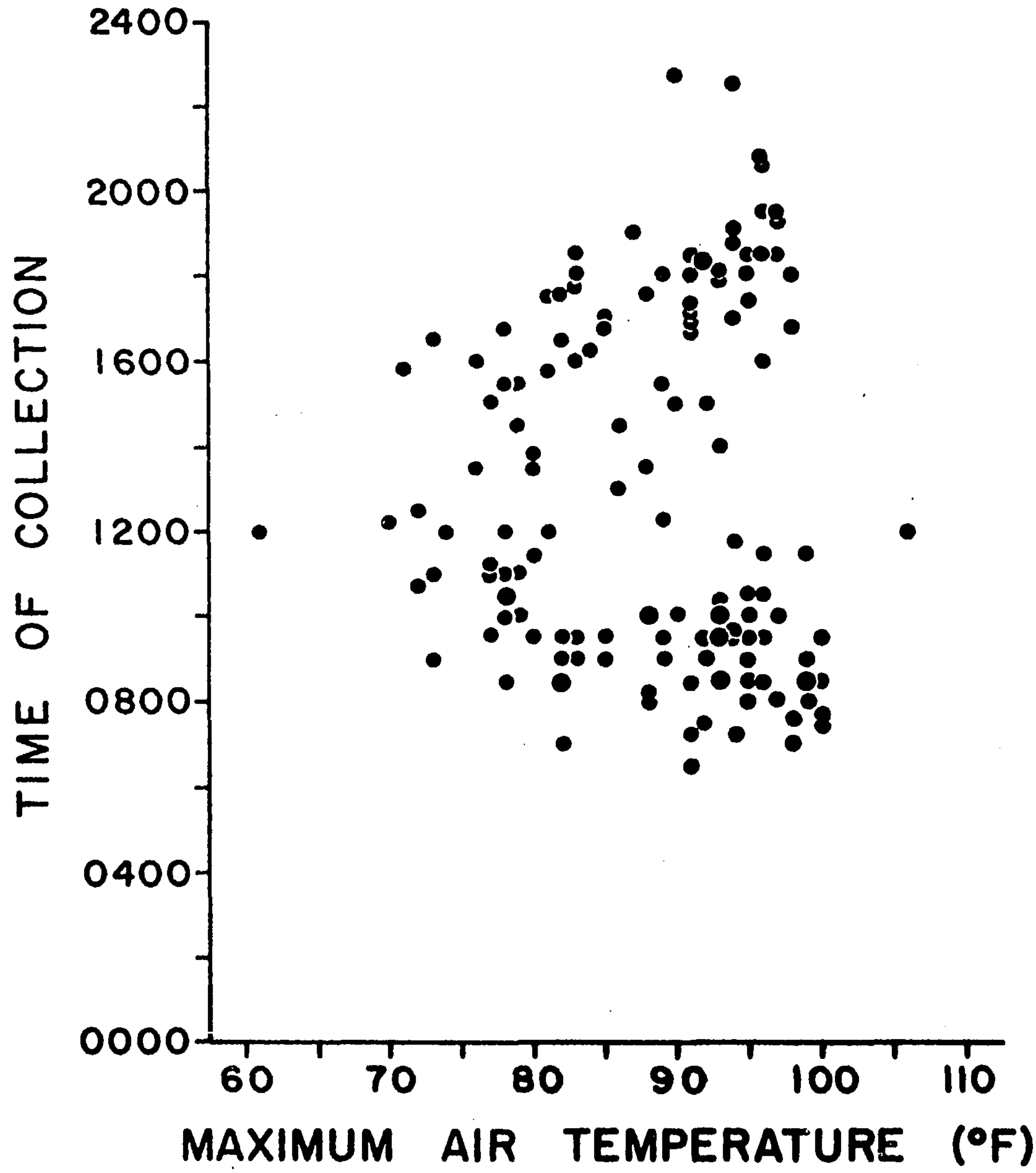

Figure 4. Time of Collection, Morning and Afternoon, Preceding and Following the Onset of the Summer Rains Against Macro-Environmental Thermal Maximum for the Date of Collection for the Period 1959-1964

At the time of emergence in March, field locomotory activity is restricted to mid-day. It is apparent by inspection that sub-optimal macroenvironmental temperature is the factor limiting activity at any other time of day. Through the months of March, April, and May the daily thermal maxima and minima exhibit a steady warming trend (Table 2, Appendix B) which has a very real effect on the diurnal orientation of the *Heloderma* activity period.

At a point in April, a general shift in the time of activity is evident. This shift is directly correlated with increasing daily thermal maxima. The thermal characteristics of this shift will be discussed below; here the major concern is the resultant shift in activity orientation.

This shift can be readily seen in the scatter diagrams of time of collection graphed for individual years (Appendix C, data in Appendix D) and for the period 1959-1964 (Figure 4). Locomotory activity shifts toward both the dawn and dusk photic transitions. This, then, is the first indication that crepuscularity is to be assumed. At the May activity peak, during hot weather and shortly before the influence of the summer drouth is felt, activity is predominantly crepuscular in orientation.

In October-November, as the seasonal activity period is approaching an end, an activity orientation shift - similar to, but in an opposite direction from, that observed in the spring - is shown in the record. This shift toward diurnality is assumed to be similar to the spring shift in that it too is dictated by temperature. Daily thermal minima at this time of year become limiting to locomotory activity and the natural consequence is a shift in activity by those individuals still active to a more diurnal orientation. As in the spring macroenvironmental temperature is the most important environmental factor contributing to the immergence and subsequent termination of the activity period.

The six year mean fall decrease in both daily thermal maxima and minima are listed in Table 2. The same data for individual years (1959-1964) are included in Appendix B.

Precipitation

There exists in the record a significant decrease in activity during June (Figure 1). Two climatological factors are found to be correlated with this: maximum temperature and lack of precipitation.

June is the warmest month of the active period and one of the driest. The lack of available moisture, which, with supra-optimal temperature, forces *Heloderma* into estivation, is related in important part to the marked decrease in precipitation during May and June (Figure 5).

Relative humidity to as low as 1% has been recorded. Under such conditions, evapo-transpiration and general evaporation can draw most of the available water from the environment and can introduce rather severe physiological stress in *Heloderma*. Thus, during this period, *Heloderma* is forced to estivate underground where lower temperature and higher relative humidity prevail.

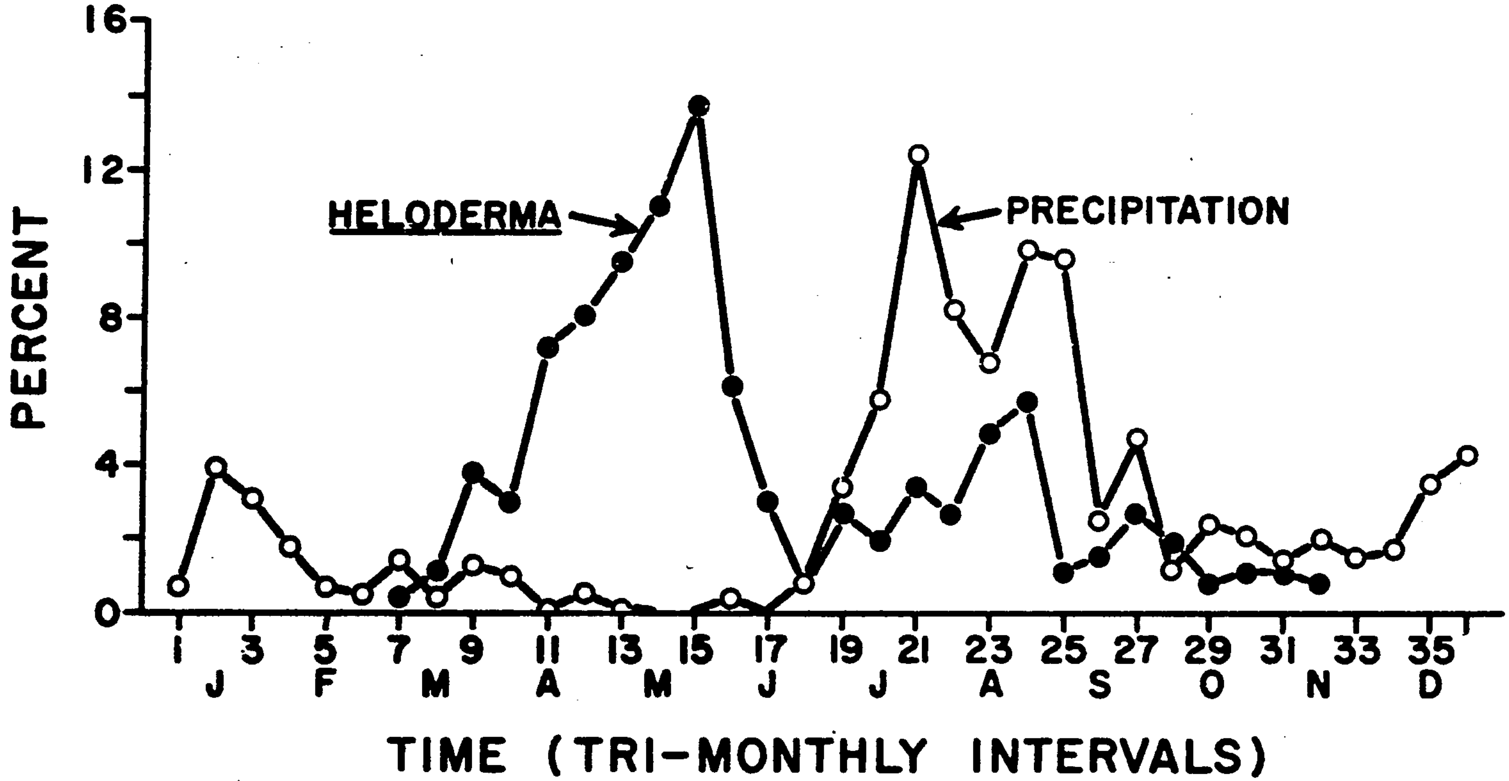

Figure 5. Six Year (1959-1964) Mean Precipitation Values Expressed as Per Cent of Total Rainfall and Six Year _Heloderma_ Field Activity Expressed as Per Cent of Collection Against Tri-Monthly Intervals

In the middle of June, moist monsoon air from the Gulf of Mexico begins to accumulate over the study area and the humidity rises significantly. This rise is not reflected, however, in an increase in Heloderma activity. At this point the daily thermal maxima are still limiting to locomotory activity.

At a point when the amount of precipitable water vapor in the atmosphere reaches a critical level, usually during early July, the summer rains commence and Heloderma activity is reinitiated although at a lesser intensity (at the population level) than during April-May. At this time locomotory activity reassumes the crepuscular orientation previously evidenced in May, occurring about both the dawn and dusk photic transitions. Sporadic activity is not uncommon during the middle of the day, but this is, in most instances, associated with the occurrence of precipitation and concommitant decrease in microenvironmental temperature and increase in micro-environmental relative humidity.

Light

It has been reported that Heloderma maintains a nocturnal activity orientation (Bogert and Del Campo, 1956), particularly during the summer months. The data clearly show that this is not the case. Most of these animals, indeed, the great majority, are active following the dawn twilight and preceding the dusk twilight (Figure 6, data in Appendix D). These data are presented graphically for individual activity seasons (1959-1964) in Appendix E. The time of

Table 4. Six Year (1959-1964) Precipitation Analysis for Tucson International Airport

TRI-MONTHLY INTERVAL	TOTAL RAINFALL	MEAN RAINFALL	PER CENT OF TOTAL	NUMBER OF INTERVALS WITH MEASURABLE RAINFALL
Jan 1	0.46	0.08	0.70	3 (1, 2, 5)
2	2.60	0.43	3.93	3 (2, 4, 5)
3	2.05	0.34	3.10	3 (3, 4, 6)
Feb 4	1.17	0.20	1.77	3 (1, 2, 5)
5	0.48	0.08	0.70	4 (2, 3, 5, 6)
6	0.33	0.06	0.50	1 (4)
Mar 7	0.95	0.16	1.44	5 (2, 3, 4, 5, 6)
8	0.28	0.05	0.42	2 (4, 5)
9	0.83	0.14	1.26	5 (2, 3, 4, 5, 6)
Apr 10	0.66	0.11	1.00	1 (6)
11	0.01	0.002	0.02	1 (1)
12	0.33	0.06	0.50	2 (5, 6)
May 13	0.08	0.01	0.12	1 (2)
14	0.00	0.00	0.00	0
15	0.00	0.00	0.00	0
Jun 16	0.25	0.04	0.38	1 (2)
17	0.00	0.00	0.00	0
18	0.52	0.09	0.79	3 (3, 4, 6)
Jul 19	2.26	0.38	3.42	6
20	3.83	0.64	5.79	5 (1, 3, 4, 5, 6)
21	8.22	1.37	12.43	6
Aug 22	5.40	0.90	8.16	5 (1, 2, 3, 5, 6)
23	4.50	0.75	6.80	6
24	6.50	1.08	9.83	6
Sep 25	6.33	1.06	9.57	5 (2, 3, 4, 5, 6)
26	1.68	0.28	2.54	4 (3, 4, 5, 6)
27	3.12	0.52	4.72	3 (2, 4, 6)
Oct 28	0.79	0.13	1.19	3 (1, 2, 4)
29	1.61	0.27	2.43	4 (2, 4, 5, 6)
30	1.39	0.23	2.10	4 (1, 2, 3, 5)
Nov 31	0.94	0.16	1.42	5 (1, 2, 3, 5, 6)
32	1.30	0.22	1.97	4 (1, 4, 5, 6)
33	0.99	0.17	1.50	4 (2, 3, 4, 5)
Dec 34	1.15	0.19	1.74	5 (1, 2, 3, 4, 5)
35	2.32	0.39	3.51	4 (1, 3, 4, 6)
36	2.82	0.47	4.26	4 (1, 2, 4, 6)

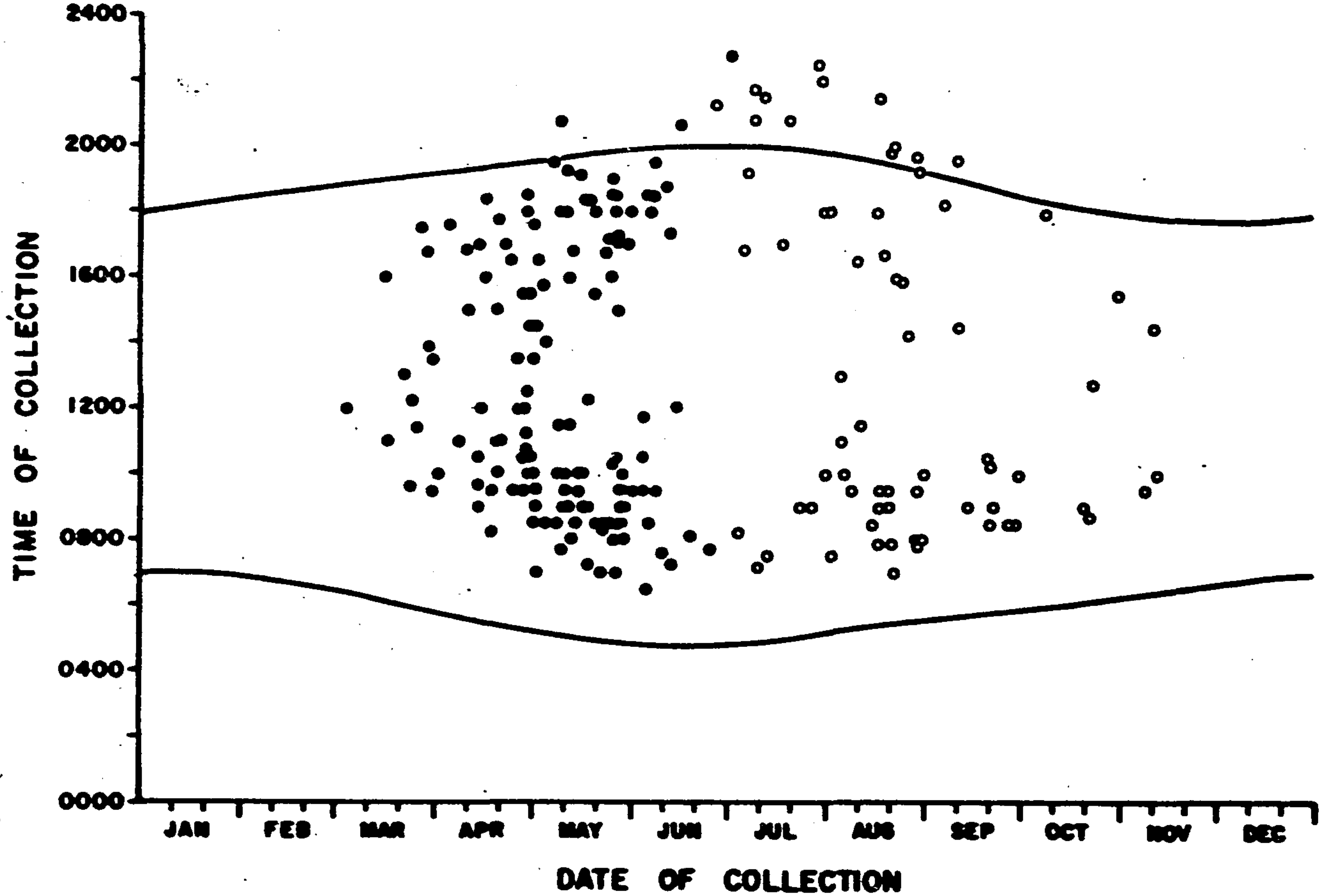

Figure 6. Time of Collection, Morning and Afternoon, Preceding (Closed) and Following (Open) the Onset of the Summer Rains, Against Date of Collection

The curves indicate the time of civil twilight. The data are in Appendix D and the Tables of Sunrise, Sunset, and Twilight. Similar graphs for individual years (1959-1964) may be found in Appendix E.

twilight utilized was that of civil twilight, the time at which the sun is 6° below the horizon. Civil twilight has been shown to be biologically significant in regard to the activity of other vertebrates, particularly in relation to initiation and cessation of song and roosting in birds (Leopold and Eynon, 1961).

It is likely that the few reported instances of nocturnal observations of Heloderma are the result of one or more of the following situations, all of which have been observed to affect the usual periodicity.

1. There are reported observations of copulating pairs following dusk (Ortenburger and Ortenburger, 1926; Kauffeld, 1943). It would appear likely that once penetration had been achieved and copulation was progressing that the pair would continue the act until completion. Therefore, if copulation commenced shortly before dusk, it might not be completed until long after the photic transition.
2. The animals appear to be occasionally forced out from a nocturnal refuge by heavy rains and wash outs. If this happens at night, the individual may wander blindly for some time until adequate refuge is located.
3. Emaciated individuals have been observed moving after civil twilight. While such individuals are infrequently encountered, the activity pattern that they exhibit is essentially the same. A starved animal moves more or less unidirectionally

for relatively long time intervals, presumably driven by hunger. No animals in this condition were included in the analysis. A more complete discussion of locomotory activity in the non-feeding Heloderma is presented below.

General Laboratory Locomotory Activity

The concept per se of the natural period (= free-running period, spontaneous period) is not considered herein. It is obvious, however, that some indicator of rhythmicity is useful or necessary in an analysis of this type and for that reason the measure of natural period has been utilized. It is rapidly becoming apparent that the classical concept of the natural period as a constant unit within populations is no longer valid and that natural period values tend to vary among individuals of the same species, assuming a value of approximately 24-hours for any adequate sample from any given population (see Lowe et al., 1967). Further, under constant conditions of light, temperature, and relative humidity the natural period can be made to vary with any of these functions. Thus, while the natural period is obviously something more than an indicator of rhythmicity, its significance, value to populations, and adaptive values will not be discussed here to any great extent.

The natural period length of Heloderma suspectum has been found to vary between 22.5 and 25.3 hours. This range of values has been derived from natural period length determinations made throughout the entire seasonal activity period and under a variety of environmental conditions, but predominantly from spring and fall individuals.

There is no apparent difference in the length of the natural period due to age or sex, but an indication of a seasonal difference in the length of the period does exist. This difference, which may occur as a response to environmental light intensity, is, however, not quantifiable with the data at hand.

In early March, Heloderma emerges from underground winter refugia and is confronted with an ambient temperature regime which, as a result of the daily thermal minima, dictates complete diurnality. Upon emergence some individuals exhibit an arhythmicity which persists in the laboratory under constant conditions of temperature, light (DD), and relative humidity. This acyclic activity is characterized by the lack of definite activity periods and by complete phase lability when individuals are placed on a light cycle (L:D, 12:12). At this time, however, there is a definite expression of diurnality in the record.

An apparent shortening of the natural period occurs through March and April, resulting finally in an activity rhythm in May with a period length of less than 24-hours. This change in period length is most likely associated with the shift in locomotory activity orientation shown in Figure 6. It has been shown that the period length in light is greater than the period length in dark for Heloderma (Lowe et al., 1967). Therefore, the longer natural period lengths in March-April are a response to the higher light intensity of the diurnal orientation. In May-August, with lower light intensity in the crepuscular orientation, the length of the natural period shifts to a value of less than 24-hours. Very few late fall (October-November) animals were run, and the data does not demonstrate that a reverse shift toward a greater than 24-hour natural period length occurs at that time. One would expect, however, that this would occur as *Heloderma* is forced back into a diurnal activity orientation by the cool thermal regime during that period.

Figure 7 illustrates the record of an animal which was entering winter estivation in November. The running wheels were exposed to the natural above-ground environmental thermoperiod and photoperiod. Continuous records of both body temperature and ambient temperature were made.

Only 4.3 days of records were obtained before the individual shown in Figure 7 entered winter torpor. These days were characterized by an activity period centered about the warmest part of the day (1400-1500 hours). There is a significant decrease in the duration of activity during each successive 24-hour period resulting from the associated advance of cessation time and a delay of the time of initiation of activity. This finally results in a totally diurnal orientation of the activity period and eventual extinction of activity. On the sixth day of the experiment no revolutions of the running wheel were recorded. On the seventh day there occurred a slight ambient warming trend and the running wheel was revolved for a total of eight revolutions, between 1530 and 1600 hours.

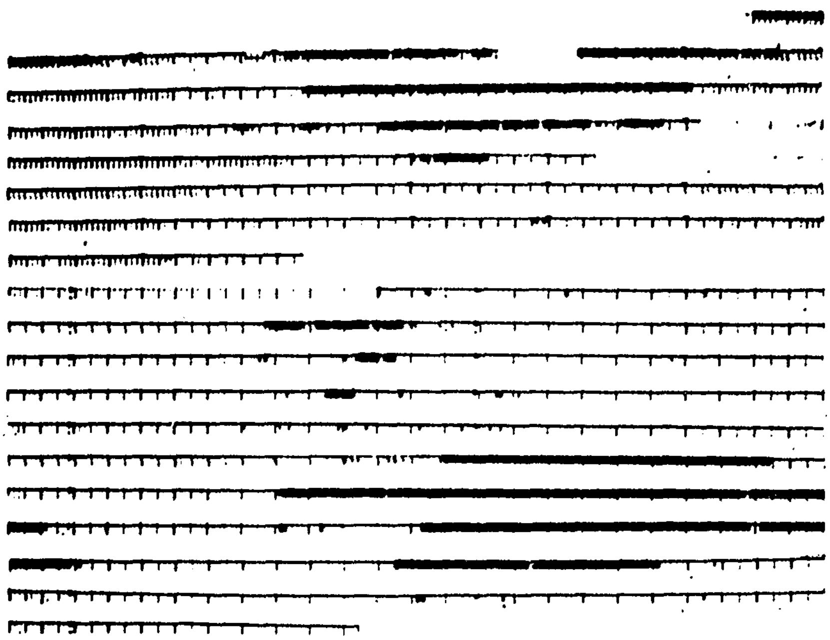

Figure 7. Extinction of Locomotory Activity by Environmental Thermoperiod

Each horizontal strip represents a single 24-hour period beginning and ending at midnight. The dark areas along a strip represent a period of wheel turning activity. See text for an explanation of this activity composite.

On the eighth day of recording the animal was removed from environmental thermoperiod to an area with a constant temperature of $25.0 \pm 1.0^{o}C$. The photoperiod, however, remained essentially unchanged. There followed five days of intermittent, acyclic activity characterized by sporadic revolutions of the wheel during the daylight hours. On the sixth day following transfer to constant temperature, the activity period assumed a rather vigorous crepuscular orientation not unlike that observed at the beginning of the experimental period.

Of three animals observed during this period, all exhibited the same arhythmicity upon transfer to constant temperature. It is assumed that this arhythmicity is not unlike that exhibited by those individuals collected in early March shortly after emergence.

The seasonal changes discussed above can be summarized as follows:

1. Emergence from winter estivation
    a. The first days after emergence are often characterized by arhythmicity, followed by activity which exhibits a natural period length of greater than 24-hours.
    b. Activity orientation is completely diurnal.
2. Activity during spring
    a. The length of the natural period is approaching or less than 24-hours.
    b. Activity orientation is predominantly crepuscular.

3. Activity during summer

   a. The length of the natural period is less than or equal to 24-hours.

   b. Activity orientation is predominantly crepuscular.

4. Activity during fall

   a. The length of the natural period is $\approx$ 24-hours.

   b. Activity orientation is predominantly crepuscular, tending toward diurnality.

5. Entrance into winter estivation

   a. The length of the natural period is $\approx$ 24-hours.

   b. Activity is completely diurnal.

Modification of Laboratory Locomotory Activity by Temperature and Light

The temperature-independence of biological rhythms would appear outwardly to be a finalized issue. It has become increasingly apparent during the course of this study that the Van't Hoff-Arrhenius relationship (Q_{10}) may not be a particularly valid measure to apply to the activity rhythms of ectothermic animals. It was not initially intended to be applied to a gross phenomenon such as locomotory behavior, and because it does not appear to adequately describe the relationship between two natural period values at different temperatures it has been eliminated as a criterion for determining the presence of a temperature-compensating mechanism at the level of the biological clock within the experimental animals. Models for temperature compensation mechanisms have been proposed (e.g., Hastings and Sweeney, 1957). These models are primarily concerned with inhibiting biochemical reactions at a subcellular level and, as such, are in a position for analysis by the Q_{10} method.

A case in point is found in a comparison of two studies of activity rhythms in ectothermic animals. Roberts (1960) in a discussion of temperature compensation describes an experiment in which a single cockroach (Leucophaea) was subjected to different temperature regimes. A similar experiment was conducted with Heloderma.

Figure 8 demonstrates the effect of a temperature change of 5°C, under otherwise constant conditions of light and relative humidity, on the length of the natural period of a Heloderma. The difference in the length of the two periods at 22.5°C and 27.5°C is significant ($t=2.45$, $P < 0.05$).

Roberts' calculated Q_{10} for the periods at 20°C and 25°C was 1.06. Under a similar 5°C temperature change (22.5° to 27.5°C) a calculated Q_{10} for Heloderma was 1.08. This latter Q_{10} value agrees with other lizard studies (Hoffman, 1957a) in which Lacerta sicula exhibited a Q_{10} of 1.02. Thus, by Q_{10} determination, one may conclude that the activity rhythm in Heloderma is temperature compensated.

By subjecting the Heloderma data to analysis by regression statistics and then calculating the significance of the difference between regression coefficients at 22.5° and 27.5°C, it can be demonstrated that the two periods differ significantly ($t=2.45$, $P < 0.05$).

Figure 8. Effect of Environmental Temperature Change on the Length of the Natural Period

Each horizontal strip represents a single 24-hour period beginning and ending at midnight. The dark areas along a strip represent a period of wheel turning activity.
This animal was on the following environmental regime: Day 1-Day 9, 27.5^{o}C, DD; Day 10-Day 11, 21.5^{o}C, DD; Day 12-Day 20, 22.5^{o}C, DD. Note the difference in slope between the times of activity initiation on Day 1-Day 9 and Day 12-Day 20. See the text for a discussion of this difference. It is interesting that for this individual a thermal regime of 21.5^{o}C eliminated locomotory activity on Days 10 and 11, but that the timekeeping mechanism continued to operate as is evidenced by the time of activity initiation on Day 12.

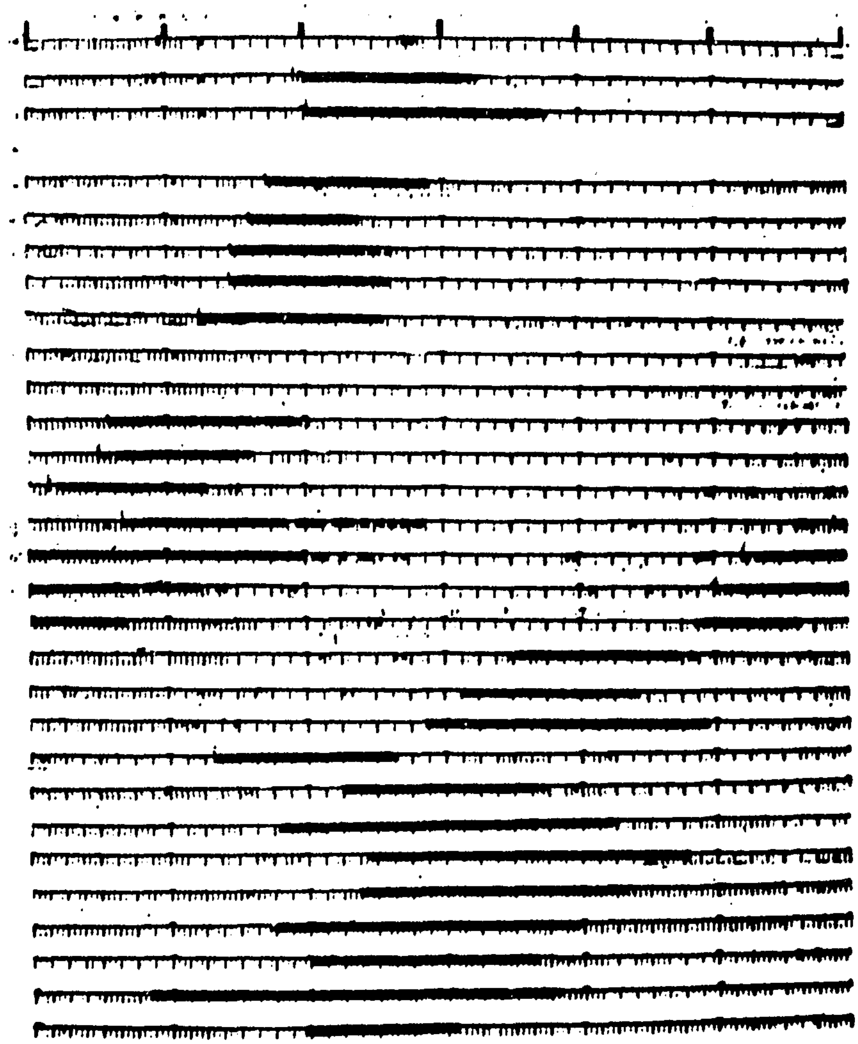

Figure 8. Effect of Environmental Temperature Change on the Length of the Natural Period

The essential problem here is the relationship between light and temperature in the operation of the biological clock. This relationship includes many factors, among which are the mechanism of temperature compensation, the mechanism of photoreception and eventual transfer of information to the clock, the mechanism of adjustment within the clocks which results in the phenomenon of entrainment, and the final pathways from the clock to various effect or mechanisms. Here one is not concerned as much with the mechanisms themselves; rather, the problem lies in the final expression of the clock - overt locomotory activity - and the environmental relationships of this rhythm.

The present investigation concerns activity rhythms in ectothermic vertebrates and it is assumed that ambient, and therefore body, temperature will have a very real effect on any activity rhythm that is measured. Therefore, the generalization of temperature independence within <u>ecological limits</u> (Pittendrigh, 1960) takes on a very special meaning. Further, we are better prepared to discuss the ecological limits of our experimental animals than have been the great majority of the investigators who have preceded us.

It is possible for us to make a generalization of our own and then proceed to hypothesize using this general statement as a base. This generalization was first stated by Lowe (personal communication, 1962): "the poikilotherm's body temperature-dependent activity (as in its habitat) is light-clocked by a clock mechanism that is temperature-independent." Although this statement still holds, it becomes a bit vague in regard to the relative independence of the light-clocked mechanism and it is perhaps better stated as: the poikilotherm's body temperature-dependent activity (as in its habitat) is light-clocked by a clock mechanism that is capable of short-term compensation to temperature, but which demonstrates long-term adjustments to environmental photoperiod and thermoperiod.

The latter point, the inclusion of both photoperiod and thermoperiod, is of some importance. It is conceivable that the presence of an extremely (relatively) short photoperiod in conjunction with a thermoperiod that is dominated, ecologically, by daily thermal minima which are low enough to inhibit activity, may alter the clock mechanism in such a manner as to significantly change the measurable components of locomotory activity from what they were at the summer solstice (June 21) when a long photoperiod, in conjunction with a thermoperiod dominated by daily thermal maxima of a critical (to the animal) nature, is experienced.

Regardless of what in the final analysis is true, it is impossible at this time to eliminate the combined effects of light and temperature from consideration.

Modification of Locomotory Activity in the Non-feeding Heloderma

Figure 9 demonstrates the effect of food deprivation on locomotory activity. Generally speaking, good activity orientation is maintained through the first fifteen days of the records, at which

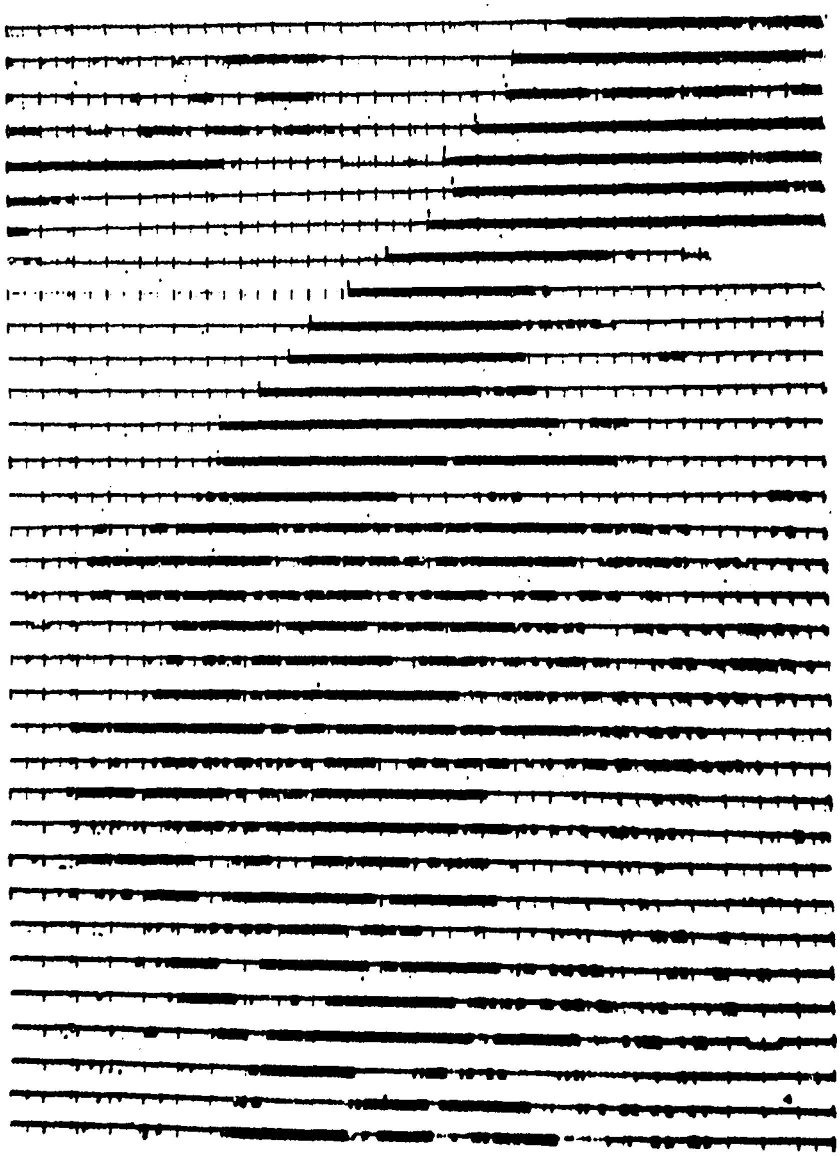

Figure 9. Effect of Food Deprivation on Locomotory Activity

Each horizontal strip represents a single 24-hour period beginning and ending at midnight. The dark areas along the strip represent a period of wheel turning activity. See text for an explanation of this activity composite.

point the animal's timekeeping ability appears to be influenced by hunger. All phase relationships of the natural period seem to be lost and locomotory activity becomes sporadic and extended in duration unless the animal is fed.

This situation is similar to the problem of food deprivation discussed above in the section on field activity. If this animal were in the field, the observed long duration, sporadic activity would appear as a unidirectional, plodding, locomotion which might cause the animal to be active past dusk civil twilight. Lowe (personal communication) describes an individual *Heloderma* observed in the field following dusk civil twilight which kept moving in a straight course, not changing direction for any obstacle. If it came upon a creosotebush, the animal would climb through the bush, often with difficulty, rather than circumnavigate it. This individual was described by Lowe as emaciated, and it died in captivity 3 days later. In this condition, *Heloderma* is obviously not responding to internal or external temporal cues and most probably would not, until its deprivation state had been alleviated.

Relationship of the Electroencephalogram to Laboratory Locomotory Activity

As a result of a relative lack of correlation of the time of initiation of locomotory activity in respect to time in *Heloderma* and other reptiles such as *Lacerta* (Hoffmann, 1957a), an attempt was made to establish a more reliable criterion for initiation of activity.

Since all locomotory activity is under the direct influence of the central nervous system, the measure taken was that of circadian variation in gross cerebral potentials.

Electrodes were surgically implanted in the manner previously described. The animals were given time to recover from the immediate effects of the operation and then placed in running wheels. All records obtained were analyzed for variation in the frequency and amplitude of the cerebral potentials.

Hunsaker and Lansing (1962) provide the only work which correlates EEG pattern with behavioral responses. Working with *Sceloporus olivaceus*, *S. mucronatus*, and *Crotaphytus collaris* they indicate that a typical saurian EEG record will include three distinct components. They are supported in this respect by Gusel'nikov (1963) and Gusel'nikova and Gusel'nikov (1963). These components, based on frequency characteristics, are as follows.

1. Basic slow potentials; 2-5 cps
2. Dominant potentials; 7-12 cps
3. Faster potentials; 15-25 cps

Heloderma EEG records agree with the above analysis. The slow waves tended to be recorded during quiescent periods and the faster waves tended to be present in the record of an alert or active animal.

Various artifacts (bioelectric potentials of other than cerebral origin) were found to be present in the record. These were typically referable to some other activity within the animal. The artifacts most commonly encountered are listed below.

1. Muscle potential: these potentials of high amplitude and frequency were recorded following the initiation of locomotory activity. They may, on occasion, have been associated with electrode movement which began to occur following several days of wheel turning activity with stress being applied to the acrylic electrode cap.

2. Tongue movement: <u>Heloderma</u> is dependent upon a chemoreception mechanism involving the tongue. As many of the EEG recordings were made in darkness, there were typically a large number of environmental samplings by the tongue. The artifact produced by this activity is extremely slow and of high voltage, easily separated from cerebral potentials. The number of tongue movements tended to increase as the time of initiation of activity drew closer. Tongue movement artifacts were recorded only when the electrode implanted in the premaxilla was coupled with one of the cerebral electrodes.

3. Eye blink: this artifact was not so common as the tongue movement artifact, but was just as easily recognized. It was a diphasic wave of approximately one-half the voltage of the tongue artifact.

4. Cardiac potentials: ECGs were recorded occasionally in some of the trial animals in which the reference electrode was placed in soft tissue rather than bone. It was not encountered in the final preparations described above.

Table 5. Relationship of EEG Amplitude to Locomotory Activity State

The mean amplitude when active is 2.64 ± 0.27.
The mean amplitude when passive is 1.76 ± 0.14.
These are significantly different ($t=2.973$; $P < 0.01$).
At 16 and 17 hours muscle potential obscured the EEG.

Time (hours)	Mean Amplitude of EEG (mm.)	Activity State
1	0.96	
2	1.45	
3	1.59	
4	1.23	
5	1.86	
6	2.76	
7	2.33	+
8	1.90	
9	2.09	
10	2.64	
11	2.01	
12	2.48	+
13	2.74	+
14	2.01	+
15	2.39	+
16	- -	+
17	- -	+
18	4.10	+
19	3.90	+
20	2.29	+
21	1.46	
22	1.65	
23	3.01	+
24	2.23	+
25	1.08	+

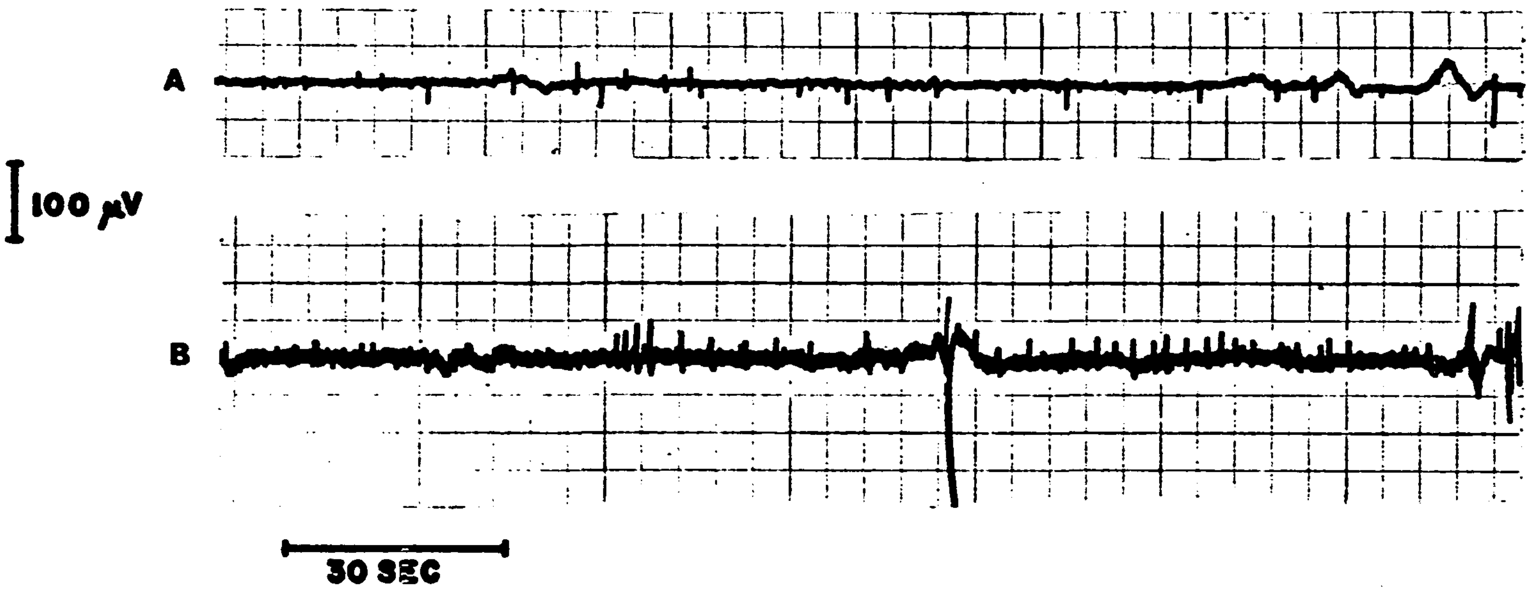

Figure 10. EEG Traces Recorded from <u>Heloderma</u> Housed in Running Wheel

Trace A represents the EEG recorded from a resting <u>Heloderma</u>. Trace B represents the EEG recorded from the same individual just before the initiation of locomotory activity.

An analysis of the type and occurrence of the various cerebral potentials was not critical to this study, except as they acted as indicators of the state of alertness of the experimental animals. More important were the gross changes in cerebral activity over periods of 24-hours or longer. The most common measure of activity state taken was the change in the amplitude of the recorded signal over a 24-hour period. This was then correlated with overt locomotory activity as recorded from the running wheel.

One such analysis is shown in Table 5 and Figure 10. Here it can be seen that there is a gradual increase in the amplitude of the recorded signal followed by a significant increase denoting complete wakefulness preceding the initiation of locomotory activity.

Extended periods of measurement were not possible because the animals tended to loosen the electrodes after several days of recording. The results of these recording periods, therefore, do not lend themselves well to analysis. The indication, however, is that the initiation of high amplitude cerebral potentials - indicating a high level of central nervous system excitation - occurs at more constant intervals than the initiation of overt locomotory activity.

SUMMARY AND CONCLUSIONS

Diurnal rhythmicity in the locomotory activity of *Heloderma suspectum* was investigated in freshly collected individuals and in thermally acclimated series under constant conditions of light, temperature, and relative humidity. The natural period (= free-running or spontaneous period) as determined by regression analysis has been utilized as the measure of rhythmicity for locomotory activity.

Collection records maintained for over a decade indicate that *Heloderma* is normally active from early March through early November. These records further indicate that there is a gradual transition in the diel relationship of the activity pattern from complete diurnality (spring and fall) to a crepuscular orientation (summer). Laboratory investigation has indicated that there is a change in the length of the natural period from greater than 24 hours (spring) to less than 24 hours (summer and fall).

An analysis of macro-climatological data for Tucson, Arizona has been correlated with the time of collection for periods when the latter data were available. The significance of the shift of activity patterns and of the seasonal change in the length of the natural period is discussed in relation to these climatological factors and to the known ecology of Heloderma.

Natural period measurements made in the laboratory were found to vary with temperature, light, the physiological state of the individual, and with the time of year at which the animal was collected. In all freshly captured animals (= field acclimated) the natural period was found to vary between 22.5 and 25.3 hours in length. No difference was found in the length of the natural period due to age or sex.

Under otherwise constant conditions of light and relative humidity, a change in environmental temperature was reflected as a change in the length of the natural period. The difference in the length of two periods at 22.5°C and 27.5°C was significant at the 5% level. The importance of these results in light of the temperature-independence hypothesis is discussed.

Food and water deprivation, during periods of locomotory activity recording, tended to destroy rhythmicity and to increase total activity.

Electrophysiological studies have indicated that the normal electroencephalogram (EEG) pattern for Heloderma does not differ from that of other reptiles studied. Potentials recorded from electrodes resting on the dura mater of the cerebral hemispheres were found to increase in amplitude prior to the initiation of locomotory activity.

APPENDIX A

HELODERMA FIELD ACTIVITY PLOTTED AGAINST TIME FOR THE PERIOD 1959-1964

Included in this section are six graphs of Heloderma field activity plotted against tri-monthly intervals for the six years, 1959-1964. The data are to be found in Appendix B. One should observe that even with limited samples the presence of primary and secondary activity peaks remains consistent through the six year period. Figure 1 in the text represents these data graphed for the total six year period.

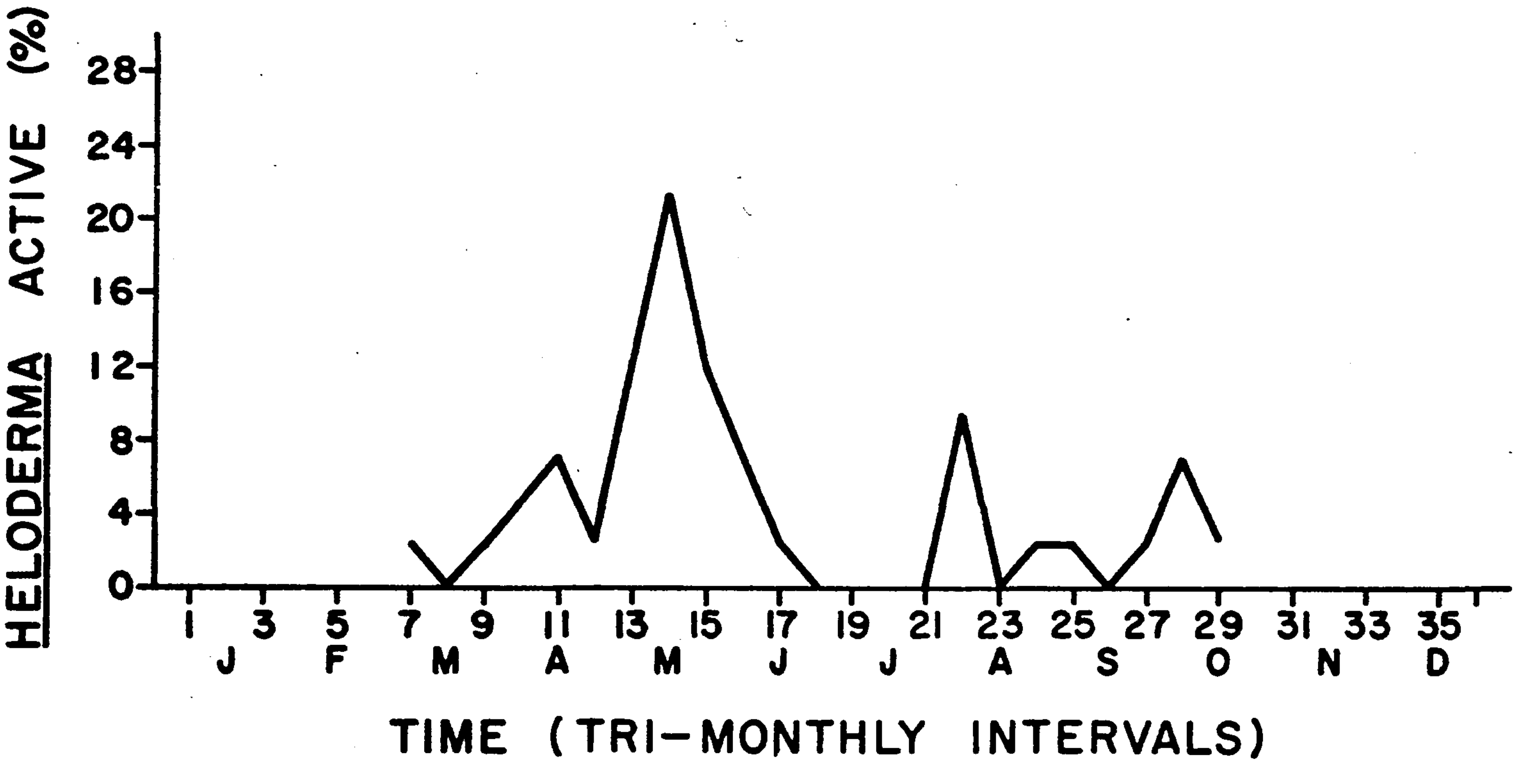

Figure A-1. <u>Heloderma</u> Field Activity Expressed as Per Cent of Total Collection Against Tri-Monthly Intervals - <u>1959</u>

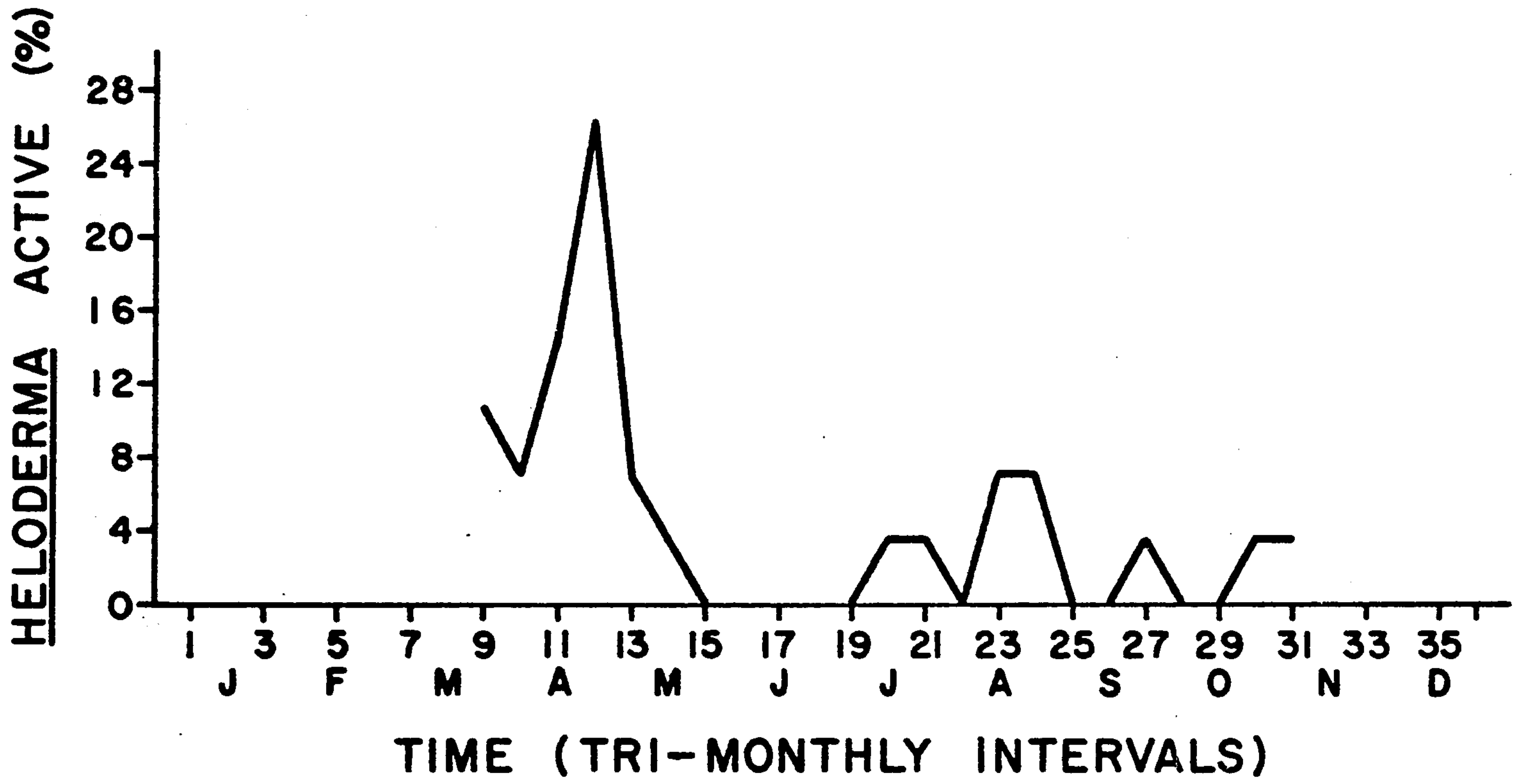

Figure A-2. Heloderma Field Activity Expressed as Per Cent of Total Collection Against Tri-Monthly Intervals - 1960

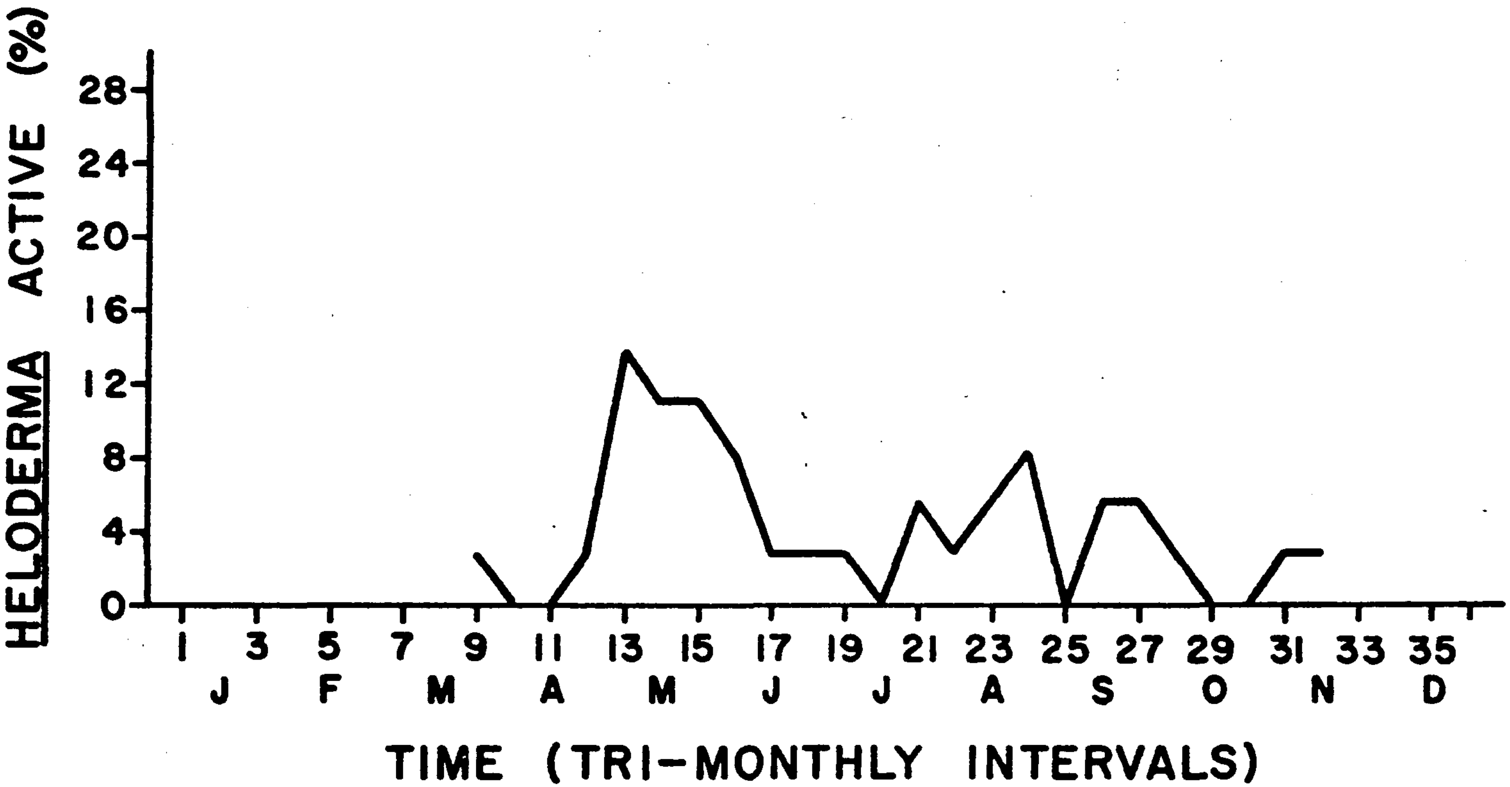

Figure A-3. Heloderma Field Activity Expressed as Per Cent of Total Collection Against Tri-Monthly Intervals - 1961

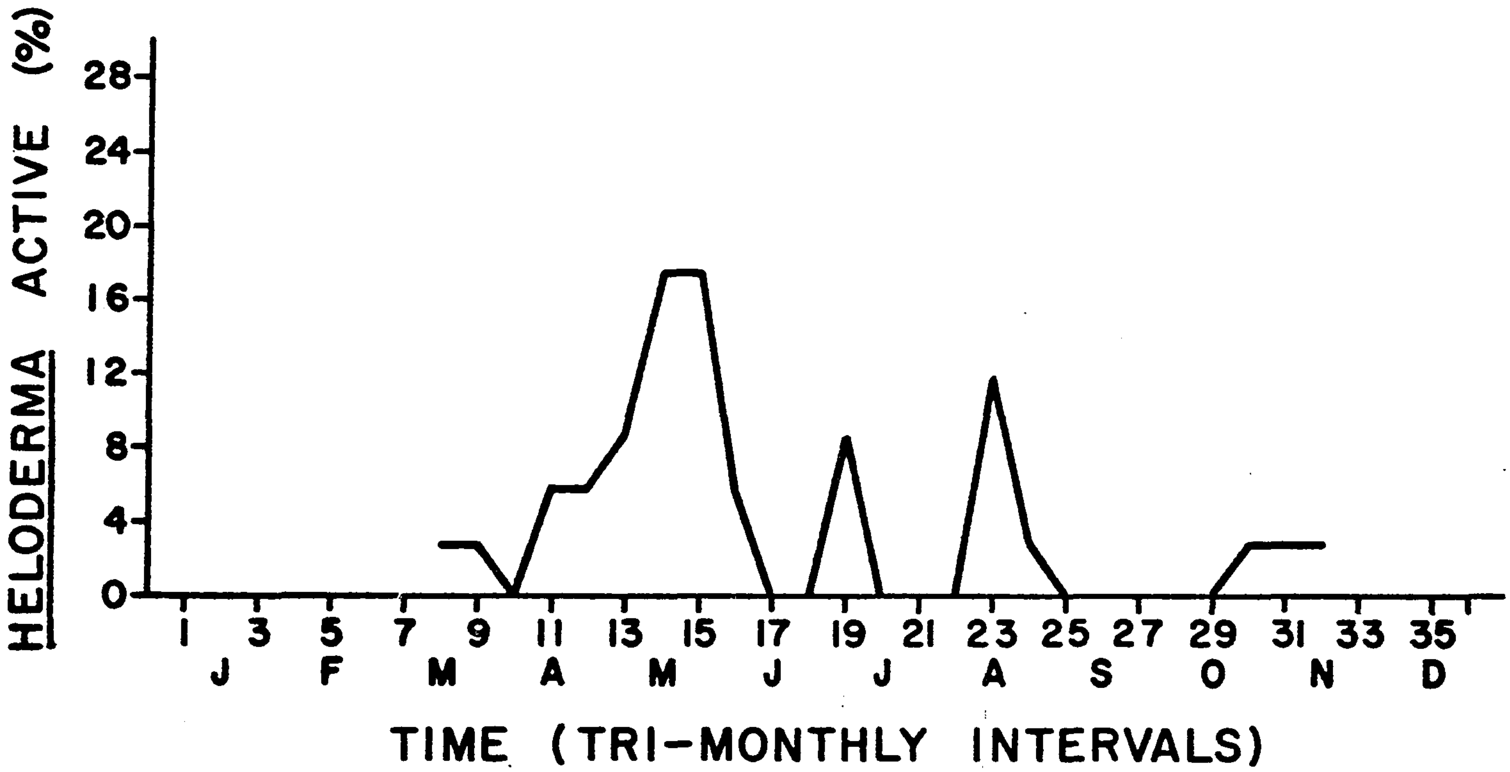

Figure A-4. Heloderma Field Activity Expressed as Per Cent of Total Collection Against Tri-Monthly Intervals - 1962

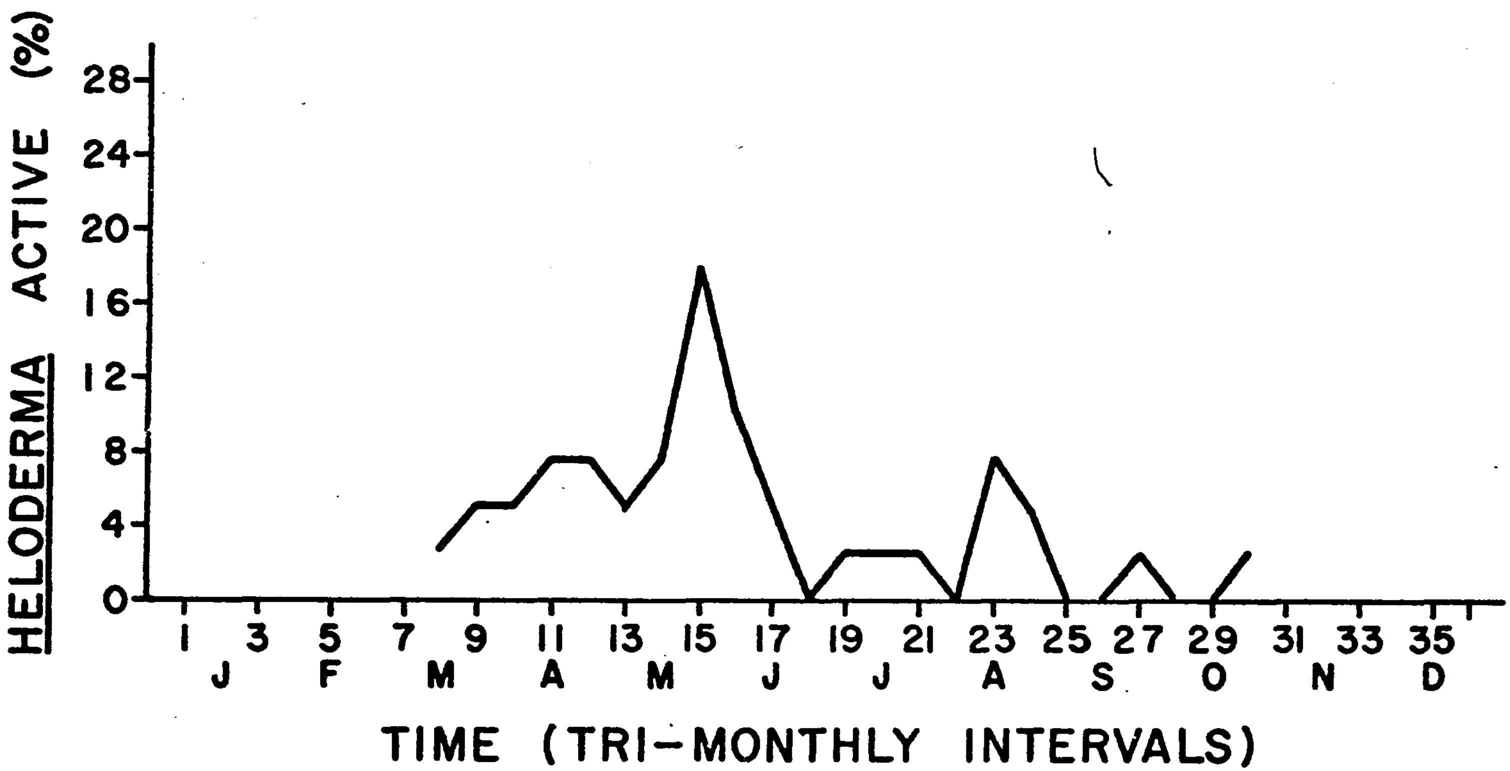

Figure A-5. Heloderma Field Activity Expressed as Per Cent of Total Collection Against Tri-Monthly Intervals - 1963

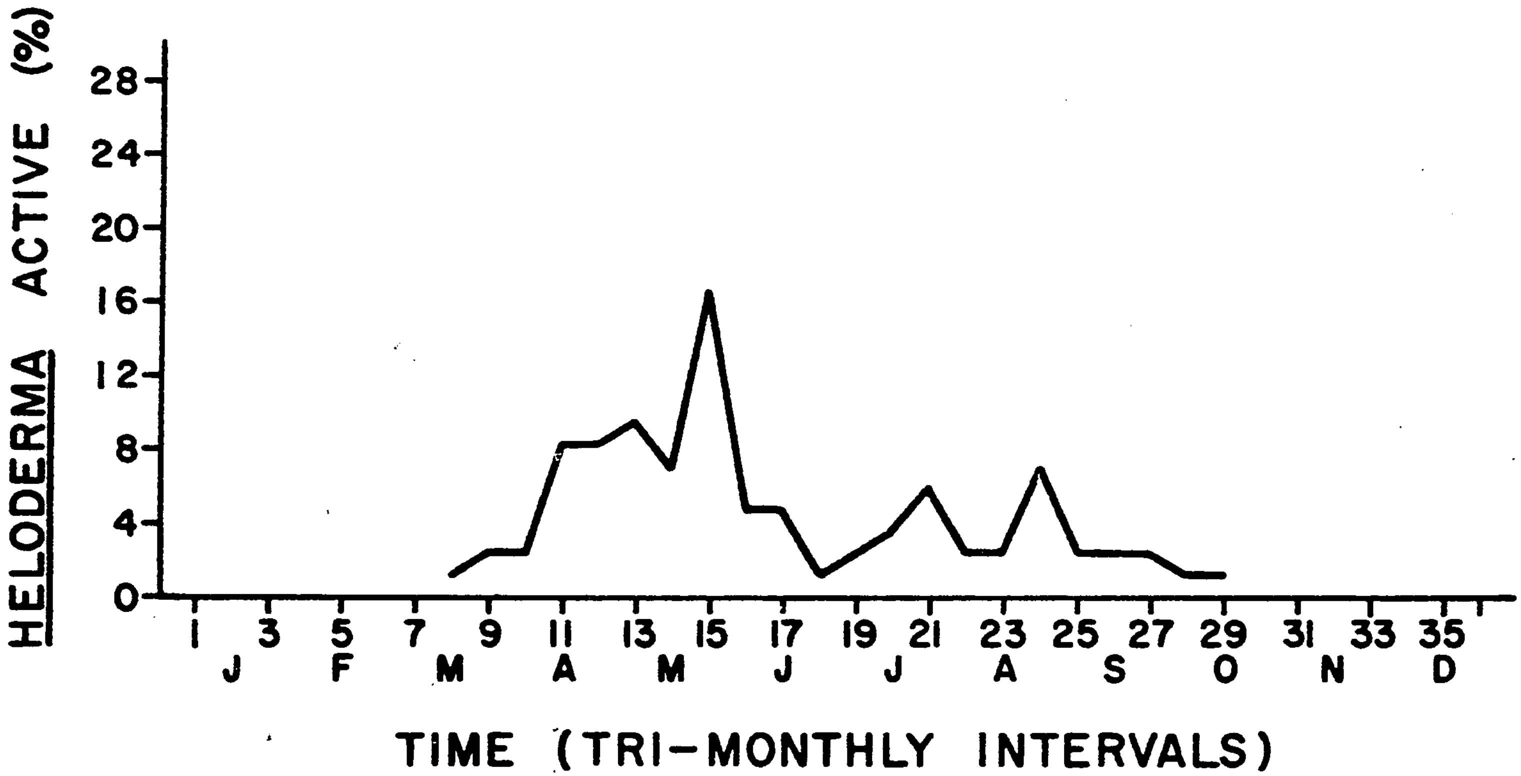

Figure A-6. Heloderma Field Activity Expressed as Per Cent of Total Collection Against Tri-Monthly Intervals - 1964

APPENDIX B

<u>HELODERMA</u> FIELD ACTIVITY AND MACRO-CLIMATIC CORRELATES FOR THE PERIOD 1959-1964

The tables included in this section consist of a compilation of field activity records for <u>Heloderma</u> during the six years, 1959-1964. Included with the field activity data are the mean thermal maxima and minima listed by tri-monthly interval. The field activity data are graphed in Appendix A. The mean field activity and mean thermal data for the six year period are found in Table 2 in the text.

Table B-1. _Heloderma_ Field Activity and Macro-Climatic Correlates by Tri-Monthly Intervals - 1959

TRI-MONTHLY INTERVAL	NUMBER OF HELODERMA COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (^{o}F)	DAILY THERMAL MINIMUM (^{o}F)
Jan 1	0	0.0	68.1 ± 1.74	38.5 ± 1.87
2	0	0.0	72.9 ± 1.77	41.5 ± 1.26
3	0	0.0	67.9 ± 1.64	34.1 ± 1.25
Feb 4	0	0.0	62.2 ± 2.02	33.8 ± 1.63
5	0	0.0	69.2 ± 1.66	41.6 ± 2.22
6	0	0.0	66.4 ± 1.75	35.8 ± 2.17
Mar 7	1	2.4	72.2 ± 2.28	39.0 ± 1.71
8	0	0.0	75.1 ± 1.58	41.0 ± 1.89
9	1	2.4	75.7 ± 1.48	45.6 ± 1.00
Apr 10	2	4.8	85.3 ± 2.01	53.0 ± 1.99
11	3	7.1	79.4 ± 0.89	52.4 ± 1.41
12	1	2.4	88.6 ± 1.54	56.6 ± 1.73
May 13	5	11.9	83.6 ± 2.00	54.1 ± 2.27
14	9	21.4	93.3 ± 1.40	59.6 ± 1.21
15	5	11.9	89.3 ± 1.36	55.1 ± 0.84
Jun 16	3	7.1	97.4 ± 0.91	63.2 ± 1.00
17	1	2.4	102.3 ± 0.90	75.6 ± 1.34
18	0	0.0	99.3 ± 1.85	73.9 ± 0.84
Jul 19	0	0.0	99.2 ± 0.95	75.0 ± 0.73
20	0	0.0	99.6 ± 0.82	73.1 ± 0.90
21	0	0.0	98.8 ± 1.09	73.5 ± 0.53
Aug 22	4	9.5	92.8 ± 1.31	72.5 ± 0.62
23	0	0.0	89.4 ± 0.79	70.7 ± 0.37
24	1	2.4	93.1 ± 1.32	71.8 ± 0.71
Sep 25	1	2.4	99.4 ± 1.14	73.1 ± 0.90
26	0	0.0	91.5 ± 1.22	66.3 ± 1.61
27	1	2.4	90.3 ± 0.82	60.3 ± 0.86

Table B-1, Continued

TRI-MONTHLY INTERVAL	NUMBER OF HELODERMA COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Oct 28	3	7.1	79.9 ± 2.56	52.6 ± 1.29
29	1	2.4	88.7 ± 0.65	58.2 ± 0.66
30	0	0.0	83.3 ± 1.94	55.4 ± 2.33
Nov 31	0	0.0	71.3 ± 1.53	48.7 ± 1.23
32	0	0.0	70.6 ± 1.43	50.3 ± 1.10
33	0	0.0	70.9 ± 1.43	39.0 ± 1.00
Dec 34	0	0.0	68.6 ± 1.92	43.3 ± 1.96
35	0	0.0	62.2 ± 2.60	37.2 ± 1.43
36	0	0.0	58.6 ± 1.60	39.0 ± 2.21

Table B-2. *Heloderma* Field Activity and Macro-Climatic Correlates by Tri-Monthly Intervals - 1960

TRI-MONTHLY INTERVAL		NUMBER OF *HELODERMA* COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (^{o}F)	DAILY THERMAL MINIMUM (^{o}F)
Jan	1	0	0.0	55.5 ± 3.33	31.4 ± 2.42
	2	0	0.0	51.6 ± 2.18	38.8 ± 2.07
	3	0	0.0	68.5 ± 0.86	38.6 ± 1.43
Feb	4	0	0.0	64.7 ± 1.92	36.3 ± 1.43
	5	0	0.0	59.0 ± 1.63	32.4 ± 0.85
	6	0	0.0	67.2 ± 2.22	33.4 ± 2.51
Mar	7	0	0.0	77.5 ± 2.57	44.8 ± 1.36
	8	0	0.0	74.8 ± 2.45	40.3 ± 2.26
	9	3	10.7	78.6 ± 1.73	49.4 ± 0.96
Apr	10	2	7.1	84.4 ± 2.41	49.0 ± 1.71
	11	4	14.3	85.5 ± 2.10	51.1 ± 1.68
	12	7	25.0	77.1 ± 2.15	46.9 ± 2.73
May	13	2	7.1	85.3 ± 2.62	52.3 ± 1.47
	14	1	3.6	90.7 ± 2.41	56.8 ± 1.99
	15	0	0.0	90.6 ± 1.47	55.5 ± 1.65
Jun	16	0	0.0	94.1 ± 1.15	62.7 ± 1.12
	17	0	0.0	102.3 ± 1.41	68.4 ± 1.39
	18	0	0.0	103.2 ± 0.79	70.0 ± 0.98
Jul	19	0	0.0	97.5 ± 1.46	72.2 ± 1.25
	20	1	3.6	102.3 ± 0.79	74.8 ± 2.05
	21	1	3.6	96.6 ± 1.01	72.6 ± 0.70
Aug	22	0	0.0	98.6 ± 1.17	74.2 ± 1.00
	23	2	7.1	97.5 ± 0.98	72.5 ± 1.08
	24	2	7.1	93.1 ± 1.91	69.8 ± 0.57
Sep	25	0	0.0	95.2 ± 1.24	71.1 ± 0.46
	26	0	0.0	95.9 ± 0.55	68.3 ± 0.63
	27	1	3.6	94.4 ± 1.02	61.9 ± 0.85

Table B-2, Continued

TRI-MONTHLY INTERVAL	NUMBER OF HELODERMA COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Oct 28	0	0.0	86.0 ± 1.64	60.4 ± 0.48
29	0	0.0	74.2 ± 1.44	49.7 ± 1.26
30	1	3.6	81.4 ± 0.94	52.2 ± 0.86
Nov 31	1	3.6	77.0 ± 1.59	50.3 ± 2.38
32	0	0.0	71.9 ± 1.27	42.0 ± 1.26
33	0	0.0	72.7 ± 1.81	41.2 ± 2.29
Dec 34	0	0.0	56.9 ± 3.91	32.7 ± 3.55
35	0	0.0	64.0 ± 1.66	34.0 ± 1.24
36	0	0.0	67.7 ± 2.07	38.5 ± 1.09

Table B-3. *Heloderma* Field Activity and Macro-Climatic Correlates by Tri-Monthly Intervals - 1961

TRI-MONTHLY INTERVAL	NUMBER OF *HELODERMA* COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Jan 1	0	0.0	64.4 ± 0.96	38.3 ± 1.66
2	0	0.0	68.2 ± 1.01	39.8 ± 1.76
3	0	0.0	60.4 ± 1.24	43.7 ± 1.53
Feb 4	0	0.0	65.1 ± 1.64	37.1 ± 0.88
5	0	0.0	69.6 ± 2.91	42.7 ± 1.53
6	0	0.0	66.8 ± 1.97	35.9 ± 0.85
Mar 7	0	0.0	73.0 ± 2.08	42.7 ± 1.78
8	0	0.0	72.9 ± 2.47	43.4 ± 1.34
9	1	2.8	71.6 ± 2.90	45.5 ± 1.35
Apr 10	0	0.0	80.4 ± 2.07	51.9 ± 2.13
11	0	0.0	82.5 ± 1.80	50.6 ± 1.57
12	1	2.8	83.4 ± 2.32	48.5 ± 1.32
May 13	5	14.0	87.3 ± 2.55	52.5 ± 1.79
14	4	11.1	89.8 ± 2.35	56.2 ± 1.52
15	4	11.1	92.2 ± 0.72	59.1 ± 0.62
Jun 16	3	8.3	94.4 ± 1.11	59.7 ± 1.40
17	1	2.8	102.0 ± 0.92	72.1 ± 1.32
18	1	2.8	102.4 ± 0.95	77.5 ± 0.50
Jul 19	1	2.8	98.7 ± 1.49	72.3 ± 1.65
20	0	0.0	102.1 ± 0.78	77.1 ± 1.02
21	2	5.5	94.7 ± 1.14	71.8 ± 0.78
Aug 22	1	2.8	96.2 ± 1.42	70.8 ± 0.53
23	2	5.5	92.7 ± 1.60	70.3 ± 0.54
24	3	8.3	92.0 ± 1.19	69.1 ± 1.12
Sep 25	0	0.0	90.8 ± 0.83	69.9 ± 1.62
26	2	5.5	86.8 ± 0.85	66.5 ± 2.88
27	2	5.5	90.3 ± 1.07	60.9 ± 1.12

Table B-3, Continued

TRI-MONTHLY INTERVAL	NUMBER OF *HELODERMA* COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Oct 28	1	2.8	85.1 ± 2.49	57.3 ± 2.15
29	0	0.0	86.4 ± 0.58	57.9 ± 2.27
30	0	0.0	74.9 ± 3.74	50.3 ± 2.18
Nov 31	1	2.8	66.5 ± 1.90	45.3 ± 1.15
32	1	2.8	64.2 ± 2.31	39.8 ± 1.44
33	0	0.0	68.7 ± 2.00	41.6 ± 1.73
Dec 34	0	0.0	63.6 ± 2.22	44.1 ± 1.39
35	0	0.0	52.7 ± 1.61	38.0 ± 1.45
36	0	0.0	66.7 ± 0.83	37.2 ± 0.98

Table B-4. Heloderma Field Activity and Macro-Climatic Correlates by Tri-Monthly Intervals - 1962

TRI-MONTHLY INTERVAL		NUMBER OF HELODERMA COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Jan	1	0	0.0	62.3 ± 2.15	37.1 ± 2.43
	2	0	0.0	57.2 ± 2.72	31.8 ± 2.14
	3	0	0.0	61.5 ± 2.63	43.0 ± 1.36
Feb	4	0	0.0	74.4 ± 1.21	44.0 ± 1.10
	5	0	0.0	67.4 ± 2.20	45.5 ± 2.18
	6	0	0.0	56.6 ± 2.58	37.0 ± 1.62
Mar	7	0	0.0	65.7 ± 2.22	40.6 ± 2.09
	8	1	2.9	64.0 ± 2.42	35.0 ± 1.66
	9	1	2.9	72.9 ± 3.03	41.7 ± 1.74
Apr	10	0	0.0	82.5 ± 1.09	49.9 ± 1.22
	11	2	5.9	93.0 ± 1.28	56.3 ± 1.17
	12	2	5.9	83.8 ± 1.45	54.9 ± 1.56
May	13	3	8.8	93.6 ± 1.05	60.2 ± 1.73
	14	6	17.7	85.4 ± 2.09	54.0 ± 2.01
	15	6	17.7	84.9 ± 1.71	51.8 ± 2.42
Jun	16	2	5.9	93.8 ± 0.80	61.3 ± 2.45
	17	0	0.0	94.8 ± 2.18	59.8 ± 1.58
	18	0	0.0	100.8 ± 1.65	71.3 ± 1.17
Jul	19	3	8.8	96.2 ± 1.35	74.6 ± 1.15
	20	0	0.0	99.2 ± 0.84	71.0 ± 1.33
	21	0	0.0	95.4 ± 0.80	73.1 ± 0.78
Aug	22	0	0.0	99.7 ± 1.11	72.9 ± 0.96
	23	4	11.8	102.3 ± 1.25	75.4 ± 0.65
	24	1	2.9	99.5 ± 0.90	72.0 ± 0.91
Sep	25	0	0.0	94.1 ± 1.27	70.3 ± 1.22
	26	0	0.0	97.3 ± 1.67	72.6 ± 0.81
	27	0	0.0	86.1 ± 1.58	67.0 ± 1.65

Table B-4, Continued

TRI-MONTHLY INTERVAL	NUMBER OF *HELODERMA* COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Oct 28	0	0.0	86.6 ± 1.40	59.2 ± 1.54
29	0	0.0	81.7 ± 2.59	56.9 ± 1.74
30	1	2.9	83.8 ± 0.86	55.5 ± 1.42
Nov 31	1	2.9	82.5 ± 0.56	52.4 ± 0.79
32	1	2.9	70.1 ± 3.29	46.3 ± 2.90
33	0	0.0	71.7 ± 1.78	45.6 ± 1.28
Dec 34	0	0.0	68.8 ± 1.81	41.5 ± 1.34
35	0	0.0	67.7 ± 1.51	47.5 ± 0.87
36	0	0.0	60.1 ± 1.80	38.9 ± 0.96

Table B-5. *Heloderma* Field Activity and Macro-Climatic Correlates by Tri-Monthly Intervals - 1963

TRI-MONTHLY INTERVAL	NUMBER OF *HELODERMA* COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Jan 1	0	0.0	61.6 ± 1.13	40.9 ± 1.14
2	0	0.0	51.6 ± 2.31	28.3 ± 1.97
3	0	0.0	69.5 ± 1.52	36.5 ± 1.60
Feb 4	0	0.0	78.3 ± 2.34	49.3 ± 0.86
5	0	0.0	61.8 ± 2.01	38.5 ± 1.14
6	0	0.0	74.1 ± 1.38	43.6 ± 0.60
Mar 7	0	0.0	67.9 ± 2.12	38.8 ± 1.40
8	1	2.6	69.0 ± 0.68	40.2 ± 1.11
9	2	5.1	79.5 ± 1.17	49.2 ± 1.55
Apr 10	2	5.1	79.6 ± 2.53	48.9 ± 2.04
11	3	7.7	81.0 ± 2.92	47.5 ± 1.68
12	3	7.7	77.6 ± 1.99	49.1 ± 1.60
May 13	2	5.1	91.7 ± 0.97	60.4 ± 2.25
14	3	7.7	94.5 ± 1.46	62.1 ± 1.75
15	7	17.9	93.5 ± 0.80	61.0 ± 1.34
Jun 16	4	10.3	90.3 ± 0.75	60.6 ± 1.64
17	2	5.1	98.3 ± 1.29	65.6 ± 1.33
18	0	0.0	101.1 ± 0.62	66.7 ± 0.73
Jul 19	1	2.6	99.2 ± 1.08	74.9 ± 1.04
20	1	2.6	102.1 ± 0.98	77.1 ± 1.10
21	1	2.6	98.3 ± 1.85	74.3 ± 1.04
Aug 22	0	0.0	92.2 ± 1.09	71.3 ± 0.88
23	3	7.7	93.1 ± 1.25	73.9 ± 1.27
24	2	5.1	91.5 ± 0.85	71.3 ± 0.88
Sep 25	0	0.0	94.1 ± 1.82	70.5 ± 0.82
26	0	0.0	94.3 ± 1.26	69.1 ± 0.86
27	1	2.6	96.1 ± 0.67	70.4 ± 1.89

Table B-5, Continued

TRI-MONTHLY INTERVAL	NUMBER OF HELODERMA COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Oct 28	0	0.0	94.1 ± 0.41	63.4 ± 0.64
29	0	0.0	84.7 ± 2.21	58.0 ± 1.53
30	1	2.6	82.9 ± 1.03	56.7 ± 0.88
Nov 31	0	0.0	74.5 ± 2.43	48.0 ± 1.67
32	0	0.0	75.6 ± 2.62	48.6 ± 1.69
33	0	0.0	65.3 ± 1.67	43.7 ± 1.69
Dec 34	0	0.0	69.9 ± 2.86	44.3 ± 1.10
35	0	0.0	63.4 ± 1.86	35.0 ± 1.46
36	0	0.0	67.2 ± 1.33	36.8 ± 1.26

Table B-6. *Heloderma* Field Activity and Macro-Climatic Correlates by Tri-Monthly Intervals - 1964

TRI-MONTHLY INTERVAL	NUMBER OF *HELODERMA* COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (°F)	DAILY THERMAL MINIMUM (°F)
Jan 1	0	0.0	62.8 ± 2.41	32.0 ± 1.59
2	0	0.0	59.3 ± 1.97	30.0 ± 2.46
3	0	0.0	64.3 ± 2.30	36.0 ± 1.80
Feb 4	0	0.0	63.5 ± 2.84	32.0 ± 1.59
5	0	0.0	62.0 ± 1.74	33.4 ± 2.00
6	0	0.0	62.2 ± 1.43	32.8 ± 1.03
Mar 7	0	0.0	59.5 ± 2.32	34.1 ± 1.20
8	1	1.2	71.7 ± 0.93	44.1 ± 2.08
9	2	2.4	73.5 ± 2.83	45.0 ± 1.44
Apr 10	2	2.4	70.4 ± 2.93	45.4 ± 2.01
11	7	8.3	85.0 ± 2.03	54.4 ± 2.12
12	7	8.3	76.9 ± 1.37	47.5 ± 1.30
May 13	8	9.5	78.4 ± 1.91	48.2 ± 1.34
14	6	7.1	94.6 ± 0.96	59.3 ± 1.08
15	14	16.7	94.2 ± 1.31	63.5 ± 1.61
Jun 16	4	4.8	95.1 ± 1.02	65.4 ± 1.28
17	4	4.8	96.9 ± 0.80	64.0 ± 0.86
18	1	1.2	100.1 ± 0.91	70.5 ± 1.84
Jul 19	2	2.4	101.2 ± 2.07	76.8 ± 1.26
20	3	3.6	98.5 ± 1.50	74.0 ± 0.92
21	5	6.0	94.5 ± 2.15	72.5 ± 0.81
Aug 22	2	2.4	91.3 ± 2.05	72.0 ± 0.61
23	2	2.4	92.9 ± 1.14	71.0 ± 0.76
24	6	7.1	92.8 ± 1.50	69.4 ± 0.89
Sep 25	2	2.4	90.4 ± 2.74	69.4 ± 0.73
26	2	2.4	86.6 ± 1.67	65.5 ± 1.45
27	2	2.4	85.1 ± 1.47	60.7 ± 0.91

Table B-6, Continued

TRI-MONTHLY INTERVAL	NUMBER OF HELODERMA COLLECTED	PER CENT OF TOTAL COLLECTION	DAILY THERMAL MAXIMUM (^{o}F)	DAILY THERMAL MINIMUM (^{o}F)
Oct 28	1	1.2	92.1 ± 0.46	63.6 ± 1.02
29	1	1.2	81.9 ± 2.43	59.4 ± 1.20
30	0	0.0	82.9 ± 1.30	53.4 ± 0.83
Nov 31	0	0.0	76.6 ± 1.62	47.7 ± 1.11
32	0	0.0	58.1 ± 2.47	36.6 ± 1.55
33	0	0.0	70.9 ± 1.14	41.4 ± 1.60
Dec 34	0	0.0	62.3 ± 1.80	38.2 ± 1.67
35	0	0.0	65.6 ± 1.49	38.8 ± 1.27
36	0	0.0	65.8 ± 2.31	43.3 ± 1.32

APPENDIX C

TIME OF COLLECTION PLOTTED AGAINST THE THERMAL MAXIMUM FOR DATE OF COLLECTION FOR THE PERIOD 1959-1964

The figures included here represent an analysis of the relationship of the time of collection plotted against the maximum air temperature at the Tucson International Airport for the date of collection. The graphs illustrate the fact that as the daily thermal maximum increases in magnitude, Heloderma field activity becomes oriented toward the dawn and dusk photic transition and away from the thermal maxima of midday. The data are to be found in Appendix D and are graphed for the entire six year sample in Figure 4 in the text.

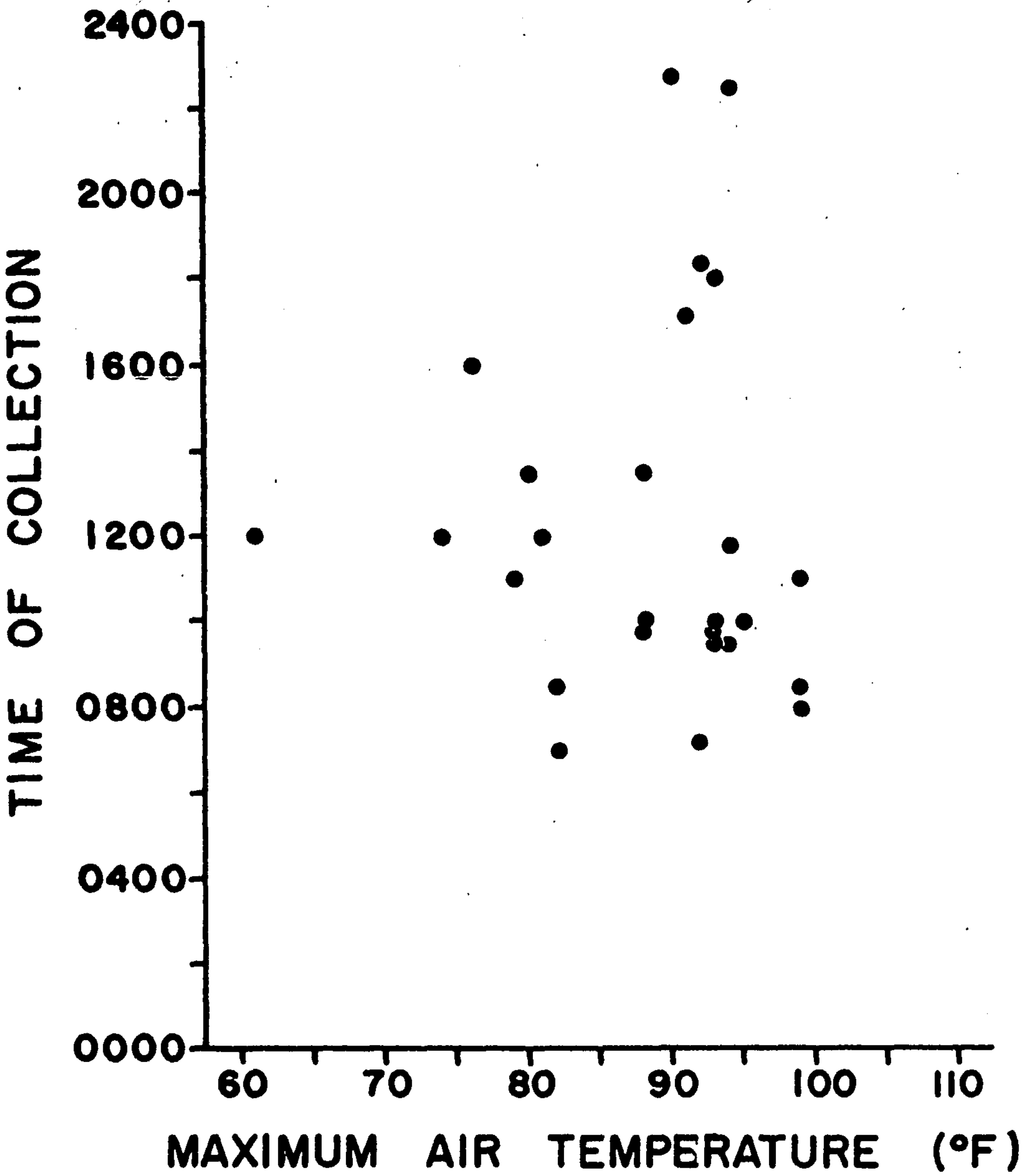

Figure C-1. Time of Collection, Morning and Afternoon, Preceding the Onset of the Summer Rains, Against Macro-Environmental Thermal Maximum for Date of Collection - 1959

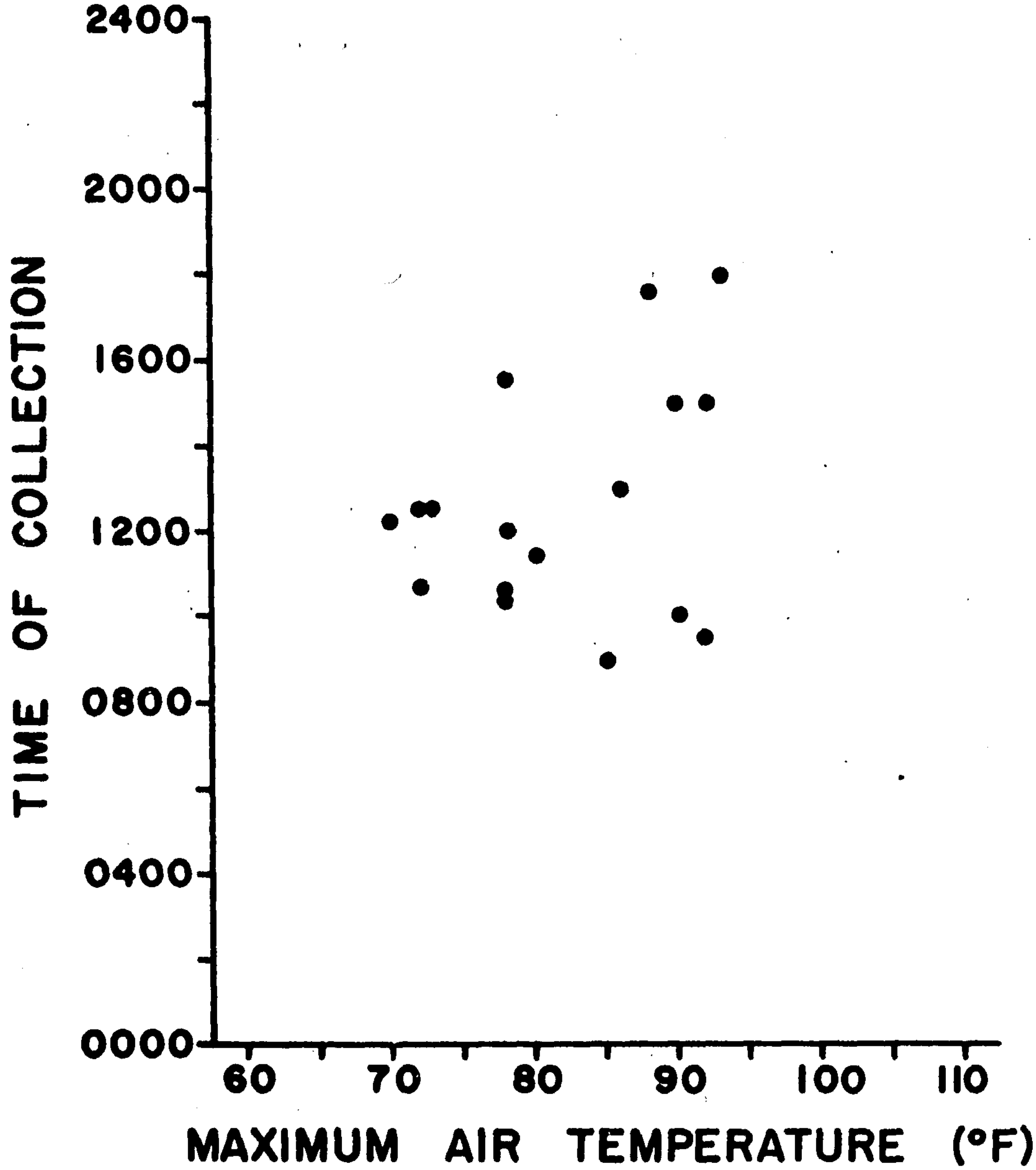

Figure C-2. Time of Collection, Morning and Afternoon, Preceding the Onset of the Summer Rains, Against Macro-Environmental Thermal Maximum for Date of Collection - 1960

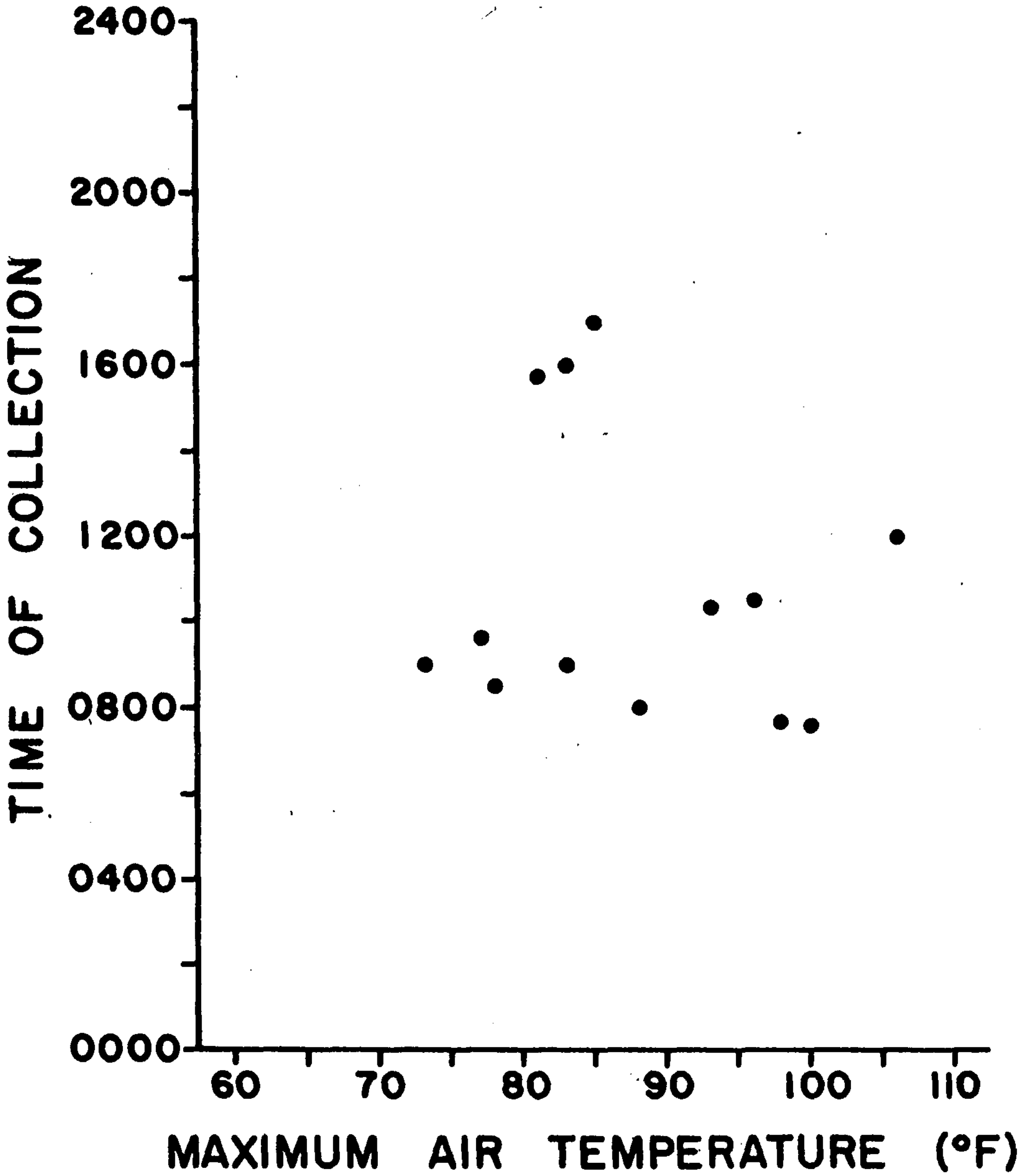

Figure C-3. Time of Collection, Morning and Afternoon, Preceding the Onset of the Summer Rains, Against Macro-Environmental Thermal Maximum for Date of Collection - 1961

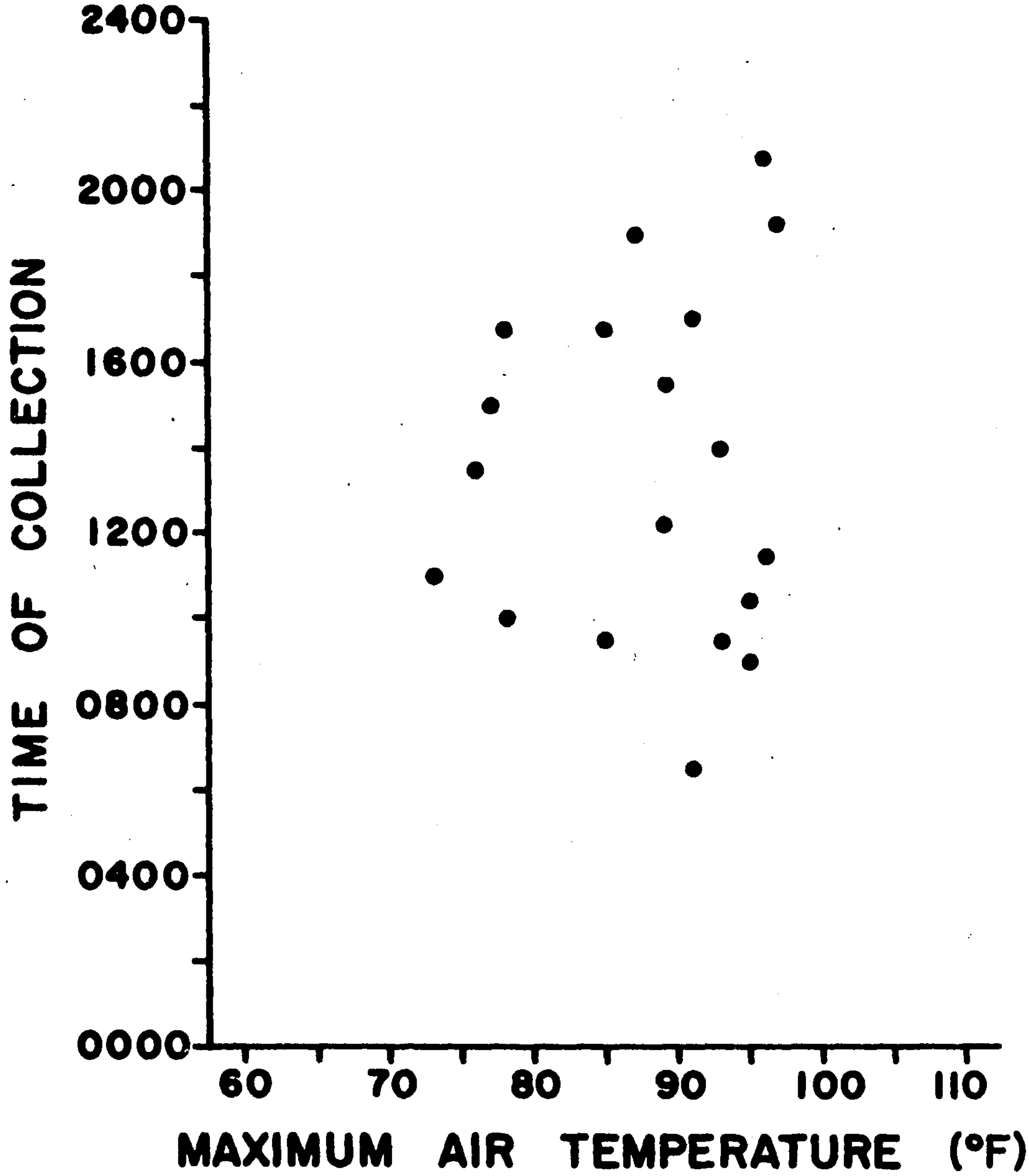

Figure C-4. Time of Collection, Morning and Afternoon, Preceding the Onset of the Summer Rains, Against Macro-Environmental Thermal Maximum for Date of Collection - 1962

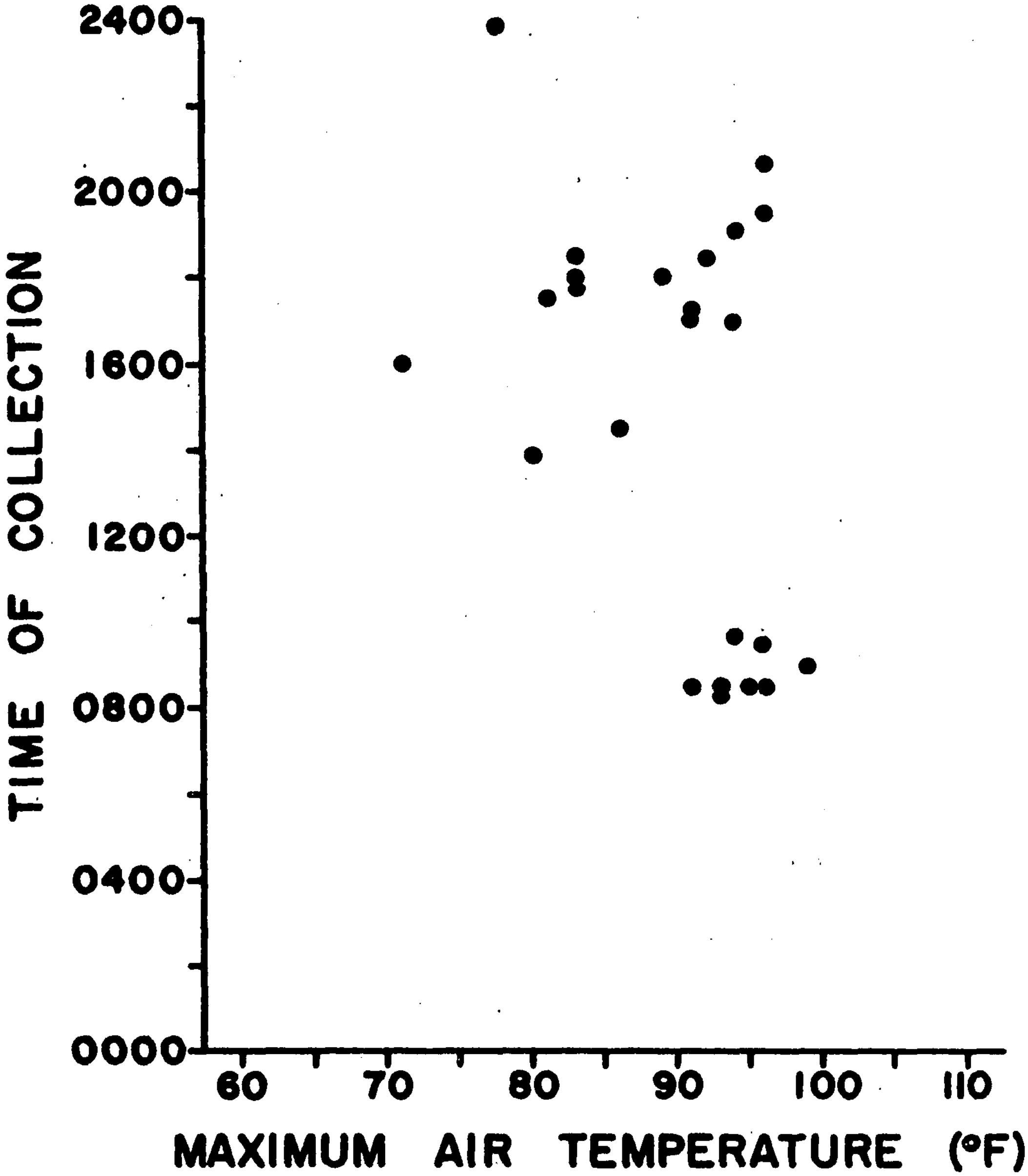

Figure C-5. Time of Collection, Morning and Afternoon, Preceding the Onset of the Summer Rains, Against Macro-Environmental Thermal Maximum for Date of Collection - 1963

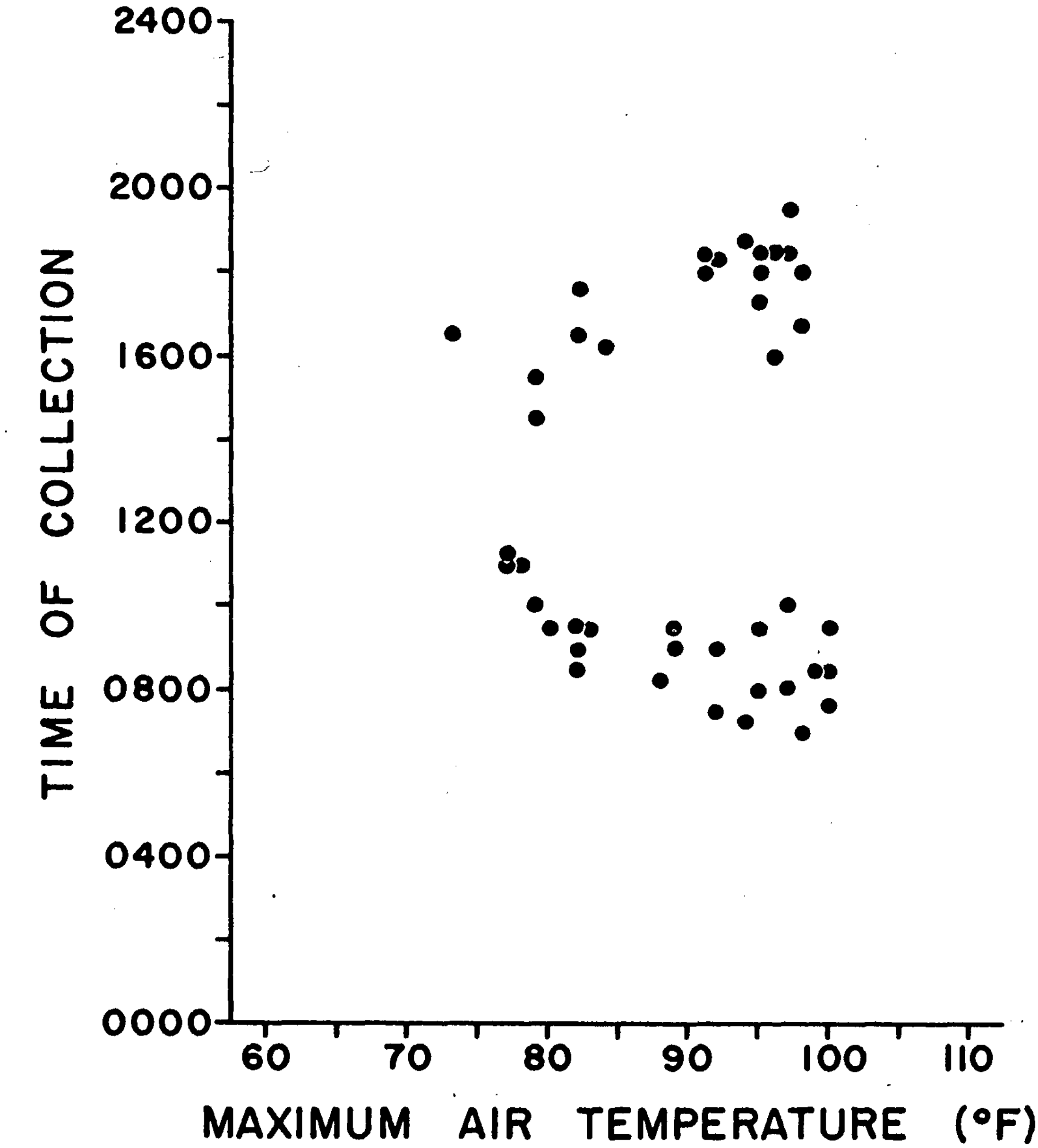

Figure C-6. Time of Collection, Morning and Afternoon, Preceding the Onset of the Summer Rains, Against Macro-Environmental Thermal Maximum for Date of Collection - 1964

APPENDIX D

DATE OF COLLECTION, TIME OF COLLECTION, AND MACRO-ENVIRONMENTAL AIR TEMPERATURE DATA FOR THE PERIOD 1959-1964

The tables included here list the date of collection, the time of collection and the thermal maximum for the date of collection for the entire Heloderma sample for the six years, 1959-1964. These data are graphed in Figures 4 and 6 in the text and in Appendices C and E.

Table D-1. Date of Collection, Time of Collection, and Macro-Environmental Air Temperature at Time of Collection - 1959

The break in the table indicates the period preceding the onset of the summer rains during which Heloderma field activity is restricted. Activity following the onset of the summer rains is indicated by those data listed below the break.

TRI-MONTHLY INTERVAL	DATE OF COLLECTION	TIME OF COLLECTION	THERMAL MAXIMUM FOR DATE OF COLLECTION
7	Mar 4	1200	61
9	31	1330	80
10	Apr 2	1000	93
11	15	1200	74
11	16	1600	76
11	19	1100	79
12	27	1200	82
13	May 1	1000	88
13	1	1330	88
13	2	0700	82
13	9	1000	88
14	11	0930	93
14	11	1000	93
14	11	1800	93
14	12	1130	99
14	13	0800	99
14	14	0830	99
14	18	0715	92
14	18	1820	92
15	24	1710	91
15	25	0830	82
15	29	1000	95
16	Jun 4	0930	94
16	4	2230	94
16	4	1145	94
18	30	2245	90
22	Aug 2	0730	93
22	5	1100	86
22	5	1300	86
22	10	1630	96
24	29	0930	95
27	Sep 21	1015	93

Table D-2. Date of Collection, Time of Collection, and Macro-Environmental Air Temperature at Time of Collection - 1960

The break in the table indicates the period preceding the onset of the summer rains during which *Heloderma* field activity is restricted. Activity following the onset of the summer rains is indicated by those data listed below the break.

TRI-MONTHLY INTERVAL	DATE OF COLLECTION	TIME OF COLLECTION	THERMAL MAXIMUM FOR DATE OF COLLECTION
9	Mar 22	1300	86
9	24	1215	70
9	26	1125	80
10	Apr 5	1735	88
11	11	1500	92
11	18	0930	92
11	20	1000	90
11	20	1500	90
12	28	1030	78
12	28	1030	78
12	28	1200	78
12	28	1530	78
12	29	1045	72
12	29	1230	72
13	May 9	1758	93
14	18	0900	85
21	Jul 27	2200	100
23	Aug 16	2130	94
23	20	0900	94
24	24	1555	93
24	29	0800	98
30	Oct 31	1530	77

Table D-3. Date of Collection, Time of Collection, and Macro-Environmental Air Temperature at Time of Collection - 1961

The break in the table indicates the period preceding the onset of the summer rains during which Heloderma field activity is restricted. Activity following the onset of the summer rains is indicated by those data listed below the break.

TRI-MONTHLY INTERVAL	DATE OF COLLECTION	TIME OF COLLECTION	THERMAL MAXIMUM FOR DATE OF COLLECTION
9	Mar 24	0935	77
12	Apr 22	1700	85
13	May 4	1545	81
13	5	0830	78
13	10	0740	98
14	12	0900	83
14	12	1600	83
14	13	0900	73
15	26	1020	93
15	27	1030	96
15	29	0800	88
16	Jun 10	0738	100
17	14	1200	106
18	26	2115	103
19	Jul 4	0815	95
21	27	0900	98
21	30	1800	89
23	Aug 11	1130	93
23	20	1950	96
24	21	0755	97
24	22	0700	93
24	31	1000	89
26	Sep 20	1030	84
27	27	0830	94
27	27	0830	94
32	Nov 11	1430	71

Table D-4. Date of Collection, Time of Collection, and Macro-Environmental Air Temperature at Time of Collection - 1962

The break in the table indicates the period preceding the onset of the summer rains during which Heloderma field activity is restricted. Activity following the onset of the summer rains is indicated by those data listed below the break.

TRI-MONTHLY INTERVAL	DATE OF COLLECTION	TIME OF COLLECTION	THERMAL MAXIMUM FOR DATE OF COLLECTION
8	Mar 17	1100	73
9	29	1615	78
11	Apr 14	0900	95
11	14	1030	95
12	25	0930	85
12	26	1330	76
13	May 5	1400	93
13	9	1130	96
13	9	2045	96
14	11	1915	97
14	13	1645	85
14	15	1000	78
14	18	1215	89
14	20	1530	89
15	25	1900	87
15	27	1500	77
15	30	1700	91
16	Jun 1	0930	93
16	5	0630	91
19	Jul 5	1650	97
19	6	1910	100
19	8	2145	96
23	Aug 15	0830	104
23	17	0900	100
23	17	0930	100
23	18	1640	97
24	28	1940	101
30	Oct 23	1245	88
31	Nov 8	0930	83
32	12	1000	86

Table D-5. Date of Collection, Time of Collection, and Macro-Environmental Air Temperature at Time of Collection - 1963

The break in the table indicates the period preceding the onset of the summer rains during which Heloderma field activity is restricted. Activity following the onset of the summer rains is indicated by those data listed below the break.

TRI-MONTHLY INTERVAL	DATE OF COLLECTION	TIME OF COLLECTION	THERMAL MAXIMUM FOR DATE OF COLLECTION
8	Mar 16	1600	71
9	27	1730	81
9	30	1350	80
11	Apr 14	0940	94
11	14	1700	94
11	20	1745	83
12	29	1800	83
12	29	1830	83
12	30	1430	86
13	May 7	1930	96
13	8	0830	95
14	15	1907	94
14	17	0900	99
15	23	0830	93
15	23	0830	93
15	27	0830	91
15	27	1707	91
15	27	1715	91
15	28	0830	96
15	28	0933	96
16	Jun 5	1830	92
16	6	1800	89
17	15	2040	96
20	Jul 11	2130	95
23	Aug 20	0930	95
24	21	2000	88
24	22	1600	90
27	Sep 22	0900	99
30	Oct 22	0840	82

Table D-6. Date of Collection, Time of Collection, and Macro-Environmental Air Temperature at Time of Collection - 1964

The break in the table indicates the period preceding the onset of the summer rains during which Heloderma field activity is restricted. Activity following the onset of the summer rains is indicated by those data listed below the break.

TRI-MONTHLY INTERVAL	DATE OF COLLECTION	TIME OF COLLECTION	THERMAL MAXIMUM FOR DATE OF COLLECTION
9	Mar 31	0930	83
10	Apr 8	1100	78
10	10	1615	84
11	16	1825	91
11	18	0815	88
12	21	1100	77
12	24	1630	73
12	28	0930	80
12	29	1115	77
12	30	1000	79
12	30	1430	79
12	30	1530	79
13	May 1	0830	82
13	1	1735	82
13	2	0900	82
13	2	0930	82
13	2	1630	82
14	15	0930	95
14	16	1000	97
14	17	1820	92
14	20	1800	98
15	21	0830	99
15	22	0700	98
15	23	1645	98
15	24	0930	100
15	25	1600	96
15	25	1830	96
15	26	0800	95
15	26	1800	95
15	26	1830	95
15	27	0730	92

Table D-6, Continued

TRI-MONTHLY INTERVAL	DATE OF COLLECTION	TIME OF COLLECTION	THERMAL MAXIMUM FOR DATE OF COLLECTION
15	May 27	0900	92
15	28	0900	89
15	31	1800	91
16	Jun 6	0830	100
16	7	1830	97
16	7	1930	97
16	8	0930	89
17	11	1845	94
17	12	1720	95
17	13	0715	94
17	19	0805	97
18	25	0740	100
19	Jul 8	2050	89
19	10	0710	99
20	13	0730	92
20	17	1700	102
20	19	2050	102
21	23	0900	98
21	28	2230	104
21	30	1800	89
21	31	1000	78
22	Aug 6	1000	95
22	8	0930	96
23	16	1800	97
23	17	0753	93
24	26	1415	87
24	29	0800	90
24	29	1915	90
24	31	0800	95
25	Sep 6	1815	91
25	10	1935	72
26	11	1430	87
26	14	0900	90
27	21	0830	87
27	30	1000	90
28	Oct 8	1800	92
29	20	0900	81

APPENDIX E

TIME OF COLLECTION PLOTTED AGAINST DATE OF COLLECTION FOR THE PERIOD 1959-1964

The six graphs included in this section demonstrate the response of <u>Heloderma</u> to increasing environmental temperature within the limits imposed by the dawn and dusk photic transitions. Very few individuals were collected before dawn civil twilight or after dusk civil twilight, both of which are biologically important photic transitions. One can also observe the effect of the summer rains on <u>Heloderma</u> activity. Those animals collected in the middle of the day following the onset of the summer rains were most typically collected during or after a rain. Finally, the gradual transition towards a midday activity orientation at the end of the seasonal activity period can be observed in the graphs for years for which there are sufficient data. The data for these graphs are in Appendix D and the entire six year (1959-1964) sample is plotted in Figure 6 in the text.

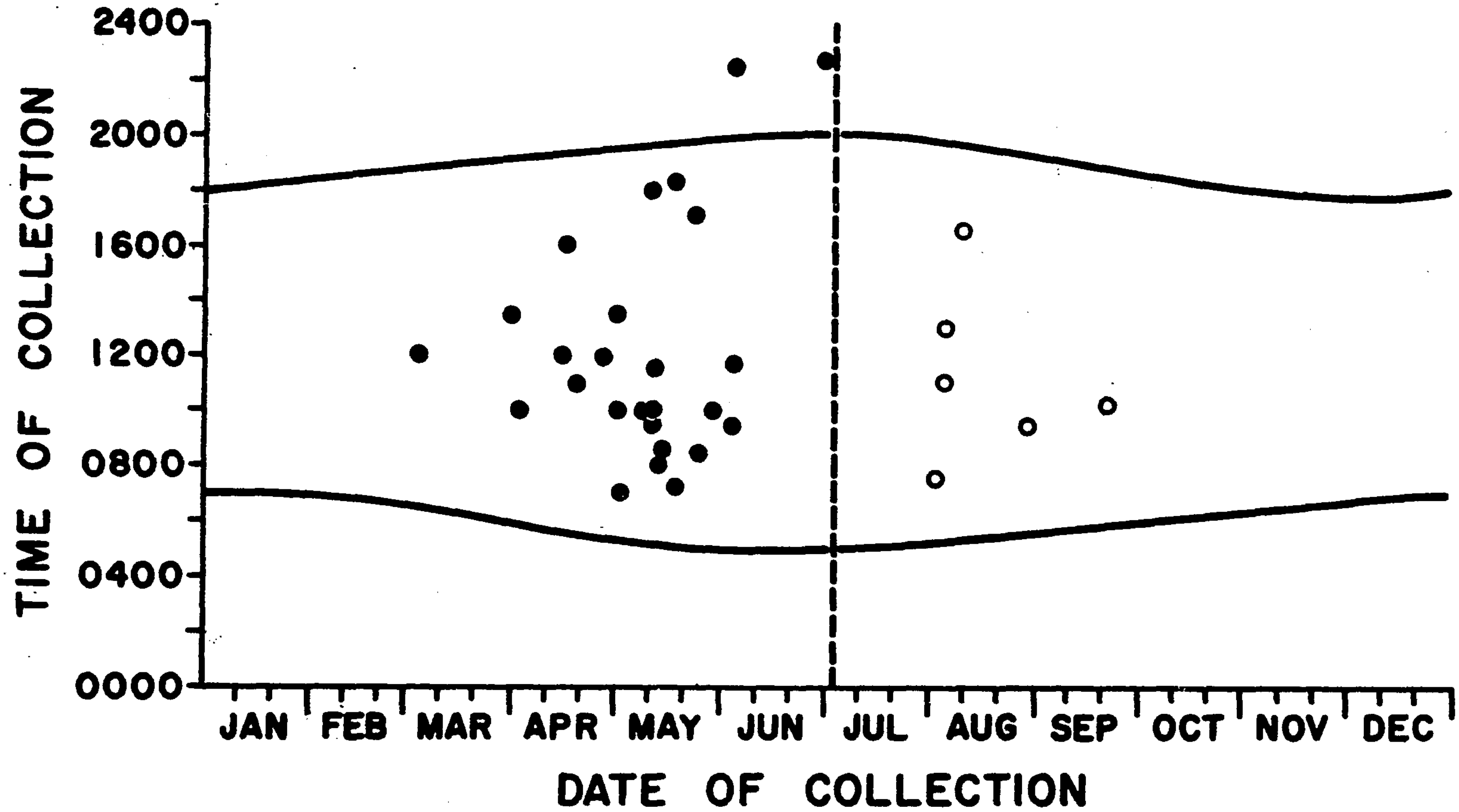

Figure E-1. Time of Collection, Morning and Afternoon, Preceding and Following the Onset of the Summer Rains, Against Date of Collection - 1959

The curves indicate the time of civil twilight and the vertical dashed line indicates the approximate date of the onset of the summer rains.

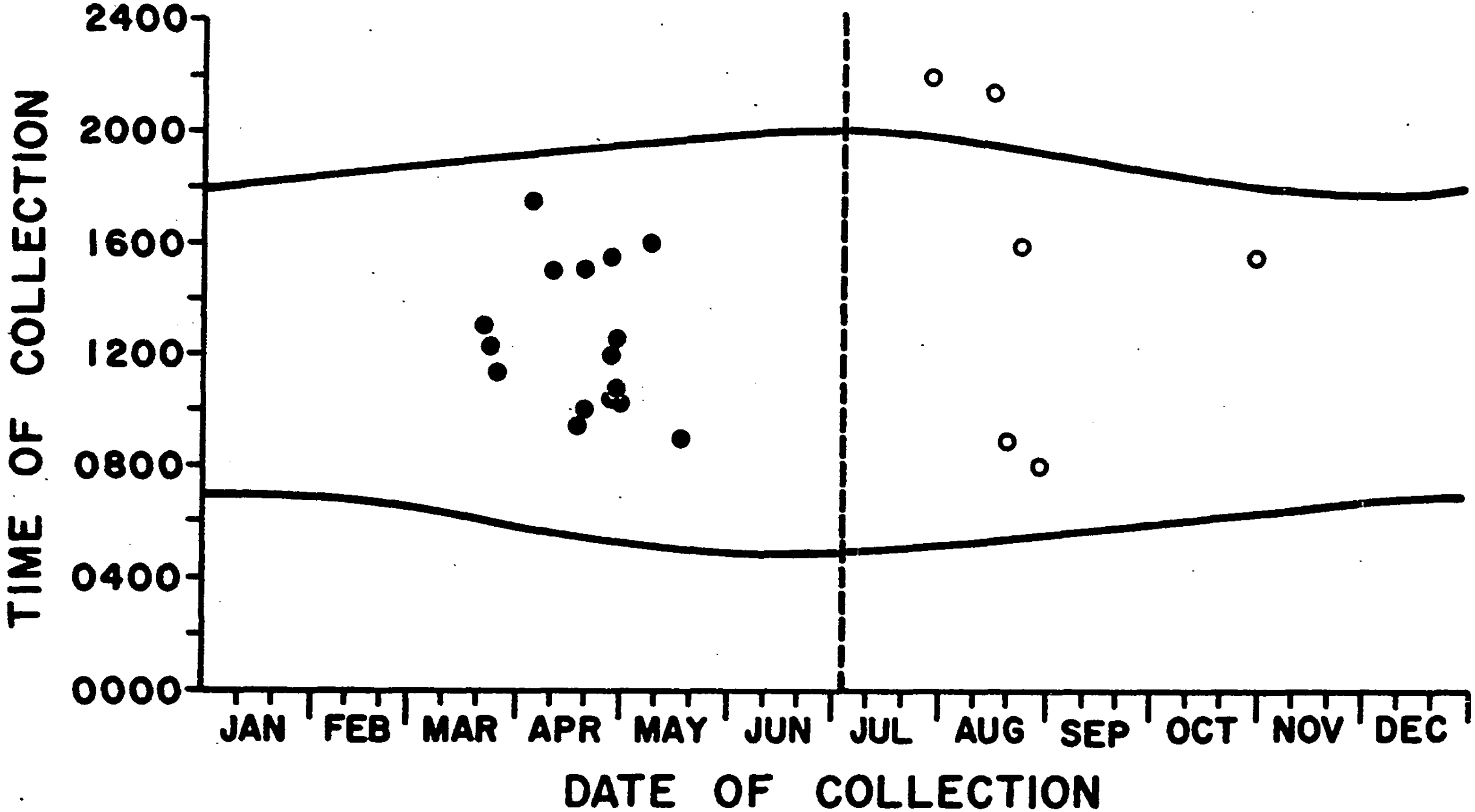

Figure E-2. Time of Collection, Morning and Afternoon, Preceding and Following the Onset of the Summer Rains, Against Date of Collection - 1960

The curves indicate the time of civil twilight and the vertical dashed line indicates the approximate date of the onset of the summer rains.

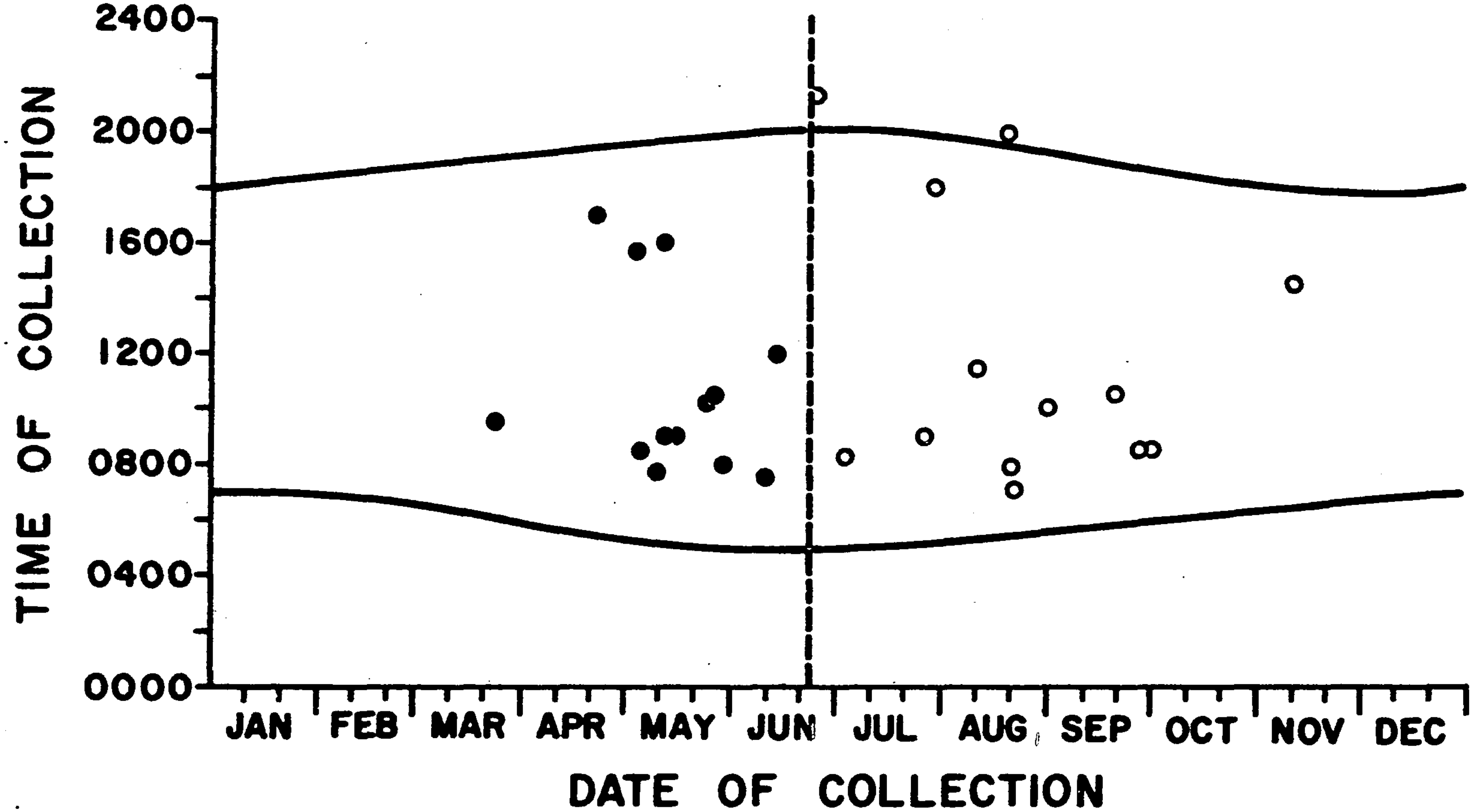

Figure E-3. Time of Collection, Morning and Afternoon, Preceding and Following the Onset of the Summer Rains, Against Date of Collection - 1961

The curves indicate the time of civil twilight and the vertical dashed line indicates the approximate date of the onset of the summer rains.

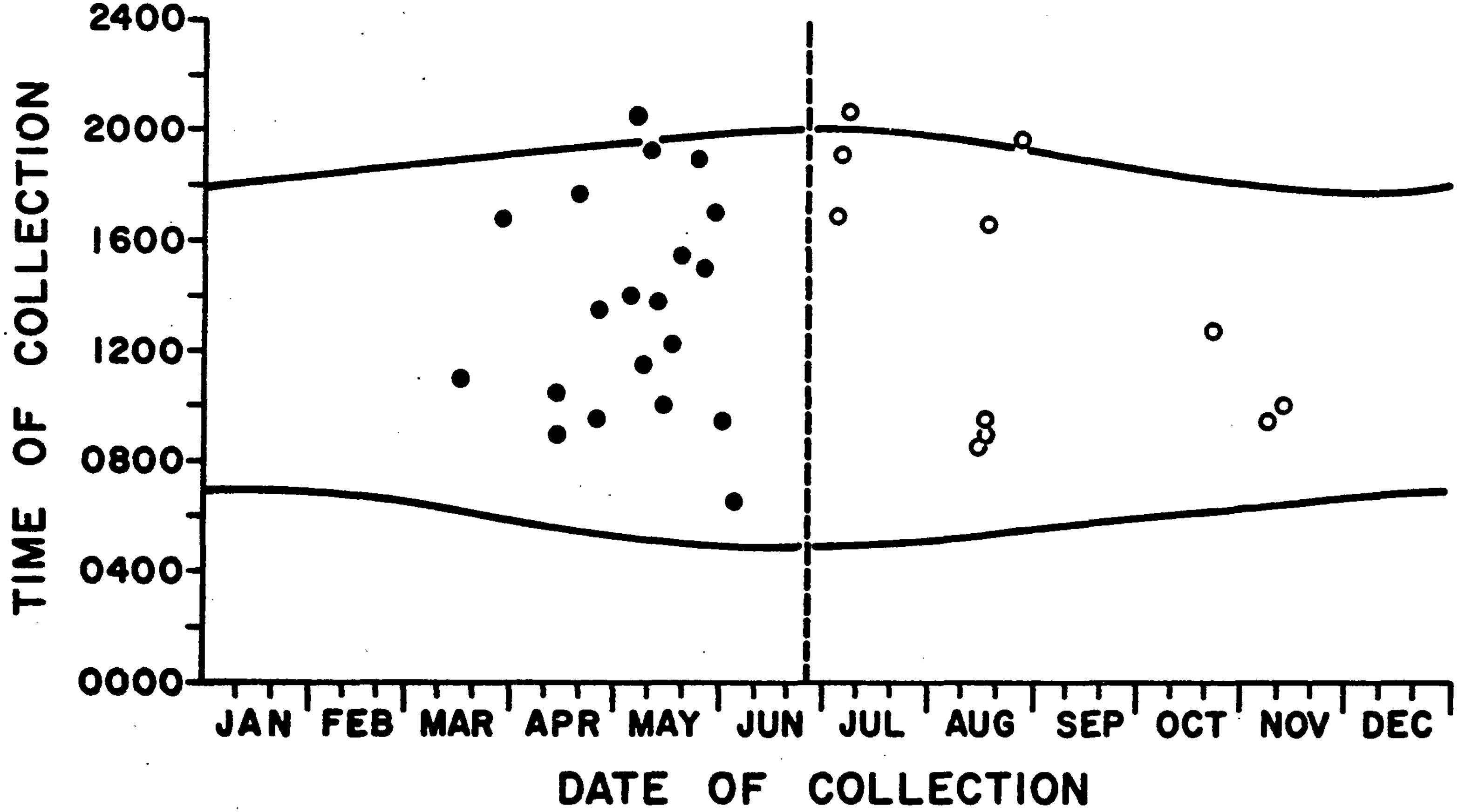

Figure E-4. Time of Collection, Morning and Afternoon, Preceding and Following the Onset of the Summer Rains, Against Date of Collection - 1962

The curves indicate the time of civil twilight and the vertical dashed line indicates the approximate date of the onset of the summer rains.

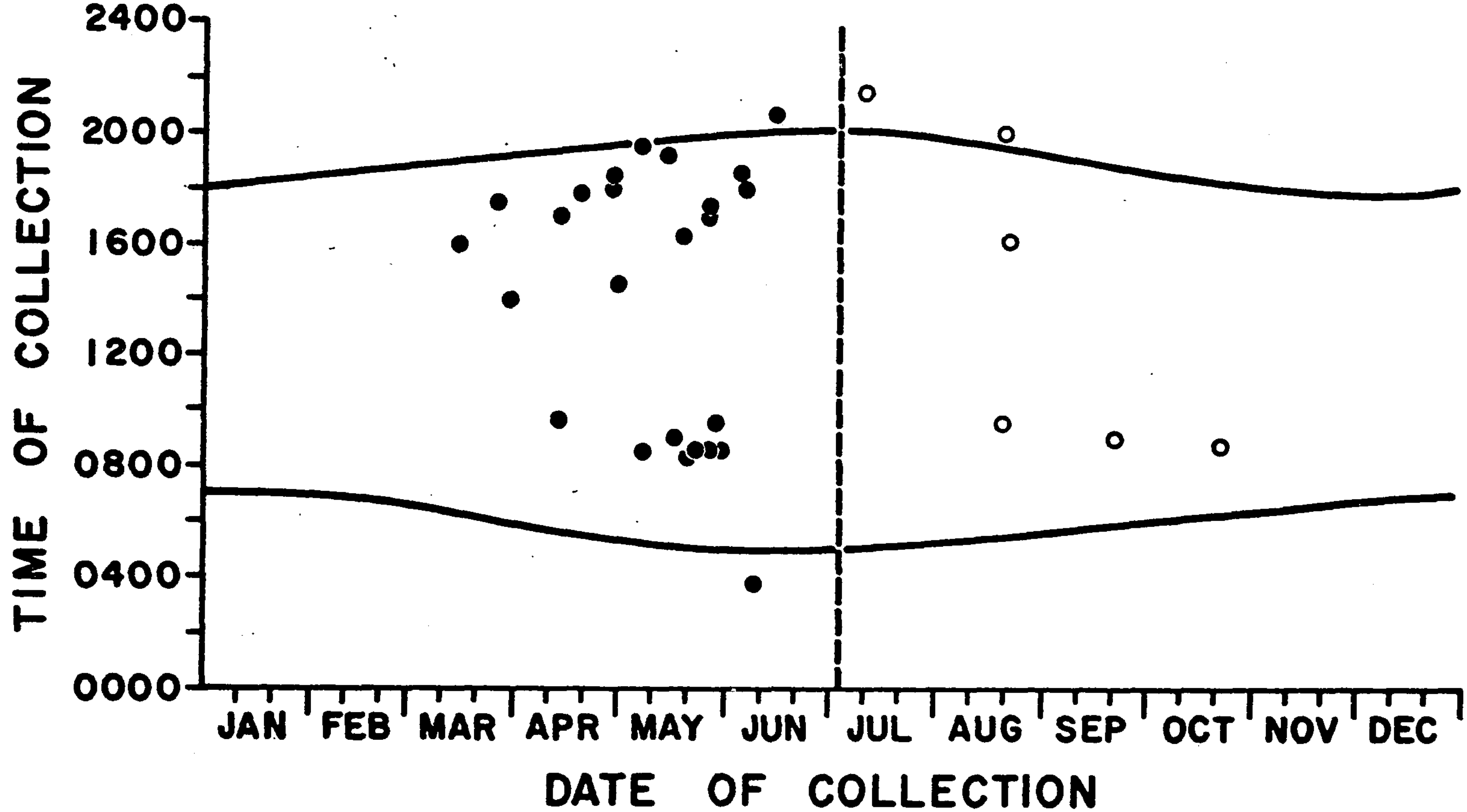

Figure E-5. Time of Collection, Morning and Afternoon, Preceding and Following the Onset of the Summer Rains, Against Date of Collection - 1963

The curves indicate the time of civil twilight and the vertical dashed line indicates the approximate date of the onset of the summer rains.

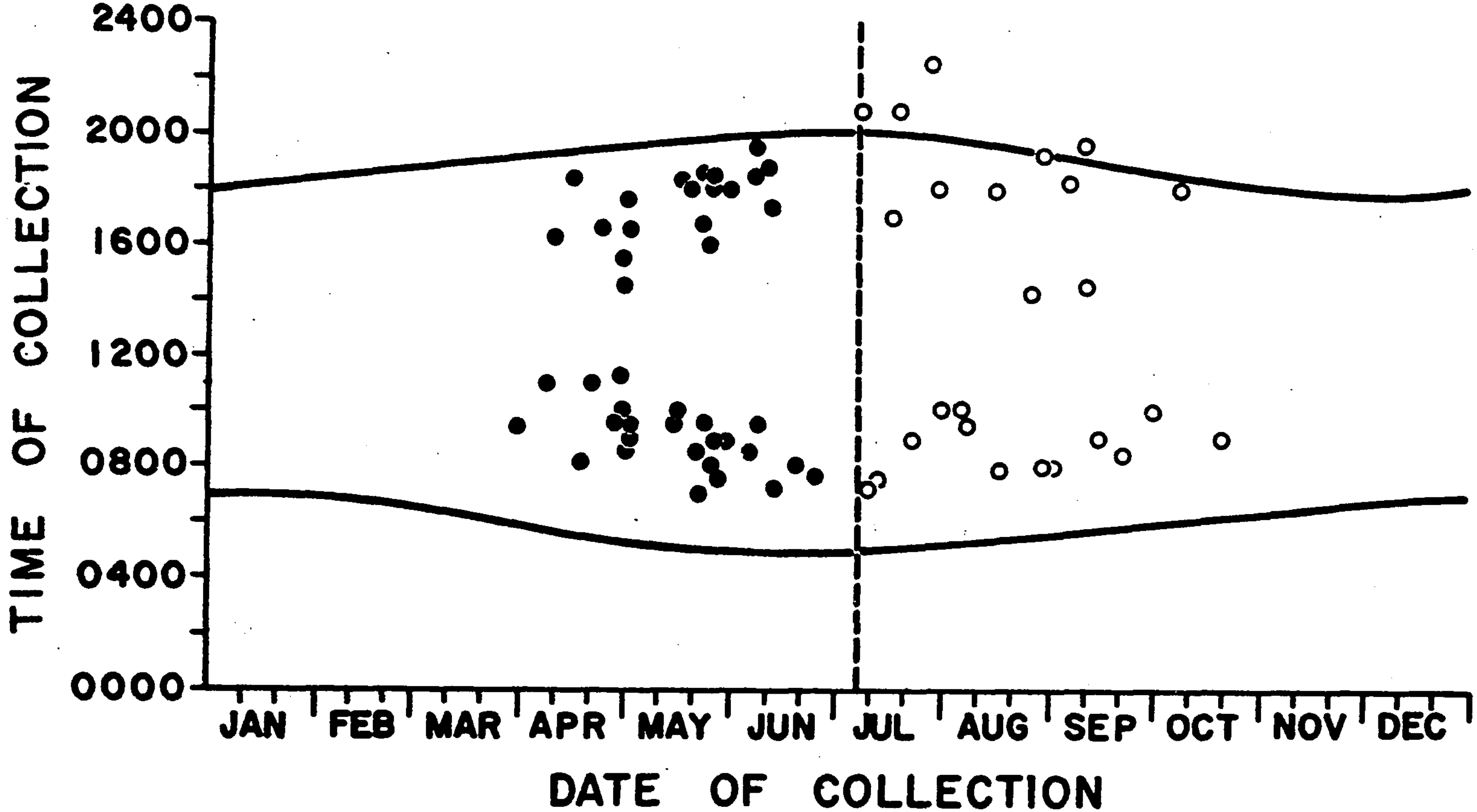

Figure E-6. Time of Collection, Morning and Afternoon, Preceding and Following the Onset of the Summer Rains, Against Date of Collection - 1964

The curves indicate the time of civil twilight and the vertical dashed line indicates the approximate date of the onset of the summer rains.

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