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Evaporative Water Loss in Two Northwest Snakes: *Pituophis catenifer* and *Crotalus viridis*

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EVAPORATIVE WATER LOSS IN TWO NORTHWEST SNAKES:

Pituophis catenifer and Crotalus viridis

A Thesis

Presented to

the Graduate Faculty

Central Washington State College

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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APPROVED FOR THE GRADUATE FACULTY

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INTRODUCTION

Traditionally, the skin of reptiles has been considered to be totally impermeable to fluids. The earliest challenge to this assumption came from Noble and Mason (1932), who stated that "lizards absorb moisture through their skin." Chew and Dammann (1961) demonstrated that the western diamondback rattlesnake (Crotalus atrox) and the Mojave rattlesnake (Crotalus scutellus) lose water at a "ratio of about 70:30," when measuring evaporative water loss from the head (principally the respiratory tract) as opposed to that lost from the skin. Later studies indicate that the skin is the major avenue of evaporative water loss. Bentley and Schmidt-Nielsen (1966), using a crocodilian (Caiman sclerops), two turtles (Pseudemys scripta and Terrapene carolina) and two lizards (Iguana iguana and Sauromalus obesus) and Schmidt-Nielsen and Bentley (1966), using the desert tortoise (Gopherus agassizi), have shown this to be the case "in all animals examined."

Snakes comprise a unique and highly specialized group of animals. Though all are highly specialized, many are apparently quite tolerant with respect to their physiological environment (humidity, temperature, etc.). Snakes have been well-examined morphologically and behaviorally. In contrast, relatively little conclusive information is

available concerning the physiology of these animals. The most recent activities which concern evaporative water loss, in particular, have come from a study by Pange and Schmidt-Nielsen (1968) and a study by Gans, Krakauer and Paganelli (1968). Pange and Schmidt-Nielsen (1968) dealt with the brown water snake (Natrix taxispilota) and the Sonora gopher snake (Pituophis catenifer affinis). In this study, also, it was found that the skin, not the respiratory tract, was the major avenue of evaporative water loss. In addition, it was concluded that "the (rate of) total evaporation (cutaneous plus pulmonary) of the brown water snake was 3.3 times greater than that of the desert-dwelling Sonora gopher snake". Gans, Krakauer and Paganelli (1968) dealt with twenty-six species of snakes representing five families. They found that the "rate of water loss was proportional to surface area" and that water loss correlates with the aridity of the selected habitat. No comparative information resulting from the direct separation of the pulmonary and cutaneous avenues of water loss is currently available. Also, no direct comparisons of this separation in sympatric species are available. These comparisons are the basis for this study.

METHODS, MATERIALS AND RESULTS

Four gopher snakes (Pituophis catenifer) and four western rattlesnakes (Crotalus viridis) were kept in terraria with water available at all times. The subjects were collected in Grant and Kittitas Counties, Washington. The gopher snakes, all females, were collected in the spring and early summer of 1970. The rattlesnakes, all males, were collected in September, 1970. For the most part, the snakes ate sparingly if at all in captivity although each was provided with a mouse following each experiment in which it was subjected.

Separation of the water lost through pulmonary and cutaneous components required the construction of a specialized chamber (figure 1). Opaque polypropylene pipe was used for the "body section" and clear glass pipe was used for the "head section". Rubber sheeting was used as the separating diaphragm. Condoms were used to close the gap created by the overlapping of the head and body sections.

The snake was pulled backward into the desiccation chamber through a carefully cut hole in the separating diaphragm until only the head protruded. Once inside the chamber, the snake was essentially immobile. Not only did the diameter of the chamber restrict movement, for it was only one-fourth to three-eighths inches larger in diameter

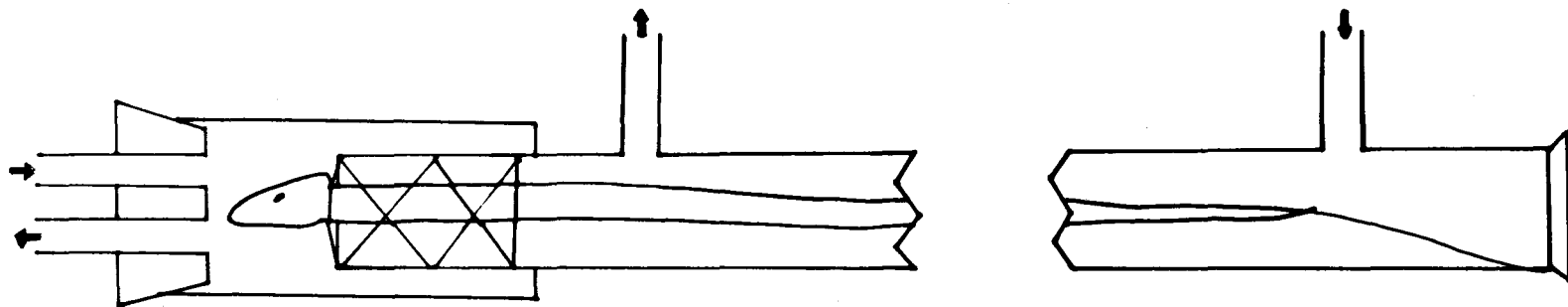


Fig. 1. Desiccation Chamber

than the diameter of the subjects used, but the subjects were secured to each end of the "body section" using cotton string for the tail and plastic tape for the neck.

Compressed air was passed through a series of four Drierite (CaSO_4 , anhydrous) columns of decreasing size (figure 2). The last container in the series was weighed before and after each six hour experimental run. In this way, the relative humidity of the air was assumed to be zero percent as it entered the desiccation chamber. Because the desiccation chamber consisted of two compartments, the air line was split before it entered the flowmeters with the rate of flow kept constant at 468.3 milliliters per minute. From the flowmeters, the air passed to the desiccation chamber. The water in the now-humidified air was collected from the exhausts of the desiccation chambers, now considering each section as a separate chamber, by passing it through Drierite columns. The changes in weight of these columns were due to the weight of the absorbed water.

A large aquarium was used as a constant temperature water bath (figure 2). A thermo-regulator, four small aquarium heaters, a small mechanical stirrer and a "Precision" Porta-cool unit were used to maintain the desired temperatures plus or minus 1°C . The glass sides of the aquarium were insulated with foam rubber pads. Each individual was subjected to temperatures of 9°C , 19°C and 29°C . The container with the subject inside was allowed to equilibrate and flush for eighteen hours at the 9°C and thirty

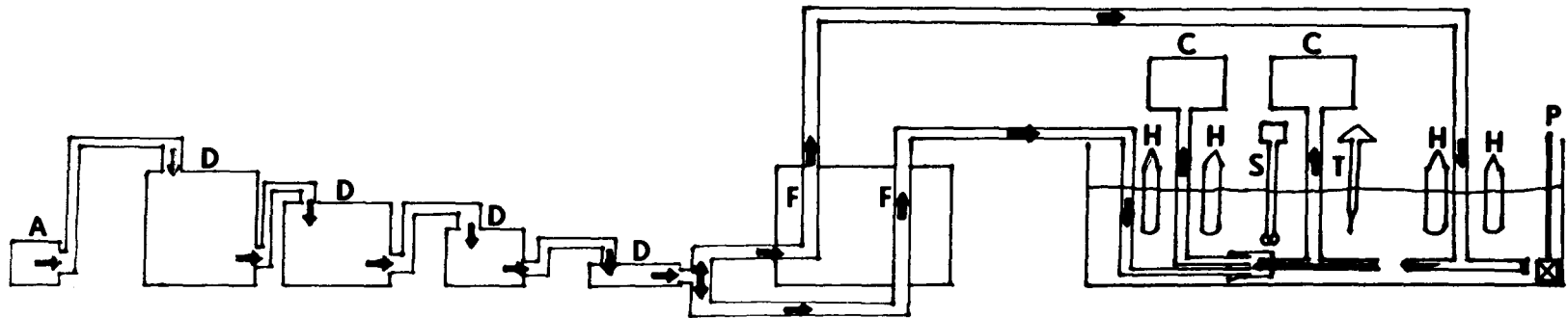


Fig.2. Desiccation Apparatus

A - Air Supply

D - Drying Columns

F - Flowmeters

H - Heaters

C - Collecting Columns

S - Mechanical Stirrer

T - Thermo-regulator

P - Cooling Unit

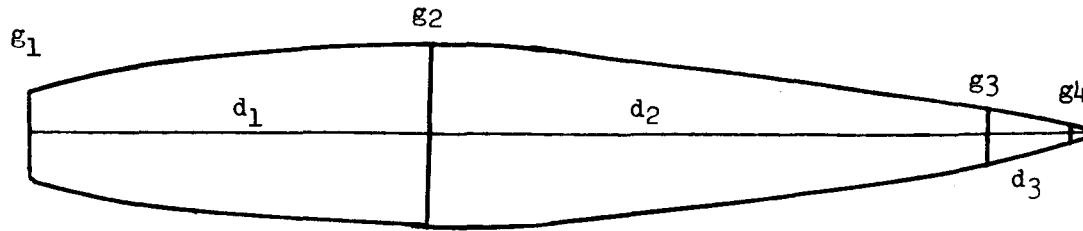
minutes to one hour at 19°C and 29°C. The actual duration of each experimental run was six hours.

The weight of each animal was taken before and after each experimental run. Snout-vent length and girth of the obviously largest portion of the body were taken prior to the series of experiments. Surface area was measured from the flattened skin after all other desired data had been obtained. It was found that the methods for calculating the surface area of a snake, using Benedict's formula ($\text{cm}^2 = 12 \text{ BW}^{2/3}$) where cm^2 represents the surface area of the individual in square centimeters and BW represents the body weight in grams (Benedict, 1934) and the modification of Benedict's formula ($\text{cm}^2 = 10 \text{ BW}^{2/3}$) (Bentley and Schmidt-Nielsen, 1966) were only to be relied upon to produce about 83 percent accuracy. This is not sufficiently accurate for detailed comparisons. For precise laboratory experiments a further refinement must be made. While it is best to measure the surface area directly, it is quite time consuming, especially in the field. The following formula has been developed:

$$A = g_2 (d_1 + d_2) - \left(\frac{2d_1}{g_2^2 - g_1^2} \right) \left(\left(\frac{g_2^3}{6} - \frac{g_1^2 g_2}{2} + \frac{g_1^3}{3} \right) \right) - \left(\frac{2d_2}{g_2^2 - g_3^2} \right) \left(\left(\frac{g_2^3}{6} - \frac{g_3^2 g_2}{2} + \frac{g_3^3}{3} \right) \right) + 2/3 d_3 g_4.$$

Figure 3 gives a graphic as well as a written description of the formula. The formula, called the Lowe Formula for Fastine Snakes, carries with it an accuracy of about 98 percent. Although accurate, the Lowe formula is rather time consuming, especially for one working in the field where time is valuable. In view of this, the Roloff formula, $cm^2 = 1.05 L^{1.26}$, where cm^2 represents the surface area of the individual in square centimeters and L represents the snout-vent length of the individual in centimeters, has been devised. Though less accurate than the Lowe formula, it is faster and, compared to Benedict's formula and the modification to Benedict's formula, it is more accurate. It can be relied upon to produce an accuracy of about 90 percent.

Rates of desiccation depend on a number of factors: speed of the air moving over the body of the individual, ambient and substrate temperature, size and activity of the animal, and relative humidity of the air. In this series of experiments, all the above factors, with the exception of size, were carefully controlled. Size was taken to mean either the weight or the surface area of the animal. Weight provides an insight into the total amount of disposable water available to the animal while the relative efficiency of the skin as a water evaporating device can be demonstrated using surface area. Excretory water loss was not permitted to interfere with the final results; if excretion occurred during the run, the run was repeated.



$$A = g_2 (d_1 + d_2) - \frac{2d_1}{(g_2^2 - g_1^2)} \left(\left(\frac{g_2^3}{6} - \frac{g_1^2 g_2}{2} + \frac{g_1^3}{3} \right) \right) - \frac{2d_2}{(g_2^2 - g_3^2)} \left(\left(\frac{g_2^3}{6} - \frac{g_3^2 g_2}{2} + \frac{g_3^3}{3} \right) \right) + \frac{2}{3} d_3 g_4$$

- 1) g_1 represents the circumference in centimeters at the neck region
- 2) g_2 represents the circumference in centimeters at the obviously largest portion of the body
- 3) g_3 represents the circumference in centimeters at the vent
- 4) g_4 represents the circumference in centimeters a short distance from the vent
(In rattlesnakes, the measurement would be taken just before the first button on the string of rattles; in others, the measurement would be taken at the point where a measurement of, at least, 1 centimeter could be obtained.)
- 5) d_1 represents the distance in centimeters from g_1 to g_2
- 6) d_2 represents the distance in centimeters from g_2 to g_3
- 7) d_3 represents the distance in centimeters from g_3 to g_4

Figure 3-Explanations of Symbols for the

Lowé Formula for Fasting Snakes

Comparative information was computed in four different ways (table 1). (1) The total water loss (pulmonary plus cutaneous) in milligrams was computed as a percentage of the mean body weight per day (% BW/day). (2) The milligrams of pulmonary water loss per mean body weight per hour ($(\text{mg}(\text{gm-hr})^{-1})$) were computed. (3) The milligrams of cutaneous water loss per surface area per day ($\text{mg}/\text{cm}^2/\text{day}$) were computed. (4) The milligrams of cutaneous water loss as a percentage of total water loss in milligrams (% Total) were computed. In (1) and (2), measurements were based on the supposition that pulmonary water loss is a function of weight. In (3) and (4), the cutaneous water loss was considered to be a function of the surface area.

Analysis of variance was used to compare differences in the effects of temperature and species (table 2). For all confidence levels of significance the 5% level was utilized. There were no significant species differences; both species lost water at the same rate. Also, there was no significant difference due to the two species in the relative importance of cutaneous versus pulmonary evaporation. There was a significant difference due to temperature; both species lost water more rapidly as the temperature increased. Finally, when considering the interaction of species and temperature, no significant difference was found.

"Student's" t-test showed that a 55 cutaneous : 45 pulmonary ratio may be expected instead of the 70 pulmonary :

Table 1-RAW AND COMPUTED DATA

	Temperature (°C)	Snout-vent (cm=A)	Surface Area (cm ² =B)	Ratio (A:B)	Mean Body Weight (grams)	Total Water Lost per run		Pulmonary Water Lost per run		Cutaneous Water Lost per run		% Total
						µl/day	*	mg	mg(gm-hr) ⁻¹	mg/cm ² /day	*	*
Pituophis catenifer												
A	9	82.0	369.75	.222	112.64	475.7	1.69	210.3	.311	265.4	2.87	55.8
B	9	86.0	374.99	.229	158.83	264.8	.67	139.4	.146	125.4	1.34	47.4
C	9	87.0	380.50	.229	131.25	408.8	1.25	177.7	.226	231.1	2.43	56.5
D	9	99.0	585.39	.169	228.19	159.1	.28	45.1	.033	114.0	.78	71.7
Crotalus viridis												
1	9	44.0	189.00	.233	62.01	175.4	1.13	59.2	.159	116.2	2.46	66.2
2	9	55.0	249.00	.221	87.31	212.7	.97	55.8	.107	156.9	2.52	73.8
3	9	62.5	298.00	.210	167.42	40.3	.10	11.5	.011	28.8	.39	71.5
4	9	63.0	452.00	.139	207.07	248.7	.48	79.6	.064	169.1	1.50	67.9
Pituophis catenifer												
A	19	82.0	369.75	.222	110.59	1175.3	4.25	351.3	.529	824.0	8.91	70.1
B	19	86.0	374.99	.229	135.69	694.8	2.05	231.1	.284	463.7	4.95	66.7
C	19	87.0	380.50	.229	111.15	893.9	3.22	440.5	.661	453.4	4.77	50.7
D	19	99.0	585.39	.169	213.72	937.9	1.76	435.5	.340	502.4	3.43	53.6
Crotalus viridis												
1	19	44.0	189.00	.233	52.20	701.4	5.37	383.5	1.224	317.9	6.73	45.3
2	19	55.0	249.00	.221	94.61	711.7	3.01	472.7	.833	239.0	3.64	33.6
3	19	62.5	298.22	.210	138.76	717.3	2.07	526.4	.632	190.9	2.56	26.6
4	19	63.0	452.00	.139	190.84	880.9	1.85	682.5	.596	198.4	1.76	22.5
Pituophis catenifer												
A	29	82.0	369.75	.222	111.94	1785.8	6.38	668.9	.996	1116.9	12.06	62.5
B	29	86.0	374.99	.229	132.06	1213.9	3.68	380.4	.480	833.5	8.89	66.7
C	29	87.0	380.50	.229	110.65	1804.6	6.32	672.9	1.014	1131.7	11.90	62.7
D	29	99.0	585.39	.169	218.06	1717.2	3.15	841.2	.643	876.0	5.99	51.0
Crotalus viridis												
1	29	44.0	189.00	.233	59.02	1135.3	7.69	467.3	1.320	668.0	14.14	58.6
2	29	55.0	249.00	.221	91.52	908.6	3.97	405.2	.736	503.4	6.09	55.4
3	29	62.5	298.22	.210	136.23	1556.9	4.57	752.4	.921	804.5	10.79	51.7
4	29	63.0	452.00	.139	185.87	1438.0	3.09	861.0	.772	577.0	5.11	40.1

* = Computed Data

Table 2-Factorial Analyses

Percent of Mean Body Weight per Day					
Variation due to:	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
A	1.0668	1	1.0668	2.8030	N. S.
B	96.5710	2	48.2855	16.1480	S.
Interaction	3.5250	2	1.7625	1.6966	N. S.
Error	53.8237	18	2.9902		

Pulmonary Water Loss per Mean Body Weight per Day

Variation due to:	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
A	.0971	1	.0971	2.6730	N. S.
B	2.1132	2	1.0566	26.9541	S.
Interaction	.2144	2	.1072	2.7347	N. S.
Error	.7052	18	.0392		

Cutaneous Water Loss per Surface Area per Day

Variation due to:	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
A	3.0033	1	3.0033	1.3917	N. S.
B	252.1120	2	126.0560	21.5462	S.
Interaction	4.3160	2	2.1580	2.7111	N. S.
Error	105.3084	18	5.8505		

Cutaneous Water Loss expressed as a Percentage of Total Water Loss

Variation due to:	Sum of Squares	Degrees of Freedom	Mean Square	F	Remarks
A	448.0704	1	448.0704	4.3047	N. S.
B	1277.2609	2	638.6305	6.1355	S.
Interaction	1635.6808	2	817.8404	7.8572	S.
Error	1873.5875	18	104.0882		

A = species

B = temperature

N. S. = Not Significant

S. = Significant with 2.18 difference

30 cutaneous ratio found by Chew and Dammann (1961) (table 3). This demonstrates that the skin is the major avenue of evaporative water loss in these species and that the water lost through the skin of Pituophis catenifer is of a lesser magnitude than that which has been suggested by Pange and Schmidt-Nielsen (1968).

Table 3-"Student's" t-test values for
Cutaneous Water Loss expressed as a Percentage of Total Water Loss

\bar{M} (%)	t-test values	Remarks
50	8.5331	S.
51	6.9757	S.
52	5.5184	S.
53	3.8610	S.
54	2.3037	S.
55	.7463	N. S.
56	.8111	N. S.
57	2.3684	S.
58	3.9258	S.
59	5.5832	S.
60	7.0405	S.

S. = Significant

N. S. = Not Significant

"Student's" t-test:
$$t = \frac{\sum x - M}{\frac{\sqrt{\sum x^2 - \frac{(\sum x)^2}{N}}}{N - 1}}$$

N = 24

DISCUSSION

The relationship of water loss to mortality is shown in figure 4. It was constructed to determine the length of time required for an average individual to reach the critical limit of desiccation, providing that no food or water is consumed. Assuming that if an animal loses up to 25 percent of his body weight in the form of evaporation, death will shortly ensue, it indicates that the time required for death due to desiccation to occur decreases as the temperature increases.

Snakes, being primarily uricotelic, lose very little water through excretion because less water is needed to eliminate the non-water-soluble uric acid. This holds true especially for xerophilous, terrestrial forms such as desert-dwelling bullsnakes, rattlesnakes and racers.

Snakes alter their behavioral patterns as the ambient temperature changes; indeed, they must do so in order to stay alive. These animals, for the most part, are diurnal. However, during the summer months, when the temperature is elevated and the humidity is reduced, this pattern may be altered considerably. The individuals may become crepuscular, remaining in underground burrows during the day with a significant reduction in water loss. A reduction would be especially noticeable if the relative humidity of

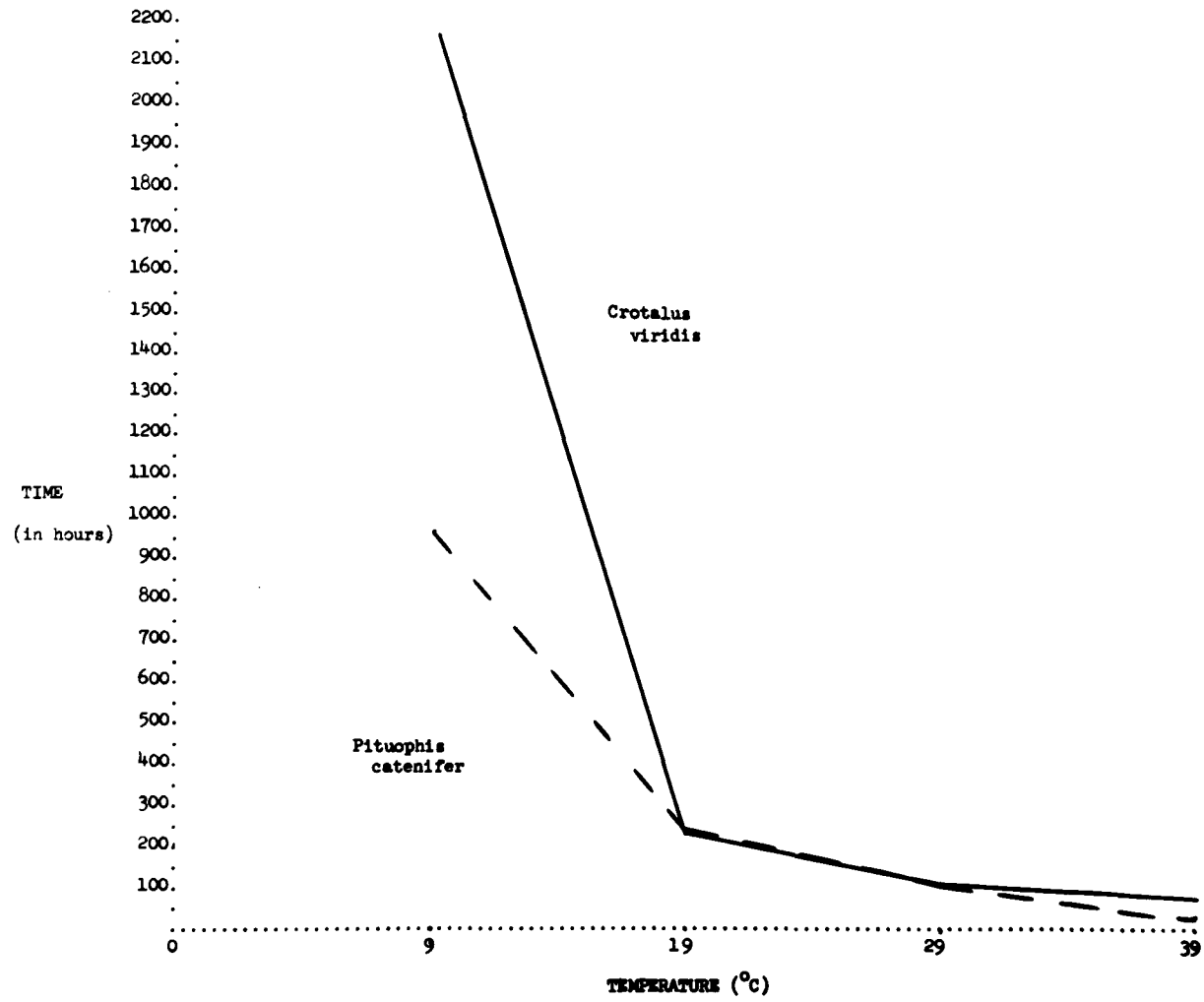


Figure 4—Time for Death to Occur

the burrow were high. The snakes usually become completely nocturnal during the period of extremely elevated summer temperatures. In this way, water is conserved during surface activity because of a lower body temperature and a higher atmospheric relative humidity. In both of the ways mentioned above, water is conserved in a habitat that, in all probability, has a minimum of available water.

As was demonstrated previously, snakes lose less water at lower temperatures. An increased rate of evaporation with increased temperature may be due to an increased rate of respiration and blood flow, etc., as well as an increase in the drying power of the warmer air. To compensate for this loss, preformed water must be found. Because water lost by evaporation tends to exceed the water formed in metabolism (Schmidt-Nielsen, 1964), especially as the temperature increases, snakes, like reptiles in general, must acquire free water in order to maintain proper water balance. This phenomenon was noted repeatedly in the present study. Although quantitative measurements were not recorded, it was observed that the snakes either drank more water or tended to immerse themselves in their water dishes after a dehydration run and as the room temperature increased; it will be recalled that all individuals refused to eat during the series of experiments. In view of these observations, it can be assumed that food, moisture and metabolic water are less of a requirement than is free water.

It has been suggested that there is a high mortality rate during the cold over-wintering months (Hirth, 1966). Figure 4 indicates that this mortality could not be due to desiccation providing that the individual entered the den fully hydrated. According to the figure, it would take an average, fully prepared rattlesnake almost 90 days to lose enough water for death to occur in a hibernaculum at 9°C and near-zero relative humidity. However, it is unlikely that a temperature as high as 9°C and a relative humidity approaching zero percent would be found in the den. Instead of the 34 percent over-wintering mortality in Crotalus viridis being due to excessive amounts of water loss, more probable suggestions are that it is due to frozen tissues occurring during the colder periods or an insufficiency of stored fats for entry into the seasonal denning period. The same reasoning would hold true for Pituophis catenifer. Though no data are currently available, it seems improbable that excretory water levels in urine or feces are high. Excretory levels would probably be minimal, except possibly for the initial stages, due to the lower metabolic activities. Moreover, the information presented by Hirth (1966) indicates that the high mortality reflects primarily that of the young born previous to the fall denning. These young simply may not have had sufficient feeding opportunity to develop adequate stored-energy levels.

In summary, measurements of evaporative water loss

taken directly indicate that the skin, not the respiratory tract, is the major avenue of water loss. This study, also, indicates that free water is more essential to compensate for dehydration stress than is metabolic water or food moisture. The suggestions of hibernation mortality are observed as an indication of frozen tissues or poor preparation for the denning period instead of excessive dehydration during hibernation. Finally, no significant difference between these geographically and ecologically sympatric species in rates of water loss was observed.

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