THE UNIVERSITY OF MANITOBA

Resistance to Cold Stress in the Red-Sided Garter Snake *Thamnophis sirtalis parietalis*

by

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Abstract

Cold-acclimated garter snakes, *Thamnophis sirtalis*, endure cold stress (-10° C) longer than warm-acclimated snakes and for both groups resistance is greater in fall than in spring. Increased cold resistance occurs within 42 hours of cold acclimation (4° C) and is re-established after 18 hours exposure to 4° C. No diurnal effects of cold tolerance could be detected. Light-temperature interaction elevated resistance in warm acclimated snakes (30° C) and decreased it in cold acclimated snakes (4° C).

Male spring snakes have body temperatures of 31-33° C on substrates above lethal temperatures ($>41^{\circ}$ C) but still retain a high level of cold resistance.

Acknowledgements

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This project was supervised by Dr. K. W. Stewart who supplied me with various methods of interpretation and approaches to the problem. His help in construction and organization of the manuscript was invaluable.

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Introduction

The garter snake, Thamnophis sirtalis, has a wide latitudinal distribution in North America from the Gulf Coast to Fort Smith, N.W.T.. This range encompasses many environments each with its own range of temperatures. The southern environments have a mean annual temperature between 20° and 27° C and practically no winter while the northern environments have lower mean annual temperatures and longer and colder winters. In Manitoba the mean annual temperature is about 10° C with eight months below freezing. In this environment prolonged cold could be detrimental to the snake's survival. Fitch (1965), by taking cloacal temperatures of snakes refrigerated for several hours, found that northern garter snakes could tolerate body temperatures between -2° and -3° C. By contrast ringneck snakes, racers, and copperhead snakes could not tolerate body temperatures below 0° C. These temperatures are referred to as the lower critical temperatures.

The concept of lower critical temperature (LCT) or critical thermal minimum (CTMin) implies the condition of ecological death. At this body temperature a poikilotherm loses its ability to respond to an external stimulus. The upper critical temperature (UCT) or critical thermal maximum (CTMax) is defined as the warmest body temperature at which co-ordination is lost. It is usually preceded by spasmodic muscle contractions and mouth gaping (Mahoney

& Hutchison 1969).

In the reptiles and amphibians studied, higher acclimation temperatures produce higher LCT and UCT than do lower acclimation temperatures (Brattstrom & Lawrence 1962, Mahoney & Hutchison 1969, Jacobson & Whitford 1970, Kour & Hutchison 1970, and Hutchison & Ferrance 1970). These results can be interpreted in terms of seasonal changes in temperature acclimation of the species studied, but give little information regarding the survival value of resistance to extreme temperatures over shorter periods. In most studies, the different acclimation temperatures which ranged from 10° to 30° C, alter the critical temperatures by about 1° C. This explains the shift in tolerance range in reptiles and amphibians as a result of seasonal changes in temperature. In a climate where periods of brief cold occur during the active season, the tolerance shift only explains part of the cold compensation. It does not explain how snakes survive short cold periods when caught away from shelter.

Thamnophis sirtalis ssp. may sun themselves on or near snow patches in early spring (Fitch, 1965). Stewart (1965) has caught twenty-six Thamnophis sirtalis concinnus emerged and basking during three winters. Stewart, like most authors, explained the activity of spring snakes using the concept of LCT. He found that garter snakes acclimated at 8° C and then left at room temperature for ten to twelve hours decreased their LCT by 0.9° C relative to snakes cold stressed immediately after acclimation. He believed that short exposure to warmth decreased the LCT so that snakes which were subjected to cold were able to find shelter due to the decreased LCT before they lost their ability to move. Stewart (1965) like Fitch (1965) measured cloacal temperatures.

The decrease in LCT after acclimation and re-warming may explain cold tolerance. In any case, garter snakes are active on cold days when other reptiles are not seen. For survival in the north they must be able to remain active for several hours during cool temperatures. The purpose of this study was to investigate the phenomenon of short-term cold tolerance in garter snakes. The thermal conditions which initiate increased cold resistance, the duration of the resistance and the minimum period necessary for the establishment of the increased cold resistance have been determined experimentally and related to conditions encountered by garter snakes in a cold climate.

Materials and Methods

Materials

Snakes were caught near Inwood, Manitoba, 80 Km north of Winnipeg. Snakes used in successive experiments were fed on perch fillets and kept at 24° C and 12L:12D for more than two weeks before the start of another experiment.

A YSI electric thermometer and physiological thermistor probes YSI #402 and #44004 were used to take temperature readings in laboratory and field observations respectively.

Temperature Methods

Cold tolerance was measured in a stainless steel tank partially filled with 50% ethylene glycol solution at -10°C circulated through a Neslab Bath Cooler PBC-2. Eight test chambers consisting of 5-l metal cans weighted with lead and with a sheet of paper covering the bottom were partially immersed in the ethylene glycol. A small centrifugal pump near the cooler outlet helped circulate the refrigerant maintaining a constant air temperature of -8° C in the chambers during experiments. Illumination was by fluorescent lights.

In preliminary experiments, cloacal, oral and dorsal anterior body surface temperature (in the heart region) were measured. Later, the dorsal surface temperature was used.

Each snake had a YSI physiological thermistor probe #402 taped to its back above its heart without affecting blood circulation.

Before acclimation all snakes were sexed, weighed, measured and placed in numbered jars. From a field sample large enough for two or three experiments, snakes were randomly selected for each test within each experiment.

Snakes were acclimated for two weeks at 4°C and complete darkness except for three experiments, 30°C acclimated snakes, the effect of light during acclimation and the short-term acclimation experiments. All experiments were statistically analysed using analysis of variance except for effect of light during acclimation which was analysed by factorial analysis.

After acclimation the jars were placed in a 30°C warm bath. The temperature of 30°C was chosen as the reacclimation temperature (RAT) since this is the optimum body temperature of garter snakes (Fitch, 1965).

Preliminary work showed that the tolerance lasted about 8 hours of reacclimation time (RAti) so two hour intervals would give a good representation of the phenomenon and still prevent the necessity of having to perform parts of two successive tests simultaneously.

The time each snake was in the cold bath, duration of tolerance, was recorded to the nearest minute. In the cold bath each snake was checked every 2 to 3 minutes for response to an external stimulus. If the snake did not move, it was tapped four more times on the head to insure that it had lost responsiveness. The body temperature at the point of no response is the lower critical temperature (LCT). The data collected for each snake were LCT, length of time in the cold bath (duration of tolerance), body weight before acclimation, weight loss during acclimation, snout-vent length, and sex.

Results

Laboratory Data

In all experiments no snakes died during testing but about 5% died 24 hours later.

The variables of weight, snout-vent length, sex and weight loss during acclimation were not related to the duration of tolerance to cold stress in any experiment (Table I). The LCT means of RAti values were found to be not significantly different at $P<.05$ (Table II).

Preliminary Experiments

Snakes were acclimated to 4° C for two weeks and tested in an ice and salt bath which varied between -8°C to -15°C. A sample of 4-5 snakes was tested in the first preliminary experiment at 4-hour intervals from 0 to 52 hours RAti. In the latter three experiments testing was in 2 hour intervals from 0 to 12 hours for two experiments and 0 to 8 hours for the third.

The results (Fig. 1) showed that the resistance lasts about eight hours of reacclimation time (RAti). Preliminary experiment I showed that the normal level of tolerance for spring snakes lies between 15 and 20 minutes.

During these experiments it was also noted that cloacal temperatures were as much as 8° C lower than oral

Table I

Analysis of variables that could affect the duration of tolerance based on one experiment of sample size 39

A) Comparison of Sex

$\bar{x}$ (females) 30.8

$\bar{x}$ (males) 29.5

$F = 0.128$

$F_{2,36(.05)} = 3.26$

B) Regressions	n	b (slope of line)
Duration vs Weight Loss	39	-0.149
Duration vs Initial Weight	39	0.092
Duration vs LCT	39	0.001
Duration vs Snout-Vent Length	39	0.030

Table II

LCT of Spring Snakes

RAti	0	2	4	6	8
$\bar{x}$	-1.13	-1.06	-0.73	-1.61	-1.38
s	0.42	0.86	1.29	0.92	0.77
n	6	7	7	8	8

Analysis of Variance

	df	SS	MS
Treatments	4	3.329	0.832
Error	31	24.691	0.796
Total	35	28.020	

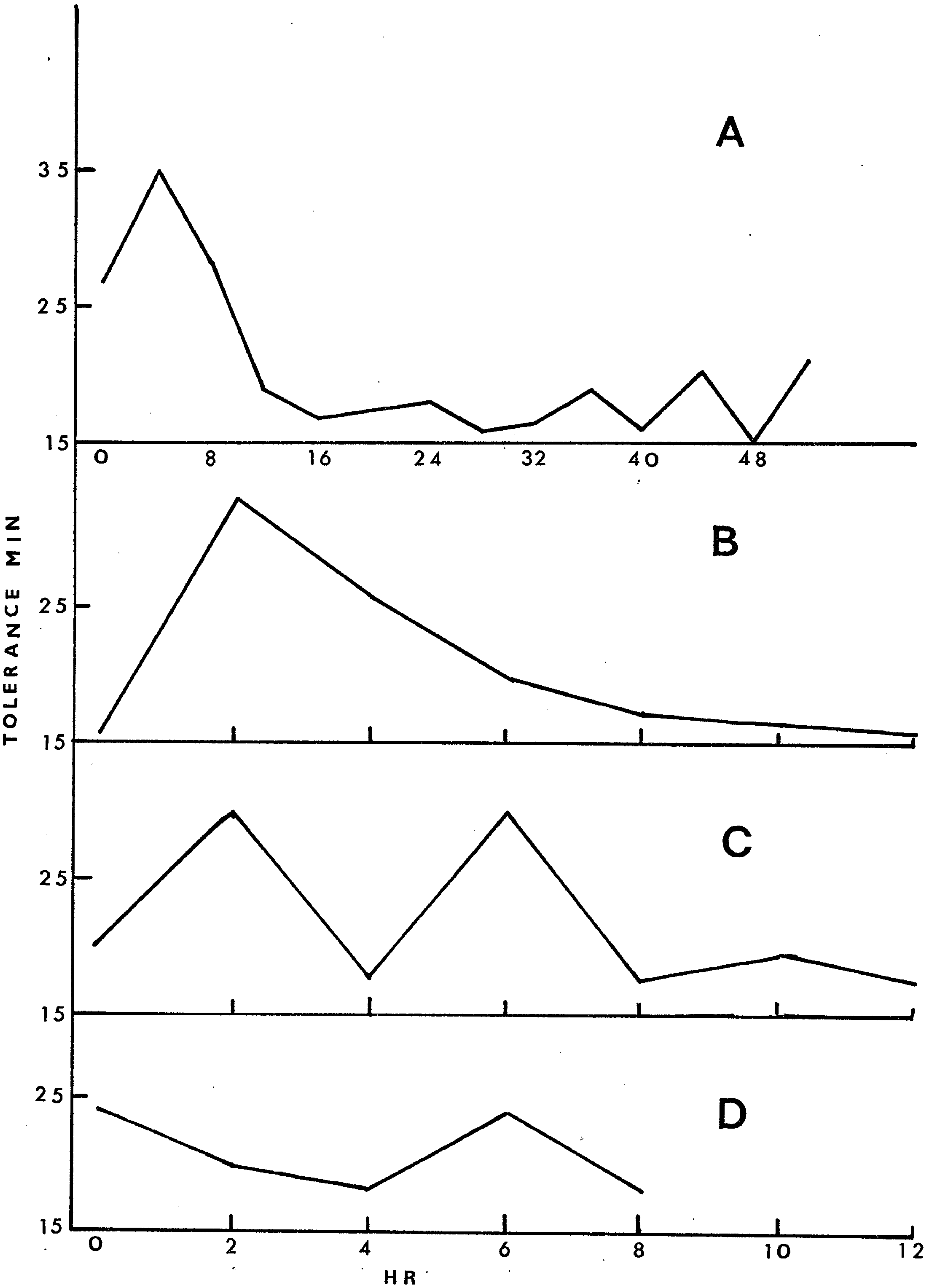
$$F = \frac{MST}{MSE} = 1.045$$

$$F_{4,31(.05)} = 2.68$$

∴ the LCT do not differ significantly from RAti to RAti

Fig. 1 A) Tolerance time of spring snakes acclimated to 4°C for two weeks and tested at 4 hour intervals from 0 to 52 hours RAti in a -10±3°C bath. Each point is a mean of 6 snakes.

B-D) Tolerance times of spring snakes acclimated to 4°C for two weeks and tested at 2 hour intervals from 0 to 12 hours for B & C and from 0 to 8 hours for D. Each point is a mean of 5 snakes.

A
35
25
15
0
8
16
24
32
40
48
B
25
15
C
25
15
D
25
15
0
2
4
6
8
10
12
TOLERANCE MIN
HR

temperatures and varied considerably during testing. This necessitated the use of dorsal body surface temperature since oral temperatures interferred with the test for movement. A dorso-ventral temperature gradient was also noted with the dorsal surface 0.5°C warmer than the esophagus and 1°C warmer than the ventral body surface.

After the snakes were placed in the cold bath their oral temperature decreased to 5°C and their cloacal temperature decreased to 1°C in ten to fifteen minutes. Here they remained upto thirty minutes before they began to decrease again to the LCT.

Resistance of Warm Acclimated Snakes

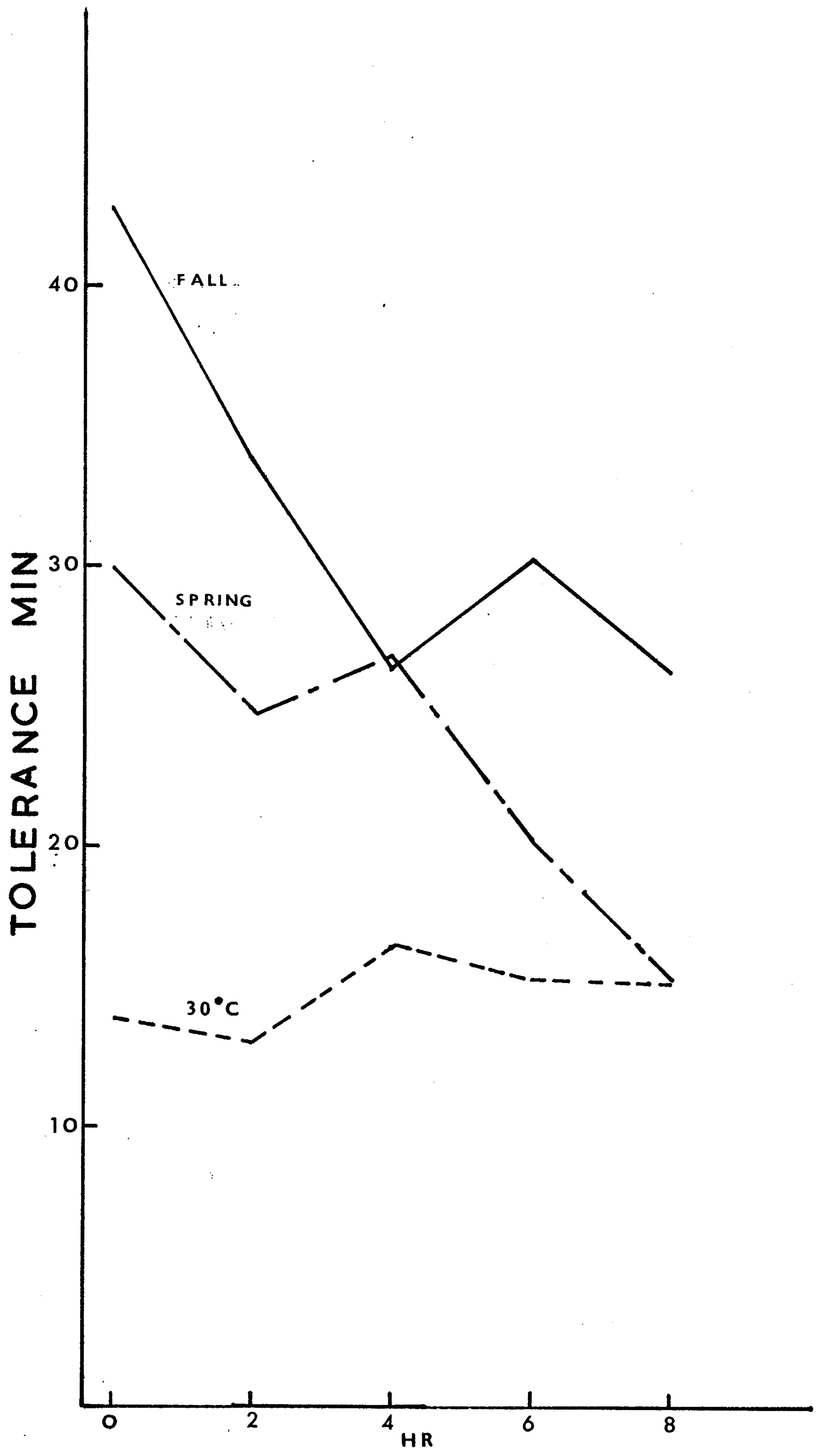
Fall snakes were acclimated for two weeks at 30°C and darkness. Eight snakes were tested at 0, 2, 4, 6 and 8 hours in light and at 30°C. The mean resistances of the RAti of these warm acclimated snakes were between 13 and 18 minutes and were not significantly different at $P<.05$ (Fig. 2).

Resistance of Spring and Fall Snakes

Snakes were acclimated to 4°C for two weeks and a sample of 6-8 snakes was tested at 0, 2, 4, 6 and 8 hours RAti.

The resistance of spring snakes was 30 minutes at 0 hour and decreased to 15 minutes at 8 hours. However,

Fig. 2 Tolerance times of spring and fall snakes acclimated to 4° C for two weeks and of fall snakes acclimated to 30° C for two weeks were tested at 2 hour intervals from 0 to 8 hours RAti. Each point is a mean of 7-8 snakes. Mean standard errors were for spring snakes 0.5 minutes, for fall snakes 0.3 minutes and for 30° C acclimated snakes 0.2 minutes.


TOLERANCE MIN
40
30
20
10
FALL
SPRING
30°C
0
2
4
6
8
HR

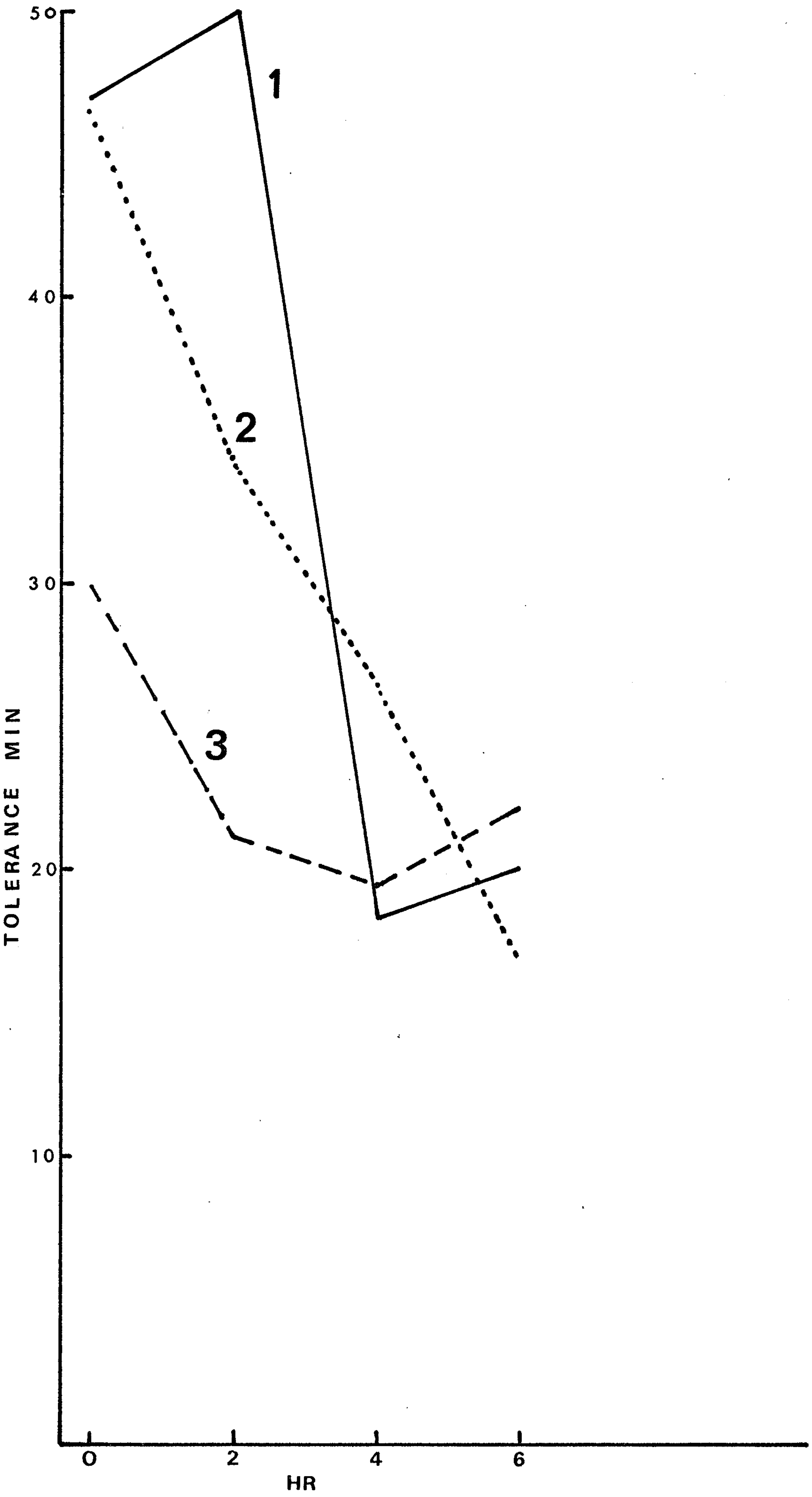
the resistance of fall snakes was 42.5 minutes at 0 hour and decreased to 26 minutes at 8 hours (Fig. 2). The mean resistances of the RAti values were significantly different for spring snakes at $P<.05$ and for fall snakes at $P<.01$.

Re-establishment of Cold Tolerance

To determine whether snakes could re-establish their cold tolerance after one night's exposure to cool temperatures, three groups of snakes were acclimated to 4°C for two weeks and a sample of 5 snakes was tested at 0, 2, 4 and 6 hour RAti. Group 1 was tested after acclimation at 4°C while groups 2 & 3 were subjected after acclimation at 4°C to the 30°C bath for 6 hours followed by 18 hours at 4°C and darkness. Group 2 was then tested while group 3 followed the same procedure as the previous day before testing.

All three groups showed decreasing resistance times with increase RAti (Fig. 3). The resistances for groups 1 and 3 were initially quite high, 47 minutes, and decreased to 18-20 minutes at 6 hours. The resistance of group 2 started considerably lower at 30 minutes and decreased to 22 minutes at 6 hours. By analysis of variance the means of the RAti values for group 1 and 3 were significant at $P<.01$. Analysis of variance of the means of the RAti values for group 2 was not significant at $P<.05$.

Fig. 3 Tolerance times of snakes subjected to 0 (group 1), 1 (group 2) or 2 (group 3) 6 hour periods of 30°C and light followed by 18 hours at 4°C and darkness after two weeks acclimated at 4°C and darkness. Snakes were tested at 2 hour intervals from 0 to 6 hours out of the cold room. The points are means of 5 snakes. Mean standard error for all points of groups 1 & 2 is 0.06 minutes and of group 3 is 0.10 minutes.


50
40
30
20
10
TOLERANCE MIN
1
2
3
0
2
4
6
HR

Short-Term Acclimation

For survival in the fall snakes must be able to establish their resistance in one or two nights. Two groups of snakes were acclimated to 4°C for 18 hours and 42 hours and samples of 7 snakes were tested at 0, 3 and 6 hours RAti.

The resistance for 18 hours of acclimation started at 24 minutes, increasing to 27.5 minutes at 3 hours RAti and then decreasing to 23 minutes at 6 hours (Fig. 4). Means of the RAti's were not significantly different at $P<.05$. The resistance for 42 hours of acclimation started at 43 minutes and decreased to 20 minutes at 6 hours RAti (Fig. 4). The means of the RAti's were significantly different at $P<.01$.

Lack of Diurnal Cycle of Tolerance

Fall snakes were acclimated to 24°C and 12L:12D centered at 1500 hours and not fed during acclimation. Six snakes were tested at 900, 1100, 1300, 1500 and 1700 hours. The mean resistance times of each sample were between 21 and 27 minutes and were not significantly different at $P<.05$ (Fig. 5).

Influence of Daily Illumination during Acclimation

To study the effect of light during acclimation, four groups of 8 snakes were acclimated for two weeks at 4°C & 24D, 4°C & 12L:12D, 30°C & 24D and 30°C & 12L:12D.

Fig. 4 Tolerance times of snakes acclimated to 4°C for 18 and 42 hours were tested at 0, 3 and 6 hours out of the cold room. Each point is a mean of 7 snakes. The mean standard errors are 0.5 minutes for 18 hours and 0.4 minutes for 42 hours of acclimation.

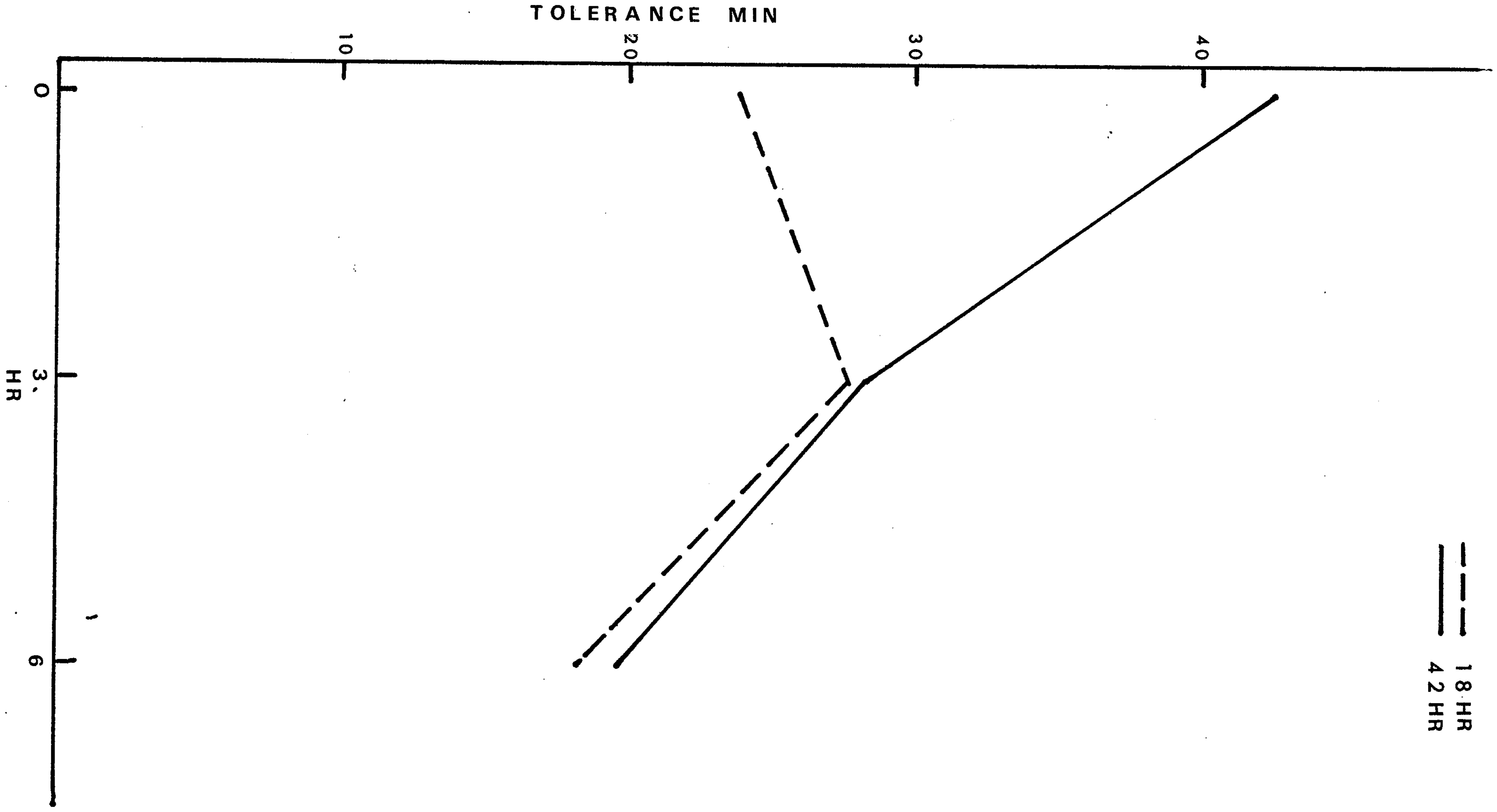
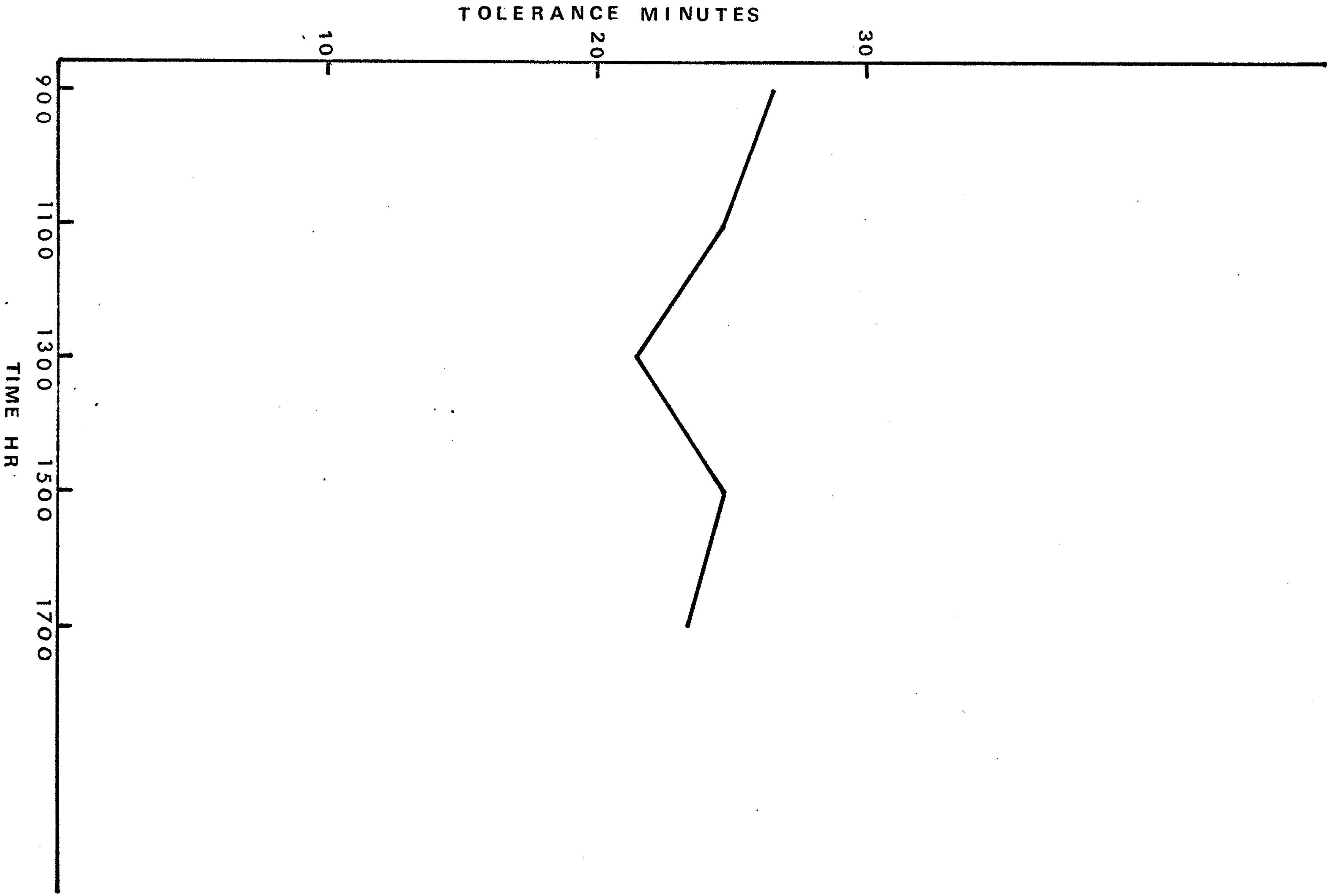

TOLERANCE MIN
10
20
30
40
0
3
HR
6
18 HR
42 HR

Fig. 5 Tolerance time of fall snakes acclimated to 24°C and 12L:12D (Diurnal Cycle) centered at 1500 hours were tested at 900, 1100, 1300, 1500 & 1700 hours. Each point is a mean of 8 snakes. Mean standard error for all points is 0.4 minutes for the diurnal cycle.


TOLERANCE MINUTES
10
20
30
TIME HR
900
1100
1300
1500
1700

All snakes were tested after 2 hours in a 30°C bath in light.

The results showed that acclimation temperature had a marked effect upon the duration of tolerance ($P<.01$) (Table 3). Light had no effect upon the duration but the interaction of light and temperature did have a significant influence ($P<.05$) on duration. Comparing means (Table 2) within each acclimation temperature it appears that light markedly reduced the cold tolerance at acclimation temperature of 4°C but slightly increased the tolerance at 30°C acclimation.

Effect of Handling at 0 Hour RAti

Twenty-four snakes were acclimated for two weeks at 4°C and tested immediately upon removal from the cold room. Twelve snakes had probes taped to their backs (handled) and twelve snakes had nothing taped to them (not handled).

The effect of handling snakes at 0 hour RAti was not significant at $P<.05$. The data are below.

	Handled	Not Handled
$\bar{x}$	49.7	52.4
n	12	12

Table III Effect of Light during Acclimation

Data transformed to $\frac{1}{\sqrt{\text{Duration}}}$

Factor Analysis

Factor 1	light --	level 1	darkness	a_1
		" 2	12L:12D	a_2
Factor 2	temp. --	level 1	4°C	b_1
		" 2	30°C	b_2

	a_1b_1	a_1b_2	a_2b_1	a_2b_2
	4°C & 24D	30°C & 24D	4°C & 12L:12D	30°C & 12L:12D
$\bar{x}$	0.162642625	0.2578147142	0.204245125	0.22039575
Back transformed $\bar{x}$	38.08 min.	15.04 min.	23.97 min.	20.60 min.

		n	Pooled Mean	S.D.
Light level 1		15	0.21	0.06
2		16	0.21	0.05
Temperature level 1		16	0.18	0.06
2		15	0.24	0.03
Light & temp.	a_1b_1	8	0.16	0.04
	a_1b_2	7	0.26	0.03
	a_2b_1	8	0.20	0.07
	a_2b_2	8	0.22	0.02

Analysis of Variance

Source	df	SS	MS	F
Light	1	0.0000	0.0000	0.01
Temperature	1	0.0234	0.0234	11.00
Light & temp. interaction	1	0.0116	0.0116	5.47
Within cells	27	0.0574	0.0021	
Error due to approx.		-0.0010		
TOTAL	30	0.0914		

$F_{1,27.05} = 4.21$

$F_{1,27.01} = 7.68$

Acclimation temperature level is highly significant, having the greatest effect upon the duration.

The temp-light interaction is significant at the $P<.05$ level, so this combination has an effect upon duration.

Field Data

The mean cloacal temperatures of male garter snakes caught during the spring breeding season are listed in Table IV.

The paired t-test on the oral and cloacal temperatures of snakes caught on warm sunny days showed no significant difference at $P<.05$ while for those caught on cool days there was a significant difference at $P<.05$. The mean difference was only 0.20°C. The oral temperature can be as much as 2.2°C below the cloacal temperature and as much as 2.0°C above the cloacal temperature on clear sunny days. On cool cloudy days the range is narrower, being -0.5° to 1.1°C.

Analysis of variance showed no significant difference ($P<.05$) between cloacal temperatures of males engaged in mass courtship and non-courting males. Female snakes were not measured due to insufficient numbers.

On sunny days, the activity of males consisted either of courtship and mating or apparent constant movement around the den. Courtship and mating always occurred while snakes were beneath rocks or in shaded areas on such days.

Some cloud cover or slightly cool weather reduces the cloacal body temperature by 5° to 7°C. Cool and cloudy weather reduces this temperature further, by 14° to 16°C but the snakes are still out even though no courting behaviour, was seen. If the substrate is also wet the snakes are found beneath rocks and the cloacal temperatures

Table IV
Body Temperature of Male Garter Snakes Under Natural Conditions in the Spring

Date (1971)	Weather Conditions	Type of Temp.	Snake Conditions	n	Mean $\overline{x}$	s^2	S	Air Temp.	Substrate Surface Temp. Litter	Rock
Apr 22	S	Cl	M & Cu	27	30.97	2.818	1.678	20.9		
May 2	S	Cl	M & Cu	25	31.04	1.954	1.397	18.0	37-39	32.0
May 6	S	Cl	M	25	32.16	3.457	1.859	23.8	45.5	32.0
May 6	S	Cl	Cu	25	32.65	2.285	1.511	23.8	45.5	29.0*
May 13	S	O	M & Cu	25	30.44	1.755	1.324	23.5	41.0	29.0*
May 13	S	Cl	M & Cu	25	30.53	2.689	1.639	23.5	41.0	17.0
Apr 30	S & Co	Cl	M & Cu	27	25.31	27.454	5.239	12.1	17.0	
Apr 26	S & C	Cl	M & Cu	33	25.19	6.082	2.466	12.0	13.5	
Apr 29	C & Co	Cl	M & Cu	25	16.29	2.733	1.653	11.5	10.5	
May 10	C, Co & wet	O	M & Cu	20	9.86	0.265	0.514	8.0	9.5-10 **	
May 10	C, Co & wet	Cl	M & Cu	25	9.51	0.476	0.689	8.0	9.5-10 **	

* on open black earth, temperature was 39.0 C

** these temperatures were taken under rocks where the snakes were lying

Legend

S -- Sunny
C -- Cloudy
Co -- Cool
Cl -- Cloacal

O -- Oral
M -- Moving
Cu -- Courting

are very close to the substrate temperatures. In all cases the cloacal temperatures of active snakes were 5° to 20°C higher than the air temperature.

It must be noted that one female snake which was half emerged from a den opening had an oral temperature of 26°C and a cloacal temperature of 10°C.

At a natural den the ground temperatures at bedrock, 56 cm deep, went as low as -1.0°C from mid January to mid March (Fig. 6).

The two snakes that were monitored during the winter of 1970-71 may have survived temperatures of -3.0°C and -2.0°C but, unfortunately, neither snake was recovered. One snake was possibly eaten and the other snake seems to have shed its skin and the probes with it in the spring.

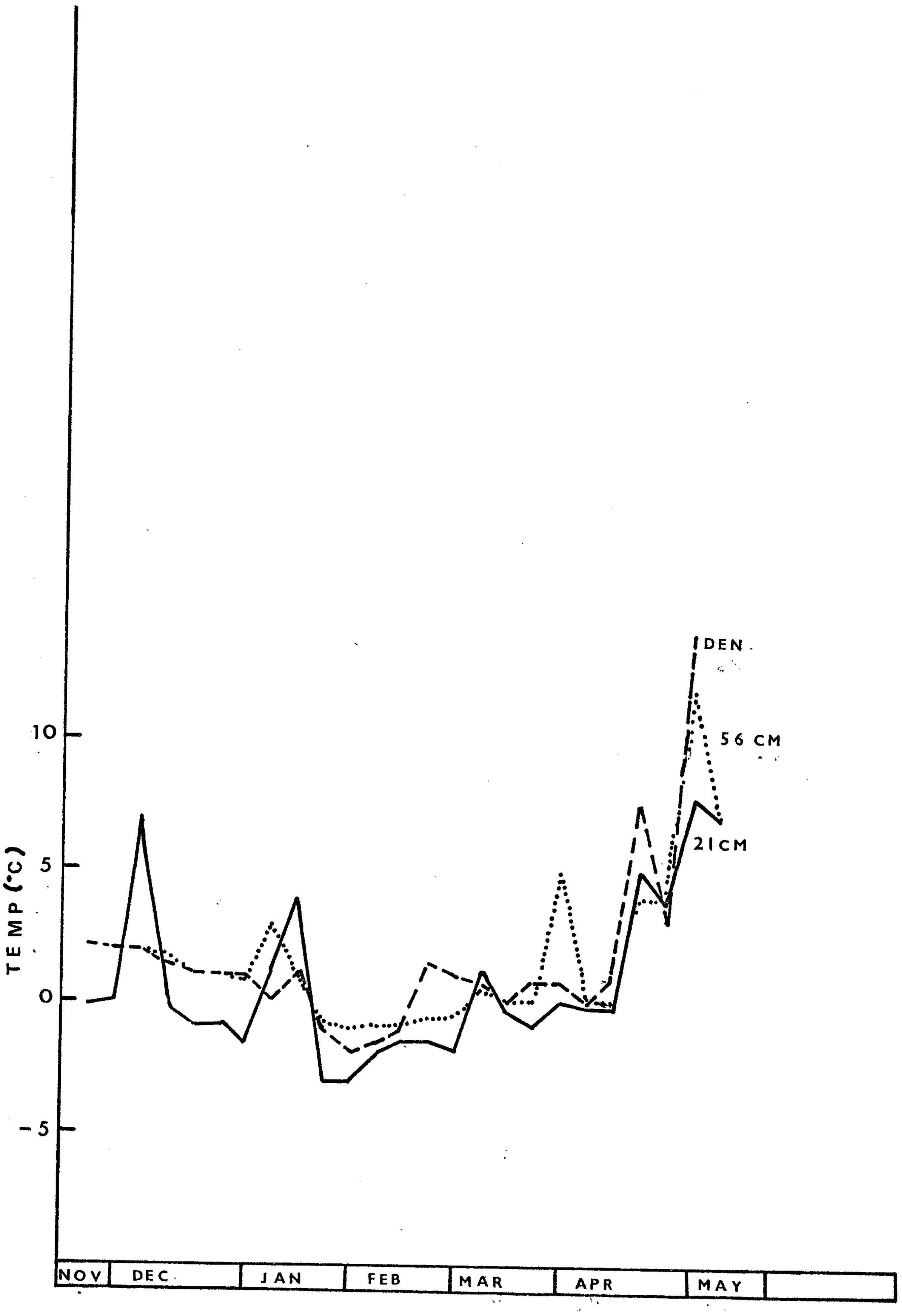
From the artificial den it appears, even though only five snakes emerged, that the inversion of the temperature gradient causes snakes to emerge from the den in spring (Fig. 7a&b). Snakes were found out of the den only after the temperature gradient started to invert itself and as the inversion penetrated deeper into the den more snakes emerged.

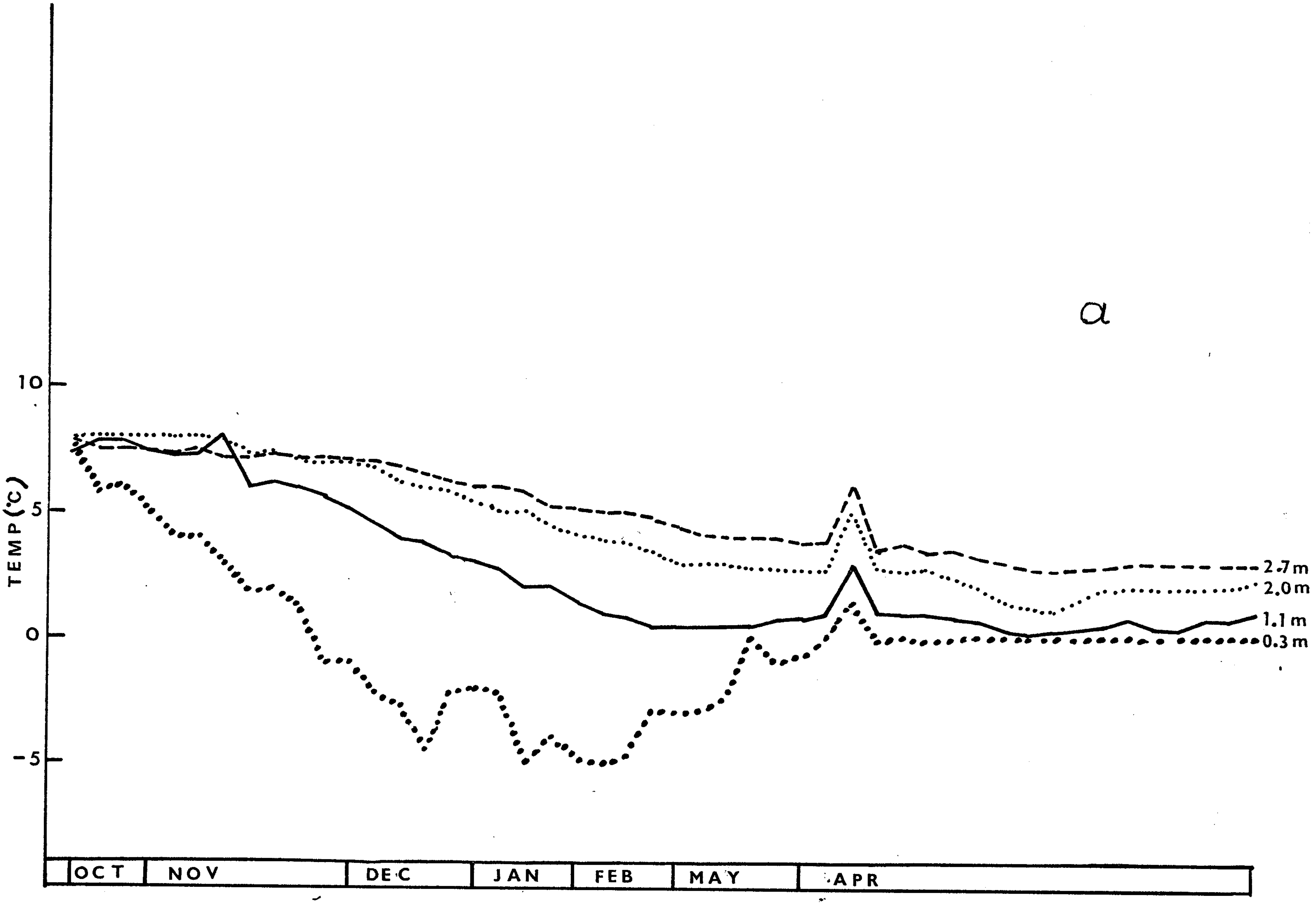
Discussion

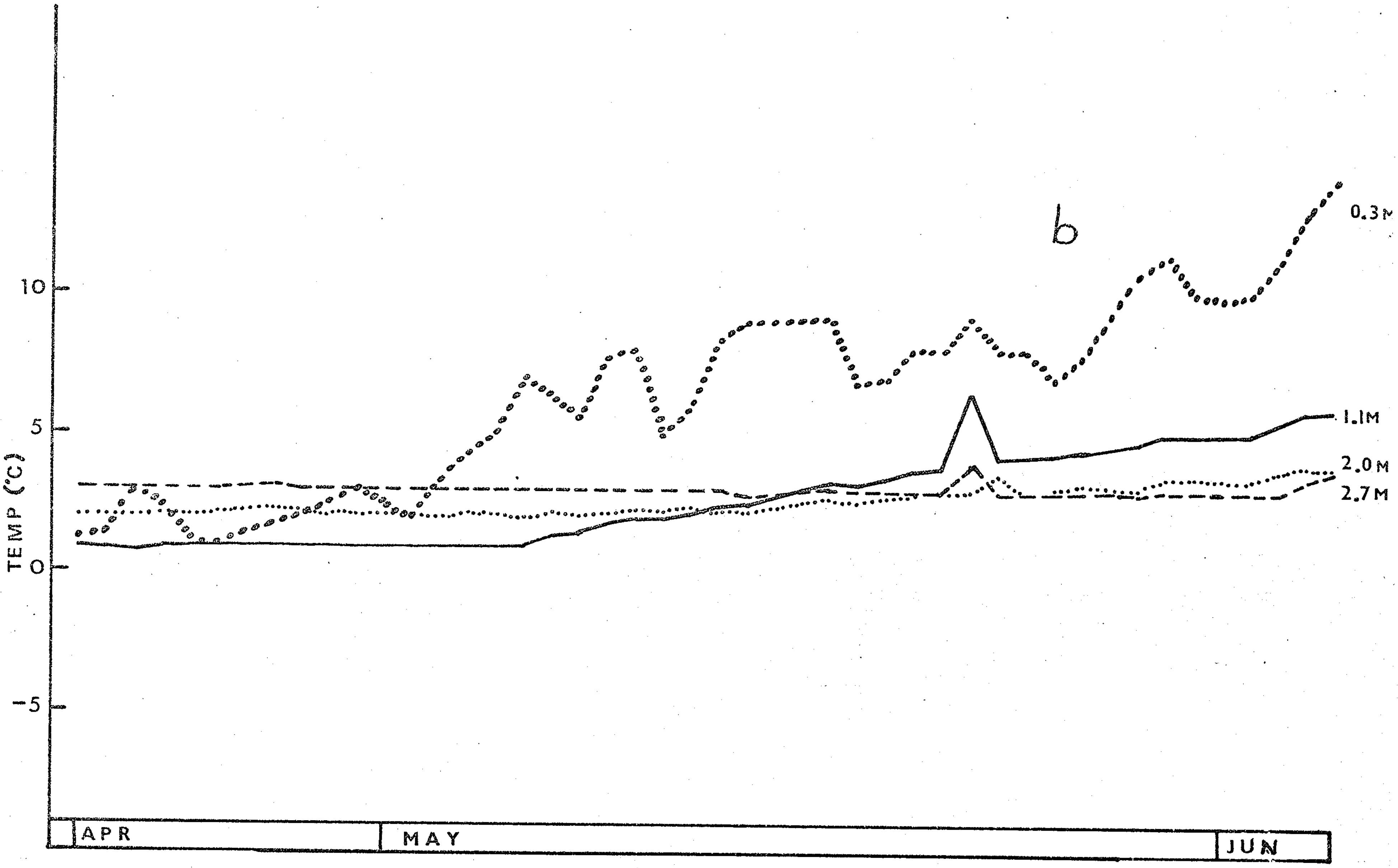
The garter snake has increased cold tolerance after acclimating to a low temperature. This tolerance is expressed not as a change in LCT but rather in the time that the snake can be active in the stress condition. This endurance implies that cold acclimated snakes are able to

Fig. 6 Ground temperatures at 21 and 56 cm at a natural den and the air temperature in the den at an unknown depth were recorded once a week during November to May.

Fig. 7a&b Substrate temperatures in an artificial constructed limestone den measured at 0.3 m, 11 m, 2.0 m and 2.7 m were monitored 3 times a week in October and November, once a week upto April and then daily upto mid June.


TEMP (°C)
10
5
0
-5
NOV
DEC
JAN
FEB
MAR
APR
MAY
DEN.
56 CM
21CM


a
10
5
0
-5
TEMP (°C)
2.7m
2.0m
1.1m
0.3m
OCT
NOV
DEC
JAN
FEB
MAY
APR


b
0.3M
1.1M
2.0M
2.7M
10
5
0
-5
TEMP (°C)
APR
MAY
JUN

resist cooling. The resistance appears near +1°C for cloacal temperatures and +5°C for anterior body temperatures where the temperatures may remain for up to 30 minutes before they begin to decrease to the LCT.

The tolerance lasts between 6 and 8 hours after removal from cold acclimation. The tolerance might extend over a considerably longer period under less severe conditions, since, in most cases, the stress temperature would be near freezing (0°C) and not -10°C, so, the resistance might be greater both in duration per RAti and in the number of hours that it lasts under natural conditions.

Spring Cold Tolerance

Spring snakes have an initial tolerance to cold upon emergence from the den (Fig. 2). This is lost after a few hours. Its ecological importance is closely associated with the ability to re-establish the tolerance level after one cool night submerged in the den.

Fall Cold Tolerance

Fall snakes have a higher tolerance level than spring snakes which could be associated with the fall movement to the dens. It is vital that they have a higher degree of tolerance in order to find shelter at the onset of a cold period, thus, avoiding immobility resulting in death from either predation or the severity of the cold period. Gregory (per. com.) has found on a few occasions several snakes frozen in funnel traps. This emphasizes the importance of finding shelter at the onset of cold periods. This tolerance maybe acquired from short exposures to cool nights. From laboratory experiments it appears to be established after two such nights (42 hours) but after one night (18 hours) the level of tolerance is equivalent to that of spring snakes.

The resistance graph of fall snakes is not an artifact of sampling since three separate experiments using fall snakes showed very similar graphs (Figs. 2-4).

The resistance graph of spring snakes is also not an artifact of sampling since five separate experiments, four of which are preliminary experiments with cold bath temperatures near -8°C, showed 0 hour resistance ranging from 20-33 minutes (Fig. 1) however, in three of the four experiments, 2 hours RAti showed an increase in resistance ranging from 7-16 minutes over the initial value of 0 hour. The bath temperature at 2 hours RAti had decreased by 2°C upto -10°C. Since the initial bath temperature of the preliminary experiments was higher than in later experiments, one would expect a higher tolerance level for spring snakes in the preliminary experiments, but the results are the same in both preliminary and later experiments.

In all later experiments the results were compared to the condition in which the snakes were acclimated to 30°C. As a result, increased tolerance is assumed to occur if tolerance exceeds 20 minutes. Tolerance less than this is considered the normal state of warm acclimated snakes. However, the snakes acclimated at 30°C were kept under unnatural conditions of darkness and starvation. Further information on the resistance of warm acclimated snakes can be gained from the preliminary experiment covering 52 hours of reacclimation. Although this series of snakes were also starved, they were acclimated at 4°C in darkness prior to testing which would approximate normal overwintering conditions. In this experiment, the mean duration of resistance after 12 hours fluctuated between 14 and 21 minutes. Analysis of variance of the means of the RAti were not significantly different at $P<.05$. The agreement of the 52 hour preliminary experiment with the observations at 30°C and the lack of correlation of resistance time with weight loss during acclimation seems to reinforce the contention that the warm-acclimated resistance is between 15 and 20 minutes and that starvation and darkness under warm temperature conditions had no significant effect on the 30°C acclimation values. However, when the 30°C acclimated fall snakes are compared with the fall snakes acclimated at 24°C & 12L:12D it becomes obvious that the 30°C acclimated snakes were under some stress but not starvation since neither group was fed. The differences between them were daily illumination, temperature and availability of space for movement, since the 30°C snakes were in 16 oz. jars while the 24°C snakes were in a 20 gal.

tank. In any case it is still my contention that normal cold tolerance of spring snakes lies between 15 and 20 minutes since the 52 hour preliminary experiment consisted of 11 replicates of six snakes within this range.

The difference in tolerance of spring and fall snakes may be due to different physiological conditions. The fall snakes are in better condition since they have been feeding upto the time of movement back to the dens. Spring snakes have just emerged from a 6-month hibernation during which no food was available.

No diurnal variation in tolerance to cold stress could be detected in the experimental data, but the level of tolerance was a lot higher than the 52 hour preliminary experiment (Fig. 1a&5). The snakes used were fall snakes which undoubtedly had an effect upon the level of tolerance. There may also be seasonal changes in the tolerance level of warm-acclimated garter snakes. Brett (1944) found that the bullhead in Ontario showed a seasonal change in the UCT, increasing as summer approached and decreasing as winter approached. Hutchison and Ferrance (1970) noted that Rana pipiens acclimated to fluctuating temperatures had UCT's which were higher than those held under a steady state acclimation and similar results were found in bullheads (Brett, 1944). These two factors may act upon the duration of tolerance in garter snakes so that fall snakes would have a higher warm-acclimated tolerance level than spring snakes.

Light during acclimation by itself had no effect upon duration of tolerance but in association with temperature, light tended to reduce the tolerance level at low acclimation temperatures and increased the tolerance level at high acclimation temperatures (Table III). This trend of increased tolerance level would be advantageous in the fall when snakes start moving back to the dens, while daytime temperatures are still quite high.

It seems that fall snakes have a higher warm-acclimated tolerance level than spring snakes (Fig. 1a&5). Since the light effect experiment showed some increased tolerance with light at high acclimation temperatures, light may have an influence in the seasonal change in duration of tolerance. The influence of fluctuating environmental temperatures may also have an effect on the seasonal differences of warm acclimated snakes. Thus, seasonal changes in tolerance levels of the garter snake may be based on physiological conditions of the snake, photoperiodism and acclimation to fluctuating fall temperatures.

In the re-establishment experiment (Fig. 2) group 2 showed a tolerance graph similar to that of warm-acclimated fall snakes, (Figs. 3&5) yet group 3 showed the expected fall tolerance level for cold acclimated snakes. The difference may be due to sampling error even though two samples of snakes are involved.

Field

The fact that garter snakes seek shelter during cold periods as seen on cold days at Inwood, would cause them to submerge into the opening of crevices of the den. The depth to which they submerge may depend upon the temperature and duration of the cold period. Progressively colder nights in the fall could drive the snakes deeper underground. As the surface ground temperatures fall below those of the lower levels, they would probably locate themselves in the warmest part of that gradient. It is known that garter snakes will follow a temperature gradient (Stewart 1965, Fitch 1965). This area is shifted deeper underground due to the colder temperatures and the chances of the snakes emerging in the fall are reduced and finally non-existant, even though the surface temperatures on some days may be quite warm.

In spring the warming of the surface layers by the sun begins to invert the ground temperature gradient established during the winter (Fig. 7a&b). When the warmer temperatures reach the level occupied by the snakes, they may follow the gradient of increasing temperatures back to the surface. In dens which drain meltwater from the surrounding countryside, the water passing through the den causes a condition of isothermy at all levels in the den. After the water is gone, solar heating can rapidly induce a temperature gradient in the den since it does not have to reverse one which is already present.

During the spring breeding period the mean body temperature of snakes is entirely dependant upon the amount of radiant energy and the air temperature. It appears that normal behavioural thermoregulation is overridden by the reproductive drive, since snakes are actively moving about on above lethal substrate temperatures (>41° C) (Lueth, 1941). In order to compensate for this the snakes are continually moving in and out of shade and in and out of the cool tunnels of the den. The mean cloacal temperature of these snakes is 1° to 2° C higher than that quoted by Fitch (1965). On warm sunny days, the greatest proportion of snakes had cloacal body temperatures above 31° C while that of Fitch was between 29° - 30° C. Thus, it appears that male garter snakes during the breeding season are under a stress condition of high temperatures.

These results re-enforce the use of 30° C as the reacclimation temperature since it appears that male garter snakes will raise their body temperature to 30° C or higher if weather conditions permit.

Conclusions

The low temperature resistance pattern found in Manitoba garter snakes constitutes an important part of their adaptation to northern conditions. One such northern condition is freezing night temperatures associated with spring and fall. To overcome this stress the garter snakes can acquire a resistance with cold acclimation as short as 42 hours, however, this resistance is not expressed in a LCT change under the experimental conditions but, rather, in an increased endurance time to the stress temperature. The tolerance level can be maintained each day as a result of cool nights which re-establish the tolerance level. Snakes in the fall are more likely to be subjected to cold exposure while removed from shelter than they are in the spring. They also have a higher resistance to cold, whether cold acclimated or not. This may be induced by their better physiological condition, photoperiod effects, fluctuating environmental temperatures or a combination of these. In any case, the increased fall resistance is adaptive since it occurs at the time of greatest need.

The submergence and emergence of garter snakes from their wintering dens are probably controlled by alterations of the surface to subterrainean temperature gradient. During winter the gradient is warmest at greater depths but in spring the reverse is true with the ground surface being the warmest part of the gradient. The emergence of snakes is dependant upon the depth at which they are and whether water flows through the den which would create a condition of isothermy in the den, and thus, decrease the length of time to establish the spring temperature gradient.

The effect of the gradient is a conservative one in that the snakes in the fall submerge early and remain there due to the rapid establishment of the winter temperature gradient. This avoids the rapid and severe changes in temperature associated with late fall. In spring however, the snakes are drawn out after the spring warming is well progressed, thus minimizing the chances of being subjected to cold weather.

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Appendix 1

During the winter of 1970-71 ground temperatures were recorded in an artificial and a natural overwintering den. The artificial den was 3 m deep. It was later found that the ground temperatures do not show annual variation below 2 m in Manitoba (Thayhewich per. com.).

The purposes of monitoring the artificial and natural dens were to show whether seasonal changes in ground temperature gradients caused the submergence and emergence of snakes and to find out at what depth snakes hibernate. Snakes in both dens had thermistor probes with lead wires taped to their tails. In addition to temperature readings the depth to which they submerged could be guaged by the amount of lead wire pulled down into the den as winter progressed. Unfortunately the lead wires were quite thin and as a result were quickly broken by the snakes. Two more snakes were probed and freed in natural den openings but the lead wires were now too thick and became stiff in cold weather. In order to compensate for this stiffness a lot of slack was left off the reel. From these results it became obvious that string would have been a better choice since it does not stiffen in cold weather.

I had also hoped to get some idea at what depth snakes hibernated and to compare this with the temperature gradient inversion and emergence times of snakes to see if monitoring ground temperatures could give any indication of the depth at which snakes hibernate in natural dens. Since I was unable to obtain the information on the depth of hibernation, I was unable to determine whether the temperature gradient inversion caused immediate emergence or if a delay exists after the inversion has reached the snake.

Since the annual variation of the ground temperature below 2 m is almost non-existant in Manitoba it may be assumed that snakes do not hibernate below this depth unless the snakes have other cues than the ground temperature gradient. This is possible in dens which drain meltwater which creates a condition of isothermy in the den tunnels. The tunnels and underground streams of such dens could draw currents of warm surface air into the tunnels which would arouse the hibernating snakes. Since the air coming in is quite warm, it cools as it moves downwards and thus producing an air temperature gradient which the snakes follow to the surface. In addition, snakes have been seen emerging from dens which are completely submerged in water along stream banks (Carpenter, 1953). In this case, the temperature gradient would be in a water medium.

It appears since the dens seem to lack any other stimuli that the snakes are cued by temperature gradients for spring emergence. These gradients can be through soil, air, or water media. The type of media involved, or combination, could speed up the process of emergence and thus being beneficial to the snake population.

Appendix 2

Direct observation of the variances in all experiments revealed definite heterogeneity. Each experiment had only four or five variances which were considered inadequate for the use in Taylor's Power Law for a good transformation, thus all variances were used. To insure a correct transformation for each experiment the variances of the transformed data were tested for homogeneity using Bartlett's test.

In experiment XII on re-establishment the transformation did not work. Taylor's Power Law was applied to the experiment to find another transformation which did work. Experiment XI, light effect did not have its data transformed into logarithms but had Taylor's Power Law applied to the data directly. The transformation was also checked using Bartlett's test.

Taylor's Power Law on all Variances

$\bar{x}$	$\ln \bar{x}$	s^2	$\ln s^2$
29.8	3.39451	98.20	4.58701
32.0	3.46574	130.00	4.86753
31.2	3.44042	115.90	4.75359
30.0	3.40120	180.67	5.19850
21.0	3.04452	41.14	3.71601
15.7	2.75366	10.21	2.32239
43.7	3.77735	109.57	4.70048
35.0	3.55535	125.81	4.83628
27.6	3.31782	8.14	2.09679
29.7	3.39115	47.90	3.86912
26.6	3.28091	25.28	3.23080
14.6	2.68102	24.84	3.12084
13.2	2.58776	7.36	1.99606
18.9	2.93916	68.70	4.22975
15.5	2.74084	13.43	2.59525
15.4	2.73437	11.41	2.43361
15.7	2.75366	16.90	2.82731
42.2	3.74479	186.50	5.22843
31.9	3.46261	403.84	6.00141
22.0	3.09104	64.60	4.15888
50.3	3.91801	37.90	3.63495
42.8	3.75654	296.57	5.69373
37.6	3.62700	505.62	6.22654
23.0	3.13549	45.67	3.82210
23.5	3.16125	17.62	2.86790
34.4	3.53806	81.30	4.39815
34.2	3.53223	43.70	3.77735
31.9	3.46261	79.81	4.37952
24.0	2.17805	33.50	3.51155
26.7	3.28466	91.47	4.51634
52.4	3.95891	300.81	5.70711
49.7	3.90600	358.97	5.88332
47.8	3.86703	83.70	4.42724
52.0	3.95124	220.00	5.39363
18.6	2.92316	13.30	2.58776
21.4	3.06339	60.30	4.09933
35.8	3.57795	387.70	5.96101
21.0	3.04452	15.50	2.74084
20.6	3.02529	62.80	4.13996
23.2	3.14415	55.70	4.01998
46.2	3.83298	82.70	4.41522
37.8	3.63281	213.20	5.36129
24.5	3.19867	120.33	4.78749
17.0	2.83321	2.00	0.69315

Taylor's Power Law on all Variances cont'd.

Regression $y = a + bx$

where $y = \ln s^2$
$x = \ln \bar{x}$

slope $b = 2.06 \approx 2.0$

Taylor's Law $z = x^p$

where $P = 1 - \frac{1}{2}b$
$= 1 - \frac{1}{2}(2)$
$= 0$

$\therefore \quad z = x^0$

which is a log transformation.

EXPERIMENT I Normal Test of Spring Snakes

0 Hour		2 Hour		4 Hour		6 Hour		8 Hour	
Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.
41	3.71357	23	3.13549	12	2.48591	12	2.48519	19	2.94444
31	3.43399	37	3.61092	14	2.63906	33	3.49651	18	2.89037
15	2.70805	43	3.76120	33	3.49651	22	3.09104	12	2.48491
39	3.66356	24	3.17805	28	3.33220	21	3.04452	18	2.89037
25	3.21888	30	3.40120	41	3.71357	22	3.09104	15	2.70805
41	3.71357	18	2.89037	49	3.89182	25	3.21888	19	2.94444
		12	2.48491	33	3.49651	15	2.70805	11	2.39790
						18	2.89037	14	2.63906
$\bar{x}_2$	3.40860333		3.208877142		3.293511428		3.0031650		2.7374425
s^2	0.15568531		0.18926637		0.283413156		0.09649728		0.046010625

Bartlett's Test

s^2	0.155685326	0.18926637	0.283413156	0.09649728	0.046010625
$\ln s^2$	-1.85993	-1.61312	-1.26085	-2.33815	-3.07887
df	5	6	6	7	7
1/df	.20000	.170667	.176667	.14286	.14286

$$\bar{s}^2 = \frac{\Sigma fiSi^2}{\Sigma fi}$$

$$= 1/31\ (0.778426630 + 1.135548286 + 1.700478436 + 0.675480330 + 0.322074375)$$

$$= 0.148776082$$

$$\ln \bar{s}^2 = -1.90532$$

$$M = (\Sigma fi \ln \bar{s}^2 - \Sigma fi \ln Si^2)$$

$$= -59.06492 - (-9.29965 - 9.67872 - 7.56510 - 16.36705 - 21.55209)$$

$$= -59.06492 + 64.46261$$

$$= 5.39769$$

$$C = 1 + \frac{1}{3(a-1)}\left[\Sigma\frac{1}{fi} - \frac{1}{\Sigma f}\right]$$

$$= 1 + \frac{1}{12}\left[0.78680\right]$$

$$= 1.06556$$

$$\frac{M}{C} = \frac{539769}{1.06556}$$

$$= 5.06558$$

EXPERIMENT I cont'd.

$\chi^2_{4,.05} = 9.49$

variances are homogeneous

Analysis of Variance

	df	SS	MS
Treatments	4	2.04073413	0.51018353
Error	31	4.61205848	0.14877608
Total	35	6.65279261	

$F = \frac{MST}{MSE} = 3.42920400$

$F_{.05}\ (4,31) = 2.68$

$F_{.01}\ (4,31) = 3.995$

significant at the 5% level

EXPERIMENT II Diurnal Cycle

900 hrs.		1100 hrs.		1300 hrs.		1500 hrs.		1700 hrs.	
Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.
40	3.68888	26	3.25810	25	3.21888	32	3.46574	19	2.94444
19	2.94444	25	3.21888	32	3.46574	34	3.52636	32	3.46574
19	2.94444	26	3.25810	20	2.99573	31	3.43399	33	3.49651
21	3.04452	33	3.49651	14	2.63906	25	3.21888	26	3.25810
31	3.43399	22	3.09104	27	3.29584	19	2.94444	22	3.09104
37	3.61092	18	2.89037	17	2.83321	15	2.70805	14	2.63906
$\bar{x}$	3.27786500		3.202166666		3.074743333		3.216243333		3.149148333
s^2	0.116205665		0.040548141		0.095387786		0.107595792		0.107646612

Bartlett's Test

s^2	0.116205665	0.040548141	0.095387786	0.107595792	0.107646612
$\ln s^2$	-2.15240	-3.20534	-2.34982	-2.22938	-2.22891
df	5	5	5	5	5
1/df	0.2		0.2	0.2	0.2

$$\bar{s}^2 = \frac{\Sigma Si^2}{a} = 0.093476801$$

$$\ln \bar{s}^2 = -2.37006$$

$$M = f(a \ln \bar{s}^2 - \Sigma \ln Si^2)$$
$$= 5\ (-11.85030 + 12.16585)$$
$$= 1.5775$$

$$\chi^2_{4,.05} = 9.49$$

variances are homogeneous

Analysis of Variance

	df	SS	MS
Treatment	4	0.13999168	0.03499792
Error	25	2.33691997	0.09347679
Total	29	2.47691165	

$$F = \frac{MST}{MSE} = 0.37440224 \qquad F_{.05}\ (4,25) = 2.76$$

means are considered equal

EXPERIMENT III Normal Test of Fall Snakes

	0 Hour		2 Hour		4 Hour		6 Hour		8 Hour	
	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.
	49	3.89182	31	3.43399	26	3.25810	43	3.76120	34	3.52636
	36	3.58352	24	3.17805	20	2.99573	32	3.46574	30	3.40120
	62	4.12713	57	4.04305	35	3.55535	25	3.21888	28	3.33220
	44	3.78419	30	3.40120	23	3.13549	39	3.66356	23	3.13549
	40	3.68888	29	3.36730	34	3.52636	24	3.17805	22	3.09104
	46	3.82864	33	3.46574	38	3.63759	32	3.46574	29	3.36730
	29	3.36730	43	3.76120	17	2.83321	23	3.13549	20	2.99573
$\bar{x}$		3.75306857		3.52150428		3.27740428		3.41266571		3.26418857
s^2		0.05792295		0.08274088		0.09435463		0.05991360		0.03689621

Bartlett's Test

s^2	0.05792295	0.08274088	0.09435463	0.05991360	0.03689621
$\ln s^2$	-2.84865	-2.49205	-2.36054	-2.81486	-3.29969
df	6	6	6	6	6
1/df	0.167	0.167	0.167	0.167	0.167

$$\bar{s}^2 = \frac{\Sigma Si^2}{a} = 0.066365654$$

$$\ln \bar{s}^2 = -2.71260$$

$$\begin{aligned} M &= f(a \ln \bar{s}^2 - \sum \ln Si^2) \\ &= 6(-13.56300 - (-13.81579)) \\ &= 1.51674 \end{aligned}$$

$$\chi^2_{4,.05} = 9.49$$

variances are homogeneous

Analysis of Variance

	df	SS	MS
Treatment	4	1.13807970	0.28451992
Error	30	1.9909672	0.06636565
Total	34	3.12904942	

$F = \frac{MST}{MSE} = 4.287156$ $F_{.05}\ (4,34) = 2.65$

$F_{.01}\ (4,34) = 3.93$ means are different

EXPERIMENT IV Acclimation to 30°C and Darkness

0 Hour		2 Hour		4 Hour		6 Hour		8 Hour	
Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.
23	3.13549	13	2.56495	28	3.33220	13	2.56495	14	2.63906
9	2.19722	12	2.48491	22	3.09104	20	2.99573	15	2.70805
21	3.04452	11	2.39790	14	2.63906	14	2.63906	12	2.48491
16	2.77259	15	2.70805	10	2.30259	15	2.70805	14	2.63906
12	2.48491	9	2.19722	19	2.94444	14	2.63906	20	2.99573
13	2.56495	18	2.89037	11	2.39790	21	3.04452	17	2.83321
12	2.48491	14	2.63906	14	2.63906	17	2.83321	11	2.39790
11	2.39790	14	2.63906	33	3.49651	10	2.30259	20	2.99573
$\bar{x}$	2.63531125		2.5651900		2.8553500		2.715896250		2.71170625
s^2	0.10499369		0.04384248		0.18718938		0.057934145		0.04808305

Bartlett's Test

s^2	0.104993694	0.04384248	0.187189388	0.057934145	0.048083052
$\ln s^2$	-2.25385	-3.12719	-1.67564	-2.84846	-3.03483
df	7	7	7	7	7

$$\bar{s}^2 = \frac{\Sigma Si^2}{a} = 0.088408671$$

$$\ln \bar{s}^2 = -2.42580$$

$$\begin{aligned} M &= f(a \ln \bar{s}^2 - \Sigma \ln Si^2) \\ &= 7(-12.12900 + 12.93997) \\ &= 5.67679 \end{aligned}$$

$$\chi^2_{4,.05} = 9.49$$

variances are homogeneous

Analysis of Variance

	df	SS	MS
Treatment	4	0.15729720	0.03932430
Error	35	2.68697921	0.07677083
Total	39	2.84427641	

$$F = \frac{MST}{MSE} = 0.5122971 \qquad F_{.05(4,35)} = 2.64$$

means are equivalent

EXPERIMENT V Effect of Light During Acclimation

	30°C & Darkness	4°C & 24D	4°C & 12L:12D	30°C & 12L:12D
	21	50	34	19
	21	52	33	18
	11	50	58	24
	13	44	18	19
	12	51	13	18
	17	50	64	24
	15	19	26	18
		22	9	28
$\bar{x}$	15.7	42.3	31.9	21.0
s^2	16.9	186.5	403.8	14.6

Bartlett's Test

s^2	16.9	186.5	403.8	14.6
$\ln s^2$	2.92731	5.22575	5.99894	2.68102
df	6	7	7	7
1/df	0.17	0.14	0.14	0.14

$$\bar{s}^2 = \frac{\Sigma fiSi^2}{\Sigma fi}$$
$$= 1/27\ (101.4 + 1305.5 + 2826.6 + 102.2)$$
$$= 160.58$$

$$\ln \bar{s}^2 = 5.08140$$

$$M = (\Sigma fi\ \ln \bar{s}^2 - \Sigma fi\ \ln Si^2)$$
$$= 137.19780 - (16.96386 - 36.58025 - 41.99258 - 18.76714)$$
$$= 22.89397$$

$$\chi^2_{3,.05} = 7.81$$

variances are heterogeneous

EXPERIMENT V cont'd.

Taylor's Power Law

$\bar{x}$	$\ln \bar{x}$	s^2	$\ln s^2$
15.7	2.75366	16.9	2.8273
42.25	3.74242	186.5	5.22575
31.87	3.46261	403.8	5.99894
22.00	3.09104	64.0	4.15888

regression with $\ln \bar{x} = x$ & $\ln s^2 = y$

slope $b = 2.79 \approx 3$

Taylor's Law = $z - x^p$

where
$$\begin{aligned} P &= 1 - \tfrac{1}{2}b \\ &= 1 - \tfrac{1}{2}(3) \\ &= 1 - 3/2 \\ &= -\tfrac{1}{2} \end{aligned}$$

$$\therefore z = x^{-\frac{1}{2}}$$

$$= \frac{1}{\sqrt{x}}$$

EXPERIMENT V cont'd. Light Effect

30°C & 24D		4°C & 24D		30°C & 12L:12D		4°C & 12L:12D	
Dur.	$\frac{1}{\sqrt{x}}$	Dur.	$\frac{1}{\sqrt{Dur.}}$	Dur.	$\frac{1}{\sqrt{Dur.}}$	Dur.	$\frac{1}{\sqrt{Dur.}}$
21	0.218217	50	0.141421	19	0.229415	34	0.171498
21	0.218217	52	0.138675	18	0.235703	33	0.174077
11	0.301511	50	0.141421	24	0.204124	58	0.131306
13	0.277350	44	0.150755	19	0.229415	18	0.235702
12	0.288675	51	0.140028	18	0.235702	13	0.277350
17	0.242535	50	0.141421	24	0.204124	64	0.125000
15	0.258148	19	0.229415	18	0.235702	26	0.196116
		22	0.2.3200	28	0.188982	9	0.333333
$\bar{x}$	0.2578147142		0.1620426250		0.2203957500		0.2042451250
s^2	0.0011021540		0.0013699639		0.0003402715		0.0052748628

Bartlett's Test

s^2	0.0011021540	0.0013699639	0.0003402715	0.0052748628
$\ln s^2$	-6.83703	-6.81280	-6.90776	-5.24821
df	6	7	7	7
1/df	0.16667	0.14286	0.14286	0.14286

$$\bar{s}^2 = \frac{\Sigma f_i S_i^2}{\Sigma f_i} = 0.002058744$$

$$\ln \bar{s}^2 = -6.19196$$

$$M = [\Sigma f_i \, \bar{s}^2 - \Sigma f_i S_i^2]$$
$$= -167.18292 - (-41.022118 - 47.68960 - 36.73747 - 48.35432)$$
$$= 6.62065$$

$$\chi^2_{.05,3} = 7.81$$

variances are homogeneous

Factor Analysis

Factor 1	light --	level 1	darkness	a_1
		" 2	12L:12D	a_2
Factor 2	temp. --	level 1	4°C	b_1
		" 2	30°C	b_2

EXPERIMENT V cont'd.

	a_1b_1	a_1b_2	a_2b_1	a_2b_2
	4° C & 24D	30°C & 24D	4° C & 12L:12D	30°C & 12L:12D
$\bar{x}$	0.162642625	0.2578147142	0.204245125	0.22039575

		n	Pooled Mean	S.D.
Light level	1	15	0.21	0.06
	2	16	0.21	0.05
Temperature level	1	16	0.18	0.06
	2	15	0.24	0.03
Light & temp.	a_1b_1	8	0.16	0.04
	a_1b_2	7	0.26	0.03
	a_2b_1	8	0.20	0.07
	a_2b_2	8	0.22	0.02

Analysis of Variance

Source	df	SS	MS	F
Light	1	0.0000	0.0000	0.01
Temperature	1	0.0234	0.0234	11.00
Light & temp.	1	0.0116	0.0116	5.47
Within cells	27	0.0574	0.0021	
Error due to approx.		-0.0010		
TOTAL	30	0.0914		

$F_{1,27.05}=4.21$ $F_{1,27.01}=7.68$

Acclimation temperature level is highly significant, having the greatest effect upon the duration.

The temp-light interaction is significant at the $P<.05$ level, so this combination has an effect upon duration.

EXPERIMENT VI   Re-establishment

Bartlett's Test

s^2	$\ln s^2$	df	1/df
0.048117995	-3.03412	4	.25
0.090549052	-2.40133	4	.25
0.045773122	-3.08410	4	.25
0.163388492	-1.81163	4	.25
0.513294622	-0.66691	4	.25
0.04192155	-3.17009	4	.25
0.117454431	-2.14171	4	.25
0.089529615	-2.41320	4	.25
0.059187970	-2.82706	4	.25
0.0276517959	-3.58821	4	.25
0.22694960	-1.48304	3	.33
0.006565543	-5.02882	3	.33

$$\bar{s}^2 = \frac{\Sigma fiSi}{\Sigma fi} = \frac{1}{46}(4.787674576 + 0.700545429)$$
$$= 0.11930913$$

$$\ln \bar{s}^2 = -2.12604$$

$$M = \Sigma fi \ln \bar{s}^2 - \Sigma fi \ln Si^2$$
$$= -97.79784 + 100.51344 + 19.53558$$
$$= 22.25118$$

$$\chi^2_{.05,11} = 19.68$$

$$C = 1 + \frac{1}{3(a-1)}\left[\frac{1}{\Sigma fi} - \frac{1}{\Sigma fi}\right]$$

$$= 1 + \frac{1}{33}(3.1666 - 0.2174)$$

$$= 1 + \frac{1}{33}(2.9492)$$
$$= 1.089$$

$$\frac{M}{C} = \frac{22.25118}{1.089}$$

variances are heterogeneous

$$= 20.43$$

EXPERIMENT VI Re-establishment cont'd.

Taylor's Power Law

$\bar{x}$	$\ln \bar{x}$	s^2	$\ln s^2$
47.8		83.7	4.42724
52.0	3.86703	220.0	5.39363
18.6	3.95124	13.3	2.58776
24.4	2.92316	60.3	4.09933
35.8	3.19458	287.7	5.96101
21.0	3.57795	15.5	2.80336
20.6	3.04452	62.8	4.13996
23.2	3.02529	55.7	4.01998
46.2	3.14415	82.7	4.41522
38.8	3.83298	213.2	5.36129
24.5	3.63281	120.3	4.78749
17.0	3.19867	2.0	0.69315
	2.83321		

regression line $y = a\ bx$

where $y = \ln s^2$

$x = \ln \bar{x}$

slope $b = 2.64 \approx 3$

Taylor's Law $z = x^p$

where $p = 1 - \frac{1}{2}b$

$= 1 - \frac{1}{2}(3)$

$= -\frac{1}{2}$

$z = x^{-\frac{1}{2}}$

transformation $\sqrt{\frac{1}{x}}$

EXPERIMENT VI Re-establishment

DAY I

0 Hour		2 Hour		4Hour		6Hour	
Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$
50	0.14142	71	0.11867	13	0.27735	26	0.19611
49	0.14285	42	0.15430	21	0.21821	11	0.30151
53	0.13736	59	0.13018	20	0.22360	31	0.17960
55	0.13484	33	0.17407	17	0.24253	22	0.21320
32	0.17677	55	0.13484	22	0.21320	17	0.24253
$\bar{x}$	0.146648		0.142412		0.2349780		0.226590
s^2	0.00029369		0.00047854		0.00068433		0.00229586

DAY II

0 Hour		2 Hour		4 Hour		6 Hour	
Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$
11	0.30151	16	0.25000	34	0.17149	35	0.16903
51	0.14002	27	0.19245	14	0.26726	18	0.23570
47	0.14586	21	0.21821	16	0.25000	17	0.24253
52	0.13867	25	0.20000	21	0.21821	20	0.22360
18	0.23570	20	0.22360	18	0.23570	26	0.19611
$\bar{x}$	0.192352		0.2168520		0.2285320		0.21339400
s^2	0.00539410		0.00050640		0.00134311		0.00092938

DAY III

0 Hour		2 Hour		4 Hour		6 Hour	
Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$	Dur.	$1/\sqrt{D}$
37	0.16439	50	0.14142	30	0.18257	19	0.22941
51	0.14002	34	0.17149	18	0.23570	17	0.24253
36	0.26666	14	0.26726	37	0.16439	16	0.25000
51	0.14002	44	0.15075	13	0.27735	16	0.25000
64	0.12500	47	0.14586				
$\bar{x}$	0.147218		0.175356		0.2150025		0.242985
s^2	0.00031753		0.00277209		0.00264304		0.00009430

EXPERIMENT VI Re-establishment

Analysis of Variance

DAY I

	df	SS	MS
Treatment	3	0.03432127	0.01144042
Error	16	0.01493845	0.00099589
Total	19	0.04925972	

$F = \frac{MST}{MSE} = 11.48763$

$F_{.05}(3,16) = 3.24$

$F_{.01}(3,16) = 5.29$ means are significantly different

DAY II

	df	SS	MS
Treatment	3	0.00341191	0.0011373
Error	16	0.03269200	0.00204325
Total	19	0.03610391	

$F = \frac{MST}{MSE} = 0.5566$

$F_{.05}(3,16) = 3.24$

$F_{.01}(3,16) = 5.29$ means are equivalent

DAY III

Treatment	3	0.02391964	0.00797321
Error	14	0.02057050	0.00146932
Total	17	0.04449014	

$F = \frac{MST}{MSE} = 5.4264$

$F_{.05}(3,14) = 3.34$

$F_{.01}(3,14) = 5.56$ means are significantly different at $P < .05$

EXPERIMENT VII Short Term Acclimation, 18 Hours

0 Hour		3 Hour		6 Hour	
Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.
12	2.48491	18	2.89037	15	2.70805
27	3.29584	27	3.29584	21	3.04452
51	3.93183	20	2.99573	16	2.77259
24	3.17805	33	3.49651	25	3.21888
40	3.68888	36	3.58352	20	2.99573
21	3.04452	24	3.17805	15	2.70805
13	2.56495	44	3.78419	17	2.83321
$\bar{x}$	3.169854285		3.31774285		2.89729000
s^2	0.286116800		0.104416964		0.037677854

Bartlett's Test

s^2	0.286116800	0.104416964	0.037679859
$\ln s^2$	-1.25135	-2.25939	-3.27876
df	6	6	6

$$\bar{s}^2 = \frac{\Sigma Si^2}{a} = 0.142737206$$

$$\ln \bar{s}^2 = -1.94676$$

$$M = f(a \ln \bar{s}^2 - \Sigma \ln Si^2)$$
$$= 6(-5.84028 + 6.78950)$$
$$= 5.69532$$

$$\chi^2_{2,.05} = 5.99$$

variances are homogeneous

Analysis of Variance

	df	SS	MS
Treatment	2	0.63687059	0.31843529
Error	18	2.56926966	0.14273720
Total	20	3.20614025	

$F = 2.23092011$

$F_{.05(2,18)} = 3.55$

means are equivalent

EXPERIMENT VII cont'd.

Short Term Acclimation, 42 Hours

0 Hour		3 Hour		6 Hour	
Dur.	ln Dur.	Dur.	ln Dur.	Dur.	ln Dur.
51	3.93183	28	3.33220	22	3.09104
50	3.91202	27	3.29584	18	2.89037
54	3.98898	33	3.49651	30	3.40120
33	3.49651	55	4.00733	19	2.94444
36	3.58352	25	3.21888	16	2.77259
51	3.93183	15	2.70805	15	2.70805
31	3.43399	28	3.33220	22	3.09104
$\bar{x}$	3.754097142		3.341572857		2.985532857
s^2	0.0568673		0.147651672		0.054688112

Bartlett's Test

s^2	0.0568673	0.147651672	0.054688112
$\ln s^2$	-2.86706	-1.91290	-2.91751
df	6	6	6

$$\bar{s}^2 = \frac{\Sigma Si^2}{a} = 0.086402361$$

$$\ln \bar{s}^2 = -2.44876$$

$$\begin{aligned} M &= f(a \ln \bar{s}^2 - \Sigma \ln Si) \\ &= 6(-7.34628 + 7.69747) \\ &= 2.10714 \end{aligned}$$

$$\chi^2_{2.05} = 5.99$$

variances are homogeneous

Analysis of Variance

	df	SS	MS
Treatment	2	2.07114092	1.03557046
Error	18	1.55524247	0.08640235
Total	20	3.62638339	

$F = 11.9854$

$F_{.01(2,18)} = 6.01$

means are different

Analysis of Variance of Non-courting & Courting Male Snakes

April 26

Source	df	SS	MS
treatments	1	6.534	6.534
error	28	172.346	
total	29	178.880	

$F = 1.061$

$F_{.05\ 1,28} = 4.20$

∴ no difference in cloacal temperatures

May 6

Source	df	SS	MS
treatments	1	2.928	2.928
error	48	137.836	2.871
total	49	140.764	

$F = 1.019$

$F_{.05\ 1,48} = 4.04$

∴ no difference in cloacal temperatures between courting and non-courting male snakes

Paired t-test on Oral and Cloacal Temperatures of Male Snakes

	May 6	May 10	May 13
Weather	Clear & Sunny	Cloudy & Cool	Clear & Sunny
n	13	20	25
Σd	-1.000	4.100	-2.100
Σd^2	14.840	3.290	16.190
$\frac{(\Sigma d)^2}{n}$	0.076	0.840	0.176
s^2	1.230	0.128	0.667
$\frac{s}{\sqrt{n}}$	0.307	0.080	0.163
t	0.250	2.562	-0.515
$t_{n-1,.05}$	2.179	2.093	2.064
significance	not	is	not