THE UNIVERSITY OF MANITOBA

Environmental Control of Seasonal Reproductive Events in the Red-sided Garter Snake (*Thamnophis sirtalis parietalis*)

by

Alexander Wilson Lewis Hawley

A Thesis

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ENVIRONMENTAL CONTROL OF SEASONAL REPRODUCTIVE EVENTS IN THE RED-SIDED GARTER SNAKE (Thamnophis sirtalis parietalis)

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Abstract

The annual testis weight cycle of *Thamnophis sirtalis parietalis* is characterized by a major peak in early July and a lesser peak in early September. These two peaks appear to be associated with gametogenesis and androgenesis respectively.

Testicular recrudesence under experimental laboratory conditions was unaffected by photoperiod but was markedly influenced by temperature. Testicular recrudesence of animals held at 30°C was similar to that of field animals, while at 20°C recrudesence was initiated but spermatogenesis was not completed. An endogenously timed testis cycle was absent at 25°C, 12L12D. These results suggest that under natural conditions the initiation of a new testicular cycle is environmentally controlled and the completion of spermatogenesis is dependent on high summer temperatures.

Mating behaviour of *Thamnophis sirtalis parietalis* is induced by exposure to increased temperatures immediately following emergence from hibernation. The thermal threshold for courtship varied widely among individuals, and ranged from about 2°C (as determined by extrapolation of data) to between 20°C

and 25°C. The lowest thermal threshold for copulation was between 5° and 10°C. Both courtship and copulation increased quantitatively with increasing environmental temperature, and reached maximal levels at 25°C and 30°C respectively. These increases appeared to be the result of an increase in sexual motivation. The existence of low thermal thresholds for mating behaviour and a capacity for a rapid increase in body temperature result in a very rapid onset of courtship and copulation following emergence from hibernation. Possible mechanisms of thermal induction of spring mating behaviour are discussed.

Acknowledgements

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Part I

The Influence of Photoperiod and Temperature on Seasonal Testicular Recrudesence in the Red-sided Garter Snake (*Thamnophis sirtalis parietalis*)

Introduction

The influence of photoperiod and environmental temperature on gonadal cycles has been investigated in a variety of reptiles. The results of several studies suggest that photoperiod may be important in the timing of gonadal recrudesence in certain species (for reviews of the literature see Bartholomew 1959; Licht 1972a), but often the interpretation of experimental results is complicated by heliothermy (heat gain primarily from incident radiation) and the capacity of reptiles for behavioural thermoregulation. Several other studies (Bartholomew 1950; 1953; Fischer 1968a, b; 1970; Licht 1966; 1967a, b; 1969; 1971; 1973; Tinkle and Irwin 1965) have demonstrated that temperature is an important environmental factor controlling the timing of gonadal recrudesence of some species. Indeed, in certain temperate climate species, environmental temperature is the primary regulating factor (Licht, Hoyer and van Oordt 1969; Marion 1970).

Little attention has been given to the environmental control of gonadal cycles in reptiles from cold climates. Several species of the genus *Thamnophis* inhabit

cold-climate areas. Testicular recrudesence in *Thamnophis* spp. from relatively mild climates (California, U.S.A.) was investigated by Fox (1952;1954). He suggested that temperature was the primary environmental factor controlling seasonal testicular development and that temperatures near the bottom of the "normal activity range of temperature" represented a minimum below which spermatogenesis would not proceed (Fox 1954).

The range of the red-sided garter snake (*Thamnophis sirtalis parietalis*) extends as far north as the Northwest Territories, Canada (Longier and Toner 1961). In the Interlake (south-central) area of Manitoba, Canada, it approaches the northeastern limit of its distribution. The species experiences very short summers in this part of its range. Because of the short summer, the timing of testicular recrudesence is critical to ensure its completion in one season. Thus the environmental regulation of testicular recrudesence is likely to be extensive. High temperatures in the spring may be important in timing the onset of testicular recrudesence. The experiments reported here were undertaken to determine the pattern of seasonal testicular recrudesence in a northern population of *Thamnophis sirtalis parietalis* and to examine the effects of temperature and photoperiod on this cycle.

Materials and Methods

Field Collections

Red-sided garter snakes were collected from the Interlake area of Manitoba, Canada (latitude approximately 51°N) at approximately biweekly intervals from May to October, 1973 (except during July when only one sample was taken). Animals were sacrificed within 24 hours of collection with the exception of the sample in mid July and that in early August. Snakes collected for these two samples were held at room temperature and sacrificed within 4 days of capture. The reproductive condition of field animals was determined as described below. All males were sexually mature.

Photoperiod and Temperature Regimes in the Laboratory

Animals were collected during late May, 1973 at a hibernaculum in the Interlake area and held at 2 ± 1°C and dark until transfer to experimental conditions on June 13, 1973. Ninety sexually mature males were chosen without bias and established at each of two temperatures (15 ± 1° and 25 ± 1°C)

and two photoperiods (12L12D and 4L20D) at each temperature. Fifteen degrees centigrade was selected as the lower environmental temperature in order to approximate the lowest temperature of their "normal activity range" as discussed by Fox (1954). However, the animals would not feed at this temperature. Therefore, on July 2 the environmental temperatures were raised from 15° to 20 ± 1°C and from 25° to 30 ± 1°C. The animals fed at 20°C, but not as extensively as the animals at 25° or 30°C. Thirty degrees centigrade is around the preferred body temperature of these animals (Beachum, unpublished results; Carpenter 1956; Fitch 1965). Light was provided by fluorescent bulbs at an intensity of approximately 470 lux (45 f.c.) as measured with a Gossen Lunasix 3 light meter. The animals were held in large brown cardboard boxes, the bottoms of which were covered with wood shavings. Under these conditions the dorsal anterior body surface temperature of the snakes was usually within ±1°C of the environmental temperature and never differed by more than ±2°C. Water was available at all times. The snakes were fed approximately every five days with an excess of

diced ocean perch fillets occasionally supplemented by live chorus frogs (*Pseudacris triseriata*) and wood frogs (*Rana sylvatica*).

Biweekly samples of ten individuals, chosen without bias, were taken from each experimental group for 14 weeks (from the initiation of the experiment in mid June to October 1, 1973) and examined as described below to determine their reproductive condition. Mortality resulted in decreased sample sizes toward the end of the experimental period.

In addition, the possible existence of an endogenous rhythm of testicular recrudesence was investigated by sampling sexually mature animals held at 25 ± 2°C, 12L12D throughout an entire year. The snakes were collected from the Interlake area during the spring and fall of 1972 and established at these light and temperature conditions. The cages were illuminated by fluorescent bulbs at an intensity of approximately 350 lux (32 f.c.). Diced ocean perch was provided as food. Samples of five individuals, chosen without bias, were taken at monthly intervals from February, 1972 to January, 1974. At the time of sampling all animals had been under these constant conditions for at least seven months.

Analysis of Testicular Cycles and Sperm Counts

On the day of sacrifice, the body weight and snout-vent length of each individual were recorded. The large number of animals sampled prevented examination of reproductive organs on the day of sacrifice. Therefore, the animals were frozen individually in plastic bags for later determination of reproductive condition. This involved removal of the right testis and weighing it to the nearest 0.1 mg. One half of this testis was fixed in Bouin's solution for subsequent histological examination. The remaining half was used to make a testis smear which was examined for the presence of spermatozoa. The presence of sperm flagella in the testis smears was used as an indication that sperm were present in the testis. These sperm may not have been fully matured spermatozoa, but they were in a highly advanced stage of spermatogenesis. Therefore, this method served as an adequate indication that spermatogenesis was complete or nearly so. Sperm in the anterior (immediately caudad to the testis) and posterior (immediately anterior to the cloaca) right vas deferens were quantified by removing a one-centimetre long section of the vas deferens

from the appropriate area and flushing the tubular contents into 0.5 ml of a 0.9% NaCl solution. The sperm within 0.02 cubic millimetres of this suspension were counted using a Spencer AO Neubauer heamocytometer. The reliability of this method of sperm extraction was demonstrated by making occasional detailed examinations of the section of vas deferens after removal of the tubular contents.

Certain of the samples fixed in Bouin's solution (as indicated by asterisks (*) in Fig. 1-4) were selected for histological examination. A sub-sample of five fixed testes was randomly chosen from each of these samples. These testes were imbedded in paraffin, sectioned at 4 microns, stained with Ehrlich's haematoxylin and counter-stained with eosin. The sections were examined to determine the relative spermatogenic advancement of the germinal epithelium and tubular diameter for comparative purposes.

Results

Testicular Cycles Under Field Conditions

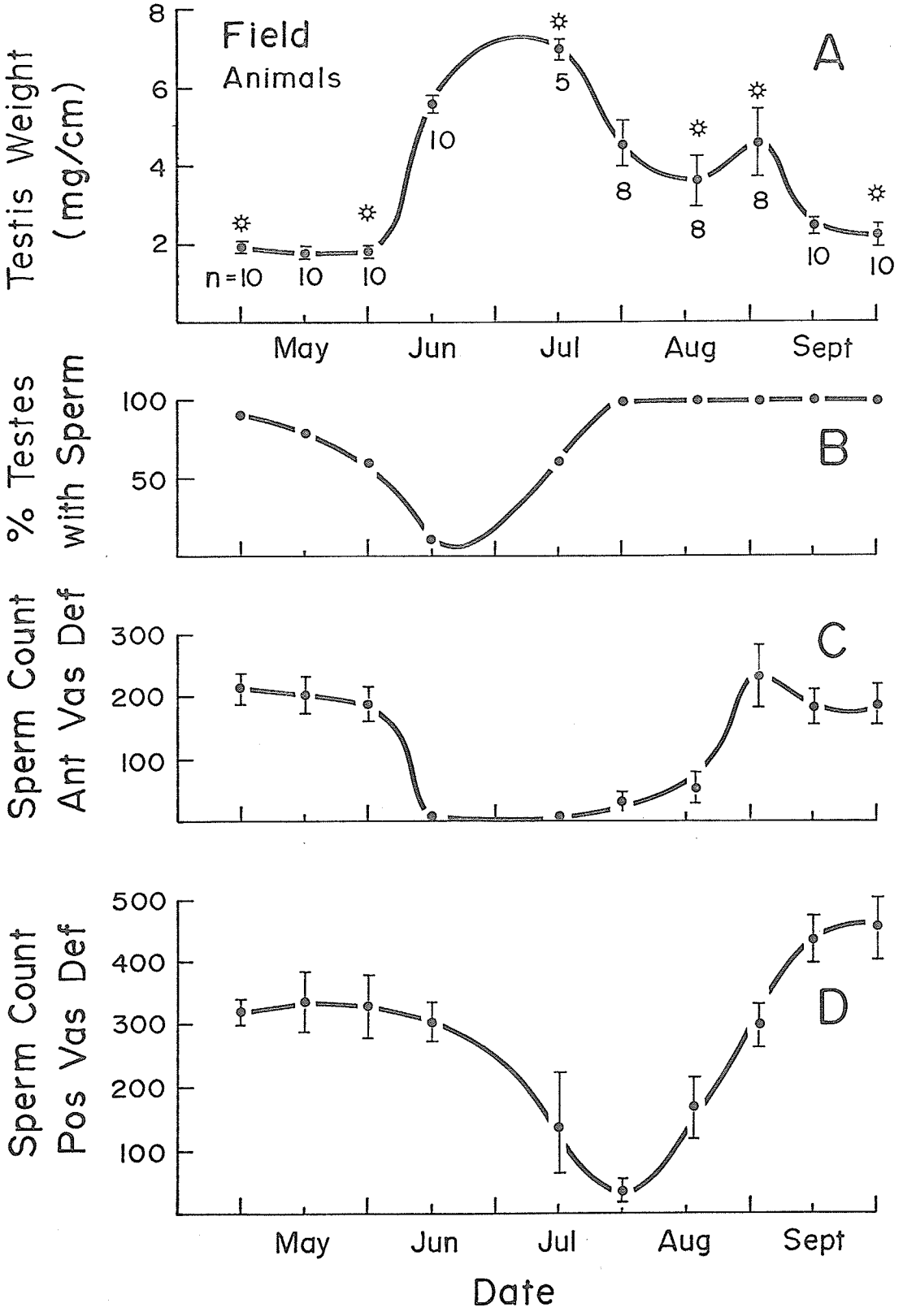
It was necessary to correct testicular weights for variations in body size. Correlation coefficients calculated for the weight of the right testis (in mg) as a function of snout-vent length (in cm) of field animals were significant ($P < 0.05$) for samples taken from July to October inclusive (Table 1). In all samples the line calculated by the method of least squares had a positive slope. Therefore, in all cases testis weight was divided by snout-vent length to reduce variation in testis weight resulting from variation in body size.

The testicular cycle and sperm counts for field animals are presented in Fig. 1. The testis weight cycle had two peaks (Fig. 1A). The first and larger peak occurred in late June and early July. The second peak occurred early in September. The right testis of almost all animals possessed sperm immediately after emergence from hibernation, but testes became aspermic by June (Fig. 1B). Testicular sperm reappeared in all animals examined by early August, just after the first peak in the testicular weight cycle. The

Table 1. Correlation coefficients (r) and slopes (b) of the equation $y = bx + a$, calculated for right testis weight (mg) as a function of snout-vent length (cm) of field-collected garter snakes. Asterisks (*) indicate statistically significant ($P < 0.05$) correlation coefficients.

Sample Period	Number of Animals	r	b (mg/cm)
May	10	0.3449	25
	10	0.4580	3
June	10	0.6206	4
	10	0.4966	9
July	5	0.8987*	9
August	8	0.8530*	13
	8	0.9266*	13
September	8	0.7309*	16
	10	0.8760*	8
October	10	0.6378*	13

Figure 1. Testicular cycles and sperm counts of the anterior vas deferens (Ant Vas Def) and posterior vas deferens (Pos Vas Def) of field-collected garter snakes. Curves were fitted to the data by eye. Numbers in graph A indicate sample sizes for all four graphs. Vertical bars represent ± one standard error about the means. Asterisks (*) indicate samples that were examined histologically.


Field
Animals
A
B
C
D
Testis Weight (mg/cm)
% Testes with Sperm
Sperm Count Ant Vas Def
Sperm Count Pos Vas Def
n=10 10 10 10 5 8 8 8 10 10
May
Jun
Jul
Aug
Sept
Date

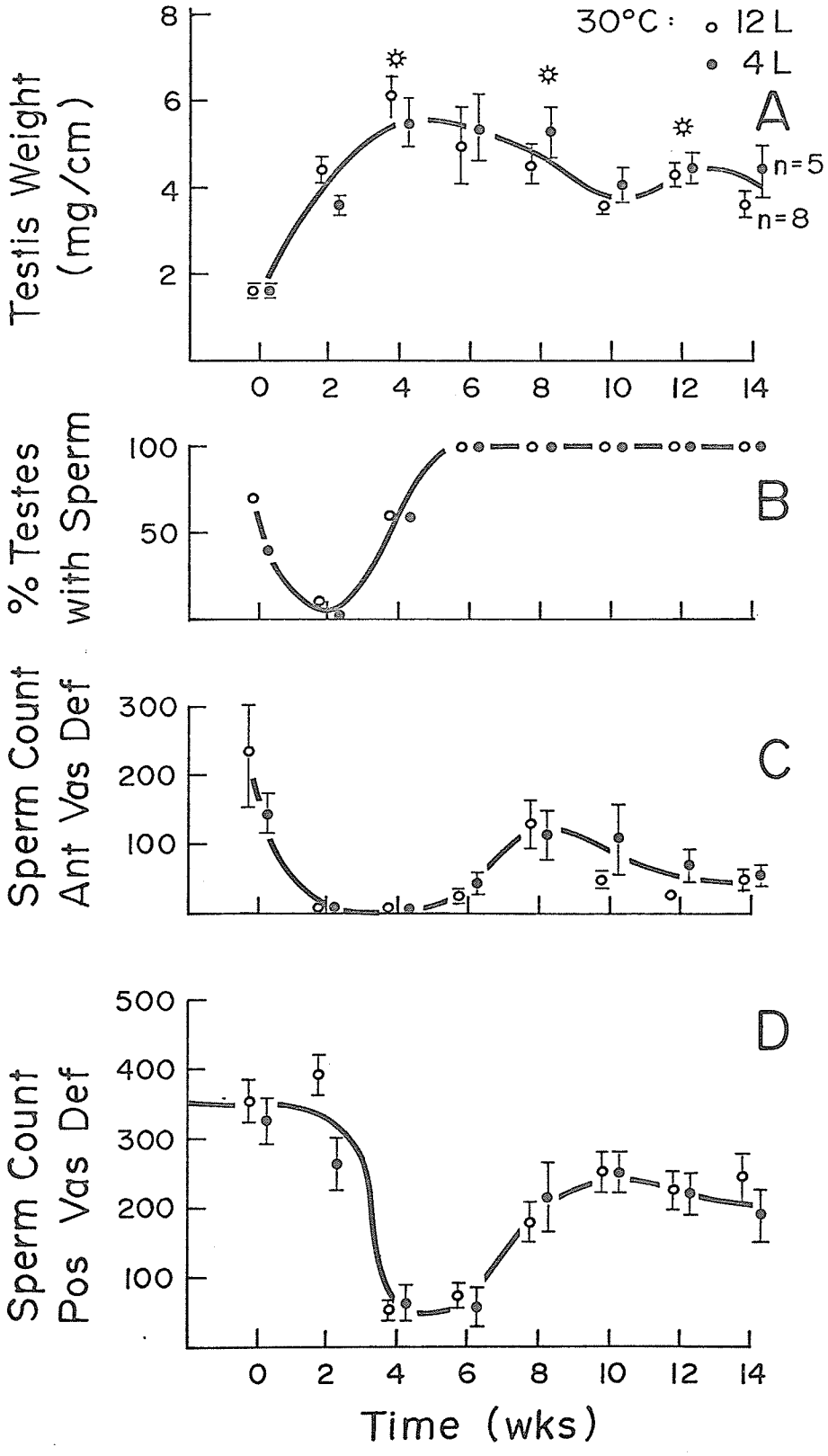
migration of sperm through the reproductive tract was indicated by a slight time lag between the reappearance of testicular sperm (Fig. 1B) and the fall increase in the number of sperm in first the anterior vas deferens (Fig. 1C) and subsequently the posterior vas deferens (Fig. 1D). By early September all animals examined possessed abundant sperm in both the anterior and posterior vas deferens.

Histological examination disclosed a reduction in seminiferous tubules between May 2 and June 1. The germinal epithelium was reduced and the tubules collapsed. Recrudesence had begun by July 14. There was an increase in the thickness of the germinal epithelium and tubular diameter. On August 18 the tubules were still in this hypertrophied condition. By September 5 regression had begun. The tubules were still quite large in diameter but there was a reduction in the thickness of the germinal epithelium. By October 1 the germinal epithelium had regressed further and the tubules were much reduced in size.

Testicular Cycles at 20° and 30°C

The testicular cycles of animals maintained at 30°C (Fig. 2) were similar to those characterizing

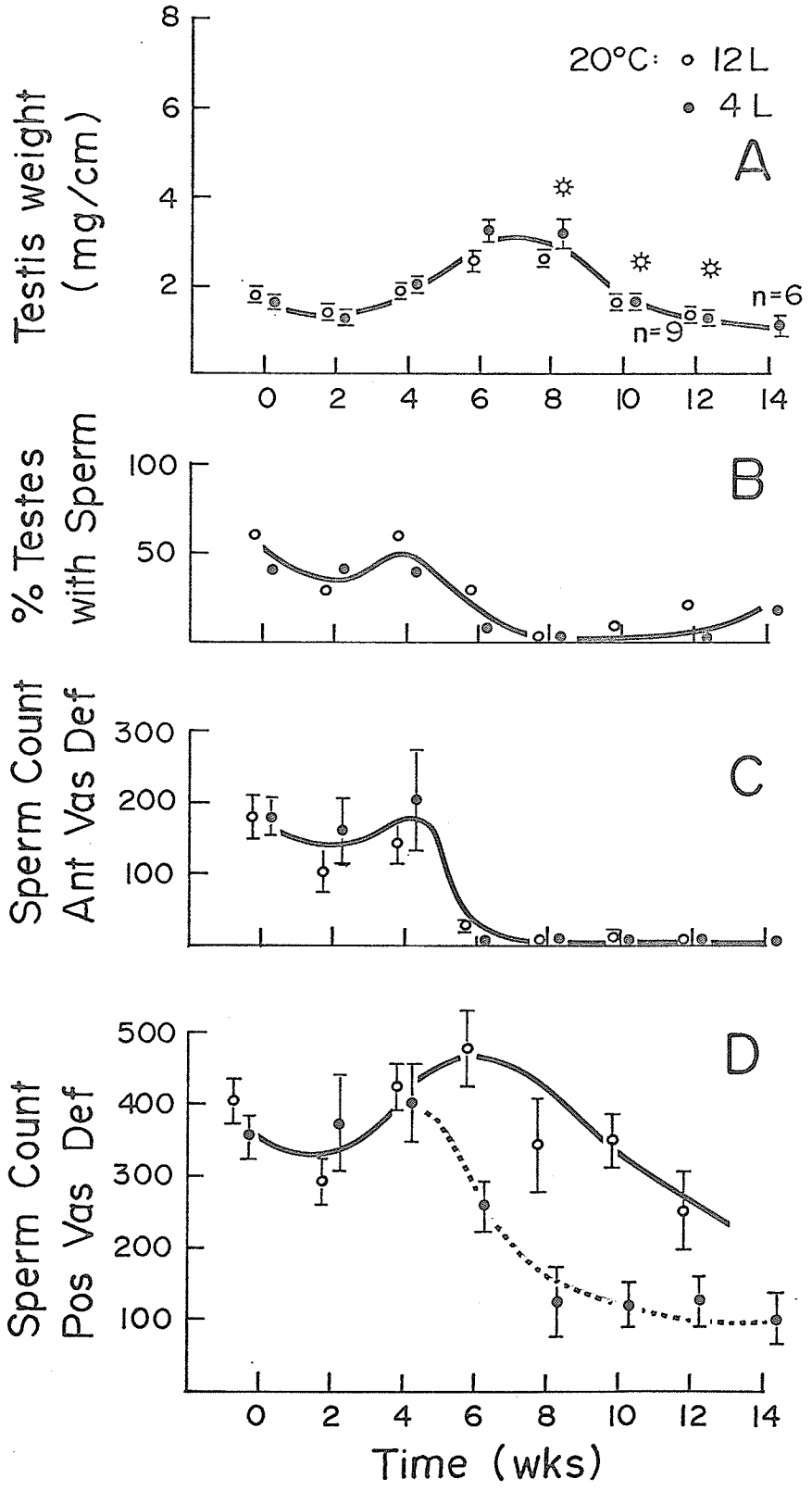
Figure 2. Testicular cycles and sperm counts of the anterior vas deferens (Ant Vas Def) and posterior vas deferens (Pos Vas Def) of garter snakes held at 30°C. Curves were fitted to the data by eye. Sample sizes were 10 unless indicated otherwise. Vertical bars represent ± one standard error about the means. Asterisks (*) indicate samples that were examined histologically (only 12L12D groups).


30°C: ○ 12 L
● 4 L
A
n=5
n=8
Testis Weight (mg/cm)
8
6
4
2
0 2 4 6 8 10 12 14
% Testes with Sperm
100
50
B
Sperm Count Ant Vas Def
300
200
100
C
Sperm Count Pos Vas Def
500
400
300
200
100
D
Time (wks)

field animals. It is clear that photoperiod did not appear to affect testicular cycles at this temperature. Because of the apparent absence of a photoperiodic effect a single curve was fitted to the combined data of both photoperiod groups. The testis weight cycle again had two peaks, the first of which was the largest (Fig. 2A). The appearance of sperm in the testis, and anterior and posterior vas deferens (Fig. 2B, C and D) followed patterns similar to those observed in field animals. The histological condition of the tubules was also very similar to field animals when compared at corresponding periods of the testis cycle.

The testicular cycles displayed by animals held at 20°C differed greatly from those of the previous groups. Unlike the field animals and both groups at 30°C, the animals held at 20°C displayed a very small and brief increase in testis weight. This single peak occurred between the sixth and eighth weeks of sampling (Fig. 3A). As was the case at 30°C, photoperiod did not appear to affect the testis weight cycle of animals held at 20°C. There was some separation between the two photoperiods in the values of sperm counts in the posterior vas deferens (Fig. 3D), but both groups followed a similar pattern. Very few animals at

Figure 3. Testicular cycles and sperm counts of the anterior vas deferens (Ant Vas Def) and posterior vas deferens (Pos Vas Def) of garter snakes held at 20°C. Curves were fitted to the data by eye. Sample sizes were 10 unless indicated otherwise. Vertical bars represent ± one standard error about the means. Asterisks (*) indicate samples that were examined histologically (only 12L12D groups).


Testis weight (mg/cm)
20°C: ○ 12L
● 4L
A
n=6
n=9
% Testes with Sperm
B
Sperm Count Ant Vas Def
C
Sperm Count Pos Vas Def
D
Time (wks)

20°C developed testicular sperm (Fig. 3B) after the testis became aspermic (eighth week of sampling). Only four individuals (3 in the 12L12D group and 1 in the 4L20D group) of a total of 45 examined possessed testicular sperm after the peak in the testis weight cycle. At the beginning of the experiment the mean counts in the anterior vas deferens were similar to counts obtained for animals at 30°C, but they decreased to a very low level by the sixth week and did not increase. The mean sperm counts in the posterior vas deferens increased slightly in both photoperiod groups but declined from the sixth week to the end of the experiment.

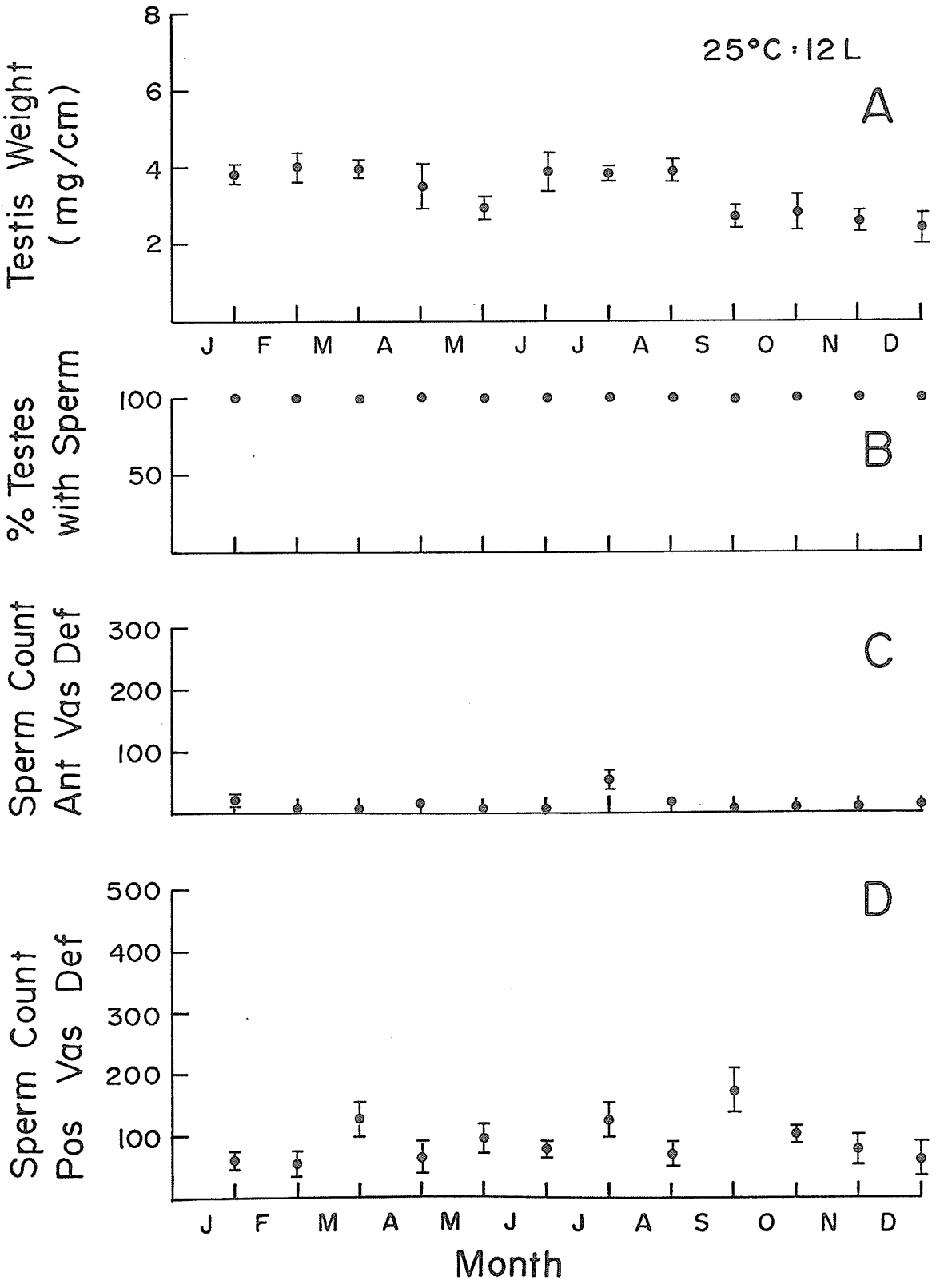
Although spermatozoa were not produced at 20°C, histological examination of the 12L12D group indicated that there was some spermatogenic advance. The seminiferous tubules of these animals on the eighth week appeared slightly advanced relative to the field animals in early June (germinal epithelium less advanced and tubules smaller in the latter group). Loss of cellular integrity resulting from freezing of the material prevented determination of the exact stage at which spermatogenesis was arrested. However, it appeared that several generations of germ cells had

developed and the tubules had begun to enlarge before the cycle was arrested. There was little tubular change at 20°C between the eighth and tenth week of sampling, but by the twelfth week the tubules had regressed. At this time the tubules were smaller and the germinal epithelium reduced relative to the condition on the eighth and tenth weeks.

Long Term Studies of Testicular Cycles at 25°C, 12L12D

There were no gonadal cycles evident in the animals held at 25°C, 12L12D for at least 7 months and sampled throughout the year. There was no distinct peak in the testis weight cycle (Fig. 4A). The right testis of every animal examined throughout the year possessed testicular sperm (Fig. 4B). The mean sperm counts of the anterior vas deferens were very low, with a slight increase in this value in August (Fig. 4C). Similarly, the mean sperm counts in the posterior vas deferens were relatively very low, with a slight increase in October (Fig. 4D). However, the magnitudes of these increases were negligible when compared to the increases experienced by field animals and animals held at 30°C. The seminiferous tubules of these animals in January appeared similar to the

Figure 4. Testicular cycles and sperm counts of the anterior vas deferens (Ant Vas Def) and posterior vas deferens (Pos Vas Def) of garter snakes held at 25°C, 12L12D throughout one year. Sample size in each month was 5. Vertical bars represent ± one standard error about the means.


25°C : 12 L
A
Testis Weight (mg/cm)
8
6
4
2
J F M A M J J A S O N D
B
% Testes with Sperm
100
50
C
Sperm Count Ant Vas Def
300
200
100
D
Sperm Count Pos Vas Def
500
400
300
200
100
J F M A M J J A S O N D
Month

tubules of field animals in mid August and early September and were not as regressed as the tubules of field animals in early October.

Body Weight and Nutritional State

The mean relative body weight (body weight/ snout-vent length, gm/cm) was used as an indication of nutritional state and general physical condition. There was little change in the mean relative body weights of field animals sampled during the course of the summer (Table 2). Similarly, animals at 20°C changed little in mean relative body weights, although there was a slight decrease toward the end of the sampling period (Table 3). The mean relative body weights of animals sampled from 30°C increased between the sixth and eighth weeks of sampling. The values before and after this period were fairly constant (Table 3). For the first six weeks of sampling, the mean relative body weights of animals held at 20°C were similar to those held at 30°C. However, for the last six weeks the animals at 30°C had significantly greater mean relative body weights (Table 3) (factorial analysis of variance, $P < 0.05$). At both 20° and 30°C there

Table 2. Mean relative body weights (body weight/snout-vent length, gm/cm) of field-collected garter snakes. Two samples were taken each month except July. Sample sizes are as in Fig. 1. Numbers in parentheses indicate one standard error about the mean.

Month	May		June		July	August		September		October
Mean Body Weight (gm/cm)	.691 (.038)	.569 (.030)	.565 (.039)	.698 (.039)	.547 (.095)	.422 (.043)	.513 (.064)	.638 (.059)	.618 (.045)	.697 (.028)

Table 3. Mean relative body weights (body weight/snout-vent length, gm/cm) of garter snakes held at 30°C and 20°C under two photoperiods. Sample sizes are as in Fig. 2 and 3 respectively. Numbers in parentheses indicate one standard error about the mean.

Weeks	0	2	4	6	8	10	12	14
30°C 12L12D	.640	.616	.584	.514	.772	.757	.748	.638
	(.045)	(.052)	(.044)	(.071)	(.058)	(.040)	(.048)	(.042)
30°C 4L20D	.567	.527	.489	.538	.810	.810	.723	.698
	(.025)	(.029)	(.046)	(.060)	(.070)	(.041)	(.040)	(.034)
20°C 12L12D	.636	.505	.546	.522	.583	.481	.436	----
	(.044)	(.042)	(.040)	(.036)	(.055)	(.019)	(.045)	----
20°C 4L20D	.528	.496	.584	.431	.580	.444	.473	.391
	(.038)	(.053)	(.027)	(.019)	(.041)	(.037)	(.069)	(.063)

was no significant difference between photoperiods in mean relative body weights at each sample period (factorial analysis of variance, $P < 0.05$). The animals held at 25°C, 12L12D were in very good condition and tended to have larger relative mean body weights than the field animals (Tables 2 and 4).

Table 4. Mean relative body weights (body weight/ snout-vent length, gm/cm) of garter snakes held at 25°C, 12L12D throughout the year. Sample size was 5. Numbers in parentheses indicate one standard error about the mean.

Month	Mean Body Weight (gm/cm)	
February	.800	(.059)
March	.951	(.039)
April	.946	(.089)
May	.913	(.070)
June	.915	(.081)
July	.766	(.038)
August	.762	(.028)
September	.667	(.036)
October	.770	(.061)
November	.585	(.059)
December	.814	(.070)
January	.737	(.071)

Discussion

The Influence of Temperature and Photoperiod on Testicular Cycles

Under the experimental conditions of this study, testicular recrudesence was strongly influenced by environmental temperature. At 30°C the patterns of testicular growth and spermatogenesis were similar to those obtained in field animals, while at 20°C testicular recrudesence was inhibited. This is in agreement with the observations of Fox (1954) for *Thamnophis elegans terrestris*. He observed that snakes held at temperatures below 20°C (near the bottom of their "normal activity range of temperature") for a month or more during the period of sexual activity underwent testicular atrophy. The period of sexual activity is in the spring, before testicular recrudesence begins. His observations did not permit the conclusion that complete testicular development was prevented at 20°C. The results reported here indicate that such inhibition occurred in *Thamnophis sirtalis parietalis*.

In contrast to the effect of temperature, the different photoperiods used in this study had no effect on gonadal cycles at either temperature with the possible exception of sperm degeneration in the posterior vas

deferens at 20°C. However, even in this latter case a similar pattern existed at both photoperiods. Despite the absence of a photoperiodic effect in these experiments, it cannot be concluded that photoperiod never influences testicular recrudesence in this species. These animals can greatly alter their body temperature through heliothermy. Thus, in nature the duration and intensity of sunlight could indirectly influence testicular cycles by influencing body temperature. Also, it has been shown in several species of lizards that the relative importance of photoperiod in regulating testicular cycles varies seasonally and depends on both thermal and photoperiodic regimes (Licht 1966; 1967a, b; 1969; 1971; 1973). Further study is required to determine if this is also true in *Thamnophis sirtalis parietalis*.

Some exposure to fall or winter conditions (i.e., low temperature and/or short photoperiod) appeared to be necessary to induce cessation of a testicular cycle and initiation of a new one. The testis of animals held at a constant 25°C, 12L12D throughout the year were arrested in a state corresponding to that of the field animals in late summer. The mean testis weights of the laboratory

animals throughout the year were similar to the mean testis weights of field animals in late summer (Fig. 1A and 4A). All the animals held under 25°C, 12L12D possessed testicular sperm throughout the year. All field animals in late summer also possessed testicular sperm. The seminiferous tubules of the animals held in the laboratory and sacrificed in January resembled those of field animals sampled in late August and early September. At that time, the germinal epithelium of field animals had regressed from its condition in mid August but was still more advanced than the condition in October. Therefore, exposure to fall and/or winter conditions appeared to be necessary to induce complete testicular regression and to allow the commencement of a new cycle. After exposure to fall and/or winter conditions, high temperature stimulated initiation of a new testicular cycle.

Licht (1972b) stimulated spermatogenesis in *Thamnophis sirtalis* from Louisiana in October by exposure to 28-30°C, 10L14D. Before the experiment the testes of these animals were in a highly regressed state. Presumably these snakes had already experienced the environmental stimulus which permits the initiation of a new spermatogenic wave. This suggests that the required stimulus, perhaps decreasing photoperiod, occurs

during the fall. The control of gonadal recrudesence in reptiles by temperature and the termination of this development by photoperiod has been reviewed by Licht (1972a).

The apparent requirement of some environmental stimulus to induce complete testicular regression in *Thamnophis sirtalis parietalis* is in contrast with the requirements of several species of temperate climate lizards that can terminate one gonadal cycle and permit the initiation of a new one endogenously (Fischer 1968a, b; 1969; Licht 1972a; 1973; Licht, Hoyer and van Oordt 1969; Marion 1970). *Uta stansburiana* may also possess this capacity (Tinkle and Irwin 1965).

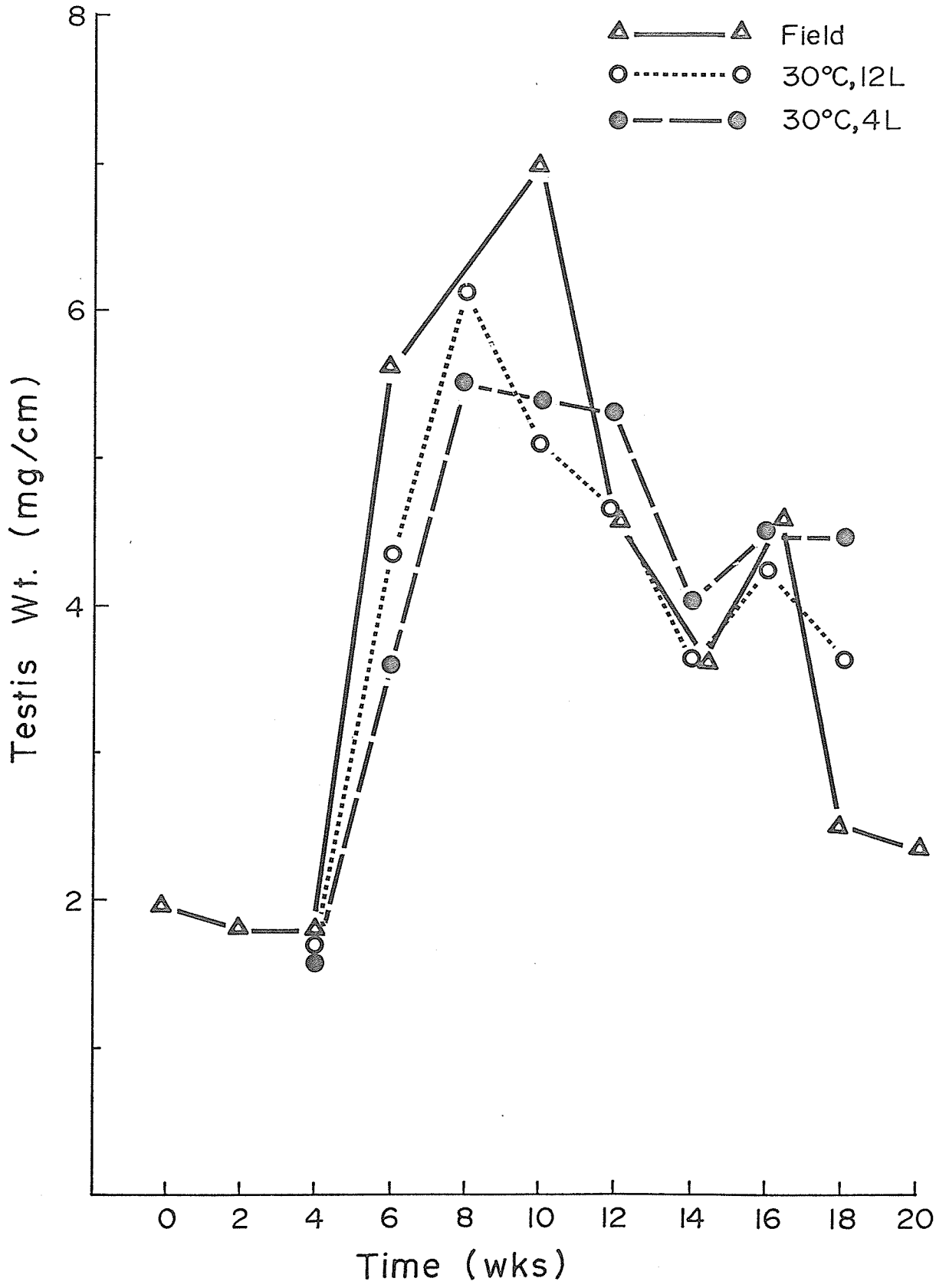
The stimulation of spermatogenesis in *Thamnophis sirtalis parietalis* by an increase in body temperature following emergence is highly adaptive, since the rapidity of the stimulus allows almost immediate commencement of the processes associated with the initiation of spermatogenesis (e.g., synthesis and release of gonadotropin(s)). The earliest possible commencement of the testicular cycle helps ensure that the cycle will be completed in the short summer season.

The Significance of Two Peaks in the Testis Weight Cycle

The presence of two peaks in the testis weight cycle reported here for field animals is in contrast to several reports of testis weight cycles with single peaks in *Thamnophis* spp. (Fox 1952; 1954; Gregory 1974). However, Cieslak (1945) observed two peaks in the testis weight cycle of *Thamnophis radix*. In that species, as in *T. sirtalis parietalis*, the first peak was the largest. Despite the large sample variances in the data presented here, the reality of the bimodality of the cycle is supported by the fact that the cycles of both groups held at 30°C were also bimodal. The testis weight cycles of the three groups are plotted together in Fig. 5. In the graph, curves for the 30°C groups have been shifted two weeks to the left to superimpose the beginnings of the cycles. The samples on which the curves are based were taken from three completely separate groups of animals, each of which experienced different environmental conditions during the sampling period. Each cycle has two peaks, both of which correlate very closely among cycles.

The presence of two peaks in the testis weight cycle appears to be associated with a temporal separation of the two primary functions of the testis: the

Figure 5. Mean relative testis weights of garter snakes sampled from the field, 30°C, 12L12D and 30°C, 4L20D. Sample sizes are as in Fig. 1 and 2.


Field
30°C, 12L
30°C, 4L
Testis Wt. (mg/cm)
8
6
4
2
0
2
4
6
8
10
12
14
16
18
20
Time (wks)

production of gametes, and the production and secretion of androgens. The appearance of sperm in the testis during and just after the first peak in the testis weight cycle indicates that spermatogenesis probably began concomitantly with or slightly before the initial increase in testis weight (Fig. 1A and 1B). Histological examination supported this conclusion. Cieslak (1945) in *Thamnophis radix* and Fox (1952; 1954) in several species of the genus *Thamnophis* also found the initiation of spermatogenesis was associated with the initial increase in testis weight. Cieslak (1945) suggested that the presence of two peaks in the testis weight cycle of *Thamnophis radix* resulted from a reduction of weight caused by the loss of sperm and fluids from the seminiferous tubules. This does not adequately explain why there was a subsequent increase in testis weight. The second peak in the cycle does not appear to be associated with an increase in spermatogenic activity. In both *Thamnophis radix* and *T. s. parietalis*, the second peak occurred more than a month after the first peak, when spermatogenesis occurred. At the time of the second peak, the germinal epithelium in *T. sirtalis parietalis* was regressing. Thus, the second increase in testis weight appears to be the result of something other than an increase in the germinal epithelium.

It is probable that the second peak in testis weight is the result of an increase in testicular interstitial tissue. Fox (1952; 1954) observed a single peak in testis weight but two peaks in the diameter of testicular interstitial cells of the species he studied. Cieslak (1945) also reported a bimodal cycle in the size of the testicular interstitial cells of *Thamnophis radix*. In this species, the second peak in interstitial cell size was much greater than the first and was synchronous with the second peak in testis weight. In *Thamnophis sirtalis parietalis*, an increase in blood testosterone (which is produced and secreted by the interstitial cells) occurs in the fall, at the same time as the second peak in the testis weight cycle (Hawley, Aleksiuk and Whitehead, in prep.). Thus, it is probable that the first peak in these bimodal testis weight cycles represents an increase in weight of the seminiferous tubules associated with spermatogenesis and proliferation of the germinal epithelium, while the second peak represents an increase in interstitial tissue associated with the production of androgen. Temporal dissociation of spermatogenic and interstitial cell cycles in other reptiles has been discussed by Licht (1972d).

Spermatogenic Failure at Low Temperature

At 20°C testicular weight increase was very slight and of short duration. Spermatogenesis was incomplete in all but four animals. It is highly unlikely that spermatogenesis would have been observed if exposure to 20°C was prolonged since the very slight testis weight cycle was complete by the fourteenth week, with the testis having regressed in weight to below the spring level (Fig. 3A). By the fourteenth week the seminiferous tubules had regressed relative to their condition on the eighth and tenth week. Rather than increasing in the fall, the mean sperm counts of both the anterior and posterior vas deferens declined (Fig. 3C and 3D). The pattern was one of degeneration rather than recrudesence.

Feeding was retarded at 20°C relative to that at 30°C and thus it is possible that inadequate nutrition contributed to testicular failure at 20°C. There is evidence that inadequate nutrition can retard testicular recrudesence in mammals and birds (Turner and Bagnara 1971; van Tienhoven 1968). Cuellar (1973) found a similar effect of nutrition in the lizard *Anolis carolinensis*. However, there are several observations suggesting that this is not

the reason for the absence of complete testicular recrudesence at 20°C in the experiments reported here.

First, when spermatogenesis occurred in animals at 30°C (around the fourth week of sampling) and in field animals (in July), these individuals possessed relative body weights similar to the relative body weights of the snakes at 20°C during their period of increased testicular weight (around the sixth week) (Tables 2 and 3). Spermatogenesis should have been completed during this period of increased testicular weight, when the seminiferous tubules were most advanced. Even after the testes of animals at 20°C had regressed in weight (around the tenth week), the mean body weight of these animals had not decreased markedly (Table 3).

Secondly, if spermatogenesis was inhibited by decreased body weight, the presence of sperm in the testis of animals at 20°C which did complete spermatogenesis should correlate with larger relative body weights. This was not the case. The relative body weights of the four males at 20°C which possessed testicular sperm after the spring regression (after the eighth week) were 0.542 gm/cm, 0.406 gm/cm, 0.381 gm/cm and 0.317 gm/cm. In each sample in

which these males occurred, there were always males with relative body weights greater than these values. Some of these animals had quite large relative body weights (e.g., 0.954 gm/cm, 0.840 gm/cm, 0.720 gm/cm), but did not possess testicular sperm. Furthermore, there were animals sampled from 30°C and from the field with very low relative body weights (e.g., 0.278 gm/cm, 0.283 gm/cm, 0.294 gm/cm, 0.322 gm/cm, 0.325 gm/cm). They did possess testicular sperm in August and later, and therefore had completed spermatogenesis.

Thirdly, data from the small amount of literature dealing with the effect of feeding on testicular cycles in reptiles suggest that some species have the capacity to preferentially shunt energy reserves to the gonads. In *Anolis carolinensis* held at 32 ± 1°C, a decrease in body weight caused by limited feeding was accompanied by an inhibition of testicular weight increase (Cuellar 1973). However, feeding sufficient to allow even a slight increase in body weight resulted in a marked increase in testicular weight. It is possible that assimilation of ingested material and mobilization of stored fats and carbohydrates were inhibited in snakes held at 20°C. Bartholomew (1953) found that the lizard *Xantusia vigilis* did not feed at 8°C, yet this did not

prevent an increase in testis weight induced by a long photoperiod over a period of seven weeks. Furthermore, a reduction of testis weight gain does not necessarily imply inhibition of spermatogenesis. In fact, inhibition of the increase in testis weight in *Anolis carolinensis* apparently did not inhibit spermatogenesis (Cuellar 1973).

Finally, Fox (1954) observed atrophy of the testis of *Thamnophis elegans terrestris* at 20°C which he concluded could not have been induced by starvation.

The above discussion suggests that decreased feeding did not directly prevent spermatogenesis at 20°C although it may have contributed to the absence of a large increase in testis weight at this temperature. Exposure to constant low temperature probably prevented the completion of spermatogenesis through some other mechanism.

In *Thamnophis sirtalis parietalis*, spermatogenesis is normally completed before entrance into hibernation. In several species of reptiles, post-nuptial spermatogenesis is arrested during hibernation and resumed the following spring (Licht 1972d; Licht, et al. 1969; Lofts 1968; 1969; Prestt 1971; Viitanen 1967). In the present

study complete spermatogenesis was inhibited by constant low temperature in the laboratory. There are several other reports of the absence of spermatogenesis in reptiles held at low temperature (Bartholomew 1953; Licht, et al. 1969). The reason for this testicular inhibition is unknown, although Licht, et al. (1969) suggested that failure to complete testicular development in *Lacerta sicula* at low temperatures was the result of either metabolic depression or hormonal imbalance. The arrest of spermatogenesis during hibernation observed in some species of reptiles and the absence of complete spermatogenesis at low temperature in *Thamnophis sirtalis parietalis* might be caused by related physiological mechanisms.

There is some evidence suggesting that spermatogenic failure in *Thamnophis sirtalis parietalis* at 20°C was due to the absence of adequate gonadotropic stimulation of the testis. The exact roles the gonadotropins and gonadal androgens play in the regulation of spermatogenesis are unclear. In most vertebrates studied, follicular stimulating hormone (FSH), luteinizing hormone (LH, or interstitial cell hormone, ICSH) and testosterone all appear to be

necessary to varying degrees for normal spermatogenesis to occur (Nalbondov 1964; Turner and Bagnara 1971; van Tienhoven 1968).

Spermatogenic activity in several species of snakes has been shown to depend on stimulation from the pituitary (Chiu and Lynn 1971; Cieslak 1945; Schaefer 1933). Cieslak's (1945) histological examinations of the pituitary and testes of *Thamnophis radix* indicated that the spring onset of spermatogenesis and the increase in testis weight correlated with an increase in basophilic cell number and activity. Hartmann (1944) found that the basophilic cell number in *Thamnophis sirtalis sirtalis* was greatest in the spring when spermatogenesis would be expected to begin.

Saint Girons (1970) described both FSH and LH (ICSH) gonadotropic cells in the pituitary of reptiles. The observations of Eyeson (1971) suggest that an FSH-like gonadotropin regulates spermatogenesis while an LH-like gonadotropin stimulates testicular interstitial tissue in the lizard *Agama agama*. However, there is evidence that all stages of spermatogenesis and androgenesis in snakes and lizards are controlled by a single FSH-like

gonadotropin (Licht 1972b, c, d; 1974; Licht and Papkoff 1971; Licht and Pearson 1969; Licht and Rosenberg 1969; Licht and Stockwell-Hartree 1971). Licht (1972b) was able to reverse the effect of hypophysectomy on the gonads of *Thamnophis sirtalis* by administration of mammalian FSH. This hormone permitted complete spermatogenesis and also stimulated the Leydig cells. Tsui and Licht (submitted) obtained a similar result.

The initiation of spermatogenesis in *Thamnophis sirtalis parietalis* at 20°C suggests that there was some gonadotropic stimulation of the germinal epithelium. Different stages of spermatogenesis may have different thermal requirements, only the earlier stages occurring at low temperature. The study by Licht and Pearson (1969) indicated that the various stages of spermatogenesis in *Andis carolinensis* may have different thermal requirements and also different gonadotropin dosage requirements. In the present study, the constant low temperature at which the animals were held may have also inhibited the production and release of gonadotropin or reduced the sensitivity of the testis to the influence of the hormone. Independent of the possible absence of some environmental cue (e.g.,

high temperature), the general metabolic depression induced by constant low temperature could have inhibited biochemical processes sufficiently to inhibit spermatogenesis. Failure to induce complete spermatogenesis in *Thamnophis sirtalis* at 8°C by injection of mammalian FSH (Tsui and Licht, submitted) suggests the thermal effect, at least at this very low temperature, is on something other than the pituitary.

Whatever mechanism was involved, temperature had a dramatic effect on testicular recrudesence of *Thamnophis sirtalis parietalis*. Until the influence of different combinations of photoperiod and temperature regimes are investigated, the exact thermal requirements for spermatogenesis in this species will remain uncertain. It is possible that a minimum thermal threshold for spermatogenesis exists close to 20°C. Further investigations of testicular recrudesence around "threshold temperatures" should help elucidate the mechanism of temperature control of gonadal cycles in cold climate reptiles.

Part II

Thermal Regulation of Spring Mating Behaviour in the Red-sided Garter Snake (*Thamnophis sirtalis parietalis*)

Introduction

The red-sided garter snake (*Thamnophis sirtalis parietalis*) is the most northerly occurring reptile in North America (Longier and Toner 1961). In the Interlake (south-central) area of Manitoba, Canada, the species hibernates communally in fissured areas and sinks in limestone bedrock. The seasonal activity and migratory movements of *Thamnophis sirtalis parietalis* from this area have been described by Aleksiuk and Stewart (1971) and Gregory (1971, 1974).

Mating in this species occurs within minutes following emergence from hibernation in the spring. This behavioural sequence is common in species within the genus *Thamnophis* (Blanchard and Blanchard 1941; Carpenter 1952; Cieslak 1945; Fitch 1965, 1970; Fox 1952, 1954; Wright and Wright 1957). Other observations of spring mating in species within this genus (Finneran 1949; Gardner 1955; Noble 1937) and in other genera (Fitch 1963a, b; 1970; Fitch and Fleet 1970;

Noble 1937; Platt 1969; Trapido 1940; Wright and Wright 1957) indicate that mating occurs if not immediately then at least shortly after emergence in many species within the family Colubridae in North America. Occasional summer and fall matings have been reported in most species, but generally spring is the major mating period.

Despite the prevalence of post-emergent mating behaviour, the timing and induction mechanisms involved are not known. Reversal of the thermal gradient in the ground and the subsequent increase in body temperature have been correlated with emergence of *Vipera berus* from hibernation in Finland (Viitanen 1967) and have been suggested as the mechanism for *Thamnophis sirtalis parietalis* (Aleksiuk 1970). The close temporal association of emergence and mating may mean that an increase in body temperature elicits both activities.

Mating behaviour has been observed following an increase in body temperature in a variety of vertebrate species. An increase in water temperature initiates spawning in many species of fish (Aronson 1957, 1965). Reproductive behaviour of some amphibians appears to correlate with an increase in

temperature (Aronson 1965; Noble 1931). Among mammals, mating in several species of the genus *Citellus* occurs very shortly after emergence from hibernation (Alcorn 1940; Mitchell 1959; Moore, et al. 1934; Sadlier 1969; Wells 1935).

Similar correlations between a change in temperature and mating behaviour have been observed in several species of snakes. Noble (1937) found that transfer from 7°C and dark to approximately 25°C and light in the spring induced courtship in the brown snake (*Storeria dekayi*). Blanchard and Blanchard (1941) suggested the mating behaviour of *Thamnophis sirtalis sirtalis* was somewhat temperature dependent. Aleksiuk and Gregory (1974) induced courtship in *Thamnophis sirtalis parietalis* in the spring by transfer from 5°C, 0L24D to 25°C, 12L12D. Other experiments (Hawley and Aleksiuk, in prep.; Noble 1937) have indicated that although light may contribute to the induction and maintenance of spring reproductive behaviour, temperature appears to be the primary controlling factor. The purpose of the present study was to elucidate in detail the thermal regulation of spring reproductive behaviour in *Thamnophis sirtalis parietalis*.

Materials and Methods

In May, 1973, red-sided garter snakes were collected at a hibernaculum near Narcisse in the Interlake area of Manitoba, Canada (latitude approximately 51°N). Snakes which had not yet emerged from hibernation were obtained by hand excavation of the den and were immediately put into ice-filled coolers at 1-3°C. On the day of collection the animals were transported to the laboratory, where they were transferred to a controlled environment room and maintained under constant darkness at 1.5°C (± 1°C).

Ambient temperatures and body (approximately 4 cm into the intestine from the cloaca) temperatures of many unemerged and emerged snakes were recorded at the den site in early May using a YSI model 42SC telethermometer with a No. 408 probe which had been fitted and calibrated with a small thermistor. Although the animals used in these experiments were all collected from a single den, body and environmental temperatures were recorded at this and a second adjacent and topographically similar den. Environmental temperatures were recorded on two days in the early afternoon in both sunny and shaded areas in and around the dens. Two temperature recordings approximately

fifteen minutes apart were made at each of three levels: on the ground surface, 0.5 metre above the ground and 1.0 metre above the ground. Body temperature was recorded within ten seconds of the snake's capture. Precaution was taken to prevent heat from the experimenter's hand from influencing the reading. All days when temperature recordings were made at the den were sunny.

Mating experiments were performed in large brown cardboard or fiberglass containers (approximate area $0.7m^2$). During each day of the mating trials the snakes were illuminated for an 8-hour photoperiod (0900 to 1700) with fluorescent light at an intensity of approximately 470 lux (45 f. c.) within the observation tanks as measured with a Gossen Lunasix-3 light meter. The bottoms of the observation tanks were covered with wood shavings. Water was available at all times, but food was not provided.

All mating trials were performed between May 5 and June 10, 1973, within the normal breeding period of snakes observed at the hibernaculum during that year. The evening before each mating experiment commenced, twelve individuals of each sex were chosen without bias for each mating trial and marked on their dorsal surface

with fingernail polish for individual identification. During the marking process these animals were exposed to dim light for several minutes, and then returned to total darkness. Mating trials were paired, so that two separate trials involving 12 males and 12 females each, were always performed concurrently. A single pair of mating trials (a total of 24 males and 24 females) was performed at each of 5° and 30°C (± 1°C). Two pairs of mating trials (a total of 48 males and 48 females) were performed at each of 10°, 15°, 20° and 25°C (± 1°C).

At the beginning of an experiment, snakes to be used in a pair of trials were transferred from 1.5°C and darkness to test conditions at 0900. Commencing at 0930, the snakes of both trials were observed for 10-minute periods at hourly intervals for the entire lighted period. Snakes used in a second experiment involving similarly paired trials were transferred from cold and dark at 0910 and first observed at 0940. Additional concurrent experiments (up to a total of 3) were similarly staggered by 10 minutes. Observations were continued for five days.

During each 10-minute observation period, any courtships and copulations observed were recorded for individual snakes. The mating behaviour of *Thamnophis sirtalis parietalis* is virtually identical to that described by Noble (1937) and Blanchard and Blanchard (1941) for *Thamnophis sirtalis sirtalis*. These authors have described the details of the behavioural sequences which were here regarded as courtship. Courtship was identified by (A) the male "palping" the back of the female with his chin and aligning his body parallel to that of the female; (B) the male tucking his anal vent under the female's body; and (C) waves of peristaltic-like contractions along the male's body while aligned parallel to the female. Behavioural patterns B and C were very distinct actions and were most frequently used as indications of courtship. Pattern A rarely occurred without B or C. When it did occur in isolation, courtship was recorded only when the pattern was persistent enough (i.e., alignment of most of the male's body continuously for several minutes) to unquestionably be courtship. Behavioural patterns A, B and C occurred chronologically in this sequence, as courtship progressed from initial (A) to advanced (C)

stages and finally culminated in copulation. Thus pattern C represented a greater advancement or extent of courtship than either patterns A or B. These differences in extent were not considered when identifying courtship. Copulation was identified by intromission of a hemipenis into the female's cloaca.

Parameters measured here dealing with observed courtship and copulation are binomial in character. They are solely an indication of whether or not an individual was sexually aroused at least to the point of courtship. Differences in the vigour and/or extent of courtship were noted, but were not quantified. Very few copulations were observed during the scheduled observation periods. Since parameters based on the hourly observations were primarily concerned with the presence or absence of sexual activity, copulating individuals were included in the count of courting individuals. Each set of binomial data from individual trials at a given temperature was tested for differences between trials using the Chi-square test or tables based on the Fisher-Irwin exact test (Owen 1962). Over the five days of each trial there were no significant differences ($P > 0.05$) among trials at each temperature. Therefore, results from individual trials at a given temperature were pooled.

The number of mated females was determined at the end of each day's photoperiod by inspection of the females for copulatory plugs as described by Fitch (1965) for this species. Examination of over 40 females after copulation of each female was witnessed revealed the presence of a copulatory plug in all cases but one. These plugs usually persisted for two or more days. Therefore daily examination for copulatory plugs was a reliable method for determining the number of mated females. Snout-vent lengths of both males and females were measured at the termination of each five-day mating trial.

During February and March 1973 and March 1974, experiments were performed to determine the rate of body temperature change and the equilibrated body temperature of both sexes under the conditions of the mating experiments. At each environmental temperature, dorsal anterior body surface temperatures of six males and six females were recorded with a recalibrated YSI model 42SC telethermometer using a small thermistor attached to a No. 408 probe by three feet of fine microphone wire. The probe was taped to the back of the snake above the heart. This method of recording body temperature was used to permit

continual monitoring of the snake's body temperature while minimizing disturbance of the animals. Furthermore, Vincent (1971) found that the dorsal anterior body surface temperature tended to be slightly higher than either oral, cloacal, or ventral body surface temperatures. Therefore, measurement of the former parameter is very useful for determining if the snake's body temperature is at all above the ambient temperature. The probe was attached to the snake while the animal was still at an environmental temperature of 1-2°C. The snake was left at this temperature until body temperature re-equilibrated after handling (at least 30 minutes), at which time the body temperature was recorded. The snake was then transferred to the test conditions, and its dorsal anterior body surface temperature was monitored for one hour. At each temperature one or two snakes were left under these conditions for 24 hours, at which time body temperature was again measured.

Results

Emergence activity in 1973 at the hibernaculum from which the snakes were collected was first observed in mid April. Body temperatures of unemerged and emerged snakes and environmental temperatures measured in early May at both dens were combined and are presented in Table 1. Since snakes were observed courting in both sunny and shaded areas in and around the dens, environmental temperatures recorded at all sites were pooled. Temperature recordings from the three levels above the ground were also pooled to give an overall mean environmental temperature on each day. The predominance of male records on May 1 and May 3 reflects the sex ratio of emerged snakes at that time. It is evident from Table 1 that emerged males and females could attain body temperatures considerably above ambient temperatures. The body temperatures of unemerged snakes appeared to decrease as the depth at which the snakes were found increased. By May 8, several hundred unemerged snakes had been collected. Most of these animals were found within the top 0.5 m of debris within the limestone sink. Very few snakes were found between 0.5 m and 1.5 m in depth. Therefore,

Table 1. Mean body temperatures of emerged and unemerged garter snakes and mean ambient temperatures with ± one standard error about the means. Recordings made at two adjacent dens were combined. Recordings of environmental temperatures measured at three levels (1.0 m, 0.5 m and ground surface) were pooled.

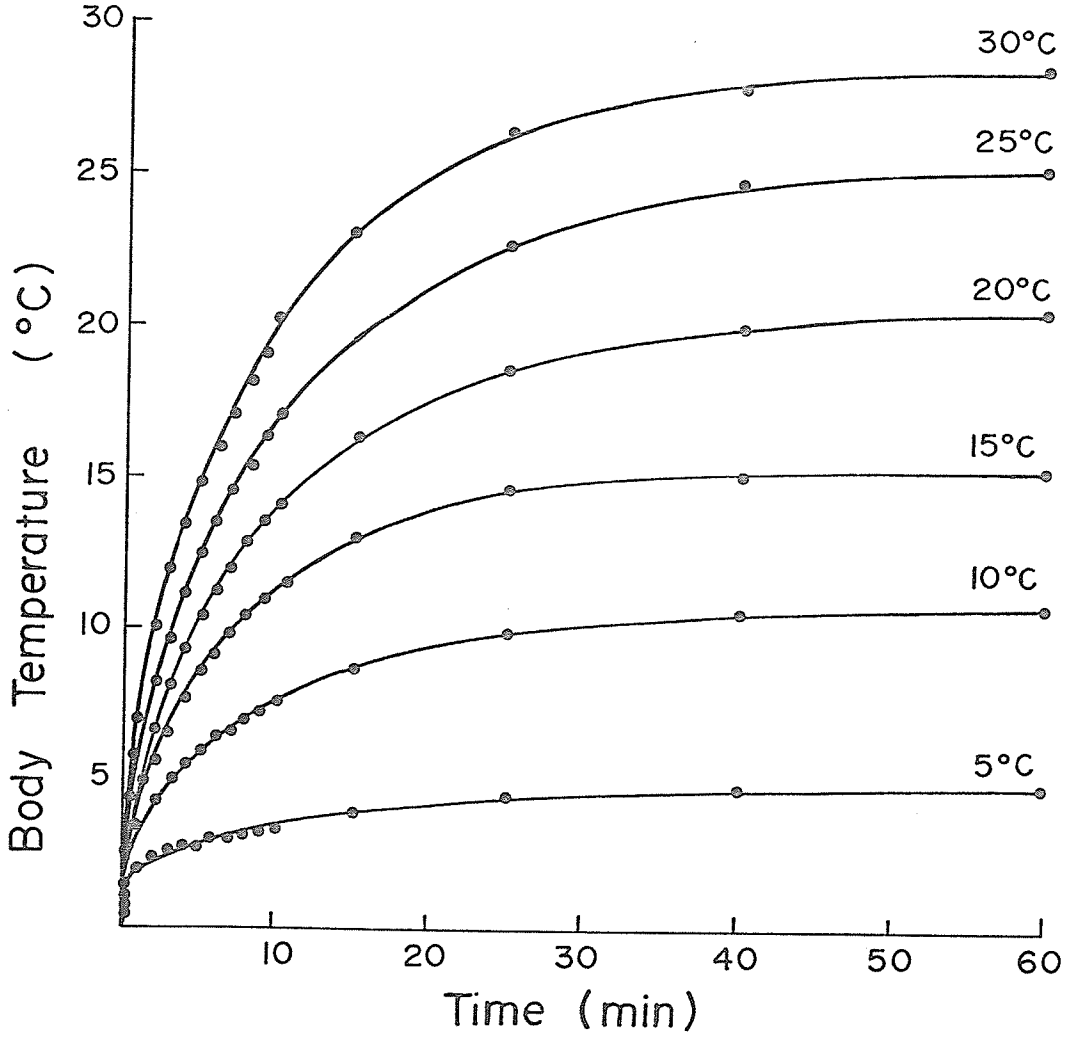
	Date					
	May 1		May 3		May 8	
	♂	♀	♂	♀	♂	♀
Mean Body Temperature Unemerged Snakes (˚C)	6.9 ± 0.3 (n = 14)	5.6 ± 0.3 (n = 11)	8.1 ± 0.3 (n = 16)	6.3 ± 0.4 (n = 8)	9.0 ± 0.4 (n = 5)	8.3 ± 0.4 (n = 5)
Mean Body Temperature Emerged Snakes (˚C)	26.7 ± 0.5 (n = 14)	24.0 ± 0.6 (n = 5)	29.3 ± 0.2 (n = 15)	25.7 ± 1.3 (n = 4)	-------	-------
Mean Ambient Temperature (˚C)	———		16.7 ± 0.4 (n = 18)		18.1 ± 0.9 (n = 18)	

since the total number of snakes overwintering at this den is estimated at several thousand (Aleksiuk, pers. comm.), the majority of snakes were presumably hibernating at depths exceeding 1m. Since the excavated snakes were very near the surface with no snakes closely beneath them, they were probably in the process of emergence when collected.

In the laboratory, the dorsal anterior body surface temperature of both sexes at all temperatures studied equilibrated near environmental temperature within one hour after transfer from 1-2°C to higher temperature (Fig. 1). After this time the body surface temperatures of snakes held under the conditions of the mating trials were usually within ± 1°C of the environmental temperature, and never differed from it by more than ± 2°C. Thus under these experimental conditions, environmental temperature and body temperature can be considered equivalent.

All male snakes collected at the den and used in the experiments were sexually mature. When the sizes of the male and female snakes used in mating trials were compared among temperatures, using a one-way analysis of variance (homogeneity of variances tested with Bartlett's test), there were no significant

Figure 1. Mean dorsal anterior body surface temperatures of garter snakes, plotted as a function of time following transfer from 1-2°C to higher temperatures (the latter are indicated at the right hand side of the graph). Each point is the mean of recordings from 6 males and 6 females at each temperature. Standard errors were very small and ranged from 0.63°C at 30°C to 0.07°C at 5°C.


30
25
20
15
10
5
Body Temperature (°C)
30°C
25°C
20°C
15°C
10°C
5°C
10
20
30
40
50
60
Time (min)

differences among temperatures for either sex ($P > 0.05$). Therefore there were probably no significant differences in average age or maturity. Thus comparison of sexual activity at different temperatures should be unaffected by any influence age or maturity may have had on sexual performance.

The cumulative percentage of males sexually active (i.e., observed courting at least once) at various temperatures is plotted as a function of time for the five days of observation (Fig.2). Values are plotted hourly for day 1, since on this day the number of hours of observation equalled the number of hours of exposure to elevated temperature. All mating trials began with 12 males; however, there was slight mortality over the five days. The total numbers of snakes alive on the last day of all mating trials at each temperature are indicated (Fig. 2).

When the total percentage of males that were sexually active within 5 days was plotted as a function of temperature, an exponential function of the form $y = (x-b)^{1/a}$ was obtained. When this parameter was plotted against the log of environmental temperature, a straight line ($r = 0.9974$ with 3 degrees of freedom) resulted (Fig. 3). The equation of this line, as calculated by the method of least squares, was $y = 86.125 \log x - 18.085$. Extrapolation to the

Figure 2. Cumulative number of males observed courting, expressed as a percentage of the number of live males present at each temperature and plotted as a function of time following transfer from 1.5°C. For the first day, this percentage is plotted hourly, while for the remaining days a single point represents the cumulative percentage achieved by the end of each day's photoperiod. Numbers in parentheses represent the total number of males alive at the end of all trials at each temperature.

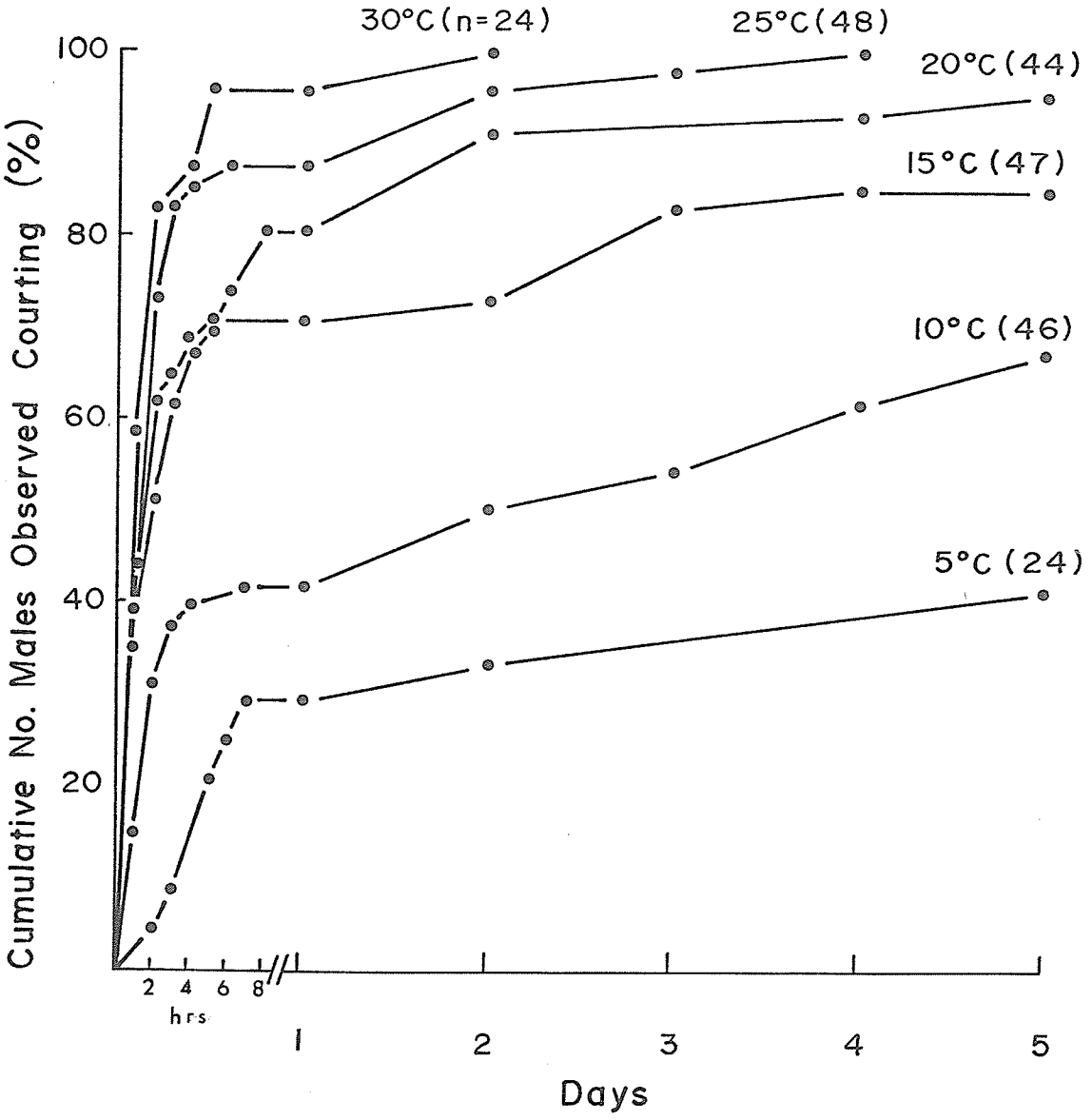
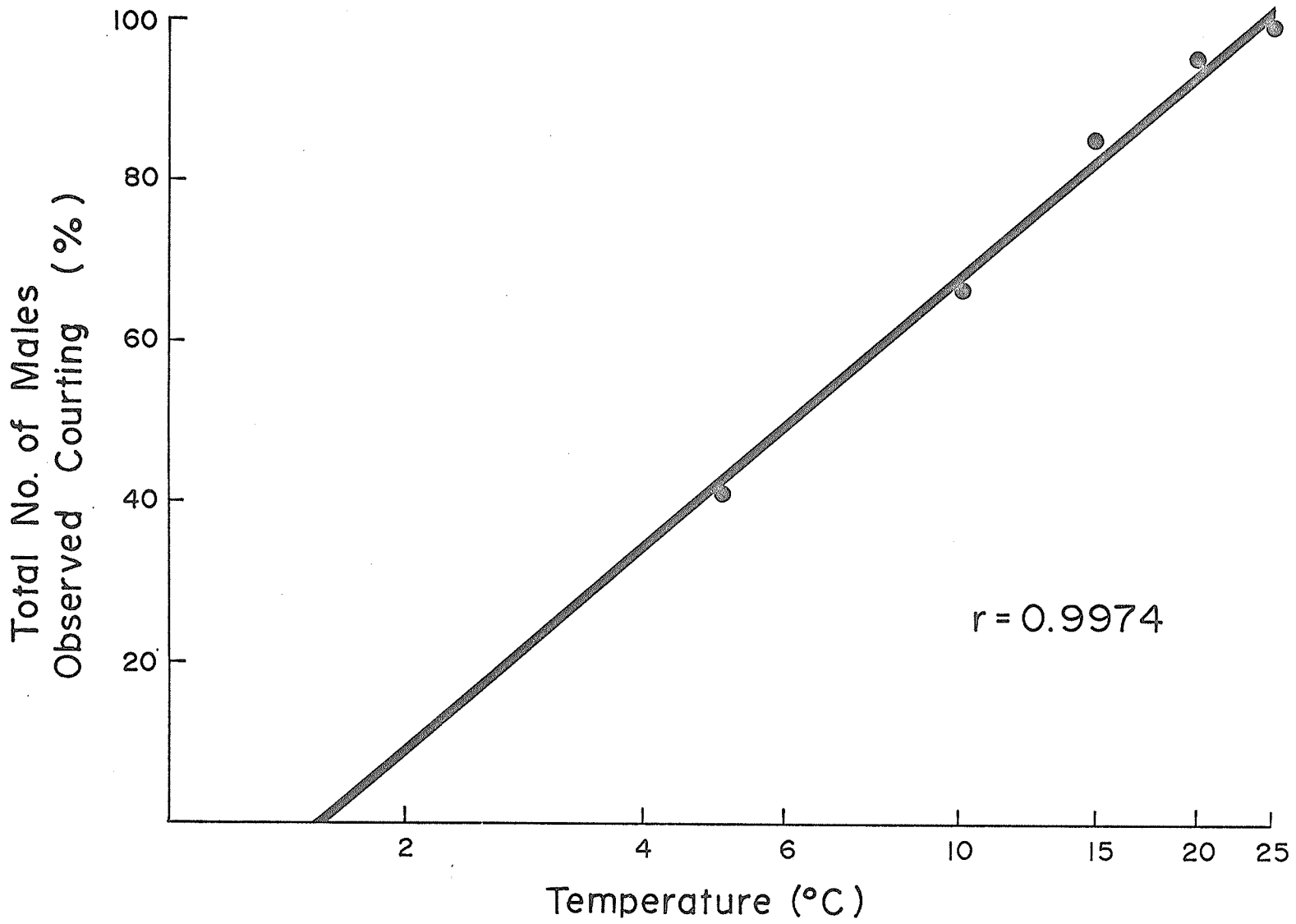

30°C (n=24)
25°C (48)
20°C (44)
15°C (47)
10°C (46)
5°C (24)
Cumulative No. Males Observed Courting (%)
100
80
60
40
20
2
4
6
8
hrs
1
2
3
4
5
Days

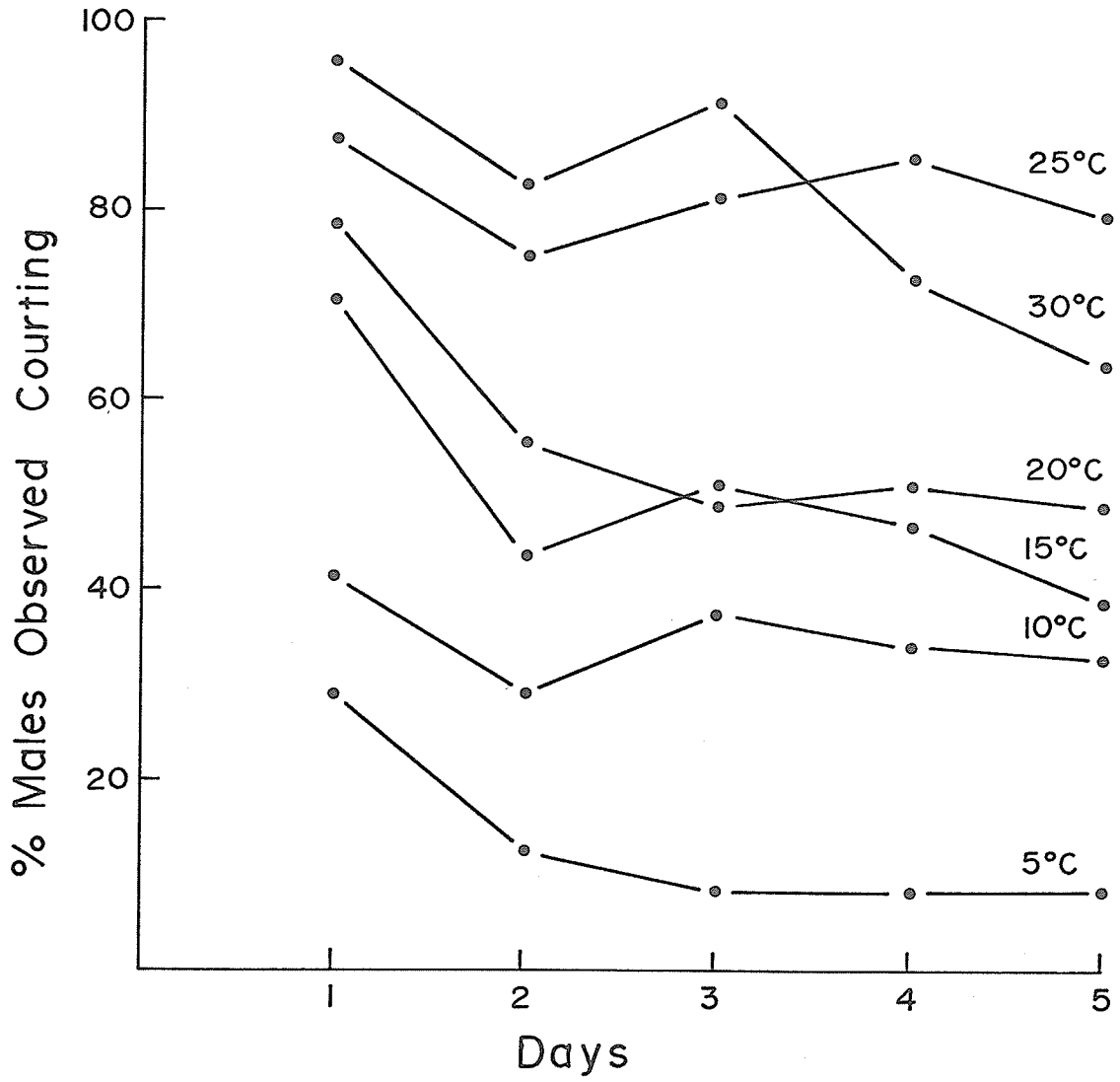
Figure 3. Total number of males observed courting during 5 days, expressed as a percentage of the total number of males present and plotted as a function of temperature. The value at 30°C was excluded because 100% of the males were sexually aroused at 25°C. Sample sizes are as in Fig. 2.


Total No. of Males
Observed Courting (%)
100
80
60
40
20
r = 0.9974
2
4
6
10
15
20
25
Temperature (°C)

x-intercept yields a minimum thermal threshold for courtship of 1.6°C. Differences among temperatures in the percentage of males which were courting were significant (Chi-square test, $P < 0.05$). There was no apparent relationship between the size of a male and whether or not the male became sexually active.

The percentage of males courting each day followed a similar temporal pattern at all temperatures tested, with the exception of 5°C (Fig. 4). The greatest number of males were courting on the first day after transfer. There was a marked decline on day 2, followed by an increase on day 3 (day 4 at 20°C) and a subsequent decline by day 5. At 5°C, day 1 also had the maximum percentage of males courting and was followed by a marked decline on day 2. However, there was a further decline on day 3 to 8.33%, which was maintained through day 5. The percentage of males courting per day was partially dependent on the percentage of males which became sexually active (these two parameters were equal on day 1), but it was also dependent on the number of days on which a given male was observed courting. The mean number of days on which males were seen courting increased linearly ($r = 0.9515$ with 4 degrees of freedom) with

Figure 4. The number of males observed courting each day, expressed as a percentage of the total number of males present at each temperature and plotted as a function of time following transfer from 1.5°C. Sample sizes are as in Fig. 2.


100
80
60
40
20
% Males Observed Courting
25°C
30°C
20°C
15°C
10°C
5°C
1
2
3
4
5
Days

temperature (Fig. 5). Males that died before termination of the experiment were excluded from these calculations. Differences among temperatures were significant (one-way analysis of variance, $P < 0.05$; homogeneity of variances tested with Bartlett's test).

The daily number of courtship sightings per male was calculated by dividing the total number of individual courtships observed per day by the total number of males observed courting on that day. At each temperature, this value varied little over the five days (Table 2). The daily values were averaged for all trials over all five days to give an overall index of daily courting activity per sexually active individual at each temperature (Fig. 6). It can be seen that the amount of courting activity per courting individual within a single day also increased linearly ($r = 0.9149$ with 4 degrees of freedom) with temperature (Fig. 6). Differences in this index among temperatures were significant (one-way analysis of variance, $P < 0.05$; homogeneity of variances tested with Bartlett's test).

There were temporal differences in courtship and copulation at different temperatures. The durations of individual courtships and copulations increased at

Figure 5. The mean number of days (out of 5) on which each sexually active male was seen courting, plotted as a function of temperature. Only males that were observed courting at least once within the five days and which were alive at the end of the mating trial were included. Numbers of these types of males at each temperature are indicated. Vertical bars represent ± one standard error about the means.

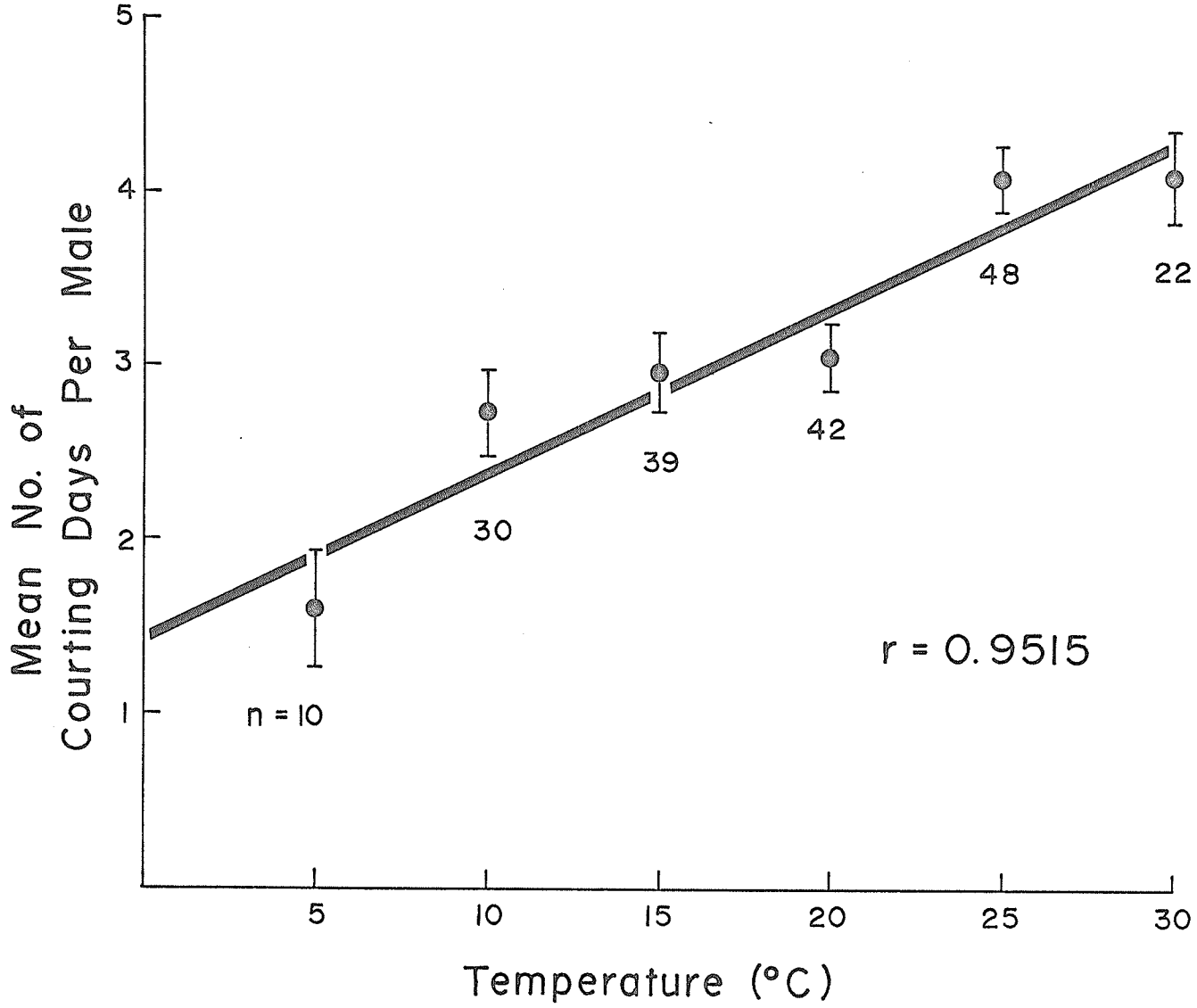
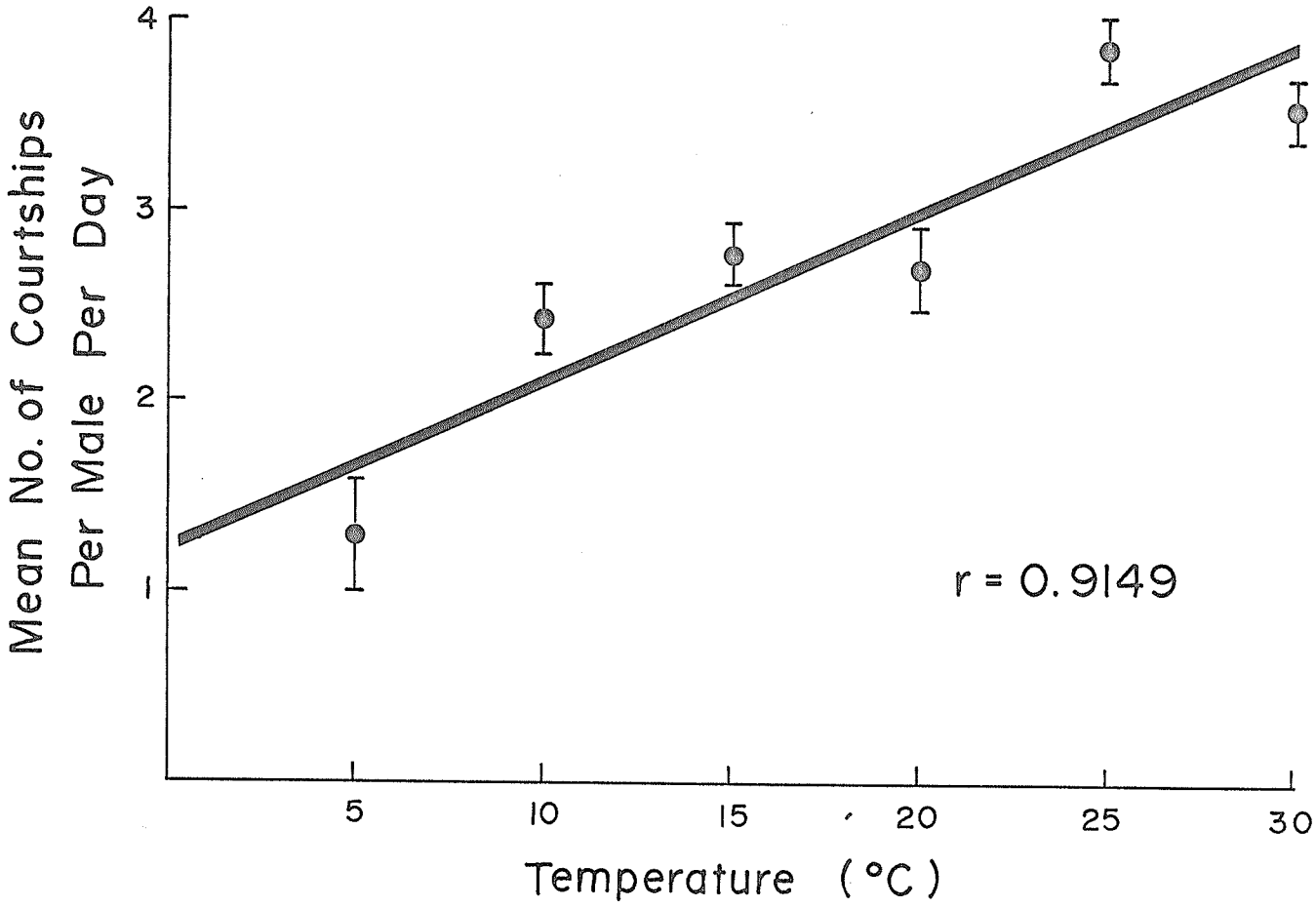

Mean No. of
Courting Days Per Male
5
4
3
2
1
n = 10
30
39
42
48
22
r = 0.9515
5
10
15
20
25
30
Temperature (°C)

Table 2. Daily number of courtship sightings per courting male (total no. of courtships/ no. of courting males) averaged among trials at each temperature.

	Day				
Temperature (°C)	1	2	3	4	5
30	3.8	3.3	3.4	3.5	3.5
25	3.1	3.9	4.0	3.9	4.3
20	2.9	2.3	2.4	2.8	3.3
15	3.0	2.2	2.9	2.8	2.9
10	3.4	2.7	2.2	2.4	2.0
5	1.2	1.5	1.5	1.3	1.0

Figure 6. The mean number of courtships per sexually active male per day, plotted as a function of temperature. Values are the means of 10 daily values at 5°C and 30°C (2 trials over 5 days) and 20 daily values at all other temperatures (4 trials over 5 days). Vertical bars represent ± one standard error about the means.


Mean No. of Courtships
Per Male Per Day
4
3
2
1
r = 0.9149
5
10
15
20
25
30
Temperature (°C)

lower temperatures. Above 20°C, a single courtship rarely lasted for an hour. Below this temperature, single courtships often lasted for two or more hours. Thirty-seven pairs of snakes were observed in copulation during the observation periods of these experiments. One or two copulations at each temperature were observed in their entirety. Mating pairs tended to remain in copulo for longer periods at lower temperatures. Only once did an observed copulation last for one hour or longer. This prolonged copulation occurred at 10°C, the lowest temperature at which any copulations occurred. Also, at lower temperatures courtship appeared less vigorous. The peristaltic-like contractions of the males during courtship at 5°C occurred much less frequently and with less vigour when compared to those at higher temperatures. Palping with the chin and tucking of the anal vent under the female appeared to be slower at 5° and 10°C than at higher temperatures, but in other ways they were normal.

Courtship usually involved a single female and one or two males, but occasionally courtships involving up to four males with one female occurred. These multiple courtships often involved larger females.

Courtship of females after they had mated was common, but multiple copulation of a female was observed only once (i.e., a single female was mated by two different males at different times).

There was a minimum size requirement of the female for copulation (Appendix 1). Only females with a snout-vent length exceeding 45 cm copulated. Therefore, only females of this size were considered in the analysis of the effect of temperature on copulation.

The percentage of females with snout-vent length greater than 45 cm that were mated within five days, as indicated by the presence of copulatory plugs, is plotted as a function of temperature in Fig. 7 ($r = 0.9583$ with 4 degrees of freedom). Differences among temperatures were significant (Chi-square test; $P < 0.05$). At all temperatures most copulations occurred within the first two days after transfer (Table 3), but there was a delay in copulations at lower temperatures. Fewer copulations occurred on the first day and copulations occurred on later days as temperature decreased.

Figure 7. The total number of females mated, expressed as a percentage of the number of females with snout-vent length greater than 45 cm and plotted as a function of temperature. Numbers indicate the number of females of this size at each temperature.

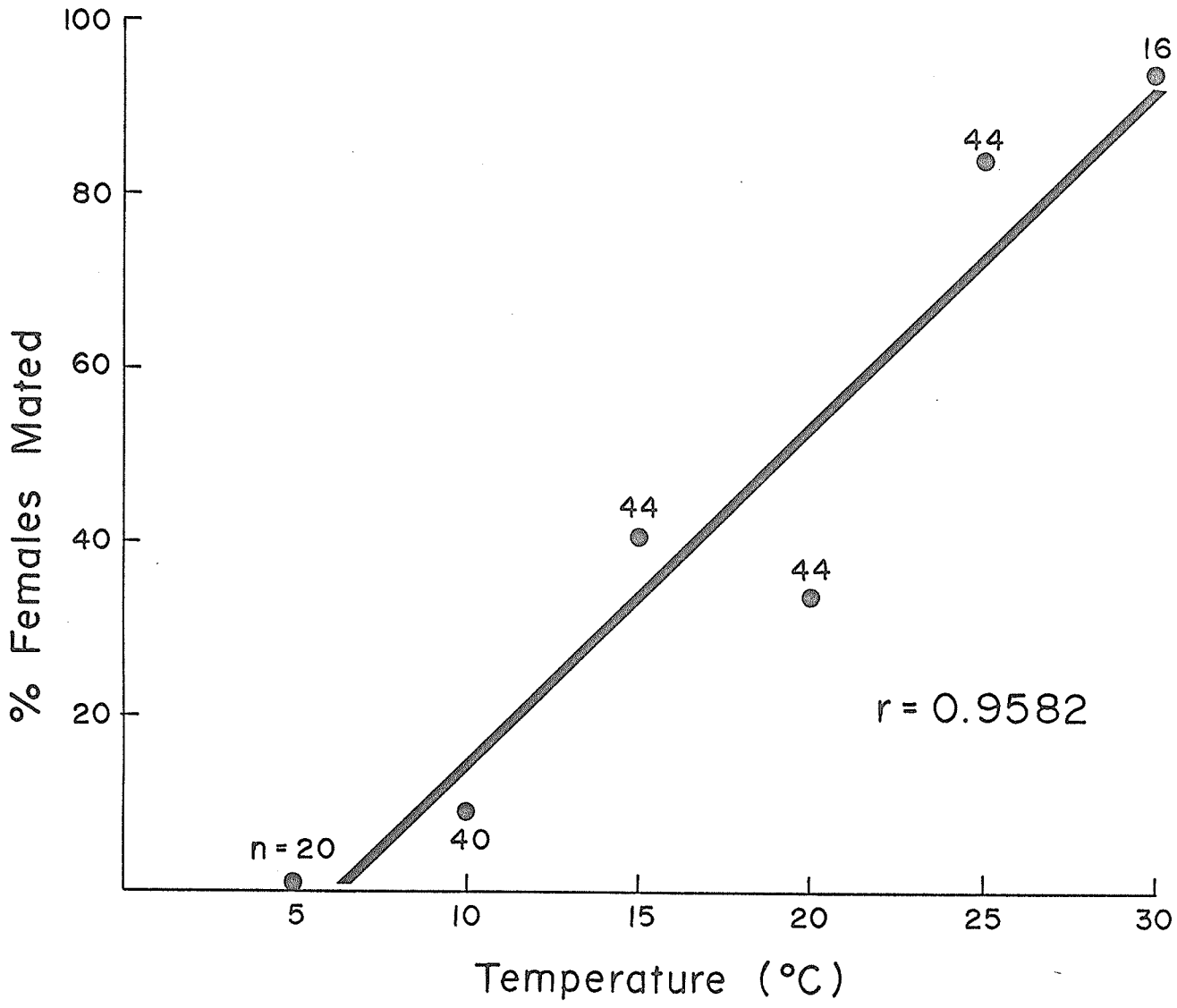

100
80
60
40
20
% Females Mated
16
44
44
44
n = 20
40
r = 0.9582
5
10
15
20
25
30
Temperature (°C)

Table 3. Cumulative number of copulations that occurred by the end of each day's photoperiod, expressed as a percentage of the total number of copulations that occurred by the end of day 5 at each temperature.

	Cumulative Percentage of Copulations				
	Day 1	Day 2	Day 3	Day 4	Day 5
Temperature (°C)					
30	87.0	100.0			
25	86.5	95.0	100.0		
20	80.0	87.0	100.0		
15	38.9	72.3	94.5	94.5	100.0
10	50.0	75.0	75.0	100.0	
5	0	0	0	0	0

Discussion

The Influence of Temperature on Spring Mating Behaviour

The amount and quality of spring sexual behaviour displayed by *Thamnophis sirtalis parietalis* was highly temperature dependent. Courtship and copulation increased with increased temperature. As evidenced by the wide range of thermal thresholds required to elicit courtship, the sexual responses of male snakes were quite variable. Noble (1937) also noticed great variability in the extent and vigour of sexual activity of male *Thamnophis sirtalis sirtalis* and *T. butleri*. Body temperatures of about 25°C must be achieved before all males are sexually active, since the range of the thermal thresholds for courtship had a maximum between 20° and 25°C. In the field, heliothermy permits achievement of body temperatures in excess of these higher thresholds very early in the spring (Table 1). Even at ambient temperatures of about 17°C, all males could have body temperatures equal to those at which full sexual activity was achieved in these experiments. Blanchard and Blanchard (1941) observed *Thamnophis sirtalis sirtalis* mating on sunny days at air temperatures in excess of 15°C. However, they

apparently did not measure body temperatures under these conditions or make extensive observations of the snakes at air temperatures below 15°C. Given sufficient incident radiation, *Thamnophis sirtalis* might be able to mate at much lower ambient temperatures than indicated by Blanchard and Blanchard (1941) and the present study.

Despite variability among individuals in the temperature required to elicit courtship, most males became sexually active very quickly after exposure to temperatures above this threshold. At all temperatures used in the present study, most of the males that were eventually observed courting were courting females on the first day of exposure to elevated temperature (Fig. 2). The rate at which the body temperature of snakes increased under these experimental conditions (Fig. 1) and the existence of a very low thermal threshold for courtship in some individuals (Fig. 2 and 3) explains the very rapid onset of first courtship in these experiments. It appeared that males became sexually active more quickly at higher temperatures, but because of the nature of the observations this cannot be concluded with certainty. In the field, heliothermy could

increase the rate of warming after emergence at any given temperature. Also, a large percentage of males had attained a body temperature at which courtship was elicited in these experiments before they had completed emergence (Table 1). These factors account for the almost "immediate" mating activity observed in some individuals after emergence from hibernation (Aleksiuk and Gregory 1974).

Although the extrapolated minimum thermal threshold for courtship was just under 2°C, it is unlikely that courtship would be observed at that temperature. A male might be stimulated to court at 2°C, but the depression of general activity could prevent courtship. A depression of physical activity at 5°C might have contributed to a reduction in the vigour and extent of courtship at that temperature. However, in view of the decrease in sexual activity with decreasing temperature between 30° and 10°C, it is likely that the decrease in sexual activity at 5°C was at least partly due to decreased sexual motivation.

Except for the number of courtships per sexually active male per day(Table 2) (which varied little over the five days), all measures of reproductive activity were greatest or had the greatest increase on the day

of transfer from cold and dark conditions. The decline in the number of courting males after day 1 (Fig. 4) may represent sexual satiation or fatigue. Although the total number of males courting per day varied over the five days, the daily amount of courtship per courting male varied little (Table 2). Therefore the amount of courting displayed by individual males on different days was similar at a given temperature.

Interpretation of the effect of temperature on female receptivity is difficult because depression and/or deletion of parts of the courtship pattern possibly contributed to the occurrence of fewer copulations (Fig. 7) and to the reduced rate at which copulations occurred (Table 3) at lower temperatures. Aleksiuk and Gregory (1974) suggested a passive role for the female during both courtship and copulation. From my observations and others (Blanchard and Blanchard 1941; Fitch 1968; Noble 1937) it is evident that copulation will not occur unless the female is actively receptive. At low temperatures, courtship may have been inadequately stimulating to the female and/or the male's sexual capacity too limited for copulation regardless of the female's receptivity. This view is supported by the

fact that copulation was totally absent only at the temperature (5°C) at which the pattern of courtship was noticeably different (paucity of peristaltic-like contractions). Female receptivity might have varied with temperature similarly to the sexual activity of males. However, the linear increase in the number of females mated with increase in temperature (Fig. 7) may have been an indirect result of the effect of temperature on male sexual activity. It is probable that both male courting inadequacy (at least at 5°C) and reduced female receptivity contributed to the occurrence of fewer copulations at lower temperatures.

In the field, females were also able to elevate their body temperature above ambient temperature (Table 1) in a manner similar to the males. Thus, after emergence both males and females have the capacity to attain body temperatures high enough to ensure that almost all individuals which are capable of being sexually active are sexually active early in the spring.

Although many of the parameters used here to quantify sexual behaviour decreased linearly with decreasing temperature (Fig. 3, 5, 6, 7), there was a relative elevation at 15°C in several instances (Fig. 5, 6, 7). A similar upward shift at about

that temperature has been observed in enzyme activity (Aleksiuk 1971b; Hoskins and Aleksiuk 1973), standard metabolism (Aleksiuk 1971a) and *in vitro* heart rates (Aleksiuk 1970) in red-sided garter snakes from the same geographic region. Aleksiuk (1970, 1971a, b) interpreted these shifts as manifestations of mechanism(s) of instantaneous cold-compensation. It is possible that the relative increase in sexual activity around 15°C is a manifestation of a similar mechanism.

The Mechanism Involved in Thermal Induction of Spring Mating Behaviour

The amount of sexual behaviour observed in this study was clearly temperature dependent. A general increase in activity accompanying an increase in body temperature may have influenced the amount of sexual activity observed at different temperatures. However, the increase in sexual activity with increasing temperature cannot be entirely attributed to the effect of temperature on general activity, since primarily sexual behaviour increased with an increase in temperature, and not other types of behaviour. The influence of temperature on these other types of

behaviour (e.g., exploratory activity) appeared to be much less than its influence on sexual behaviour *per se*. Indeed, an increase in the amount of observed sexual behaviour was at the expense of other types of behaviour. Moreover, the number of courting males increased with an increase in temperature. Under the experimental conditions, males and females were sufficiently close that only short distances (usually less than 40-50 cm) had to be crossed to bring individuals together, and even at 5°C locomotory activity of both males and females appeared sufficient to allow courtship to occur. Therefore, the increase in the number of courting males with an increase in temperature was probably due to an increase in sexual activity of the individual *per se*. Both the selective enhancement of sexual activity and the increase in the number of courting males with increasing temperature indicate that the increase in sexual activity was not due solely to an increase in general activity. Therefore, it appears that sexual motivation increased as body temperature increased.

It is as yet unclear whether it was a change in temperature or the level of temperature *per se* that was stimulatory. In contrast to my observation of

courtship at 5°C, 8L:16D after transfer from 1.5°C and dark, Aleksiuk and Gregory (1974) did not observe courtship after transfer from 5°C and dark to 5°C, 12L12D, suggesting a temperature change, at least at very low temperatures, is important. However, this result may have been due to infrequent observation (Aleksiuk, pers. comm.).

The nature of the receptor(s) stimulated by an increase in temperature is uncertain. However, the rapidity of the onset of mating behaviour suggests a direct neural effect. Experiments designed to determine the rate of onset of sexual behaviour (Hawley, unpublished) indicated courtship occurred within as little as seven minutes after transfer from 1° to 25°C, and much of this time was spent by the male locating and approaching the female. Copulation sometimes occurred in less than fifteen minutes following the transfer. In view of the rapid mating response, it seems likely that the increase in body temperature induced mating behaviour directly via the central nervous system (possibly mediated by peripheral thermal receptors).

High temperature has a stimulatory effect on general locomotory activity of *Thamnophis* spp. (Heckrotte 1967; Lueth 1941), implying heightened

neural function. Neural activity of several species of reptiles, as indicated by electroencephalograms (Andry, et al. 1971; Hunsaker and Lansing 1962; Luttges and Gamon 1970) and somatic sensory sensitivity (Proske 1969), also increase with increased body temperature. Undoubtedly such general stimulation contributes to emergence from hibernation and the display of sexual behaviour observed following emergence in *Thamnophis sirtalis parietalis*. However, there must be some mechanism for determining that sexual behaviour specifically, and not some other goal-oriented behaviour (e.g., feeding, which is depressed (Aleksiuk and Gregory 1974)) will occur in response to an increase in body temperature.

The overwhelming number of demonstrations of the association of sex steroids with seasonal sexual behaviour in both sexes in all classes of vertebrates (reviews include: Altman 1966; Aronson 1965; Bastock 1967a,b; Beach 1948; 1964; Forbes 1961; Goldstein 1957; Lehrman 1964; Pfaff 1973; Robson 1940; van Tienhoven 1968; Young 1961) suggests that these hormones are involved with the reproductive response of *Thamnophis sirtalis parietalis*. It probably would have been necessary for an increase in levels of hormones associated with

sexual behaviour to have occurred in the summer or fall before entrance into hibernation. This conclusion is based on a) the mating response is immediate after emergence and b) the depressed metabolism at low hibernation temperatures (Aleksiuk 1971) would probably have prevented the production of hormone(s) during the winter.

Rahn (1940) was of the opinion that the endocrine basis of the female mating urge in *Thamnophis sirtalis sirtalis* was not associated with follicular growth. The correlation between female sexual receptivity, female size, and the presence of developing follicles (ova to be ovulated the same year) in the spring in *Thamnophis sirtalis* as indicated by Fox (1954), Gregory (1974) and the present study (Appendix 1) suggests the opposite. Whether the endocrine contribution is gonadotropic, gonadal, or of some other hormone type is unknown.

It is unlikely that spring mating behaviour in males is induced directly by gonadotropin(s), since it appears that these hormones are involved directly with spermatogenesis but only indirectly with spring sexual behaviour. Cieslak's (1945) histological examination of the pituitary of male *Thamnophis radix* indicated

that basophilic cell number and activity correlated with the onset of spermatogenesis. Maximum basophilic activity occurred after the spring mating period. An indirect influence of gonadotropin(s) on sexual behaviour would be mediated by gonadal steroids.

The amount and extent of male sexual behaviour is, within limits, often correlated with the level of gonadal steroids (Altman 1966; Bastock 1967a, b; Lehrman 1964; Young 1961). However, radioimmunoassay of serum testosterone (Hawley, Aleksiuk and Whitehead, in prep.) indicated that circulating testerone levels were very low in field-collected male *Thamnophis sirtalis parietalis* during the spring mating period, relative to other times of the year. Levels are highest in the fall just prior to entrance into hibernation and apparently drop during the winter. Evidently, elevated testosterone levels were not necessary in the spring for the courtship response to occur. This hormone might nevertheless be involved by inducing an adjustment or change in the central nervous system during the period of elevated circulating levels in the summer and fall. According to this hypothesis this change is maintained until the following spring, influencing behavioural patterns at that time.

"Sex centres" have been localized in the brain (i.e., thalamus, hypothalamus) of many animals which a) apparently govern sexual behaviour b) preferentially concentrate sex steroids and c) are active to greater or lesser degrees depending on the presence or absence of sex steroids (reviews include Bastock 1967a, b; Beach 1964; Hinde 1970; Pfaff 1973; van Tienhoven 1968; Vaughn and Fisher 1962; Young 1961). Presumably, it is not the amount of sex hormone per se that influences behavioural patterns, but the degree of the influence the hormone has on the neural target sites. The classical concept has been that sex hormones alter thresholds for the release of mating behaviour reflexes, either by exciting neural circuits and centres responsible for the behaviour or releasing inhibition of mating reflexes (Bastock 1967a, b; Beach 1964; Pfaff 1973; Vaughn and Fisher 1962; Young 1961). An increase in neural activity caused by an increase in temperature could preferentially stimulate sexual behaviour in the spring as a result of hormonal sensitization of sexual centres in the fall. Increasing temperature would then result in an observed increase in sexual behaviour. Alternatively, sexual behaviour could be elicited by sexual stimuli such as the scent of the female or

palping by the male. An increase in body temperature could increase the sensitivity of neural centres to these stimuli, resulting in increased sexual motivation and activity once appropriate stimuli are present. Because males which have not been exposed to a female since emergence sometimes court each other and even themselves (Hawley, unpublished), it is unlikely this second alternative is the sole mechanism in this sex. However, both mechanisms may be operating.

If there is a hormonal influence, the snakes are refractory to it in the fall when testosterone levels are very high. Relative to the spring, courtships in the fall are rare and fall copulations occur only very rarely. These infrequent fall courtships and copulations might be a result of malfunction of the refractory mechanism (loss of inhibition?). The loss of refractoriness appears to be environmentally controlled. Cold-conditioning during the winter is required to elicit courtship after exposure to 25°C in the spring (Hawley and Aleksiuk, in prep.).

Adaptive Significance of the Spring Mating Response

Populations of *Thamnophis sirtalis parietalis* are dispersed widely during the summer and aggregate in

large numbers in the fall just prior to hibernation (Gregory 1971; 1974). Large spring mating aggregations of snakes are associated with cool-temperate climates (Noble 1937) and are particularly well developed in *Thamnophis sirtalis parietalis*. The spring mating response of this species ensures that copulation occurs during a portion of the year when the entire population is organized into large aggregations, and thus allows the successful copulation of females with a minimum amount of time and energy spent in pair formation. It also allows fertilization of eggs immediately following ovulation in June (Gregory 1971; 1974) and thus permits the earliest possible commencement of embryonic development. These are important advantages in a region where the period available for reproduction, feeding and growth is restricted to about four months of the year.

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Appendix 1

There was a minimum size requirement of the female for copulation. Fig. 1 shows the female size-class frequencies (1A), the mean frequency of courtships per female in each size class (1B) and the percentage of females per size class that were mated (1C) for the mating trials performed at 25° and 30°C. To calculate the mean frequency of courtships per female for each size class (Fig. 1B) the number of times each female was observed being courted per day was totalled over the 5 days of each mating trial, and averaged for the females within each size class. Only trials at 25° and 30°C were considered for this analysis to ensure full male and female sexual activity. Only females with a snout-vent (S.V.) length exceeding 45 cm were mated (Fig. 1C), despite the fact that at least ten of the twelve females less than 45 cm in S.V. length were courted. One of the females mated at 20°C was 37.9 cm in S.V. length. This was the only exception to the 45 cm minimum female size requirement. It is uncertain whether this particular female subsequently gave birth to young, but most, and perhaps all of the mated females gave birth during the summer of 1973.

These data indicate that there was a female minimum length requirement for copulation of around 45 cm. However, examination of the correlations among follicular development, body length and sexual receptivity (see below) indicate that female sexual receptivity was not dependent on the size of the female per se, but rather on the reproductive state of the female. Gregory (1974) and Fox (1954) found correlations between S.V. length and the presence of large (length greater than 3 to 4 mm in the former study) developing follicles in the ovaries of female *Thamnophis sirtalis parietalis* and *T. s. tetrataenia* respectively. Ova from follicles of this size in the spring were presumably ovulated the same summer. Therefore, Gregory (1974), viewed these females as "potentially reproductive". In his study, no females with a S.V. length less than 45 cm had developing follicles. As S.V. length increased to 65 cm, the percentage of females that were potentially reproductive also increased. All females with S.V. length greater than 65 cm examined were potentially reproductive. This pattern of increase in the percentage of potentially reproductive females with increase in size is the same as that followed by the percentage of females mated at 25° and 30°C (Fig. 1C). Furthermore, all mated

females that Gregory examined were 47 cm or greater in snout-vent length and all had large developing follicles. Females that exceeded 45 cm in S.V. length but did not have developing follicles were not mated. These data indicate that female receptivity was directly dependent on reproductive state rather than size (age or maturity).

Noble (1937) suggested the qualities of female *Thamnophis sirtalis sirtalis* stimulatory to a male were dependent on an "estrous" condition. This implies a correlation between sexual attractiveness and reproductive condition. This was apparently not the case in the present study. Whether or not a female was ever courted did not depend on her sexual receptivity. All but one of the unmated females with S.V. lengths between 45 and 65 cm, and all but two with S.V. lengths less than this, were courted. Furthermore, the increase in the frequency with which a female was courted with increase in size (Fig. 1B) cannot be attributed to the sexual state of the female since a) there was more courtship directed toward females in the 55 to 60 cm size class than the 50 to 55 cm class (Fig. 1B), yet more females in the former class were sexually receptive and presumably in a reproductive state (Fig. 1C), b) in each size class

from 45 to 65 cm the mean number of courtships per unmated female was not significantly different (students t-test, $P > 0.05$) from the mean number of courtships per mated female (Table 1), c) females less than 45 cm in snout-vent length were courted, and d) the linearity of the increase in the mean number of courtships per female from the smallest size class to the largest suggests that the amount of courtship was correlated with the size *per se* rather than with sexual condition. This correlation seems likely since larger females would probably be more noticeable to the males than smaller females. Visual and possibly olfactory stimuli would increase with increasing size, and both of these are important in eliciting courtship (Noble 1937; and indirect evidence from Finneran 1949; Gardner 1955; 1957; Wright and Wright 1957). Thus, although sexual receptivity of females appeared to be dependent on their reproductive state, their attractiveness to males was independent of sexual condition.

Figure 1. Size-frequency histograms of the total number of females used in the mating trials (A), the mean number of courtships per female in each size category over 5 days (B), and the percentage of females mated in each size category (C). Only females from the mating trials at 25° and 30°C were used in this analysis.

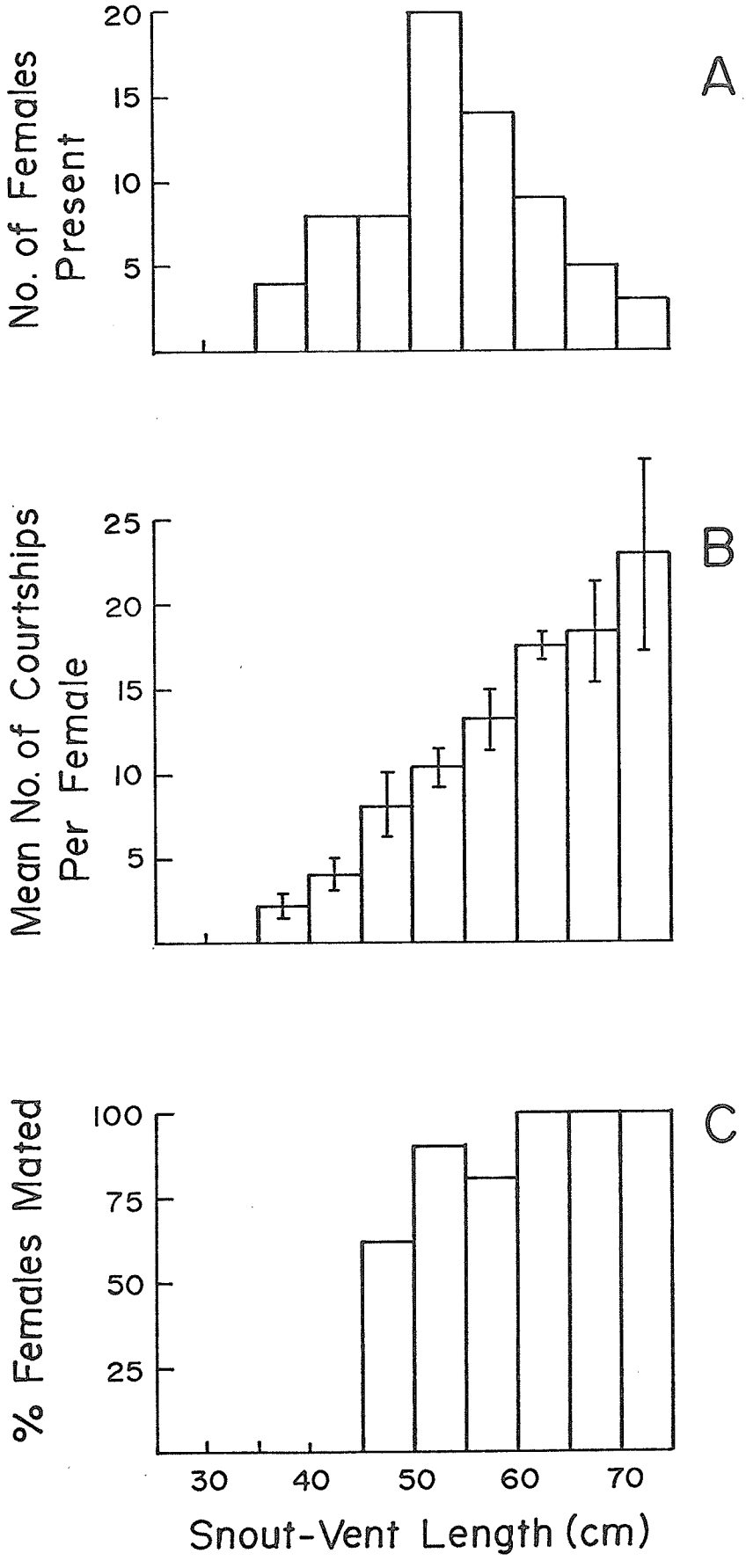
No. of Females Present
20
15
10
5
A
Mean No. of Courtships Per Female
25
20
15
10
5
B
% Females Mated
100
75
50
25
C
30
40
50
60
70
Snout-Vent Length (cm)

Table 1. Mean number of courtships per mated and unmated female over five days ± one standard error about the mean. Numbers in parentheses indicate the sample size. Differences between mated and unmated females in each size class were not significant (student t-test, $P > 0.05$).

	Size Class (cm)		
	45-50	50-55	55-60
unmated females	5.3 ± 3.2 (n = 3)	9.0 ± 1.0 (n = 2)	10.3 ± 3.0 (n = 3)
mated females	9.8 ± 2.6 (n = 5)	10.5 ± 1.2 (n = 18)	14.0 ± 2.1 (n = 12)