Role of light and temperature in the regulation of reproduction in the red-sided garter snake, *Thamnophis sirtalis parietalis*

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The influence of photoperiodic manipulation on sexual behavior and ovarian recrudescence of male and female red-sided garter snakes (*Thamnophis sirtalis parietalis*) was examined over a 4-year period. Snakes were exposed to photoperiodic manipulations before, during, and after a 17-week cold temperature dormancy; sexual behavior of males and females and ovarian recrudescence were observed after emergence from cold temperature dormancy. In the 1st year (1982), males were exposed to two conditions representing minimum and maximum exposure to daylight: (*i*) 6 weeks of a short-day prehibernation period (10L:14D, 28:18°C), followed by 17 weeks of hibernation in complete darkness (0L:24D, 4°C) and emergence into warm dark conditions (0L:24D, 28:18°C); and (*ii*) 6 weeks of a long-day prehibernation period (14L:10D, 28:18°C), followed by 17 weeks of hibernation with exposure to light (12L:12D, 4°C) and emergence into warm, long days (14L:10D, 28:18°C). Males in both conditions exhibited intense courtship behavior on emergence from hibernation. Females in 1982 were significantly influenced only by long-day prehibernation conditions (14L:10D, 28:18°C); under these conditions, ovarian recrudescence on emergence was inhibited. Long prehibernation photoperiod did not significantly influence female receptive behavior on emergence, indicating that neuroendocrine control of ovarian activation and sexual behavior may be separate in this species. In three subsequent years (1983, 1984, 1985) none of the photoperiodic conditions significantly influenced male or female sexual behavior or ovarian recrudescence. Slight differences in experimental protocol in these subsequent years that may account for differences in results from 1982 are discussed. Finally, ovarian development was found to be clearly tied to the duration of cold temperature dormancy in this species. Females receiving 7 or 17 weeks of exposure to cold (4°C) underwent vitellogenesis at similar frequencies. Most females receiving 0 or only 4 weeks of exposure to cold (4°C) did not become vitellogenic. Mating on emergence was not a requirement for the initiation of vitellogenesis in this study.

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Les effets de manipulations de la photopériode sur le comportement sexuel et le développement ovarien ont été examinés chez des mâles et des femelles de la Couleuvre rayée *Thamnophis sirtalis parietalis* au cours d'une période de 4 ans. Les couleuvres ont été soumises à des manipulations de photopériode avant, pendant et après une période de dormance de 17 semaines au froid; le comportement sexuel des mâles et des femelles et le développement ovarien ont été suivis après cette période de dormance. Durant la 1ère année (1982), les mâles ont été exposés à deux types de conditions, photopériode minimale et photopériode maximale : (*i*) 6 semaines de pré-hibernation à photopériode courte (10L : 14O, 28°C : 18°C) suivies de 17 semaines d'hibernation à obscurite totale (0L : 24O, 4°C) et sortie d'hibernation dans des conditions d'obscurité à une température chaude (0L : 24O, 28°C : 18°C); (*ii*) 6 semaines de pré-hibernation à photopériode longue (14L : 10O, 28°C : 18°C), suivies de 17 semaines d'hibernation avec exposition à la lumière (12L : 12O, 4°C) et sortie d'hibernation dans des conditions de jours longs et chauds (14L : 10O, 28°C : 18°C). Dans les deux séries de conditions, les mâles sont sortis d'hibernation en manifestant des comportements de cour très marqués. Les femelles en 1982 ont été influencées significativement seulement par les conditions de pré-hibernation à photopériode longue (14L : 10O, 28°C : 18°C); dans ces conditions, le développement ovarien à la sortie d'hibernation a été inhibé. La longue photopériode durant la pré-hibernation n'a cependant pas influencé le comportement réceptif des femelles à la sortie d'hibernation, ce qui indique que le contrôle neuroendocrinien de l'activation ovarienne est peut-être séparé du contrôle du comportement sexuel chez cette espèce. Au cours des trois années subséquentes (1983, 1984, 1985), les variations de photopériode n'ont influencé significativement ni le comportement sexuel des mâles, ni celui des femelles, ni le développement ovarien. De légères différences dans le protocole expérimental au cours de ces années subséquentes sont peut-être responsables des différences dans les résultats de 1982. Le développement ovarien est très clairement relié à la durée de la dormance à basse température chez cette espèce. La vitesse de la vitellogenèse était la même chez les femelles exposées à 7 ou à 17 semaines de froid (4°C). La plupart des femelles exposées à 0 ou seulement 4 semaines de froid (4°C) n'ont pas subi de vitellogenèse. L'accouplement ne s'est pas avéré essentiel au déclenchement de la vitellogenèse au cours de cette étude.

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Introduction

Many studies on the environmental control of reptilian reproduction have indicated that both temperature and photoperiod may be used as proximate cues for gonadal maturation and the expression of sexual behavior, although temperature alone appears to be the more common cue (see Crews and Garrick 1980; Duvall *et al.* 1982; Licht 1984; Whittier and Crews 1987 for reviews). Generally, high temperatures serve to initiate gonadal maturation and sexual behaviors (see Licht 1984 for review). However, constant warm conditions following breeding often fail to stimulate renewal of gonadal development or sexual behavior, providing evidence for postbreeding refractory periods in a few species (Gavaud 1983; Licht 1984). Species exhibiting refractory periods require exposure to set periods of

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low temperatures, and possibly to dark conditions, to reinitiate sexual responsiveness on exposure to warm conditions.

The red-sided garter snake (*Thamnophis sirtalis parietalis*) provides a unique model for investigating the role of photoperiod and temperature in the regulation of reproduction. This species has a dissociated reproductive cycle, in which maximal gonadal development, sex steriod secretion, and sexual behavior are dissociated (Crews 1984). We know very little about how environmental cues are integrated to regulate reproduction in species that exhibit dissociated reproductive cycles. It is evident that reproduction in garter snakes is cued by shifts in photothermal conditions (Hawley and Aleksiuk 1975, 1976; Garstka *et al.* 1982; Bona-Gallo and Licht 1983). Further, only direct neural responses to photothermal shifts have been shown to influence male sexual behavior (Crews *et al.* 1984; R. Krohmer and D. Crews, unpublished data). This is in contrast to other reptiles, such as the green anole lizard (*Anolis carolinensis*), in which photothermal conditions influence sexual behavior via the neurohypothalamohypophysial gonadal axis (Crews 1980; Licht 1984). Finally, red-sided garter snakes also exhibit refractoriness to prolonged exposure to warm conditions; both males and females require a period of exposure to low temperatures to reinitiate sexual responsiveness on reexposure to warm conditions (Hawley and Aleksiuk 1975; Bona-Gallo and Licht 1983; Camazine *et al.* 1980).

Before describing the experimental procedures used to study the relative contributions of photoperiod and temperature in the regulation of reproduction of the red-sided garter snake, it is useful to detail the photothermal environment that this species encounters under natural conditions. The red-sided garter snake is the northernmost reptile in North America and is found throughout south central Manitoba, Canada (50–55° N). There has been much interest in the life history of these animals (Gregory 1977; Garstka *et al.* 1982). The annual cycle of the red-sided garter snake has been studied in detail (Aleksiuk and Gregory 1974; Hawley and Aleksiuk 1975, 1976; Gregory 1977; Garstka *et al.* 1982; Crews and Garstka 1982). Briefly, males emerge from underground hibernacula before the females in the spring. Males remain in the vicinity of the hibernacula for 3–5 weeks, courting females as they emerge. Females generally mate with a single male and then disperse to summer feeding grounds. Male courtship behavior is stimulated by vernal environmental conditions, particularly increasing temperatures on emergence. Female sexual receptivity and the initiation of ovarian development are also stimulated by conditions at emergence.

During the period of spring emergence photoperiod is approximately 17L:7D and ambient temperatures range from −3.7 to 17.3°C (Environment Canada, Broad Valley site, 1982). Although vernal day length is long at this latitude, the animals limit their activity to days of bright, sunny weather with little wind; the snakes are underground and inactive in the early morning hours and return underground by late afternoon. Under conditions of cloud cover and wind, garter snakes tend to remain underground. In some years, ambient weather conditions are consistently cool, cloudy, or windy and the activity of the animals, and hence exposure to daylight, is restricted to as little as 1 week. Thus, although day length may be a predictable cue in this northern latitude environment, the effective exposure of the garter snakes to this cue during the period of emergence is very irregular and may be as brief as 8L:16D. Thus, while temperature of the air may vary widely during this time, ground temperature exhibits a gradual warming and may be more predictable than day length (Hawley and Aleksiuk 1975).

Dispersal of the snakes from the hibernacula occurs in late May and early June, well before summer solstice. We presume that during migration and on arrival at summer feeding grounds garter snakes are exposed to ambient light and temperatures. Beginning in late August and extending through September and October, during the time approaching and just after fall equinox, the snakes begin to return to the hibernacula from summer feeding grounds. At this time photoperiod is rapidly changing from 13L:11D to 11L:13D. Ground temperature is higher than air temperature and the snakes' body temperatures are higher than air temperature (Environment Canada; J. Whittier and R. Mason, unpublished data). Migration to the hibernacula may be stimulated by decreasing temperatures at night. On arrival at the hibernacula in the fall the snakes aggregate and eventually move underground and do not reemerge on a daily basis. During hibernation the animals are exposed to complete darkness and low (estimated 3–6°C) constant ground temperature for approximately 7 months.

In this paper we are interested in determining the relative contributions of photoperiod and temperature to the regulation of sexual behavior and ovarian recrudescence in the red-sided garter snake. Before this study was initiated, we knew that male courtship behavior, female receptivity, and ovarian development could be stimulated in the laboratory by exposing animals to 17 weeks of 4°C in total darkness (Camazine *et al.* 1980; Bona-Gallo and Licht 1983). This dormancy period could be abbreviated to not less than 8 weeks for the stimulation of male courtship behavior (Garstka *et al.* 1982). Comparable information on the duration of dormancy needed to stimulate female receptivity or ovarian development was not available. The studies reported here were designed to examine the influence of photoperiodic manipulations before, during, and after hibernation on male courtship behavior, female sexual receptivity, and ovarian development.

Materials and methods

Animals

Sexually mature (see Gregory 1977) male (SVL (snout–vent length) 38.0–56.0 cm) and female (SVL 55.0–70.0 cm) red-sided garter snakes (*T. s. parietalis*) were obtained from two sources. In the fall of 1982 animals were purchased from Lemberger Associates (Oshkosh, Wisconsin). In 1983, 1984, and 1985 snakes were collected in the field near Narcisse Community Pasture, Manitoba, Canada, in September and October. All animals were transported by air to Texas. Each replicate of this study spans from the fall of one calendar year (e.g., 1982) to the spring of the next year (e.g., 1983). For the sake of simplicity we refer to each replicate by the year of initation.

Maintenance

Before and after emergence from winter dormancy, animals were offered chopped fish (mackerel and smelt) fortified with vitamins (Petco, Inc.) twice weekly. Animals were housed according to treatment group in divided wire mesh cages (31 × 31 × 47 cm). Water was available for soaking and drinking.

Behavioral testing

Behavioral tests were conducted as described in Camazine *et al.* (1980). In tests for male courtship, males were presented with two newly emerged stimulus females in succession. Male courtship was scored on a scale from 0 to 3 with 0 representing no courtship and 3, mating. The courtship score of individual males was noted at the end of 2 min. Males were scored as courting on the test day if they received a courtship score of 1.5 or higher with either of the two stimulus females. All males were repeatedly tested to monitor levels of behavior over time. Repeated testing of males does not inhibit the expression of courtship behavior (D. Crews, unpublished observations). All males were tested at the same time of day (09:00–11:00 a.m.). In tests for

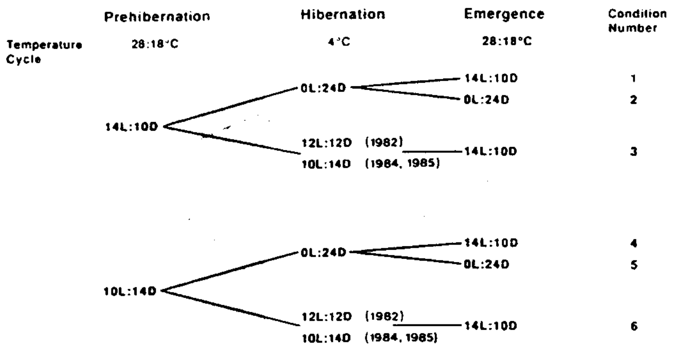

FIG. 1. Experimental design of treatment conditions. At each branch males were divided equally between conditions. Females were selected randomly and assigned to conditions. In 1982, animals exposed to light during hibernation received 12 h of light each day. In 1984 and 1985, animals exposed to light during hibernation received 10 h of light each day. In 1983 all animals were hibernated in total darkness only.

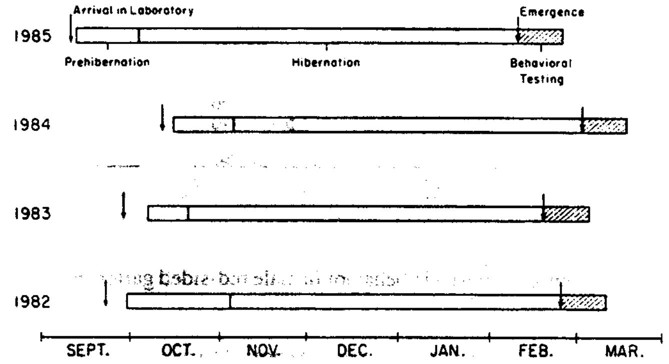

FIG. 2. Time period of three treatments (prehibernation, hibernation, and emergence) during a 4-year study on the influence of photoperiod on reproductive function of red-sided garter snakes, *Thamnophis sirtalis parietalis*.

female receptivity, females were placed in cages containing five sexually active males for 2 h. Intromission was used as a confirmation of receptivity as it is the only reliable measure indicative of receptivity in this species. Ovarian recrudescence was determined by laparotomy and (or) palpation at 3 and 6 weeks postemergence. Both of these measures of ovarian recrudescence were used to determine a single measure of presence or absence of vitellogenesis during the experiment. Females tested for receptivity were monitored for ovarian condition; additional females were monitored for ovarian condition only. There was no difference in the incidence of vitellogenesis among these two groups of females and data are combined for respective treatment groups. This procedure was conducted to give a better estimate of ovarian responses, which tend to vary more than behavioral responses.

Influence of photoperiod before, during, and after hibernation on male courtship and on female receptivity and ovarian development

On arrival in the laboratory snakes were placed for 6 weeks (1982) or 3 weeks (1983, 1984, 1985) in one of two prehibernation conditions (either 14L:10D or 10L:14D, corresponding daily thermal cycle of 28:18°C, and constant relative humidity of 60% (see Figs. 1 and 2). Females were not divided equally into treatment groups because some females were used for other nonconflicting experiments (not presented here). Thus, sample sizes in some female groups were larger (see Tables 1–4). A minimum of 15 animals were used for each treatment (see Fig. 1). In 1982, 116 males and 75 females were placed under treatments 1, 2, 3, 4, 5, and 6; in 1983, 38 males and 60 females were placed under treatments 1, 3, 4, and 6; in 1984, 76 males and 52 females were placed under treatments 1, 3, 4, and 6; and in 1985, 60 males were placed under treatments 1, 3, 4, and 6 while 30 females were placed under treatments 1 and 4 because a limited number of animals were available (Fig. 1). A group of animals was subjected to treatment 4 in every year of the study. The treatment group 4 (10L:14D prehibernation, 0L:24D hibernation, 14L:10D emergence) most closely approximates natural conditions. This treatment has become the standard treatment used in our laboratory to stimulate sexual behavior in males. In 1982, snakes were housed under one of two treatment conditions during dormancy: hibernation in total darkness or hibernation in a photoperiodic environment (12L:12D) (see Fig. 1). (Under these hibernation conditions, mortality averaged 16.0 ± 1.5%; no particular treatment induced greater mortality than any other.) In 1983, snakes hibernated in total darkness only. In 1984, males and females hibernated in total darkness or in a photoperiodic environment (10L:14D). In 1985, males hibernated in total darkness or a photoperiodic environment (10L:14D); because of the small number of females available that year, females were placed in hibernation only in total darkness. (In these subsequent years (1983, 1984, 1985) mortality during hibernation was uniformly low and less than 2%.) No males were maintained under constant warm conditions (rather than a 4°C dormancy period) as these conditions result in high rates of mortality. Animals subjected to constant darkness were housed in burlap bags in light-tight cardboard boxes; snakes subjected to a daily photoperiod were housed in clear plastic boxes. High humidity in the boxes and chambers was maintained by placing moistened sponges in the bags. In all years the period of enforced dormancy was 17 weeks.

At the end of 17 weeks of hibernation in 1982, male snakes were placed in one of two conditions: (*i*) exposure to 28:18°C and 14L:10D with testing for sexual behavior beginning immediately and continuing for 15 days (treatments 1, 3, 4, and 6; Fig. 1), or (*ii*) exposure to 28:18°C and 0L:24D with testing for sexual behavior beginning immediately and continuing for 10 days (treatments 2 and 5; Fig. 1). Limitations of laboratory facilities did not permit further testing of the latter animals. Females that were placed under 28:18°C and 14L:10D conditions on their emergence were tested for receptivity and then monitored through pregnancy. In 1983, 1984, and 1985, all male and female garter snakes were placed under 14L:10D and 28:18°C conditions after 17 weeks of hibernation (treatments 1, 3, 4, and 6; Fig. 1). Male courtship was monitored for 15–22 days postemergence. Monitoring of behavior was extended in these years because males continued to court females after 15 days. Receptivity of females (except in 1985) was tested 1 and 2 days postemergence. Ovarian development of females was monitored 3–6 weeks postemergence. Arrival time of animals in the laboratory varied slightly from year to year causing the start and end dates of the experimental protocols to vary slightly from year to year (Fig. 2).

Statistical methods

Significant differences among frequencies of courtship by males were determined on day 5 using contingency table and χ^2 analyses. When cell frequencies were too small for χ^2 analyses, Fisher's exact probability estimates were used. Receptivity and ovarian responses of females were tested in the same manner. Cells of similar tendencies were pooled to examine overall effect of prehibernation and hibernation on measures of female behavior and ovarian response. A difference in treatment was considered significant if $P < 0.05$. Although the study was conducted over several years and ideally we would have liked to test for between-year differences, we have not tested for such differences. We feel that there were sufficient differences in between-year experimental conditions to preclude the validity of such comparisons.

Influence of duration of hibernation on ovarian development of red-sided garter snakes on emergence

A second experiment in 1984 examined the influence of duration of hibernation at 4°C and in complete darkness on the incidence of ovarian recrudescence among females. On arrival in the laboratory, females were placed on a 3-week prehibernation (28:18°C, 10L:14D) schedule. Females were then randomly selected and assigned to one of four treat-

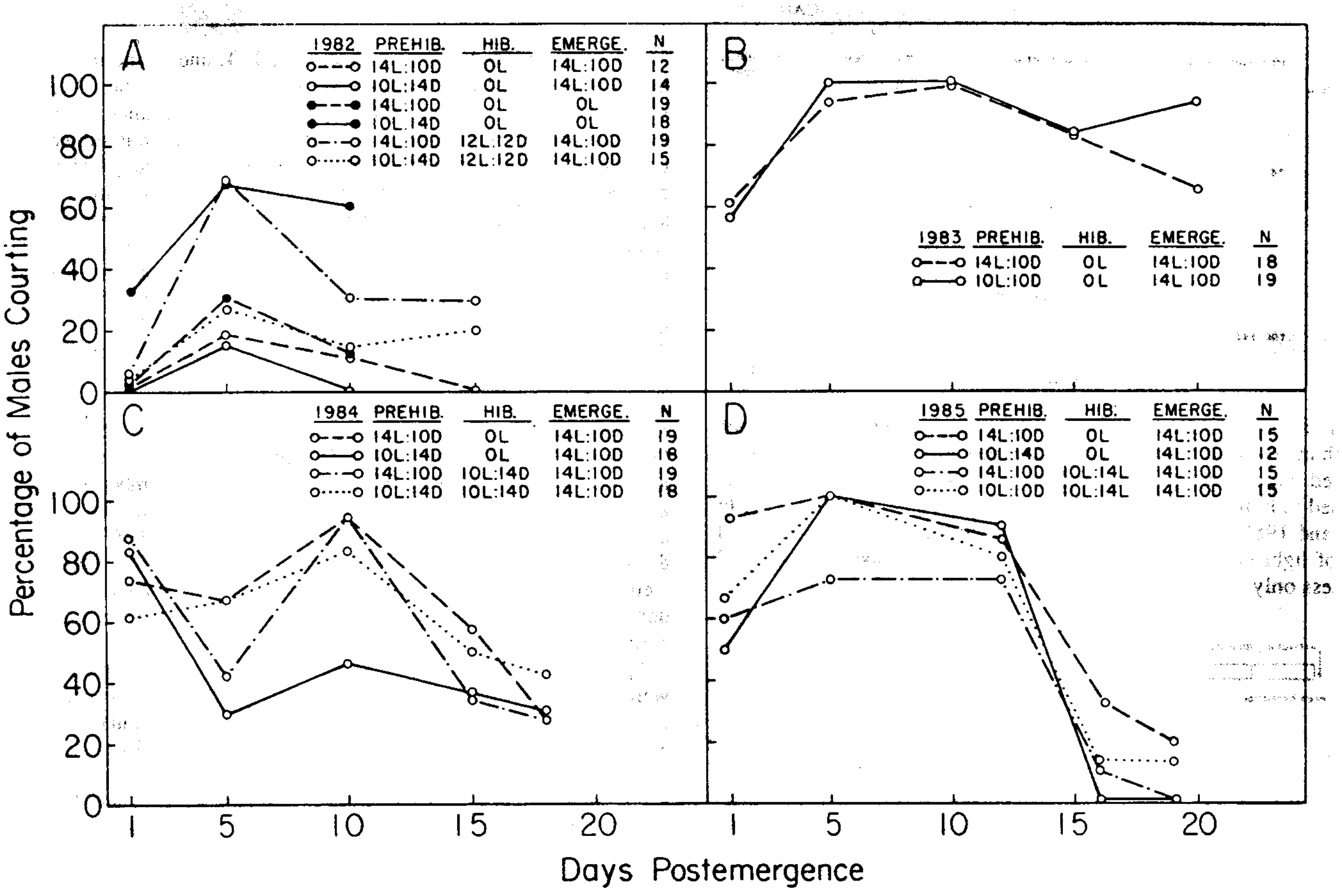

FIG. 3. Influence of prehibernation, hibernation, and emergence photoperiod on incidence of courtship behavior in male red-sided garter snakes, *Thamnophis sirtalis parietalis*.

ment groups. One group was kept at 28:18°C and 14L:10D for the entire treatment period (0 weeks in hibernation; $N = 34$). The second, third, and fourth groups experienced similar hibernation conditions (4°C, 0L:24D) but the length of hibernation varied (4 weeks, $N = 14$, 7 weeks, $N = 32$, or 17 weeks, $N = 73$). Sample sizes for each treatment differed because some animals were used for subsequent studies after the conclusion of this experiment (not presented here). We include results from all treatments rather than limiting the N values to the smallest sample size. Dates of entry into hibernation were staggered such that all females emerged from hibernation during the same week in early spring. On emergence from hibernation the females were kept at 28:18°C and 14L:10D. The females were not exposed to males nor were they allowed to mate on emergence. Ovarian development was monitored for 3–6 weeks after emergence as outlined previously.

Results

Effects of photoperiod before, during, and after hibernation

Male courtship behavior

No consistent effect of photoperiod during prehibernation, hibernation, or emergence was seen on courtship behavior of males over the 4-year study. Nevertheless, some of the effects of photoperiod on courtship during individual years are worth noting. In 1982 (Fig. 3A) courtship observed in two treatment groups was significantly elevated over that of other groups (groups 3 and 5; $\chi^2 = 17.09$, $P < 0.001$). One group consisted of males that underwent a long-day prehibernation (14L:10D) followed by exposure to light during hibernation (12L:12D) and long days (14L:10D) on emergence (group 3). These males were exposed to the maximum amount of light in the study. The second group of males that exhibited significantly elevated incidence of courtship in 1982 were exposed to a short prehibernation photoperiod (10L:14D) followed by exposure to complete darkness during hibernation (0L:24D) and emergence (group 5; tested under red light illumination, Fig. 3A). These males were exposed to the minimum amount of light during the study. These latter males required only a shift in temperature, and not of light, to initiate courtship on emergence. All other groups of males, including males exposed to 10L:14D prehibernation, 0L:24D hibernation, and emergence on 14L:10D (group 4) exhibited low levels of courtship on emergence in 1982. However, it should be noted that these other groups (and in particular, group 4) had relatively little courtship compared with comparable treatments in all other years.

In subsequent years, subsets of the treatments used in 1982 were repeated. In 1983 (Fig. 3B) only prehibernation photoperiod was varied. Most males exposed to either short or long days during the prehibernation period courted on emergence ($\chi^2 = 1.60$, $P > 0.05$). The high levels (greater than 60%; Fig. 3B) of courtship exhibited by these males in 1983 contrast with the low levels (less than 30%; Fig. 3A) of courtship observed among males placed under similar conditions in 1982.

In 1984 (Fig. 3C) both prehibernation photoperiod and hibernation photoperiod were varied. Significant differences in courtship of males were seen among the four treatment groups ($\chi^2 = 8.02$, $P = 0.05$). However, courtship was more variable over time among these groups, with a depression on day 5. Frequency of males courting females in 1984 was intermediate between that of males in 1982 and 1983.

In 1985 (Fig. 3D), again both prehibernation and hibernation photoperiod were varied. No significant differences in courtship

TABLE 1. Effect of prehibernation photoperiod on receptivity of female red-sided garter snakes, *Thamnophis sirtalis parietalis*

	Prehibernation condition	*N*	% receptive	*P*
1982	14L:10D	8	25	0.37
	10L:14D	17	41	
1983	14L:10D	12	58	>0.50
	10L:14D	29	62	
1984	14L:10D	16	25	>0.50
	10L:14D	35	37	

TABLE 2. Effect of prehibernation photoperiod on ovarian recrudescence of female red-sided garter snakes, *Thamnophis sirtalis parietalis*

	Prehibernation condition	*N*	% vitellogenic	*P*
1982	14L:10D	11	0	0.001
	10L:14D	23	61	
1983	14L:10D	20	50	>0.10
	10L:14D	37	73	
1984	14L:10D	16	38	>0.50
	10L:14D	33	37	
1985	14L:10D	15	7	>0.10
	10L:14D	16	19	

TABLE 3. Effect of hibernation photoperiod on receptivity of female red-sided garter snakes, *Thamnophis sirtalis parietalis*

	Hibernation condition	*N*	% receptive	*P*
1982	12L:12D	15	37	>0.50
	0L:24D	15	37	
1984	10L:14D	17	18	>0.10
	0L:24D	34	41	

among the four treatment groups were found. High levels of courtship were sustained for the first 12 days of the postemergence period and declined thereafter. The two treatment groups that were kept in complete darkness during hibernation in 1985 (Fig. 3D) showed courtship patterns nearly identical with males in these same treatment groups in 1983 (Fig. 3B).

These results suggest that males will court females on emergence from a low-temperature hibernation regardless of photoperiodic cues tested in this study. This includes light cycles received 3–6 weeks before, during, or after hibernation. One of the most interesting results was that males exhibited high levels of courtship in complete darkness if transferred to warm temperatures. This suggests that males rely primarily on temperature cues to synchronize courtship on emergence from hibernation.

Female receptivity and ovarian development

Prehibernation photoperiod—There was no significant effect of prehibernation photoperiod (pooled across hibernation treatments) on the receptivity of female garter snakes on emergence (Table 1). Prehibernation photoperiod (pooled across hibernation treatments) did significantly affect ovarian recrudescence of females on emergence in 1982 (Table 2). In that year, no females that had been maintained on an unseasonably long prehibernation photoperiod (14L:10D) underwent ovarian recrudescence on emergence. However, this effect was not observed in three subsequent replicates of the experiment (1983, 1984, 1985; Table 2).

TABLE 4. Effect of hibernation photoperiod on ovarian recrudescence of female red-sided garter snakes, *Thamnophis sirtalis parietalis*

	Hibernation condition	*N*	% vitellogenic	*P*
1982	12L:12D	19	26	>0.50
	0L:24D	55	35	
1984	10L:14D	17	35	>0.50
	0L:24D	25	32	

Hibernation photoperiod—The influence of exposure to light during hibernation of females maintained on 10L:14D prehibernation was examined in 1982. Exposure to light during hibernation did not significantly affect the receptivity of females or the incidence of ovarian recrudescence on emergence (Tables 3 and 4). In 1984, the influence of hibernation on female receptivity was examined, pooling the similar frequencies of females exposed to the two prehibernation conditions. Again no significant differences were detected (Tables 3 and 4).

These results indicate that, like males, female red-sided garter snakes are not influenced by exposure to unseasonable photoperiod before or during hibernation. It suggests that the only cue necessary for the synchronization of reproductive behavior and ovarian development is an increase in temperature after sustained low temperature dormancy.

Influence of duration of hibernation on ovarian development of red-sided garter snakes on emergence.

Of 153 females examined, a large majority of those exposed to at least 7 weeks of low temperature underwent ovarian development (Fig. 4). Only 3 of 48 females exposed to shorter durations of low temperatures became vitellogenic (0 and 4 weeks). Females that were maintained at least 7 weeks in hibernation exhibited levels of ovarian development similar to those of females maintained for 17 weeks in hibernation. This minimum duration of low temperature dormancy required to activate ovarian growth is similar to the requirement of 8 weeks of low temperature to activate courtship behavior in males (Camazine *et al.* 1980).

Discussion

These studies demonstrate that both male and female red-sided garter snakes rely primarily on temperature cues to synchronize their annual reproductive cycle. This result experimentally confirms the observations of Aleksiuk and his associates at the University of Manitoba (Hawley and Aleksiuk 1975, 1976). Photoperiodic manipulation before, during, or after low temperature hibernation had no consistent effect on male courtship, female receptivity, or ovarian recrudescence.

Although photoperiodic manipulations had no consistent effects on reproductive function in male or female garter snakes, both males and females were significantly influenced by

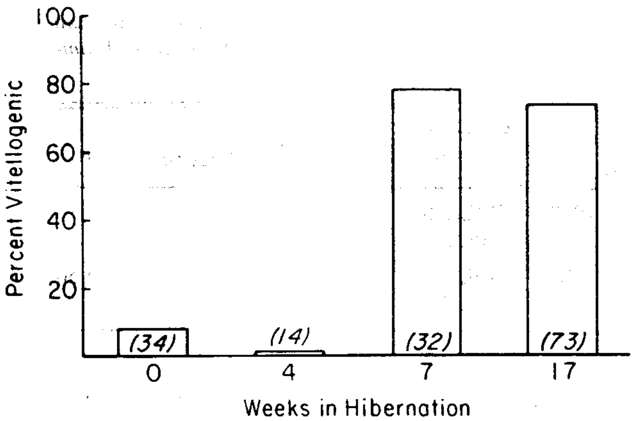

FIG. 4. Influence of duration of hibernation on the incidence of ovarian recrudescence in female red-sided garter snakes, *Thamnophis sirtalis parietalis*. Numbers in parentheses indicate sample size.

photoperiod in 1982. In the case of male courtship behavior, two photoperiodic treatments, one that exposed males to the maximum amount of light and one that exposed males to the minimum amount of light, significantly elevated levels of courtship over those of other treatments. However, in 1982 the level of courtship of males placed on a short-day prehibernation, dark hibernation, and long-day emergence schedule (group 4) was depressed relative to all other years before (Camazine *et al.* 1980; Bona-Gallo and Licht 1983) and after 1982 (this study). The depression of courtship among these males makes interpretation of the result in 1982 problematical.

In females, ovarian development in 1982 was also significantly influenced by photoperiod. In this case, all females exposed to an unseasonably long prehibernation photoperiod in the autumn of 1982 did not develop large vitellogenic follicles on emergence in 1982. However, this effect of prehibernation photoperiod had no effect on the female's receptivity. Thus, as has been noted in other studies, receptive females, i.e., females that mated, were no more likely to develop follicles on emergence than females that did not mate in spring (Whittier and Crews 1986). Females that do not mate in the spring most probably rely on stored sperm from previous matings to activate the ovary or fertilize ova (see Whittier and Crews 1986). These findings contrast sharply with those of an earlier study on the same species in which vitellogenesis appeared to be highly dependent on copulation following emergence from hibernation (Bona-Gallo and Licht 1983). Recent results (Whittier and Crews 1986) suggest that vitellogenic recrudescence is dependent on the presence of oviducal sperm; thus, the discrepancies among studies may reflect the extent of mating that occurred in the preceding fall. In this study we assume that females that became vitellogenic (and later delivered offspring) but had not mated on emergence had mated in the previous year.

As with male courtship behavior we are not certain why photoperiodic manipulation significantly influenced ovarian development in 1982 but not in subsequent years of the study. The study in 1982 did differ from subsequent studies in three respects: (*i*) animals in 1982 were obtained from animal suppliers whereas in all subsequent years, animals were collected; (*ii*) the hibernation photoperiod in 1982 was 12L:12D whereas in subsequent years, the photoperiod was shortened to 10L:14D; and (*iii*) the prehibernation period was 6 weeks long in 1982, rather than 3 weeks in subsequent years. At this time we do not know the relative importance of these factors on sexual function at the time of emergence; in addition, the treatment history of the commercially supplied animals is unknown.

We would like to emphasize that these studies may be interpreted only with due attention to the precise levels of daylight that were manipulated. Longer days could influence reproductive function on emergence; these were not tested in the study. Furthermore, this study does not test, nor was it designed to test, the sole influence of temperature on the reproductive cycle of male and female red-sided garter snakes (see also discussion in Bona-Gallo and Licht (1983)). While others have concluded that temperature is the only cue (Aleksiuk and Gregory 1974) used by red-sided garter snakes, we feel that this conclusion is not based on solid evidence. No studies to date have eliminated the alternative hypothesis that male courtship behavior is regulated by an endogenous cycle that can be synchronized by temperature.

In this study, most female red-sided garter snakes examined required a minimum exposure of 7 weeks at 4°C in midwinter to stimulate ovarian development on emergence. In addition, exposure to 4°C for 7 weeks resulted in similar incidences of vitellogenesis (after emergence) as were observed among females exposed to 4°C for 17 weeks. Exposure to 4°C for 4 weeks (for the last 4 weeks before emergence of all females) or exposure for 17 weeks to summerlike conditions (28:18°C on a 14L:10D light cycle) with few exceptions did not stimulate vitellogenesis when females were placed or maintained under summerlike conditions. Thus we confirmed the observations of Bona-Gallo and Licht (1983) that most female red-sided garter snakes exhibit ovarian refractoriness to high temperatures.

A threshold of sensitivity to 4°C must exist between a duration of 4 and 7 weeks in females and between 0 and 8 weeks in males (Camazine *et al.* 1980). This observation of a requirement for a set duration of cold (4°C) is remarkably similar to that observed by Gavaud (1983) in *Lacerta vivipera*. In *Lacerta*, it has further been demonstrated that the degree of low temperature is linked to the duration of low temperature; together the "total amount of cold" contributes the effective cue (Gavaud 1983). This mechanism would represent an important alternative evolutionary tactic used to time reproduction from that observed and theorized to exist in primarily photoperiodic organisms (see Gwinner 1971). Our choice of the single low temperature was based on conditions expected to resemble those normally encountered in nature. Previous studies showed that maintenance at constant temperatures of 17 and 30°C over winter prevented ovarian recrudescence in garter snakes (Bona-Gallo and Licht 1983). Although other intermediate temperatures (between 4 and 17°C) might not be ecologically relevant, a more complete investigation of temperature and the duration and timing of hibernation in the red-sided garter snake may provide further insights into the mechanisms of the thermal dependency. Further, the neuroendocrine and physiological mechanisms that regulate these types of responses in this and other species need to be examined.

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