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The Effects of Photoperiod-Temperature Interactions on Testicular Regression in the Green Anole, Anolis Carolinensis

Teri Wickelhaus

Western Kentucky University

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Wickelhaus,

Teri Lynn

1982
THE EFFECTS OF PHOTOPERIOD-TEMPERATURE INTERACTIONS ON TESTICULAR REGRESSION IN THE GREEN ANOLE, ANOLIS CAROLINENSIS

A Thesis
Presented to
the Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment of
the Requirements for the Degree
Master of Science

by
Teri Lynn Wickelhaus
December, 1982
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THE EFFECTS OF PHOTOPERIOD-TEMPERATURE INTERACTIONS ON TESTICULAR REGRESSION IN THE GREEN ANOLE, ANOLIS CAROLINENSIS

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Dean of the Graduate College
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I would like to dedicate this thesis to my parents, Marvin and Joyce Wickelhaus, whose encouragement and support made this work possible.

I would also like to thank the Graduate Student Research Committee for funding this project.
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THE EFFECTS OF PHOTOPERIOD-TEMPERATURE INTERACTIONS ON TESTICULAR REGRESSION IN THE GREEN ANOLE, *ANOLIS CAROLINENSIS*

Teri Lynn Wickelhaus

Directed by: Drs. Blaine R. Ferrell, D. Hugh Puckett, and Joe E. Winstead

Department of Biology

Western Kentucky University

Interrupted-night photoperiod experiments were carried out in green anoles (*Anolis carolinensis*) in July and August 1981 in order to explore the possibility that seasonal timing of testicular regression regulated by day length involves circadian rhythms. The influence that temperature has on testicular responsiveness to light was also assessed. Testicular weight responses to different interrupted-night photoperiod treatments were similar in anoles kept at constant warm temperatures but varied in anoles kept at constant cold temperatures. Rates of decline of spermatogenesis in response to different interrupted-night photoperiod treatments were, on the other hand, similar in anoles kept at constant cold temperatures but varied in anoles kept at constant warm temperatures. Testicular regression occurred at a faster rate in anoles held in environmental chambers set at constant warm temperatures or at a thermocycle of warm-cold temperatures compared with the rate in anoles kept at constant cold temperatures or at a thermocycle of cold-warm temperatures regardless of photoperiodic conditions.

Results of this study support the concept that the mechanism
whereby day length influences the timing of testicular regression does involve circadian rhythms. The results further indicate that the temperature experienced, particularly during day light hours, can modify the influence of day length on the timing of testicular regression.
Anolis carolinensis is a temperate zone lizard species found in abundance throughout the southern coastal states from Florida to Texas between 30°N and 35°N latitude (Licht, 1967a). As in most temperate zone vertebrates, reproductive and fattening conditions change seasonally in anoles. Testicular recrudescence (i.e., development of spermatogenesis) is initiated and progresses slowly during the winter months (Licht, 1967a), a time of year when cool temperatures are experienced in nature. Spermiogenesis is completed and spermiation occurs with the advent of warmer temperatures in spring (Licht, 1967a). In the summer the testes begin to regress when day lengths decrease below 13.5 hours and are fully quiescent by early fall (Licht, 1971a). A reciprocal annual cycle of fattening is expressed in anoles. Fat stores increase in summer reaching a peak level in fall preparation for hibernation. The testes are declining in development during this time period (Dessauer, 1955a). It is apparent that seasonal physiological and behavioral events occur coincident with appropriate environmental conditions. For example, reproductive development is completed in spring, a time of year when climate and food resources are suitable for reproductive success (i.e., development of the young) (Dessauer, 1955a; Fox and Dessauer, 1957). Fat stores accumulated in the late
summer, on the other hand, reach peak levels just prior to hibernation when anoles become inactive and do not feed (Dessauer, 1955b; Fox and Dessauer, 1957). Because the expression of seasonal conditions of reproduction and fattening require anticipatory physiological changes, predictive environmental cues are likely to be involved in timing the onset of these changes. Photoperiod and temperature are two environmental factors important in timing the occurrence of these events in anoles.

Many experiments have been carried out in order to test the effects that different photoperiod-temperature combinations have on timing the occurrence of seasonal events of the annual reproductive cycle in *Anolis carolinensis* (Fox and Dessauer, 1958; Licht, 1966, 1967a, b, 1969a, 1971a, b; Noeske and Meier, 1977; Underwood, 1978; Ferrell and Meier, 1981) and in other lizard species (Bartholomew, 1953; Licht et al., 1969; Botte et al., 1978). Most of these studies have been carried out during the progressive phase (i.e., recrudescence) of the annual reproductive cycle. The mechanism whereby photoperiod regulates testicular recrudescence may be explained according to a hypothesis of photoperiodism proposed by Bunning (1936, 1960). According to this hypothesis, light acts as an entrainer of a circadian rhythm of testicular photosensitivity. If day length is such that light occurs coincident with the light sensitive phase (i.e., photoinducible phase) of this photosensitivity rhythm then
some event, such as testicular growth, is induced. If light occurs coincident with a photoinhibitory phase of this photosensitivity rhythm then some event (i.e., testicular growth) is inhibited. Once this photosensitivity rhythm is entrained by a period of light, it continues to be expressed on a circadian basis for several days without further entrainment. This hypothesis has been used to explain photoperiodic effects observed during studies carried out in avian species (Hamner, 1963, 1964, 1965; Farner, 1964; Gwinner and Ericksson, 1977; Turek, 1974), in photoperiodic teleosts (Baggerman, 1973), in mammals (Elliot et al., 1972) and during the recrudescent phase of testicular development in anoles (Ferrell, 1982). Temperature influence on the regulation of seasonal fattening in anoles can be explained according to a variation of this mechanism (Ferrell and Meier, 1981). Although the mechanism whereby day length and temperature affect the timing of events of the annual reproductive and fattening cycles in anoles has been explored (Licht, 1971b; Underwood, 1978; Ferrell, 1982), a critical evaluation has not been carried out during the regressive phase of the annual testicular cycle.

Understanding the mechanism whereby day length and temperature affect testicular regression is important for understanding physiological mechanisms involved in the regulation of this phase of the reproductive cycle. Because the mechanism whereby photoperiod and temperature
influence the timing of the regressive phase of the reproductive cycle remains unclear, this study was carried out in anoles entering the regressive phase of the reproductive cycle in July and August to explore the possibility that this mechanism is based on circadian rhythms. Day length is reported to have an important influence on the timing of testicular regression during this phase (Licht, 1971a). The testes regress when the day length falls below a critical threshold, 13.5 hours.
MATERIALS & METHODS

Male *Anolis carolinensis* were obtained from a commercial dealer in Louisiana on 1 and 2 July 1981. Only sexually mature males were used in these experiments (i.e., snout-vent length of 58mm or greater, Dessauer, 1955a). Experimental groups of anoles were housed individually in cardboard boxes equipped with screen tops. Food (crickets) and water (in gravel filled petri dishes) were available at all times. The cages were placed in environmental chambers which were divided into three sections using black plastic covered cardboard partitions. Light was provided by 20-watt fluorescent bulbs mounted above the cages in each section. Light from this source is considered sufficient to produce photoperiodic effects in *Anolis carolinensis* (Licht, 1969 a,b). Each light source was regulated by an automatic timer which turned the lights on and off abruptly.

Temperature in each environmental chamber was maintained at either constant 20±2°C (CC), which is the minimum temperature at which anoles are active and readily feed (Licht, 1966), or constant 30±2°C (WW), which corresponds to their mean preferred body temperature (Licht et al., 1966; Licht, 1968). In one experiment thermocycles of these temperatures were established within environmental chambers. Anoles not used immediately in the first set of
experiments were held under LD 10:14 (L, hours of light; D, hours of darkness) in an environmental chamber set at CC for use in subsequent experiments.

Anoles in one group in each experiment were killed before experimental conditions were initiated in order to determine the initial paired testis weight, stage of spermatogenesis, and weight of fat bodies. Anoles were denied food two days prior to taking weight measurements in order to allow voiding of the gut.

Each experiment covered a period of 3 weeks. At the end of each experiment all animals were killed. Both testes and paired fat bodies (i.e., discrete deposits of fat found in the coelomic cavity that represent 60% of the stored lipid in anoles, Dessauer, 1955a) were removed and weighed separately using a Mettler balance. The right testes and vas deferens were fixed in Bouin's solution, embedded in paraffin, sectioned at 10μ, stained with hematoxylin and eosin and examined under a microscope for the progressive stages of spermatogenesis described by Licht (1967a) as follows:

**Stage 1** - Seminiferous tubules are involuted with only spermatogonia present and the epididymus is atrophic and empty.

**Stage 2** - Primary spermatocytes are evident in seminiferous tubules and the epididymus is atrophic and empty.

**Stage 3** - Secondary spermatocytes are evident and early spermatids are abundant and the epididymus is atrophic and empty.

**Stage 4** - Spermatids are transforming with a few spermatozoa and the epididymus is
Stage 5 - Spermatids and spermatozoa are abundant and the epididymus is hypertrophied and empty.

Stage 6 - Spermatozoa are abundant and the epididymus is hypertrophied with many sperm.

Stage 7 - Spermatozoa are abundant but spermatids and spermatozoa are greatly reduced and the epididymus is hypertrophied with many sperm.

Experiment I was initiated on 1 July 1981. One group of lizards was kept at CC and another group at WW under LD 10:14. Each temperature treatment group was divided into three light treatment groups. Each of these light treatment groups received one additional hour of light nightly at 11, 14, or 18 hours after the onset of lights.

Experiment II was initiated on 13 July 1981. One group of lizards was kept at a daily thermocycle of CW, (i.e., 20±2°C during the photoperiod and 30±2°C during the dark period) and a second group at a daily thermocycle of WC, (i.e., 30±2°C during the photoperiod and 20±2°C during the dark period). Each of these thermocycle treatment groups was divided into three light treatment groups. These light treatment groups were exposed to the same nightly light treatments as in Experiment I.

Experiment III was initiated on 5 August 1981. One group of lizards was kept at CC and the other group at WW temperatures. Each temperature treatment group was divided into three light treatment groups. Each of these
light treatment groups was exposed to the same nightly light treatments as in Experiment I. However, one group in this experiment received light 11.8 hours instead of 11 hours after the onset of light nightly as in Experiment I.

Experiment IV was also initiated on 5 August 1981. Two groups of anoles were kept at WW temperatures and exposed to greater than the critical 13.5 hours of light reportedly necessary to maintain spermatogenesis (i.e., LD 12.8:11.2 plus an additional hour of light at either 14 or 18 hours after the onset of lights = 13.8 hours).

During all experiments locomotor activity of three lizards housed in activity cages from each experimental group was recorded using an Esterline-Angus event recorder. Each activity cage consisted of a plastic container with a metal strip at each end. A wire mesh screen covered by a piece of cardboard (to prevent shocking the anole) was used as the bottom of the cage. The wire mesh was suspended by a copper wire one half inch above the plastic floor such that only one end of the wire mesh was touching the bottom of the cage at any one time. This arrangement resulted in a seesaw effect. The copper wire was attached to the event recorder. As the anole moved from one end of the cage to the other, the wire mesh was depressed onto a metal strip thereby closing an electrical circuit. The closing of the circuit caused lizard movements to be recorded as dashes on the paper chart of the recorder. The
paper chart moved at a fixed rate, and the accumulation of
dashes produced a record of the daily activity pattern of
each anole. For analytical purposes, each hour representa-
tion on the chart was divided into six ten minute periods,
and the amount of activity in each of these periods was
analyzed and assigned a value based on a scale of activity
units, 0, 0.5, or 1.0. A value of 1.0 represented continu-
ous activity throughout the ten-minute period and a value
of 0 represented no activity during that period. The sum
of these values represented the total amount of locomotor
activity of the anoles per given hour and was expressed in
activity units ranging from 0 to 6.

Data from all experiments were analyzed statistically
by Analysis of Variance using an SPSS computer program
(Nie et al., 1975). Significant differences between
photoperiodic treatment means within a given temperature
treatment group were determined using Student-Newman-Keul's
ranking procedure. Differences among treatment means were
considered significant at the 95% confidence level (P<0.05).
RESULTS

Experiment I was performed in order to test for the presence of a daily rhythm of testicular responsiveness to light and to explore the possibility that temperature has an important influence on testicular photosensitivity during the regressive phase of the reproductive cycle in anoles. Results of this experiment are presented in Table 1 and Figure 1. The paired testes weight, stage of spermatogenesis, and nondissectable fat bodies of the initial group of anoles were found to be similar to measurements of these parameters of seasonal conditions reported in other studies carried out in anoles in July (Dessauer, 1955a; Licht, 1967a,b, 1971a,b). No daily rhythm of testicular growth responsiveness to light was apparent in anoles kept at WW, however in anoles kept at CC there was a daily rhythm in the growth responsiveness of the testes to light. The stages of spermatogenesis in response to the different light pulse treatments varied slightly between anoles held at WW and CC (Table 1). The testes in anoles of all photoperiodic treatment groups held at CC contained sperm. This finding corresponds with findings reported in previous studies carried out in anoles at other times of the year wherein cold temperatures maintained testicular development (Licht, 1967a,b). Testes from anoles held at WW, however, had seminiferous tubules
TABLE 1. CHANGE IN BODY WEIGHT, PAIRED TESTES WEIGHT, STAGE OF SPERMATOGENESIS, AND PAIRED FAT BODY WEIGHT IN RESPONSE TO DIFFERENT INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS IN ANOLIS CAROLINENSIS HELD AT CONSTANT COLD (CC) OR WARM (WW) TEMPERATURES. INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS WERE INITIATED 1 JULY 1981 AND CARRIED OUT FOR 3 WEEKS.

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a The number of anoles in each group at the end of the experiment.

b Mean ± one standard error about the mean.

c The stage of spermatogenesis found most often.

d Analysis of variance (ANOVA) among photoperiod treatment groups within each temperature treatment group.

e Ranking of treatment means according to Student-Newman-Keul's procedure. Means without a letter in common are statistically different at the 95% confidence interval.
Figure 1. Response of paired testes and fat bodies to interrupted-night photoperiod treatments in anoles kept at CC or WW. Experimental treatments were carried out daily for 3 weeks beginning 2 July 1981. Responses to interrupted-night photoperiod treatments are depicted directly above the photoperiod treatments. The hash-marked circle represents the paired testes weight of anoles prior to experimental treatment. Fat bodies were not dissectable in anoles of this group. S.E.M. = one standard error about the mean. Numbers beside the circles represent the most frequent stage of spermatogenesis observed (see Materials & Methods for details).
with and without sperm present. The WW group of anoles that received light 14 hours after the onset of the daily photoperiod had sperm present in all tubules. Fat stores increased in anoles kept at WW compared with fat stores in anoles kept at CC regardless of photoperiodic treatments (Table 1 and Figure 1). However, there was no daily rhythm in fattening responsiveness to light in anoles at either temperature. Locomotor activity patterns of anoles are presented in Figures 2 and 3. The anoles were active mainly during the photoperiod in both WW (Figure 2) and CC (Figure 3). However, the overall amount of activity of the anoles in WW was greater than the activity of anoles in CC. Locomotor activity of anoles in both temperature treatment groups was reduced during the dark period except where it became elevated coincident with the nightly-light pulse treatments, particularly in anoles kept at WW. However, such an activity increase was not observed in response to light pulse treatments occurring 14 hours after the onset of lights in anoles kept at CC (Figure 2).

Experiment II was performed concurrently with Experiment I in order to explore the possibility that temperature influences testicular responsiveness to light through its affect on either the entrainable or the inducible phases of a daily rhythm of photosensitivity. The results of this experiment are presented in Table 2 and Figure 4. No daily rhythm in testicular responsiveness to light appeared in anoles held in WC, whereas in anoles kept in CW there was
Figure 2. Daily locomotor activity patterns in anoles of Experiment I at CC under different photoperiodic treatments. Bars beneath the graph represent the light-dark cycles with the shaded area representing the dark period and the unshaded area representing the light period of each cycle. The total amount of locomotor activity of anoles per hour is expressed as activity units. See Materials and Methods for a description of activity units.
Figure 3. Daily locomotor activity patterns in anoles of Experiment I at WW under different photoperiodic treatments. See legend to Figure 2 for a description of light-dark cycles.
Time (hours after onset of light)
TABLE 2. CHANGE IN BODY WEIGHT, PAIRED TESTES WEIGHT, STAGE OF SPERMATOCENESIS, AND PAIRED FAT BODY WEIGHT IN RESPONSE TO DIFFERENT INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS IN ANOLIS CAROLINENSIS HELD AT THERMOCYCLES OF COLD-WARM (CW) OR WARM (WC) TEMPERATURES. INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS WERE INITIATED 13 JULY 1981 AND CARRIED OUT FOR 3 WEEKS.

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<th>STAGE OF SPERMATOCENESIS</th>
<th>PAIRED FAT BODY WEIGHT (% BODY WEIGHT)</th>
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\textsuperscript{a} The number of anoles in each group at the end of the experiment.

\textsuperscript{b} Mean + one standard error about the mean.

\textsuperscript{c} The stage of spermatogenesis found most often.

\textsuperscript{d} Analysis of variance (ANOVA) among photoperiod treatment groups within each temperature treatment group.

\textsuperscript{e} Ranking of treatment means according to Student-Newman-Keul's procedure. Means without a letter in common are statistically different at the 95% confidence interval.
Figure 4. Response of paired testes and fat bodies to interrupted-night photoperiod treatments in anoles kept at CW or WC. Experimental treatments were carried out daily for 3 weeks beginning 13 July 1981. See the legend to Figure 1 for a description of symbols.
a daily rhythm in the testicular responsiveness to light. The response of the testes to nightly light treatments in this experiment appeared to mimic the response of the testes to the same nightly light treatments in Experiment I. That is, the response of the testes in anoles kept at CC was similar to that in anoles kept at CW, whereas the testicular response in anoles kept at WW was similar to that in anoles kept at WC. Testicular weights were smaller in anoles in response to thermocycle treatments compared with testicular weights in anoles kept at constant temperatures except in anoles at CW that received light 18 hours after the onset of the photoperiod. The testicular weights in anoles of this group were maintained. The testes in anoles kept at CW contained sperm. However, in anoles of this treatment group stage 2 (i.e., no sperm present) was also detected. Seminiferous tubules with and without sperm were present in anoles kept at WC regardless of photoperiodic treatments. Fat stores were heavier in anoles kept at WC compared with fat stores in anoles kept at CW regardless of photoperiodic treatment. Again, there was no daily rhythm in fattening responsiveness to light. Locomotor activity patterns of anoles in this experiment are presented in Figures 5 and 6. Locomotor activity occurred mainly during the photoperiod in anoles kept at CW (Figure 5) and WC (Figure 6) except in anoles kept at WC that received an additional hour of light nightly at 11 hours after the onset of the photoperiod.
Figure 5. Daily locomotor activity patterns in anoles of Experiment II at CW under different photoperiodic treatments. See legend to Figure 2 for a description of light-dark cycles.
Figure 6. Daily locomotor activity patterns in anoles of Experiment II at WC under different photoperiodic treatments. See legend to Figure 2 for a description of light-dark cycles.
activity of anoles in this group was dramatically elevated coincident with this nightly light interruption. However, the amount of this activity is much greater in anoles kept at WC in comparison with anoles kept at CW. Anoles in both thermocycle treatment groups were also active during the dark period. However, activity became elevated coincident with the nightly-light pulse treatments in WC only.

Experiment III was essentially a repeat of Experiment I except that it was performed in August which is later in the reproductive cycle of anoles. The results of this experiment are presented in Table 3 and Figure 7. Testicular weight decreased except in response to nightly-light pulse treatments received 18 hours after the onset of lights in anoles at WW. No daily rhythm in testicular responsiveness to light was present in anoles at CC or WW. Active stages of spermatogenesis (i.e., sperm present) were evident in testes of anoles kept in CC temperatures, whereas both active and inactive stages (i.e., no sperm present) were present in anoles kept in WW. As in Experiment I, fat stores increased to a greater extent in anoles kept at WW compared with fat stores in anoles kept at CC. Again, there was no daily rhythm in fattening responsiveness to light. The daily locomotor activity patterns of anoles in this experiment are presented in Figures 8 and 9. As in Experiments I and II, anoles were mainly active during the photoperiod at WW. Anoles kept at CC were not active. Anoles kept at WW were also active during
TABLE 3. CHANGE IN BODY WEIGHT, PAIRED TESTES WEIGHT, STAGE OF SPERMATOCENESIS, AND PAIRED FAT BODY WEIGHT IN RESPONSE TO DIFFERENT INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS IN ANOLIS CAROLINENSIS HELD AT CONSTANT COLD (CC) OR WARM (WW) TEMPERATURES. INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS WERE INITIATED 5 AUGUST 1981 AND CARRIED OUT FOR 3 WEEKS.

<table>
<thead>
<tr>
<th>PHOTOPERIODIC SCHEDULE</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CHANGE IN BODY WEIGHT (g)</th>
<th>PAIRED TESTES WEIGHT (mg)</th>
<th>STAGE OF SPERMATOCENESIS</th>
<th>PAIRED FAT BODY WEIGHT (% BODY WEIGHT)</th>
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<tr>
<td></td>
<td>CC</td>
<td>WW</td>
<td>CC</td>
<td>WW</td>
<td>CC</td>
</tr>
<tr>
<td>INITIAL GROUP</td>
<td>5</td>
<td>----</td>
<td>40±4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3,4&lt;sup&gt;c&lt;/sup&gt;,5</td>
<td>0±0</td>
</tr>
<tr>
<td>LDDLD 10:1:8:1:11:2</td>
<td>7</td>
<td>6</td>
<td>-4±.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32±4</td>
<td>14±3&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDDLD 10:4:1:9</td>
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<td>7</td>
<td>.0±.1</td>
<td>40±3</td>
<td>20±7&lt;sup&gt;y&lt;/sup&gt;</td>
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<tr>
<td>LDDLD 10:8:1:5</td>
<td>6</td>
<td>7</td>
<td>-.2±.3</td>
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<td>28±4&lt;sup&gt;y&lt;/sup&gt;</td>
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<td>ANOVA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P&lt;0.05</td>
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</tr>
</tbody>
</table>

<sup>a</sup>The number of anoles in each group at the end of the experiment.

<sup>b</sup>Mean ± one standard error about the mean.

<sup>c</sup>The stage of spermatogenesis found most often.

<sup>d</sup>Analysis of variance (ANOVA) among photoperiod treatment groups within each temperature treatment group.

<sup>e</sup>Ranking of treatment means according to Student-Neuman-Keul's procedure. Means without a letter in common are statistically different at the 95% confidence interval.
Figure 7. Response of paired testes and fat bodies to interrupted-night photoperiod treatments in anoles kept at CC of WW. Experimental treatments were carried out daily for 3 weeks beginning 5 August 1981. See the legend to Figure 1 for a description of symbols.
Figure 8. Daily locomotor activity patterns in anoles of Experiment III at CC under different photoperiodic treatments. See the legend to Figure 2 for a description of light-dark cycles.
Locomotor activity (Clivityum's)

Time (hours after onset of light)
Figure 9. Daily locomotor activity patterns in anoles of Experiment III at WW under different photoperiodic treatments. See the legend to Figure 2 for a description of light-dark cycles.
the dark period. Again, this activity became elevated coincident with the nightly-light pulse treatments except during the light treatments occurring 11.8 hours after the onset of lights.

Experiment IV was performed to determine whether day length per se or the time of day that light occurs is important for the effect of photoperiod on spermatogenesis. The results of this experiment are presented in Table 4 and Figure 10. The rate of testicular regression with respect to spermatogenic activity was accelerated in those anoles that received light 14 hours after the onset of the photoperiod compared with the rate of regression in those that received light at 18 hours. Both inactive and active stages of spermatogenesis were present in testes from anoles of each photoperiodic treatment group. However, inactive stages appeared more often in those anoles that received an additional hour of light nightly 18 hours after the onset of the photoperiod. Testicular size decreased by the same amount in both treatment groups. No daily rhythm was present with regard to fattening responsiveness to light treatments. Once again, fat stores were increased. Locomotor activity patterns are presented in Figure 11. Locomotor activity occurred mainly during the photoperiod. Some nocturnal activity was evident, however it did not coincide with the nightly light pulses.
TABLE 4. CHANGE IN BODY WEIGHT, PAIRED TESTES WEIGHT, STAGE OF SPERMATOGENESIS, AND PAIRED FAT BODY WEIGHT IN RESPONSE TO DIFFERENT INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS IN ANOLIS CAROLINENSIS HELD AT CONSTANT WARM (WW) TEMPERATURES. INTERRUPTED-NIGHT PHOTOPERIOD TREATMENTS WERE INITIATED 5 AUGUST 1981 AND CARRIED OUT FOR 3 WEEKS.

<table>
<thead>
<tr>
<th>PHOTOPERIODIC SCHEDULE</th>
<th>N</th>
<th>CHANGE IN BODY WEIGHT (g)</th>
<th>PAIRED TESTES WEIGHT (mg)</th>
<th>STAGE OF SPERMATOGENESIS</th>
<th>PAIRED FAT BODY (%) BODY WEIGHT</th>
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<tr>
<td>INITIAL GROUP</td>
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<td>-----</td>
<td>40±4</td>
<td>3,4,5</td>
<td>0±0</td>
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<td>LDLD 12:8:5.2:1:5</td>
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<td></td>
<td>N.S.</td>
<td></td>
<td>P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

a The number of anoles in each group at the end of the experiment.

b Mean ± one standard error about the mean.

c The stage of spermatogenesis found most often.

d Analysis of variance (ANOVA) among photoperiod treatment groups within each temperature treatment group.

e Ranking of treatment means according to Student-Newman-Keul's procedure. Means without a letter in common are statistically different at the 95% confidence interval.
Figure 10. Response of paired testes and fat bodies to interrupted-night photoperiod treatments in anoles kept at WW. Experimental treatments were carried out daily for 3 weeks beginning 5 August 1981. See the legend to Figure 1 for a description of symbols.
Paired testes weights (mg)

Fat bodies (% body wt.)

Time (hours after onset of light)
Figure 11. Daily locomotor activity patterns in anoles of Experiment IV at WW under different photoperiodic treatments. See the legend to Figure 2 for a description of light-dark cycles.
DISCUSSION

Results of this study support the concept that the mechanism whereby day length influences the timing of testicular regression in anoles involves circadian rhythms. A daily rhythm of testicular responsiveness to interrupted-night photoperiods was observed in anoles kept at CC (Experiment I) or CW (Experiment II). Testicular weights in anoles kept at CC or CW were greater in response to an additional hour of light occurring nightly 18 hours after the onset of the daily photoperiod compared with testicular weights in response to light pulse treatments occurring 14 hours after the onset of the daily photoperiod. The fact that a daily rhythm of testicular photosensitivity with regard to weight occurred in anoles kept at CC was surprising in that CC inhibited the expression of a daily rhythm of testicular photosensitivity expressed in anoles kept at WW during the recrudescence phase of the annual reproductive cycle (Ferrell, 1982). However, it must be realized that neither CC or CW conditions would be experienced during the time of year (i.e., summer) that testicular regression normally takes place in anoles. Although a statistical analysis of spermatogenic responses could not be performed, a daily rhythm of testicular photosensitivity with regard to spermatogenesis appears to be expressed in anoles kept at WW (Experiments III and IV). A difference
in the predominant stage of spermatogenesis in response to the different interrupted-night photoperiod treatments was observed. This latter result is consistent with results from previous interrupted-night photoperiod studies carried out in anoles during the recrudescent phase of the annual reproductive cycle (Ferrell, 1982).

Results of this study also indicate that the mechanism involved in regulating the expression of a daily rhythm of testicular sensitivity to light can be influenced by temperature during the regressive phase of the annual reproductive cycle in anoles. As mentioned previously, a daily rhythm of testicular photosensitivity with regard to weight was expressed in anoles kept at CC or CW. No such daily rhythm of testicular responsiveness to light, in contrast, was evident in anoles kept at WW. A daily rhythm of testicular photosensitivity with regard to spermatogenesis was observed in anoles kept at WW but not in those kept at CC. The latter finding was again in accord with results reported in studies carried out in anoles during the recrudescent phase of the annual reproductive cycle (Ferrell, 1982). This finding, however, was not in accord with findings of similar interrupted-night photoperiods (Licht, 1971b) and resonance (Underwood, 1979) experiments carried out in anoles during the regressive phase of the annual reproductive cycle. Although no clear explanation for this discrepancy presents itself, several possibilities exist.
One possible explanation for the discrepancy between results of this study and previous interrupted-night photoperiod experiments (Licht, 1971b) with regard to the mechanism involved in photoperiodic time measurements is based on differences in experimental protocol. Licht (1971b) did not examine the influence of interrupted-night photoperiods in anoles kept at CC. It was in anoles kept at this temperature that the daily rhythm of testicular growth responsiveness to different interrupted-night photoperiodic treatments was expressed in this study. Therefore, the results are not directly comparable.

Furthermore, differences in testicular photosensitivity with regard to spermatogenesis in lizards kept at WW are somewhat subjectively analyzed and therefore open to different interpretations. Licht (1971b) reported differences in the spermatogenic activity in response to different interrupted-night photoperiod treatments. However, these differences were not felt to be significant. This might be true of the spermatogenic responses to different interrupted-night photoperiod treatments in Experiments I and II of this study, but not in Experiment IV. There is a clear difference between the predominant stages of spermatogenesis observed in lizards exposed to an additional hour of light nightly at 14 hours or 18 hours after the onset of the daily photoperiod.

The hourglass model explaining photoperiodism has been invoked to account for the influence of interrupted-night
photoperiodic (Licht, 1971b) and resonance photoperiodic (Underwood, 1978) schedules on testicular regression in anoles. According to this hypothesis, the length of light (or dark) is measured by the amount of a substance that accumulates. If the length of light (or dark) period is sufficient that threshold levels of the substance is accumulated, then a photoperiodic response (e.g., gonadal growth) is initiated. Therefore, testicular regression would occur when day length fell below (or night lengths increased above) some critical number of hours. This critical day length is reportedly 13.5 hours in anoles during the regressive phase of the reproductive cycle (Licht, 1971a). Testicular regression is initiated in summer when day lengths fall below this critical length of light. Day lengths (LD 14:10) above this critical length maintain testicular weight and spermatogenic activity in anoles at this season (Licht, 1966, 1967a,b, 1969a, 1971a). Testicular weight and spermatogenic activity were not maintained in animals of Experiment IV exposed to an amount of light (13.8 hours) greater than the reported critical day length. Therefore, it seems likely that results of this study can not be accounted for by the hourglass hypothesis of photoperiodism. These photoperiodic schedules were not explored in previous studies (Licht, 1971b; Underwood, 1978). Furthermore, in that testicular recrudescence is influenced by day length according to a photoperiodic mechanism involving circadian rhythms (Ferrell, 1982), it seems
unlikely that anoles would utilize a totally different mechanism in the regulation of testicular regression.

Testicular growth and spermatogenesis appear to be regulated by separate circadian based mechanisms. Testicular weights were significantly reduced in anoles at WW that received nightly light pulse treatments at 11 or 14 hours after the onset of the photoperiod compared with initial testicular weights in Experiment III. Testicular weights in lizards at WW that received the nightly light pulse interruptions at 18 hours after the onset of the photoperiod were not significantly reduced compared with initial testicular weights. Spermatogenic activity was reduced to a greater extent in anoles at WW that received light 18 hours after the onset of the photoperiod than in anoles exposed to light 11 or 14 hours after the onset of the daily photoperiod. Therefore, an inverse relationship between the effects of these photoperiodic treatments was expressed with regard to testicular growth and spermatogenesis. Testicular weights in anoles of Experiment IV were not different in response to the two photoperiodic treatments, whereas spermatogenic activity in response to these two treatments was different. These results are in accordance with the concept that testicular growth and spermatogenesis are regulated by two mechanisms described in reports of previous studies carried out in anoles at other seasons (Licht, 1967a,b, 1969a).

Reportedly, anoles become photosensitive during late
June and testicular regression is initiated in response to decreasing day lengths (Licht, 1967a, 1969b, 1971a). The concept that short day lengths initiate the regressive phase of the annual reproductive cycle is based on the misconception that the photoperiod drives physiological processes. Rather, light acts as a synchronizer that can only influence physiological processes in the context of the immediate physiological state of the organism. In fact, recent findings from studies carried out in birds (Moore et al., 1982) indicate that testicular regression is timed by day lengths experienced in spring and that day length at the time of testicular regression has little influence on this event. It has been hypothesized (Gwinner, 1973) that day lengths in spring synchronize an endogenous mechanism with the annual cycle of environmental conditions. Once synchronized, this endogenous mechanism regulates the orderly expression of seasonal physiological and behavioral events such that they coincide with appropriate environmental conditions. Results of this study are more easily interpreted in this light.

Although temperature is perhaps not a major factor affecting the endogenous mechanism in endotherms (e.g., birds), it may be quite important in ectotherms such as anoles. Warm temperatures would be expected to accelerate the progression of seasonal events, whereas cold temperatures would retard this progression. Such appears to be the case in this study. Testicular regression occurred at a faster
rate in anoles kept at WW or WC (Experiments I, II, III) compared with the rate of testicular regression in anoles kept at CC or CW, respectively. This finding is consistent with previous reports of studies carried out in anoles at various seasons (Licht, 1967a, b; Noeske and Meier, 1977; Ferrell, 1982). Temperatures present during day light hours appear to be particularly important in that CW mimics CC and WC mimics WW with respect to the effects on seasonality in anoles (Licht, 1971a). The influence of warm temperature on accelerating the rate of expression of seasonality in anoles might be due in part to its effect on increasing the rate of metabolic processes involved in the endogenous mechanism. However, based on results of this study part of the influence that WW or WC has on seasonality involves an interaction with photoperiod. Day lengths greater than 13.5 hours (e.g., LD 14:10) reportedly prevent testicular regression from occurring in anoles kept at WW in June or July (Licht, 1966, 1967a, b, 1969a, 1971a). In this study testicular regression with respect to spermatogenesis did not occur in lizards kept at WW and exposed to an additional hour of light nightly at 14 hours after the onset of the daily photoperiod (Experiment I). Regression did occur in several anoles of other treatment groups. The fact that testicular regression did occur in anoles kept at WC and exposed to an additional hour of light nightly at 14 hours after the onset of the daily photoperiod (Experiment II) indicates further that temperatures experienced during day
light hours are important in determining the testicular responsiveness to day length. These results indicate that the onset of testicular regression does not result from anoles becoming photosensitive. Rather, anoles are already photosensitive at this season. Testicular regression apparently results when day lengths are not such that light coincides with a photoinducible phase of the photosensitivity rhythm. Warm temperatures must coincide with this photoinducible phase as well in order for the influence of photoperiod to be expressed. This concept is not only supported by results of this study but by unpublished results of previous studies carried out in birds in our labs.

Fat body weights of lizards in all experiments were greater in WW or WC as compared with fat body weights of anoles in CC or CW, but there was no daily rhythm in fattening responsiveness to light. This finding is consistent with previous findings (Licht, 1971b; Noeske and Meier, 1977; Ferrell and Meier, 1981). Apparently the effects of temperature and photoperiod on reproductive development did not result because of differences in health of anoles in that fat stores increased despite a slight loss in body weight in many of the anoles.

Locomotor activity was monitored in anoles in order to determine the effects that photoperiod and temperature had on circadian rhythms of activity in relation to reproduction, this observation does not necessarily mean that the two
rhythms are coupled. For example, in anoles kept at WW (Experiment I) activity rhythms were present in all photoperiodic treatments. However, in these same anoles no testicular rhythm with regard to size was present in response to the light treatments. The finding that the activity and reproductive rhythms are not necessarily coupled is consistent with a previous finding in birds (Enright, 1965). Activity was timed by the photoperiod rather than temperature. Photoperiod appears to be the major entrainer of locomotor activity and temperature influences the intensity of activity.
Results of this study support the concept that the mechanism whereby day length influences the timing of testicular regression in anoles involves circadian rhythms. A daily rhythm of testicular growth responsiveness to interrupted-night photoperiods was observed in anoles kept at CC or CW.

Results also indicate that the mechanism involved in regulating the expression of a daily rhythm of testicular sensitivity to light can be influenced by temperature during the regressive phase of the reproductive cycle. No daily rhythm of testicular growth responsiveness to light was evident in lizards kept at WW. However, a daily rhythm of testicular photosensitivity with regard to spermatogenesis was observed in WW but not CC. The latter finding was in accord with results reported in studies carried out in anoles during the progressive (i.e., recrudescence) phase of the annual reproductive cycle. This finding, however, was not in accord with findings of similar interrupted-night photoperiod and resonance experiments carried out in anoles during the regressive phase of the annual reproductive cycle.

Testicular growth and spermatogenesis appear to be regulated by separate circadian based mechanisms. An inverse relationship between the effects of the photoperiodic
treatments was expressed with regard to testicular growth and spermatogenesis. These results are in accordance with previous reports carried out in anoles at other seasons.

Temperature is not considered a major factor affecting the endogenous mechanism in endotherms but may be important in ectotherms such as anoles. Warm temperatures were found to accelerate the progression of seasonal events, whereas cold temperatures retarded this progression. However, part of the influence that WW or WC has on seasonality involves an interaction with photoperiod. Temperatures experienced during day light hours are important in determining the testicular responsiveness to day length. The results also indicate that the onset of testicular regression does not result from anoles becoming photosensitive. Rather, anoles are already photosensitive at this season. Testicular regression apparently results when day lengths are such that light does not coincide with a photoinducible phase of the photosensitivity rhythm. Furthermore, warm temperatures apparently must coincide with this photoinducible phase as well in order for responses to photoperiodic treatments to be expressed. This finding is similar to that found in previous studies carried out in birds.


