# RESPONSE OF THE MALE REPRODUCTIVE SYSTEM OF LIZARDS (ANOLIS CAROLINENSIS) TO UNNATURAL DAY-LENGTHS IN DIFFERENT SEASONS

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In this study we have examined the response of the male reproductive system of the green anole, *Anolis carolinensis,* to unnatural day-lengths at different seasons of the year. Evidence of the photoperiodic control of reproduction in reptiles has been slowly accumulating. Burger (1937) found that artificially increased day-length stimulated a new spermatogenic cycle in the red-eared turtle, *Pseudemys scripta elegans.* Clausen and Poris (1937) reported unseasonal gonadal hypertrophy and spermatogenesis in green anoles exposed daily to 18 hours of light. Bartholomew (1950, 1953) described similar gonadal recrudescence in the desert night lizard, *Xantusia vigilis,* maintained at 16-hour day-lengths.

The above investigators have been primarily interested in the condition of the gonads. Descriptions of accessory sex organs have not been detailed enough to allow for a positive statement that reptiles can be brought into full breeding condition by artificially increasing day-length. In the experiments to be described we have examined both gonads and certain accessory sex organs to obtain a more complete measure of breeding condition. Further, the influence which the condition of the reproductive system at the beginning of an experiment has upon its response to day-length alterations has not been thoroughly examined in all seasons. We have studied the effect of short days prior to and during the breeding season, as well as the effect of long days during and following the breeding season. To our knowledge there has been no previous effort to determine whether a seasonal "refractory period," characteristic of passerine birds (for review see Hammond, 1954), is present in reptiles or if a period of exposure to short days is necessary before a new reproductive cycle can be initiated (Miller, 1954).

## Methods

Animals were collected as needed over a period of three years in the vicinity of New Orleans. Adult animals (61 to 71 mm. snout-vent length), that had probably been through a previous reproductive period, were separated from immatures or sub-adults (51 to 60 mm.). The latter had not been through a previous complete reproductive hypertrophy but were due to become sexually mature in the next normal breeding season (Fox, 1958).

Methods of animal care have been reported previously (Fox and Dessauer, 1957). Cage temperatures were maintained at 28 ± 2° C. Artificial lighting was supplied by means of daylight fluorescent lamps mounted above the cages. These were regulated by automatic time switches to supply 18L (hours of light per

24-hour period), 16L, 14L, 9L or 6L. Animals exposed to natural day-length were placed in front of a north-facing window.

Experiments were conducted for approximately 60 days unless otherwise stated. At the end of each experiment lizards were killed with ether, and their fat bodies, liver and left testis weighed. Loss of body weight during the course of an experiment, and liver and fat body weights at autopsy were useful in estimating the degree of starvation of unresponsive lizards. The right testis with attached epididymis, the ductus deferens, and the right kidney were fixed in Bouin's solution, embedded in paraffin, sectioned at 10 micra, and stained with Harris' hematoxylin and eosin. The state of spermatogenesis and the cytology of interstitial cells and accessory sex organs were studied microscopically. Measurements of the diameter of seminiferous tubules, the diameters and epithelial heights of the ductus epididymidis, ductus deferens, and the sexual segment of the kidney were made with a calibrated ocular micrometer. All mensural data were plotted as histograms and subjected to the "*t* test" for possible significance. The size of each sample, mortality and number of starving animals (fat bodies less than 1% of body weight) are presented in Table II. Column "N" in Table II is the number used in calculating standard deviations and "*t*" values for determining the level of significance of the data when it seemed justifiable to eliminate the measurements on starving animals. Differences are accepted as significant at the 5% level and highly significant at the 1% level of probability.

In reporting the results, frequent reference is made to arbitrarily delimited stages of the normal reproductive cycle of wild male *Anolis*. These stages are characterized briefly in Table I; detailed descriptions are presented by Fox (1958).

Although considerable effort was made to locate and measure interstitial cells of the testes, it was felt that these data were unsatisfactory for valid statistical analyses. Data on the sexual segment of the kidney, however, afford an index to androgenic activity (Reynolds, 1943). The sexual segment of *Anolis,* like that of *Sceloporus* (Forbes, 1941), includes the entire set of uriniferous collecting ducts and ureter (Fox, 1958). Measurements were taken in the distal, middle, and proximal regions of the collecting ducts, but only those of the distal end are recorded in Table II.

## Results

### *Experiment 1. September–November: Adult lizards exposed to 18L and natural day-length*

At the beginning of the experiment adults had just completed a breeding season and the gonads and accessory sex organs were in their most atrophic state

Table I

*Arbitrary stages in the normal reproductive cycle of Anolis*

| | Spermatogenesis | Accessory sex organs |
|---|---|---|
| Stage I | Dividing spermatogonia | Atrophic |
| Stage II | Primary spermatocytes predominate | Slight hypertrophy |
| Stage III | Maximum development; large numbers of spermatocytes and spermatids | Near maximum hypertrophy |
| Stage IV | Large numbers of spermatids; spermatocytes reduced | Maximum hypertrophy |
| Stage V | Only spermatids numerous | Partial atrophy |
| Stage VI | Expulsion of all active cells | Atrophic |

TABLE II

| Experiment | Light exposure | Number | Mortality | Starving | N | Seminiferous tubule diameter μ | Spermatogenic stage | | | | | | Ductus epididymidis epithelial height μ | Ductus deferens diameter μ | Sexual segment epithelial height μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I | II | III | IV | V | VI | | | |
| 1 Sept.–Nov. Adults | 18L | 19 | 2 | 2 | 15 | 120–290† 206±48.9†† | 2 | 9 | 4 | | | | 11–35† 20.0±6.7†† | 40–100† 59±15.1†† | 13–22† 16.1±2.9†† |
| | ND* | 14 | 0 | 0 | 14 | 50–170 110±26.2 | 14 | | | | | | 8–13 10.8±1.5 | 40–60 53±8.0 | 13–16 14.0±1.1 |
| 2 Sept.–Nov. Immatures | 18L | 33 | 12 | 2 | 21 | 90–280 174±57.6 | 7 | 9 | 5 | | | | 10–30 18.8±6.1 | 40–140 62±27.2 | 13–25 17.7±3.6 |
| | ND | 16 | 5 | 2 | 11 | 70–170 107±31.6 | 11 | | | | | | 10–16 13.7±2.1 | 30–60 48±7.7 | 13–22 16.9±2.7 |
| 3 Oct.–Dec. Immatures | 18L | 45 | 9 | 6 | 30 | 90–290 173±62.0 | | 9 | 15 | 6 | | | 8–32 18.4±8.4 | 50–180 81 ±38.0 | 16–38 23.3±5.0 |
| | 16L | 14 | 5 | 3 | 6 | 90–260 156±67.5 | | 2 | 1 | 3 | | | 12–38 21.7±10.5 | 50–210 120±68.8 | 16–32 20.4±3.5 |
| | 9L | 29 | 9 | 3 | 17 | 80–190 127±29.2 | 10 | 7 | | | | | 6–13 11.6±2.1 | 40–70 54±8.8 | 13–25 19.1±2.3 |

* Natural day-length (maximum = 14 hours, minimum = 10 hours).
† Range.
†† Mean and standard deviation.

TABLE II—*Continued*

| Experiment | Light exposure | Number | Mortality | Starving | N | Seminiferous tubule diameter μ | Spermatogenic stage | | | | | | Ductus epididymidis epithelial height μ | Ductus deferens diameter μ | Sexual segment epithelial height μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I | II | III | IV | V | VI | | | |
| 4 Nov.–Jan. Adults | 18L | 26 | 5 | 3 | 18 | 110–300<br>245±50.1 | 1 | 1 | 10 | 6 | | | 12–30<br>25.6±6.6 | 90–220<br>167±45.3 | 20–50<br>35.8±9.8 |
| | ND | 21 | 0 | 8 | 13 | 90–280<br>176±56.1 | | 9 | 4 | | | | 10–18<br>13.1±2.4 | 30–60<br>45±12.6 | 12–20<br>16.9±2.6 |
| 5 Dec.–Feb. Adults | 18L | 8 | 4 | 2 | 4 | 240–305<br>271±31.2 | | | 1 | 3 | | | 22–29<br>24.8±3.4 | 67–130<br>106±30.6 | 17–32<br>22.5±6.6 |
| | 16L | 26 | 6 | 2 | 18 | 240–310<br>269±15.5 | | | 5 | 13 | | | 19–38<br>27.6±6.6 | 100–190<br>150±30.8 | 25–50<br>37.6±8.1 |
| | 9L | 20 | 0 | 4 | 16 | 230–300<br>269±24.3 | | 1 | 11 | 4 | | | 16–35<br>22.5±4.8 | 80–230<br>142±52.4 | 16–40<br>24.9±7.6 |
| 6 Jan.–March Immatures | 18L | 26 | 6 | 2 | 18 | 160–330<br>256±38.1 | | | 3 | 13 | 2 | | 21–38<br>30.0±6.6 | 120–300<br>185±49.5 | 25–51<br>37.3±8.7 |
| | 9L | 25 | 15 | 3 | 7 | 180–300<br>246±40.9 | | 1 | 5 | 1 | | | 18–32<br>22.4±4.9 | 100–210<br>142±38.9 | 21–32<br>26.0±3.9 |

TABLE II—*Continued*

| Experiment | Light exposure | Number | Mortality | Starving | N | Seminiferous tubule diameter μ | Spermatogenic stage | | | | | | Ductus epididymidis epithelial height μ | Ductus deferens diameter μ | Sexual segment epithelial height μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I | II | III | IV | V | VI | | | |
| 8 May–July Adults | 18L | 10 | 4 | 0 | 6 | 176–300<br>212±48.3 | | | 1 | 3 | 2 | | 16–32<br>24.0±5.4 | 70–192<br>149±49.4 | 13–32<br>22.0±8.2 |
| | ND | 11 | 2 | 1 | 8 | 210–262<br>239±17.4 | | | 1 | 7 | | | 19–36<br>28.3±4.9 | 160–196<br>179±12.6 | 22–51<br>37.0±9.1 |
| | 9L | 8 | 4 | 0 | 4 | 77–120<br>100±18.7 | | | | | | 4 | 9–16<br>13.4±2.7 | 50–85<br>63±15.4 | 12–17<br>13.7±1.8 |
| 9 July–Sept. Adults | 18L | 22 | 3 | 2 | 17 | 112–290<br>222±40.2 | 2 | 3 | 7 | 5 | | | 13–32<br>23.9±6.6 | 77–215<br>150±42.5 | 18–51<br>33.3±10.1 |
| | ND | 10 | 0 | 0 | 10 | 55–118<br>81±22.1 | | | | | | 10 | 10–20<br>14.9±3.6 | 40–96<br>70±20.1 | 13–20<br>15.9±2.5 |
| 10 Aug.–Oct. Adults | 18L | 15 | 1 | 3 | 11 | 80–250<br>189±70.1 | 2 | 1 | 4 | 4 | | | 10–35<br>24.5±8.7 | 50–230<br>178±70.7 | 13–35<br>22.3±7.9 |
| | 14L | 28 | 7 | 3 | 18 | 70–280<br>146±71.1 | 5 | 10 | 3 | | | | 10–38<br>21.2±9.2 | 60–150<br>100±21.4 | 13–26<br>18.1±4.1 |
| | ND | 33 | 6 | 0 | 27 | 75–135<br>120±17.9 | 27 | | | | | | 12–23<br>16.1±2.9 | 45–75<br>62±9.5 | 12–19<br>15.9±2.5 |

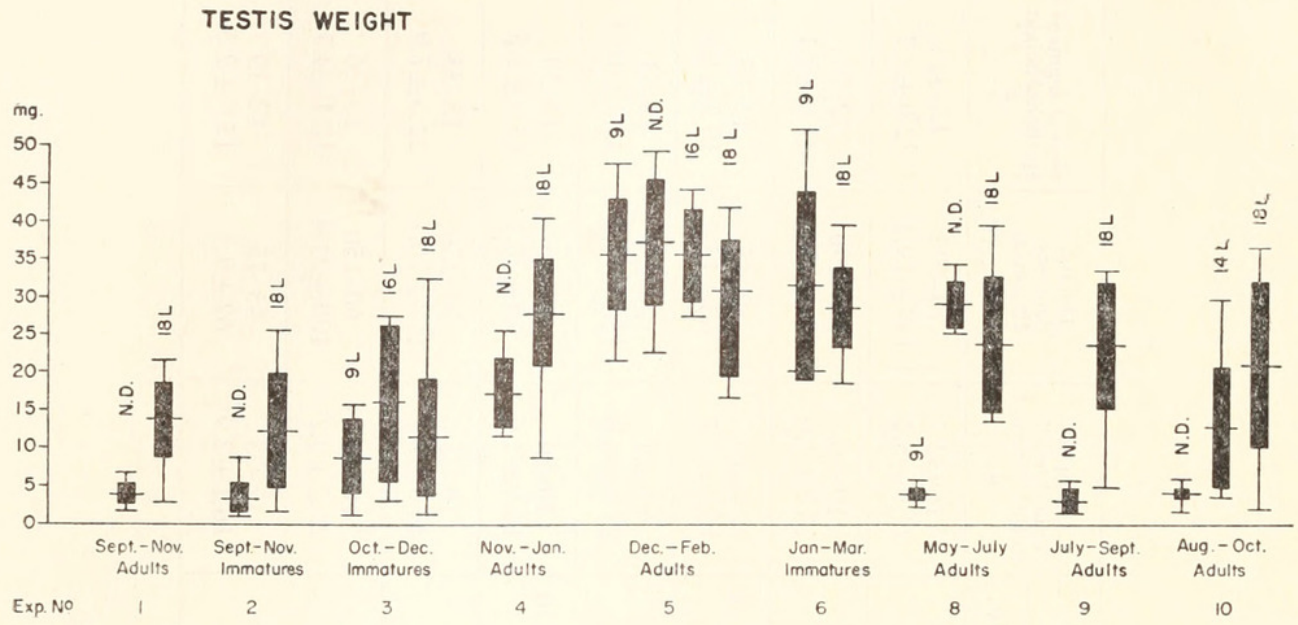


FIGURE 1. Testis weights demonstrating the seasonal gradient in testicular response to exposure to long photoperiods. The terminal horizontal bars indicate the range in sample weights, the middle horizontal bar indicates the sample mean and the solid rectangle represents the standard deviation plotted on both sides of the mean. N. D. = exposure to natural day-lengths; L = hours of artificial light per 24 hours.

(end of Stage VI). By the end of the experiment, animals in the wild had initiated spermatogonial mitoses but their accessory sex organs were still atrophic (Stage I). The 18L sample underwent a highly significant increase in testis weight (Fig. 1) although two apparently healthy animals did not respond to the light treatment. Control animals retained small gonads that averaged significantly less in weight than those of wild animals in November (Fig. 2). Seminiferous tubule diameters were greater and spermatogenesis more advanced in the experimental sample (Table II). Whereas none of the control animals advanced further than spermatogenic Stage I, four of the experimentals were classified as early Stage III. The total cell number, however, was about half that present in a normal Stage III gonad.

The accessory sex organs were partially stimulated by exposure to 18-hour day-lengths at this season (Table II). The ductus epididymidis underwent a very significant increase in diameter and epithelial height. In half of the sample the cells contained secretion granules (Stages II and III); in six animals sperm were present in the lumen (beginning of Stage III). Secretion granules and sperm were lacking in all the controls (Stage I). Neither the average of epithelial height nor diameter of the ductus deferens significantly increased in the 18L sample (Table II). In three animals, however, sperm had entered this organ and it was significantly enlarged, convoluted and contained secretion granules (Stage III). The ductus deferens of the controls remained atrophic (*viz.*, empty, not convoluted, and with a pseudostratified agranular epithelium).

Measurements of the sexual segment (Table II) did not reveal significant differences between the means of the experimental and control samples. Four experimental animals, however, showed slight hypertrophy and the presence of eosinophilic granules at the tips of the epithelial cells of the ureter and the distal

ends of the collecting tubules (Stage II). No hypertrophy or secretion occurred among the controls.

*Experiment 2. September–November: Immature lizards exposed to 18L and natural day-length*

Although the reproductive organs of these young animals had achieved a partial maturity during the previous summer, they were completely atrophic at the beginning of the experiment. Wild animals in this age group were at minimal reproductive development at the close of the experiment (Stage I).

Immature lizards on 18L underwent a highly significant gonadal response. Testis weights were markedly greater ($P < 1\%$) than the controls (Fig. 1). Four were more responsive to the light treatment than adults during the same period. Spermatogenesis (Table II) almost reached peak activity (Stage III) in a few individuals, whereas none of the controls progressed beyond Stage I.

Of the accessory sex organs studied, only the ductus epididymidis proved to be significantly hypertrophied ($P < 1\%$) by the long light treatment. Over half the animals showed secretory granules and one-third had sperm in the epididymis. In all controls the epididymis was atrophic and without sperm. Three experimental animals showed enlargement of the ductus deferens (Stage II). They had a few sperm in the lumen, the epithelium was taller and the cytoplasm granular. The ductus deferentia of the controls were atrophic (Stage I).

Five experimental animals showed a slight hypertrophy of the sexual segment of the kidney with secretion granules at the tips of epithelial cells near the ureter (Stage II). The sexual segment of a few controls underwent a minor hypertrophy but no signs of secretion were present.

*Experiment 3. October–December: Immature lizards exposed to 18L, 16L and 9L*

At the beginning of this experiment the animals were in a stage of minimal development. The healthy animals on 16L and 18L showed a highly significant increase in testis weight over those kept at 9L (Fig. 1). Animals that lost weight did not respond to the light treatment. Many kept on the long days had very large seminiferous tubules and had progressed into Stages III and IV of spermatogenic development (Table II), without achieving, however, the high volume of sperm production characteristic of adults during the breeding season. Nearly half of the 9L animals attained the spermatogenic development of adult wild animals for December (Stage II), the others remained in Stage I.

Both samples on 18L and 16L showed highly significant or significant hypertrophy of the various accessory sex organs when compared to those on 9L. The differences between the 18L and 16L samples were not statistically significant. The epididymis of the animals on long days contained many sperm. The cytoplasm of the ductus epididymis was granular and the cell height and diameter much enlarged (Stages II and III). The epididymes of controls were all in Stage I. Significant numbers of animals on long day treatment had sperm in the ductus deferens. In these individuals the diameter of the ductus was increased, the epithelium hypertrophied and the cytoplasm granular (Stage III). A few animals remained in Stage I or II.

The sexual segment underwent a significant hypertrophy on the 18L ($P = 1\%$) and 16L ($P = 5\%$) regimes. Secretion occurred in only five animals on 18L and one on 16L. In these animals the epithelium of the ureter and the distal end of the collecting tubules exhibited eosinophilic granules in the apical ½–⅔ of the cells (Stage II). The 9L series showed only slight hypertrophy, and no signs of secretion (Stage I).

*Experiment 4. November–January: Adult lizards exposed to 18L and natural day-length*

During November the reproductive system of *Anolis* is in Stage I. Lizards on 18 hours of day-length underwent marked hypertrophy of the testis ($P < 1\%$) compared to controls on natural day-length (Fig. 1). The testes of the controls enlarged also, but the sample mean did not differ significantly from that of wild animals killed in January (Fig. 2).

Most of the experimental animals reached the peak of seminiferous tubule diameter and spermatogenic development (Stage III). A few passed the peak and advanced to Stage IV. Four control animals progressed to early Stage III but these were far from attaining peak development. The remainder of the controls were in spermatogenic Stage II.

The accessory sex organs of the experimentals were greatly hypertrophied ($P < .01$; Table II). In most of the sample the ductus epididymidis was filled with sperm and the epithelial cytoplasm completely filled with eosinophilic granules (Stages III and IV). Four controls had a secretory epididymis with a few sperm in the ductus (Stage II). The ductus deferentia of the 18L sample were very convoluted, filled with sperm and highly secretory (Stages III and IV). Those of the control sample were largely atrophic and empty (Stages I and II).

The sexual segment of the experimentals was hypertrophied (Table II) and highly secretory at its distal end in all animals (Stage II) and in its middle portion in half the sample (Stage III). Control animals showed no signs of secretion although wild animals at this time show slight activity (Stage II).

*Experiment 5. December–February: Adult lizards exposed to 18L, 16L and 9L*

During this period the reproductive system of wild animals progresses through Stage II with some individuals reaching early Stage III. The distribution and sample means of testis weights were very similar for animals exposed to 9L and 16L (Fig. 1). Testes averaged smaller in the 18L sample. There were no significant differences in seminiferous tubule diameters (Table II). All healthy animals in the 16L and 18L series reached peak spermatogenic development (Stage III), and two-thirds passed on to Stage IV. Animals in the 9L sample were largely in Stage III, but a few merited assignment to Stage IV. When subjected to the Chi-square test, the 16L and 18L samples proved to be very significantly advanced beyond the 9L sample.

In the 16L sample, but not in the 18L sample, the epithelial cells of the ductus epididymidis were significantly taller than those of the 9L sample. Diameters of both the ductus epididymidis and ductus deferens were similar in the 9L and 16L samples, but averaged significantly smaller in the 18L sample. All were secretory

and contained sperm. The ductus deferens appeared to be more highly convoluted in the 16L sample than in the 9L sample, indicating greater sperm storage. Those of the 18L animals were less convoluted and contained considerably fewer sperm than either of the two other samples.

The sexual segment was hypertrophied in both the 9L and 16L samples. In the 16L sample Stage III was reached whereas in the 9L sample the sexual segment remained in Stage II. The distal ends of the collecting tubules were hypertrophied and secretory in both samples. The middle portions of the collecting tubules were secretory in thirteen animals on 16L but in only one on 9L. All sexual segments of the 18L sample were atrophic except that of one animal in which the distal end was secretory.

Data on testes weights were available for a series of captive animals exposed to natural day-length during this period. The mean testis weight was comparable to that found in the 9L and 16L series (Fig. 1).

*Experiment 6. January–March: Immature lizards exposed to 18L and 9L*

At the beginning of this experiment smaller immature male lizards were in Stage I and larger ones in Stage II. At the end of this experiment the mean testis weight of the 9L sample was greater (Fig. 1), although not significantly so, than the 18L sample. However, animals on 18L were far advanced beyond those on 9L in respect to spermatogenesis (Table II). Most of them appeared to have passed the peak of gonad development (Stage IV).

The ductus epididymidis was secretory and filled with sperm in both samples. Epithelial height of the 18L sample (Table II) was significantly greater. The ductus deferens was secretory, convoluted and filled with sperm in most animals of both samples. Those of the 18L sample had a significantly greater diameter, appeared to be more convoluted and to contain more sperm. In three animals of the 9L sample the ductus deferens was pseudostratified, non-secretory and contained very few or no sperm.

The sexual segment was very significantly hypertrophied in the 18L sample. In over half of this sample it was secretory in the ureter and distal ends of the collecting tubules, and in ⅓ it was secretory through the middle portion (Stage III). Most sexual segments in the 9L sample remained in Stage I; in two animals they were secretory through the middle segment (Stage III) and in one secretion occurred in the ureter and distal end only (Stage II).

Regardless of the length of light exposure, most animals that underwent marked testicular development and hypertrophy of the accessory sex organs were over 55 mm. snout-vent length at the start of the experiment and grew to over 60 mm. during the two months period.

*Miscellaneous winter experiments*

Data available on a few animals maintained for longer than the standard 60-day period are pertinent to this study. Eight immature lizards kept at 9L from December 10 to April 4 underwent considerable hypertrophy of the reproductive system despite the short day-length. Testes ranged from 8 to 33 mg. with a mean of 22 mg. Spermatogenesis reached Stages III and IV in the larger gonads.

These same individuals had sperm in the ductus epididymidis and ductus deferens, both of which were hypertrophied and secretory. The sexual segment of two larger and faster growing individuals was secretory through the middle portion (Stage III). Those lizards that reached 60 mm. snout-vent length had the largest gonads and the most hypertrophied accessory sex organs. In those less than 60 mm. the extent of testicular hypertrophy was closely correlated with body growth.

Four animals of the same size group survived 18L exposure during the above period. The range in testicular size (3 to 33 mg.) was similar to that in the group exposed to 9L but the gonads averaged considerably smaller (16.5 mg.). Only two possessed large gonads with spermatogenic activity equivalent to Stage IV. One of the latter animals, that had grown more than the rest, had hypertrophied accessory sex organs (Stage III), the other had atrophic accessory sex organs and a spermatic granuloma of the epididymis. The remaining two animals were in spermatogenic Stage VI. The epididymis and ductus deferens were either empty or contained cellular debris. None of the accessory sex organs was secretory (Stage V). Four adult lizards on 9L from January 9 to April 4 were in spermatogenic Stage IV and the accessory sex organs approached Stage III. Similarly, ten adults on 9L from February 3 to April 4 were in spermatogenic Stages III and IV and their accessory sex organs in Stage III.

*Experiment 7. April–June: Adult lizards exposed to 6L and natural day-length*

On April 30, 26 adult male lizards were placed on a regime of 6L. Five of these animals were sacrificed on June 3 and compared to a series of 24 control animals maintained on natural day-length. Testis weight was very significantly less in the 6L sample (22 to 33 mg; 29.5 ± 3.1 mg.)[1] than in the controls (24 to 52 mg.; 37.0 ± 6.5 mg.), but was larger than that of two samples of wild animals taken in June (Fig. 2). Spermatogenesis in the 6L animals was in late Stage IV typical of wild animals in July, whereas the controls were in spermatogenic Stages III and early IV. The accessory sex organs of the 6L sample were secretory and were not significantly different from those of the controls.

Nine additional animals from the 6L sample were sacrificed on June 21 and compared with a sample of nine newly captured animals. Testis weights were very significantly less (4 to 22 mg.; 14.9 ± 5.5 mg.) than those of wild animals (16 to 33 mg.; 23.7 ± 5.6 mg.). Eight of the 6L animals were in spermatogenic Stage V (typical of August animals) and one in Stage VI. All wild animals were in spermatogenic Stage IV. The epididymis and ductus deferens were significantly reduced in size and in secretory activity in a few experimental animals. All experimental animals showed significant reduction in epithelial height of the sexual segment, but a few retained secretory activity in the distal end of the tubules.

An attempt was made to determine whether the precocious termination of the sexual cycle, which was brought about by exposure to short days, resulted in a temporary gonadal refractoriness to stimulation by long day-lengths. Six animals which had been maintained on 6L from April 30 to June 20, were exposed to 18L from June 21 to September 18. Three responded markedly and three did not. Testis weights (3 to 17 mg.; 10.8 ± 7.2 mg.) were significantly heavier than those of a sample of eight controls maintained on natural day-length from June 4 to

[1] Range; mean and standard deviation.

September 18 (2 to 6 mg.; 3.5 ± 0.6 mg.) and a sample of nine wild animals killed September 22 (1.5 to 4 mg.; 2.3 ± 0.7 mg.). Controls and wild animals were at the end of the annual spermatogenic cycle (Stage VI) and all accessory sex organs were atrophic. Of the six experimental animals two were in spermatogenic Stage VI, one advanced to Stage I, and three advanced to Stage II. The latter three individuals exhibited a precocious spermiogenesis and the epididymis was hypertrophied and contained sperm. The ductus deferens and sexual segment were secretory (Stage II) in only a single specimen in which sperm had reached the ductus deferens. The two unresponsive animals were seriously starved.

*Experiment 8. May–July: Adult lizards exposed to 18L, natural day-length and 9L*

At the initiation of this experiment the reproductive system of newly captured animals was near peak development (Stage III). During July wild lizards undergo a regression of spermatogenesis (Stage IV), but the accessory sex organs are still near maximum development. The two experimental samples for this period had a relatively high mortality due to starvation (Table II). Spermatogenesis was exhausted (Stage VI) in the 9L sample (Fig. 1) and the accessory sex organs were atrophic (Table II). Considerable variation existed in the 18L sample. Its average testis weight (Fig. 1) was very significantly less than that of controls exposed to natural daylight, but it was nearly equal to the average for wild animals in July. Only one individual was markedly stimulated by the excessive light treatment. Its reproductive organs were at peak activity and comparable to those of an animal in April or May. Except for a slight increase in the number of primary spermatocytes, the gonads of three animals were comparable to those of control or wild animals in July; the sexual segment of the kidney, however, was less hypertrophied. The two remaining lizards had smaller gonads and an atypical spermatogenic pattern. The germinal elements closest to the lumina of the seminiferous tubules were all advanced spermatids (Stage V) whereas the outer layers revealed a proliferation of spermatogonia and primary spermatocytes (Stage I). In one of these animals the ductus deferens and ductus epididymidis were moderately hypertrophied and contained sperm, in the other these organs were atrophic. The sexual segment was inactive in both. Since these animals gained weight, the poor response to light did not appear to be due to starvation.

*Experiment 9. July–September: Adult lizards exposed to 18L and natural day-length*

The sample means of the experimental animals were very significantly greater for all measurements taken (Fig. 1 and Table II). The testes of the controls were lacking in spermatogenic activity (Stage VI). The testes of the experimentals varied from 5 to 34 mg. and were judged to be in spermatogenic Stages I–IV.

Control and wild animals sacrificed in September have atrophic accessory sex organs. Among animals maintained on 18L, the ductus epididymidis and ductus deferens were highly convoluted, filled with sperm and secretory in twelve; nearly empty and much reduced in three; empty and atrophic in two. The sexual segment of the kidney was hypertrophied and secretory in the ureter and distal ends of the

ducts in six, and non-secretory in the others. The animals that were least responsive to the light treatment possessed fat bodies weighing around 100 mg. (5% of body weight). This represented half or less the weight of fat bodies of animals that yielded a good response.

*Experiment 10. August–October: Adult lizards exposed to 18L, 14L, and natural day-length*

This experiment was initiated at a time when rapid involution was occurring in all reproductive organs. By the end of the experiment both wild animals and controls had completed a reorganization of these organs and were ready to initiate a new cycle. The testes of most animals in both experimental groups were markedly ($P < 1\%$) enlarged (Fig. 1). Both the maximum and average responses of the 14L sample were considerably less than those of the 18L sample in respect to testis weight and spermatogenic development (Table II). On the basis of a highly convoluted and sperm-packed ductus deferens, four lizards on 18L were classified in spermatogenic Stage IV. The fat bodies of the unresponsive lizards on 18L weighed half as much as those that responded well. Most of the unresponsive animals on 14L had very large fat bodies.

Hypertrophy of the accessory sex organs of the 18L sample tested to be very highly significant except for the sexual segment of the kidney for which $P = .02$. Only the four animals classified in spermatogenic Stage IV showed secretion in the middle and distal portions of the collecting ducts. The hypertrophy of the accessory sex organs of the 14L sample proved to be significant, although no secretion was found in the sexual segment of the kidney.

## Discussion

*Degree of response of Anolis to artificially lengthened days.* In the fall, when gonads are smallest, a 60-day exposure to 18L was sufficient to produce numerous spermatozoa in the larger testes and a precocious, but limited, spermiogenesis in the smaller ones. Although the size of the gonads was only about half that of animals during the breeding season, the relative increases were the greatest of the entire series. Assuming that testes weights were equivalent to those of wild anoles at the beginning of the experiments, gonadal weights of both adults and immatures underwent at least a six-fold increase (Experiments 1 and 2). The closer to the breeding period (April through August) an experiment was started, the more complete was gonadal response. The relative increase in weight, however, was proportionately less.

A similar seasonal gradient in responsiveness was observed in the accessory sex organs. The sexual segment is a more reliable index of this gradient than are the epididymis and vas deferens in which the apparent activity varies with sperm content. Exposure to 18L for 60 days starting in September or October (Experiments 1 to 3) did not bring the sexual segment of adult or immature lizards into a secretory condition typical of the breeding period. However, in Experiment 4, started November 14, half of the adults developed secretory sexual segments typical of April breeding animals. Progressively greater responses were obtained in each succeeding experiment through the winter (Table II).

Our data on the completeness of the testicular response correspond reasonably well with the results of others working with reptiles and birds (for references see reviews by Hammond, 1954 and Farner, 1955). Only rarely do experimental, photoperiodic-stimulated gonads achieve the size that is typical of the particular species during the breeding season; further, the largest gonads are invariably induced just prior to the natural time for hypertrophy. The data of Vaugien (1955) on testis size and bill color of immature English sparrows best illustrate the seasonal gradient of response.

Several factors have been suggested to account for the seasonal variation in the response of the reproductive system. Vaugien (1955) considered the increasing responses which he observed to be due, in part, to the increasing age of the immature sparrows. In immature anoles the degree of response appears to be correlated with growth rate as well as age (as determined by size). Vaugien also demonstrated that in immature English sparrows the longer the exposure to short days prior to capture, the greater the testicular response to long day-lengths. Exposure to a period of short days in the fall is apparently essential for spermatogenesis in a number of species of birds. In adult anoles, exposure to long day-lengths during or following the involution of the testes (Experiments 9, 10, and 1) elicited a response without prior exposure to short photoperiods.

It is generally accepted that the annual variation of the reproductive systems of wild vertebrates is primarily determined by the cyclic nature of pituitary secretions. However, Van Oordt (1956) demonstrated a seasonal difference in the sensitivity of spermatogonia to pituitary gonadotropins in the frog, *Rana temporaria*. On the basis of thyroxin injections, Vaugien (1955) postulated that during short days the testes of immature English sparrows become increasingly susceptible to stimulation. It is possible, however, to explain adequately the data on *Anolis* without assuming a seasonal change in sensitivity on the part of the reproductive system. The gradient of response of the testes can be accounted for by the number of spermatogonia present at the beginning of each experiment, there being fewest in September and progressively more during the winter. The gradient observed in the accessory sex organs appears to depend both upon the state of their respective cells and the state of the interstitial cells at the beginning of each experiment. In September the reproductive organs are atrophic and secretory interstital cells are virtually absent (Fox, 1958). At this time, a long period of stimulus appears necessary since the interstitial cells must be brought into activity before their secretions can secondarily stimulate the accessory sex organs. During later months hypertrophied interstitial cells are increasingly more abundant. These probably immediately release androgenic hormone which, in turn, would bring about progressively greater enlargements of the already partially hypertrophied accessory sex organs.

The above explanation could account for the marked response of some individuals in Experiments 9 and 10. Although the reproductive organs and interstitial cells of these animals were declining at the start of the experiments, they were not atrophic and could respond rapidly to a new stimulus. Conversely, the results of Experiment 7, in which lizards hastened into an early atrophy of the reproductive organs by exposure to 6L responded only mildly to exposure to 18L, could be explained by the degree of atrophy at the time the long day stimulus was applied.

On the basis of data obtained by exposing normal and pinealectomized anoles to normal and long days, Clausen and Poris (1937) have suggested that the pineal eye inhibits the testicular cycle. It is interesting to compare data from our Experiment 4 with their data since the two experiments were conducted at approximately the same time of the year. The testes weights of our animals on either long or short days during this period appear to be nearly identical to those of their pinealectomized animals on similar light regimes. Of the two samples they maintained on normal day-lengths the unoperated controls averaged 0.8 g. less in weight at the beginning of the experiment. The average weight of this sample (3.92 g.) suggests that it was composed largely of sub-adult animals. Since testis weight in *Anolis* has been shown to regress significantly with body size (Fox, 1958), the differences between the two samples could be due to inequalities in sampling. Similarly, in their two samples kept on a long day program, the unoperated animals averaged 0.7 g. lighter than the operated. However, even without this consideration it is doubtful whether the minor difference of 1.5 mg. between the mean testis weights would prove to be statistically significant. In our opinion, the data of Clausen and Poris (1937) do not afford adequate proof that the pineal eye acts as an inhibitor of the male reproductive system in *Anolis*.

Studies on *Anolis* and other species emphasize the importance of examining reproductive structures other than the testes or sperm-storing organs when determining the breeding status of an animal. The presence of viable sperm may not always be a reliable indicator as to whether or not an individual is in full breeding condition. Experiments 1, 2, and 3 indicate that the response of the accessory sex organs may lag behind spermatogenesis. Further, in many species the male normally stores sperm during a non-breeding stage of the reproductive cycle (Fox, 1952; Harrington, 1956). To determine the true breeding status some reliable test for the presence of androgenic secretions should accompany tests for viable sperm. In the English sparrow the color of the bill serves this purpose (Keck, 1933). In many species the emergence of characteristic behavioral patterns or, more directly, the histology of the interstitial cells has been correlated with androgenic activity. We have made only cursory observations on the above criteria in this study. The nuchal crest, a secondary sex character, appeared to enlarge in animals brought into full breeding condition. Although no detailed records of behavior were made, we observed frequent attempted copulations between males exposed to long days in the winter and spring experiments.

The histology of the sexual segment of the kidney in lizards can be used as an accurate measure of androgenic activity and the stage of the breeding cycle. First, cell height and tubule diameter can be measured with precision. Second, the progressive spread of secretion granules can be traced both intracellularly (from the apices to the basal nuclei of the tall columnar cells) and from the ureter and distal ends of the collecting ducts to the proximal collecting ducts.

If the presence of viable sperm alone were used as an indicator it would appear that male anoles were brought into breeding condition by exposure to 18L for 60 days at any season of the year. However, examination of the sexual segment reveals that none were brought into breeding condition in experiments started in September and October and only half of those in experiments started in November.

*Refractoriness to photoperiodic stimulation.* Passerine birds characteristically

exhibit a refractoriness to stimulus by long photoperiods at the close of the breeding season. This refractoriness persists until there has been a period of exposure to long nights (Wolfson, 1952). The refractory period allows for the physiological reorganization of the gonadal and fat cycles (Wolfson, 1954) and is probably caused by seasonal reduction in pituitary activity (Miller, 1948, 1949; Farner and Mewaldt, 1955).

We have been unable to find a period during which *Anolis* is completely refractory to photoperiodic stimulation. The testes seem to respond, to some extent, at all seasons of the year (Fig. 1, Table II). In all experiments except Nos. 5 and 6, which were started near the peak of testicular development (Stage III), a longer photoperiod resulted in either greater mean or maximum testis weights. In Experiments 5 and 6, although there was no quantitative increase, there was a premature progression to a more advanced stage of spermatogenesis. Experiments 8, 9, and 10, initiated after the peak of testicular size had been achieved, yielded variable results. Normally, animals at this time should have progressed from spermatogenic Stages III or IV towards Stages V or VI with the number of dividing spermatogonia and spermatocytes rapidly decreasing. In some individuals, however, all classes of germinal cells were well represented so that the gonad appeared to be in Stage III. In other individuals spermatogenesis was maintained at about a Stage IV level without showing the expected decline. In still others, germinal elements characteristic of Stages I and V were present in the same seminiferous tubule, although intervening stages were missing. This suggests a premature beginning of a new cycle before the completion of the old.

The sexual segment of the kidney appeared to be refractive in experiments started in September and only mildly responsive in October. Although this could be due to a difference in the cyclic nature of the two pituitary gonadotropins as suggested by Farner and Mewaldt (1955), we believe that the poor response of the sexual segment can be accounted for by the delay in arousing the atrophic interstitial cells.

*Inherent rhythm.* In studying the natural reproductive cycle of male anoles, Fox (1958) noted that spermatogenesis is initiated in the fall and makes considerable progress during the winter despite the short day-lengths. We sought to determine if there was any period during the year in which constant exposure to short days would disrupt the normal cycle. Nine hours of artificial light were chosen instead of 10 (day-length at time of the winter solstice at New Orleans) since 10 hours of bright light obviously are not available during the winter. Nine hours did not disrupt the cycle of immature lizards exposed from October through December and from January through March, nor that of adults exposed from December through February. The accessory sex organs likewise were not retarded by the short days. In fact, in adults of the 9L sample killed in February, both the testes and accessory sex organs were more advanced than those of wild lizards at that date. At the time of writing, additional data were furnished us by Anthony Dimaggio of our Biochemistry Department. Six immature males (49–57 mm.), maintained at 6L for 60 days ending March 1, appeared to have normally active testes (left testis ranged 12–40 mg., mean = 24.3 ± 11.4 mg.). One must conclude that reasonably short day-lengths are not very effective in disrupting the inherent reproductive rhythm of male anoles in the fall, winter and early spring when gametogenesis is on the upswing.

Fox (1958) also stated that the peak of spermatogenesis for most individuals of this species was achieved in April. Normally, all maintained quite active spermatogenesis through July. Experiment 7 showed that six-hour day-lengths, initiated at the end of April, produced a significant reduction in testis weight within 34 days, and highly significant atrophy in both the testes and accessory sex organs within 52 days. Nine hours of light (Experiment 8) from May to July also precipitated complete involution. Thus, it appears that short days will end a reproductive cycle prematurely after the cycle has neared or passed maximum development.

*The stimulating effects of different day-lengths.* Since our original choice of 18 hours for a long day stimulus was arbitrary, we designed three experiments to test whether shorter periods might be equally stimulating but have fewer detrimental effects upon the animals. In Experiment 3 all average measurements of the 16L sample tended to be greater than the 18L sample. However, the larger gonads and more secretory sexual segments occurred in the 18L sample. In Experiment 5 a few adults on 16L gave a better response than any on 18L. The smaller gonads of the 18L sample were judged on a histological basis to be more advanced than those of the controls, but there does not appear to be justification to similarly appraise the smaller accessory sex organs. We believe the data for the 18L sample reflect the exhausting features of long hours of wakefulness imposed upon animals with initially low fat reserves, rather than a lack of stimulation. Experiment 10 demonstrated that exposure to 14L from August to October was stimulating (Fig. 1) to the testes but had little effect on the accessory sex organs. On the other hand, 18L was very stimulating to both gonads and accessory sex organs.

These data indicate that, in general, the longer the day-length the greater the response of the reproductive organs of *Anolis*. A similar relationship between day-length and gonadal size of the white-crowned sparrow has been thoroughly analyzed recently by Farner and Wilson (1957). Eighteen-hour day-lengths appeared detrimental to many anoles (see mortalities and starvation, Table II). For this reason a 16-hour day-length is probably more satisfactory to use as a long day stimulus for *Anolis*. Fourteen-hour day-lengths (the maximum day-length for New Orleans) are definitely stimulatory to *Anolis*, but the response may not be sufficiently rapid to be detected in short-term experiments. Dessauer (1953) found no differences in the metabolism of anoles exposed for three weeks to 10L and 14L. It now seems likely, in view of the considerable individual variation he obtained, that three weeks may not have been sufficient or the light regimes not sufficiently different to produce marked contrasts in metabolism.

*The response of immature lizards compared to adults.* The usefulness of immature anoles as experimental animals was impaired by their high mortality rate which resulted from starvation. However, since they were more abundant than adults in the wild it was easier to collect a large series. They responded to the long day-length exposure very much as did the adults without any indication of a refractory period. Comparison of the September experiments (I and II) indicates that the non-starving immature lizards responded as well as or better than the adults. Likewise, the maximal measurements recorded in Experiment 6 (Table II, Fig. 1) compare favorably with the best results on adults in any experiment. The most striking responses occurred in animals above 55 mm. (snout-vent

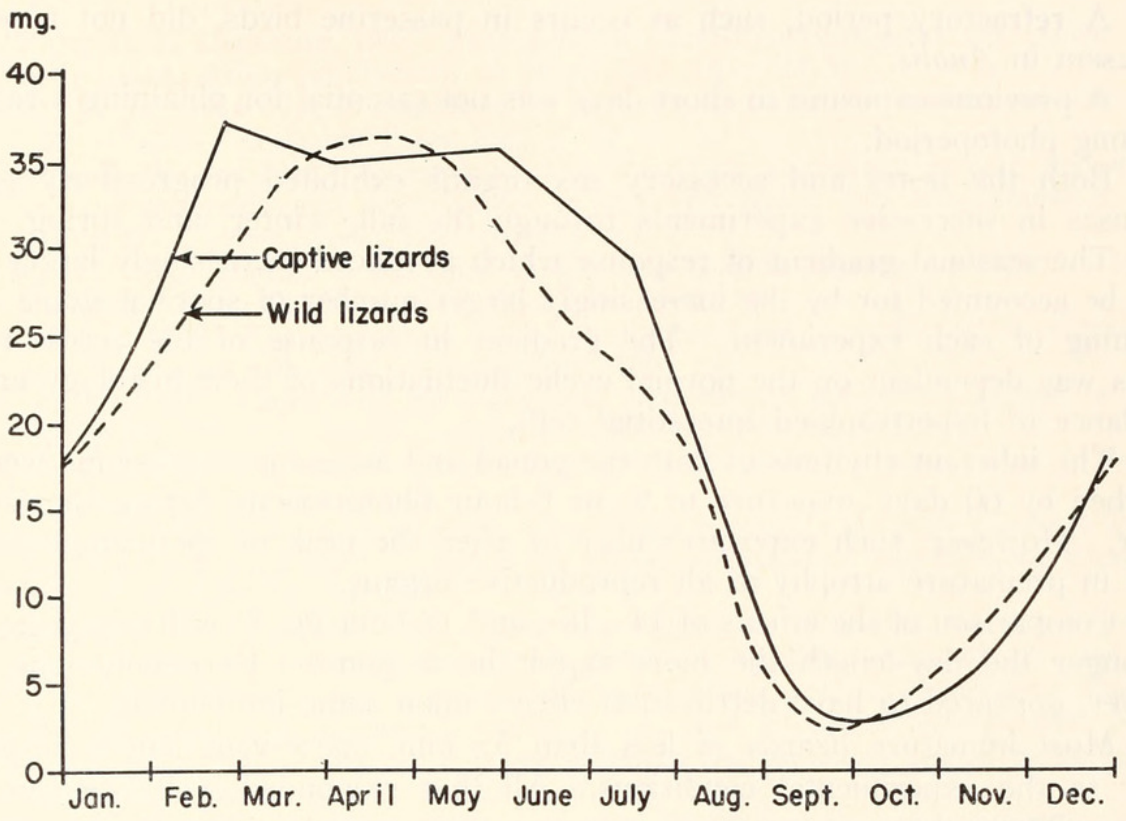


FIGURE 2. Curves comparing the annual variation in testis weights of freshly captured wild anoles (Fox, 1958) with those of laboratory controls exposed to natural day-lengths for 60 days.

length). All individuals with markedly active reproductive organs grew at least several millimeters and usually reached 60 mm. in snout-vent length.

Data on animals maintained for periods longer than two months suggest that unseasonal long photoperiods are most stimulating during the first two months. Over a period of four months the controls matched the earlier achievements of the experimentals and the experimentals regressed.

*Captive lizards exposed to natural day-lengths compared to wild lizards.* The marked weight difference between testes of two-month captive lizards exposed to natural day-lengths and wild lizards can be seen in Figure 2. From February through most of July the testes of captive controls usually averaged significantly heavier than those of wild animals sacrificed at approximately the same date. Bartholomew (1950) found similar differences in captive and wild yucca night lizards (*Xantusia vigilis*). He suggested that the differences were due to the higher temperature of the laboratory. In the case of *Anolis,* this could easily account for the differences during the winter and spring but it is doubtful whether it would account for the differences in June and July.

## SUMMARY

1. The reproductive system of adult male anoles was stimulated by artificially lengthened days at all seasons of the year. At least a few males were brought into full breeding condition in experiments initiated from November through August. Sperm were produced at all seasons.

2. A refractory period, such as occurs in passerine birds, did not appear to be present in *Anolis*.

3. A previous exposure to short days was not essential for obtaining a response to a long photoperiod.

4. Both the testes and accessory sex organs exhibited progressively greater responses in successive experiments through the fall, winter, and spring.

5. The seasonal gradient of response which produced increasingly larger testes could be accounted for by the increasingly larger number of spermatogonia at the beginning of each experiment. The gradient in response of the accessory sex organs was dependent on the normal cyclic fluctuations of their histology and the abundance of hypertrophied interstitial cells.

6. The inherent rhythms of both the gonad and accessory sex organs were not disturbed by 60 days' exposure to 9- or 6-hour photoperiods during the fall and winter. However, such exposures near or after the peak of spermatogenesis resulted in premature atrophy of all reproductive organs.

7. Comparison of the effects of 14-, 16-, and 18-hour day-lengths suggested that the longer the day-length the more rapid the response. Extremely long days, however, appeared to have detrimental effects upon some individuals.

8. Most immature lizards of less than 55 mm. snout-vent length responded poorly to the experimental conditions. All that responded well were growing rapidly. Those that grew to 60 mm. or more had reproductive organs as large or larger than older adults.

9. Captive lizards exposed to natural day-length for 60 days at any time between February and July tended to have larger testes than wild lizards sacrificed on the same date.

## LITERATURE CITED


Bartholomew, G. A., Jr., 1950. The effects of artificially controlled temperature and day length on gonadal development in a lizard, *Xantusia vigilis*. *Anat. Rec.*, **106**: 49–59.

Bartholomew, G. A., Jr., 1953. The modification by temperature of the photoperiodic control of gonadal development in the lizard *Xantusia vigilis*. *Copeia*, **1953**: 45–50.

Burger, J. W., 1937. Experimental sexual photoperiodicity in the male turtle, *Pseudemys elegans* (Wied.). *Amer. Nat.*, **71**: 481–487.

Burger, J. W., 1949. A review of experimental investigations on seasonal reproduction in birds. *Wilson Bull.*, **61**: 211–230.

Clausen, H. J., and E. G. Poris, 1937. The effect of light upon sexual activity in the lizard, *Anolis carolinensis*, with especial reference to the pineal body. *Anat. Rec.*, **69**: 39–50.

Dessauer, H. C., 1953. Hibernation of the lizard, *Anolis carolinensis*. *Proc. Soc. Exp. Biol. Med.*, **82**: 351–353.

Farner, D. S., 1955. The annual stimulus for migration: experimental and physiologic aspects. *In:* Recent Studies in Avian Biology, Ed. by A. Wolfson, Publ. by Amer. Ornithol. Union, Univ. Ill. Press, Urbana.

Farner, D. S., and L. R. Mewaldt, 1955. The natural termination of the refractory period in the white-crowned sparrow. *Condor*, **57**: 112–116.

Farner, D. S., and A. C. Wilson, 1957. A quantitative examination of testicular growth in the white-crowned sparrow. *Biol. Bull.*, **113**: 254–267.

Forbes, T. R., 1941. Observations on the urogenital anatomy of the adult male lizard, *Sceloporus*, and on the action of implanted pellets of testosterone and esterone. *J. Morph.*, **68**: 31–69.

Fox, W., 1952. Seasonal variation in the male reproductive system of Pacific Coast garter snakes. *J. Morph.*, **90**: 481–553.

Fox, W., 1958. Sexual Cycle of the male lizard, *Anolis carolinensis*. *Copeia*, **1958**: 22–29.

Fox, W., and H. C. Dessauer, 1957. Photoperiodic stimulation of appetite and growth in the male lizard, *Anolis carolinensis*. *J. Exp. Zool.*, **134**: 557–575.

Hammond, J., Jr., 1954. Light regulation of hormone secretion. *Vitamins and Hormones*, **12**: 157–206. Academic Press Inc., N. Y.

Harrington, R. W., Jr., 1956. An experiment on the effects of contrasting daily photoperiods on gametogenesis and reproduction in the centrarchid fish, *Enneacanthus obesus* (Girard). *J. Exp. Zool.*, **131**: 203–223.

Keck, W. N., 1933. Control of bill color of the male English sparrow by injection of male hormone. *Proc. Soc. Exp. Biol. Med.*, **30**: 1140–1141.

Miller, A. H., 1948. The refractory period in light-induced reproductive development of golden-crowned sparrows. *J. Exp. Zool.*, **109**: 1–11.

Miller, A. H., 1949. Potentiality for testicular recrudescence during the annual refractory period of the golden-crowned sparrow. *Science*, **109**: 546.

Miller, A. H., 1954. The occurrence and maintenance of the refractory period in crowned sparrows. *Condor*, **56**: 13–20.

Reynolds, A. E., 1943. The normal seasonal reproductive cycle in the male *Eumeces fasciatus* together with some observations on the effects of castration and hormone administration. *J. Morph.*, **72**: 331–377.

Van Oordt, P. G. W. J., 1956. The role of temperature in regulating the spermatogenetic cycles in the common frog (*Rana temporaria*). *Acta Endocrinol.*, **23**: 251–264.

Vaugien, L., 1955. Sur les réactions testiculaires du jeune moineau domestique illuminé a diverses époques de la mauvaise saison. *Bull. Biol. France et Belgique*, **89**: 218–243.

Wolfson, A., 1952. The occurrence and regulation of the refractory period in the gonadal and fat cycles of the junco. *J. Exp. Zool.*, **121**: 311–326.

Wolfson, A., 1954. Production of repeated gonadal, fat, and molt cycles within one year in the junco and white-crowned sparrow by manipulation of day length. *J. Exp. Zool.*, **125**: 353–376.