ROLE OF LIGHT IN THE PROGRESSIVE PHASE OF THE PHOTOPERIODIC RESPONSES OF MIGRATORY BIRDS [1]

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Two conspicuous changes in physiological state precede the initiation of northward migration in late March in the slate-colored junco (*Junco hyemalis*)—gonadal recrudescence and fat deposition. The occurrence of these changes is regulated by day-length and in two separate phases. The first phase, called the preparatory phase, occurs in the fall and requires short days for its completion. The second phase, called the progressive phase, occurs in late fall and winter, and the rate at which it proceeds is a function of the daily photoperiod and is extremely rapid under long days. The summation of different degrees of physiological response to the daily light-dark cycles has been postulated to explain the events in these phases. (See Wolfson, 1959a, 1959b for review and references.)

The observations that interruption of the long night of a short day with a brief period of light during the progressive phase results in a rate of response comparable to that of a long day (Kirkpatrick and Leopold, 1952; Jenner and Engels, 1952) and that administration of the same total photoperiod in smaller doses induces a more rapid response (Wolfson, 1953), raised the question of the roles of light and darkness. The experiments reported here are concerned with the progressive phase and are part of an extensive series which was designed to determine the roles of light and darkness in both the progressive and preparatory phases. A brief summary of the results of all previous experiments and a preliminary report of one aspect of the present experiments have been reported (Wolfson 1959a, 1959b, 1959c).

The roles of light and darkness were explored in the present study by combining effective daily photoperiods with inhibitory dark periods and testing their effects in the winter and spring.

Materials and Methods

The species used was the slate-colored junco. Its geographic distribution and migration and the sequence of events in its annual cycle have already been described (Wolfson, 1952a). The birds were trapped during the period of fall and spring migration and were held in captivity under natural day-lengths (including civil twilight) until the experiment began. Artificial illumination only was used in all experimental rooms and was provided by 40-watt white fluorescent bulbs. Intensity varied from approximately 15 to 60 foot candles, depending on the dis-

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tance of the cages from the light source. Temperature was not controlled and the rooms were unheated. In the winter experiment, the temperature ranged from 67°–79° F.; in the spring experiment it ranged from 60°–80° F. and was usually in the low 70's. Conditions of captivity, feeding, and care were similar to those already reported. Observations were made on fat deposition, body weight, and reproductive activity using methods described previously (Wolfson 1952a, 1954).

Experiments and Results

Experiment 1. In this experiment a stimulatory photoperiod, 12 hours, was combined with an "inhibitory" dark period, 16 hours, to give a 28-hour cycle. The experiment began on December 11 with 19 juncos and was continued to

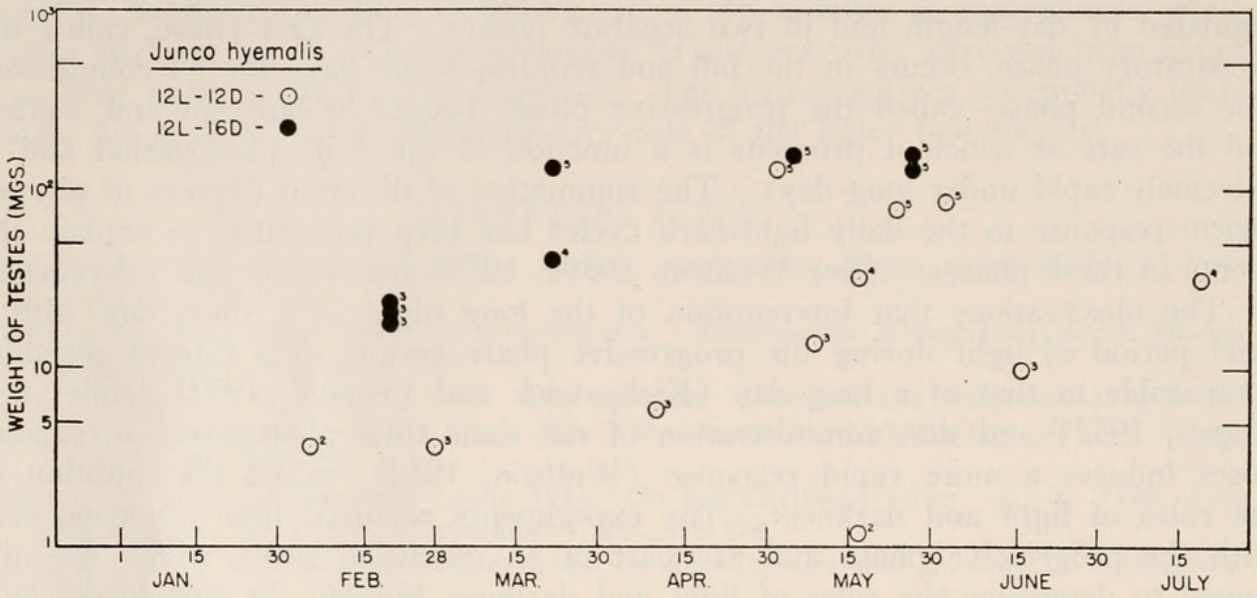

Figure 1. Testicular response in the light-dark cycles indicated. Weight of testes on logarithmic scale. Numbers adjacent to points indicate stage of spermatogenesis; stage 1 is minimum and 5 is maximum.

May 27, well beyond the normal time of response to experimental treatment, in order to determine the duration of the response, which is diagnostic for a 12-hour photoperiod. By the starting date, the birds had completed the preparatory phase.

Sixteen (11 ♂♂, 5 ♀♀) of the 19 birds showed a gonadal response. Figure 1 shows the rate of response for the responsive males and permits comparison of this rate with that of juncos treated with 12L–12D cycles beginning December 4 (Winn, 1950). The rate of response was clearly greater in the present experiment. The duration of response, however, was similar and was longer than that in birds treated with approximately 16-hour photoperiods, in which testicular activity ends by March 15 (Wolfson 1959b). The seminal glomera (vesicles) were not well developed, not even in the three birds autopsied in May which showed complete spermatogenesis (Stage 5). Histological study revealed normal spermatogenesis except perhaps in two males (autopsied on May 27) in which the germinal epithelium was extremely low and the lumens of the tubules extremely large. The mean ovarian weight for four responsive females autopsied in April and May was 26.3 (21.0–36.2) grams.

Seventeen of the 19 birds also showed a typical vernal fat response and concomitant increase in body weight. Figure 2 shows the mean body weights for the females and two groups of males, one of which responded sooner. The mean increase in weight (and range) from initial weight to maximum response weight (when birds were in the heavy fat class) was 5.2 (2.7–6.6) grams for 12 males and 5.4 (4.7–6.1) grams for 5 females. For the three groups graphed in Figure 2, the mean increases for each were, respectively, 5.6, 5.6, and 5.4 grams, and each represented a 31 per cent increase from the initial weight. Using the median date between successive weighings when the birds first showed moderate or heavy fat deposits, it took from 15–83 days for the fat response; the mean for the central

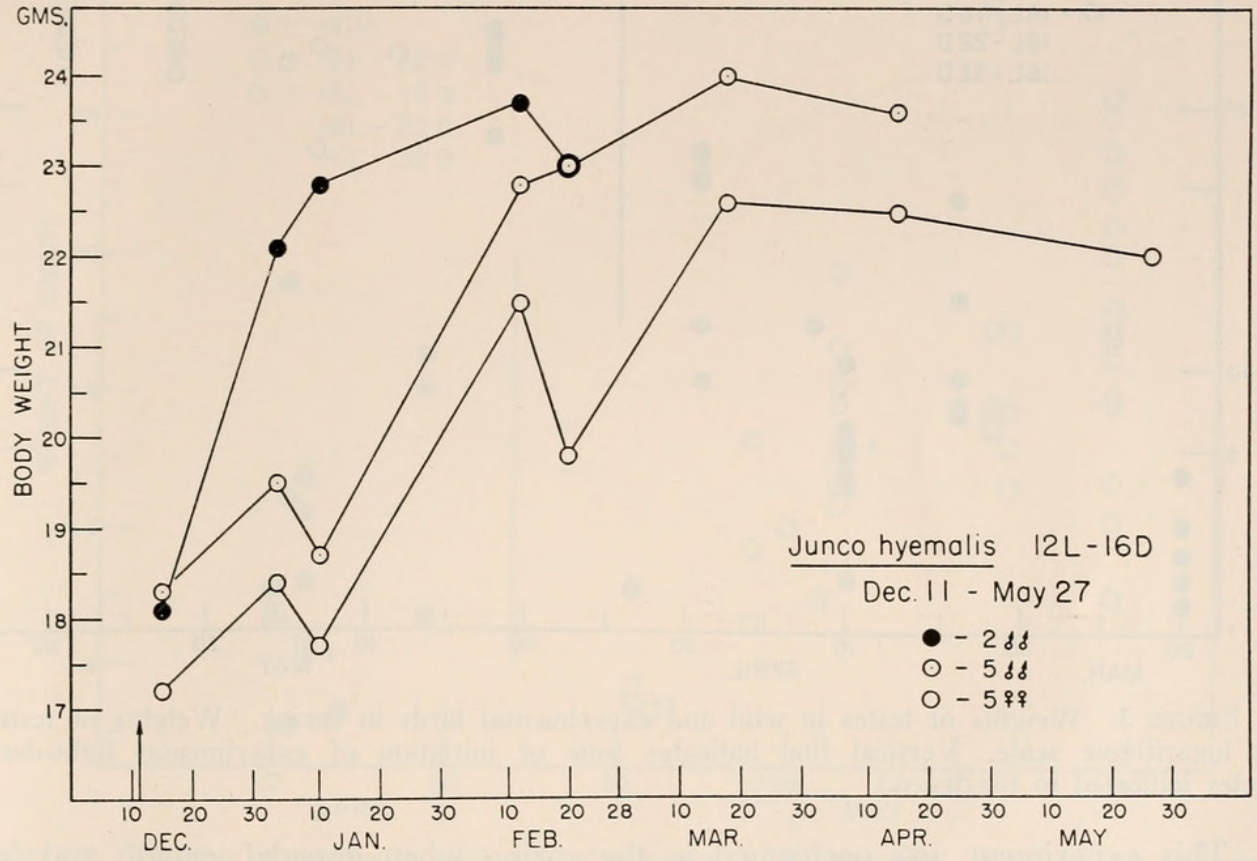

FIGURE 2. Changes in body weight in the 12L–16D group from December 11 to May 27. Each point is the mean weight for the three groups shown in the legend.

group of seven males was 54 days; for a similar group of four females it was 48 days. Comparable figures from the previous 12L–12D experiments (two separate experiments, and without separation of data for each sex) were 61 days and 79 days, and in these experiments only two males in a group of 23 responded in less than 55 days. The mean increase in weight for 12 birds in one experiment was 5.6 grams, which is almost identical with that in the present experiment.

Experiment 2. From the results of experiment 1 it seemed likely that the photoperiod determined the response and that the dark period was not inhibitory. But a possible weakness in that experiment was the ratio of light to darkness, which was close to one, and hence simulated a 12L–12D cycle. In an 8L–16D schedule, when the dark period appeared to be inhibitory, the ratio of dark to light was 2:1. To test further, therefore, the following schedules were selected,

using longer dark periods: 12L–20D; 16L–16D; 16L–22D; 16L–32D. The 16-hour photoperiod was selected because it is more stimulating than a 12-hour photoperiod; the 16L–32D cycle was chosen to obtain the same ratio of dark to light as in the nonstimulatory 8L–16D cycle. The lights were controlled automatically by time switches prepared specially for this study by the Aemco Corporation (Mankato, Minnesota).[2]

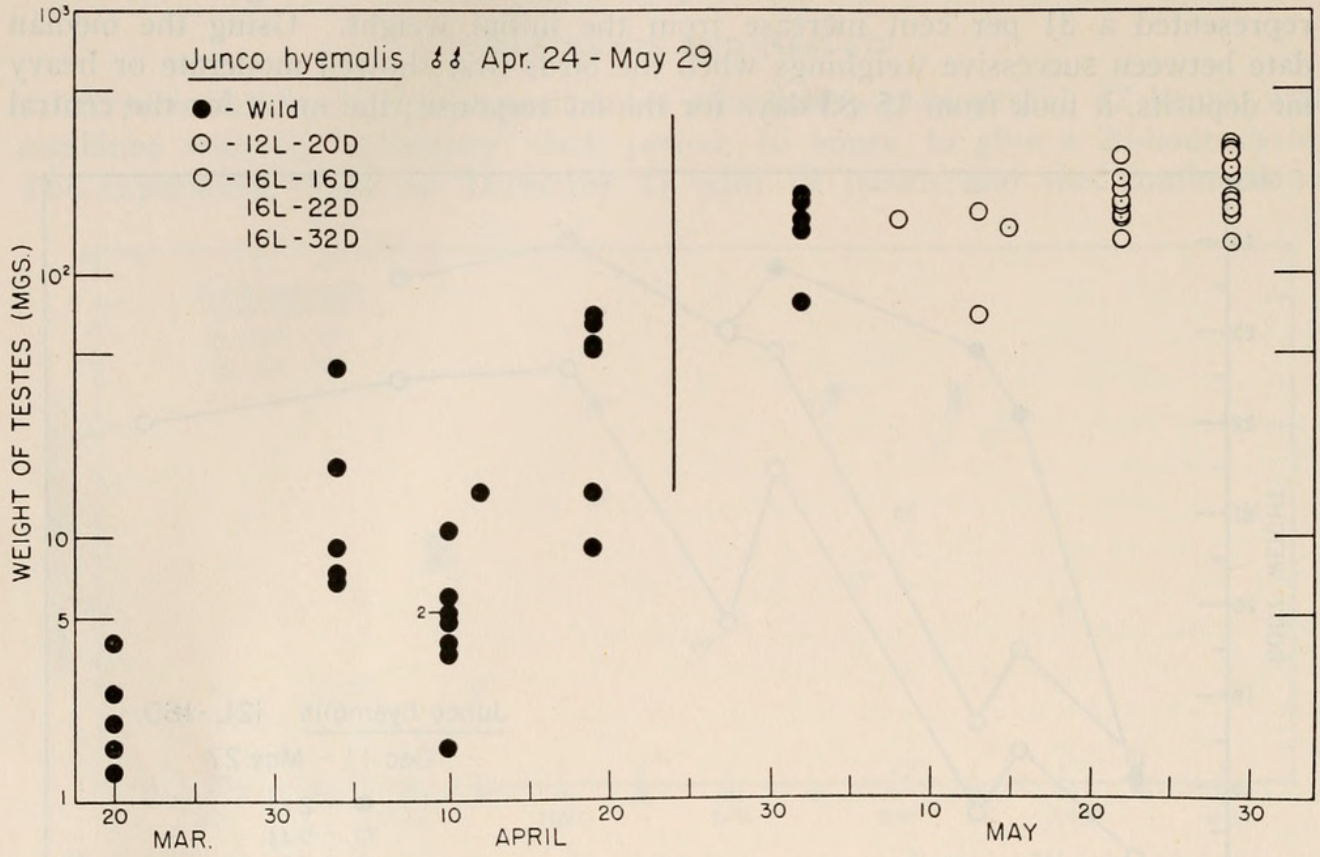

FIGURE 3. Weights of testes in wild and experimental birds in spring. Weights of testes on logarithmic scale. Vertical line indicates time of initiation of experimental light-dark cycles indicated in the legend.

This experiment was performed in the spring when gonadal growth and fat deposition were already underway, but continuation and maintenance of these responses are also regulated by photoperiod. For example, treatment with long daily dark periods, as in a 9L–15D cycle, beginning in late April, results in gonadal regression and loss of fat deposits within a few weeks; in cycles of 12L–12D, 20L–4D, and under natural day-lengths the responses continue, with a slower rate and a protracted response under 12-hour photoperiods.

The experiment began on April 24 and ended May 29; 68 juncos were used. The number and sex of the birds in each group were as follows: 12L–20D—11 ♂♂, 10 ♀♀; 16L–16D—3 ♂♂, 8 ♀♀; 16L–22D—6 ♂♂, 11 ♀♀; 16L–32D—2 ♂♂, 12 ♀♀.

Gonadal data were obtained for 22 ♂♂ and 41 ♀♀. The response of the males is shown in Figure 3, that of the females in Figure 4. Also shown are gonadal

[2] In a later study it was discovered that the light period in the 16L–32D cycle was 20–25 minutes shorter and the dark period correspondingly longer. This difference does not affect the design or results of the experiments, and for convenience the cycle is still referred to as 16L–32D.

data from wild birds and birds held under natural day-lengths to indicate the normal rate of growth and the condition of the gonads when the experiment began. It is clear from Figure 3 that all of the males maintained their reproductive activity. Histological study showed that spermatogenesis was normal except perhaps in a few cases where the lumens of the tubules were larger than normal because of the decreased depth of the germinal epithelium. There was a slight retardation in spermatogenesis in three birds, since they attained only stage 4 by the end of May. One occurred in each of the groups except the 16L–16D. The

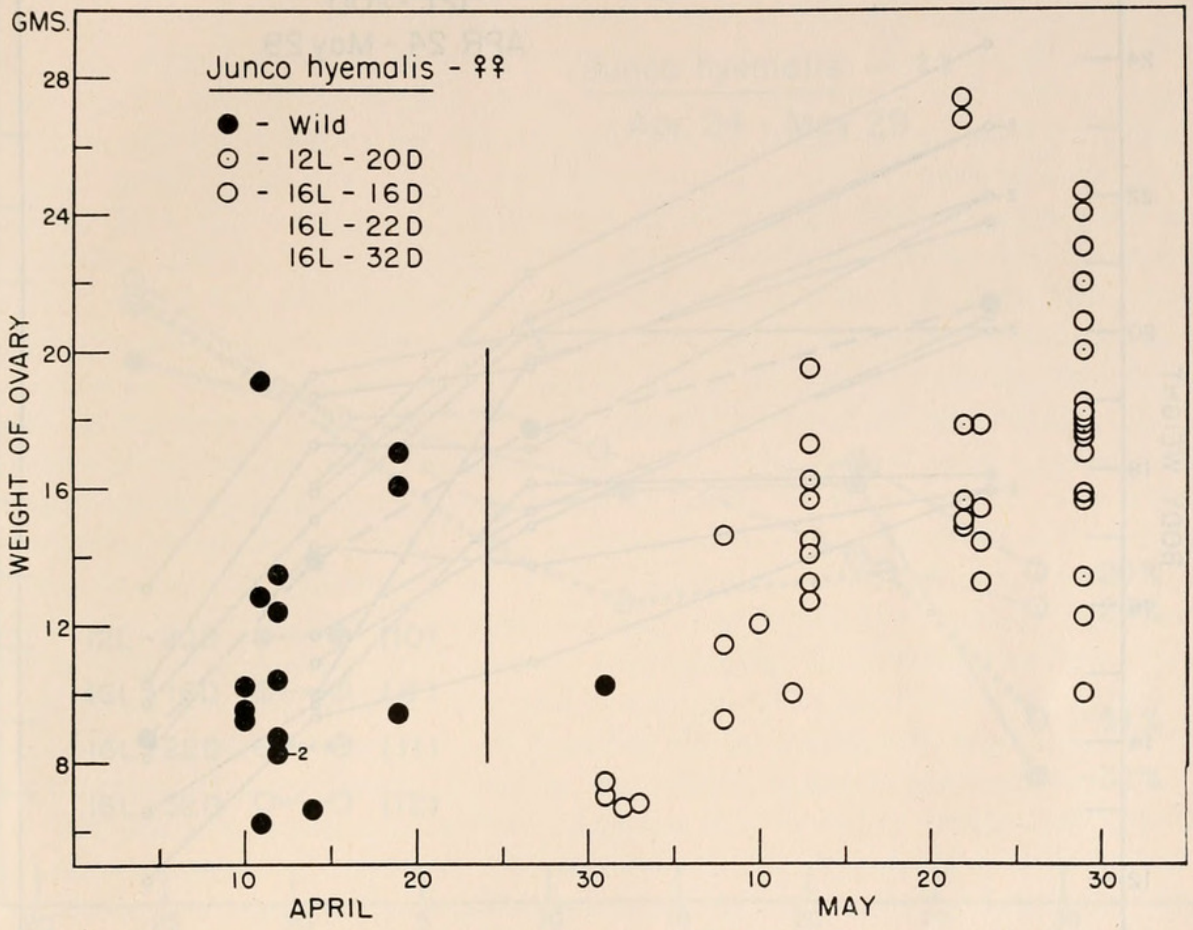

FIGURE 4. Weights of ovaries in wild and experimental birds in spring. Vertical line indicates time of initiation of experimental light-dark cycles.

seminal glomera were at maximal or submaximal size by May 29, except in a few cases. The testes of birds which received only 12 hours of light daily were as well developed as those in birds which received 16 hours of light.

The response of the females (Fig. 4) was more variable, but the results clearly indicate continuing growth of the ovary and maintenance of activity in all birds. The size of the largest follicles ranged from 1–2 millimeters by late May; this is the normal maximum size in captive birds. Inactive ovaries normally weigh less than 10 milligrams. The mean weight (and range) for four inactive ovaries obtained in the first 10 days of the experiment was 7.0 (6.6–7.4) milligrams.

Of the 68 birds in the experiment, 64 lost weight and reached below-normal weights which ranged from 12–15 grams. Only four birds failed to show abnormal losses in weight, and three of these were in the 16L–16D group. Represent-

ative data for individual birds are presented in Figure 5, which shows the weight changes in the males in the 12L–20D group. The individual curves show the uniformity of response. The weight loss, to about 18 grams, was due mostly to loss of fat from subcutaneous and intraperitoneal depots. The mean loss was about 6 grams and the percentage lost (from the initial mean weight) was about

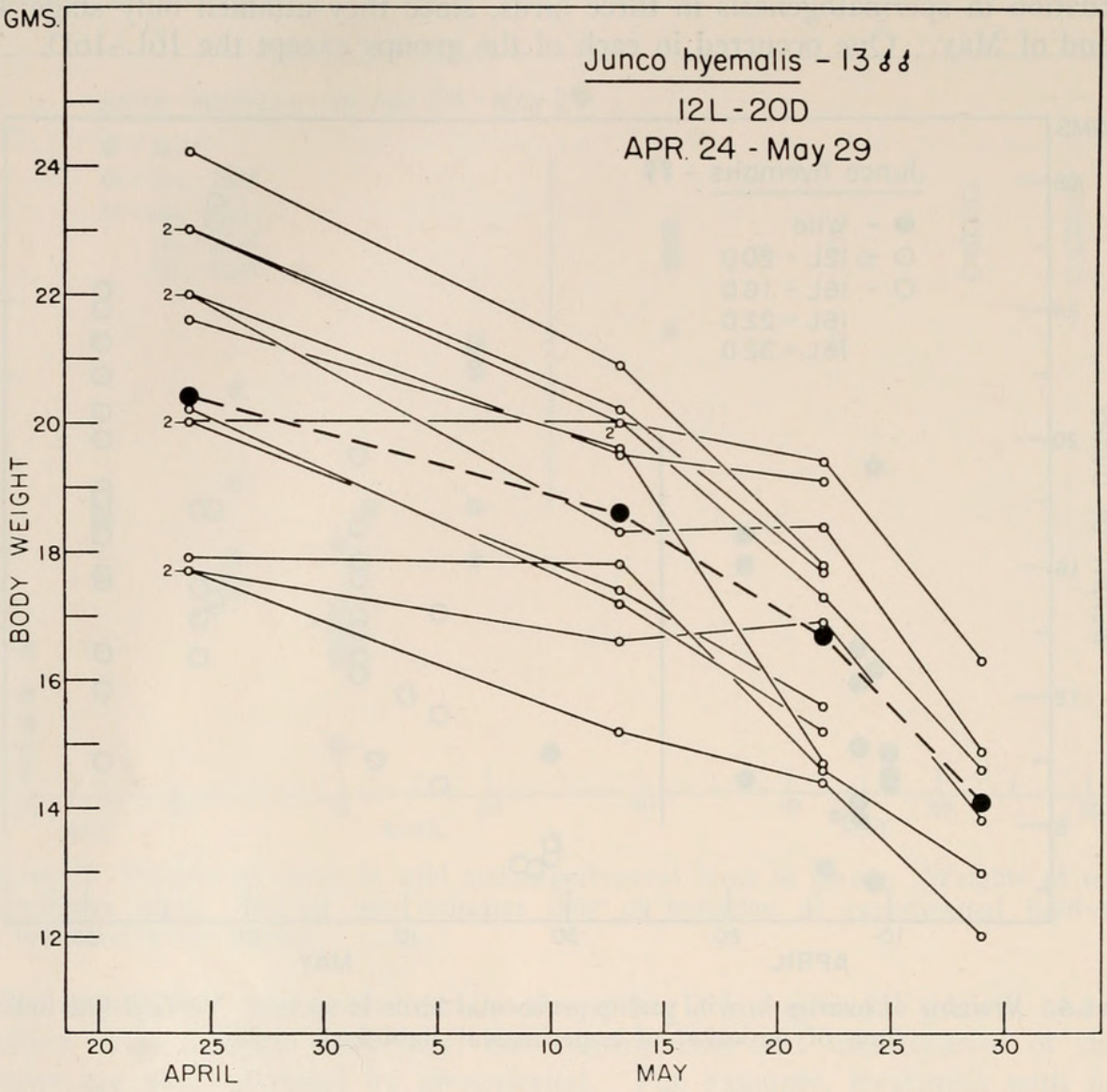

FIGURE 5. Changes in body weight in individual male juncos treated with 12L–20D cycles in spring. Mean for the group shown by large points and dashed line.

31 per cent. Adequate numbers of females occurred in all groups to permit calculation of the mean weight curves which are given in Figure 6. Marked losses occurred in all groups, reaching abnormally low levels especially in the 12L–20D and 16L–22D groups. The weight loss of 32 per cent in 12L–20D females was almost identical to that in the males.

It is evident that the gonadal response continued despite excessive losses in weight. For 12 birds that showed the greatest gonadal weights on May 22 and May 29, the mean weight loss was 5–6 grams except in the 16L–16D group where it was 2–3 grams. No. 464 in the 16L–22D group had the largest testes of all

the birds on May 29 (312.6 milligrams); its body weight was 13.4 grams, which was 6.6 grams less than its initial weight.

Discussion and Conclusions

From the results of their studies Kirkpatrick and Leopold (1952) and Jenner and Engels (1952) assigned an inhibitory role to the long dark period of short winter days. On the basis of the experiments reported here it seems unlikely

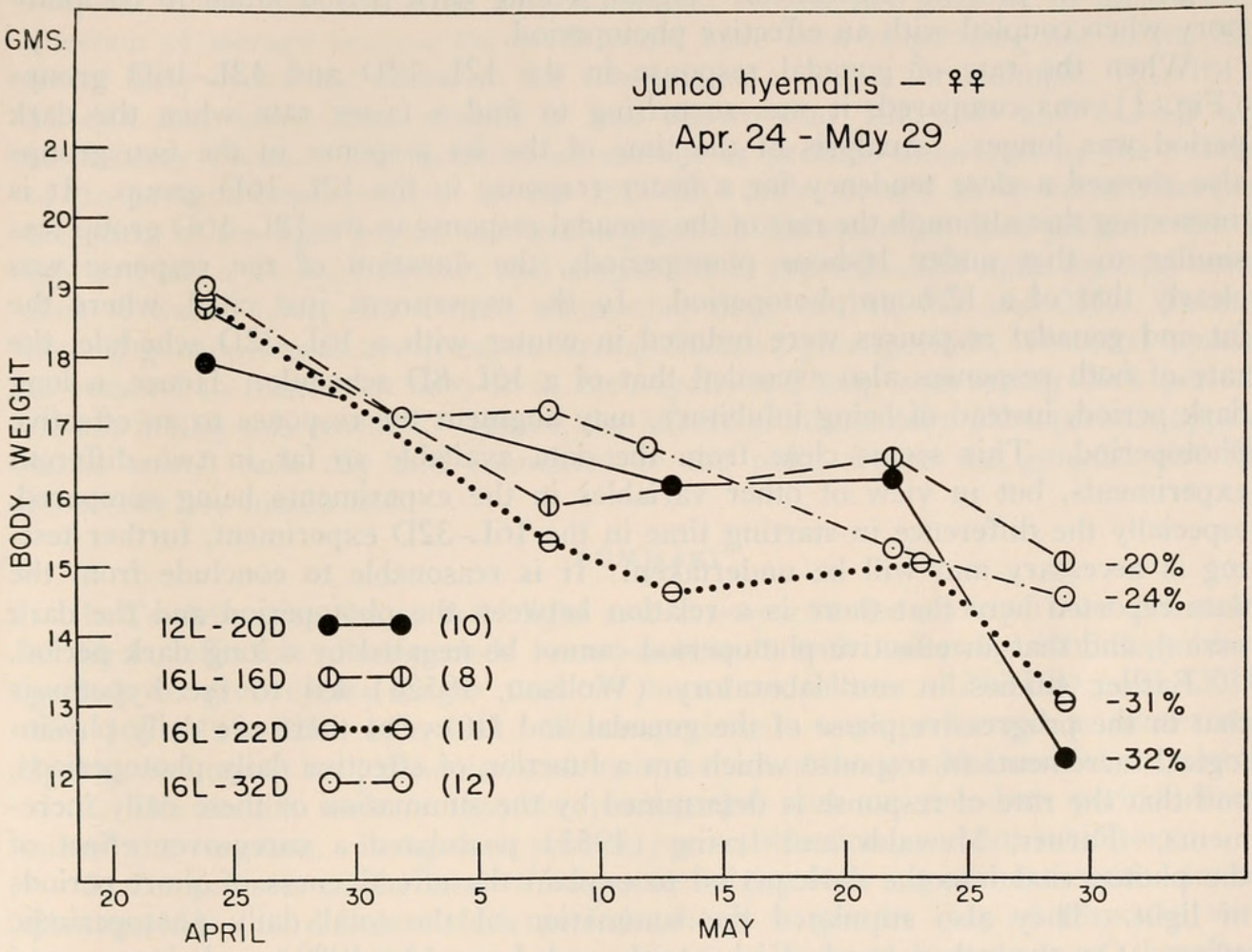

Figure 6. Changes in mean body weight in females in the light-dark cycles indicated. Number of birds in each group is given in parentheses in the legend. Percentage lost is based on mean initial weight.

that the dark period is inhibitory. The responses simulated those obtained with similar photoperiods rather than similar dark periods. This was brought out particularly by the 12L–16D schedule in the first experiment. Not only did the birds respond, but the duration of the response was longer than normal and characteristic of 12-hour photoperiods (Wolfson, 1952b, 1959b). Also, the annual molt was not induced and this also is characteristic of exposure to 12-hour photoperiods. By contrast, birds treated with approximately 16-hour or longer photoperiods beginning in December showed a shorter duration of response and were usually molting by April 1.

In the spring experiments, long dark periods also failed to inhibit gonadal

activity. It is clear that an effective photoperiod, for example, 12 or 16 hours long, can maintain gonadal activity despite long intervening dark periods, even when the dark period is twice as long as the light period, as in the 16L–32D schedule. The ability of the birds to maintain gonadal activity under this schedule raised the question of whether such a schedule could induce the gonadal response in winter. This was tested recently, and not only was a gonadal response obtained but also a fat response (Wolfson and Winchester, unpublished data). The occurrence of the fat response was completely unexpected, since the birds lost weight in the present experiment. Again, a long dark period failed to be inhibitory when coupled with an effective photoperiod.

When the rate of gonadal response in the 12L–12D and 12L–16D groups (Fig. 1) was compared, it was surprising to find a faster rate when the dark period was longer. Analysis of the time of the fat response in the two groups also showed a clear tendency for a faster response in the 12L–16D group. It is interesting that although the rate of the gonadal response in the 12L–16D group was similar to that under 16-hour photoperiods, the duration of the response was clearly that of a 12-hour photoperiod. In the experiment just cited, where the fat and gonadal responses were induced in winter with a 16L–32D schedule, the rate of both responses also exceeded that of a 16L–8D schedule. Hence, a long dark period, instead of being inhibitory, may augment the response to an effective photoperiod. This seems clear from the data available so far in two different experiments, but in view of other variables in the experiments being compared, especially the difference in starting time in the 16L–32D experiment, further testing is necessary and will be undertaken. It is reasonable to conclude from the data reported here that there is a relation between the photoperiod and the dark period, and that an effective photoperiod cannot be negated by a long dark period.

Earlier studies in our laboratory (Wolfson, 1952b) led to the hypothesis that in the progressive phase of the gonadal and fat cycles there are daily physiological increments of response which are a function of effective daily photoperiods, and that the rate of response is determined by the summation of these daily increments. Farner, Mewaldt and Irving (1953) postulated a carry-over effect of the photoperiod into the dark period to explain the effectiveness of short periods of light. They also stipulated the summation of the total daily photoperiodic effect. On the other hand, Kirkpatrick and Leopold (1952) and Jenner and Engels (1952) assigned an active regulatory role to the dark period. All of these interpretations have been derived indirectly from the manifested response of the "effectors"—the gonads and the fat depots. Very little is known about the dynamics of the specific events which regulate the gonadal and fat cycles, but two organs are clearly involved, the hypothalamus and the pituitary (see Wolfson, 1959b, and Farner, 1959, for review and summary). The results of the present experiments lend support to the primary role of the photoperiod, and there is no evidence for an inhibitory role of darkness. When there is no manifest response in a particular photoperiodic schedule, for example 8L–16D, the failure appears to be caused by an ineffective photoperiod, for when the photoperiod was increased to 12 hours a response occurred, even though the dark period remained the same. The difference in rate of response in the 12L–12D and 12L–16D groups suggests that the daily response may be a function of the photoperiod and the subsequent

dark period. The dark period could function as a period of time which permits the effect of the photoperiod to continue, as postulated in the carry-over effect, but this would not explain the difference in rate of response in the 12L–12D and 12L–16D groups if the duration of the carry-over effect is the same for a given photoperiod.

One of the questions which was asked in previous studies (Wolfson, 1959a) was whether the bird could "store" the effects of stimulatory light-dark cycles when nonstimulatory cycles intervened. Cycles of 8L–16D were alternated with 16L–8D cycles and the birds responded, but it was not possible to answer the question of storage because the birds could have been responding not to the different daily cycles but rather to the schedule as a whole, for example, 16L–16D–8L–8D, and from other studies it was known that 16L–16D and 8L–8D are stimulatory cycles. The question of storage is perhaps answered by the results of the present experiments. In the 16L–32D cycle, to use an extreme example, the effect of the light period was not negated by the extended dark period. Or, if the bird has a 24-hour rhythm and responded to the 16L–32D cycle on that basis, then it would have experienced alternating days of 16L–8D and 24D. In any case, if gonadotropins are released during effective photoperiods, the testes appear to respond to them each day, or in each cycle, and long intervening dark periods do not inhibit this reaction. Hence, the effects of stimulatory photoperiods appear to be stored each day and summated to the point where the gonadal and fat responses are manifested.

Summary

1. To determine the roles of light and darkness in the photoperiodic responses of birds, effective photoperiods were combined with normally inhibitory dark periods to give cycles longer than 24 hours as follows: 12L–16D; 12L–20D; 16L–16D; 16L–22D; 16L–32D. The experiments were performed in winter and spring, and tested both induction and maintenance of the gonadal and fat responses.

2. The results indicate that the photoperiod and not the dark period determined the response. Long dark periods *per se,* therefore, are not inhibitory.

3. Comparison of the rate of response in previous experiments with similar photoperiods but different dark periods shows a greater rate of response with longer dark periods. The response to a given light-dark cycle may, therefore, be a function of the photoperiod and the subsequent dark period.

4. The results also indicate that the gonadotropic and lipogenetic effects of each stimulatory light-dark cycle are "stored," or cannot be negated by long intervening dark periods.

5. The relation of these findings to theories of the mechanism of response to light and dark is discussed.

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