EVIDENCE FOR A REFRACTORY PERIOD IN THE DICKCISSEL (*SPIZA AMERICANA*)

by

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Not only is photoperiodic control of annual gonadal cycles well documented for many Temperate Zone species of birds (see Farner, 1959, 1961, 1964a, 1964b, 1967, 1970; Wolfson 1959a, 1960, 1966; Farner and Follett, 1966), but temporal patterns and rates of photoinduced gonadal development have been described and quantified as well (Farner et al., 1966). Although equatorial migrants also have well-defined annual reproductive cycles, there are few experimental studies that demonstrate photoperiodic control of gonadal growth and refractoriness. Because such birds experience little or no change in daylength, endogenous factors may play an important role in control of the annual cycle (Curry-Lindahl, 1958; Marshall and Williams, 1959; Marshall, 1960; Miller, 1965). Lofts (1962, 1964) showed that gonadal growth in an equatorial weaver finch (Quelea quelea) is due largely to an endogenous rhythm, and demonstrated (1962) the presence of a well-defined refractory period which is independent of the photoperiod. Additional studies have demonstrated the effect of environmental factors such as drought and rainfall on reproduction of tropical species (see Wolfson, 1959a; Miller, 1960). However, as Wolfson (1959a) emphasizes, daylength cannot be ruled out a priori as a fundamental regulator of migration and breeding cycles in tropical species simply because it is relatively constant. It has been demonstrated (Marshall and Disney, 1956) that testicular growth in Quelea quelea can be induced by long daily photoperiods, even though in nature gonadal growth is due largely to an endogenous reproductive rhythm in conjunction with

other environmental factors occurring after rainfall. Through natural selection a species can adapt to the photoperiod it experiences by changing the rate of response to stimulation (Wolfson, 1959b), by including a refractory period of appropriate length (Marshall, 1960), or both.

In captive Dickcissels (Spiza americana) premigratory activity, fat deposition, and molt are largely internally regulated, but require exogenous environmental information to stay in phase with wild populations (Zimmerman, 1966). The gonadal response, however, has not been examined experimentally. The purposes of this investigation were to determine the dependence of the annual gonadal cycle of the Dickcissel on photoperiod and to quantify the growth and refractory phases.

Materials and Methods

Preliminary Experiment. Male Dickcissels were captured with mist nets from postnuptial populations near Manhattan, Kansas, between 28 July and 1 September 1969. Birds were retained indoors in windowless aviaries on 15-hour (06:30-21:30 CDT) daily photoperiods (15L:9D) until 13 September when they were divided into three groups (PR4, PR6, and PR8) of five birds each and placed on 12-hour (06:30-18:30 CDT) daily photoperiods (12L:12D). Two birds (which later died) were laparotomized on 13 September to obtain an estimate of testicular condition at beginning of experimental treatment, and two were retained on 15L:9D as long-day controls. Groups PR4, PR6, and PR8 were retained on 12L:12D for 4, 6, and 8 weeks, respectively, before being returned to 15L:9D. Testicular development of birds in each group (including long-day controls) was assessed by laparotomy at selected intervals between 13 September 1969 and 17 January 1970.

Main Experiment. Male Dickcissels were captured with mist nets from breeding and postnuptial populations near Manhattan, Kansas, between 5 June and 10 August 1970 and placed singly in small cages (27 x 18 x 27 cm or 39 x 22 x 27 cm) on approximately natural daily photoperiods. Light was provided at a minimum intensity of 320 lux by overhead fluorescent lamps. Food (a mixture of millet seed, parakeet feed, and finches' seed mix) and water were freely available. The daily photoperiod was reduced weekly in accordance with the decreasing daylength of late summer (Figure 1) until 21 September 1970 (autumnal equinox) when 30 experimental birds were divided equally into groups

designated SD6, SD7, and SD8 and placed on 12-hour daily photoperiods. This light regime approximates that experienced on tropical wintering grounds. Birds of Groups SD6, SD7, and SD8 were retained on 12L:12D for 6, 7, and 8 weeks, respectively, before they were returned to 15L:9D. Five birds were retained for 91 days on 12L:12D as short-day controls.

Birds of Groups SD6, SD7, and SD8 were sacrificed by decapitation at weekly intervals during the 5 weeks' exposure to 15L:9D. Immediately after sacrifice, the pars distalis was removed, blotted on a paper saturated with Ringer's solution, and weighed to the nearest 0.1 ug on an electrobalance. Testes were removed and placed in an aqueous solution of acetic acid, formalin, ethanol (AFA) for 5 days before being transferred to 70% ethanol (Farner et al., 1966). Ten days after sacrifice, testes were freed of extraneous tissue and weighed to the nearest 0.01 mg on a torsion balance. Testes exceeding 25 mg were weighed to the nearest 0.1 mg.

Testicular condition of short-day controls was assessed by laparotomy under Nembutal anesthesia (Donovan, 1958). Selected short-day controls were laparotomized on 21 September and when Groups SD6, SD7, and SD8 were transferred from 12L:12D to 15-hour daily photoperiods. Then each bird was laparotomized at monthly intervals until termination of the experiment.

Testicular development of birds in Groups SD6, SD7, and SD8 was assessed microscopically using the index of Bartholomew (1949).

I. Resting spermatogonia only

II. Spermatogonia dividing, but only a few spermatocytes present

III. Spermatocytes

IV. Spermatocytes with spermatids

V. Spermatids with a few sperm

VI. Full spermatogenic activity with many sperm

Regression lines for adenohypophysial and testicular weights, and 95 per cent confidence intervals about the slopes, ordinate intercepts (Tables 1 and 2), and predicted mean and single weights (Tables 5 and 6) were calculated using formulae described by Simpson et al. (1960). Adenohypophysial and testicular weights were transformed into logarithms to improve the linear relationship between weight and time.

A linear relationship between the logarithm of testicular weight and time has been observed for several Temperate Zone species (Wilson, 1968). In order to determine if a similar relationship between testicular weight and time exists in Dickcissels, testicular growth in birds exposed to 15-hour daily photoperiods was examined using the model

$$\log \underline{W}_t = \log \underline{W}_o + \underline{k}\underline{t} \qquad (1)$$

where $\underline{W}_o$ is combined testicular weight at beginning of treatment with long daily photoperiods, $\underline{W}_t$ is the weight at $\underline{t}$ days, $\underline{t}$ is time in days on long daily photoperiods, and $\underline{k}$ is the slope of the regression line (logarithmic testicular growth-rate constant) in days^{-1}. Similarly the presence of a growth response in the pars distalis was tested against the model.

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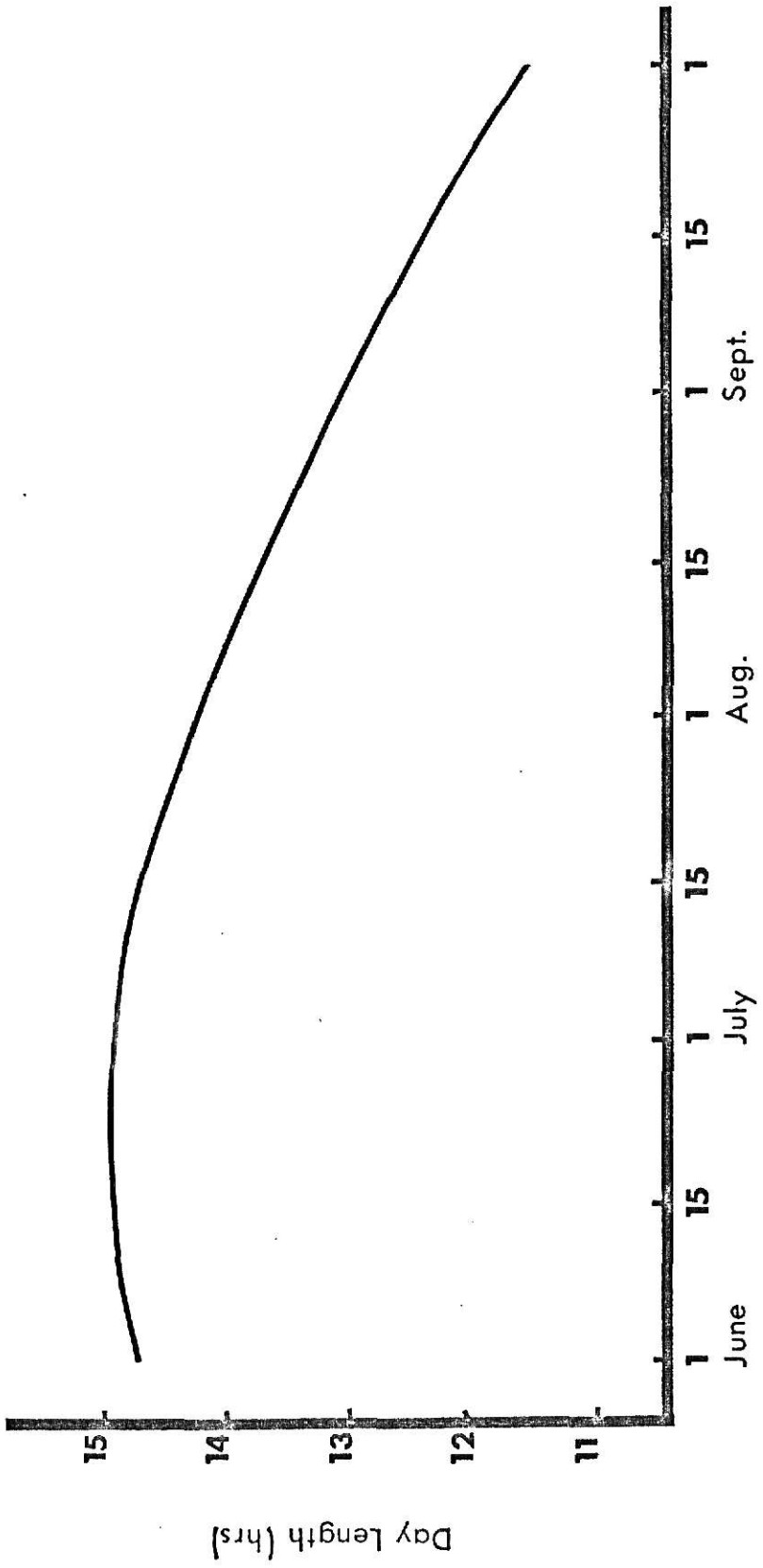
Figure 1. Natural photoperiod (excluding civil twilight) at Manhattan, Kansas (39° 12' N.L., 96° W.L.).


Day Length (hrs)
15
14
13
12
11
1
15
1
15
1
15
1
15
1
June
July
Aug.
Sept.
Time

Results

In the preliminary experiment, no testicular growth occurred in the two birds retained on 15L:9D. One bird in each of Groups PR4 and PR6 showed slight testicular growth. Four of five birds in Group PR8 showed testicular growth (Figure 2). These results served as a basis for the following experiment.

Main Experiment. During the 5-week period when birds were exposed to 15L:9D, no significant regression in combined testicular weight occurred in birds of Group SD6 or SD7 (Table 1; Figure 4 and 6). However, birds of Group SD8 showed significant regression in testicular weight (Table 1; Figure 8). Four of five short-day controls showed no testicular development. However, one bird had large testes on the day Group SD7 was placed on 15L:9D (Table 3). Testes of this bird regressed until termination of the experiment. It was assumed that testes of all birds would be completely regressed at the beginning of treatment with 15-hour daily photoperiods. The fact that one short-day control had large testes on the day Group SD7 was placed on 15L:9D, and the fact that one bird in Group SD6 had sperm present in the seminiferous tubules after only 7 days on 15L:9D, indicate that this assumption was invalid.

Testes of selected birds in each group were examined microscopically. Although no significant regression in testicular weight occurred in Group SD6 or SD7, many birds in each group had large testes. In Group SD8 all birds showed some testicular development. All testes examined in which the

logarithm of combined testicular weight was 1.9 or greater were in at least Stage V of development. In each group the maximum stage of development was Stage VI (Figure 10). The minimum stage of development in Groups SD6 and SD7 was Stage I (Figure 9). In Group SD8 the minimum stage of development was Stage III.

There was no significant increase in weight of the pars distalis in birds of Group SD6 (Table 2, Figure 3), but the pars distalis increased significantly in birds of Groups SD7 and SD8 (Table 2; Figures 5 and 7).

Figure 2. Left testis length as a function of time (in days) in photorefractory Dickcissels exposed to 15-hour daily photoperiods (upper); in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour photoperiods for 4 or 6 weeks (Groups PR4 and PR6, respectively) (middle two graphs); in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour photoperiods for 8 weeks (Group PR8) (bottom). Symbols represent individual birds.

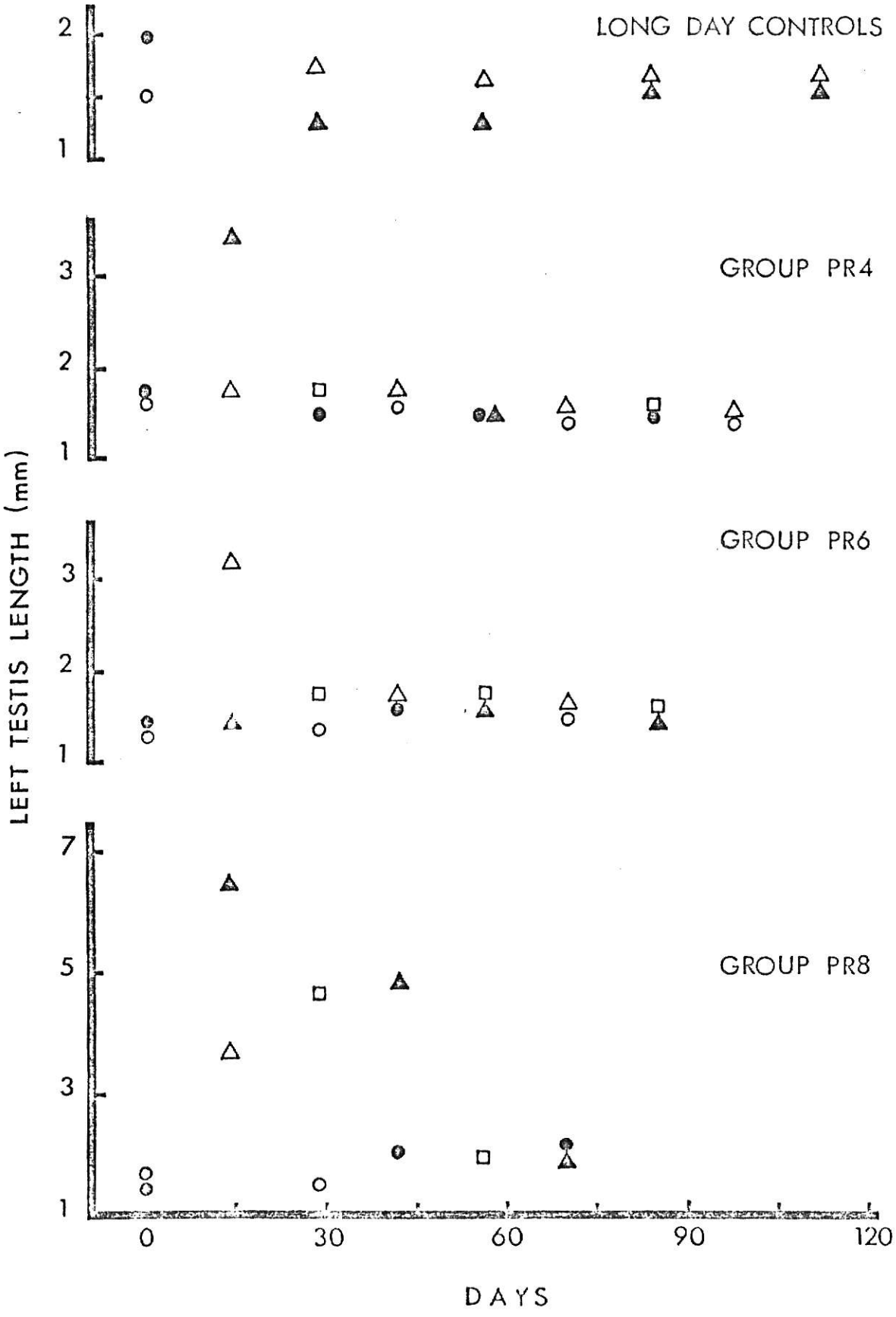
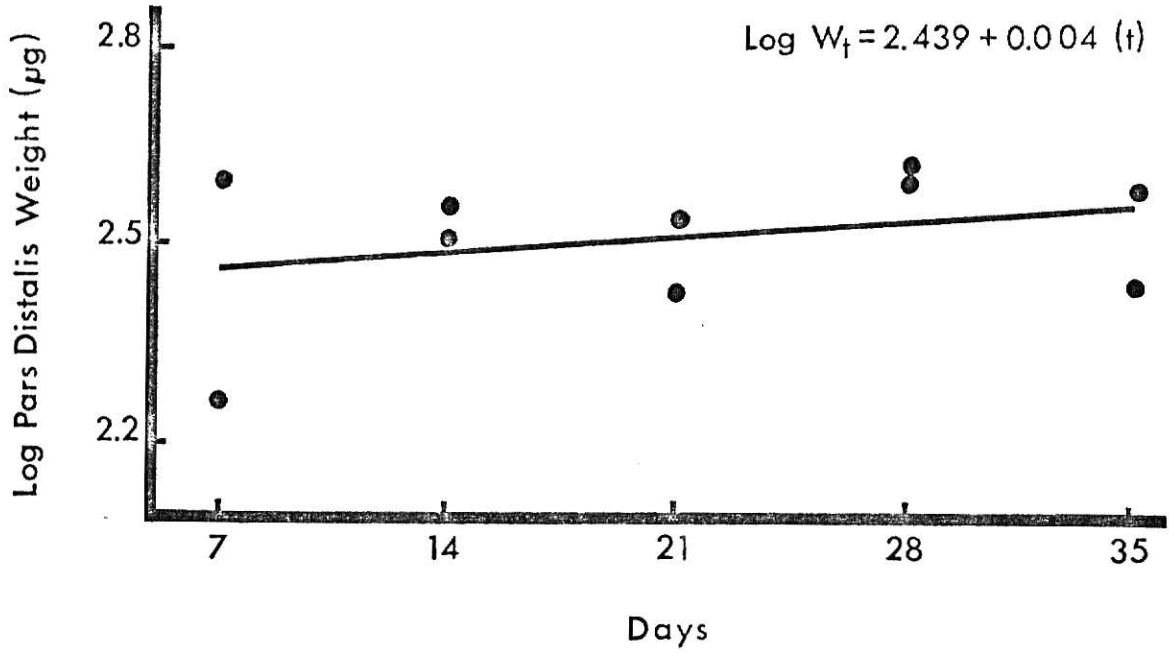

LONG DAY CONTROLS
GROUP PR4
GROUP PR6
GROUP PR8
LEFT TESTIS LENGTH (mm)
DAYS
0
30
60
90
120

Figure 3. Relationship between weight of the pars distalis and time in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour daily photoperiods for 6 weeks (Group SD6).

Figure 4. Relationship between testicular weight and time in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour daily photoperiods for 6 weeks (Group SD6).


Log $W_t = 2.439 + 0.004\ (t)$
Log Pars Distalis Weight (µg)
2.8
2.5
2.2
7
14
21
28
35
Days

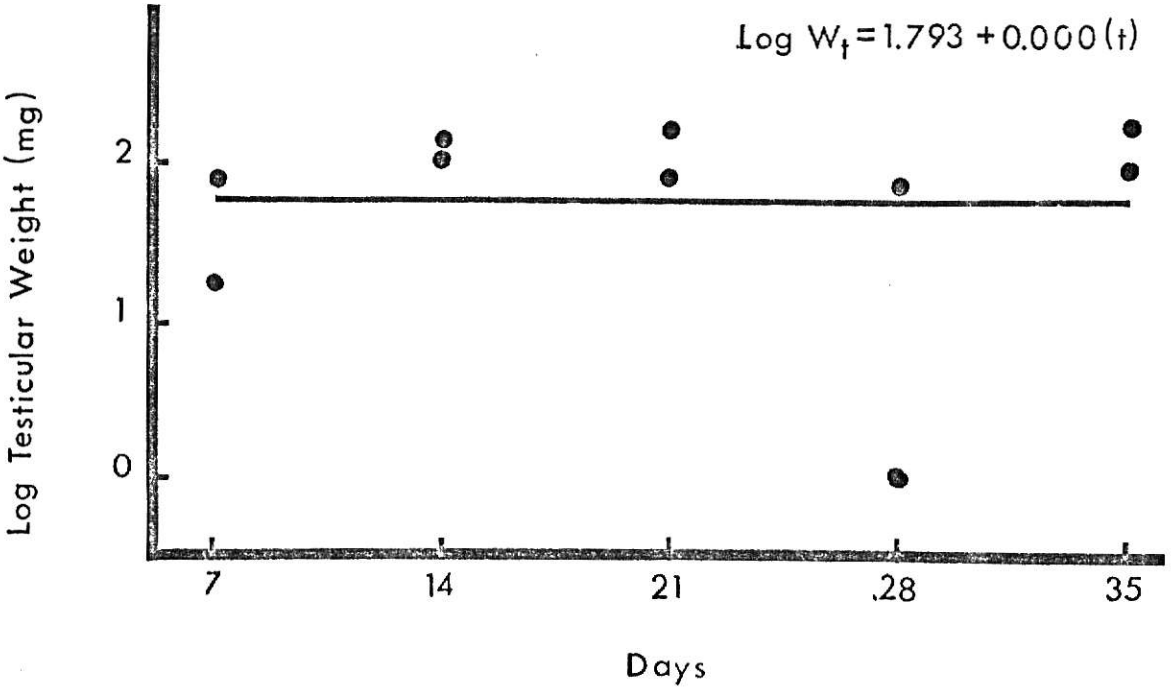
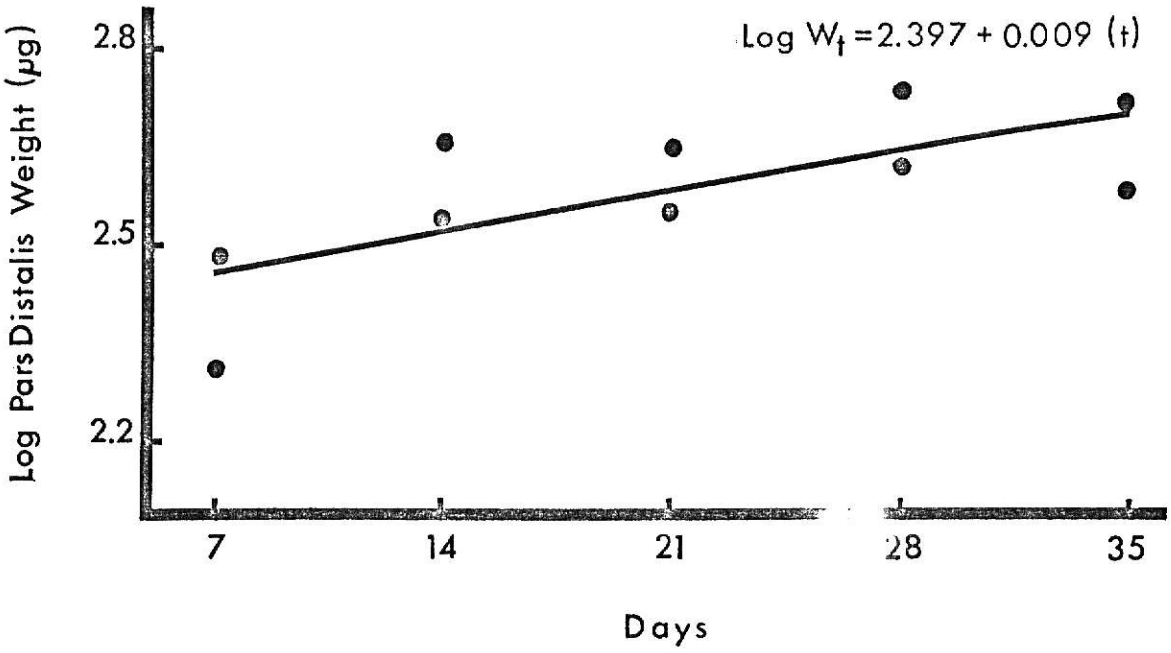

Log $W_t = 1.793 + 0.000\ (t)$
Log Testicular Weight (mg)
2
1
0
7
14
21
28
35
Days

Figure 5. Relationship between weight of the pars distalis and time in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour daily photoperiods for 7 weeks (Group SD7).

Figure 6. Relationship between testicular weight and time in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour daily photoperiods for 7 weeks (Group SD7).


Log W_t = 2.397 + 0.009 (t)
Log Pars Distalis Weight (µg)
2.8
2.5
2.2
7
14
21
28
35
Days

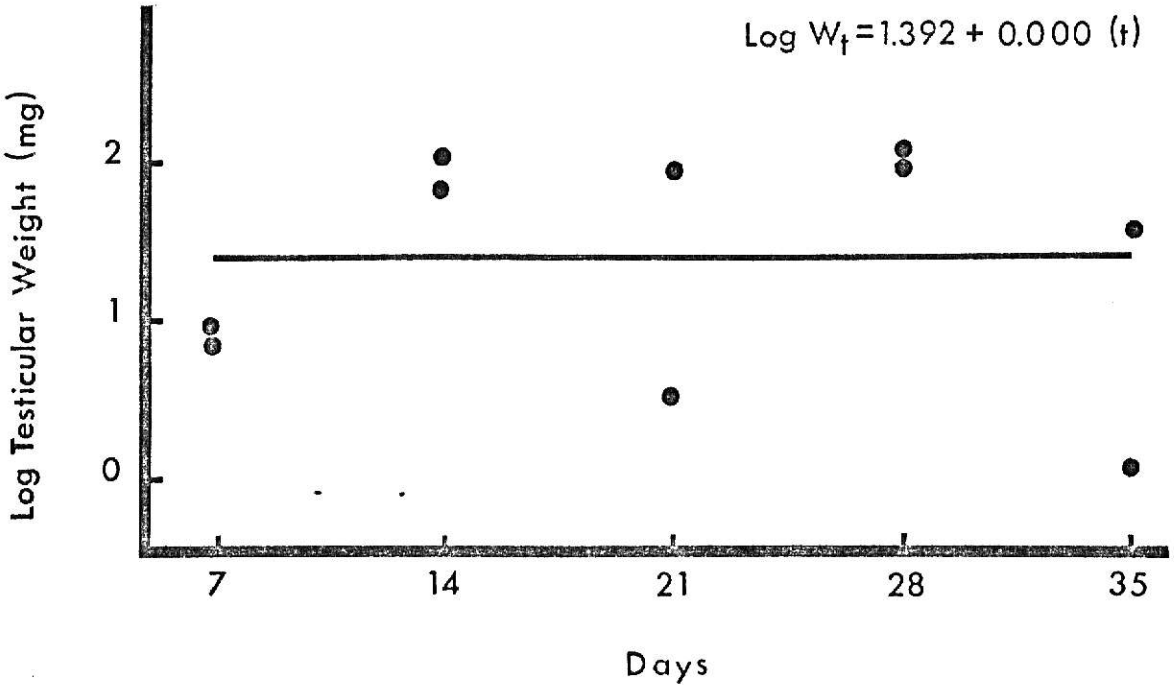
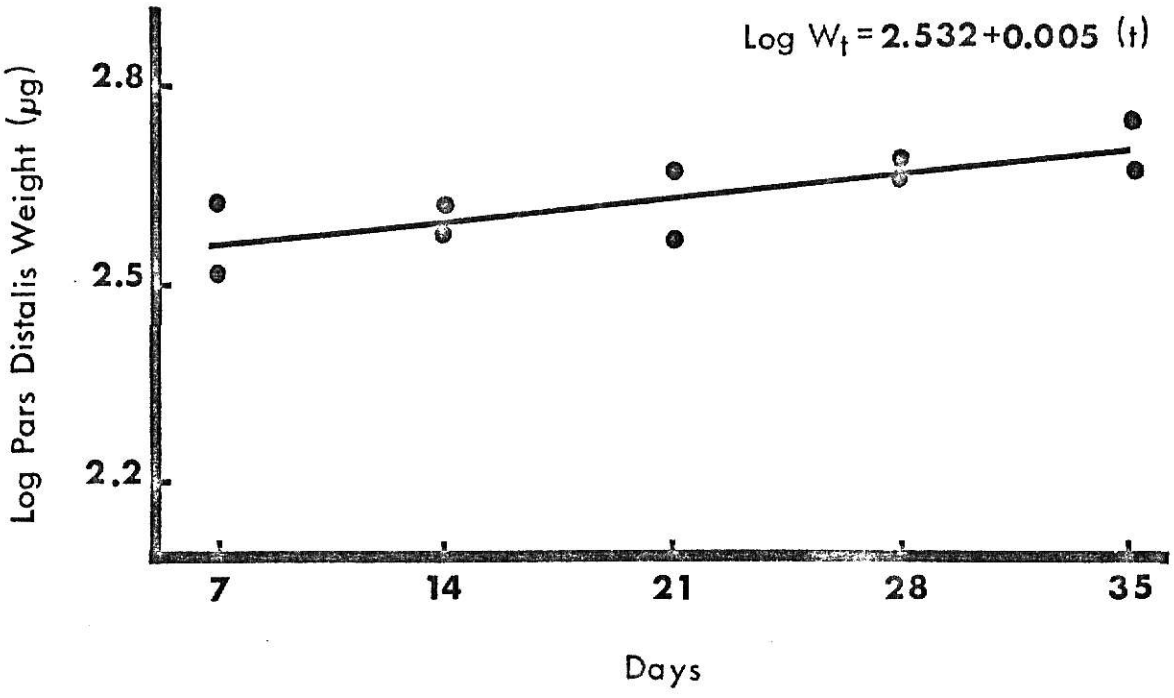

Log W_t = 1.392 + 0.000 (t)
Log Testicular Weight (mg)
2
1
0
7
14
21
28
35
Days

Figure 7. Relationship between weight of the pars distalis and time in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour daily photoperiods for 8 weeks (Group SD8).

Figure 8. Relationship between testicular weight and time in Dickcissels exposed to 15-hour daily photoperiods after being retained on 12-hour daily photoperiods for 8 weeks (Group SD8).


Log $W_t = 2.532 + 0.005\ (t)$
Log Pars Distalis Weight (µg)
2.8
2.5
2.2
7
14
21
28
35
Days

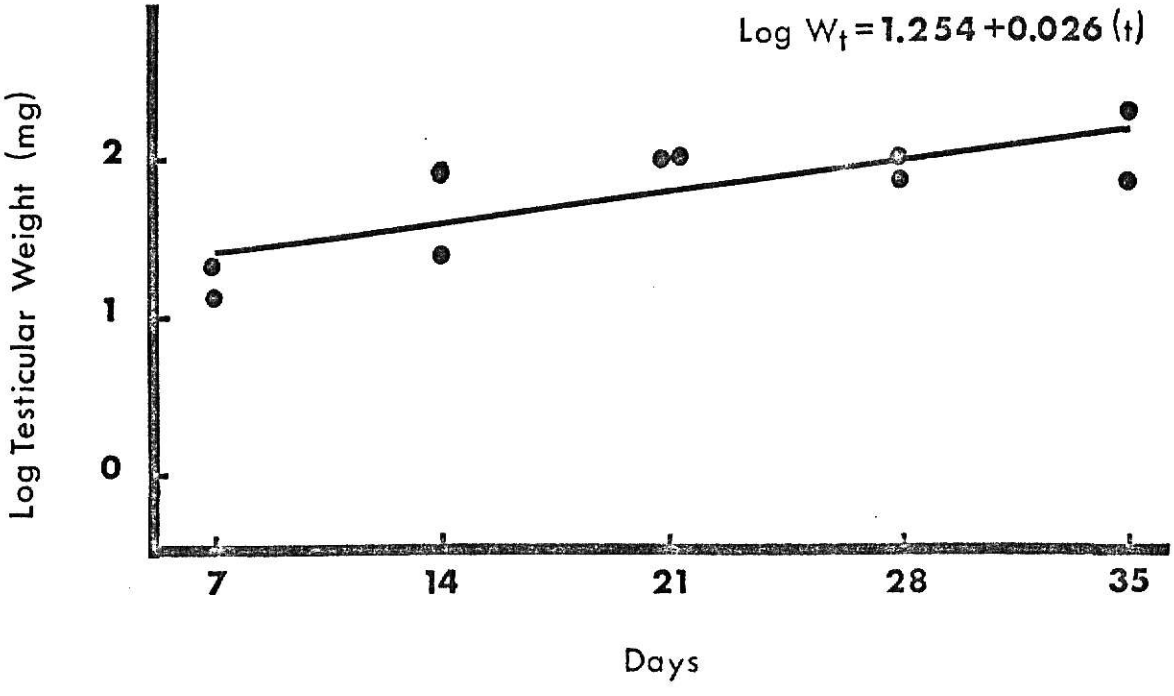

Log $W_t = 1.254 + 0.026\ (t)$
Log Testicular Weight (mg)
2
1
0
7
14
21
28
35
Days

Figure 9. Testis of photorefractory, photostimulated Dickcissel with resting spermatogonia in each seminiferous tubule (Stage I of spermatogenesis; note abundant interstitium). Sa50, SD6. AFA fixative, Hematoxylin-Periodic Acid-Schiff's Reagent. X430

Figure 10. Testis of a photosensitive, photostimulated Dickcissel with seminiferous tubules in Stage VI of spermatogenesis (note bunched spermatozoa). The interstitium has been largely displaced by tubule expansion. Sa47, SD6. AFA fixative. Hemotoxylin-Periodic Acid-Schiff's Reagent. X430

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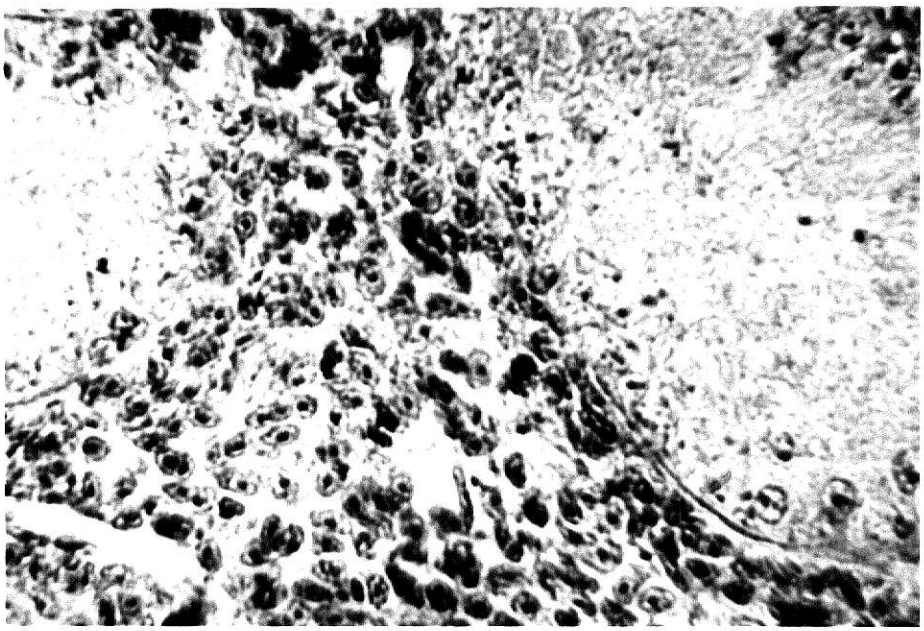

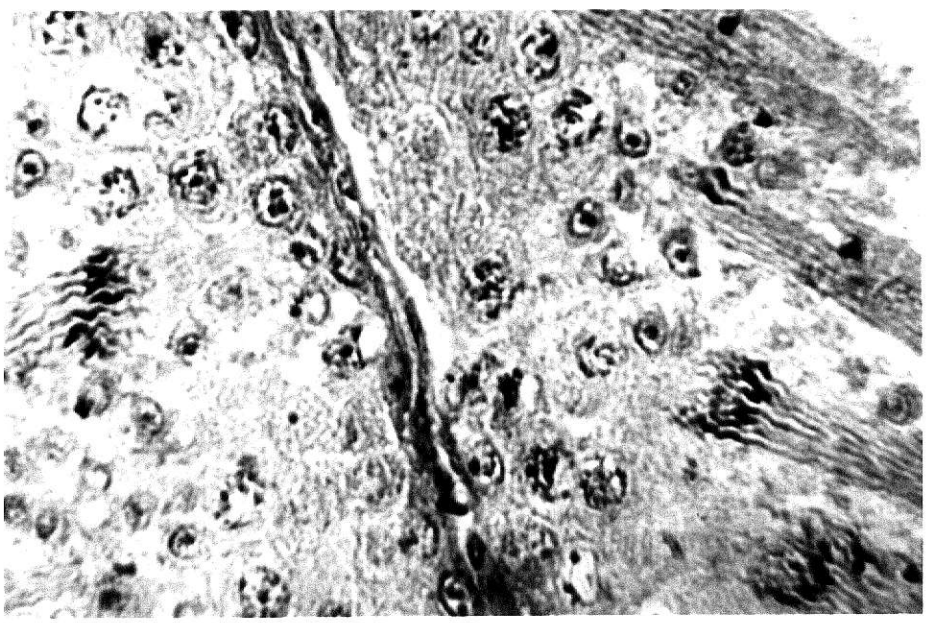

Table 1

Relationship Between Testicular Weight and Time in Dickcissels Exposed to 15-Hour Daily Photoperiods for 5 Weeks

Group	N	Days on 12-hour daily photoperiods	Stage of maximum testes	log $\underline{W}_o$ ± 95% confidence interval	$\underline{k}$, days^{-1}
SD6	10	42	VI	1.793 ± 0.507	0.000 ± 0.051
SD7	10	49	VI	1.392 ± 0.548	0.000 ± 0.055
SD8	10	56	VI	1.254 ± 0.077	0.026 ± 0.008

Table 2

Relationship Between Weight of the Pars Distalis and Time in Dickcissels Exposed to 15-Hour Daily Photoperiods

Group	N	Days on 12-hour daily photoperiods	Days on 15-hour daily photoperiods	log W_o ± 95 per cent confidence interval	k, days^{-1} ± 95% confidence interval
SD6	10	42	35	2.439 ± 0.082	0.004 ± 0.008
SD7	10	49	35	2.397 ± 0.065	0.009 ± 0.007
SD8	10	56	35	2.532 ± 0.029	0.005 ± 0.003

Table 3

Left Testis Lengths of Short-day Controls

Days on short photoperiods (12L:12D)	Left testis lengths (mm)	
0	1.90	1.80
42[1]	1.25	1.55
49[2]	1.40	5.75
56[3]	1.75	-
77	1.40	2.95
84	1.50	-
91	1.25	1.60

[1]Day Group SD6 was transferred to 15L:9D.

[2]Day Group SD7 was transferred to 15L:9D.

[3]Day Group SD8 was transferred to 15L:9D.

Table 4

Confidence Intervals (95%) for

Predicted Mean and Single Testicular Weight

DAYS	Group SD6		Group SD7		Group SD8	
	Mean	Single	Mean	Single	Mean	Single
7	±0.878	±1.828	±0.949	±1.974	±0.134	±0.279
14	±0.621	±1.719	±0.671	±1.857	±0.095	±0.262
21	±0.507	±1.681	±0.548	±1.816	±0.077	±0.256
28	±0.621	±1.719	±0.671	±1.857	±0.095	±0.262
35	±0.878	±1.828	±0.949	±1.974	±0.134	±0.279

Table 5

Confidence Intervals (95%) for

Predicted Mean and Single Pars Distalis Weight

	Group SD6		Group SD7		Group SD8	
DAYS	Mean	Single	Mean	Single	Mean	Single
7	±0.141	±0.294	±0.113	±0.235	±0.051	±0.105
14	±0.010	±0.277	±0.080	±0.221	±0.036	±0.099
21	±0.082	±0.271	±0.065	±0.216	±0.029	±0.097
28	±0.010	±0.277	±0.080	±0.221	±0.036	±0.099
35	±0.141	±0.294	±0.113	±0.235	±0.051	±0.105

Discussion

Results of the preliminary experiment suggest the presence of a refractory period in some Dickcissels which is similar to that in many Temperate Zone species and indicate that testicular growth can be induced by 15-hour daily photoperiods. The fact that four of five birds in Group PR8 showed testicular growth suggests that between 6 - 8 weeks' exposure to 12-hour daily photoperiods terminates refractoriness in the majority of birds. These data suggest that, under natural conditions, termination of refractoriness should occur around mid-November; certainly by late November. These results are comparable to those found for many Temperate Zone species (for reviews, see Wolfson, 1958; Farner, 1959) and a transequatorial migrant, the Bobolink (Dolichonyx oryzivorus) (Engels, 1959, 1961, 1962).

An examination of testicular weights in birds of Groups SD6 and SD7 indicate a lack of conformance with equation 1. It should be noted that, although k for both groups is zero (Table 1) the majority of birds in each group showed testicular development (Figures 4 and 6). Testes of one bird in Group SD7 sacrificed after only 7 days' exposure to 15-hour daily photoperiods were in Stage V of development. This and the fact that one short-day control had large testes (Table 3) indicate that some birds in these groups were released from refractoriness and had begun testicular development before they were placed on long daily photoperiods. These results seem to contradict the hypothesis that a refractory period exists in the Dickcissel.

However, Engels (1962) has demonstrated that, in Bobolinks, photoperiods of 12.5 and 12.75-hours can act as short days and terminate refractoriness. Just prior to initiation of experimental treatment Dickcissels were retained for 1 week (Figure 1) on 12.5-hour daily photoperiods. If a daily photoperiod of 12.5 hours can serve as a short day in releasing the Dickcissel from photorefractoriness also, then birds of Groups SD6, SD7, and SD8 actually received 7, 8, and 9 weeks' exposure to short days, respectively. In light of this, and the fact that in the preliminary experiment one bird in Group PR4 showed some testicular growth after only 4 weeks' exposure to short days, it would be anticipated that some birds would be released from refractoriness several weeks before being placed on 15L:9D. Hamner (1968) demonstrated that, in the House Finch (Carpodocus mexicanus), recovery from photorefractoriness occurs naturally on daylengths of or exceeding 12 hours. In the testicular response of this species there appears to be a circadian rhythm of two approximately 12-hour phases of differing sensitivity to light. The duration of these two phases of differing light sensitivity can be altered by at least five hours by photoperiods of different duration in a 24-hour cycle, so that a 12-hour photoperiod which can serve as a short day in terminating refractoriness can also be stimulatory in nonrefractory birds. If a similar mechanism exists in Dickcissels, then some birds which were released from refractoriness early in the experiment may have begun to respond to 12-hour photoperiods. The fact that gonadal recrudescence in Dickcissels begins on the wintering grounds on approximately 12-hour daily photoperiods and that other elements of the annual cycle

such as fat deposition, molt, and migratory behavior likewise occur on 12-hour daily photoperiods supports this hypothesis.

The value of k for birds of Group SD8, 0.026 ± 0.008 days^{-1}, (Fringilla montefringilla), Chaffinch (Fringilla coelebs), Greenfinch (Chloris chloris) (Dolnik, 1963) and for the White-crowned Sparrow (Zonotrichia leucophrys gambelii) (Farner and Wilson, 1957) exposed to 15-hour daily photoperiods. Engels (1964) has demonstrated that Bobolinks respond very slowly to 14-hour photoperiods (k near zero) in sharp contrast to Temperate Zone migrants which show a measurable response (k at least 0.04 days^{-1} in White-crowned Sparrows). Although Bobolinks show little response to 14-hour daily photoperiods, when exposed to 18-hour photoperiods k was comparable to that of White-crowned Sparrows (Engels, 1964). It is not known whether a similar relationship between the value of k and duration of the daily photoperiod exists in Dickcissels. However, the Dickcissel, like the Bobolink, is never exposed to 18-hour photoperiods in nature, the photoperiod of the extreme northern range being approximately 16-hours. This slow rate of response to 14-hour and 15-hour photoperiods exhibited by the Bobolink and Dickcissel, respectively, may indicate differences in processing photoperiodic information between transequatorial and equatorial migrants and Temperate Zone species. If Dickcissels are released from refractoriness by late November and are capable of responding to 12-hour daily photoperiods, then the rate of testicular development on 12-hour photoperiods must be extremely slow, or perhaps

environmental factors of the wintering grounds function to retard the onset of growth, because in nature testicular development is not observable until late March. As stated, Zimmerman (1966) demonstrated that circennial endogenous cycles are involved in the regulation of migratory behavior, fat deposition, and molt in the Dickcissel. It seems likely (although not demonstrated) that a similar endogenous circennial element may be involved in the gonadal cycle of this species. If this is true, then this slow rate of response to 12-hour daily photoperiods or this retardation of testicular growth by environmental factors of the wintering grounds could serve to keep this presumed circennial cycle in phase with the environment. Ability to "predict" oncoming seasons sufficiently in advance for appropriate physiological adjustments would have definite selective advantages. A more adequately controlled experiment determining the value of $\underline{k}$ for Dickcissels exposed to 12-hour photoperiods is needed.

It is important to note that 12-hour photoperiods do not terminate refractoriness in some populations of Slate-colored Juncos (Junco hyemalis) (Engels, 1961) or in White-throated Sparrows (Zonotrichia albicollis) (Wolfson, 1958; Shank, 1959; Engels, 1961). This may indicate a basic difference between some Temperate Zone species and equatorial migrants in the maximum daylength capable of eliminating photorefractoriness. Since the shortest photoperiod to which equatorial and transequatorial migrants are exposed is 12 hours, ability to overcome refractoriness on intermediate daylengths probably represents a basic adjustment in the photoperiodic mechanism to

equatorial and transequatorial migration. Hamner (1968) suggests that, in transequatorial migrants, the photoperiodic threshold is shifted permanently beyond the 12-hour point, so that days as long as 14-hour as in the House Finch, can act as short days to control the annual cycle.

It has been shown that the avian pars distalis increases in weight and gonadotropic potency during maximal gonadal growth (for reviews, see Wolfson and Kobayashi, 1962; Uemura, 1964; Tanaka et al., 1965; Kobayashi and Farner, 1966; Follett and Farner, 1966a, 1966b). A similar increase in weight of the pars distalis was found in Groups SD7 and SD8 (Table 2). These increases are significant because the 95% confidence intervals do not include zero. A significant increase in weight of the pars distalis did not occur in Group SD6. Although this is unreliable evidence, it may indicate that the photoperiodic response mechanisms of birds in Groups SD7 and SD8 were released from photorefractoriness and were responding to photostimulation.

Acknowledgments

I am grateful to Dr. John L. Zimmerman for his assistance in the field and for his guidance throughout the entire study and in preparation of this thesis. I am also grateful to Dr. Fred E. Wilson for use of his laboratory equipment and for his helpful suggestions. Appreciation is also expressed to Dr. Donald J. Ameel for his critical reading of the manuscript. I wish to thank Dr. S. C. Kendeigh for the Dolnik translation; Dr. Dayton for suggestions concerning the statistics; Mr. Harold Schuler for testes microtechnique; Dr. Steve Fretwell for assistance in the field; and Mr. Thomas G. Shane and Mr. Edward K. Cusick for technical assistance during the study. Special thanks are due to my wife, Louise, for helping prepare the manuscript. This investigation was supported in part by a National Science Foundation Grant GB-6087 to Dr. John L. Zimmerman.

Summary

The testicular cycle of Dickcissels was examined in order to determine if the photoperiod played an important role in its regulation.

Results of a preliminary experiment suggested that a refractory period (similar to that found in Temperate Zone species) exists in some Dickcissels, that refractoriness could be terminated by 6 - 8 weeks' exposure to 12-hour photoperiods, and that testicular development could be induced by 15-hour photoperiods.

In light of these results an attempt was made to quantify the testicular growth and refractory phases of this species.

Testicular growth in photosensitive birds exposed to 15-hour daily photoperiods, after being held on 12-hour daily photoperiods for 8 weeks, approximated a logarithmic function of time between 7 and 35 days. Rate of photoinduced testicular growth in this equatorial migrant was less than that in several Temperate Zone species exposed to the same photoperiod at approximately the same time of year. This difference may indicate differences in processing photoperiodic information between equatorial migrants and Temperate Zone residents.

Although no significant regression in testicular weight occurred in birds which were retained on 12-hour photoperiods for 6 or 7 weeks before being placed on long daily photoperiods, many birds in these groups showed testicular development. It is thought that some birds in these groups were released from

photorefractoriness and had begun testicular development before being placed on 15-hour photoperiods. The fact that one bird retained on 12-hour photoperiods for 49 days had large testes, and that the testes of one bird were in Stage V of development after only 7 days exposure to 15-hour photoperiods, supports this hypothesis. Because of the great variability in response of these birds, the refractory period could not be defined further.

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EVIDENCE FOR A REFRACTORY PERIOD IN THE DICKCISSEL (*SPIZA AMERICANA*)

by

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In order to determine the presence of a photorefractory phase and to determine the dependence of the annual testicular cycle of the Dickcissel on the photoperiod, initially photorefractory birds were exposed to 15-hour daily photoperiods after being retained on 12-hour photoperiods for 4, 6, or 8 weeks. In birds retained on 12-hour photoperiods for 4 or 6 weeks, one of five birds in each group showed slight testicular growth when exposed to 15-hour daily photoperiods. Four of five birds showed testicular growth when exposed to 15-hour photoperiods, after being exposed to 12-hour photoperiods for 8 weeks. Two birds continued simultaneously on 15-hour photoperiods for 112 days showed no testicular growth. These results suggest that a refractory period exists in the Dickcissel, that refractoriness can be terminated by 6 - 8 weeks' exposure to 12-hour photoperiods, and that testicular development can be induced by 15-hour photoperiods.

In order to quantify the testicular growth and refractory phases, initially photorefractory Dickcissels were retained on 12-hour photoperiods for 6, 7, or 8 weeks, before being placed on 15-hour photoperiods. Testicular growth in photosensitive birds exposed to 15-hour photoperiods, after being held on 12-hour daily photoperiods for 8 weeks, approximated a logarithmic function of time between 7 and 35 days. No significant regression in testicular weight occurred in birds exposed to 15-hour photoperiods after being exposed to 12-hour photoperiods for 6 or 7 weeks. However, many birds in these groups had developed testes. It is thought that some birds in these groups were

released from refractoriness and developed testes while still on 12-hour photoperiods. The fact that one control bird had large testes while exposed to 12-hour photoperiods supports this hypothesis. Because of the design of this experiment the refractory period could not be defined further.