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SOME PHYSIOLOGICAL ASPECTS OF
BREEDING BIOLOGY OF BLUE-WINGED
TEAL.

Iowa State University, Ph.D., 1969
Zoology

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SOME PHYSIOLOGICAL ASPECTS OF BREEDING BIOLOGY OF BLUE-WINGED TEAL

by

Hallett Jacob Harris, Jr.

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Zoology (Animal Ecology)

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1969

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INTRODUCTION

A preponderance of drakes in spring populations of some North American waterfowl has been recognized for some time (Hochbaum, 1959:15). Although this phenomenon is most marked in the inland diving ducks (tribe Aythyini) it also occurs among dabbling ducks (Anatini) such as the blue-winged teal Anas discors (Sowls, 1955:164; Bellrose et al., 1961). From analysis of band returns, Bellrose and Chase (1950) concluded that there was a higher rate of loss among mallard hens than among mallard drakes. Further analysis of year class band data suggested that greater hen mortality occurred outside of the hunting season; this mortality was attributed to high vulnerability of hens during the reproductive season. Bellrose et al. (1961) later suggested that the differential mortality of hens may be due to stress as defined by Selye (1956:3). Bellrose postulated that the physiological demands of reproduction "places the hens in much greater jeopardy to stress than the drakes, which experience marked depletion of energy only through the period of the post-nuptial molt." Bellrose, however, pointed out that little is known about the effects of stress as described by Selye in relation to waterfowl mortality. Hanson (1962), in a study of condition factors and seasonal stresses of Canada geese, referred to the low liver weights of females at the beginning of incubation as being suggestive of the stress of mobilization of body resources for egg production. He further suggested that the molt period, following the stress of reproduction, constitutes the greatest period of metabolic stress in the life of birds.

According to Selye (1956) the stress syndrome is a manifestation of increased adrenal activity. Adrenal weight and relative adrenal weight have been suggested as indices of adrenal activity. Höhn et al. (1965) found an increase in mean relative adrenal weight for hen mallards during reproduction. Their review of literature demonstrated similar findings by investigators working with other species. However, there appears to be some question whether adrenal hypertrophy serves as a valid index to increased adrenal activity (Gorbman and Bern, 1962:331; Bhattacharyya et al., 1967). Raitt (1968), working with the Gambel Quail, suggested that a histometric measurement of interrenal tissue may be a more meaningful index of adrenal activity than relative adrenal weight. However, an increase in cell size and activity does not necessarily mean there is an increase in secretory rate.

After reviewing available information on avian adrenocortical response, Bhattacharyya et al. (1967) remarked that reports on avian manifestations of the so-called alarm reaction have been inconsistent and distinctly varied. In addition to these uncertainties, there remains the fact that these indices of adrenal activity require sacrifice of the bird, which is not always feasible or desirable.

Inductively, it was hypothesized that if female waterfowl undergo stress during the breeding season as a result of energy demands of laying or restricted feeding during incubation, then alteration of the level of plasma constituents and formed elements of the blood should be associated with increased adrenal activity. More specifically, it was proposed that increased adrenal corticoid secretion would be reflected by increased

levels of plasma non-protein nitrogen (NPN), free fatty acids (FFA), glucose, ketone bodies, free amino acids (FAA), and acidophilia. Body weight loss also should be expected to accompany utilization of energy stores.

The feasibility of the hypothesis was examined by studying both wild waterfowl under natural conditions and wild captives under experimental conditions. The blue-winged teal (Anas discors) was chosen as the study species because of availability and ease of handling. Certain physiological parameters were followed in wild hens during all periods of the reproductive cycle. For comparative purposes, some additional information was collected on birds in fall. Selected physiological parameters were followed in experimental birds in response to exogenous corticosterone administration and fasting. Additional experiments were conducted to examine the effect of corticosterone administration on hematocrit, hemoglobin levels and blood volume.

VARIATION OF BODY WEIGHT, PLASMA CONSTITUENTS AND ACIDOPHIL LEVELS OF WILD HENS

Field studies were carried out during the spring and summer of 1966 and 1967 to examine the suitability of selected physiological parameters for detecting stress in wild blue-winged teal hens. Blood samples and physical measurements were obtained from hens during prelaying, laying and incubation periods. Birds were trapped on Dewey's Pasture, located in Clay and Palo Alto Counties of northwest Iowa. The study area has been described in detail elsewhere (Hayden, 1943).

Methods

The field methods utilized in this study are essentially those described by Strohmeyer (1967). However, a brief account of these methods is appropriate to clarify methods peculiar to this study.

Traps and trapping

To secure birds continuously from spring to fall migration, several different methods of trapping were employed. Birds were captured during spring and fall migration by using single lead, baited funnel traps. At times, as many as fifty birds were taken at one tending. During spring, when blood samples were secured from bait trapped hens, trapping was confined to late morning hours. During the fall bait traps were tended at mid-morning (8-10 A.M.) and early evening (4-6 P.M.).

Nesting hens were captured with the use of two types of traps, the Weller nest-trap and the Salyer nest-trap. The Salyer trap (Salyer, 1962) was used to capture laying hens since it could be easily concealed and

thus inconspicuous to the wary hen. The Weller trap (Weller, 1957b) proved highly effective in capturing incubating hens and could be set in less time than the Salyer trap.

Procedure followed for trapping nesting hens was to set the traps between 4:30 and 6:30 A.M. At this time laying hens had not yet arrived at the nest although incubating hens often were found on the nest between these hours. Traps usually were sprung manually about 11:00 A.M. or sometimes automatically by the return of an incubating hen to a Weller trap.

Night-lighting, as described by Leitch (1958) and Cummings and Hewitt (1964), was employed to capture some blue-wings at the time of brood rearing and molting.

Nest location

Nests were found by observation from blinds and searching. Nests were located by observation from 5:00 to 8:00 A.M. at which time hens were going to their nests to lay. The landing site of the hen was marked and the area was searched later in the morning and the hen generally could be flushed. This method proved most effective when two observers, in communication by walkie-talkie, could get a "fix" on the same hen.

Nests also were located by the method described by Sowls (1949) of dragging a weighted rope through the vegetation to flush laying or incubating hens. This method was most effective between the hours of 8:00 and 11:00 A.M.

Marking

All hens captured during the nesting season were marked with a colored plastic "nasal saddle" and banded with a U.S. Fish & Wildlife Service band. Birds captured outside the nesting season were banded but were not individually marked. The use of nasal saddles for marking individuals is described in detail by Strohmeyer (1967).

Weights and measurements

Hens were weighed and measured at the time of capture. Weight was recorded to the nearest gram on a "Hanson" model 1440 dietetic scale which was calibrated with a standard 200 gm weight before each use.

Culmen and keel measurements were taken with Glogau's vernier caliper to the nearest tenth of a millimeter. The chord of the culmen length was taken along the dorsal median line with the tip of the nail to the "V" of feathers on the forehead. The keel was measured from the anterior tip to the posterior margin along the ventral median surface of the sternum. The length of the flattened wing was measured from the anterior margin at the distal end of the humerus to the tip of the longest primary. Measurement was made to the nearest tenth of a centimeter.

Also noted at the time of capture was the number of eggs in the nest. In addition, the hen was palpated in the ventral abdominal region to ascertain whether there was an egg in the uterus. Eggs were field candled using the technique described by Weller (1956) to establish the stage of embryonic development. Eggs of known stage of incubation were used as a standard.

Blood sampling and handling

Blood samples were drawn as soon after capture as possible. When samples could not be taken immediately, the hens were held in a covered retaining box where they rested quietly. All materials needed for blood sampling, marking, weighing and measuring were carried in two specially designed field kits. One of these, constructed as described by Denington and Lucas (1955), contained red blood cell pipettes and other materials needed for cell count preparation. The other kit contained all other materials and instruments needed.

Each blood sample was drawn from the brachial vein into a 5 ml heparin treated syringe, transferred to a clean glass culture tube, sealed and immediately chilled on ice. A sample was taken at this time for acidophil counts, and a blood smear was made.

After birds trapped during the morning had been sampled, the blood was taken to the field laboratory where the plasma was separated by centrifugation. The pipetted plasma samples were placed in hard plastic vials and stored in deep freeze at -15 degrees centigrade. Plasma samples remained frozen until analyzed.

Acidophil counts

Acidophils (both heterophils and eosinophils) were counted by the semi-indirect method of Wiseman (1931). The dilute solution (3 mg dye/100 ml) was used in conjunction with a 4 to 5 hour staining time. Mixing was accomplished with ten minutes minimum mixing time in a Bryan-Garrey pipette rotor. Counts were made with a Neubauer "bright-line" counting chamber using the average of the counts of two chambers as the

estimated number. If counts from both chambers did not agree within 10 percent, a second preparation was made and recounted. Values were converted and expressed in acidophils per cubic millimeter.

Analysis of plasma constituents

Plasma analyses of Acetoacetate (AcAc), free amino acids (FAA), nonprotein nitrogen (NPN), free fatty acids (FFA) and glucose were conducted. Although desirable, the quantity of plasma available was insufficient to permit duplicate analysis on all plasma samples. As an alternate method of estimating precision and accuracy, control "plasma" purchased under the name of Lab-trol was analyzed in conjunction with the unknowns. This procedure, although inferior to duplicate samples, provided a quality check for NPN and glucose.

Plasma acetoacetate was estimated colorometrically by the method of Schilke and Johnson (1965). Analysis of free amino acids was performed using the modified nenhydrin colorimetric method described by Fisher et al. (1963). The modified Folin and Wu method outlined by Henry (1965) was used for the determination of blood glucose. Nonprotein nitrogen was estimated employing wet digestion with H_2SO_4 and the Berthelot color reaction (Henry, 1965). Plasma free fatty acids were determined using the modified method of Duncombe as described by Itaya and Ui (1965).

Results

Weight changes during the reproductive cycle

Body weight change provided a gross index of metabolic reserve. Body weight was plotted against the stage in the nesting cycle of each hen,

i.e., number of eggs in the nest or estimated day of incubation. Regression lines then were fitted to the resulting points (Figure 1). A separate regression line was fitted to the points from egg 2 to egg 7. This interval was chosen on the basis of Dane's (pers. com.)[1] observation that the reproductive tract of blue-wing teal hens starts regression after the seventh day. The interval from egg 8 to day 5 of incubation provided the points for the second regression and was separated on the basis of Phillips and van Tienhoven (1962) observation that the reproductive tract of pintails has completely regressed by day 5 of incubation. The third regression line was fitted to points from day 6 incubation to day 22 incubation. The regression equation of body weight on number of eggs or day of incubation for the three separate intervals are: egg 2 thru egg 7, $\hat{Y} = 410.7 + (-1.75X)$; egg 8 thru day 5 incubation, $\hat{Y} = 436.7 + (-6.2X)$; day 6 incubation thru day 22 incubation, $\hat{Y} = 336.8 + (-0.64X)$. The most rapid weight loss, approximately 6 grams per day, occurred during the interval from the 8th egg thru day 5 of incubation.

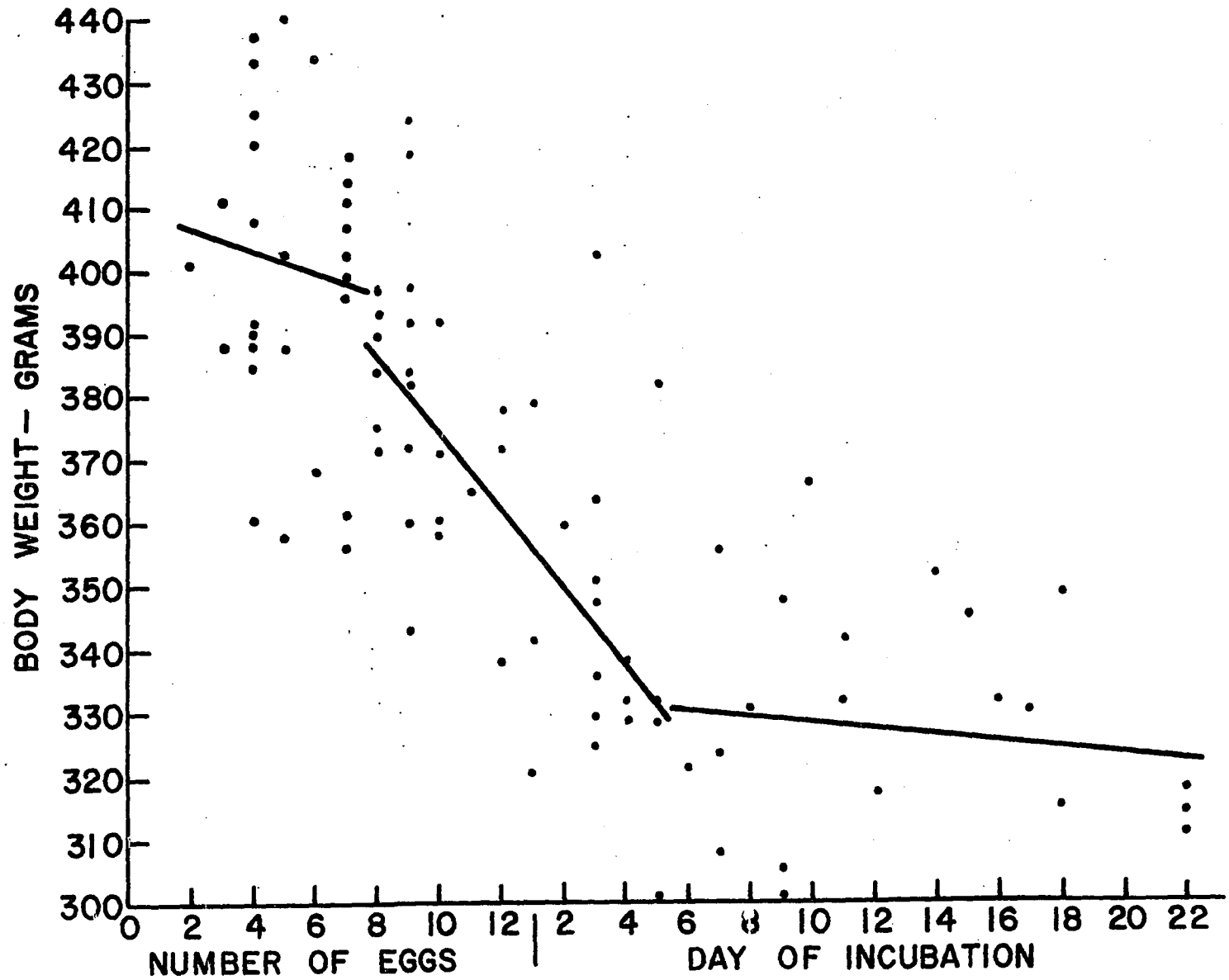
Weights of reproductive organs

Since birds could not be sacrificed in this study, the weights of female reproductive organs were estimated by indirect means. Ovary, and ova weight at ovulation for blue-winged teal were estimated from known

---

[1] Dane, Charles W., Northern Prairie Wildlife Research Center, Jamestown, North Dakota. Body weights and organ weights of blue-winged teal. Personal communication. 1969.

Figure 1. Regression of body weight on number of eggs in nest or day of incubation for birds in early laying, late laying and early incubation and late incubation


BODY WEIGHT— GRAMS
440
430
420
410
400
390
380
370
360
350
340
330
320
310
300
0 2 4 6 8 10 12
NUMBER OF EGGS
2 4 6 8 10 12 14 16 18 20 22
DAY OF INCUBATION

oviduct weight of blue-winged teal (Dane, pers. com.)[1] and body weight ovary weight and ova weight of lesser scaup (Trauger, pers. com.)[2]. These data are presented in Table 1.

Table 1. Known and estimated mean weight (in grams) of reproductive structures of lesser scaup Aythya affinis and blue-winged teal

	Body weight 7th egg	Ovary weight 7th egg	Oviduct weight 7th egg	Ova weight at ovulation
Scaup	850	31	25	20
BWT	400	Unknown	12	Unknown
Estimated BWT		15[a]		10

[a]Ovary weight of BWT was estimated by constructing a simple proportion between known oviduct weights of scaup and BWT and ovary weight of scaup then solving for the unknown: $\frac{25}{12} = \frac{31}{x}$. Utilization of known body weights and ovary weight of scaup yielded the same estimate. Ova weight was estimated in a similar manner.

Condition index

It is evident from the scatter diagram (Figure 1) that there was considerable variation of body weight between individuals at the same point in their reproductive cycle. It was theorized that some of this variation was due to structural differences of individual hens. Connell et al. (1960) studied the fat-free weight of some passerine birds and found that

[1]Dane, Charles W., Northern Prairie Wildlife Research Center, Jamestown, North Dakota. Body weights and organ weights of blue-winged teal. Personal communication. 1969.

[2]Trauger, David L. Department of Zoology and Entomology, Iowa State University, Ames, Iowa. Body weights and organ weights of lesser scaup. Personal communication. 1969.

structural variation, as measured by wing length, was the most important factor influencing the fat-free weight. Therefore, it was thought that some compensation for structural variation could be made by creating a structural index and dividing it into the body weight. The structural index was taken as the product of the bill length times the keel length. The quotient resulting from the division of the body weight by the structural index was called the condition index. As can be seen in the scatter diagram (Figure 2), structural variation is inherent in the population. Thus, it was believed that the condition index would provide a more meaningful measure of the metabolic reserves than would body weight.

Variation of condition index during the reproductive cycle

Condition index varied with respect to reproductive stage. As seen in Figure 3, the highest mean condition index was observed in hens during the early weeks of the laying stage while the lowest condition index was associated with the late weeks of the incubation stage. A test of the results, when applied to the data, using Duncan's New Multiple Range Test (Steel and Torrie, 1960) is summarized in Table 2.

Table 2. Summary of mean condition indices and significant[a] differences between means at different stages of the reproductive cycle

Laying		Molt	Prelay	Incubation	
Early	Late			Early	Late
13.5	12.7	12.5	12.0	11.7	10.9

[a]Any two means not underscored by the same line are significantly different; .01 special protection level employed.

Figure 2. Apparent association of body weight and condition index of prenesting hens

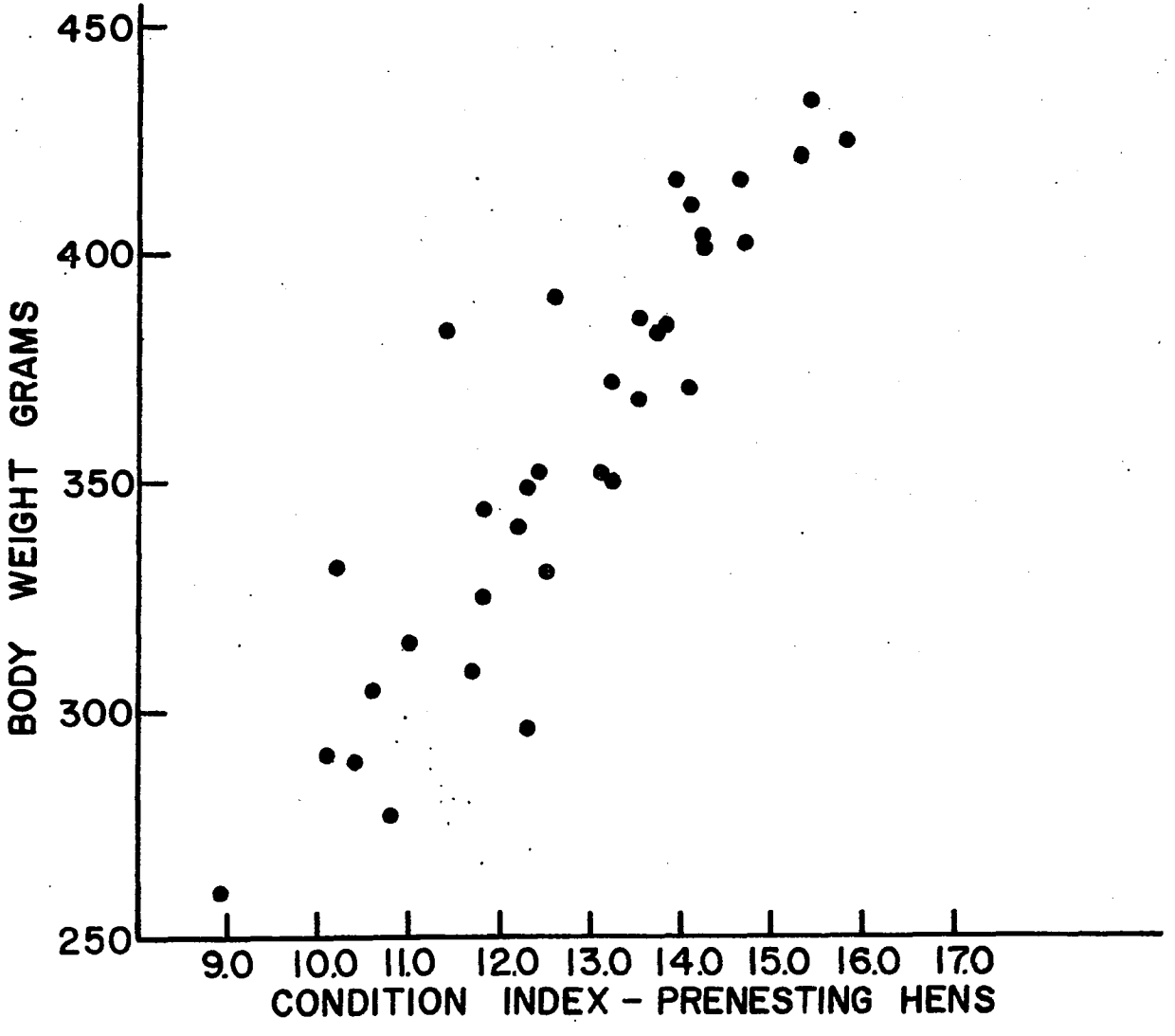
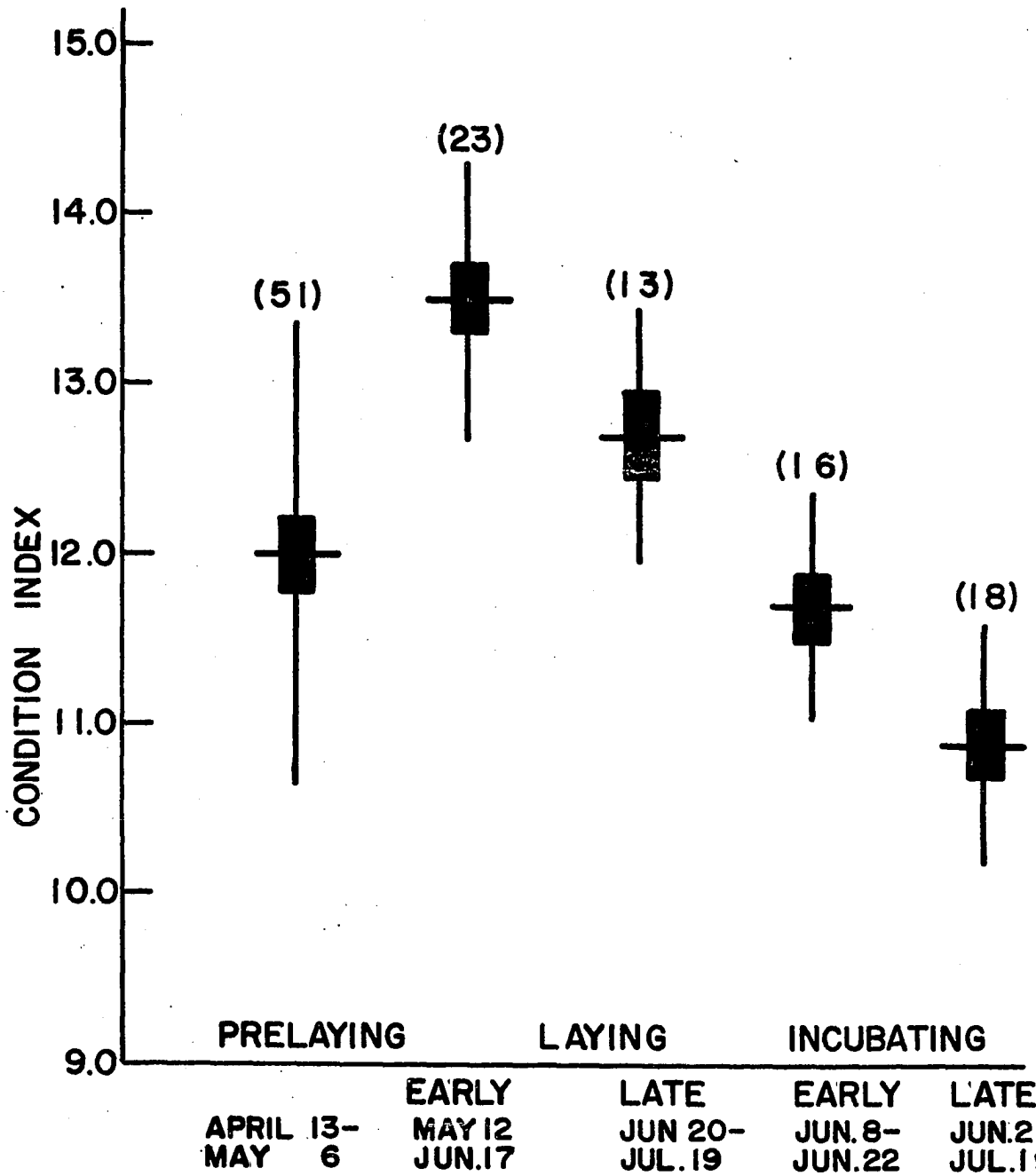

450
400
350
300
250
BODY WEIGHT GRAMS
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
CONDITION INDEX - PRENESTING HENS

Figure 3. Standard deviation and mean standard error of condition index for blue-winged teal hens at various reproductive stages (number of observations in parenthesis)


15.0
14.0
13.0
12.0
11.0
10.0
9.0
CONDITION INDEX
(51)
(23)
(13)
(16)
(18)
PRELAYING
LAYING
INCUBATING
EARLY
LATE
EARLY
LATE
APRIL 13-
MAY 6
MAY 12
JUN.17
JUN 20-
JUL. 19
JUN. 8-
JUN. 22
JUN.24
JUL. 19

Variation of plasma constituents

Mean nonprotein nitrogen (NPN) and free fatty acid (FFA) levels were higher in incubating hens than in laying or prelaying hens (Table 3). However, there appeared to be little difference in plasma free amino acid (FAA) levels of laying and incubating hens while plasma glucose levels were markedly higher in prenesting hens (Table 2). Only trace levels of acetoacetate were detected in the plasma of all birds collected during the study.

Table 3. Level of plasma constituents during different stages of reproductive cycle

	Prenesting	Laying	Incubating
FAA mg/100 ml	----------	6.28 ± 0.18[a](27)	6.41 ± 0.26(25)[b]
NPN mg/100 ml	17.2 ± 0.54(23)	--------	19.0 ± 0.63(32)
FFA µeq/liter	----------	557.8 ± 2.47(36)	669.1 ± 1.89(42)
Glucose mg/100 ml	384.9 ± 1.98(48)	259.2 ± 0.66(32)	275.7 ± 0.67(33)

[a]Mean standard error.

[b]Number of observations.

It was thought that mobilization of metabolic reserves probably would be reflected in changing levels of NPN, FFA and glucose during incubation since this period was associated with the lowest condition index (Figure 3). Therefore, most effort was concentrated on analysis of plasma constituents of incubating hens. Plasma levels of NPN in incubating hens

were influenced by condition index as seen in Figure 4. The regression of NPN on condition index is in the form of $\hat{Y} = 28.0 + (-0.86X)$; the negative coefficient is significant as seen in Table 4.

Table 4. Linear regression of NPN on condition index of incubating hens

Source	df	SS	MS	F
Due to regression	1	35.28	35.28	5.38*
Deviations from regression	37	242.49	6.55	
Total	38	277.77		

*Tab F (1,37.05) = 4.11.

Plasma free fatty acid levels increased with decreasing condition index and then decreased as the condition index continued to decline (Figure 5). A linear fit of the points was not significant; however, the quadratic expression of the form $\hat{Y} = -18228 + 3061.8X - 123.47X^2$ was significant (Table 5).

Table 5. Regression of plasma FFA on condition index of incubating hens

Source	df	SS	MS	F
Due to regression	2	260676	130338.00	7.73*
Deviations from regression	39	657739	16865.12	
Total	41	918415		

*Tab F (2, 39, .01) = 5.19.

Figure 4. Linear relationship of plasma NPN and condition index of incubating blue-winged teal hens

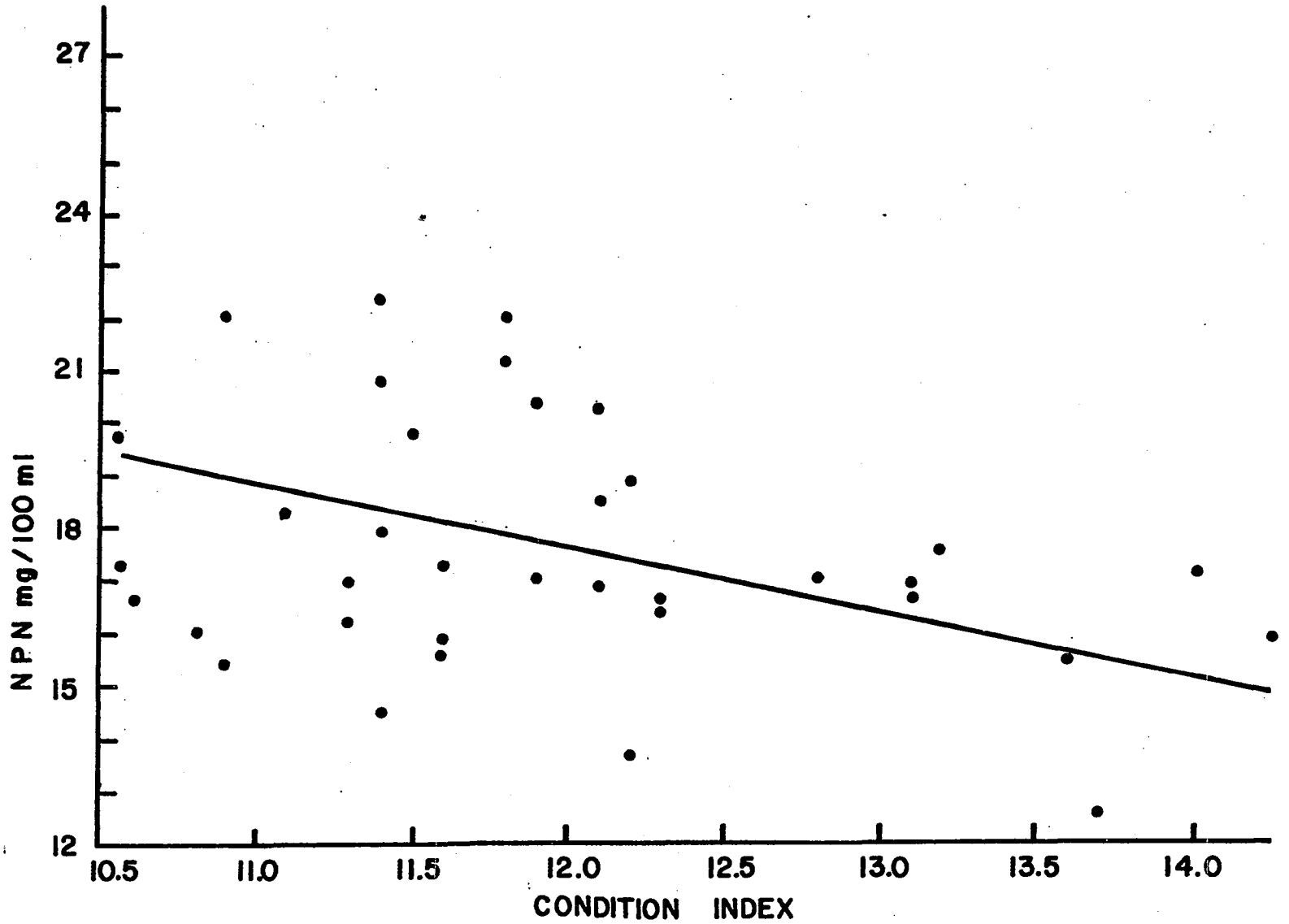
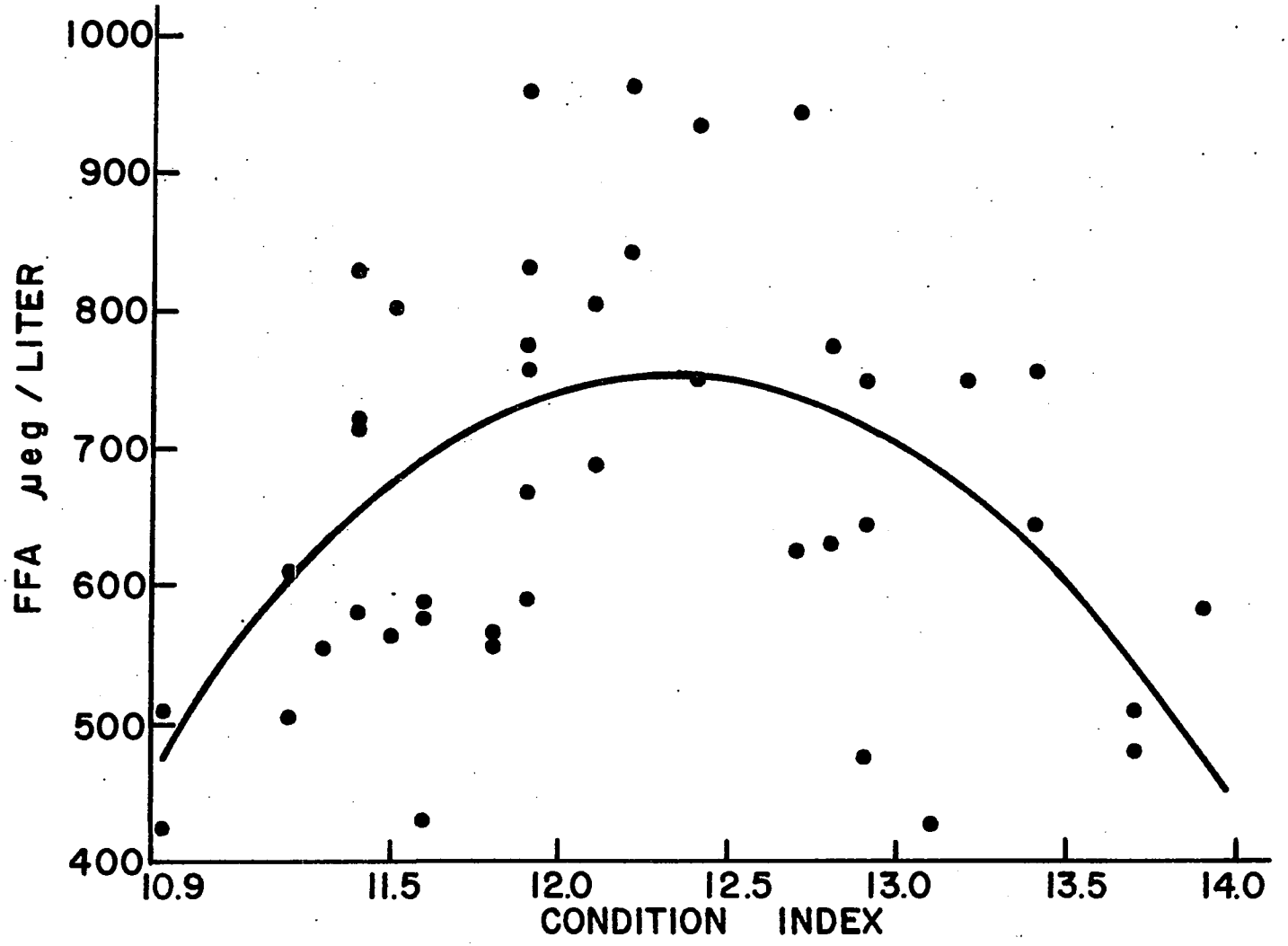

27
24
21
18
15
12
NPN mg/100 ml
10.5
11.0
11.5
12.0
12.5
13.0
13.5
14.0
CONDITION INDEX

Figure 5. Quadratic relationship of plasma FFA (μeg/liter) and condition index of incubating blue-winged teal hens


1000
900
800
700
600
500
400
FFA µeg / LITER
10.9
11.5
12.0
12.5
13.0
13.5
14.0
CONDITION INDEX

Plasma glucose also responded to decreasing condition index (Figure 6). Although the linear fit was highly significant (Table 6), the second degree term approached significance and the points were fitted to a slight curve.

Table 6. Regression of plasma glucose on condition index of incubating hens

Source	df	SS	MS	F
Due to regression	1	11560.8	11560.8	8.47*
Deviations from regression	27	30687.2	1136.56	
Total	28	42248.0		

*Tab F (1, 27, .01) = 7.68.

Variation of acidophil levels

Mean levels of circulating acidophils were higher (Figure 7) in laying than prelaying hens, and reached peak levels in incubating hens. Mean differences were tested using Duncan's New Multiple Range Test; a summary is presented in Table 7.

Table 7. Summary of mean acidophil levels and significant[a] differences between means of various stages of reproductive cycle

Incubation		Laying		Prelaying
Late	Early	Early	Late	
8.9	8.5	7.5	6.7	5.3

[a]Any two means not underscored by the same line are significantly different; .01 special protection level employed.

Figure 6. Curvilinear response of plasma glucose of incubating blue-winged teal hens to decreasing condition index

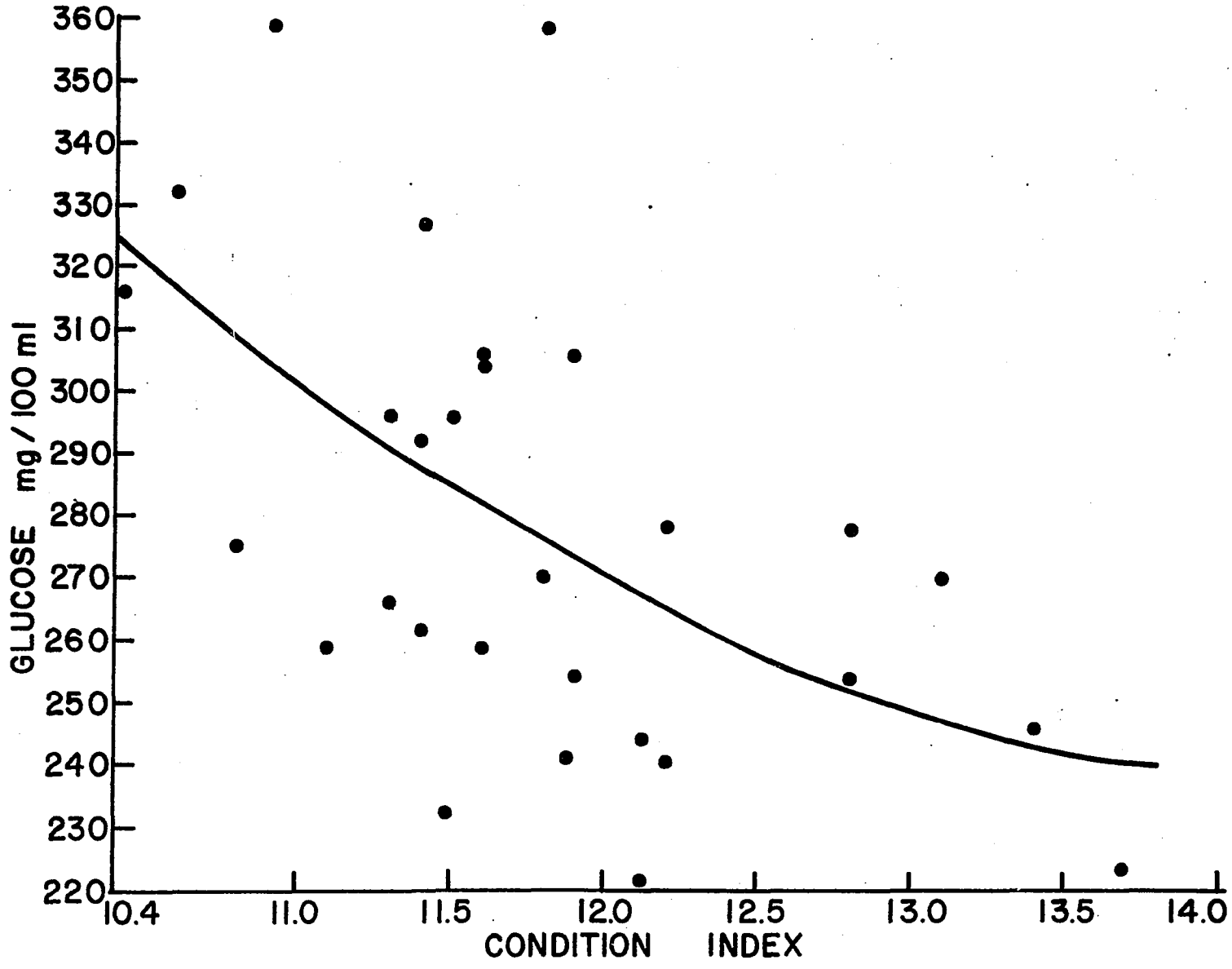
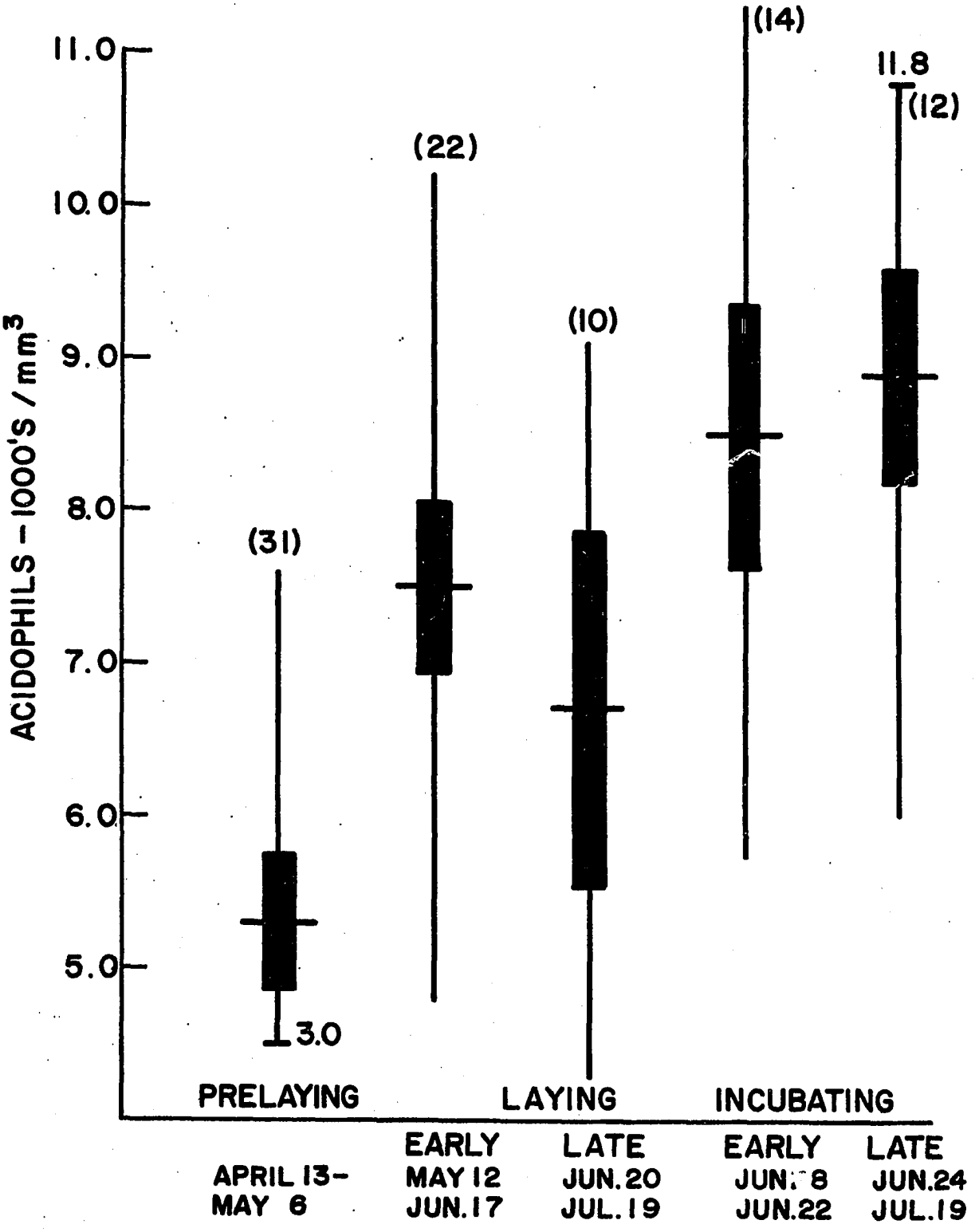

360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
GLUCOSE mg/100 ml
10.4
11.0
11.5
12.0
12.5
13.0
13.5
14.0
CONDITION INDEX

Figure 7. Standard deviation and mean standard error of circulating acidophils of blue-winged teal hens at various reproductive stages (number of observations in parenthesis)


ACIDOPHILS – 1000'S / mm3
11.0
10.0
9.0
8.0
7.0
6.0
5.0
(31)
3.0
(22)
(10)
(14)
11.8
(12)
PRELAYING
LAYING
INCUBATING
EARLY
LATE
EARLY
LATE
APRIL 13 – MAY 6
MAY 12 JUN. 17
JUN. 20 JUL. 19
JUN. 8 JUN. 22
JUN. 24 JUL. 19

Body weight variation of bait-trapped birds during spring and fall

Because body weight was used in an index as a measure of metabolic reserve, some knowledge of daily and seasonal weight fluctuation was needed to interpret weight losses observed during reproduction. Migrating blue-winged teal were trapped during the last week of August and first week of September during 1966, 1967 and 1968. Data were analyzed with respect to the effect of year, sex, week and time of day (A.M. or P.M.) on total weight variation observed. Mean body weights of birds in respective categories are presented in Figure 8. Results of the 2^4 factorial in complete random design may be seen in Table 8.

Table 8. Partitioned sums of squares, main effects and interactions of year, sex, week and time treated as factors of weight variation

Source	df	SS	MS	F
Year	1	33441.84	33441.84	16.50***
Sex	1	221425.38	221425.38	109.50***
Week	1	3249.51	3249.51	1.61
Time (A.M. or P.M.)	1	87307.54	87307.54	43.17***
Year x sex	1	2301.76	2301.76	1.14
Year x week	1	13767.47	13767.47	6.81**
Year x time	1	6103.90	6103.90	3.01
Sex x week	1	0.01	0.01	0
Sex x time	1	0.75	0.75	0
Week x time	1	118.25	118.25	0.58

*** ($P < 0.001$)

** ($0.01 < P < 0.02$)

Table 8 (Continued)

Source	df	SS	MS	F
Year x sex x week	1	5153.02	5153.02	2.54
Year x sex x time	1	4159.00	4159.00	2.01
Year x week x time	1	8218.83	8218.83	4.06
Sex x week x time	1	6589.24	6589.24	3.26
Year x sex x week x time	1	207.07	207.07	0.10
Error	701	1417558.02	2022.19	
			$R^2 = 27.66\%$	

It is evident from these data that sex and time of day accounted for a larger portion of the total weight variation observed than did year. Week was nonsignificant but there was a significant year x week interaction. The large error sum of squares suggests that only a small portion of overall weight variation can be accounted for by the 4 factors considered as seen by the small R^2 value.

Body weight variation at the end of a 24 hour period was estimated from weight changes of individual birds recaptured 24 hours after banding. These data were treated as paired samples and a 99 percent interval estimate constructed about the mean differences (Table 9).

Figure 8. Morning and evening mean body weight of immature male and female blue-winged teal trapped in a two-week period during the fall of 1966, 1967 and 1968

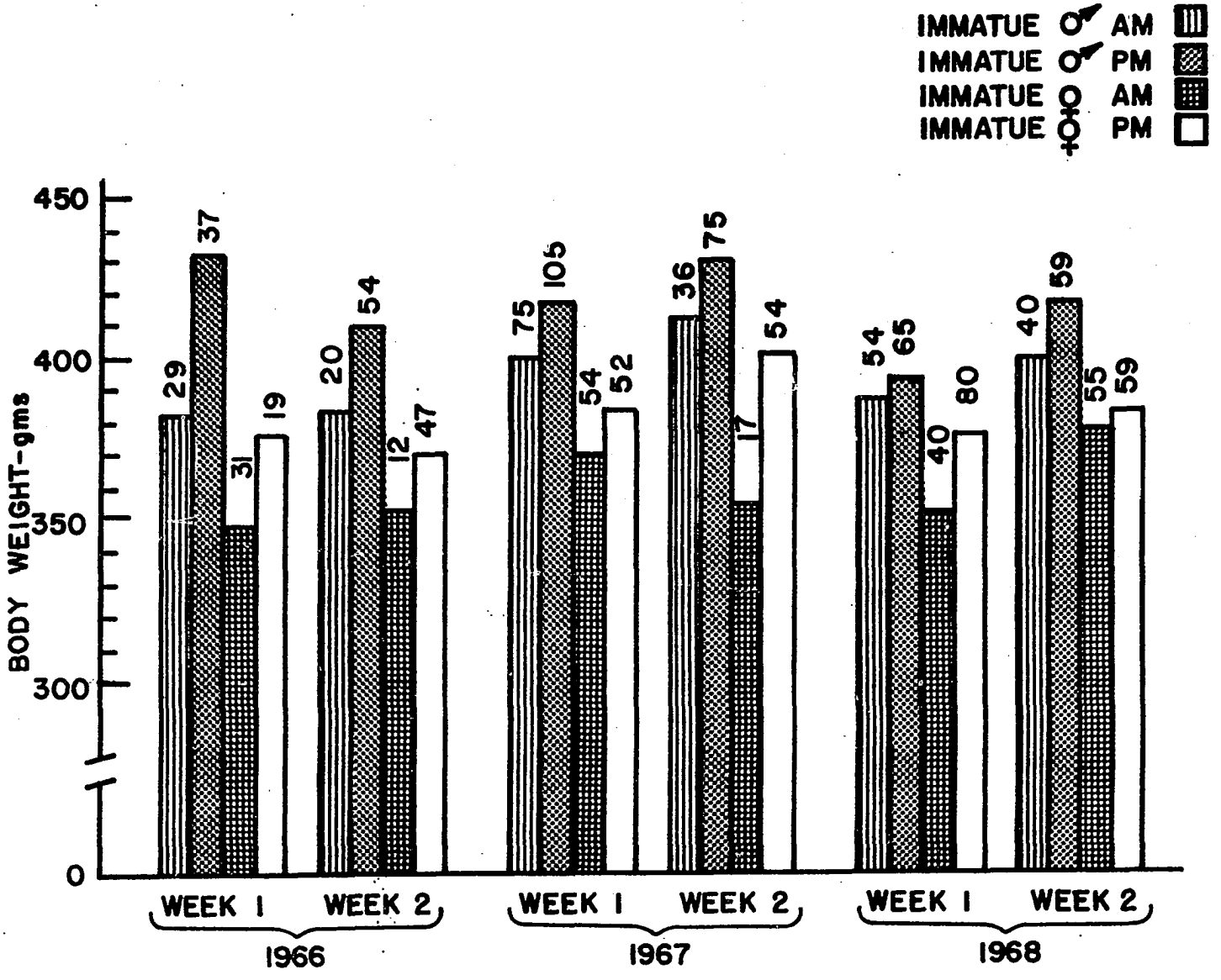

IMMATUE ♂ AM
IMMATUE ♂ PM
IMMATUE ♀♀ AM
IMMATUE ♀♀ PM
BODY WEIGHT-gms
450
400
350
300
0
29
37
31
19
20
54
12
47
75
105
54
52
36
75
17
54
54
65
40
80
40
59
55
59
WEEK 1
WEEK 2
1966
WEEK 1
WEEK 2
1967
WEEK 1
WEEK 2
1968

Table 9. Mean weight differences (grams) of blue-winged teal recaptured after 24 hours

	$\bar{d}$	$s_{\bar{d}}$	n	99% interval estimate
Immature males	0.08[a]	2.62	25	$-7.36 \leq \mu_D \leq 7.20$
Immature females	-4.57	3.53	21	$-14.61 \leq \mu_D \leq 5.47$

[a]Since body weight was measured to the nearest gram, the two decimal figure does not imply level of accuracy but represents a mean value only.

An estimate of diel weight variation was attained by estimating mean weight changes occurring between birds trapped in the morning (A.M.) and evening (P.M.) and between birds trapped in the morning and birds held in captivity overnight (Table 10).

Table 10. Mean body weight (grams) and diel weight variation of blue-winged teal captured in the fall

	A.M.	P.M.	Overnight[a]	A.M. to P.M. gain	Daylight to A.M. gain
Immature males	393	417	372	24	21
Immature females	359	381	346	22	23

[a]Overnight weight represents the mean weight of birds captured in the evening and weighed in the morning after being held in confinement overnight.

As seen in Table 10, birds appeared to gain weight between morning and evening (P.M. - A.M.); a second weight gain is apparent from the difference between mean morning weights minus mean overnight weights (Daylight to A.M. gain).

The effect of repeated trapping on changes in body weight also was investigated. This was done by comparing the observed weight change of individual birds which were trapped only twice in a five-day interval with changes which occurred in birds trapped 3 or more times in the same interval. The difference between weights at first and last trapping constituted an observation on a single bird. Mean differences were calculated for each group and tested with the "Student" t statistic using a pooled estimate of variance (Table 11).

Table 11. Effect of frequency of capture on weight changes

Trap frequency	n	$\bar{d}$	s_p^2	t
Twice only	20	12.8	1987.78	3.58
3 or more	20	-5.0		

Tabular t with 38 degrees of freedom at the 99% level is equal to 2.700. Therefore, it was concluded that repeated trapping did have an effect on weight changes biasing them in a downward direction.

Weight gains of blue-winged teal during a migratory stopover appeared to be in proportion to the length of time between recapture weights (Figure 9). This curve was constructed from recapture data collected from migrating birds during the fall of 1967 and 1968.

Mean weights of adult migratory teal, bait-trapped at the same geographic location during spring (April 1 - May 1), appear considerably lighter than fall-trapped adults (Table 12). Immature birds in the fall also appear heavier than migratory birds captured in the spring.

Figure 9. Cumulative weight gains of transient blue-winged teal trapped during the fall of 1967 and 1968

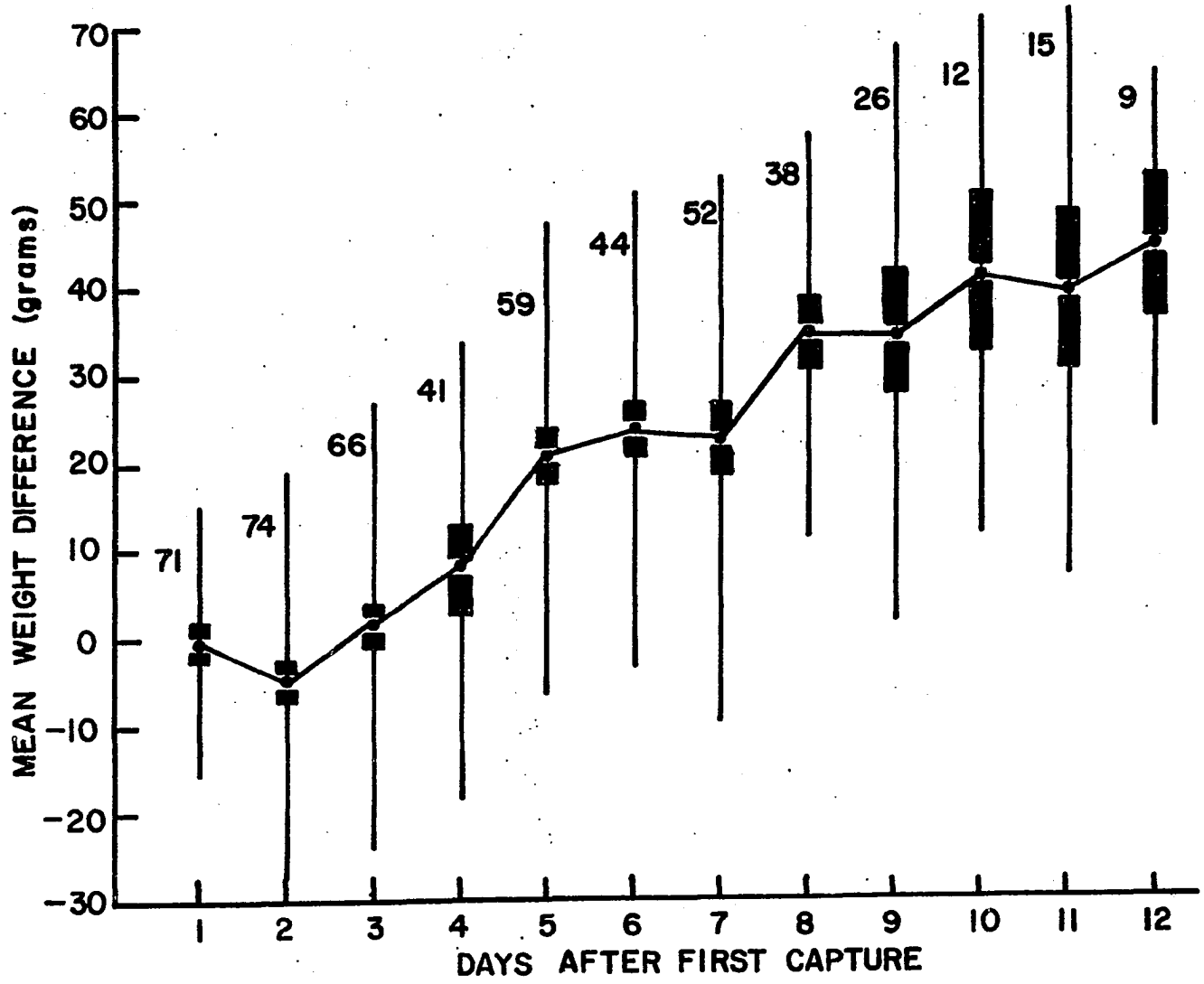

MEAN WEIGHT DIFFERENCE (grams)
70
60
50
40
30
20
10
0
-10
-20
-30
71
74
66
41
59
44
52
38
26
12
15
9
1
2
3
4
5
6
7
8
9
10
11
12
DAYS AFTER FIRST CAPTURE

Table 12. Mean body weight (grams) of bait-trapped blue-winged teal during spring and fall

	Fall	(n)	Spring	(n)
Immature males	405	(520)	--	
Adult males	444	(23)	377	(146)
Immature females	370	(364)	--	
Adult females	397	(39)	351	(47)

Discussion

Physiological correlates associated with reproduction

Rapid loss of body weight during late laying and early incubation is evident from data presented in Figures 1 and 3. What is not evident from these data is the physiological significance of this weight loss. An average of seventy grams of body weight is lost from the laying of the 7th egg until day 5 of incubation (Figure 1). Of these seventy grams, approximately thirty-seven grams (53%) can be attributed to involution of ovary and oviduct (Table 1). The remaining weight lost during this period can be considered loss of metabolic reserves. The major portion probably is due to utilization of fat reserves. Mobilization of fat reserves was reflected in increasing levels of plasma FFA as hens underwent a decrease in condition index (Figure 5). Estrogen is known to have a lipogenic effect on abdominal fat and liver lipids (Sturkie, 1965; Hawkins and Heald, 1966). Fasting in fowl promotes lipid mobilization with a resulting increase in plasma FFA (Heald et al., 1965; Gibson and Nalbandov, 1966; Lepkovsky et al., 1967). As indicated by the level of FFA, lipid

mobilization observed in blue-winged teal hens during early incubation may be due to decreasing levels of circulating estrogens coupled with reduced food intake during incubation. A decline in FFA as condition continues to decline (Figure 5) suggests a depletion of fat reserves associated with incubation.

The increasing level of glucose during incubation (Figure 6) is consistent with the hyperglycemic effect of corticosterone as demonstrated in chickens by Greenman and Zarrow (1961) and in ducks during a later portion of this study. The concomitant increase in NPN (Figure 4) is highly suggestive that gluconeogenesis is associated with the observed hyperglycemic response of incubating hens. The observed responses of NPN and glucose in incubating blue-winged teal hens also are consistent with similar responses observed in fasting pullets (Hazelwood and Lorenz, 1959).

The fact that plasma free amino acid (FAA) levels showed no apparent relation with condition index or no significant increase in incubating hens (Table 3) would seem to be inconsistent with the observed increase of plasma NPN. However, it is possible that mobilized tissue amino acids were metabolized so rapidly that no change in the level of plasma FAA occurred.

Acidophilia was associated with laying and incubating hens (Figure 7). More specifically, the highest mean acidophil level was associated with the lowest mean condition index (Figures 3 and 7) which occurred in incubating hens during the latter part of the nesting season. Undoubtedly, most hens incubating during this period (June 24 - July 19) were renesting. Acidophilia in laying and incubating birds and shifts in plasma

constituents of incubating birds examined in this study are believed to be manifestations of increased adrenal cortical activity. It is further believed that hypercortical activity, typical of the alarm reaction (Selye, 1956), is a result of the energy demands accompanying reproduction.

Physiological correlates associated with migration

It is tempting to associate the remarkably high glucose levels found in prenesting hens (Table 3) with a migratory phenomena. The fact that all prenesting hens were banded and none subsequently were recaptured as nesting hens indicates that many prenestors were migrating at the time of capture. It is possible that high glucose levels observed in these hens may have resulted from increased epinephrine output precipitated by trapping activities. However, one would have expected similar responses associated with trapping of laying and incubating hens which was not indicated by blood glucose levels. Available information (George and Berger, 1966; Goodridge, 1964; Goodridge and Ball, 1965) suggests the high mean glucose level observed in this group of birds may be attributed to high glucagon secretion during active migration.

George and Berger (1966) appear to have established the metabolic role of fat as an energy source in migrating birds. After lipids deposited in the muscle cells have been depleted, mobilization of free fatty acids from adipose tissue occurs (George and Berger, 1966). A high lipolytic activity should facilitate the release and movement of the fatty acids for oxidation. Goodridge (1964) has found glucagon to have a lipolytic effect on the adipose tissue of house sparrows. In vitro lipolysis in pigeon adipose tissue also was stimulated by glucagon and epinephrine (Goodridge

and Ball, 1965). However, according to Carlson et al. (1964), adipose tissue lipase of the chicken did not appear to respond to epinephine but did respond to glucagon. In addition to its lipolytic effect, glucagon is known to have a marked hyperglycemic effect in birds (Hazelwood and Lorenz, 1959).

From their work on migratory and nonmigratory finches, Goodridge and Ball (1965) have hypothesized "that high glucagon output inhibits lipid synthesis during nonmigratory periods. During migratory periods, the output of glucagon falls, allowing lipid sythesis to proceed at a more rapid rate". The known hyperglycemic effect of glucagon and the high blood glucose level observed in migrating hen blue-winged teal during this study would suggest that a peak level of glucagon might be expected in actively migrating birds rather than during the nonmigratory periods. This question obviously needs further attention.

There are few data on body weight of wild blue-winged teal (Bennett, 1938; Bellrose and Hawkins, 1947; Marshall and Harris, 1953; Nelson and Martin, 1953; Dane, 1965). Mean body weights of blue-winged teal, as summarized by Dane (1965), vary widely. Much of this variation undoubtedly is due to small sample size while some may be due to a failure to consider effects of age, time of day, season and temperature on weight variation. Baldwin and Kendeigh (1938) stressed the need to account for the effects of sex, age, time of day, season and temperature of the body weight of birds. It is clear from this study (Table 8) that sex, year and time of day (morning or evening) must be considered when discussing body weights of migratory waterfowl. The fact that a significant year by week interaction

was detected while week alone was nonsignificant suggests that some variable, not accounted for, also was having an effect. Temperature could cause such an effect.

Apparently, diel weight variation has never been studied in waterfowl. However, investigations of daily weight variation in small birds revealed a 5 to 10 percent weight loss during the night which was regained during daylight hours (Taber, 1928; Nice, 1929; Sumner, 1935; Baldwin and Kendeigh, 1938). In this study, both male and female blue-winged teal lost an estimated 45 grams overnight; representing approximately 11 percent weight loss from the peak evening weight. The overnight weight loss was arrived at by inference from combining the mean A.M. to P.M. weight gain with the mean daylight to A.M. weight gain (Table 10). Because the daylight to A.M. weight gain was based on the difference between mean weights of teal held overnight and morning bait-trapped teal, the 11 percent estimate of overnight weight loss may be high since the confinement of teal overnight may have caused excessive weight loss. Because teal can be expected to vary as much as 40 grams during a 24 hour period, it is somewhat remarkable that the mean weight of birds recaptured at the end of 24 hours varied within rather narrow limits from initial weights (Table 9). It is apparent that in the course of a comparative study a significant proportion of weight variation can be eliminated if birds are weighed at the same time of day.

Weight gains associated with fall migratory teal (Figure 9) would indicate that the marshes of the Ruthven area are utilized for feeding stops before continuing southward migration. As seen in Figure 9, teal

appeared to lose weight during the first two days after banding, then gained steadily until the sixth day where gains leveled off. After the seventh day, the rate of gain again increased although the sporadic nature is undoubtedly related to the small sample size beyond day nine. Two aspects of this "growth curve", which undoubtedly portrays the rate of fat deposition, appear unusual. One is the mean weight loss during the first two days; the second is the step-like pattern of weight gain. The first phenomenon has been described by several investigators studying weight changes in migratory passerines (Nisbet et al., 1963; Browne and Browne, 1956; Davis, 1962; Mueller and Berger, 1966). Some workers (Browne and Browne, 1956) reject handling as a cause for weight decline during the first two days after banding while others believe handling to be a causative factor (Mueller and Berger, 1966). Because handling of birds only twice in this study did not cause a weight decline (Table 11), an alternate hypothesis to explain the initial weight loss of recaptured birds may be considered.

It is possible that blood glucose levels play a role in controlling the level of feeding intensity in migratory birds. It has been suggested that relatively high blood glucose levels in mammals reduce the level of feeding while lower blood glucose stimulates feeding. This theory is commonly referred to as the glucostatic theory of hunger and feeding regulation (Guyton, 1966:1006). Migratory teal in this study were found to be hyperglycemic (Table 3), a condition believed related to high glucagon secretion in actively migrating birds. It is conceivable that the glucagon level declines gradually in birds during a migratory feeding stop resulting in a gradual decline of glucose and permitting hyperphagia only

after a lapse of one or two days. This could cause the initial loss of weight followed by rapid weight gains as observed in this and other studies.

The unusual step-like pattern of weight gain cannot be explained at this time. However, the fact that the rate of gain declined on days 6 and 7 during both years would suggest that some internal mechanism, rather than some environmental factor, may be responsible. When similar data are plotted for the Blackpoll warbler Dendroica striata (Nisbet et al., 1963) and Swainson's thrush Hylocichla ustulata (Mueller and Berger, 1966), a biasymptotic sigmoid curve also is suggested.

The apparent overall weight gain of approximately 45 grams in a 12 day period undoubtedly can be attributed to an increase in fat deposition. Odum et al. (1964) found that the components of the nonfat body remain essentially homeostatic in the migratory bird despite very large and rapid changes in total body weight. The 40 to 45 grams of fat accumulated during a feeding stopover represent the migratory energy reserve. If the energy required for sustained flight of teal were known, it would be possible to calculate the estimated flight range of teal after migratory fattening. Odum et al. (1961) have made such estimates for some other migratory birds.

EFFECTS OF FASTING AND CORTICOSTERONE ADMINISTRATION ON PLASMA CONSTITUENTS AND ACIDOPHIL COUNTS OF CAPTIVE HENS

As seen in the previous section, blood levels of NPN, FFA, glucose and acidophils in wild blue-winged teal hens increased during periods of laying and incubation. Because these responses are similar to those observed in fasted and corticosterone treated domestic fowl, an investigation was undertaken to determine the effect of fasting and corticosterone administration on levels of blood NPN, FFA, glucose and acidophils of captive wild blue-winged teal.

Corticosterone has been found to be the major adrenal steroid of the duck (De Roos, 1961). Cortisol, corticosterone and to some extent aldosterone are generally recognized as being gluconeogenic (Gorbman and Bern, 1962; De Roos, 1963). Greenman and Zarrow (1961) observed a marked hyperglycemic effect in hens treated with hydrocortisone and corticosterone. This response appears to be common to all vertebrates tested as indicated in a summary presented by Bentley and Follett (1965). According to Sturkie (1965), cortisone increased the blood lipids of cockerels but had no appreciable effect on other blood constituents. Heald and Rookledge (1964) found a marked increase in plasma free fatty acids of adrenocorticotrophin (ACTH) injected pullets. However, when 40IU/kg of long lasting ACTH were administered to laying hens, a decline in FFA occurred which was accompanied by a marked increase of blood glucose.

Fasting in birds has produced shifts in plasma constituents similar in some ways to those produced by corticoid administration (Hazelwood and Lorenz, 1959; Heald and Rookledge, 1964; Gibson and Nalbandov, 1966;

Lepkovsky et al., 1967). Formed elements of the blood also have been shown responsive to stressor and corticoid administration. Newcomer (1958, 1959) concluded that acidophilia in chickens is indicative of a state of acute stress. Shapiro and Schechtman (1949) noted an increase in percent of heterophils following injection of adrenocortical extract in White Leghorn laying hens.

Methods

Procedures for handling blood samples, methods used for cell counts and chemical analysis were the same as those used in examining blood samples of wild birds.

Fasting experiment

Ten adult blue-winged teal hens randomly selected from a large captive flock during February were used to determine the effects of fasting on acidophil levels, plasma NPN, glucose and body weight. The flock was housed in an unheated frame building with woodshavings as litter. The experimental birds were transferred to a heated building and individually caged one month before initiating the experiment. Purina "turkey grower" and scratch grain were fed ad libitum during this acclimation period. Fast was initiated in 8 of the 10 hens by food withdrawal. Two hens were continued ad libitum as controls. All birds had access to water during the experimental period. Air temperature ranged from 73° to 81°F during the fasting period. All birds were weighed, and a 3 ml blood sample was withdrawn from each bird. Birds were weighed again on the 2nd, 7th and 10th days; 2 ml blood samples were taken on the 2nd and 7th

day. Data were analyzed using analysis of variance and regression techniques (Snedecor, 1956).

Corticosterone injections

The effect of exogenous corticosterone on blood acidophils, plasma NPN, FFA and glucose was investigated on the basis of paired comparisons. Twelve blue-winged teal were randomly selected from the captive flock and a preinjection blood sample withdrawn after food had been withheld for eight hours. Nine birds received 2.5 mg corticosterone/day (Nutritional Biochemical Company) for seven days. The hormone was administered subcutaneously in a 0.2 ml saline Tween 80 suspension (Zarrow et al., 1964).

Three birds served as controls and received saline-Tween 80 solution only. Postinjection blood samples were drawn on the 7th day with birds again in a postabsorbative state. All birds were in a nonreproductive condition as indicated by their Nonbreeding Plumage. Data were analyzed using paired comparison technique (Simpson et al., 1960).

In a separate experiment, the dose-response relationship of acidophil level to exogenous corticosterone was investigated. Corticosterone was administered daily for seven days at levels of 0, 1 and 2 mg/day to three separate groups containing 4 birds each. Preinjection acidophil levels of each bird were compared with the levels found after seven days of treatment.

Results

Effects of fasting

Body weight loss was very rapid over the period of fast. Experimental birds lost an average of 113 grams during the ten day fast

(Table 13).

Table 13. Effects of fasting on mean blood NPN, glucose, acidophil levels and body weight of hen blue-winged teal

	Day 0	Day 2	Day 7	Day 10
NPN mg/100 ml	20.2	21.2	25.7	----
Glucose mg/100 ml	243	217	259	----
Acidophils 1000's/mm^3	5.2	4.7	5.0	----
Body weight grams	313	282	240	200

At the end of ten days, 3 of the eight experimental birds succumbed to starvation. Control birds also lost weight over the experimental period but weight loss was only one-third as great as experienced by fasting birds. Weight loss of controls was attributed to handling. Regression of body weight on number of days fasted yielded an expression of the form $\hat{y} = 320 + (-11.5X)$. The slope, or rate of weight loss was 11.5 grams per day.

Mean plasma NPN increased markedly over the seven day fasting period (Table 13). These data were analyzed using regression analysis in a single factor experiment (Snedecor, 1956:346); results are presented in Table 14. The effect of days on level of NPN was tested by partitioning the sum of square due to treatment (days) into a linear and lack-of-fit component. An F test of the linear component against the error mean square resulted in an F value of 12.56 which would indicate a significant

increase of NPN with days of fasting.

Table 14. Analysis of variance of increased NPN levels in Table 10

Source	df	SS	MS	F
Hens	7	153.52	21.93	1.91
Days	2	140.30	70.15	6.11
linear	1	139.20	139.20	12.56[a]
lack-of-fit	1	1.10	1.10	----
Error	14	160.88	11.49	

[a] $P < 0.01$.

Plasma glucose decreased on day two and had increased again by the seventh day (Table 13). An F test for lack of fit (Table 15) indicated significance where $P < 0.05$.

Table 15. Completed analysis of variance of blood glucose data of fasted blue-winged teal

Source	df	SS	MS	F
Hens	7	12433	1886	2.1
Days	2	7280	3640	4.3
linear	1	2606	2606	3.1
lack-of-fit	1	4674	4674	5.5[a]
Error	14	11834	845.3	

[a] $0.05 < P < 0.01$.

The low sum of squares found for the linear component and the significant test for lack-of-fit would indicate that the blood glucose

response of treated hens was something other than linear. The mean glucose level of control birds had increased 20 mg percent by day seven without undergoing the marked drop in blood glucose experienced by experimental birds on day two.

Effects of corticosterone injections

The change in mean plasma FFA, NPN, glucose and acidophil levels as a result of corticosterone injections may be seen in Table 16.

Table 16. Mean response of FFA, NPN, glucose and acidophils to exogenous corticosterone — t test based on change from preinjection levels

	Preinjection levels	$\bar{d}$	n	$s\bar{d}$	t	$\bar{d}$(control)
FFA μeq/liter	750-965	325.7	8	135.6	2.40	171.0
NPN mg/100 ml	12.5-15.8	8.10	8	2.06	3.93	-2.0
Glucose mg/100 ml	150-270	71.4	8	11.94	5.97	7.0
Acidophils 1000's/mm^3	1.5-5.1	4.2	8	0.63	6.60	-10.0

Free fatty acids increased an average 325.7 μeq/liter from preinjection levels; however, this increase was nonsignificant at the 0.01 probability level. The level of FFA in control birds increased appreciably over the same period. In the treated group NPN, glucose and acidophil levels underwent marked increases over preinjection levels while NPN and acidophil level of control birds experienced a decline. The mean glucose level of control birds changed only slightly over the experimental period.

Acidophil response to various levels of injected corticosterone may be seen in Table 17.

Table 17. Change in acidophil number[a] from preinjection levels — response to various levels of corticosterone

		Levels — mg corticosterone/day		
		0	1	2
Hens	1	1.5	5.1	3.7
	2	1.1	-2.4	3.7
	3	-2.0	3.3	6.9
	4	1.1	0.9	11.2
Mean		0.42	1.73	6.37

[a]Acidophils in 1000's/mm^3.

Data were analyzed using single factor regression analysis. When the sum of squares due to treatment (levels) was partitioned and tested against error a significant linear response was detected (Table 18).

Table 18. Analysis of variance of acidophil response in Table 17

	df	SS	MS	F
Hens	3	20.87	6.96	0.74
Levels	2	78.29	39.15	4.16
line	1	70.81	70.81	7.52[a]
lack-of-fit	1	7.48	7.48	0.79
Error	6	56.50	9.42	

[a]$P < 0.05$.

Discussion

It seems clear from present knowledge of the physiology of the avian adrenal (De Roos, 1963) that avian corticoid function is similar to that of mammals in controlling both mineral and carbohydrate metabolism (gluconeogenesis). The marked increase in blood glucose and NPN (Table 16) of hens treated with corticosterone in this study is consistent with the expected gluconeogenic effect (Bentley and Follett, 1965). Brown et al. (1958) have suggested that weight loss, increased uric acid and total nitrogen excretion and increased liver glycogen of ACTH treated chickens is the result of a glucocorticoid compound released by the adrenals in response to ACTH. In a recent study by Frankel et al. (1967), adrenal cortical function of the cockerel was found dependent on ACTH release although the site of adrenocortical hormone feedback was found not to be at the pituitary level.

Available information related to the effect of adrenal steroids and ACTH on plasma lipid is somewhat conflicting. ACTH has been found to both increase and decrease the level of plasma FFA depending on reproductive state of the bird and dosage of hormone administered (Heald et al., 1965; Heald and Rookledge, 1964). Nagra and Meyer (1963) found that corticosterone treatment of growing cockerels increased plasma level of total lipid and carcass lipid but decreased carcass protein and glycogen.

The significance of the effect of corticosterone administration on the plasma FFA levels of ducks in this study was not clear. Although the mean FFA levels of experimental birds increased from preinjection levels ($P < 0.05$), the magnitude of increase in control birds would suggest that the response in treated birds should be viewed with caution (Table 16).

The fact that all birds, including controls, were on full ration during the experimental period but still experienced weight loss would suggest that food consumption was curtailed. Daily handling of the birds to administer the hormone or carrier may have disrupted their feeding pattern.

The number of circulating acidophils of ducks treated with 2.5 mg corticosterone/day increased considerably from preinjection levels (Table 16). This acidophilia is consistent with the findings of Newcomer (1958, 1959) who found acidophil numbers to be sensitive to both exogenous adrenal steroids and ACTH. In the present study, when the effects of various levels of corticosterone were tested for acidophil response, a significant linear effect was detected (Table 18). The results indicate that acidophilia in blue-winged teal is associated with increased levels of corticosterone even though the form of the dose-response relationship was not revealed above 2 mg/day.

Blue-winged teal hens subjected to total fast lost an average of 36% of their body weight in a 10 day period. The three birds which succumbed on the 10th day of fast had lost between 40 and 42 percent of their initial body weight. This is less than the 50 percent weight loss found to be the critical level for mallards (Jordan, 1953). The blue-winged teal also succumbed to the effects of starvation in a much shorter time than did the mallards in Jordan's study. These differences are most likely due to low initial weights of the blue-winged teal rather than to species differences.

The effects of fasting on plasma FFA levels of blue-winged teal was not tested because of the low initial starting weights. However, fasting in chickens has been associated with increased levels of plasma FFA

(Lepkovsky et al., 1967; Heald and Rookledge, 1964; Gibson and Nalbandov, 1966). In this study, fasting was found to cause a progressive increase in plasma NPN while the mean glucose level was depressed by the 2nd day of fast and elevated above the initial level by the 7th day of fasting (Table 13). Although this apparent hypoglycemic-rebound effect was found to be nonsignificant in this study (Table 15), a similar hypoglycemic effect has been recorded in fasting domestic fowl (Hazelwood and Lorenz, 1959; Heald and Rookledge, 1964; Gibson and Nalbandov, 1966; Lepkovsky et al., 1967). Hazelwood and Lorenz (1959) suggested that the progressive rise in NPN and the secondary rise in blood glucose during prolonged fasting were due to increased gluconeogenesis. This hypothesis would appear to gain support from the fact that hypophysectomized, fasted cockerels did not respond with a secondary rise in blood glucose (Gibson and Nalbandov, 1966).

If gluconeogenesis plays a significant role in the fasting bird an increase in adrenal cortical activity would be expected, resulting, by reason of previously discussed findings, in acidophilia. However, in this study there was clearly no change in acidophil numbers in response to fasting (Table 13). Starvation in pigeons has been shown to significantly reduce hematocrit (Hazelwood and Wilson, 1962) while both hematocrit and plasma protein have been reduced in protein-depleted cocks (Wessels and Fisher, 1965). It is possible that demands on metabolic reserves of blue-winged teal hens, as a result of fasting, inhibited acidophil formation.

HEMATOLOGICAL ALTERATIONS INDUCED BY CORTICOSTERONE INJECTIONS

During the study of the effect of exogenous corticosterone on plasma constituents, it was noted that postinjection blood samples appeared less viscous than normal. A cursory comparison with controls revealed a much reduced packed cell volume in the corticosterone treated birds. It was hypothesized that apparent decrease in hematocrit could be due to an actual decrease in erythrocytes or to an increase in plasma volume. Therefore, a study was conducted to test the effects of exogenous corticosterone on erythrocyte counts, packed cell volume, hemoglobin concentration and plasma volume of blue-winged teal hens.

In mammals, it is generally accepted that cortisol increases production of red blood cells (Guyton, 1966). No information was found in the recent literature on the hematological effects of corticosterone in the avian system. However, hematological alterations have been induced in the pigeon by nonspecific stresses (Hazelwood and Wilson, 1962). Injection of adrenal cortical extract in white leghorn hens was followed by a marked leucopenia, absolute lymphopenia, a decrease in number of erythrocytes and an absolute polymorphonuclear leucocytosis (Shapiro and Schechtman, 1949). Decreased hematocrit, hemoglobin and red blood cell levels also have been observed to accompany the onset of reproductive activity in several avian species. Domm and Taber (1946) observed erythrocyte counts of domestic fowl to be lowest during peak reproductive activity while Ronald et al. (1968) found erythrocyte numbers, hematocrit and hemaglobin levels of the red-winged blackbird to be lower in August than during the spring. Hunsaker et al. (1964) found packed cell volume,

erythrocyte numbers and hemoglobin levels of female geese to vary inversely with the rate of egg production. Because increased adrenal activity has been associated with reproductive activity in the blue-winged teal a study of the effect of corticosterone on hematological alterations was of particular interest.

Methods

Birds used in this study were either wild trapped birds which had been in captivity for approximately one year or were birds one generation from wild stock. In late July, twelve hens were randomly selected from a larger flock and housed in a separate section of a frame building with wood-shavings used for litter. The teal had access to food and water as well as an outside run during the experimental period.

Hematological parameters

Plasma volumes, packed cell volumes, blood volumes and erythrocyte counts were determined for the twelve birds prior to initiation of corticosterone treatment. Plasma volumes were estimated using the dye (T-1824) method as outlined by Consolozio et al. (1951) with minor modifications. One and one-half ml of blood were drawn from the brachial vein of the wing in a heparinized syringe. This sample was used to obtain the plasma blank. Sixteen hundredths milligrams of dye (0.16) in 0.1 cc avian Ringers was injected into the leg vein (tarsometatarsus) and allowed to mix three minutes as suggested by Sturkie (1965). A second blood sample was withdrawn at the end of 3 minutes from the opposite wing vein; the plasma was separated and compared in the standard manner with the plasma blank (Consolazio et al., 1951).

Blood for hematocrits, cell counts and hemoglobin determination was obtained at the same time that blood was procurred for the plasma blank. Hematocrits were determined using the microhemotocrit technique (Cohen, 1967) while erythocyte counts were made in a Neubauer "bright-line" counting chamber using Wiseman (1931) stain. Hemoglobin was not determined for the preinjection samples but was determined on postinjection samples. The acid hematin method as described by Lucas and Jamroz (1961) was employed. Total blood volume was calculated using estimated plasma volume and venous hematocrit. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated in the standard manner (Wintrobe, 1962).

Experimental design

After preinjection parameters were estimated, the birds were divided into 3 equal groups and corticosterone was administered at levels of 0, 1 and 2 mg per day. The hormone was administered in a 0.2 ml Tween-80 saline suspension as described by Zarrow et al. (1964); controls received carrier saline only. Injections continued for 7 days and the hematological parameters again were estimated four hours after receiving the injections on day seven. Except for hemoglobin, the data were treated as paired samples and analysis performed on differences observed between pre and postinjection values.

Results

Because values for hematological parameters in blue-winged teal apparently have not been recorded, the mean and standard deviation of some parameters from preinjection blood samples are presented in Table 19.

Table 19. Hematological parameters in twelve blue-winged teal ducks[a] — preinjection levels

Parameter	Mean	Standard deviation
Erythrocyte volume, ml	13.4	±2.08
Plasma volume, ml	15.6	±1.67
Blood volume, ml	29.0	±3.56
Blood volume, ml/100g	9.4	±1.09
Venous hematocrit	46.8	±3.35
Erythocytes million/mm^3	3.02	±0.1749

[a]Mean based on a sample size of twelve for all parameters.

The effects of corticosterone injections on some hematological parameters may be seen in Table 20. No attempt was made to test differences observed between means of various injection levels (Table 20) because the experiment was designed to detect differences on the basis of paired comparisons.

Table 20. Mean[a] hematological response of blue-winged teal to a seven day treatment of corticosterone injections

Parameter	Level of corticosterone — mg/day		
	0	1	2
Plasma volume, ml	19.7	16.6	17.2
Venous hematocrit	49.0	43.3	37.5
RBC's million/mm^3	3.21	2.89	2.44
Hb grams/100 ml	15.4	14.1	12.8
MCV Cμ	151	152	155
MCH γγg	48	49	52
MCHC %	31.6	32.4	34.0

[a]Mean based on four observations at each injection level.

The effect of corticosterone injections on plasma volume, venous hematocrit number of circulating erythrocytes and mean corpuscular volume expressed as a mean difference between pre and postinjection samples is recorded in Table 21.

Table 21. Mean change[a] from preinjection levels of some hematological parameters of blue-winged teal

Parameters	Level of corticosterone — mg/day		
	0	1	2
Plasma volume, ml	4.6	1.6	0.8
Venous hematocrit	2.8	-4.5	-9.0
RBC's millions/mm^3	0.13	-0.08	-0.58
MCV C	4	-4	-4

[a]Mean change for all parameters based on observed differences within 12 birds.

Apparent changes in plasma volume, venous hematocrit and number of erythrocytes with respect to the level of corticosterone were tested using the technique of regression in a single factor experiment (Snedecor, 1956:345).

A significant change in plasma volume associated with the level of corticosterone administered was not detected as seen in Table 22. The test of linear regression, $F = 28.50/5.91 = 4.82$ shows $0.1 < P < 0.2$.

Table 22. Test of linear relationship between level of injected corticosterone and plasma volume of blue-winged teal

Source	df	SS	MS	F
Hens	3	20.47	6.82	1.15
Levels	2	31.96	15.98	2.70
linear	1	28.50	28.50	4.82
lack-of-fit	1	3.46	3.46	0.58
Error	6	35.48	5.91	

Hematocrit appeared to change linearly with respect to level of corticosterone administered (Table 21, Table 23). The overall test of levels $F = 140.6/15.3 = 9.2$ was significant where $P < 0.05$ while the test for linearity shows $0.01 < P < 0.02$.

Table 23. Test of linear relationship between level of injected corticosterone and hematocrit of blue-winged teal

Source	df	SS	MS	F
Hens	3	14.2	4.73	0.3
Levels	2	281.2	140.6	9.2
linear	1	276.2	276.1	18.0
lack-of-fit	1	5.1	5.1	0.3
Error	6	91.5	15.3	

Number of circulating erythrocytes also decreased linearly with respect to level of hormone administered (Table 24). The test of linear regression $F = 1.001/0.005 = 10.21$ shows $0.02 < P < 0.05$.

Table 24. Test of linear relationship between level of injected corticosterone and erythrocyte number of blue-winged teal

Source	df	SS	MS	F
Hens	3	0.002	0.006	0.006
Levels	2	1.056	0.528	5.39
linear	1	1.001	1.001	10.21
lack-of-fit	1	0.055	0.055	0.56
Error	6	0.587	0.098	

A strikingly linear relationship was found between hemoglobin levels and level of hormone administered. As may be seen in Table 25 the linear component accounted for the entire sum of squares due to levels and the test of linear regression shows $P < 0.01$.

Table 25. Test of linear relationship between level of injected corticosterone and hemoglobin level of blue-winged teal

Source	df	SS	MS	F
Hens	3	0.39	0.13	0.02
Levels	2	13.26	6.63	10.36
linear	1	13.26	13.26	20.72
lack-of-fit	1	--	--	--
Error	6	3.86	0.64	

Discussion

Information on blood values for blue-winged teal was not found in the literature but Hemm and Carlton (1967), in a review of duck hematology, cited blood values for some dabbling ducks comparable to those found in this study and shown in Table 19. Bond and Gilbert (1958) studied blood volumes of several avian species and found the mallard Anas platyrhynchos

and black duck Anas rubripes have an average total blood volume (ml/100 mg) approximately 2 ml higher than found for teal in this study (Table 19).

Mean hematocrit, erythrocyte number and hemoglobin values were lower in corticosterone treated birds (Table 20). Hematocrit, number of erythrocytes and hemoglobin declined linearly with corticosterone treatments 0, 1 and 2 mg/day (Tables 23, 24 and 25). The fact that no concomittant increase in the plasma fraction occurred (Tables 21 and 22) while hematocrit, number of RBC's and hemoglobin declined, would indicate corticosterone produced a real rather than apparent anemia. The apparent decrease of extracellular fluid volume (Table 21) is consistent with findings of cortisone acetate treated chickens which experienced a slight decrease in thiocyanate space (Brown et al., 1958).

Mean corpuscular values, especially mean corpuscular hemoglobin concentration (MCHC), do not appear to be consistent with reductions of hemoglobin and hematocrit in corticosterone treated birds. The increase in MCHC in the treated group is counter to what would be expected in an anemic animal. Only reductions below normal in MCHC have been observed in human anemias with the exception of hereditary sperocytosis (Wintrobe, 1962:404). The apparent disparity of the data in this study may be due to a macrocytosis occurring in the control group. The initial acute blood loss of birds in the control group coupled with a good capacity for hematopoiesis may have caused a large number of immature cells to pass into circulation. Since immature forms are generally larger (Lucas and Jamroz, 1961), the average size of cells in the bloodstream may have been increased (Table 21) and thus reduced the MCHC below normal. If

corticosterone inhibited erythropoiesis in the treated birds, as is believed to have occurred, then no change in MCHC would be expected and the increase relative to controls (Table 20) would be apparent rather than real.

It is tempting to speculate about the possible role of adrenal steroids in relation to seasonal variation of erythrocyte counts, hematocrits and hemoglobin levels. Although no reference was found relating seasonal variation of hematological parameters to adrenal activity, Kakara and Kawasima (1939) reported that incubating birds had lower erythocyte counts than did laying birds.

Reduced hematocrit, hemoglobin and red blood cell levels have also been observed to accompany the onset of reproductive activity in several avian species (Domm and Taber, 1946; Hunsaker et al., 1964; Ronald et al., 1968). Domm et al. (1943) and Hunsaker (1968) have suggested that estrogen may cause the hematological alterations accompanying the onset of reproduction.

Since there is good reason to believe that increased adrenal activity accompanies the onset of reproduction in the hen blue-winged teal, as described earlier in this study, an increase in circulating adrenal steroids as well as increased levels of estrogen would be expected. The decrease in hematocrit, erythrocyte number and hemoglobin level of corticosterone treated ducks suggests that corticosterone, as well as estrogen, may be functionally related to known seasonal variation in hematologic parameters of birds.

SYNTHESIS

It is evident from the foregoing findings that during the late stages of the reproductive cycle, wild blue-winged teal exhibit a number of characteristic effects produced in corticosterone-treated or fasted captive teal. The increase in blood level of NPN, glucose and acidophils of incubating teal hens is evidence of increased adrenal cortical activity resulting in mobilization of tissue reserves to meet reproductive energy demands. An increase in FFA levels in incubating hens during early incubation and subsequent decline associated with a reduction in condition index is believed to reflect fat mobilization precipitated by lowered estrogen levels and reduced feeding. Striking weight losses at cessation of laying can be interpreted as a loss of metabolic reserves. Similar weight changes have been recorded for the redhead Aythya americana (Weller, 1957a) and mallard duck Anas platyrhynchos (Falk et al., 1966; Höhn, 1947). A rapid decline in body weight of hen pheasants during the later stages of reproduction has been documented by several investigators (Kirkpatric, 1944; Kabat et al., 1950; Breitenbach and Meyer, 1959). Kabat et al. (1956) associated stress resistance of hen pheasants with available energy stores and found hens at their lowest level of physical condition after egg laying. Breitenbach and Meyer (1959) concluded that hen pheasants that have incubated a clutch and brooded young have depleted their energy reserves and are more vulnerable to stress factors.

It is clear that in waterfowl and gallinaceous birds, a loss of metabolic reserves is associated with the postlaying period. Although only a moderate weight loss was associated with egg production of wild

blue-winged teal, the eggs produced represent mobilized energy. Clearly, the stress of reproduction is associated with energy demands of the reproductive processes.

In vertebrates which produce polylecithal or mesolecithal eggs, protein and energy material must be mobilized and stored in the ova in a relatively short period of time. Other aspects associated with reproduction, such as migration, care of the young and reduced feeding during all or part of the reproductive process compound the energy demands of ova production. In forms subject to such rigors, the adrenal cortex is believed to play a primary adaptive role in energy mobilization.

That the demands of reproduction can exceed the adaptive capacity of the adult, resulting in death, is exemplified in some species of the Salmonidae. There is evidence to indicate (Robertson et al., 1961a) that the degenerative changes occurring in the Pacific salmon (Oncorhynchus tshawytscha) during migration and spawning are a result of hyperadrenal activity. In Pacific salmon it would appear that the catabolic effect of the adrenal steroids are of such magnitude that all reproductive individuals succumb. However, in other salmonids, only partial or no postspawning mortality occurs. Robertson et al. (1961b) compared adrenal activity and accompanying degenerative changes in spawning migratory and nonmigratory rainbow trout (Salmo gairdnerii) with Pacific salmon. He concluded that differences in food intake of the various species may have a modifying influence on the catabolic action of high levels of adrenal steroids.

Evidence from the present study on blue-winged teal hens and

previously mentioned studies on pheasant hens indicates that a phenomena may be occurring in these birds similar to that described by Robertson for the Salmonidae. The physiological information accumulated in this study suggests that the adrenal plays an adaptive role to meet the energy demands of reproduction in female teal. If adaption to meet reproductive demands leads to degenerative changes, some postbreeding hen mortality would be expected.

The fact that corticosterone administration appeared to produce anemia in captive blue-winged teal suggests that hyperadrenal activity in wild blue-winged teal hens would have a similar result. Reduced oxygen carrying capacity in wild hens would appear distinctly disadvantageous.

Lymphoid tissue (thymus) is suppressed by adrenal cortical hormones (Garren and Statterfield, 1957; Huble, 1958; Höhn, 1959; Newcomer and Connally, 1960). Since lymphoid tissue is an integral part of the immunological system, any dysfunction of this system could result in lowered resistance to disease or parasitic infections. These possible deleterious side effects may accompany increased adrenal cortical activity precipitated by the energy demands associated with reproduction. The culmination of such a phenomena may result in increased vulnerability of hen blue-winged teal to natural mortality factors.

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