ANNUAL CYCLE OF ADRENAL AND THYROID GLANDS IN GAMBEL QUAIL OF SOUTHERN NEW MEXICO

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The annual cycle of a group of populations of Gambel Quail (*Lophortyx gambelii*) in southern New Mexico was studied intensively for a period of slightly over two years in 1959–61. The main emphasis of the study was on reproduction, molts, and behavior (Raitt and Ohmart 1966), but thyroid and adrenal glands were also given attention, especially in the last year of the study. A number of studies have been made of seasonal changes in histology of avian thyroids (Höhn 1961, and Wilson and Farner 1960 have reviewed most of this work), but relatively few have studied these changes simultaneously with other seasonal phenomena. Furthermore, desert birds and wild galliforms appear not to have received attention. Annual cycles of avian adrenals have been studied in only a very few species (Lorenzen and Farner 1964; Thybusch 1965). For these reasons the normal role of these endocrine glands in free-living birds remains uncertain and, to a degree, controversial (Höhn 1961; de Roos 1963; Lorenzen and Farner 1964; Sturkie, 1965), and it appears worthwhile to explore the cycles in the quail.

Seasonal variations in weight of adrenals and in histological criteria of activity were examined in quail collected from October 1960 through September 1961. The rationale for this study involves two assumptions: (1) that morphological changes reflect changes in secretory level and (2) that, if seasonal changes in these glands are correlated with environmental variables or with other seasonal changes in the birds themselves, then these correlations may give indications as to the normal role of the products of the glands in the physiology of the birds. As Wilson and Farner (1960) and Lorenzen and Farner (1964) have correctly pointed out, the precise role of the glands and their hormones can be fully elucidated only by experimental studies. However, it would seem just as true that descriptive analytical studies of the "natural experiment" of the annual cycle can provide a framework for experimental studies, posing specific questions to which the experimentalist might address himself and suggesting relationships which require explanation.

POPULATIONS, STUDY AREA, METHODS

The quail populations, their habitat, and the general methods of the study have been discussed in detail elsewhere (Raitt and Ohmart 1966), but a brief review here seems pertinent. Monthly samples were collected among several localities in the Rio Grande floodplain within seven miles of Las Cruces, Dona Ana County. Most birds were collected by live-trapping, but some were shot in midsummer and late fall, when trapping was impractical. Trapped birds were brought alive to the laboratory, killed by thoracic compression, and weighed. Thyroids and median portions of the back were dissected, fixed in Bouin's fluid, and transferred to 70 per cent ethanol for storage. Shot birds were treated similarly as soon as possible after death.

Later, the left adrenals were dissected and weighed to the nearest 0.1 milligram on a Roller-Smith balance. Adrenals and thyroids were prepared for histological examination by sectioning at a thickness of 8 microns and stained with Harris's hematoxylin and eosin.

In this paper, only results based on full-grown birds will be included. Birds-of-the-year collected prior to December were excluded. A few December specimens that were still in terminal stages of the postjuvenal molt were also excluded.

Thyroid activity was evaluated by the method of Davis and Davis (1954), in which epithelial cells intercepted by two straight, perpendicular lines across a section from the center of the gland are assigned to one of five graded categories of increasing activity. The average rating for all cells classified constitutes an index for the gland. The index may range from 1.00 for a gland in which all cells are in the flat, inactive stage to 5.00 for one in which all are in the tall, columnar, active stage. Oakeson and Lilley (1957, 1960) believed this method to be a valid one for assessing thyroid secretory activity and relied on it in their studies of White-crowned Spar-

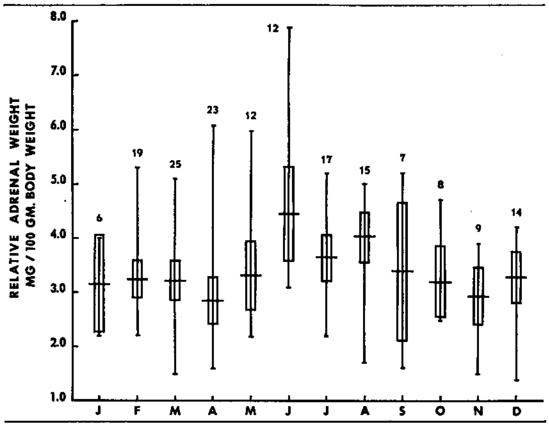

FIGURE 1. Monthly variation in relative weight of the left adrenal gland in Gambel Quail. Horizontal lines indicate means, vertical lines extremes, and the rectangles the 95 per cent confidence limits of the means. Numerals indicate sample sizes.

rows (*Zonotrichia leucophrys*); following their usage, this value will be referred to in this paper as the TAI (Thyroid Activity Index). TAI values were obtained for 152 quail.

Wilson and Farner (1960) employed a different histometric method for evaluating thyroid activity. In their method, the percentage of epithelium (%E) in the gland was measured using similar line intercepts through cross sections. Lack of suitable microprojection equipment made it impossible to rely on this method in the present study. However, several glands were evaluated by both methods and the TAI and % E for those were highly correlated.

For histometric determinations of interrenal activity the method of Lorenzen and Farner (1964) was used. Each of four sections of each gland was systematically scanned; the type of cell falling at the end of each of four ocular hairlines was recorded for 144 fields in each section. The proportion of interrenal cells among the 2304 cells thus counted was assumed to be equivalent to the fractional volume of interrenal (cortical) tissue in the gland. This index is termed the FCV (Fractional Cortical Volume). It was obtained for 90 of the birds in the sample. Qualitative changes in the histology of adrenal glands were also studied.

RESULTS

ADRENAL WEIGHTS

Variations in absolute weights and in relative weights were both examined for seasonal changes. The two measures showed very similar temporal patterns. Relative weights were chosen for detailed analysis because they are probably somewhat more meaningful than absolute ones. Before passing to this analysis it might be well to record that the mean absolute weight of the left adrenal for 168 birds was 5.60 mg, with a standard deviation of 1.70 mg and a range of 2.4 mg to 13.0 mg. For relative weights, the overall mean for 167 birds was 3.38 mg/100 g body weight, with a standard deviation of 1.00 and a range of 1.4 to 7.9.

For seasonal analysis of relative adrenal weights (and for FVC and TAI), samples were divided into monthly groups (fig. 1). A single-factor analysis of variance (Steel and Torrie 1960:112–114) confirmed the significance of variation among months (table 1). A modified Duncan's multiple range test (Duncan 1957) was employed to determine which of the monthly means were significantly different ($P < 0.05$) from each other. The mean relative weight for June was significantly higher than those of all other months except August, and June and August were significantly higher than April and November. All other combinations of monthly means

TABLE 1. Results of analysis of variance and Duncan's multiple range test of monthly variation in Gambel Quail adrenals and thyroids.

Variable	*F* Ratios[a]	Degrees of freedom	Months arranged by ranked means[b]											
Rel. ad. wt.	3.29	11, 155	Apr	Nov	Jan	Oct	Mar	Feb	Dec	May	Sept	July	Aug	June
FCV	2.82	11, 78	July	Sept	Oct	Nov	Dec	Jan	June	Mar	Aug	Apr	Feb	May
TAI	8.64	11, 140	Oct	Nov	Sept	Aug	Feb	Jan	May	Mar	July	Apr	June	Dec

[a] Ratio of mean square of variance among months to mean square of variance within months. All three F values are highly significant ($P < 0.01$).

[b] Means of months underlined by the same line are not significantly different; those of months not subtended by the same line may be regarded as significantly different (5% level).

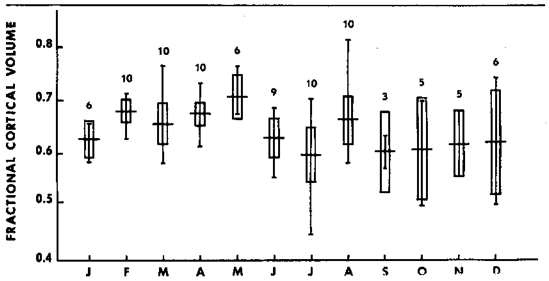

FIGURE 2. Monthly variation in proportional volume of interrenal tissue in adrenal glands of Gambel Quail. See figure 1 for explanation of symbols.

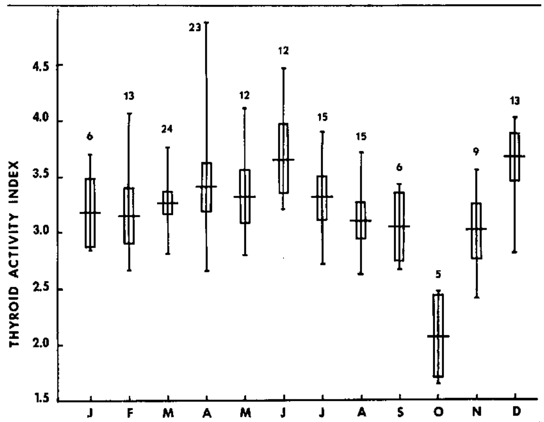

FIGURE 3. Monthly variation in histological indications of thyroid activity in Gambel Quail. See figure 1 for explanation of symbols.

were members of homogeneous sets, and therefore differences between these means were not significantly different. In summary, the statistical tests confirm what is suggested graphically in figure 1; that is, that there was significant variation through the year, with elevations in relative weight in June and August and reductions in April and November. Visual comparison of plotted values for the two sexes indicated no obvious differences, and so data were pooled to simplify analysis. Sturkie (1965:679) mentions lack of sex differences in relative adrenal weight in fowl.

ADRENAL HISTOLOGY

The general histological organization of the adrenals in these quail is very similar to that in White-crowned Sparrows as described by Lorenzen and Farner (1964). The only points of disagreement are that interrenal tissue in the quail occupies the subcapsular position at least as fully as chromaffin tissue and that clusters of lymphoid cells are absent. Otherwise the descriptions of Lorenzen and Farner are applicable in detail to the quail, and it would therefore be redundant to include description here.

FCV values were treated statistically in the same fashion as relative weights of the glands. Results appear in figure 2 and table 1. Data for the two sexes were pooled after scrutiny and showed no obvious sex differences. The data of Lorenzen and Farner (*op. cit.*: fig. 1) also fail to show differences between sexes. The overall mean FCV was 0.645 for a sample of 90; the standard deviation was 0.061; the lowest value was 0.45, the highest, 0.81. Compared with the results of Lorenzen and Farner this mean is lower and the overall range and the variation greater.

True seasonal changes are indicated by the facts that the mean for May was significantly higher than for July, September, October, November, December, January, and June; that February was significantly higher than October and July; and that July was significantly lower than April, August, and March.

Qualitative histological characteristics changed seasonally also. Again these were as found by Lorenzen and Farner. Interrenal cells of glands with high FCV values (in May and February) were tall with round nuclei and vacuolated cytoplasm and were well aligned in anastomosing cords. In glands with low FCV (in July) the tissue was generally less organized and the cells smaller and pycnotic with nuclei variable in shape. These differences were particularly evident in the centers of the glands.

THYROIDS

The mean TAI value for 152 birds was 3.265, with a standard deviation of 0.461 and a range of 1.65 to 4.88. This mean is higher and the range is greater than Davis and Davis (1954) found for House Sparrows (*Passer domesticus*) and Oakeson and Lilley (1957, 1960) found for White-crowned Sparrows. As with adrenals no sex differences were apparent, and results for males and females were treated together.

Marked seasonal changes in TAI occurred (fig. 3, table 1). Means for June and December were significantly higher than those for all other months except April; and October was significantly lower than all other months. Differences between the general histological appearance of glands with low TAI values and those with high ones were marked and similar to those described by many authors for inactive versus active thyroids.

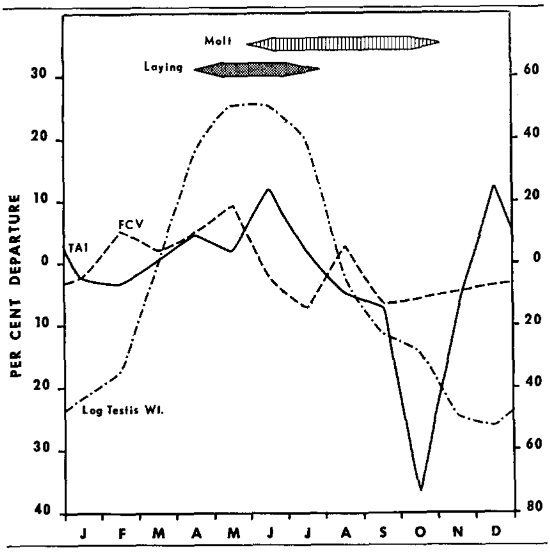

FIGURE 4. Monthly variation in adrenals (FCV), thyroids (TAI), and testes. Monthly means were converted to per cent deviation from overall mean for each parameter. Scale on left ordinate is for adrenals and testes, scale on right ordinate for thyroids.

DISCUSSION

ADRENAL CYCLE

The lack of agreement between the cycles in adrenal weight and interrenal histology is striking. The two variables fail to exhibit significant correlation. Sturkie (1965) has recently reviewed the literature on factors affecting size of avian adrenals; the results of various studies are confusing and conflicting. The general impression is that adrenal size in birds may be affected by a diversity of factors, but that size of the gland is much less responsive to stresses and to hormones than is the mammalian adrenal. It appears that size of the whole gland is very probably a less reliable index to interrenal activity than in mammals. Perhaps the failure of adrenal size to respond in a coherent pattern as in mammals is related to the internal organization of the gland and to the fact that the avian adrenal is of small size and quite irregular in shape.

At any rate, the histometric measure (FCV) is probably a better index to interrenal activity. Changes in FCV are correlated with qualitative changes in histology that appear to be related to secretory activity (Lorenzen and Farner 1964). Lorenzen and Farner also found that FCV was correlated with a quantitative cytochemical evaluation of lipid content, and Fromme-Bouman (1962) found that seasonal variation in interrenal cell nuclear diameter paralleled that in FCV. For these reasons variation in FCV rather than in weight was used in searching for correlated variables that might point to factors affecting level of interrenal secretory activity.

It must be admitted that this search was not particularly fruitful, and most conclusions concerning correlations are negative. No obvious correlation was found with environmental factors. Stress from climatic extremes, shortage of food, or high population density would appear to be ruled out as a cause of a high point in activity in May. Figure 4 shows seasonal variation in FCV with concomitant variation in other features of the annual cycle. The closest agreement appears to have been with gonadal activity. May was certainly the period of greatest reproductive activity, with highest percentage of males with fully active testes and highest percentage of laying females (fig. 4; Raitt and Ohmart 1966); and this was also the month of the highest interrenal index. Variation in FCV was analyzed on the basis of the reproductive status as well as on a monthly basis; and the principal result was that mean FCV values for individuals with active gonads were significantly higher than those with regressing ones, without regard to date. The other studies of interrenal cycles yielded conflicting results concerning the relationship between breeding and high interrenal activity. In Germany, Fromme-Bouman (1962), working with the European Blackbird (*Turdus merula*), and Thybusch (1965), working with the Common Gull (*Larus canus*), both found peaks of interrenal activity at the end of the breeding season, whereas the peak in the present study was early in the season. Lorenzen and Farner (1964) obtained still different results with White-crowned Sparrows; the peak in FCV occurred in winter and the lowest level occurred during the breeding season.

Examination of FCV, reproductive condition, and molt status of individual birds late in the general breeding season in June and July (table 2) provides no indications that decline in FCV is closely correlated with the other variables. Some experimental evidence indicates a relationship between gonads and adrenals (Höhn 1961; Sturkie 1965); however, experimental work certainly has not established the nature of this relationship or even unequivocal evidence of its existence. Thus, high interrenal activity may be related to high reproductive activity; but if so, the relationship is probably an indirect one and is not universal among birds.

Lorenzen and Farner (1964) found little

TABLE 2. Status of adrenal, thyroid, gonads, and molt of adult Gambel Quail in late breeding season.

Date	Sex	Adrenal[a]	Thyroid[a]	Gonads[b]	Molt[c]
9 June	M	43	24	early R	N
9 June	M	24	64	5	N
12 June	M	29	20	5	N
13 June	M	53	18	5	2
13 June	M	37	40	early R	1
13 June	F	43	64	R	N
17 June	M	51	40	5	3
17 June	F	51	18	medium R	N
5 July	M	31	43	early R	4
7 July	M	38	0	early R	3
7 July	F	27	43	medium R	N
7 July	M	46	1	medium R	5
8 July	F	48	35	ovulating	N
8 July	M	0	20	early R	3
13 July	F	21	21	R	N
25 July	M	39	20	5	3
27 July	F	27	11	late R	3

[a] Values for adrenal (FCV) and thyroid (TAI) expressed as percentages of departure above the lowest value for a June or July bird.
[b] For gonads "R" indicates regression and "5" denotes fully active testes (see Raitt and Ohmart 1966 for fuller description of breeding cycle).
[c] For molt "N" indicates birds that had not begun to molt; the various numbers indicate molt stages corresponding to the number of primary remiges molted (see Raitt and Ohmart 1966 for explanation).

agreement between their results on White-crowned Sparrows and those of Fromme-Bouman (1962) on *Turdus merula*, except that low periods of FCV occurred at the same season as low thyroid activity. My results show roughly similar coincidence, but the agreement between FCV and TAI is not close. Regression analysis of the two variables gave a statistically insignificant coefficient.

In summary, the results of this study and of previous ones indicate that the activity of the interrenal portion of the adrenal gland does appear to undergo seasonal changes in level, but the timing of these changes varies from species to species and is not obviously correlated with other events of the annual cycle or with environmental variables.

THYROID CYCLE

The two high periods in TAI, one in June and one in December, agree with results of some other workers. Höhn (1950, 1961), Wilson and Farner (1960), and Ringer (1965) have recently reviewed the literature on the avian thyroid. The impression from these summaries is that thyroid activity is likely to be high in winter and in the period preceding the molt. Wilson and Farner (*op. cit.*) found that thyroid activity in *Zonotrichia leucophrys gambelii* in Washington was negatively correlated with mean temperature, with low activity in summer and high in winter. They adduced evidence that in passerine species living in areas of cold winters (difference of 20°C or more between winter and summer mean temperatures) a winter increase in thyroid activity is usual. The implication is that low temperatures stimulate thyroid activity; presumably this response is related to thermoregulation. This relationship appears to explain the high mean TAI in December of this study. December was the coldest month during the study; the mean temperature was 39.7°F, which was more than 20°C lower than the mean for the hottest month of both preceding and following summers (mean for August 1960 was 78.0°F and mean for July 1961 was 80.7°F).

Aside from this correspondence of high thyroid activity to the coldest weather, the correlation between TAI and environmental temperature in this study was not close. Especially the high mean for June must be related to some factor(s) other than low temperature. High periods of thyroid activity in seasons other than cold ones are reported for the European Blackbird (*Turdus merula*) by Fromme-Bouman (1962) and for *Zonotrichia leucophrys nuttalli* by Oakeson and Lilley (1960), but these high periods occurred early in the breeding season rather than nearer the end as in this study. Höhn (1950, 1961) states that high thyroid activity preceding the molt has been found in a number of species. The June peak in this study appears to fit rather well into this pattern (fig. 4). June was the month in which breeding reached its culmination and gonadal regression began in some birds; molting also began in some birds in June. If increase in thyroid activity were closely related to either cessation of breeding activity or onset of molt, it might be expected that variation in TAI would be correlated with variation in reproductive status and molt stage, both of which are variable in June and July. However, the data of table 2 show no such correlation. This lack of correlation appears to cast doubt on a close causal relationship between increase in thyroid activity and gonal regression, as Höhn (1961:99) suggests, or to a similar relationship between thyroid secretion and molt, as a number of authors have suggested (but Höhn 1961, and Wagner 1961 seem to argue against such a relationship). Perhaps the June peak in this study was simply related to the general high level of activity—locomotor, secretory, keratin synthesis—accompanying the culmination of breeding and the onset of molt.

The fact that different species, and even different subspecies (Oakeson and Lilley 1960) differ so markedly in relative levels of thyroid activity outside of winter and in their responses to experimental manipulations such as thyroxine administration (Wagner 1961) makes it seem improbable that full explanations of annual cycles of the thyroid can be given without a large amount of additional data.

SUMMARY

Seasonal changes in adrenal and thyroid glands of Gambel Quail in southern New Mexico were studied from October 1960 through September 1961. For adrenal glands, the relative weight and a histometric measure of the proportion of interrenal tissue (FCV) both showed seasonal changes, but the two variables were not correlated. The FCV value is probably a better measure of interrenal activity because histological indications of interrenal cell size and activity were correlated with FCV. Mean FCV was high in May and low in late summer and fall. This pattern suggests a relationship to gonadal activity. These changes are different in timing from those of two other studies of FCV in other species, which were also different from each other.

High mean values of TAI (Thyroid Activity Index) occurred in December and in June. The December peak was probably caused by low temperatures in that month, which was the coldest of the study. The June peak occurred during the culmination of breeding and the onset of molting in the population, but in individual birds in this period TAI was correlated with neither gonadal condition nor molt status. High thyroid activity in spring or summer is variable among species; it is absent in some and occurs at different periods of the reproductive cycle in others.

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