Studies on migratory fattening in passerine birds

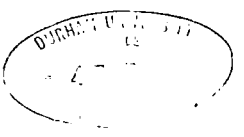
Baggott, G. K.

Studies on migratory fattening in passerine birds.

by

G.K.Baggott, B.Sc.

..... being a thesis presented in candidature for the Degree of Doctor of Philosophy in the University of Durham 1973.



Acknowledgements

I would like to thank,

Professor D Barker for providing facilities in the Department of Zoology,

Dr P R Evans for his direction and criti-cism of this study,

Miss K Flower for her expert preparation of histological material,

and my wife, Pamela, for preparing the manuscript and her many sacrifices.

Abstract

The body composition of juvenile Willow Warblers, Phylloscopus trochilus , was examined during the autumn moult and premigratory period. Premigratory birds exhibited hyperphagia and lipid deposition. Weight decreases of the pectoralis muscles and their glycogen reserves during moult are interpreted as responses to increased thermoregulatory demands, and not to utilization of muscle amino-acids for feather growth. Apparent premigratory muscle 'hypertrophy' represents merely a recovery to pre-moult values.

Mean liver weights do not vary between the post-juvenile moult and the premigratory period, but the times of day at which weight maxima of water and protein occur are earlier in premigratory birds. These birds also show an earlier increase in liver lipid levels during the day than moulting birds. Premigratory birds also have greater evening lipid concentrations than moulting birds. As the dawn lipid levels of moulting and premigratory birds do not differ, all lipid synthesized (or processed) by the latter group must be stored solely in adipose tissue. The marked diurnal cycle of liver glycogen of moulting birds is absent in the premigratory period.

Samples of Willow and Grasshopper Warblers (Locustella naevia) killed during migratory flight had different potential ranges but similar potential flight times, due to a lipid-correlated increase in body water in the latter species.

Injections of prolactin in photosensitive Bramblings, Fringilla montifringilla , showed that the stimulation of appetite by this hormone was independent of the time of its injection, whereas lipid deposition was strongly dependent. In Bramblings given restricted food body and liver weights still increased when birds were injected with prolactin, confirming that prolactin acts directly on liver lipid synthesis, independently of its effects on appetite. Changes in liver size produced by prolactin injections were similar to size changes found in photostimulated birds. It is concluded that prolactin is the probable cause of changes in liver composition of autumnal premigratory Willow Warblers.

Table of Contents

page

page

Chapter 1. Introduction

This thesis is concerned with migratory fattening chiefly in two passerine species - the Willow Warbler[1] and the Brambling. The former species is a typical insectivorous migrant, breeding in the Palaearctic and wintering in Africa; the latter is a seed-eating finch, breeding in Scandanavia and wintering in the North temperate-zone of Europe. Both species were numerous in the Durham area, the Willow Warbler in summer and the Brambling in winter; their availability was one of the main reasons for their use in the studies described in the following chapters.

Since the original demonstration that the body lipid of birds increases at the time of migration (Groebbels 1932, Rowan 1925), many investigators have examined this lipid deposition and its metabolic basis. The process of lipid deposition in migratory birds can be divided into two phases: the premigratory phase in which lipid levels in the body increase from previous low levels, and in which captive birds exhibit nocturnal restlessness, Zugunruhe, (although these two characteristics are independently controlled, King and Farner 1963, Lofts, Marshall and Wolfson 1963); and the migratory phase, in which the high lipid levels are maintained (unless used for migratory flight), and feral birds exhibit orientated migratory activity. These definitions agree with the usage of Dol'nik and Blyumental (1967), and correspond to the 'dynamic' and 'static' phases (respectively) of weight gain in lesioned White-throated Sparrows (Kuenzel and Helms 1970). These two phases must be separated in studies on migratory birds, since their characteristics are not similar (Dol'nik and Blyumental 1967).

1. Scientific names are given in Appendix 5.

Most studies have been concerned with the spring premigratory phase (Farner et.al.1961, Helms 1968, King 1961a, 1961b,Odum 1960, Yarborough and Johnston 1965), as this is easily produced in captive northern temperate-zone passerines by artifically long photoperiods (King 1961b, King and Farner 1956, 1963, Lofts and Marshall 1960, Odum and Major 1956, Wolfson 1945, 1954, 1960). The autumn premigratory phase has been less extensively investigated (Dol'nik 1966, 1967, 1968, 1970a, Dol'nik and Blyumental 1967, Evans 1969, King, Barker and Farner 1963, King, Farner and Morton 1965, Rogers and Odum 1966). The appearance of lipid deposition in autumn in the Willow Warbler is not dependent on day length, but on an internal rhythm (Gwinner 1968); this applies also to the Garden Warbler (Berthold, Gwinner and Klein 1971). Hence, autumn lipid deposition is more difficult to study in captive birds, since it cannot be produced by variation of photoperiod.

Two approaches have been used to study the metabolic changes associated with premigratory lipid deposition. In the first approach, energy balance studies have been performed that show that lipid deposition is due to increased energy intake (hyperphagia) (Helms 1968, King 1961a, 1961b, Koch and de Bont 1952, Odum 1960a, Rautenberg 1957), and not due to more efficient utilisation of the food (King 1961b); some authors have suggested that there is also a reduction in 'existence energy', but there is no conclusive evidence on this point (Kendeigh, West and Cox 1960, Koch and de Bont 1952, Pohl 1971). The disadvantage of these studies on captive birds is that the magnitude of the changes in lipid deposition are increased, probably due to the reduced activity of captives (King and Farner 1959). Consequently, studies on

energy reserves of captives (such as Farner et. al. 1961) must be treated with reserve. The alternative approach is the study of the changes of body composition of feral birds (e.g. Dol'nik and Blyumental 1967, Evans 1969, King, Barker and Farner 1963); in this case the metabolic changes are usually inferred from changes in energy reserves. The drawback has been, however, that in most passerine species studied (with one exception, the Rosy Pastor) the premigratory phase is preceded by a moult; in only a few studies have possible effects of this moult been considered, and even then not fully (Dol'nik and Blyumental 1967, King and Farner 1959).

In the first part of the investigations reported here, I have studied the metabolic changes associated with premigratory lipid deposition in autumn by following the changes in body composition of Willow Warblers during and after the moult, in July and August. The adult Willow Warbler has two complete moults per year; one in Europe before the autumn migration and another in Africa before the spring migration. Juveniles have a partial moult immediately after leaving the nest, and acquire their new body plumage before migrating south in their first autumn of life. They have a complete moult in Africa before returning on spring migration.

The moult of juveniles, and associated changes in total body weight, are described in detail in Chapters 3 and 4 of this thesis, as a preliminary to the discussion of variation in body composition, since a main aim of the study of this species has been to separate metabolic peculiarities associated with moult from the changes associated with premigratory lipid deposition. In Chapter 5, evidence is presented that the premigratory phase, identified by

hyperphagia, begins only after the moult is completed. Chapter 7 contains supporting evidence that total body lipid does not increase appreciably until the same period; while Chapter 6 shows that the lipid content of the brain is constant throughout the moult and premigratory phases, and so does not obscure changes in weight of metabolizable lipid as measured by extraction of lipid from the whole carcase. Chapter 8 considers variation in weight of certain other parts of the body during and after the moult.

It is impossible to investigate the changes in enzyme activities associated with lipid deposition in feral birds; the nearest approximation is to follow changes in energy reserves and tissue composition of those organs known to be implicated in avian lipid metabolism. In my study of feral Willow Warblers attention has been focussed on the liver (Chapter 10) and pectoralis muscles (Chapter 9). The former is the principal site of lipid synthesis in birds (Goodridge and Ball 1967a), and changes in the size of this organ have been noted in the premigratory period in White-crowned Sparrows (Oakeson 1953, 1956). The pectoralis muscles have been suggested as another area in which premigratory metabolic adaptations occur (reviewed by George and Berger 1966). Since plasma glucose and free fatty acid levels are intimately related to lipid metabolism in birds (Goodridge and Ball 1967a, Langslow et.al.1970), I have also examined these in Willow Warblers (Chapter 11). The only other species in which blood composition during the premigratory period has been examined is the Chaffinch (Dol'nik 1966,1967, Dol'nik and Blyumental 1967). Information on body composition and adaptations of migrant passerines killed during migratory flights at Bardsey lighthouse, North Wales, is given in Chapter 12, to compare with the premigratory adaptations discussed in previous chapters.

The hormonal control of premigratory lipid deposition is still a matter of controversy. As shown by experiments on lesioned White-crowned and White-throated Sparrows the response is mediated by the hypothalamus (Kuenzel and Helms 1970, Stetson 1971); lesions in the ventral hypothalamus induced lipid deposition of the same magnitude as found in the premigratory phase. Evidence from the administration of exogenous hormones has been interpreted as suggesting that prolactin is primarily responsible for lipid deposition (Meier and Farner 1964), but that its effect depends on the time of injection in relation to the daily photoperiod (Meier 1969, Meier and Davis 1967), possibly due to synergism with corticosteroids (Meier and Martin 1971a, 1971b). However, no attempt has been made to determine whether the fattening response produced by prolactin is comparable with premigratory fattening in wild birds. It is not known whether in migrants it affects the appetite, or lipid synthesis, or both. In the non-migratory pigeon, prolactin does increase both appetite (Bates et.al.1962) and lipid synthesis (Goodridge and Ball 1967b, 1967c).

In my study, experiments were performed on the effects of prolactin injections prior to spring migration in the Brambling. The results (Chapter 13) go some way towards understanding how prolactin administration leads to lipid deposition in migratory birds. This is of importance since it is conceivable that the fattening observed in migratory passerines injected with prolactin is of the same nature as that found in hypophysectomized chickens (Gibson and Nalbandov 1966a, 1966b), namely the result of an impairment of lipid mobilization from the adipose tissue, rather than a stimulation of lipogenesis. Recent reports that castration can inhibit

photoperiodically-induced fattening in White-crowned Sparrows suggest that prolactin may not be the only hormone involved (Stetson and Erickson 1972); similar results were found for White-throated Sparrows. This possibility of an interaction between gonadal steroids and prolactin was not examined in my study, but is discussed in the final section (Chapter 14).

Chapter 2 outlines general methods of carcase analysis, biochemical assay, and statistical analysis. Thereafter each chapter contains a short section on special methodology (if any), followed by results, analysis, discussion and summary. The overall conclusions are gathered together and evaluated in Chapter 14.

Chapter 2 General Methods

2.1 Carcase analysis and assays

Willow Warblers were captured in mist nets on the feeding areas near Durham City. They were removed from the nets to the laboratory, where they were killed. The maximum time between time of capture and death was 30 minutes. The composition of the samples differed in 1970 and 1971. In the latter year, only juveniles from the beginning of the premigratory period were sampled, as the birds prematurely left the feeding areas. Juveniles were captured throughout the moult in both years, but unmoulted juveniles were caught in any numbers only in 1971. In subsequent Chapters 'morning' refers to the first six hours after dawn, and 'evening' to the two hours prior to sunset. Birds could not be caught at any other time of day.

The birds were either sampled for blood first, or killed immediately and blood samples obtained by decapitation. Samples of liver and muscle tissues were removed as quickly as possible, and frozen in liquid nitrogen, usually within 10-20 seconds. The samples were then wrapped in foil, and stored on dry ice until analysis later the same day. Carcases were wrapped in foil, placed in a polythene bag, and stored in a deep freeze at $-20^{\circ}C$, until analysis at a later date.

The weight of the carcase was obtained from the difference between the weight of the dry polythene bag plus foil and the weight of the bird, foil and bag. Carcases were thawed before dissection, and the rest of the liver and pectoralis muscles removed and processed as detailed in Chapter 9.2 and 10.2. The carcase was also plucked.

The stomach was removed from the carcase and the contents washed into a tared tube. It was then replaced in the carcase. The whole carcase was homogenised in a blender with distilled water, and the homogenate poured into a tared crystallising dish. This was then dried at 40°C *in vacuo* to constant weight. Lipid was extracted by heating the dish full of technical petroleum ether to 60°C, and pouring off the extract of lipid. This was repeated three times, after which there was no further detectable decrease in weight. The lipid-extracted carcase was dried to constant weight *in vacuo* at 40°C. This weight difference gave the "residual carcase lipid weight" (Chapter 7). The weight of the remaining portion of the carcase is referred to as the "residual fat-free dry weight" (Chapter 8). Tissue components have been expressed as concentrations (e.g. mg water/100mg fat-free dry weight) or total weights, that is the total weight (for example water) in a whole muscle, liver or carcase.

Glycogen assay:

The method is based on the determination of the total amount of glucose in tissue and follows Mendel, Kemp and Myers (1954) and Kemp and Kits Van Heijningen (1954). The reagents used are a solution of 5gm trichloroacetic acid (AR) and 100mg of silver sulphate (AR) dissolved in 100 ml of deionised water, and kept in a dark bottle; and a solution of 96% (w/v) of sulphuric acid.

The tissue sample was weighed and added to 5 ml of the trichloro-acetic acid solution. The tissue was homogenised for 5 mins in a glass homogeniser. The homogeniser tube was capped with foil

and placed in a boiling water bath for 15 minutes. The tube was then cooled, and another 5 ml of the acid solution added; the tube was capped with parafilm and shaken. The contents were then transferred to a centrifuge tube, and spun at 3000g for 15 minutes. Three 1 ml aliquots of the supernatant were each heated with 3 ml of the sulphuric acid solution, for exactly 6.5 minutes. The tubes were cooled 10 minutes in a cold water bath, and the solutions read for optical density at 520 nm. A standard of glucose in trichloroacetic acid solution and a trichloroacetic acid blank was run with each batch of samples.

The slope of optical density versus µg of glucose determined from a standard curve was 0.001457. The inverse of this gives the change in glucose concentration of the 1 ml aliquots for an increase in 1 optical density unit, namely 686.3 µg/1 optical density unit. The percentage standard error of the predicted mean glucose concentration (µg/ml of aliquot) for the glucose concentrations used for the calibration was 4.9%. Glucose concentrations of the tissue on a per gm wet weight basis were calculated from the dilution factors, calibration slope, weight of the tissue, and the optical density of the standard sample run with the batch. The recoveries of glucose added to tissue samples were for liver and muscle, respectively, 88.1$\pm$.9% and 86.3$\pm$1% (n = 9 in each case).

As shown in Chapters 9.2 and 10.2 there was no disadvantage of this method in measuring changes in tissue glycogen compared with direct glycogen assay procedures.

Plasma glucose assay:

The method was based on the glucose oxidase procedure for assaying glucose; reagents of uranyl acetate (160 mg AR and 900 mg sodium chloride in 100ml of distilled water) and glucose oxidase solution were obtained from Boehringer Mannheim GMBH.

To 50 µl of plasma (obtained as detailed in Chapter 11.2) 0.5 ml of uranyl acetate solution was added. The mixture was well shaken, and centrifuged for 5 minutes. 50 µl of the supernatant was transferred to a cuvette, and 5 ml of the glucose oxidase solution was added, and mixed. The mixture was incubated at room temperature for 40 minutes, and read at 420 nm. A distilled water blank and glucose standard was run for each batch of samples.

The slope of optical density against µg of glucose per 50 µl was 0.0018. The inverse is the increase in glucose (µg per 50 µl) for a change in 1 optical density unit - 557.2 µg/1 optical density unit. The standard error of the estimated mean glucose concentration (µg per 50 µl) for this calibration curve was 1.4%. The plasma glucose level in mg% was calculated from the dilution factors, the volume of plasma, the slope of the calibration curve, and the optical density of the standard run with the sample batch.

Free fatty acid assay:

The method is a combination of the fatty acid extraction of Dole and Meinertz (1960) and the quantification procedure of Laurell and Tibbling (1967). 100 µl of plasma was shaken with 10 ml of Dole's extraction fluid (0.1 vol N sulphuric acid; 1 vol heptane; 4 vol isopropyl alcohol) plus 1.9 ml of distilled water; the latter to maintain the phase relationships. The mixture was stood

for 5 minutes. 4 ml of water and 6 ml of heptane were added. The mixture was shaken again and stood for 5 minutes for the phases to separate. A 7 ml aliquot of the upper phase was shaken with 7 ml of N sulphuric acid in a glass stoppered tube for 3 minutes, and the tube centrifuged for 3 minutes to separate the phases. Two 2.5 ml aliquots were taken from the upper phase and each transferred to a glass stoppered tube containing 2.5 ml of AR chloroform and 2 ml of a copper nitrate triethanolamine solution (10 ml of 0.5 M copper nitrate, 10 ml of 1M triethanolamine and 3.5 ml of N sodium hydroxide are diluted to 100 ml, and 33 gms of sodium chloride is dissolved in this, and the pH adjusted to 8.1 with sodium hydroxide solution). The tube was shaken for 3 minutes, and centrifuged for another five to separate the layers. A 3 ml aliquot was taken from each tube and added to a cuvette in which contained 0.5 ml of a solution of 0.4% diphenylcarbazide in absolute ethanol (0.1 ml of 1M triethanolamine is added to 10 ml of this solution 30 minutes before it is used). The contents were mixed thoroughly and read against a distilled water blank at 550 nm, ten minutes after mixing. A blank of 0.1 ml of distilled water and a standard were run with each batch, and read against the distilled water blank 10 minutes after mixing. This was necessary as the optical density increases linearly with time.

The slope of the calibration curve is 118.0 picomoles (in the final 2.5 ml aliquot) increase per 1 optical density unit. The standard error of the estimated mean fatty acid concentration of the concentrations used in the calibration is 1.3%. The plasma

free fatty acid level, in millimoles, is calculated from the dilution factors, calibration slope, and the optical density reading for the standard run with each batch of samples.

2.2 Statistical analyses

In later chapters the following abbreviations have been used for statistical parameters:

SD - standard deviation

SE - standard error

p - probability that the null hypothesis is correct

b - regression coefficient, i.e. slope of regression line

SE_b - standard error of b

r - correlation coefficient

n - sample size

H - Kruskal-Wallis H

t - Student's t

CV - coefficient of variation

Parametric tests have been used only for those samples in which normality has been established by examining the third and fourth moments (Snedecor and Cochran 1967). If homogeneity of sample variances was established by Bartlett's test, then one-way analyses of variance have been applied, and means compared by the Newman-Keuls sequential modification of the Studentised Range Test (Sokal and Rohlf 1969). Correlation analysis was used only where a bivariate normal distribution has been established; where this was not the case regression analyses have been used, or the data transformed. The two-tailed significance level at which the null hypothesis was taken as disproved was $p \leq .05$.

Where the frequency distributions of the samples were not normal, non-parametric tests have been used. Non-normality was a major

problem only for the data on liver composition, mainly due to the skewed distribution of liver water; there was a shift towards higher values in larger livers, but this was not due to a shift in the mean level of a normal distribution. Non-parametric tests applied were either the Kruskal-Wallis one-way analysis of variance, the Mann-Whitney U-test, or Kendall's rank correlation method (Siegel 1956).

The details of statistical tests are given in Appendix 1, and the numbers in parentheses in the body of the text refer to these.

Chapter 3. The post-juvenile moult of the Willow Warbler

3.1 Introduction

The juvenile Willow Warbler begins its post-juvenile moult, involving only the body feathers, almost as soon as it leaves the nest. Consequently the numbers of juvenile birds captured which had not started their moult were small. Most of these consisted of individuals still being fed by the parents and whose penultimate (ninth) primary was growing. As the first phase of moult (as defined below) lasts only a short time, there is a bias towards catching those birds in which the post-juvenile moult is well progressed.

Gwinner (1969) has shown that in captive broods the post-juvenile moult lasts 31 days (SD 3.9 days) and begins at 37 days (SD 6 days) after hatching. In my study also the moult lasted about one month, beginning in the first week of July, when the birds first fledged, and ending in the first week of August. Second broods or relays meant that there were still some moulting birds present in mid or late August.

In the subsequent account I have followed the terminology of Amadon (1966), after Dwight (1900), in designating the moult of the juvenile plumage the post-juvenile moult.

3.2 Methods

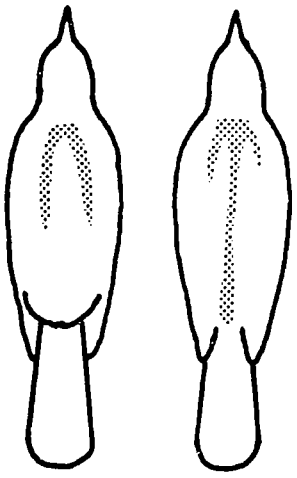
The moult was subdivided into four stages and a fifth class was used for those birds that had finished their moult. As Gwinner (1969) has shown that a second generation of nestling feather growth occurs during the first part of the post-juvenile moult,

care was taken not to confuse these, mainly peripheral, feathers with the growing feathers of the post-juvenile plumage. Fortunately the moult proceeds from the breast area in most cases, and the contrast between the new yellow feathers and the dirty white feathers of the juvenile plumage eliminates confusion. In the description of the moult which follows the naming of the feather tracts as used by Miller (1928) has been adopted.

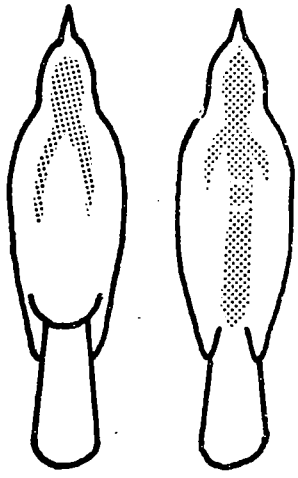
The moult starts by loss of the feathers from the sternal region, the humeral tract, the interscapular or the pelvic region. It continues with further feather replacement in these regions and by new feather growth in the coronal, submalar or interramal areas. Feather growth continues in these regions, and the next areas in which moult is initiated are the abdominal region and the tail coverts. The latter areas are, of course, the last to be fully replaced with full-grown feathers in completely moulted birds, although a few feathers in the sternal region continue growth long after the end of the moult proper.

Throughout this study the term moult has been used to refer to the process of both visible feather growth and visible feather loss, and where necessary in the text I have distinguished between the two, even though the former is the cause of the latter. The visual scoring method adopted is based on the appearance and growth of new feathers and not on the loss of old plumage; a process which is much harder to follow. At all times the following categories were strictly adhered to in an attempt to make the classification as reproducible as possible.

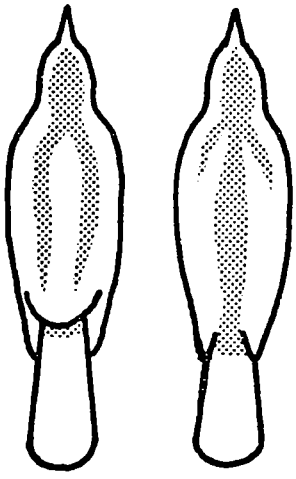
Figure 3.2.a . The progression of the post-juvenile moult of the Willow Warbler. In each pair of drawings the left is the ventral, and the right the dorsal view. Stippled areas are areas of feather growth, and hatched areas where feather growth has finished. The white areas of wing and tail are where there is no moult.



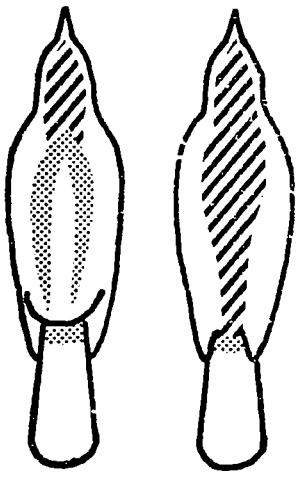
MOULT STAGE 1



MOULT STAGE 2



MOULT STAGE 3



MOULT STAGE 4

Moult stages:

Moult stage 1: all birds in this category had feathers growing only in the sternal region, the interscapular region, the pelvic region, or the humeral tract. Feather growth in any one of these areas was sufficient for inclusion in this moult stage.

Moult stage 2: all birds in this class had feathers growing in all the regions mentioned for moult stage 1. In addition birds in this category had growing feathers in the submalar, coronal or interramal regions.

Moult stage 3: all the tracts mentioned in the first two stages have growing feathers and in addition feather growth has started in the tail coverts or abdominal region.

Moult stage 4: all the previously mentioned areas except the abdominal region and the tail coverts are substantially fully grown; these latter two areas being only part way through their moult.

The progress of the post-juvenile moult is illustrated diagrammatically in Figure 3.2.a.

The fifth category contains not only moulted birds but also those individuals in which very few ventral tract feathers still had sheaths, as it seems that some of these sheaths are present for quite a long time after the finish of the moult proper.

3.3 The temporal pattern of the post-juvenile moult

Table 3.3.1 shows the numbers of birds in different stages of moult for ten-day periods from the beginning of July through to August in both 1971 and 1970. As stated in the introduction to this section it is possible to infer from this population data that the post-juvenile moult lasts for about one month; the total period over which 95% of the sample was in moult is somewhat longer, from 9 July to 18 August in 1970, and from 5 July to 15 August in 1971.

Figure 3.3.a . The percentage of juvenile Willow Warblers in the various moult classes for seven ten-day periods in 1970 (upper panel) and 1971 (lower panel). The ten-day periods are;

a. 21-30th June

b.1 - 10th July

c.11-20th July

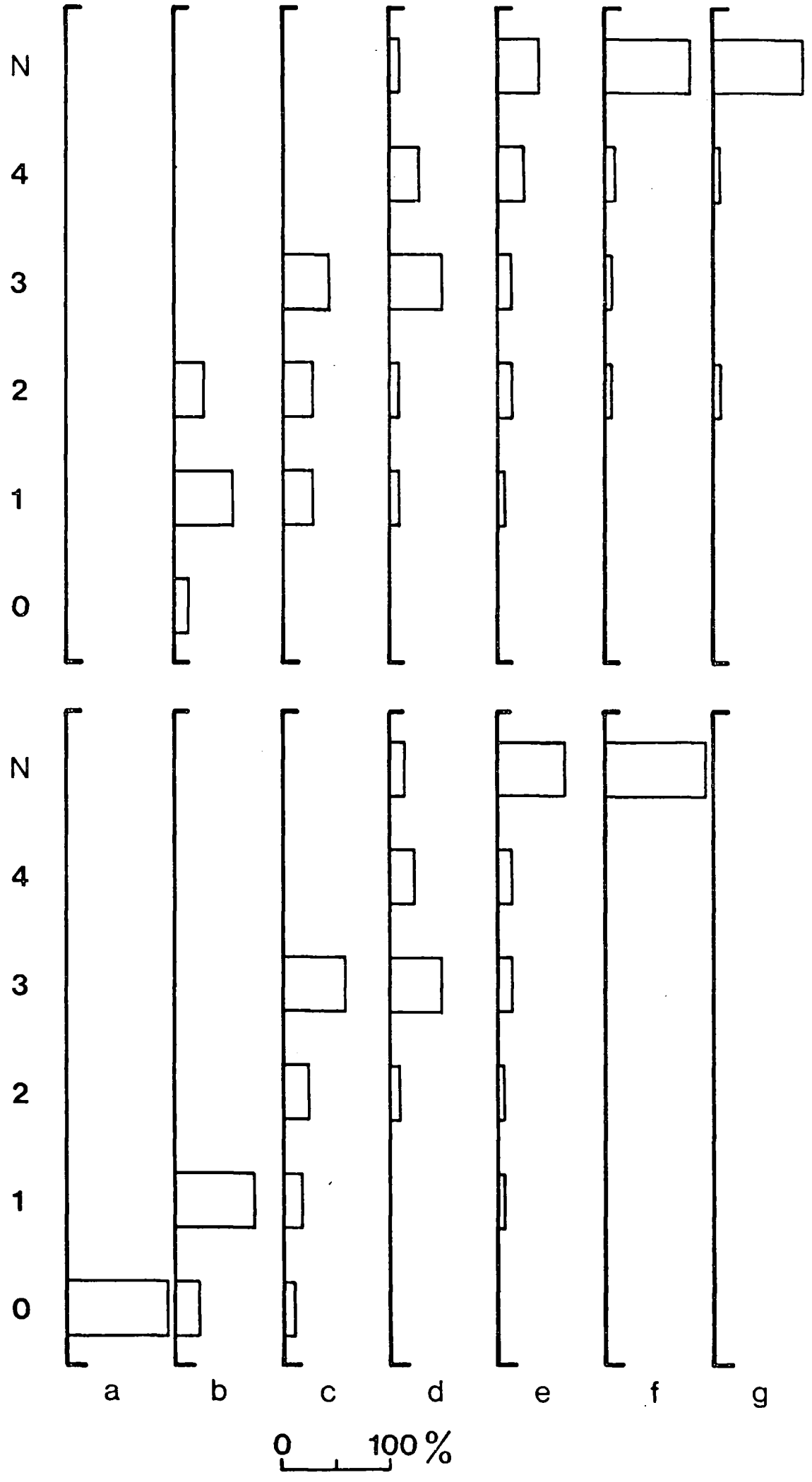
d.21-30th July

e.31st July - 9th August

f.10-19th August

g.20-29th August

The ordinate is the moult classes; 0 - unmoulted; 1,2,3,4 - the moult stages; N - moulted birds.


N
4
3
2
1
0
N
4
3
2
1
0
a
b
c
d
e
f
g
0
100 %

The median date of moult (by interpolation) does not differ much between the two years examined: 27 July in 1970 (95% confidence limits, 24 to 30 July) and the 23 July in 1971 (95% confidence limits, 19 to 26 July). The progression of the moult is illustrated in Figure 3.3.a in which the data of Table 3.3.1 are presented on a percentage basis.

Table 3.3.1 The numbers of birds in different stages of moult for ten-day periods from June to August.

1970

	Moult stage						
	Unmoulted	1	2	3	4	Moulted	Total
21-30 June							
1-10 July	1	4	2				7
11-20 July		7	7	8			22
21-30 July		3	3	18	11	3	38
31 July - 9 August		2	5	5	9	12	33
10-19 August			3	3	4	38	48
20-29 August			1		1	12	14

1971

	Moult stage						
	Unmoulted	1	2	3	4	Moulted	Total
21-30 June	7						7
1-10 July		2	6				8
11-20 July	2	4	6	16			28
21-30 July			3	13	6	4	26
31 July - 9 August		1	1	5	5	21	33
10-19 August						6	6
20-29 August							

3.4 Plumage dry weight - methods

The carcases were plucked when partially thawed, and the plumage dried to constant weight in tared tubes at 40°C *in vacuo*.

3.5 Plumage dry weights during the post-juvenile moult

In both years examined the variances of the samples of moulting and moulted birds were homogeneous (Bartlett's test, 1, 3), and so one-way analyses of variance were performed on the mean weights with respect to the stage of the moult. The means were compared by the Newman-Keuls modification of the Studentised Range Test. Since there was no reason to suspect a diurnal variation in the dry weights of plumage, birds captured at all times of day are used in this analysis.

In 1970 a significant effect due to stage of moult ($p < .001$) (2) was found by the analysis of variance. The mean for moult stage 4 was significantly greater (at $p < .05$) than the means for the samples from moult stages 1 and 2. The largest increase in the mean plumage dry weight occurred between moult stages 2 and 3, and there was a decrease in weight between moult stage 4 and fully moulted birds, though this was not statistically significant (Table 3.5.1) (Figure 3.5.a).

In 1971 also the analysis of variance showed a significant effect due to the stage of the moult ($p < .001$) (4), and at $p \leq .05$ most of the mean weights were significantly different from one another. The mean plumage dry weight for birds in moult stage 4 was significantly larger than the means for moult stages 1 to 3 and for the unmoulted birds. However, unlike 1970 the largest increase in mean plumage dry weight occurred between moult stages 3 and 4 and not between 2 and 3. (Table 3.5.1) (Figure 3.5.a) In 1971 this maximum increase in dry weight was 86mg compared with 40mg in 1970 (Figure 3.5.a).

This difference between years in the time of maximum increase in plumage dry weight is solely due to the difference in plumage dry weights in moult stage 3. This is the only statistically significant difference in mean plumage weights between years for birds in the same moult stage ($.005 > p > .001$) (5); whether this is a true difference between years (larger birds in 1970 for instance), or a product of the lack of sensitivity to weight changes on the part of the visual scoring method is impossible to say.

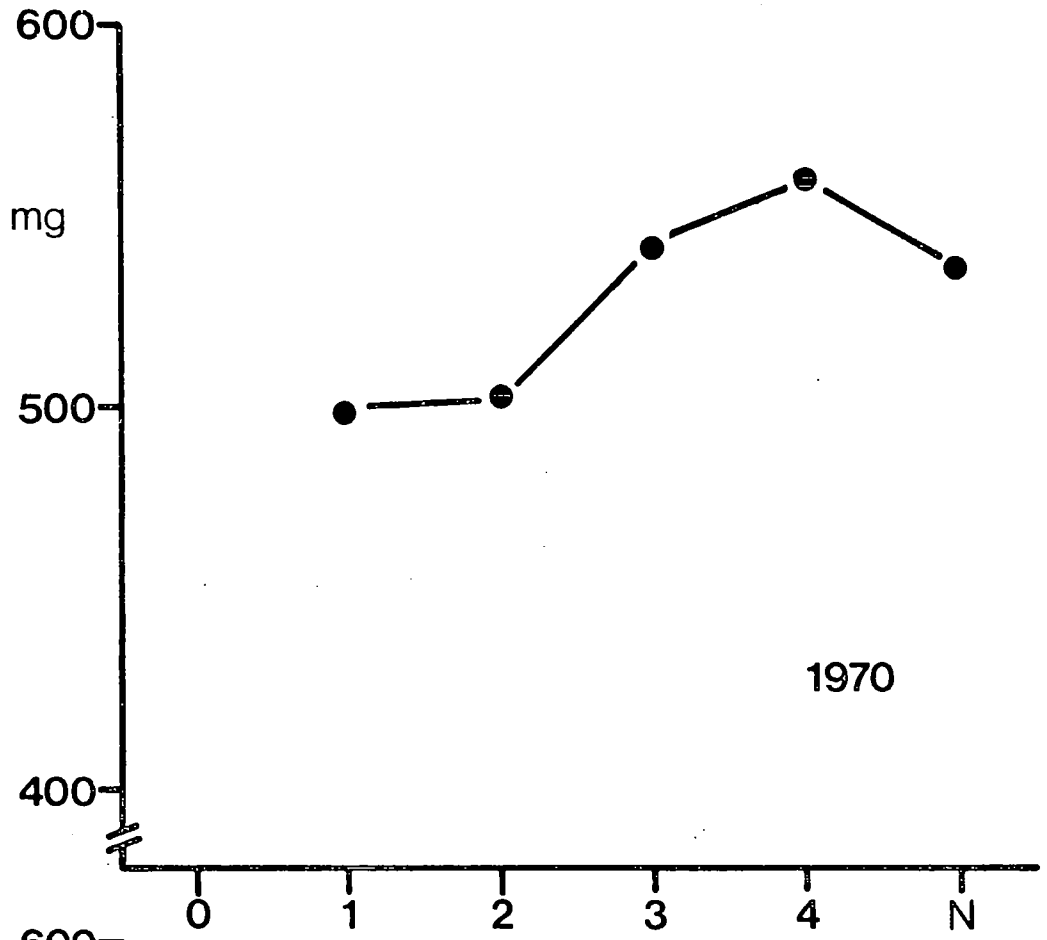
Newton (1968) found that juvenile Bullfinches more than doubled the dry weight of their plumage during the post-juvenile moult; this also applied to the wet weight. In the Willow Warbler the weight changes are by no means so large with only a 7.2% increase in dry weight between moult stage 1 and after the moult in 1970, and a 16.9% increase in 1971. As in the Bullfinch there was a decrease in plumage dry weight between the end of the moult and fully moulted birds (Figure 3.5.a). This is presumably due to loss of feather sheaths and also blood proteins when the copious blood supply to the growing feather is withdrawn (Newton 1968).

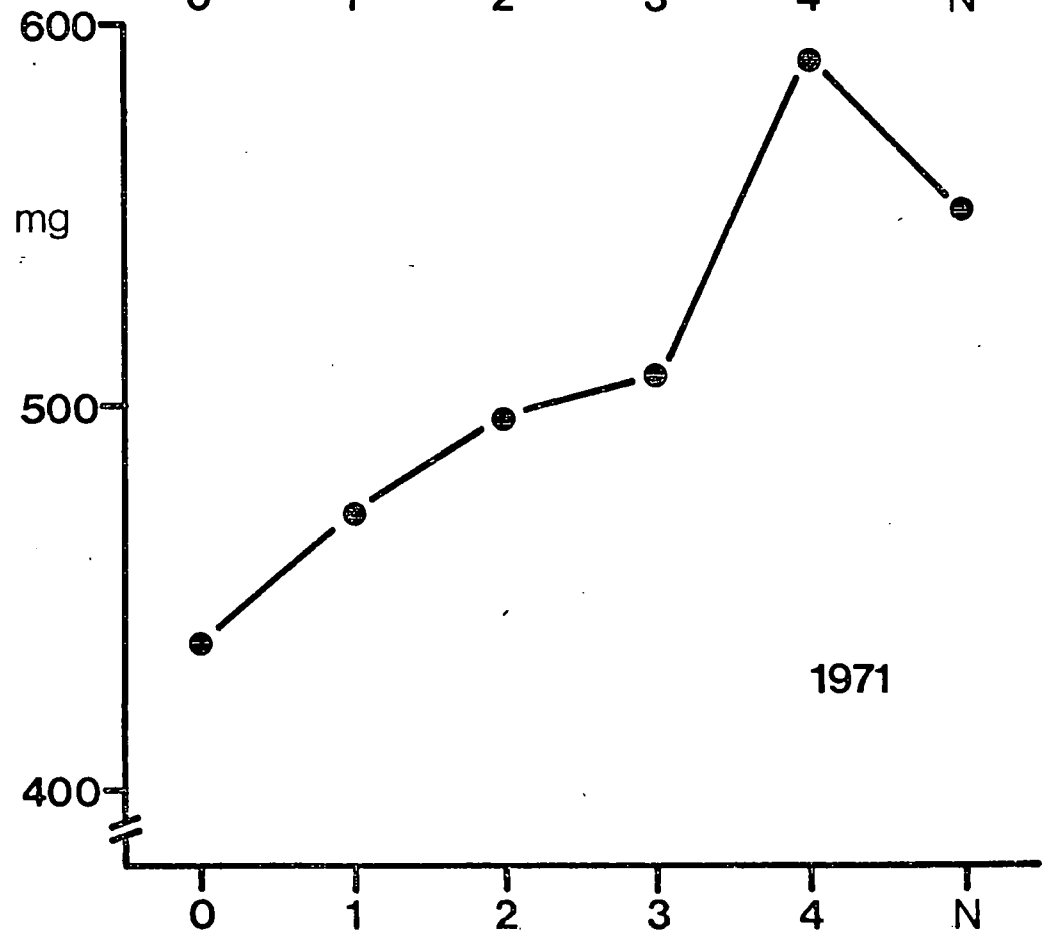
Table 3.5.1 The plumage dry weights (gms) of juvenile Willow Warblers.

	1970	1971
Unmoulted		0.437±.019 (9)
Moult stage 1	0.501±.012 (12)[a]	0.472±.013 (7)
Moult stage 2	0.503±.016 (13)	0.497±.011(15)
Moult stage 3	0.543±.008 (22)	0.507±.008(34)
Moult stage 4	0.558±.013 (18)	0.593±.017(11)
Moulted	0.537±.008 (34)	0.552±.006(33)

a. mean ± standard error (sample size)

Figure 3.5.a . The variation in the mean plumage dry weight during and after the post-juvenile moult of the Willow Warbler. Upper panel 1970, lower panel 1971. Ordinate is mg dry weight of plumage. Abscissa the state of moult; O - unmoulted; 1,2,3,4 - moult stages; N - moulted.


600
mg
500
400
1970
0
1
2
3
4
N


600
mg
500
400
1971
0
1
2
3
4
N

The data clearly demonstrate that the dry weight of the juvenile plumage is less than that of the post-juvenile plumage. The flight feathers and rectrices are not replaced at this moult, and it is not known whether these weigh less than the corresponding adult feathers, (since they are usually shorter they probably weigh less, so contributing towards the lower total plumage weights of juvenile birds).

3.6 The relation between moult stage and plumage dry weight

The mean plumage weight of unmoulted birds is lower than that of birds beginning their moult, this is presumably due to incompletely grown flight feathers. The linear relationship between the length of the penultimate primary (ninth) and the dry weight of the plumage of unmoulted birds supports this view. The regression equation linking these two variables is,

$$\text{plumage dry weight (gms)} = 0.014 + 0.0103 \text{ (length of primary nine in mm)} \quad (1)$$

76% of the plumage weight variations in unmoulted birds are associated with variations in the length of primary nine. The residual variation is presumably of the same type as found in the other stages of moult.

The visual scoring method used does not give equal increments in plumage dry weights between one moult stage and the next. In fact the mean weight for the same moult stage differed by as much as 36 mg between the two years. This is primarily due to the plumage dry weight measuring the balance between feather loss and feather growth. The visual assessment method classifies the moult into stages of feather growth, starting from few feathers growing in moult stage 1, to most feathers growing in moult stage 3, to few feathers growing

at the end of the moult in stage 4 (Figure 3.2.a). In 1970 the dry weight of the plumage follows the changes in the intensity of feather growth, whilst in 1971 this is not the case. As in this study we are primarily concerned with the intensity of feather growth all data for moulting birds has been analysed with reference to moult stage and not to plumage dry weight.

3.7 Summary

The post-juvenile moult of young Willow Warblers could be divided into four stages. The dry weight of plumage increased steadily throughout the moult, but at most the plumage after moult weighed about 17% more than at the start.

Chapter 4. The total body weights of Willow Warblers.

4.1 Introduction

The total body weight of an individual of a migratory species is of interest for two reasons; first, changes in body weight may reflect changes in body components such as lipid; and second, the weight at which a bird starts its migratory journey affects both its flight range and flight time. As an indicator of changes in weight of total body lipid, the weight of the whole bird is useful only in certain cases. King and Farner (1959) found that the total body weight of the White-crowned Sparrow during the spring premigratory period increased by the same amount as body lipid, but that during the prenuptial moult total weight was not a good measure of the change in body lipid weight, as at this time lean body weight increased. In adults of the same species, King(1963) found that although approximately the same weight of lipid was deposited in both spring and autumn premigratory periods, this deposition was not reflected in the total body weight in autumn as the mean lean weight had decreased by 1 gm compared with the spring. In Lesser Redpolls body weight is even less reliable as an indicator of changes in body lipid weight since little change was found in mean body weights in autumn (Evans 1966), even though the birds were undergoing fat deposition (Evans 1969). In part, the changes in lipid weight were masked by the reduction in total body water of both adults and juveniles at the end of their moults. Nakamura (1962) found that lipid weight increased during the spring moult in the Eastern Great Reed Warbler, but that mean weights decreased. Fry et.al. (1970) found in a number of palaearctic passerines in North Africa that body lipid weight increases were accompanied by weight increases in the water and lean dry fractions of the body, so that again the weight increase of the live bird was not an accurate measure of the change in lipid weight.

Since the weight of body lipid is constant (and low) during a moult of any sort in passerine birds, body weight changes during the moult indicate weight changes in other body components. Evans (1966) found a decrease in mean body weights of adult Lesser Redpolls during the post-nuptial moult which could be explained entirely by changes in the weight of the plumage; but Newton(1969) found much larger decreases (about 3 gms) in captive Redpolls, which from Evans' (1969) data could not be accounted for solely by a decrease in the weight of body lipid. As will be shown in later sections, body weight changes in moulting juvenile Willow Warblers also reflect changes in the weight of body components other than lipid.

The effect of body weight on migratory performance will be fully dealt with in Chapter 12.

4.2 Methods

Only birds which had not been sampled for blood were used, which restricted the samples to 1970 birds. Birds were weighed as described in the General Methods section (Chapter 2).

4.3 The total body weights of Willow Warblers caught during the morning.

The means for the samples of juvenile Willow Warblers are presented in Table 4.3.1. As the variances of these samples were homogeneous (Bartlett's test) (1) a one-way analysis of variance was performed on the five means; there was no significant effect due to the stage of the moult ($p > .25$) (2), even though there is a decrease in mean values in the middle of the post-juvenile moult (Figure 4.3.a.). Interestingly, the coefficients of variation

also decrease in mid-moult even though the means are smaller. This suggests that the sample composition at this time is more homogeneous than either at the beginning of the moult or after it is complete. In spite of lipid deposition the mean body weight of moulted birds was slightly lower than for juveniles at the start of the moult, but the standard deviation was much larger in the case of the moulted birds.

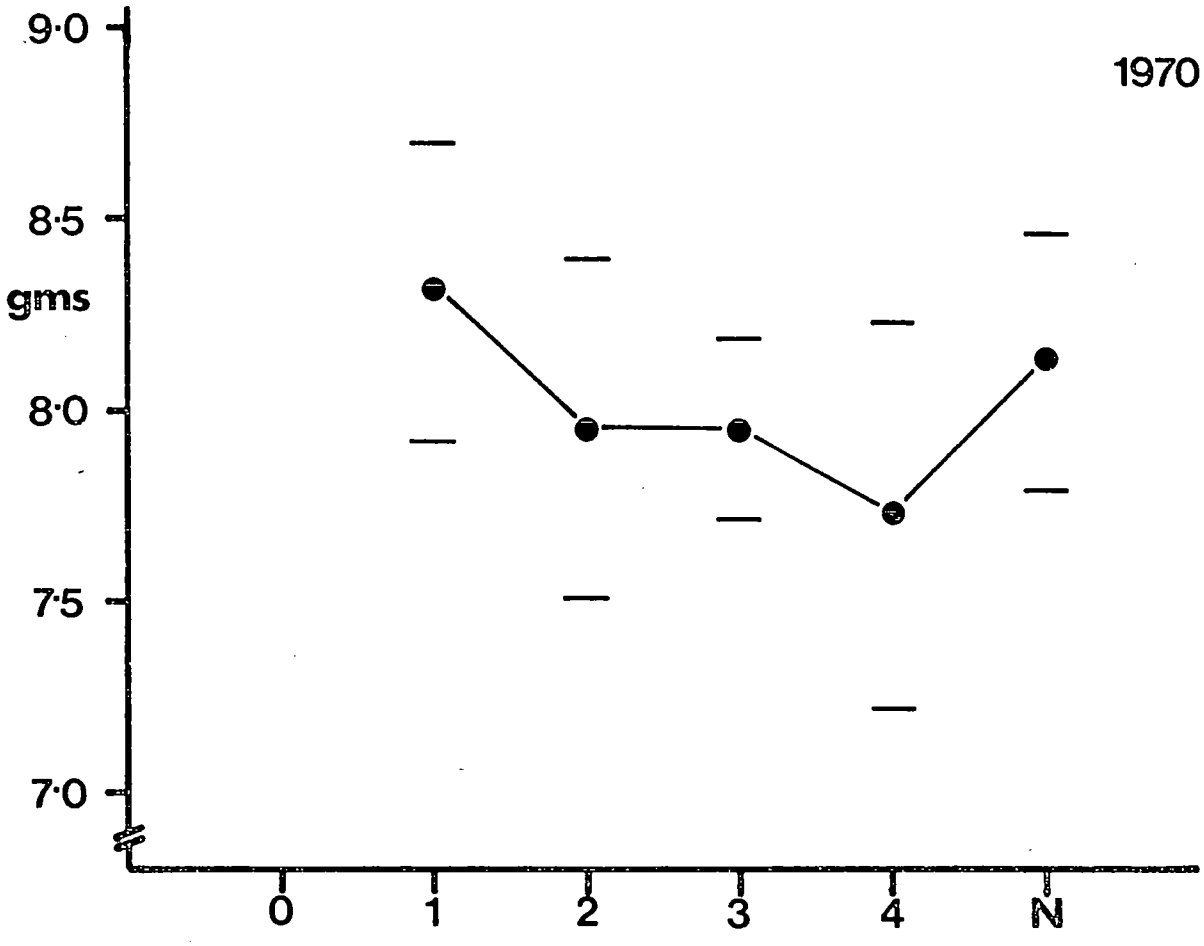
Table 4.3.1. Total body weights of Willow Warblers, 1970, in gms.

Moult stage	Mean	SD	SE	CV	n
Juveniles					
1	8.31	0.43	0.18	5.2	13
2	7.96	0.41	0.20	5.2	10
3	7.96	0.19	0.11	2.5	16
4	7.74	0.23	0.20	3.0	6
Moulted	8.14	0.70	0.16	8.6	26
Adults					
Primary moult score					
0 to 20	8.34	0.50	0.25	6.0	8
20 to 80	9.05	0.77	0.31	8.5	8
80 to 90 and moulted	8.41	0.12	0.17	1.4	4
All moulting birds	8.84	0.60	0.21	6.8	14

Primary moult score is arrived at by totalling the scores for individual feathers on both wings - 0 an old feather, 1 for a small pin or lost feather, 2 and 3 for one-third and two-thirds grown respectively, 4 for a nearly fully grown feather and 5 for a new feather (Evans 1966).

The mean body weight of any sample of adult Willow Warblers is larger than any sample of juvenile birds, and although there is no significant weight change during the post-nuptial moult ($.25 > p > .1$) (3) there are slight differences (Table 4.3.1.). The mean weight of the sample for the middle of the moult (primary moult score 20 to 80, explained in legend to Table 4.3.1) is greater than the mean for either moulted birds (primary moult

Figure 4.3.a . The mean total body weights (± two standard errors) of juvenile Willow Warblers during the post-juvenile moult. The ordinate is the weight of the body in gms; the abscissa the state of moult; 0 - unmoulted; 1,2,3,4 - moult stages; N - moulted juveniles.


9·0
8·5
gms
8·0
7·5
7·0
1970
0
1
2
3
4
N

score 80 and above), or birds at the beginning of the post-nuptial moult (unmoulted and up to primary moult score 20).

4.4 Discussion

A decrease in mean total body weight of Willow Warblers during moult has been noted before, but not on the breeding grounds. Pearson(1971) in Uganda found that Willow Warblers arrive with a mean weight of 8.3± .8 (SD) gms but that birds present during the complete moult in January and February have weights of 7.6± .4 (SD) gms and 8.1± .8 (SD) gms respectively. It is not clear whether this body weight decrease during the moult is due solely to changes in lipid weight, or even to the presence of different populations at different times of year. In my study the median lipid weight of juvenile Willow Warblers (from a single population) remained constant from moult stages 1 to 4 in 1970 (Chapter 7.4) even though body weights showed a decrease. This mean weight decrease is due to weight changes of other body components (see Chapter 9) and this might be the case on the wintering grounds also.

The difference in the pattern of weight changes during the autumn moults of juvenile and adult Willow Warblers probably has a parallel in the White-crowned Sparrow. In adult sparrows body weight increases during the pre- and post-nuptial moults (King and Farner 1959 , King and Farner and Morton 1965), whereas in immatures in autumn body weight remains constant during the post-juvenile moult. There is not sufficient information to decide whether the mid-moult weight increase of adult Willow Warblers is due to changes in lipid weight, in the lean fraction (ie lean dry weight plus water) as in the White-crowned Sparrow (King, Farner and Morton 1965), or in the water fraction alone as in the

Lesser Redpoll (Evans 1969). In 1970 adults did tend to have more body lipid than juveniles, so that the mid-moult weight increase could simply be due to this alone.

The maximum weights of about 10gms found in this study were infrequently recorded. In the premigratory period, sampling of wild migrants is not representative, in that the chances of catching an individual bird decrease as it gains weight, since the chances of it migrating increase; but it seems that migration does occur at weights below 10 gms. The maximum weights of the sample of migrating Willow Warblers taken from Bardsey lighthouse (Chapter 12.1) were again about 10 gms, with averages of 9.2 gms for juveniles and 8.7 gms for adults. These are, however, weights from the middle of the night and so would be expected to be greater than the mean morning weight of 8.1 gms found during the autumn premigratory period on the breeding grounds. Heavier Willow Warblers occur in captivity, but here inactivity may allow increased size of the lipid deposits; 17 gms has been recorded in autumn in juveniles reared from the nest (Gwinner 1969), but 12 to 14 gms is a more typical value. Birds kept for longer periods show high weights all year round, with minima of 10 gms during the moults (Gwinner 1968).

4.5 Summary

Mean total body weights of samples of juvenile Willow Warblers decreased slightly (though not significantly) during post-juvenile moult. Adults in moult did not show a decrease in weight and were heavier than juveniles at all times.

Chapter 5. The weight of the stomach contents of Willow Warblers.

5.1 Introduction

The hyperphagic basis of body weight increases in seed-eating migratory birds has been demonstrated by a number of investigators. King (1961b) was able to show that an increase in metabolisable energy (gross energy intake minus energy value of excreta) in captive White-crowned Sparrows in the spring coincided with an increase in body weight, and that these events could be reproduced by exposing birds caught in the winter to a 20 hour photoperiod. Similar events occur in captive White-throated Sparrows (Helms 1968). In the Chaffinch too, increases in body weight in the autumn coincide with increased metabolisable energy intake (Dol'nik 1968, 1970a).

As a direct measure of energy intake in insectivorous Willow Warblers was not possible, the stomach contents were weighed as a means of assessing food intake. The object was to determine whether hyperphagia could be detected by this method, and so provide supporting evidence that the samples examined included genuine premigratory birds. As Dol'nik (1966) had demonstrated differences in weights of stomach contents between moulting and migrating Garden Warblers (an insectivorous and frugivorous species) the method seemed promising.

5.2 Methods

The stomach was separated from the rest of the gut, opened out by an incision and the contents washed into a tared tube with distilled water. The contents were dried to constant weight at 40°C *in vacuo* and weighed to ± 1 mg.

5.3 Diurnal variations in the dry weight of the stomach contents of juvenile Willow Warblers

The combined data from the two years was markedly anormal, as birds caught near to sunrise frequently had almost nothing in the stomach. Consequently a Kruskal-Wallis one-way analysis of variance was applied to each moult stage to determine whether the samples from the three morning periods (0-2 hours, 2-4 hours, and 4-6 hours after sunrise) were all drawn from the same statistical frequency distribution. The medians, values of H and associated probabilities for these samples are presented in Table 5.3.1. Only in the sample of moulted birds is there any significant increase in dry weights during the morning ($.02 > p > .01$) (Table 5.3.1.). Mann-Whitney two-sample U-tests performed on pairs of samples within the morning showed that the median for moulted birds in the sample 2-4 hours after sunrise was significantly greater than the median for the 0-2 hours after sunrise period ($.02 > p > .002$) (1). The difference between the medians for 0-2 hours and 4-6 hours after sunrise was nearly significant at $p = .05$ (2); there was no difference between the medians for 2-4 hours and 4-6 hours after sunrise (3). These changes within the morning are illustrated in Figure 5.3.a. The median for evening-caught birds in moult stage 3 was no larger than the median for the last morning period ($p > .05$) (4), nor was there any difference between evening and morning medians for moulted birds ($p > .05$) (5).

When the three morning subsamples were combined for each sample, the median of the sample of moulted birds was no different from the median of any other moult stage. This is not suprising as the dry weight of the stomach contents represents the balance between

food intake and stomach clearance; if both these processes increased in rate during premigratory hyperphagia, the increased rate of food intake would not be detected. The reduced median for 0-2 hours after sunrise in the sample of moulted birds must be due to a change in the balance between the rates of these two processes. It could be due to a faster rate of clearance from the stomach, but in view of the repeated demonstrations of changes in food intake a decrease in this is more likely.

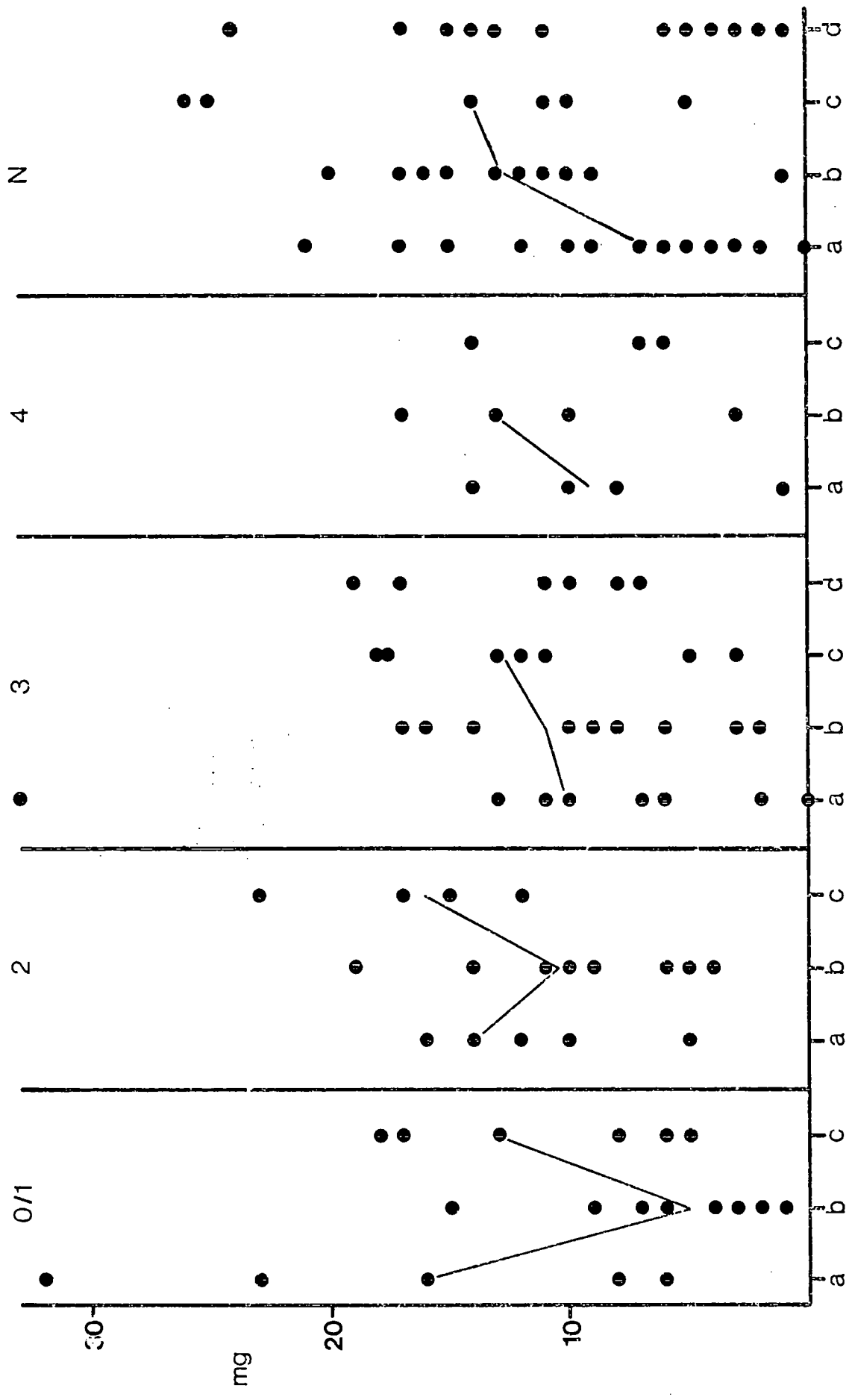
Table 5.3.1 The median dry weights of the stomach contents of morning-caught juvenile Willow Warblers (1970 and 1971 combined).

Moult stage	Hours after sunrise			H	p	n
	0-2	2-4	4-6			
Unmoulted/1	10	5	13	4.82	$p > .05$	20
2	14	11	16	4.07	$p > .05$	19
3	11	11	13	1.43	$p > .05$	32
4	9	13	-	5.20	$.1 > p > .05$	15
Moulted	7	13	14	8.02	$.02 > p > .01$	40

Median weights are in mg; H-Kruskal-Wallis H; 1,2,3,4 - moult stages; p - probability that value of H is significantly larger than zero.

As Morton (1967) has shown, when White-crowned Sparrows on an eight hour photoperiod are exposed to one of double the length the peak of food intake at 'dawn' and just after is reduced in size; so that after 20 days on the sixteen hour photoperiod, and a considerable increase in body weight, there is only a gradual increase in the amount of food eaten during the first eight hours of the photoperiod. Dol'nik and Blyumental (1962) also found that fat birds eat less than thin birds during the first hours of feeding, and Dol'nik (1966) has demonstrated a change from no diurnal rhythm of food in the stomach for moulting Garden Warblers,

Figure 5.3.a . Diurnal variations in the dry weight of the stomach contents of juvenile Willow Warblers during and after the moult.
The ordinate is mg dry weight of stomach contents.
The abscissa is time of day; a - 0-2, b-2-4 , and c-4-6 hours after dawn, d is evening-caught birds.
0- unmoulted juveniles; 1,2,3,4 - moult stages; N - moulted juveniles.
The solid lines join median values for the time periods.


mg
30
20
10
0/1
2
3
4
N
a
b
c
d

to a large diurnal increase in premigratory birds; this increase is from zero weight of stomach contents at dawn to a peak by mid-afternoon. Thus the pattern of weight changes of the stomach contents found in moulted juvenile Willow Warblers is very much the same as for the species in these other studies. As this early morning decrease in food intake is a correlate of hyperphagia, the data suggests that only moulted birds are hyperphagic and so in a premigratory state.

5.4 Summary

The weight of stomach contents of juvenile Willow Warblers did not vary significantly with time after sunrise in moulting birds, but was lower in moulted birds immediately after sunrise.
By analogy with other species, this is taken as evidence of hyperphagia in moulted birds.

Chapter 6. Brain weight and composition of Willow Warblers

6.1 Introduction

Although the removal of the M.pectoralis and the liver eliminates two major lipid sources in the body, the total lipid extracted from the remaining carcase has another potentially large contributor which is not adipose in origin; this is the brain. In order to obtain the best estimate of the birds' metabolizable lipid reserves it is essential that this non-metabolizable lipid should be subtracted from the totals (presented in Chapter 7) or at least shown to be negligible.

6.2 Methods

The brain was dissected from the thawed carcase and weighed in tared tubes to ± 1 mg. In many cases it proved impossible to remove the whole brain, so that the results are presented as mg lipid per 100 mg dry weight rather than total lipid weight per brain; an estimate of this will be made below.

The brains were dried to constant weight at 40°C *in vacuo* and weighed again to ± 1 mg to give percentage water. The samples were then ground, placed in a tared sinter glass thimble and redried to constant weight. The ground sample was extracted of lipid in technical petroleum ether by refluxing in a Soxhlet apparatus for 20 hours. The thimble plus sample was removed and again dried to constant weight at 40°C *in vacuo*. From this weight difference and the weight of dried ground sample the percentage lipid in the dried brain could be calculated.

6.3 Percentage of lipid in the brain

No differences in mg lipid per 100 mg dry weight were found between unmoulted, moulting and moulted birds. All juveniles had a mean of 20.9 mg lipid per 100 mg dry weight of brain (sample size 19). The mean for adult birds is slightly higher at 22.6 mg per 100 mg dry weight (sample size 5). The maximum dissected wet weight of the Willow Warbler brain is about 400 mg, and taking this as the true brain wet weight with a mean water content of 80.7% (sample size 23), the brain of a juvenile bird would contain about 15 mg of lipid in total; and the brain of the adult bird about 16 mg. So not only is the total amount of brain lipid small compared with the total body lipid (a minimum of 100 mg to 200 mg), it is also constant throughout moult and premigration. Note that, in spite of the small total amount of lipid due to the small size of the brain, the percentage of lipid (of dry weight) is the highest of the tissues examined, as found previously by Odum and Perkinson (1951).

6.4 Summary

Lipid weight in the brain is constant and small throughout and after the moult in both juvenile and adult Willow Warblers.

Chapter 7. Residual carcase lipid of Willow Warblers

7.1 Introduction

This quantity is a measure of the lipid remaining in the carcase after the contributions of the liver and pectoralis muscle lipid has been subtracted. As shown in Chapter 6.3 the amount of brain lipid is small and constant throughout the autumn, so the inclusion of this in the residual carcase lipid weight produces negligible error. Most of the lipid extracted from the residue of the carcase is derived from adipose tissue, although small amounts come from the remaining skeletal muscle and other tissues. Most of the adipose tissue is found in 'pads' located ventral to the abdomen, around the clavicle, and subcutaneously on the dorsal side of the body, as well as around the gut and heart. Adipose tissue is found in other parts of the body subcutaneously, and around the trachea.

7.2 Summary of previous studies

Many investigators have shown that migratory birds have greater amounts of body lipid during migration than at other times of year. This applies both in spring and autumn (Odum 1960a, Odum, Connell and Stoddard 1961). Garden Warblers and Siskins in Europe have greater amounts of body lipid during the autumn migration than during the post-nuptial moult which precedes it (Dol'nik 1966, 1967), and Willow Warblers in the eastern Baltic have as much as double the post-nuptial moult lipid levels during their autumn migration (Dol'nik and Blyumental 1967). Again, in North America White-throated Sparrows show peak levels of body lipid in both spring and autumn (Helms 1968); the spring migration lipid level is three

times the level during the early spring moult (Odum and Major 1956). However, migrants do not always have increased levels of body lipid before migration. The migratory Myrtle Warbler departs with little body lipid but levels increase as migration proceeds, following stops for feeding en route (Yarborough and Johnston 1965). Odum, Connell and Stoddard (1961) list four other North American passerine species which set out on migration when lean, but show increases in body lipid when sampled along the migratory route. Resident species such as Tree and House Sparrows show no changes in body lipid at the migration times of other European granivorous species (Dol'nik 1966); although the migratory race of the latter species (Passer domesticus bactrianus) does have higher levels of body lipid during the autumn migration compared to the level during the preceding moult (Dol'nik 1970b).

Studies on captive birds or on samples of migrants taken both before and during the migratory period confirm this picture. King (1963) found an increase in the "lipid index" (gm lipid per gm of total body weight) of up to 7% in wild White-crowned Sparrows during the autumn. Captive sparrows showed the same trend but the spring peak was more pronounced. The spring increase in body lipid produced levels more than double the non-migratory ones (King and Farner 1959, King, Barker and Farner 1963), and the time taken to reach maximum body lipid weight was about 10 days (King and Farner 1959). In the wild, lipid levels of the Chaffinch during the autumn migration are three times greater than during the preceding moult, and caged juveniles show a gradual increase in body lipid up to the migratory period (Dol'nik and Blyumental 1967). Lesser Redpolls too show an increase in body lipid as the autumn migration approaches (Evans 1969).

As pointed out by Dol'nik and Blyumental (1967) the date at which

maximum body lipid of a given species is recorded at any given place depends on the location of the sampling station along the migratory route. They found that samples of Willow and Garden Warblers at the origin of their migratory journey showed maximum body lipid weight early in the autumn; Greenfinch, Robin, Brambling, Siskin and Great Tit at the middle of their migratory route in Lithuania showed a later peak of body lipid in the samples; Bullfinch and Redpoll which terminate their journey there had high levels on arrival. Migrant Chaffinches which they examined originated from several countries and hence showed elevated body lipid in samples taken throughout a long period in autumn.

In my study Willow Warblers were sampled at the origin of their migratory route (on the breeding grounds), so that changes followed through the autumn accurately reflect the developemnt of premigratory lipid deposition.

7.3 Methods

The method of fat extraction for the carcase residue is given in the section on General Methods (Chapter 2.1). The total weight of residual body lipid, rather than a"lipid index" (gm lipid per gm body weight), has been used in this section as an index is not helpful in assessing premigratory changes, since variations occur in the weight of other body components at this time.

7.4 Variations in residual carcase lipid during the moult and premigratory period.

In both years examined, the frequency distributionsof residual carcase lipid in the samples of Willow Warblers were not normal.

This applied both to samples of moulting and moulted birds and arose from a skew towards higher lipid weights, especially in moulted birds, and from a truncation of the distributions at the lower end, presumably because a small amount of lipid in all birds is not metabolisable (e.g. in the brain). Consequently the samples were analysed by the Mann-Whitney two sample U-test.

In 1970 all the medians of the morning-caught samples of moulting juveniles (moult stages 1 to 4) were significantly smaller than the median weight for the sample of moulted birds; these medians and their significance levels are presented in Table 7.4.1 (Figure 7.4.a). None of the samples from the four moult stages were significantly different from each other (1). When the samples at the height of the post-juvenile moult (moult stages 2 and 3) were combined, an increase in median residual carcase lipid weight could be detected between the sample for 0-2 hours after sunrise and the sample for 4-6 hours ($.05>p>.02$) (2). No such difference was found for the sample of moulted birds ($p>.05$) (3). This difference between moulting and moulted birds is probably due in part to the higher lipid levels of moulted birds (compared to moulting birds) during the first two hours of daylight ($.1>p>.05$) (4). However, a diurnal increase in residual carcase lipid was present in moulted birds (even though this was not evident in the morning) as the median level for juveniles caught in the evening was significantly larger than that for the morning ($p=.002$) (5). Moulting adults in 1970 have a greater median level of carcase lipid than juvenile birds in moult stage 3 (the height of the post-juvenile moult) ($p=.032$) (6).

Figure 7.4.a . The residual carcase lipid of juvenile and adult Willow Warblers during and after the moult , 1970. The ordinate is gms of total residual carcase lipid, the abscissa - 1,2,3,4 ,post-juvenile moult stages; N, moulted juveniles; A - adults in moult.
The closed circles are birds caught in the first six hours after dawn, open triangles birds caught in the evening.

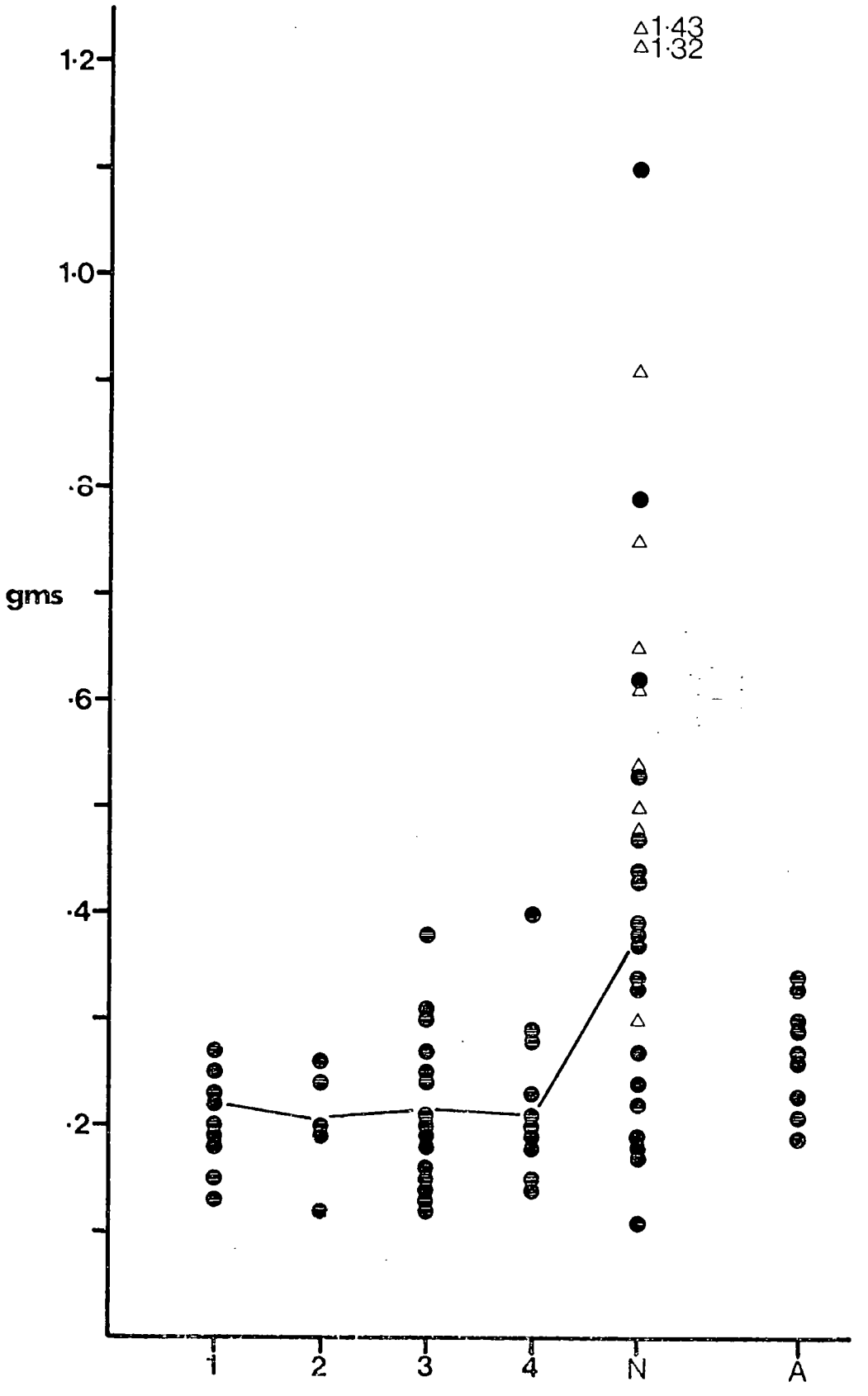

△1·43
△1·32
1·2
1·0
·8
gms
·6
·4
·2
1
2
3
4
N
A

Table 7.4.1 Residual carcase lipid of Willow Warblers in 1970 (gms).

Moult stage	Median	95%	p	n
Juveniles,morning				
1	0.220	(0.248-0.184)	.002	11
2	0.210	(0.271-0.116)	.044	10
3	0.216	(0.265-0.198)	.0004	21
4	0.213	(0.293-0.187)	.008	11
Moulted	0.380	(0.439-0.326)		26
Juveniles,evening				
Moulted	0.640	(1.339-0.481)		11
Adults,morning				
Moulting	0.275	(0.336-0.224)		15

95%-95% confidence limits for median;p-probability that the difference between the median for this moult stage and the median for moulted bird sample is significant by a two-tailed test.

Again in 1971 the median residual carcase lipid weight for the sample of moulted birds caught in the morning was significantly larger than the medians for any of the four moult stages; and the medians for these four moult stages did not differ amongst themselves (Table 7.4.2, Figure 7.4.b). Interestingly, the median value for morning-caught unmoulted birds does not differ from the median weight for moulted birds (7), suggesting that there is a difference in residual carcase lipid weight between moulting and unmoulted birds, even though this latter median is not significantly larger than the median weight for any moult stage. These recently fledged birds had more visible adipose lipid, and yolk was still present in the gut; the latter could well be the source of the extra lipid in unmoulted birds.

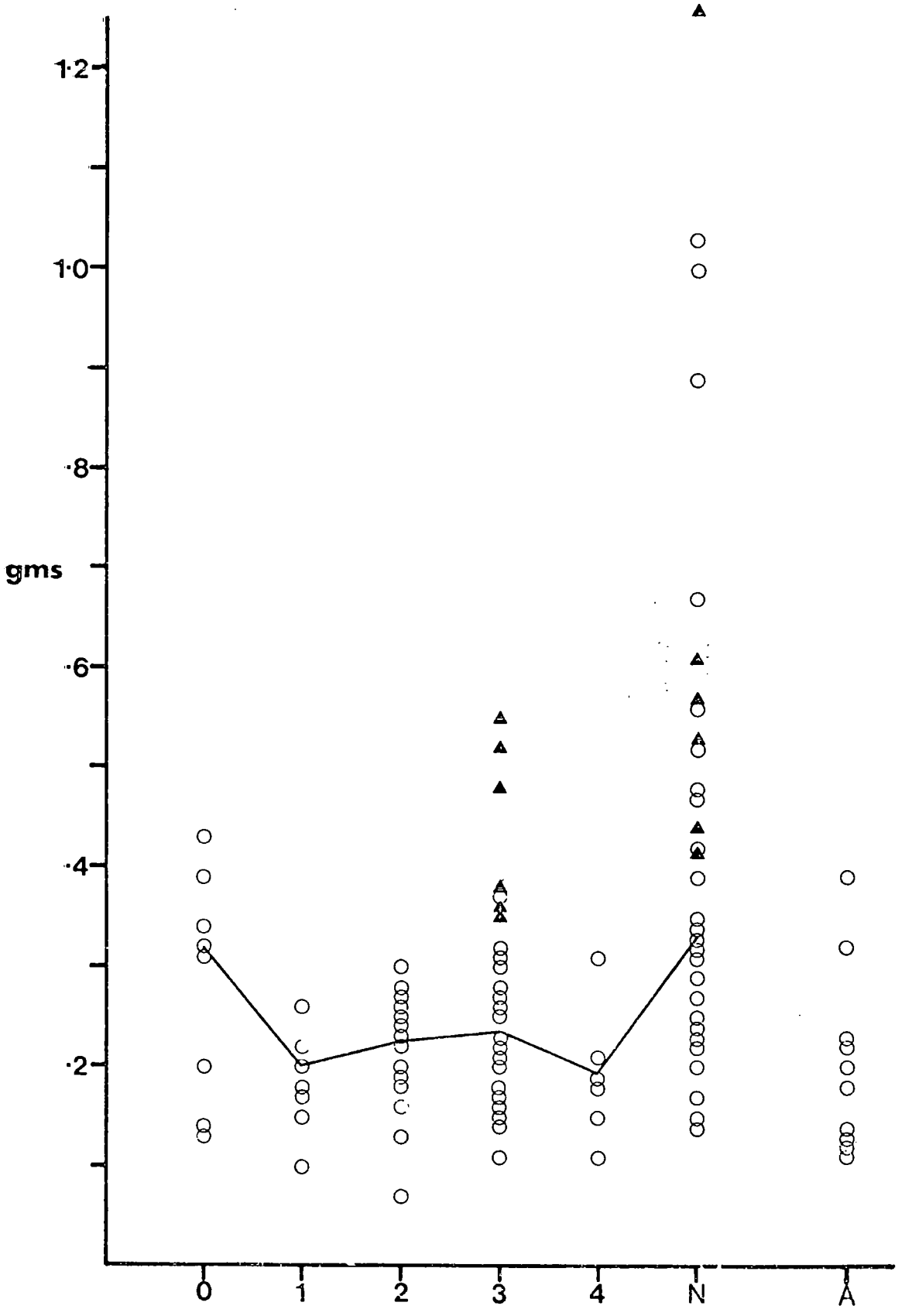
Table 7.4.2 Residual carcase lipid in Willow Warblers, 1971 (gms)

Moult stage	Median	95%	p	n
Juveniles, morning				
Unmoulted	0.320	(0.399-0.201)		9
1	0.200	(0.272-0.148)	.002	7
2	0.225	(0.264-0.184)	.0001	16
3	0.235	(0.272-0.195)	.001	24
4	0.195	(0.318-0.142)	.01	6
Moulted	0.333	(0.462-0.259)		27
Juveniles, evening				
3	0.480	(0.638-0.362)		7
Moulted	0.560	(0.908-0.448)		7
Adults, morning				
Moulting	0.210	(0.332-0.148)		10

95%-95% confidence limits for median; p-probability that the difference between the median for this moult stage and the median for moulted birds is significant by a two-tailed test.

As in 1970 an increase in residual carcase lipid weight is found during the morning in moulting birds (moult stage 2 and 3), but in this year the only significant difference is between the sample for 2-4 hours after sunrise and 4-6 hours ($.05 > p > .02$) (8). But even by the 2-4 hour period after sunrise the median residual carcase lipid weight of moulted birds is significantly larger than that for moulting birds ($.05 > p > .02$) (9). A diurnal increase in lipid weight between morning and evening is found both in moulting juveniles ($p=.00006$, moult stage 3) (10) and in moulted birds ($p=.01$)(11). Unlike the situation in 1970 the median lipid weight for moulting adults is no different from that for juveniles in moult stage 3 (12).

Figure 7.4.b . The residual carcase lipid of juvenile and adult Willow Warblers during and after the moult, 1971. The ordinate is gms of total residual carcase lipid, the abscissa - O, unmoulted juveniles; 1,2,3,4 ,the post-juvenile moult stages; N, moulted juveniles; A, moulting adults.
The open circles are birds caught in the first six hours after dawn; closed triangles birds caught in the evening.


1·2
1·0
·8
gms
·6
·4
·2
0
1
2
3
4
N
A

7.5 Discussion

As pointed out in the previous section there is reason to believe that the residual carcase lipid of recently fledged birds caught in the morning is somewhat higher than the levels found subsequently during the post-juvenile moult. The lipid weight decreased to a level of about 200 mg, which remains constant throughout all the stages of the moult. In the Bullfinch there is a fairly constant amount of body lipid during the post-juvenile moult (Newton 1968), and in the White-crowned Sparrow also body lipid is constant in weight during the post-nuptial moult (King and Farner 1959). In fact it seems that yearly minima in body lipid weight occur during the moults of a number of passerine species, with only immediate post-migrants having similar very low levels (King,Barker and Farner 1963,Johnston 1968). Examples of such species are the Chaffinch (Dol'nik and Blyumental 1967), White-throated Sparrow (Helms 1968), and White-crowned Sparrow (King,Barker and Farner 1963, King and Farner 1959). The Willow Warbler can now be added to this list.

The body lipid of Lithuanian Willow Warblers differs by as much as 300mg between moult and premigration, but this probably refers to adults rather than juveniles (Dol'nik and Blyumental 1967). The increase in median levels found in this study for juvenile birds is only 130 to 200 mg, although increases in individual birds were much larger, even five times the post-juvenile moult level.

A more difficult point to decide is whether there is any increase in residual carcase lipid weight in individual Willow Warblers during the post-juvenile moult. Obviously individuals could not be followed through the moult, but as the range of lipid weights within a moult stage increases through the moult, the data hints

that there might be fat deposition in individual juveniles (Figures 7.4a and 7.4b). In juvenile Chaffinches, for instance, body lipid increases in weight over mid-moult levels before the moult has finished (Dol'nik and Blyumental 1967).

In juvenile Willow Warblers in this study the median evening levels of residual carcase lipid were about double the morning levels, and there is no difference in this respect between moulting and moulted birds. This is not the same as in birds during the migratory period; Dol'nik and Blyumental (1967) cite Chaffinch, Garden Warbler, Willow Warbler and Siskin as migrants in which the diurnal amplitude of body lipid weight is greater during the migratory period than during the moult. However, my data does agree with Newton's (1969) finding that in the resident Bullfinch the weight of body lipid at dusk is twice the dawn weight both in and out of moult.

Little information is available on the pattern of weight changes of residual carcase lipid in adult Willow Warblers in this study. Adults tended to have higher lipid weights when moulting than juveniles; the significance of this is, however, not clear. Very few adults that had completed their post-nuptial moult were caught, suggesting that they leave the breeding grounds as soon as (or before) the moult is complete. Thus the higher lipid levels may reflect fat deposition during the moult for subsequent migration, or simply help the birds to reach feeding grounds away from the breeding areas where premigratory fat deposition may then take place.

7.6 Summary

The median lipid levels in the carcases of juvenile Willow Warblers remained constant throughout the moult but increased thereafter. Hence, most birds entered the premigratory phase only when the moult was complete. In both moulting and moulted birds, evening lipid levels were about double the morning levels.

Chapter 8. Residual fat-free dry weight of the Willow Warbler carcase.

8.1 Introduction

This quantity is the weight of the dried lipid-extracted material remaining after the pectoralis muscles, liver, stomach contents and plumage has been removed. It consists, therefore, principally of skeleton, nervous tissues, gut, heart, kidney, lung and the remaining skeletal muscle. In the birds included in these samples the supracoracoideus muscle was not removed.

8.2 Methods

The methods for obtaining the lipid-extracted residue of the carcase are detailed in the General Methods section (Chapter 2).

8.3 The weight of residual fat-free dry material during and after the moult

In both years, 1970 and 1971, the variances of the samples of morning-caught juvenile birds were homogeneous (Bartlett's test, 1,2). A one-way analysis of variance applied to the samples of juvenile birds for 1970 (moult stages 1 to 4 and moulted birds) showed that there was no significant effect due to the state of moult ($p > .25$) (3). In fact only just over 1% of the variance was associated with the mean differences due to the post-juvenile moult. As the means did not differ amongst themselves an overall mean was calculated, and this together with the five means is presented in Table 8.3.1. Means for the samples of birds caught in the evening, either fully moulted or in moult stage 4, did not differ significantly from the corresponding means for morning-caught birds (Table 8.3.1); thus there was apparently no diurnal weight

change in this parameter. The mean weight of the dried lipid-extracted residue of adult carcases (all stages of moult combined) was larger than the overall mean for the juveniles, although not significantly so ($.1 > p > .05$) (4).

Table 8.3.1 Residual fat-free dry weights (gms) of Willow Warbler carcases, 1970

Moult stage	Mean	SD	SE	CV	n
Juveniles, morning					
Unmoulted/1	1.07	.117	.035	11.0	11
2	0.99	.108	.033	10.8	10
3	1.07	.139	.030	13.0	20
4	0.99	.145	.041	14.6	12
Moulted	1.07	.125	.024	11.6	25
Overall	1.05	.130	.041	12.4	78
Juveniles, evening					
4	1.09	.156	.163	14.3	6
Moulted	1.08	.144	.045	13.3	10
Adults, morning					
Moulting	1.11	.117	.024	10.5	20

Note; 1,2,3,4 - moult stages

Again in 1971 a one-way analysis of variance applied to the samples of morning-caught juvenile Willow Warblers showed that there were no differences between the means of different moult stages, including unmoulted birds (5) (Table 8.3.2). The mean for unmoulted birds was smaller than for the later stages of the moult, but this difference was not significant. However, the

large coefficient of variation for this sample suggests, as does the smaller mean, that the growth of one or more components present in this residue was not complete on fledging. This reinforces the suggestion made in Chapter 9.8 that the pectoralis muscles may not be fully grown on leaving the nest. Perhaps in the case of the carcase residue it is the skeletal muscle which is not fully grown by fledging.

Table 8.3.2. Residual fat-free dry weights(gms)of Willow Warbler carcases, 1971

Moult stage	Mean	SD	SE	CV	n
Juveniles,morning					
Unmoulted	1.02	.184	.061	18.0	9
1	1.08	.135	.050	12.4	7
2	1.11	.171	.044	15.4	15
3	1.05	.143	.026	13.6	27
4	1.11	.085	.037	12.7	5
Moulted	1.09	.132	.024	12.1	28
Overall	1.08	.145	.014	13.5	91
Juveniles,evening					
3	1.15	.123	.046	10.7	7
Moulted	1.10	.080	.030	7.3	7
Adults,morning					
Moulting	1.18	.152	.037	12.8	16

Note; 1,2,3,4 - moult stages.

As in 1970, there are no significant differences between the means for morning and evening-caught birds (Table 8.3.2). However, the mean weight of the lipid-extracted residual carcase

of the adults is significantly larger than the overall mean for the morning-caught juveniles ($.05 > p > .02$) (6). Thus, in the two years examined the mean adult residual fat-free dry weight of the carcase was 0.06 and 0.10 gms heavier that the mean for juveniles caught at the same time of day. This, together with the difference in pectoralis muscle weights presumably accounts for a large part of total body weight differences between juvenile and adult birds, although, of course, other fractions such as total water weight may also differ.

Although no variations can be detected in the mean weights of the lipid-extracted dried carcase residue during the post-juvenile moult or in the premigratory period, the differences in the variances of the samples suggest a hidden effect. None of the variances are significantly different from one another, but in both years the highest coefficients of variation are found during the moult. In 1970 this is in moult stages 3 and 4 (Table 8.3.1) and in 1971 in moult stages 2 and 3 (Table 8.3.2). It is possible therefore, that small changes in fat-free dry residual carcase weight occur during the moult and that this is reflected in the variances.

To test this possibility fat-free dry residual carcase weights were plotted against the wet weight of the pectoralis muscles for juvenile birds caught in the morning. The ideal covariable would be the fat-free dry weight of these muscles, but as wet weight is a good measure of this (Chapter 9.8) this was used to increase the sample size. As the wet weight of these muscles are reduced

during the post-juvenile moult (Chapter 9.6), a positive correlation with fat-free dry residual carcase weight would indicate that this variable is reduced during the post-juvenile moult, and that the biggest reductions occur in those birds in which the pectoralis muscles are smallest. At the same time identical plots for all moult stages, moulted juveniles, and adults would show whether birds with larger fat-free dry residual carcase weights also have bigger muscles.

In both years neither unmoulted nor moulted birds show any correlation between muscle weight and the fat-free dry residual carcase weight (Table 8.3.3.). This also applies to moult stage 1. However, in both years one or more moult stages do show such a correlation. In 1970 it is moult stage 3 only (Table 8.3.3.); one of the moult stages in 1970 which showed a significant reduction in pectoralis muscle wet weight (Chapter 9.6). In 1971 it is moult stages 2 and 3, and again in this year the mean muscle weight for moult stage 3 was lower than that for moulted or unmoulted birds, though not significantly. In 1971 the mean muscle wet weight for moult stage 2 showed no reduction compared to unmoulted or moulted juveniles.

It seems likely therefore that one or more components of the fat-free dry residue of the carcase undergoes a weight reduction during the post-juvenile moult. This decrease is not large, for even taking the greatest slope of 0.744 gms fat-free dry residual carcase weight/gm of pectoralis muscle wet weight (moult stage 3 1970), and the largest mean decrease in muscle weight of 0.136 gms (moult stage 3 compared with moulted birds, 1970) this reduction would only amount

to about 0.1 gm (c.10%) of fat-free dry residual carcase.

Table 8.3.3. Regression statistics for a plot of fat-free dry residual carcase weight (y) against the wet weight of the pectoralis muscles (x) (both in gms).

Moult stage	b	SE_b	t	n	p
1970 juveniles, morning					
Unmoulted/1	0.577	0.329	1.75	10	.2>p>.1
2	-0.027	0.254	0.11	10	p>.5
3	0.744	0.265	2.81	18	.025>p>.01
4	0.479	0.247	1.94	12	.1>p>.05
Moulted	0.164	0.175	0.94	24	.4>p>.2
1971 juveniles, morning					
Unmoulted	0.444	0.464	0.96	7	.4>p>.2
1	0.541	0.210	2.57	6	.1>p>.05
2	0.635	0.254	2.50	13	.05>p>.025
3	0.337	0.146	2.31	24	.05>p>.025
4	0.416	0.252	1.65	7	.2>p>.1
Moulted	0.260	0.128	2.03	21	.1>p>.05
Adults all day, years combined					
Moulting	0.258	0.149	1.73	28	.1>p>.05

p-probability that regression coefficient is significantly different from zero; 1,2,3,4 - moult stages.

As shown above, all other samples show no correlation between the size of the pectoralis muscles (measured by wet weight) and the weight of the far-free dry residual carcase. This absence of

correlation is found both in morning-caught juveniles and adults. Since these two variables are essentially independent, and since variations in total body weight of juveniles are to a great extent dependent on variations in pectoralis muscle wet weights (Chapter 9.11), outside the moult period one would not expect a large part of the variation in body weight to be explained by changes in far-free dry residual carcase weight.

8.4 The contribution of fat-free dry residual carcase weight to variations in total body weight.

Since total body weight and pectoralis muscle wet weight are not correlated during the middle of the post-juvenile moult (Chapter 9.11) one might expect changes in residual carcase weight to determine total body weight variations to a large extent during this period, especially as the amount of body fat is low at this time. This is in fact the case, as 60% of the total body weight variations are associated with changes in the fat-free dry residual carcase weight during moult stages 2,3 and 4 (Table 8.4.1.). This is reduced to only 11% in moulted juveniles, and the correlation is no longer significant; most of the variations in total body weight in these birds being due to weight changes of the pectoralis muscles and adipose fat.

Interestingly, even though 63% of total body weight variations in unmoulted birds is associated with variations in pectoralis muscle wet weight (Table 9.12.1), 57% of total body weight variations are also associated with changes in the weight of the fat-free dry residual carcase. This is presumably due to the incomplete growth of both pectoralis muscles and one or more components of the fat-free dry carcase residue when the juveniles leave the nest.

Table 8.4.1. The correlation between total body weight and fat-free dry residual carcase weight (both in gms) of Willow Warblers, 1970.

Juveniles	r	%	p	n
Moult stage 1 and unmoulted	0.754	56.8	$p < .01$	11
Moult stages 2,3,4	0.777	60.3	$p < .01$	15
Moulted	0.336	11.3	$p > .05$	15
Adults				
Moulting	-0.045	-	$p > .05$	13

%-%variation in total body weight associated with fat-free dry residual carcase weight; p-probability that r is greater than zero.

Adults, as well as moulted juveniles, show no correlation between total body weight and fat-free dry residual carcase, even though the sample is of moulting birds. This is possibly due to the greater amount of adipose lipid found in moulting adults compared with moulting juveniles (Chapter 7.3).

8.5. Discussion

In studies of seasonal changes in the weight of the pectoralis muscles investigators have tried to eliminate much of the variation by obtaining some measure of body 'size' which is independent of variable components such as adipose lipid. Wing length has been used (Ward 1969), but in some species, such as the Lesser Redpoll, this is not a good indicator (Evans 1969). For a parameter to eliminate effects of body 'size' on pectoralis muscle weights two criteria must be fulfilled: first, there must be no seasonal change in this parameter, and secondly, it must be correlated with

changes in muscle weights. A likely candidate, therefore, is fat-free dry residual carcase weight, since it apparently satisfies both these criteria. However, as we have seen above, although the mean values of this variable do not vary through the autumn, there are changes in individual birds, and the correlation between this parameter and muscle wet weight probably has little to do with an absolute measure of body 'size', but is related to the physiological events during the post-juvenile moult; anyway this correlation is present for part of the post-juvenile moult only. There is no correlation in moulted birds, when it would be most useful, as muscle weights are increasing. If one takes the percentage of body weight variation explained as a measure of the ability of a parameter to indicate body size (and this is not perfect by any means), fat-free dry residual carcase weight does not have a large influence on total body weight variations in moulted Willow Warblers. In fact paradoxically the best measure of body 'size' on this criterion is pectoralis muscle wet weight itself. Consequently, no attempt has been made in this study to attempt to correct for body 'size' variations.

As already noted the mean fat-free dry residual carcase weight is remarkably constant throughout the moult and the premigratory period, although a trend towards a reduction in this weight in some moulting juveniles does agree with Ward's (1969) studies on the Yellow-vented Bulbul. In this species not all the decrease infat-free dry weight of the carcase can be explained by weight reductions of the pectoralis muscles, but the weight fluctuations of the liver were not accounted for. In spite of the slight weight reductions of this residual dry material in juvenile

Willow Warblers, its mean weight at migration is no greater than at an earlier date in the autumn. Thus birds with larger lipid reserves do not have greater fat-free dry residual carcase weights than birds with small amounts of body lipid.

8.6 Summary

Residual fat-free dry weight does not change with time of day, nor are there significant differences between mean weights of samples taken throughout and after moult. Adults were heavier than juveniles, and this difference accounts for much of the difference in total body weight between the two age groups at this time of year. Some components of the residual fat-free dry weight may have decreased in weight during moult in some individuals, and it is argued that the residual weight is not a useful measure of body 'size'.

Chapter 9. Flight muscle composition of Willow Warblers

9.1 Introduction

The flight musculature of birds consists principally of two muscles, the M. pectoralis and the M. supracoracoideus (George and Berger 1966). The latter muscle has also been referred to as M. pectoralis minor (Kuroda 1961). As has been shown by Greenewalt (1960) for a large variety of species these muscles are relatively massive compared with body size, with the M.pectoralis constituting about 15% of the birds' live weight. The size of the M. supracoracoideus is more variable and is correlated with flight habits (Hartman 1961). Anatomically M. supracoracoideus elevates the wing, and George and his co-workers (reviewed in George and Berger 1966) have shown that this muscle has fewer of the adaptations for sustained aerobic metabolism that are found in the M.pectoralis. Thus the supracoracoideus muscle contains more large diameter fibres and less fat per unit weight than the pectoralis muscle; and in the pigeon M.supracoracoideus only has half the mitochondrial content of the M. pectoralis (Harman 1956). However, the extent to which the M.supracoracoideus is equipped for aerobic metabolism varies, as in hummingbirds (in which the upstroke of the wing is important in hovering), there is little difference in histology and energy reserves between the pectoralis and supracoracoideus muscles, and no difference in succinic dehydrogenase activity, in contrast to other birds (Lasiewski, Galey, and Vasquez 1965). In these other species it seems unlikely that the supracoracoideus muscle plays a major part in sustained migratory flight, and most investigations have centered on the M. pectoralis. This study is no exception.

Two main categories of variation in pectoralis muscle composition with physiological state have been investigated: those changes associated with migration, and those associated with moult. Most studies have focussed on muscle weights and/or their energy reserves, but the number of direct investigations are few. However, indirect evidence for changes in muscle weights have come from studies of whole carcase lean dry weights, although this includes other components such as skeleton and connective tissue, as well as muscle. Child (1969), for example, found that this parameter increased during the migratory period in many species of North American migrants. In the Lesser Redpoll also, Evans (1969) found that fat birds, whether during or before migration, had significantly greater lean dry weights of the whole carcase. Fry et.al. (1972) have directly demonstrated a logarithmic relation between pectoralis muscle wet weight and the weight of carcase lipid in premigratory Yellow Wagtails at Lake Chad. But it is conceivable that this relationship simply reflects the fact that larger wagtails have larger pectoralis muscles, and these larger birds need more total lipid in order to migrate a given distance. None of these investigators has directly demonstrated an increase in pectoralis muscle weight during the premigratory period, nor have they identified what components might be responsible for such a weight change. Data presented later fulfill these criteria.

Changes in pectoralis muscle energy reserves and physiology during the spring premigratory period have been extensively studied by George and his co-workers in the Rosy Pastor, a species which does not moult before migration. They have concluded that there is a reduction in lipid utilization and an increase in lipid synthesis in the pectoralis muscle during the premigratory period (George

and Iype 1964, George and Vallyathan 1964a, 1964b, and Vallyathan 1963). This is accompanied by an increase in muscle lipid reserves and also an increase in muscle glycogen, the latter having a lipid-sparing role (George and Chandra-Bose 1967, Vallyathan 1963, and Vallyathan and George 1964). Farner and his associates have also come to the same conclusion, but on different evidence, in the White-crowned Sparrow, a species which moults before the spring migration. However, in the latter species no attempt was made to distinguish between the events due to the moult and the changes due to the premigratory state; and there is good reason to suspect that in the studies of the Rosy Pastor the adaptations recorded refer to migratory rather than premigratory individuals (George and Chandra-Bose 1967). In my study on Willow Warblers in autumn an attempt has been made to distinguish the effects of the moult from any premigratory changes; and all the moulted birds examined were premigratory, as shown in Chapters 5 and 7.

Moult is the only other event with which changes in pectoralis muscle composition have been associated. The best evidence that the composition of the M. pectoralis alters during moult comes from Ward's study (1969) of the Yellow-vented Bulbul. In this tropical species the predicted dry non-fat weight of the pectoralis muscles during the moult, based on a winglength-muscle weight relationship worked out for non-moulting birds, was always smaller than predicted. In the Bullfinch too, although no overall decrease in the mean lean dry weight of the carcase could be measured during the moult, both adults and juveniles exhibited about a 0.7 gm loss in lean dry weight overnight during the middle of the moult. In adults this overnight weight loss was not detectable either before or after

the moult, but in juveniles it persisted after completion of the moult, although the size of this weight loss was much reduced (Newton 1968). It was suggested that this lean dry material was being used in part for overnight feather growth; it is not unreasonable to suggest that some fraction of this weight decrease was due to loss of pectoralis muscle lean dry material. In my study of Willow Warbler flight muscles, the weight, composition and energy reserves of the M. pectoralis have been followed throughout the post-juvenile moult, in an attempt to account for any changes.

9.2 Methods

M. supracoracoideus:

All supracoracoideus muscles were dissected out of thawed carcases, and one muscle from each bird (the left) was weighed on a tared foil square on a torsion balance to ± 1 mg. No further analyses were conducted.

M. pectoralis:

Both muscles were dissected from thawed carcases and weighed to ± 1 mg in tared tubes. Pectoralis muscles were dissected from two types of carcase.

a) those in which muscle tissue had been excised immediately after death for glycogen analysis, and

b) those in which both M. pectoralis were intact

To obtain the total weight of the two pectoralis muscles in those birds in which the tissue had been sampled, the weight of the frozen tissue sample was added to the weight of the muscle left in the carcase. To test whether the removal of the tissue sample had significantly altered the weights of the muscle (by, for example, loss of tissue) the mean weights of the sampled and unsampled pectoralis muscles were compared within each moult stage.

There was no effect for moult stages 1 to 4 in 1970 (1), but the mean weight of sampled muscles for the moulted birds was significantly smaller than the mean weight of the muscles that had not been tissue sampled ($.05 > p > .01$) (2). Consequently, moulted birds whose pectoralis muscles had been sampled for tissue glycogen were excluded from the analyses performed later in this section. In 1971 tissue sampling of the pectoralis muscles had no effect on mean muscle weights in any sample of birds.

Muscles which had been weighed in tared tubes were dried to constant weight at 40°C in vacuo. This weight difference was taken as a measure of water in the muscle. Part of this water fraction consists of extracellular water, but as the blood volume of chick muscle is only 0.038 ml blood/gm tissue (Stearner 1958), this amounts to only 39.7 mg of blood per gm of wet tissue. As the water content of avian pectoralis muscles is about 70% (w/w, see below), most of this water must be intracellular in origin. Also with only .038ml/gm blood tissue, the contribution of blood glucose to muscle total glucose (= glycogen) will only be 0.08 mg/gm wet weight.

Muscle samples dried to constant weight for determinations of percentage water were also used for measurement of muscle lipid. These muscle samples were ground, placed in a sinter glass thimble preweighed to constant weight, and redried at 40°C in vacuo, again to constant weight. The ground sample was extracted of lipid by refluxing with technical petroleum ether in a Soxhlet apparatus for 20 hours. After this period no further measurable weight of fat could be extracted. Thimble and sample were then dried again to constant weight. When corrected for the weight loss due to grinding, this gave the weight of the lipid extracted dry material of the muscle sample - the fat-free dry weight. The weight difference between this and the dry muscle sample gave the weight

of extractable lipid in the sample.

That the fat-free dry weight of the muscle is an accurate reflection of the weight of muscle protein is supported by studies on the growth of the mouse M. biceps brachii. Goldspink (1962) found that for this muscle the dry weight approximated closely to the protein content throughout most of its growth. Indeed electron microscope studies of the avian M. pectoralis show no constituents other than fat, glycogen, mitochondria and myofibrils (Grinyer and George 1969).

For the analysis of weight variations of the pectoralis muscles of juvenile Willow Warblers only muscle weight from the morning have been used to minimize any diurnal variations.

9.3 Wet weights of the supracoracoideus muscles

Hartman (1961) found a large variation in the size of the supracoracoideus muscle, from 0.4% of body weight in buzzards to 11.5% in hummingbirds. In the juvenile Willow Warbler it constitutes about 1.6% of the total body weight, and about 11% of the pectoralis weight (Table 9.5.1.).

9.4 The relationship between the weights of the pectoralis and supracoracoideus muscles

In spite of a reduction in mean supracoracoideus weight between moult stages 1 and 2 in juveniles, there are no significant differences in mean weights for the four moult stages and moulted birds, probably because of the small samples involved. Consequently in view of the weight variations of the pectoralis muscles during the post-juvenile moult (see below, Table 9.6.1), a least squares regression analysis was performed to determine whether pectoralis

and suprcoracoideus weights were correlated. The regression equations are,

juveniles $y = 0.053\ X + 5.23$ $SE_b = 0.0059$ $n = 43$

adults $y = 0.038\ X + 20.66$ $SE_b = 0.027$ $n = 13$

with y as supracoracoideus wet weight (mg), and x as pectoralis wet weight (mg). Only the regression coefficient for the sample of juvenile birds is significantly different from zero (1). Since there are no significant variations in adult pectoralis muscle weights during the post-nuptial moult (Chapter 9.7), this probably indicates that the reduction in pectoralis muscle weight found during the post-juvenile moult also applies to the M. supracoracoideus.

9.5 Wet weights of the pectoralis muscles

Hartman 1961 has shown that the weight of the pectoralis muscles varies from 10 to 20% of body weight in a large variety of species; these figures agree with the average value of 15.5% of body weight quoted by Greenewalt (1960). In the juvenile Willow Warbler the usual figure is about 14% of body weight, although as shown in Table 9.5.1 the percentage varies through the moult. This is due to changes in pectoralis muscle composition that will be enlarged upon later.

Table 9.5.1 Flight muscle wet weights as a percentage of total body weights in juvenile Willow Warblers (data from 1970).

		M. supracoracoideus				M. pectoralis	
Moult stage	Mean body weight (gms)	Mean wt. (mg)	%BW	%PW	n	Mean wt (gms)	%BW
1	8.31	148	1.78	12.4	3	1.19	14.3
2	7.96	121	1.56	11.4	5	1.06	13.3
3	7.96	131	1.66	11.8	5	1.11	13.9
4	7.74	129	1.68	11.6	7	1.12	14.4
Moulted	8.14	128	1.58	10.2	20	1.25	15.3

%BW-percentage of body weight; %PW-percentage of pectoralis muscle weight; n-sample size of supracoracoideus muscles. Data for body weights comes from Table 4.3.1, and that for pectoralis muscle wet weights from Table 9.6.1 .

9.6 Variations in pectoralis muscle wet weights of juvenile Willow Warblers.

In 1970 the variances of the sample means for moult stages 1 to 4 and for moulted birds were homogeneous (Bartlett's test, 1). Therefore, a one-way analysis of variance was applied to these samples; there was a significant effect due to the stage of the moult ($p < .005$)(2). Comparison of the means of these samples by the Studentised Range Test showed that at the $p = .05$ level the mean for the sample of moulted birds was significantly larger than the means for moult stages 2, 3 and 4, although these means did not differ amongst themselves. Also the mean muscle weight for moult stage 1 was greater than the mean for moult stage 2, but did not differ significantly from the mean for the sample of moulted birds. These means and their standard errors are presented in Table 9.6.1.

Table 9.6.1 Variations in pectoralis muscle wet weights (mg) of juvenile Willow Warblers during and after the moult.

Moult stage	1970	1971
Unmoulted		1120.1±70.8 (7)
1	1191.2±26.8 (14)[a]	1183.2±81.3 (5)
2	1057.6±33.8 (20)	1227.5±48.3(13)
3	1109.9±23.9 (29)	1142.6±37.6(24)
4	1114.5±34.9 (16)	1211.9±49.1 (7)
Moulted	1245.0±26.1 (27)	1167.5±41.3(21)

a. mean ± standard error (sample size).

Table 9.6.2 suggests that within the morning (the first six hours of daylight) the lowest pectoralis muscle wet weights are found in the first two hours after dawn. However, a comparison of the means for the three morning periods within each moult stage shows that these differences are not statistically significant with the small samples available. If protein is being utilised overnight (as has been suggested by Newton,1968) then this is precisely the time one would expect the lowest muscle weights. This is a point that might repay further investigation.

In 1971 the variances of the sample means were homogeneous (Bartlett's test), and a one-way analysis of variance performed on the six means (see Table 9.6.1) was significant ($p < .005$)(4), although it could not be attributed to any particular pair(s) of means. The mean weight for the sample of unmoulted birds is very much smaller than the others, and as in 1970 the lowest mean weights during the post-juvenile moult are for the middle moult stages (moult stage 3). The small mean wet weight for the sample

of moulted birds in 1971 is due to sampling conditions. The birds left the catching area in 1971 much earlier than in the previous year, so that the time available for regaining lost muscle weight (see below) before departing was much shorter.

Table 9.6.2 The variations in pectoralis muscle wet weights (mg) during the first six hours after dawn in moulting juvenile Willow Warblers (1970).

	after sunrise		
Moult stage	0-2 hours	2-4 hours	4-6 hours
1	1202.5±61.5 (2)[a]	1194.4±50.4 (5)	1185.7±43.6 (7)
2	1075.2±57.4 (6)	1112.1±55.9 (8)	961.2±44.3 (6)
3	1059.5±73.1 (6)	1130.8±37.8(12)	1114.6±29.6(11)
4	1026.3±61.9 (4)	1144.3±49.2 (9)	1254.6±41.3 (3)
Moulted	1139.8±73.6 (7)	1233.9±57.4(10)	1130.0±30.2(10)

a. mean ± standard error (sample size)

9.7 The total wet weights of the pectoralis muscles of adult Willow Warblers during and after the moult

As the variances for the four samples (see Table 9.7.1) were just heterogeneous (Bartlett's test,1), a one-way analysis of variance was applied to the means from the combined data of both years. In spite of the quite substantial reductions in mean weights during the middle of the post-nuptial moult, there was no significant effect ($.25 > p > .1$) (2).

Table 9.7.1 Variations in total wet weights (mg) of the M.pectoralis of adult Willow Warblers during and after the post-nuptial moult.

Moult stage	
Unmoulted and primary moult score 0-30	1286.3 ± 49.7 (17)[a]
Primary moult score 31 - 60	1157.5 ± 34.1 (8)
Primary moult score 61 - 90	1167.3 ± 37.9 (14)
Moulted	1222.8 ± 64.4 (7)

a. mean ± standard error (sample size)
For explanation of primary moult score see Table 4.3.1.

9.8. Variations in the total water weight in the pectoralis muscles of juvenile Willow Warblers

In both 1970 and 1971 the variances of the means for the moult stages and moulted birds were homogenous (Bartlett's test, 1, 3). In 1970 a one-way analysis of variance applied to the four moult stages and the sample of moulted birds showed a significant effect due to the state of the post-juvenile moult ($p < .01$, 2) (Table 9.8.1). Comparison of the means by the Studentised Range Test demonstrated that the mean total water weight for moulted birds was significantly larger (at $p = .05$) than the means for moult stages 2, 3 and 4 (Table 9.8.1). The mean weight for moult stage 1 was not significantly different from that for moulted birds or from any other mean. Thus the increase in wet weight of the pectoralis muscles is in part due to an increase in the weight of muscle water.

An analysis of variance applied to the samples for 1971 (Table 9.8.1) showed no significant effect due to the moult ($p > .25$) (4). This permitted the calculation of an overall mean water content for the pectoralis muscles of 834.8 ± 16.8 mg (SE). In 1971

Table 9.8.1 Variations in the total water (mg) weight of the pectoralis muscles of juvenile Willow Warblers during and after the moult.

Moult stage	1970	1971
Unmoulted		803.5 ± 59.0 (6)
1	842.7 ± 25.4 (10)[a]	872.4 ± 63.0 (5)
2	768.0 ± 40.8 (8)	876.5 ± 38.2 (10)
3	781.4 ± 22.1 (16)	801.8 ± 30.6 (15)
4	805.4 ± 30.0 (13)	875.3 ± 62.4 (4)
Moulted	904.6 ± 27.5 (13)	827.8 ± 32.0 (12)

a. mean ± stanard error (sample size)

Table 9.9.1 Variations in total fat-free dry weight (mg) of the pectoralis muscles of juvenile Willow Warblers during and after the moult.

Moult stage	1970	1971
Unmoulted		283.3 ± 20.4 (6)
1	319.5 ± 9.0 (10)[a]	349.8 ± 24.8 (5)
2	284.0 ± 15.4 (7)	334.2 ± 15.3 (9)
3	299.3 ± 8.6 (16)	319.0 ± 14.7 (10)
4	304.0 ± 9.5 (13)	318.0 ± 28.3 (3)
Moulted	340.0 ± 10.2 (13)	342.1 ± 13.5 (9)

a. mean ± standard error (sample size)

there is a reduction in the mean water weight of the muscle in the middle of the moult, but this is not significant; there is, however, no increase in mean water weight for the moulted birds compared with the weights in the middle of the moult.

9.9 Variations in the fat-free dry weight of the pectoralis muscles of juvenile Willow Warblers during and after the moult.

In both 1970 and 1971 the variances of the samples were homogeneous (Bartlett's test, 1 and 3). An analysis of variance applied to the five samples for 1970 (see Table 9.9.1) showed a significant effect ($.01 > p > .005$) (2). Comparison of the means by the Studentised Range Test showed that the mean fat-free dry weight of pectoralis muscles for moulted birds was significantly larger than the means for moult stages 2,3 and 4 (at $p \leq .05$) (Table 9.9.1). The mean weight for the sample of moulted birds is not significantly different from the mean for moult stage 1.

In 1971 a one-way analysis of variance shows no effect due to the moult ($.2 > p > .1$) (4), though again there were small, though not significant, reductions in fat-free dry weight in moult stage 3 and 4 (Table 9.9.1). Interestingly the mean fat-free dry weight for the sample of unmoulted birds is smaller (as is the wet weight) than that for moult stage 1 (though not significant), suggesting that on fledging the pectoralis muscles are not fully grown.

9.10 Changes in the water concentration of the pectoralis muscles during and after the moult

As in this case we are dealing with a ratio (expressed as mg water/ 100 mg fat-free dry weight of muscle) all muscles from the sample of moulted juveniles can be used, whether sampled for tissue glycogen or not.

In 1970 only the variances for moult stages 1 to 4 are homogeneous (Bartlett's test,1), as the variance for the sample of moulted birds is significantly larger. A progressive decrease in water concentration appears to be present through the moult (Table 9.10.1), but this trend, when examined by a one-way analysis of variance applied to the four means, is not significant ($.25 > p > .1$) (2). An overall mean for these four moult stages is estimated as 263.7 $\pm$ 1.20 (SE) mg water/100 mg fat-free dry weight of muscle. When the mean for the sample of moulted birds and this overall mean are compared they are found not to differ (3), although the mean for moulted birds is almost significantly different from the mean for moult stage 1 (4) (Table 9.10.1).

The variances of the samples for 1971 show no homogeneity; and again the mean water concentrations of the pectoralis muscles show a reduction from unmoulted through to moulted birds. When the means are compared by individual t-tests, it is found that in this year the mean value of mg water/100 mg fat-free dry weight for the moulted birds is significantly smaller than the mean for birds in moult stage 3 ($.01 > p > .001$) (5). The former mean is also smaller than the mean for moult stage 2 ($p < .05$) (6). Additionally, the

mean water concentration of the muscles from unmoulted birds is not significantly different from the mean for moult stage 1, but is larger than the mean for moult stage 2 ($p < .001$)(7) (Table 9.10.1).

Table 9.10.1 Variations in M.pectoralis water (mg) per 100 mg fat-free dry weight in juvenile Willow Warblers during and after the moult.

Moult stage	1970	1971
Unmoulted		283.6 ± 2.84 (6)
1	267.3 ± 2.06 (10)[a]	269.5 ± 9.38 (5)
2	265.9 ± 1.94 (7)	265.9 ± 2.06 (9)
3	260.4 ± 2.20 (16)	263.4 ± 3.49 (10)
4	263.6 ± 2.52 (13)	263.6 ± 1.68 (3)
Moulted	259.9 ± 2.97 (22)	244.4 ± 3.69 (9)

a. mean ± standard error (sample size).

So in 1970 there is no statistical evidence that there is any change in water concentration during or after the moult; but in 1971 there is a significant reduction in mean values between unmoulted birds and birds in mid-moult, and a decrease between mid-moult and moulted birds. Either the situation in the two years differ: in both there is a reduction in the mean value of this ratio between unmoulted and moulting birds, but in 1970 there is no further decrease, whereas in 1971 there is; or the situation is the same in both years, a decrease in this ratio through the moult and after. In either case, the value of this ratio does not

follow the mid-moult reduction found for total water, total wet weight or total fat-free dry weight for these muscles.

9.11 Pectoralis muscle composition changes during the moult and the premigratory period - discussion.

The data on pectoralis weights and composition presented in the preceding sections permit a number of conclusions to be drawn about the relationship between moult, premigration and flight musculature. Firstly, in the case of total wet weight of the pectoralis muscles, there is undoubtedly an increase in mean weight between mid-moult and after the moult is complete (Figure 9.11.a). In 1970 this difference is statistically significant; in 1971 it is not. Suggested reasons for this difference have already been mentioned in Chapter 9.6, but even allowing for differences in sample composition there were fewer birds with smaller muscles during the middle of the moult in 1971. This may well be a genuine interyear effect, for it is easy to postulate (though not to demonstrate) that if muscle protein is being used as a source of amino-acids for synthesis of feather protein or as fuel for thermoregulation, then year to year differences in food supply or temperature may well influence the extent to which muscle weight reductions are seen. However, I have no evidence on this point. There does seem to be a difference between adults and juveniles with respect to the mid-moult reduction in pectoralis muscle weights, as in spite of decreases in mean weights for the adults during the moult these are not significant. This might be expected if muscle protein is being used as energy source, for juveniles would be expected to be less efficient feeders than adults and so might need an alternative source of energy.

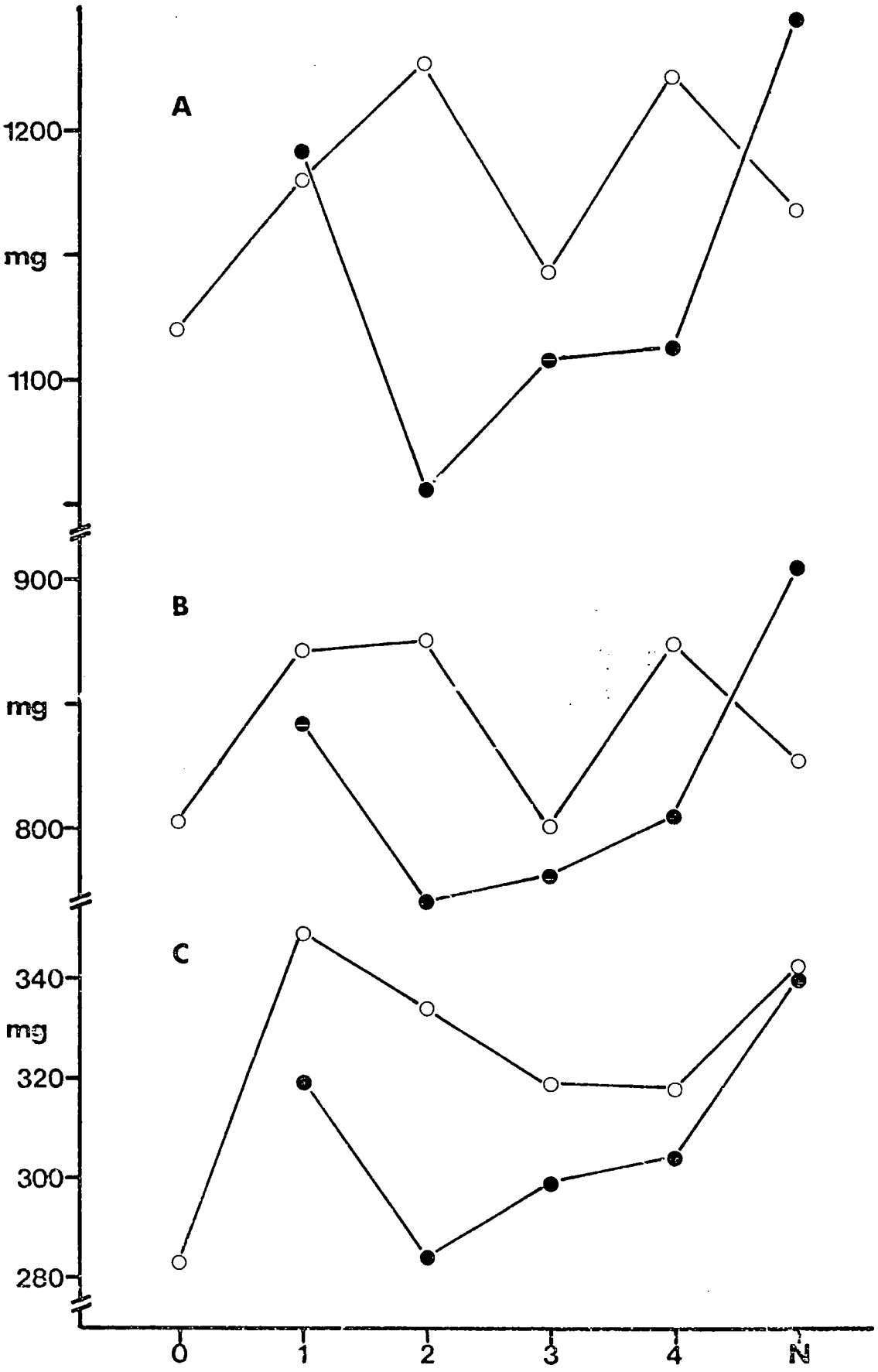
Figure 9.11.a . The changes in pectoralis muscle composition of juvenile Willow Warblers during and after the moult. Ordinate is the weight of muscle component in mg, and the abscissa the stage of moult; 0 - unmoulted; 1,2,3,4 - the moult stages; N - moulted juveniles.

A - mean wet weights of the muscles.

B - mean total water weights of the muscles.

C - mean fat-free dry weights of the muscles.

Open circles - 1971; closed circles - 1970 .


A
1200
mg
1100
B
900
mg
800
C
340
mg
320
300
280
0
1
2
3
4
N

Accepting that in some years muscle weights can be higher in moulted than in moulting birds, it remains to establish whether this is a true premigratory adaptation. In this study muscle weights and compositions were followed throughout the moult in order to answer this question. The results support the view that the muscle weights of moulted birds are drawn from the same population of values as are the muscle weights of unmoulted birds. It was possible to demonstrate in one year (1970) that the mean weight of the pectoralis muscles for moulting birds was significantly smaller than the mean weight for unmoulted birds. So in juvenile Willow Warblers the apparent premigratory 'hypertrophy' of the pectoralis muscles seems to be simply a return to pre-moult weights. Therefore, great care must be exercised in the interpretation of published studies of other temperate zone migrants, most of which moult before they migrate. The difference in lipid-extracted dry weights of the carcase between lean and fat Lesser Redpolls demonstrated by Evans(1969) might well be due in part to changes in the weights of the pectoralis muscles as suggested, but only as a post-moult recovery in weight rather than a special migratory adaptation. However, some other component of the lean dry carcase weight of Redpolls must have changed for the recovery from mid-moult muscle weight reductions amounts to only about 0.5% of body weight in Willow Warblers compared with 4.7% between fat and lean Lesser Redpolls.

Fry et.al.(1972) found a positive correlation between total body lipid and pectoralis muscle weight in Yellow Wagtails just before the spring migration from Africa; however, in this species too

there is a moult before migration (Curry-Lindahl 1960). They cited this relationship as evidence for premigratory muscle hypertrophy. Careful consideration raises two points. Firstly, there is no such relationship in juvenile Willow Warblers and it is hard to see why there should be in any species. A sample of birds taken just before migration will contain birds with widely differing amounts of body lipid; this is due to the heterogeneous nature of the sample. Such a sample will contain three categories: birds which have little lipid and are not undergoing lipid deposition; premigratory birds undergoing lipid deposition, for which there will be a variety of body lipid weights; and migratory birds in which lipid deposition is complete and are waiting to migrate - these birds will also vary in the weight of their total body lipid due to such factors as body size, time of day collected, etc. The relationship found by Fry et.al. (1972) is valid as a proof of premigratory muscle hypertrophy only if the absolute amount of body lipid is a measure of migratory preparedness. In other words it must be a valid substitute for studies on individual birds followed through the premigratory period. This is not the case. The nearest approach to this requirement is to follow the mean weights of samples of birds from before the premigratory period, and to be sure that there is no heterogeneity in the samples due to immigrant birds. In the case of Yellow Wagtails it is equally likely that larger fat reserves are found in larger birds which have larger muscles (they do, see Chapter 9.12) so that a sample of wagtails taken before the premigratory period would have shown the same distribution of muscle weights as during this period.

Secondly, there is a theoretical objection to premigratory hypertrophy of the flight muscles. Fry et.al. (1972) suggest that it supplies the extra muscular power needed to fly the extra load of lipid. In fact, as shown by Pennycuick (1969) small birds have no shortage of muscular power, and birds as heavy as 750 gms can double their body weight (with lipid) and still meet the power requirements. The only advantage of increased flight muscle weight would be the shortening of flight times, though with a corresponding reduction in range. This point will be discussed more fully later (Chapter 12).

The variation in total pectoralis weight seen during the post-juvenile moult is a reflection of changes in the fat-free dry weight of the muscle. As pointed out earlier this is a close approximation to the protein content. However, the total weight changes depends to a great extent on the weight of muscle water, since this accounts for about 60 to 70% of the weight of the muscle. As shown in Chapter 9.10 the ratio of muscle water to muscle fat-free dry weight is different in the two years examined. In 1970 there was no significant variation in the ratio and total water weight and fat-free dry weight varied in parallel. In 1971, the ratio was not constant and showed a gradual reduction through the moult, with moulted birds having the smallest ratio. In this year, therefore moult stage 3 saw a parallel reduction in mean water and mean fat-free dry weight of the muscles from moult stage 1, but the increase in fat-free dry weight found in moulted birds was not accompanied by an increase in total water weight; hence the ratio was smaller than for moult stage 4 and there was a decrease in total pectoralis muscle weights compared with this moult stage. The low value of this

ratio for moulted birds in 1971 is, however, not fortuitous, as the mean total water weight for this sample is greater than the mean for moult stage 3. The most likely explanation is that in this sample, which as explained earlier was taken right at the start of the premigratory period, the post-moult weight recoveries of these two components did not proceed at the same rates.

The largest reduction in the water/fat-free dry weight ratio is between unmoulted birds and birds in moult stage 1 (1971). Since, this ratio is a measure of the amount of water in the muscle cells, this observation corresponds to Goldspink's (1962) finding that the % inulin space of the mouse biceps brachii muscle drops during growth. This confirms, therefore, the suggestion that the pectoralis muscles of the juvenile Willow Warbler are not fully grown on fledging; and since the water/fat-free dry weight ratio is highest in the first moult stage in both years, these muscles are probably still growing during this moult stage also. In the mouse biceps brachii growth is by increase in myofibrillar size, and splitting when the myofibrillar diameter is double the usual (Goldspink 1970). The increase in dry weight is due to an increase in myofibrillar number per muscle fibre with a corresponding increase in fibre diameter. This is probably the mechanism in birds also, since Fry et.al. (1972) found that large muscles had significantly fewer fibres per cross sectional area than small muscles.

Ward (1969) and Newton (1968) have suggested that pectoralis muscle protein might be used for overnight feather growth in moulting birds. Short of demonstrating a transference of protein to the feathers from the M. pectoralis in captive birds, the nearest approach using field data is to show that the maximum reduction in muscle protein

weight occurs during the period of maximum feather growth. In the juvenile Willow Warbler this was the case. As was pointed out in Chapter 3.6, the scoring method adopted for the post-juvenile moult gives a good indication of the intensity of feather growth. Most feathers are growing in stage 3, and this is the period of the moult in which the greatest reductions in pectoralis muscle weight are found. Alternatively, muscle protein might be used for some process which is linked with the most intense period of feather growth. A likely candidate is thermoregulation, since one would expect the insulative effect of the plumage to be reduced during the moult; however, there is no published evidence on this point. A more extensive examination of the possible use of this muscle protein will be found in the final discussion (Chapter 14).

9.12 The interrelation of total body weight and pectoralis muscle wet weight

Since there is a decrease in pectoralis wet weights during the moult of juvenile Willow Warblers and a subsequent increase in moulted birds, it is of interest from the point of view of weight at migration, whether these changes affect the total body weight appreciably. As the mean differences involved are not large (about 40 mg) one would suspect this not to be the case. But individual reductions in muscle weight will be larger and so could be important in determining the total body weight of the bird.

Correlation and regression analyses were performed for pectoralis muscle weights and total body weights of morning-caught birds not used for blood sampling; only data from 1970 was available. As both body weights and muscle weights had normal frequency distrib-

utions, correlation analyses were possible, so allowing a partitioning of the variance.

As can be seen from Table 9.12.1, birds which were not moulting had 60% to 70% of their body weight variations associated with variations in pectoralis muscle weights. This contribution was reduced during the post-juvenile moult; and in moult stage 3 when most feathers were growing and muscle weights lowest, only 9% of body weight variations could be accounted for by variations in muscle weight. In fact the correlation coefficient was no longer significant. In mid-moult, therefore, there is no longer any relation between body size and muscle weight, so that large birds can have small muscles and vice versa. The conclusion is that changes in pectoralis muscle weights during the moult have little effect on body weight, but that in moulted birds the weight of the pectoralis muscles could have an important effect on the 'take-off' weight at the start of migration.

For comparison, figures for adults and moulted birds caught in the evening are included in Table 9.12.1. In these groups the percentage variation explained by variations in the weights of the pectoralis muscles is less than in the morning caught birds, presumably because of the higher body lipid of both these categories.

Table 9.12.1 The interrelation between total body weight and the wet weight of the pectoralis muscles of Willow Warblers.

	Correlation				Regression			
Moult stage	n	r	p	%	b	SE_b	t	p
Juveniles-morning								
Unmoulted/1	13	0.795[a]	p<.01	63	0.134	0.031	4.35	.005>p>.001
2	10	0.731	p<.01	53	0.196	0.065	3.03	.025>p>.005
3	16	0.303[a,c]	p>.05	9	0.085	0.072	1.19	.400>p>.200
4	6	0.792	p<.05	68	0.186	0.072	2.60	.100>p>.05
Moulted	26	0.822[c]	p<.01	68	0.157	0.022	7.07	p<.001
Juveniles-evening								
All moult stages	12	0.658	p<.05	43	0.163	0.059	2.77	.025>p>.01
Adults								
All moulting	15	0.652	p<.01	43	0.111	0.036	3.10	.01>p>.005

a. coefficients sharing superscript a are nearly significantly different (t = 1.88, p = .068)
c. coefficients sharing superscript c are significantly different (t = 2.44, p = .02)
In the regression analyses y is muscle weight and x body weight, both in gms.
p - probability that the correlation or regression coefficient is significantly different from zero.

9.13 The energy reserves of the pectoralis muscles - lipid

It has been suggested, mainly by George and his co-workers (George and Vallyathan 1964, Vallyathan 1963), that in the premigratory period there is a switch in the pectoralis muscles from lipid to carbohydrate utilisation, and that subsequent lipid synthesis is directed towards providing fuel for storage for the migratory flight. However, as pointed out by Farner et.al. (1961) the energy available from lipid in the pectoralis muscles is miniscule compared with the rest of the body's lipid reserves. It is unlikely, therefore, that the increased lipid levels of these muscles are for direct utilisation as fuel for the migratory flight(s). In the following sections the changes in muscle lipid have been measured through the moult and into the premigratory period in an attempt to find out why changes in these levels occur.

9.14 Methods

The Soxhlet extraction procedure used for measuring lipid levels in the pectoralis muscles are described in section 9.2.

9.15 Total lipid weight of the pectoralis muscles of juvenile Willow Warblers caught in the morning

In both 1970 and 1971 only the variances of the samples of birds taken during or before the moult were homogeneous (Bartlett's test 1,4). In 1970 a one-way analysis of variance applied to the four samples taken during the moult (Table 9.15.1) showed no significant effect due to the moult ($.1 > p > .05$) (2). An overall estimate of the mean lipid content of the pectoralis muscles for these four

moult stages is 16.5 ± 0.6 (SE) mg. The sample of moulted birds had a significantly larger variance than the variances for moult stages 1 to 4 due to the inclusion of the larger lipid values. The mean for this sample is significantly larger than the overall mean for the four moult stages ($.01 > p > .001$) (3)(Table 9.15.1) (Figure 9.15.a).

In 1971 also, there was no significant effect due to the stage of the moult, when a one-way analysis of variance was performed on the samples for the four moult stages and the sample for unmoulted birds ($p > .25$) (5) (Table 9.15.1). The overall mean for the four moult stages and unmoulted birds is 14.2 ± 0.5 (SE) mg. Again the variance of the sample of moulted birds was larger than any of the other sample variances, and the mean was significantly greater than the overall mean for the moult ($p > .05$) (6) (Table 9.15.1) (Figure 9.15.a).

In both years the total amount of lipid in thepectoralis muscles is greatest in moulted birds, but the level during the moult is greater in 1970 than in 1971. This partly due to an increase in mean lipid weight in moult stage 4 in the former year, and partly to the lower lipid weight found in unmoulted birds in 1971. Since the higher levels of lipid in moulted birds could simply reflect changes in muscle size the lipid concentration in these muscles was examined.

Figure 9.15.a . The total weights of the energy reserves in the pectoralis muscles during and after the post-juvenile moult of the Willow Warbler. Ordinate is the total weight in mg of lipid or glycogen. The abscissa is the stage of moult; O-unmoulted; 1,2,3,4 - moult stages; N - moulted. Closed triangles are mean glycogen weights from 1971 and 1970 combined; open squares are mean muscle lipid weights for 1970; closed squares the mean lipid weights for 1971.

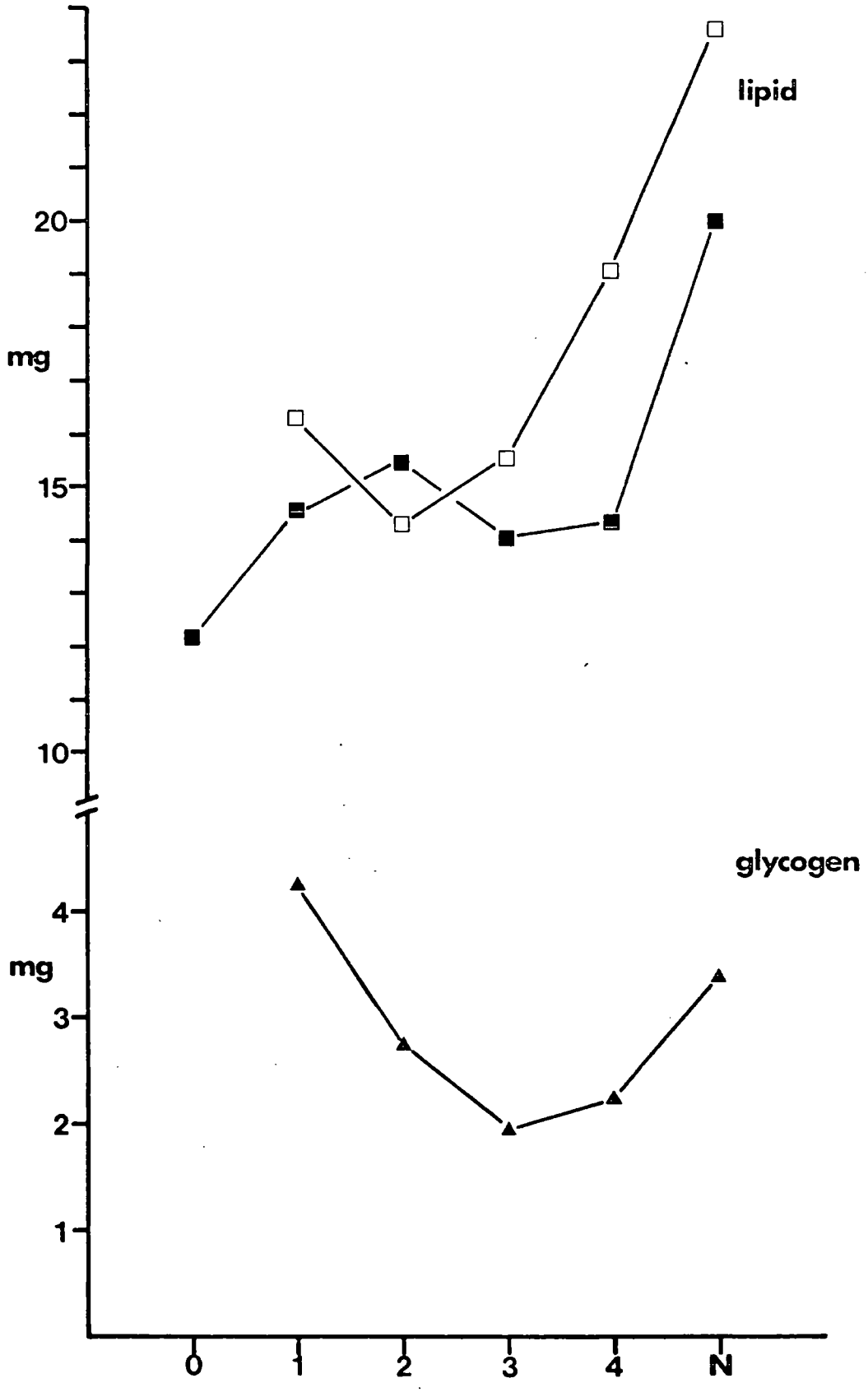

lipid
20
mg
15
10
glycogen
4
mg
3
2
1
0
1
2
3
4
N

Table 9.15.1 Variations in M.pectoralis total lipid (mg) during and after the moult of juvenile Willow Warblers.

Moult stage	1970	1971
Unmoulted		12.2 ± 0.9 (6)
1	16.3 ± 0.8 (10)[a]	14.6 ± 1.6 (5)
2	14.3 ± 1.4 (7)	15.4 ± 1.1 (9)
3	15.6 ± 1.1 (16)	14.0 ± 0.7 (10)
4	19.1 ± 1.4 (13)	14.3 ± 1.7 (3)
Moulted	23.6 ± 2.0 (13)	20.0 ± 2.1 (9)

a. mean ± standard error (sample size)

9.16 Pectoralis muscle lipid (mg)/100 mg dry weight of juvenile Willow Warblers

This measure of muscle lipid concentration has been used since it allows easy comparison with other published data and the difference between this and concentration on a per unit fat-free dry weight basis are slight (less than 5%). As this is a ratio, all birds have been used for the moulted bird sample, not just those in which there was no tissue sampling.

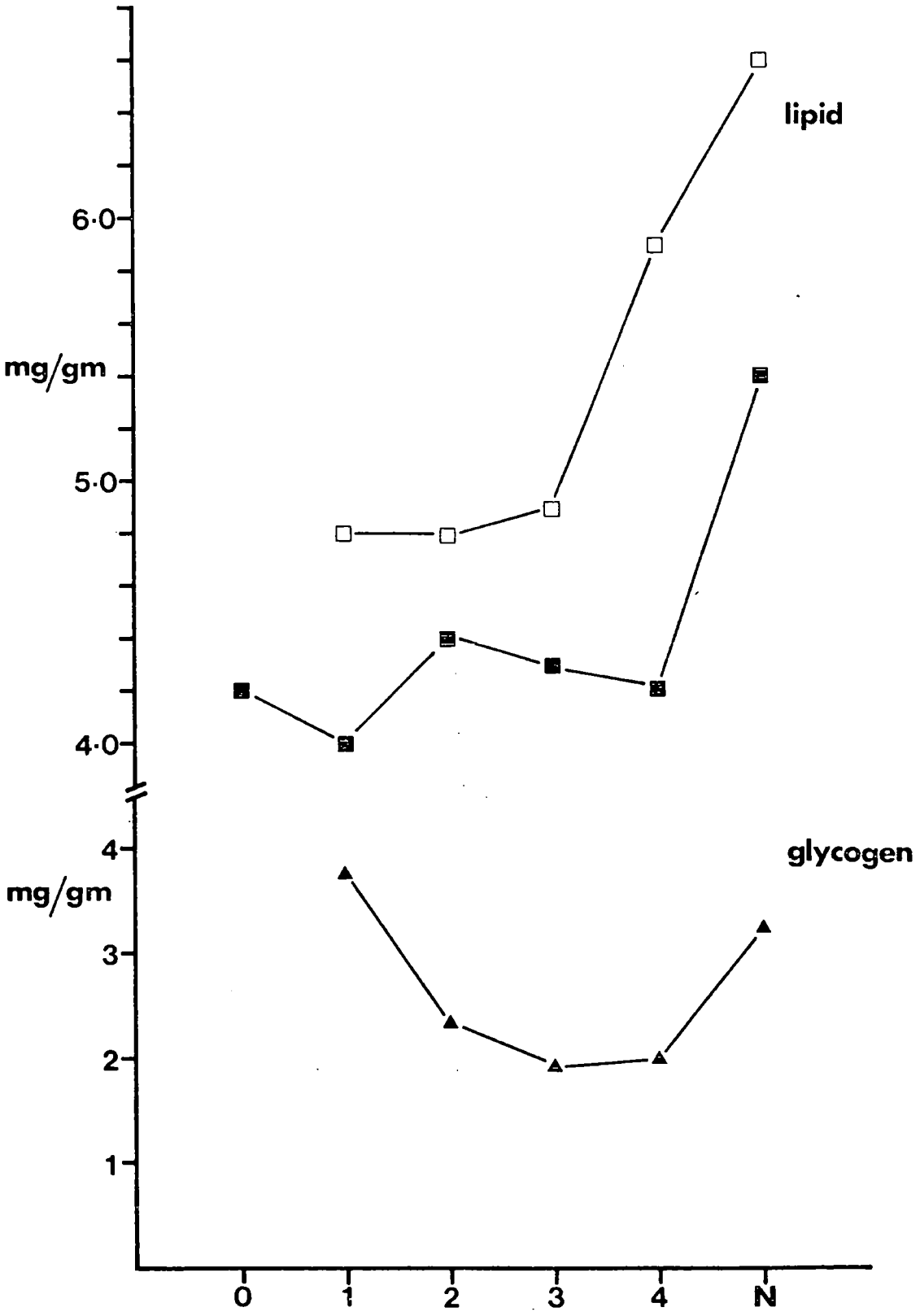
In 1970 the variances of the morning-caught samples of birds from the four moult stages were homogeneous (Bartlett's test,1), whereas the variance for the sample of moulted birds was significantly larger than any one of these. A one-way analysis of variance showed a significant effect due to the stage of the moult ($.05 > p > .02$) (2), but this could not be attributed to any particular

mean difference. However, the mean lipid concentration for moult stage 4 is larger than any of the other moult stages (Table 9.16.1). An overall mean for the four moult stages is 5.2 ± 0.2 (SE)mg/100 mg dry weight, and the mean for the sample of moulted birds is significantly greater than this overall mean ($.01 > p > .001$) (3) (Figure 9.16.a).

The variances for the four moult stages and the sample of unmoulted birds are homogeneous in 1971 (Bartlett's test,4). However, a one-way analysis of variance applied to these five samples showed there was no significant effect due to the moult ($p > .25$) (5) (Table 9.16.1). The overall mean for these five samples is 4.2 ± 0.1 (SE) mg/100 mg dry weight. Again the mean for the sample of moulted birds is significantly larger than the overall mean ($.02 > p > .01$) (6) (Figure 9.16.a).

Estimates of the pectoralis lipid concentration for evening-caught birds are presented in Table 9.16.a. In 1970 only the evening mean for birds in moult stage 4 is significantly larger than its morning counterpart ($.01 > p > .005$) (7), although the mean value for moulted birds caught in the evening is near significance ($.1 > p > .05$) (8). Thus the diurnal increase in muscle lipid concentration has decreased slightly between moult stage 4 and moulted birds, from 1.8 mg to 1.7 mg respectively. In birds in moult stage 3 there is no diurnal increase in muscle lipid concentration ($p > .05$) (9). In 1971 the diurnal increase in muscle lipid concentration in moulted birds is not significant ($.2 > p > .1$) (10), and slightly smaller than in 1970 (1.6 mg/100mg dry weight); these birds were of course collected early in the premigratory period. In no case is the amount of lipid in the muscle correlated with either muscle size or body lipid.

Figure 9.16.a . The concentrations of the energy reserves in the pectoralis muscles of Willow Warblers during and after the post-juvenile moult. Ordinate is the lipid or glycogen concentration in mg / 100 mg dry weight. Abscissa is the stage of moult; 0 - unmoulted; 1,2,3,4 - moult stages; N - moulted juveniles.
The closed triangles are the mean weights for glycogen in 1971 and 1970 combined; the open squares are the mean lipid concentrations for 1970; the closed squares the mean lipid concentrations for 1971.


lipid
6·0
mg/gm
5·0
4·0
glycogen
4
mg/gm
3
2
1
0
1
2
3
4
N

Thus the lipid concentration does increase in the premigratory period, and in 1970 this increase was apparent by moult stage 4. The higher lipid levels found in moulted birds in the morning, is reflected in the development of a diurnal cycle of muscle lipid, which is absent in moulting birds (moult stage 3).

Table 9.16.1 Variations in the lipid concentrations (mg/100mg dry weight) of pectoralis muscle of juvenile Willow Warblers

Moult stage	Morning	Evening
	1970	
1	4.8 ± 0.2 (10)[a]	
2	4.8 ± 0.3 (7)	
3	4.9 ± 0.3 (16)	5.0 ± 0.5 (6)
4	5.9 ± 0.3 (13)	7.7 ± 0.6 (5)
Moulted	6.6 ± 0.4 (22)	8.3 ± 0.4 (6)
	1971	
Unmoulted	4.2 ± 0.2 (6)	
1	4.0 ± 0.2 (5)	
2	4.4 ± 0.3 (9)	
3	4.3 ± 0.2 (10)	
4	4.2 ± 0.4 (3)	
Moulted	5.4 ± 0.5 (9)	7.0 ± 1.0 (5)

a. mean ± standard error (sample size)

9.17 Discussion

In both the White-crowned Sparrow and the Oregon Junco Farner et.al. (1961) found an increase in the lipid concentration of the pectoralis muscles of birds kept on artificially long photoperiods, a treatment that allows development of the migratory condition. Birds of both

species had about double the weight of lipid found in the muscles of birds kept on an 8 hour photoperiod. The absolute concentrations of lipid were in all cases much higher than the levels found in Willow Warblers. Even sparrows held on short-day had about 12mg lipid/100 mg dry weight compared with the 4 or 5 mg found in Willow Warblers during the post-juvenile moult. As total body lipid is reduced during the moult this might be expected, but there is even less agreement between fat birds, 24 mg per 100 mg dry weight being recorded in sparrows compared to 5 to 6 mg in wild caught Willow Warblers. This divergence is presumably due to the sparrows' confinement, as agreement between values for Willow Warblers and for feral sparrows of the same race is much better. Afternoon levels for wild sparrows are 6.8 mg/100 mg dry weight during the winter, 10.1 mg for spring migration, and 7.8 mg for autumn migration (King,Barker and Farner 1963). As these are afternoon values, the agreement with the figures found in this study are much closer than with the levels from captive birds. Vallyathan (1963) has also shown an increase in pectoralis muscle lipid before migration. In the Rosy Pastor, at dawn, the lipid increased from 14.6 mg/100 mg dry weight in February to 16.8 mg in the premigratory period in April. In none of these species have lipid levels during a moult been examined.

Diurnal variations in the lipid content of the pectoralis muscles have been noted before. Farner et.al. (1961) found a 21% increase in lipid concentration during the day in captive White-crowned Sparrows held on the long photoperiod (this agrees fairly well with the 30% and 26% increases found in Willow Warblers in moult stage 4 and after moult in 1970). They found no dirnal variation in

muscle lipid concentrations in sparrows kept on short days; correspondingly there was only a minute diurnal increase (2%) in mean lipid concentrations in moulting Willow Warblers. George and Chandra-Bose (1967), however, found a diurnal decrease in muscle lipid concentrations between morning and evening in Rosy Pastors before the premigratory period, but this was converted to an absence of diurnal change in the premigratory period itself, as the evening lipid levels increased.

As shown above both the total weight of lipid in the pectoralis muscles and its concentrations are low during the middle of the moult, and increased in moulted birds, (Figure 9.16.a). There is no correlation between the weight of muscle lipid and body lipid weight in individual birds; lipid levels of the pectoralis muscles, therefore, only follow total body lipid amounts in a general way. In the one year in which it was examined, the lipid concentration of these muscles exhibited a diurnal increase in moult stage 4; this accompanied an increase in the mean morning lipid level over earlier moult stages. This was not the case in 1971. So it does seem that lipid accumulation can begin in these muscles even before the post-juvenile moult is finished. This does suggest that the level of lipid in the muscle is a response to increased lipid levels in the body, and not a specific migratory adaptation.

9.18 The energy reserves of the pectoralis muscles - glycogen

The distribution of glycogen within the pectoralis muscles varies between taxa. Most small passerines have only one morphological fibre type (George and Berger 1966) and one type of fibre staining for lipid (Salt 1963). In warblers it is probable that the greatest

concentration of glycogen is in a thin band of the outermost fibres of the muscles (by analogy with George et.al's (1964) studies on the House Sparrow), these being specialised for anaerobic metabolism (George and Berger 1966). However, all fibres contain both lipid and glycogen, and the measurement of total glycogen in the pectoralis muscles is concerned with the glycogen levels in these aerobic fibres (the vast majority).

Although a number of studies have been made of changes in pectoralis muscle glycogen in the premigratory and migratory periods, there have been no specific investigations of variations in this metabolite during the moult. This is of particular importance in the interpretation of premigratory events in a species like the Willow Warbler in which there is a moult before the migration.

As the energy obtainable from the instantaneous glycogen reserves of the M. pectoralis is minute in comparison with the energy needs of the muscle in any form of activity (Farner et.al.1961) changes in the levels of glycogen in the muscle tend to reflect metabolic alterations rather than energetic ones. In this connection, as already explained, George and his co-workers have suggested that variations in muscle glycogen indicate a switch from lipid catabolism to carbohydrate catabolism in the premigratory period, so permitting an increase in lipid synthesis in the muscle. In the following sections I shall argue that thermoregulatory needs are the most likely cause of muscle glycogen reductions during the post-juvenile moult, and that the increase in the premigratory period is a recovery from this.

9.19 Methods

Samples of M.pectoralis tissue were excised from identical positions in the right muscle immediately after death, wrapped in foil and frozen in liquid nitrogen (whole process about 10 seconds). The frozen samples were stored on dry ice until used for analysis, which was usually later on the same day. The assay for muscle glycogen is as described in the section on General Methods (Chapter 2).

9.20 Changes in the total weight of glycogen in the pectoralis muscles of morning-caught juvenile Willow Warblers

As the samples from each year were much smaller than those used for the other variables measured, the data from the two years examined were combined. The frequency distributions of the data are not normal, and consequently Kruskal-Wallis one-way analysis of variance was performed on the samples for the four moult stages and moulted birds (Table 9.20.1). These samples were not all drawn from the same population of values ($.01 > p > .001$) (1). Medians of pairs of samples were compared by the Mann-Whitney two sample U-test; the significance levels for these tests are presented in Table 9.20.2. The lowest median levels of pectoralis muscle total glycogen occur in moult stages 3 and 4 (Table 9.20.1) (Figure 9.15.a);reference to Table 9.20.2 shows that the medians for these moult stages are significantly smaller than the medians for moulted birds or birds in moult stage 1. The percentage reduction in muscle glycogen between moult stages 1 and 3 and the subsequent rise is much the same as for the glycogen concentrations described in the next section.

Table 9.20.1 Median values of total pectoralis muscle glycogen (in mg) for morning-caught juvenile Willow Warblers during and after the moult.

Moult stage 1	4.25	(5.80 - 2.70)	(7)[a]
Moult stage 2	2.75	(6.51 - 1.74)	(17)
Moult stage 3	1.96	(4.15 - 1.59)	(20)
Moult stage 4	2.25	(3.49 - 0.77)	(9)
Moulted	3.40	(11.77 - 1.79)	(19)

a. median (95% confidence limits) (Sample size)

Table 9.20.2 Signficance levels of Mann-Whitney U-test performed on pairs of medians of total muscle glycogen of juvenile Willow Warblers caught in the morning.

	1	2	3	4
1	-	-	-	-
2	$p > .05$			
3	$p = .041$	$p = .164$		
4	$.02 > p > .002$	$p > .05$	$p = .212$	
Moulted	$p > .05$	$p > .05$	$p = .009$	$.02 > p > .002$

1 ,2,3 and 4 are stages of the moult. p-probability that the medians for not differ.

9.21 Pectoralis muscle glycogen concentration of morning-caught juvenile Willow Warblers

The measure of concentration was taken as mg glycogen per gm wet weight as this was the parameter measured by the assay and only a few of the muscles for which there are glycogen levels were extracted of lipid. However, as shown in previous sections wet weight is a good measure of changes in fat-free dry weight. Again the data for 1970 and 1971 were combined.

The frequency distributions of the samples were not normal, and so a Kruskal-Wallis one-way analysis of variance was used to test whether all the samples could have been drawn from the same distribution. This was not the case ($.05 > p > .02$) (1), demonstrating that the medians for the four samples during the moult and for moulted birds differed amongst themselves (Table 9.21.1). Pairs of medians were compared by the Mann-Whitney U-test; the significance levels are presented in Table 9.21.2. As for total muscle glycogen the smallest median levels are found in moult stages 3 and 4 (Table 9.21.1) (Figure 9.16.a), and are significantly smaller than the medians for the samples drawn from moult stage 1 or moulted birds (Table 9.21.2). There is a 49% reduction in median muscle glycogen concentration between moult stages 1 and 3, with a subsequent rise so that the median for moulted birds is only 13% smaller than the median for moult stage 1. It was not possible to demonstrate a diurnal cycle in muscle glycogen levels, but this was due to small sample sizes; there is no reason to expect that Willow Warblers are any different in this respect from other species in which such a diurnal increase has been repeatedly demon-

strated (Farner et.al.1961, George and Chandra-Bose 1967,Hissa and Palokangas 1970).

Table 9.21.1 Median values of pectoralis muscle glycogen concentrations (expressed as mg glycogen/gm wet weight muscle) for juvenile Willow Warblers caught in the morning, during and after the moult.

Moult stage 1	3.75	(5.30 - 2.10)	(7)[a]
Moult stage 2	2.33	(5.02 - 1.49)	(17)
Moult stage 3	1.93	(2.92 - 1.61)	(21)
Moult stage 4	2.00	(2.82 - 0.77)	(9)
Moulted	3.25	(4.14 - 2.34)	(19)

a. median (95% confidence limits) (sample size)

Table 9/21.2 Significance levels of Mann-Whitney U-tests performed on pairs of medians of muscle glycogen concentration (in mg/gm wet weight) of morning-caught juvenile Willow Warblers.

	1	2	3	4
1	-	-	-	-
2	$p > .05$			
3	$p = .041$	$p = .156$		
4	$.05 > p > .02$	$p > .05$	$p = .166$	
Moulted	$p > .05$	$p > .05$	$p = .024$	$.02 > p > .002$

1,2,3, and 4 are moult stages. p-probability that the medians do not differ.

9.22 The relationship between muscle size and glycogen concentration

Table 9.22.1 Correlation and least squares regression analyses between pectoralis muscle wet weight and muscle glycogen (mg) per gm wet weight in juvenile Willow Warblers caught in the morning.

0-2 hours after sunrise

	τ	SD	z	p	n
Moulting	0.41	0.20	2.03	.042	14
Moulted	0.44	0.19	2.28	.023	15

Least squares regression

Moulting	$y = 4.04\,x - 2.289$	$SE_b = 1.57$	n=14
Moulted	$y = 8.22\,x - 6.285$	$SE_b = 2.83$	n=15

2-4 hours after sunrise

	τ	SD	z	p	n
Moulting	-0.16	0.19	0.85	0.41	16
Moulted	0.14	-	-	0.72	8

4-6 hours after sunrise

Moulting	0.53			0.14	7

where, τ-Kendall's rank correlation coefficient, SD its standard error, p- the probability that τ is significantly different from zero, y is mg glycogen/gm wet weight of muscle and x is pectoralis muscle wet weight in gms.

Since there is a reduction in pectoralis muscle glycogen levels in the middle of the post-juvenile moult, it seemed likely that if this was in some way linked to the decreases found in muscle protein, then small muscles would have less glycogen (on a per gm wet weight basis) than large ones. Correlation analyses were used to test this. As the frequency distributions of muscle glycogen concent-

rations are not normal, Kendall's rank correlation coefficient has been used. Fully moulted birds have been compared with birds in moult stages 2 and 3 combined in order to increase the sample size; moult stage 4 has been omitted so as to eliminate any overlap.

As Table 9.22.1 illustrates, only samples taken in the first two hours after dawn show any significant correlation between pectoralis muscle wet weights and mg glycogen/gm wet weight of muscle. There is a significant correlation for both moulting and moulted birds. However, when a least squares regression model (for which there is no requirement of normality) is fitted to the data, it shows (Table 9.22.1) that although the intercept for moulted birds is smaller than for moulting birds, the greater slope means that most values of muscle glycogen concentration are greater for the moulted birds.

9.23 Pectoralis muscle glycogen - discussion

No previous studies on glycogen changes during the moult have been reported. The data for juvenile Willow Warblers during their post-juvenile moult clearly demonstrates that muscle glycogen, whether considered as a total weight or concentration, decreases to a minimum in moult stages 3 and 4, and is already reduced in moult stage 2. (Figures 9.15.a, 9.16.a). These reductions are statistically significant, and there is no statistical difference between birds at the start of the moult (stage1) and fully moulted birds. As pointed out in Chapter 3.6, the middle period of the moult (especially moult stage 3) is a time of intense feather growth,

whereas in stage 4 a substantial number of feathers are fully grown. However, in stage 4 there is more moult in the breast area (over the pectoralis) than in stage 2; glycogen levels are lower in stage 4 than in stage 2. This evidence strongly supports the view that the decrease in muscle glycogen is linked to the moult of the feather tracts overlying the pectoral muscles. Since the pectoralis muscle provides the main source of heat by muscle shivering in birds (Hart 1962), it is likely that exposure of these muscles during the moult would increase the energetic cost of thermoregulation, due to the increase heat loss from the ventral area. In the pigeon Chandra-Bose and George (1967) found that birds with the breast defeathered and subjected to cold stress ($2^{o}C$ compared with $30^{o}C$) showed significant reductions in muscle glycogen compared with control pigeons kept at the same low temperature. They proposed that the glycogen was being used for metabolic heat production.

However, there are no effective reserves of muscle glycogen which can be used up for thermogenesis, for as Farner et.al. (1961) and this study show, the total amounts are always very low in skeletal muscle; rather the metabolism of the muscle is altered during cold stress such that measureable levels of glycogen in the muscle decrease. Nor does this necessarily imply that carbohydrate is the primary substrate for oxidation as Chandra-Bose and George (1967) seem to suppose. The night in autumn lasts for about eight hours; glycogen reserves of the muscles could not provide enough energy for more than a small fraction of this time.

Direct evidence that most chemical thermogenesis must occur at night comes, of course, from weather records which show that, as expected, the lowest air temperatures during the autumn were found at night. Indirect evidence is found in the observation that there is a correlation between muscle glycogen concentration and muscle wet weight only during the first two hours after sunrise. As larger muscles presumably produce more heat by shivering than small ones, and if chemical thermogenesis really takes place mainly overnight, then to maintain a given body temperature smaller muscles would have to produce more heat per gm than large muscles, and so corresponding reduction in glycogen might be expected, given a fixed rate of glycogen synthesis. However, this effect is more accentuated for moulting birds, since as the slope of the regression line is smaller, individuals with muscles of the same weight as moulted birds always have smaller glycogen concentrations (Chapter 9.22).

As in the case of muscle weights the increase in muscle glycogen in the premigratory period seems to be a recovery from a reduction in levels during the moult. Increases in muscle glycogen during the premigratory period have been reported before, but in every case the base line with which this increase has been compared has been different. Farner et.al.(1961) showed that the dawn level of pectoralis muscle glycogen of the White-crowned Sparrow remained constant at 12 mg per gm dry weight irrespective of the daylength the birds were subjected to. However, the diurnal rhythm discernable in birds on an 8 hour photoperiod in the spring (up to 36.7 mg/gm dry weight in the evening) was diminished in birds on a 20 hour photoperiod (18.6 mg/gm dry weight at the end of the light period). But George and Chandra-Bose

(1967) found an enhanced diurnal rhythm of muscle glycogen in the Rosy Pastor during the premigratory period as well as an increased dawn level between March and premigration in April. In the same species Vallyathan and George (1964) found that evening levels of muscle glycogen in the premigratory period are double the values found earlier in the year in December.

Thus evidence for the postulated switch is not too sound. There is little evidence that the appropriate samples have been compared; for example, many of the results quoted by George are based on comparisons between mid-winter and the premigratory period, rather than from a detailed examination of events before and during this period. In the Willow Warbler there is no good reason to suspect a switch from lipid to carbohydrate catabolism, since changes in muscle glycogen can be adequately explained by the events of the preceding moult. As yet, there is no reason to reject George's suggestion that the pectoralis muscles have an enhanced capacity for lipid synthesis in the premigratory (or probably the migratory) period. However, an equally likely explanation of the increased levels of muscle lipid is that they result from an increase in liver lipid synthesis and increase storage around the body. The observation of Farner et.al. (1961) that storage of lipid occurs only in flight muscles may have no other significance than that pectoralis muscles are a site of lipid storage.

9.24 Summary

Both the wet and dry weights of M.pectoralis decrease during the moult, but recover to their former levels during the premigratory period. There is no evidence of muscle hypertrophy. Superimposed on these changes, the percentage of glycogen in the pectoralis muscle declines to a minimum in mid-moult and then recovers to former levels in the premigratory period. The decrease is probably

related to increased heat production needed during loss of insulative plumage. The percentage of lipid in the muscles remains constant during the moult, but increases in the premigratory period, when a diurnal increase also becomes evident. Lipid levels in the muscle probably rise in response to an increase in lipid levels in the whole body.

Chapter 10. Liver composition of Willow Warblers during and after the moult.

10.1. Introduction

Unlike the mammalian situation, avian adipose tissue is not capable of appreciable de novo fatty acid synthesis (Goodridge 1964 for the White-crowned Sparrow, Goodridge and Ball 1967a in the pigeon, O'Hea and Leveille 1968 in the chicken). The major site of fatty acid synthesis is the liver (Goodridge and Ball 1967a); in pigeons up to 96% of total fatty acid synthesis occurs in this organ. As the preparation for migration in passerine birds is marked by deposition of lipid (King and Farner 1959,1965), it is logical to examine changes in the composition of the liver during the premigratory period. Also, since it has been shown (Chandrabose, Bensadoun and Clifford 1971) that lipogenic enzymes of the avian liver have diurnal rhythms of activity, not only has liver composition of premigratory and non-migratory individuals been compared, but the diurnal variations of various parameters have been examined.

Injections of mammalian prolactin can produce fattening in migratory birds (Meier and Farner 1964), which is comparable in magnitude to natural premigratory lipid deposition. The lipogenic effect of prolactin seems to work via the liver. Goodridge and Ball (1967b) found a dose-dependent effect of prolactin on liver size, such that over five days of prolactin administration at 1 mg per day the liver wet weight doubled. The total liver nitrogen (a measure of the protein content) also doubled in weight, so that the percentage of water in the liver remained constant. This hypertrophied liver had an enhanced capacity for fatty acid synthesis

from glucose, and was better able to process preformed fatty acids. These changes were accompanied by an increase in the activities of citrate cleavage enzyme, malate dehydrogenase and 'malic' enzyme. Goodridge and Ball were unable to find a significant increase in liver fat, the excess produced being stored in their birds in the adipose tissue. Bates, Miller and Garrison (1962) also found an increase in liver weight in response to prolactin administration in hypophysectomised pigeons.

10.2. Methods

In the statistical analyses a combined sample of moult stages 2 and 3 has been used as representative of moulting juveniles. Since there is a slight increase in liver lipid in moult stage 4 (1970, Chapter 10.5), a possible increase in total body lipid (Chapter 7.5) and certainly an increase in pectoralis muscle lipid (1970, Chapter 9.16), moult stage 4 has been combined with the samples of moulted birds and compared with the combined sample of moult stages 2 and 3. The samples of birds caught in the evening are obtained from different years, juveniles in moult stages 1/2 from 1972, those in moult stage 3 from 1971, and moulted birds from both 1970 and 1971. These samples have been compared with mean levels for morning-caught birds in both 1970 and 1971.

Lipid extraction and quantification:

Since the ventral lobe of the liver was removed for glycogen analysis, the whole of the remaining liver tissue (minus gall bladder) was used for the extraction and measurement of liver lipid levels.

The following method follows Folch et. al.(1957). Liver tissue was dissected from the thawed carcase, weighed and first homogenised with 2/3 of a total 20-fold volume of chloroform:methanol(2:1,v/v) for five minutes in a glass homgeniser. Cooled chloroform:methanol

solution was used. The homogenate was stood in an ice bath for 30 minutes, and the supernatant then poured through a glassfibre filter paper moistened with the chloroform:methanol solution. The remaining protein precipitate was rehomogenised for 5 minutes with the remaining volume of chloroform:methanol, and the whole tipped onto the filter paper, and washed through with a little chloroform:methanol colution. To the crude lipid extract was then added 0.2 volumes of 0.05M sodiumchloride solution, and shaken in a glass stoppered tube. This tube was stood overnight at 4°C.

The upper phase, which contains such contaminants as peptides, sugars etc.,was aspirated with a fine-tip pipette. The purified lipid extract was transferred to a 5ml volumetric flask, and made up to this volume with chloroform:methanol solution. Four 0.5 ml aliquots were placed in foil trays (which had been pre-heated on a 60°C hot-plate, cooled and weighed) and heated for 5 minutes on the hot-plate at 60°C. The trays were removed to a dessicator containing silica gel and potassium hydroxide, and cooled for 5 minutes; they were then weighed to $\pm$ 1 µg. Batches of solvent were evaporated, as for the samples, every time a batch of samples was weighed. No weight increase in the trays due to impurities in the solvent could be found. The percentage error on the quadruplicate aliquots run for each tissue sample was 1-2%.

Glycogen analysis:

The details of the assay for tissue glycogen are given in the section on general methods (Chapter 2). However, since the method relies on the detection of glycogen by hydrolysis to glucose,and

no correction for tissue glucose was used, the quantitative errors introduced by such a procedure must be examined.

The amounts of glucose in tissues is very small; however in an organ, such as the liver, to which there is a copious blood supply, the contribution of blood sugar to the glycogen levels measured by the assay might be important. The blood value of the chick liver is 231 µl/gm wet weight (Stearner 1958); using a blood glucose level of 200 mg%, one gram of wet liver tissue would contain c, 0.4 mg of blood glucose. The reduction in plasma glucose levels between moulting and moulted birds in1970 (a maximum of 15mg%), would produce a change in the blood glucose of the liver of only 0.03mg. Both the changes in blood glucose in liver tissue, and the absolute values, are much smaller than the measured levels of total glucose in this organ.

Even though the same (ventral) lobe of the liver was used in all the analyses, no reproducible measurements could be obtained by the usual alkaline digestion, precipitation with alcohol, and quantification with anthrone method, due to rapid post-mortem glycogenolysis, a phenomenon which has apparently been overlooked by previous workers in this field. However, as we have seen, measurements of total tissue glucose levels give a good approximation to glycogen levels for liver tissue.

10.3 The wet weights of the livers of morning-caught Willow Warblers during the autumn

In both 1971 and 1970 the variances of the samples of juvenile birds taken during and after the moult were homogeneous (Table 10.3.1)(Bartlett's test 1,3). A one-way analysis of variance applied to the four moult stages and the sample of moulted birds

showed that there was no significant difference between the means ($.1 > p > .05$) (2). However, there is a slight increase in mean liver weight in moulted birds (Table 10.3.1.). A one-way analysis of variance performed on the six samples in 1971 (Table 10.3.1) also showned no significant effect due to the post-juvenile moult ($.25 > p > .1$) (4). Nor were the mean weights of the livers from adults significantly different from juvenile mean weights in either year. There was, therefore, no significant change in the total liver weights of adult or juvenile during the autumn.

Table 10.3.1 Changes in the liver wet weights of Willow Warblers during the autumn.

	Juveniles	
Moult stage	1970	1971
Unmoulted		337.7 ± 28.9 (8)
1	290.2 ± 12.1 (15)[a]	297.7 ± 15.4 (7)
2	316.2 ± 11.0 (20)	301.0 ± 9.2 (15)
3	314.3 ± 8.9 (31)	301.2 ± 7.1 (27)
4	294.5 ± 11.6 (18)	290.3 ± 18.3 (7)
Moulted	328.2 ± 8.3 (53)	286.1 ± 9.0 (27)
	Adults	
Moulting	313.6 ± 9.3 (24)	353.5 ± 35.5 (12)
Moulted	310.9 ± 16.8 (7)	

a. mean ± standard error (sample size) in mg

10.4 The fat-free wet weights of livers of morning-caught Willow Warblers during the autumn.

In both 1970 and 1971 the variances of the samples of juvenile Willow Warblers caught during the moult and the premigratory period were homogeneous (Bartlett's test, 1,3). One- way analyses of variance applied to the five samples in 1970 (Table 10.4.1) and the six samples in 1971 (Table 10.4.1) showed that in neither case

was there any significant difference between the means (in both years $.25 > p > .1$) (2,4). So, changes in liver lipid were apparently not masking a change in the lean wet weight of the liver; the constancy of mean liver weights through the moult and after is found in both total and lean weights. In no case were the mean weights for adult samples greater than any of the juvenile mean liver weights.

Table 10.4.1. Variations in the lean liver weights of Willow Warblers caught during the autumn.

Moult stage	Juveniles 1970	Juveniles 1971
Unmoulted		323.2 ± 23.7 (8)
1	267.9 ± 17.3 (9)[a]	285.4 ± 14.7 (7)
2	300.4 ± 13.2 (11)	289.8 ± 8.7 (15)
3	293.3 ± 7.5 (21)	287,6 ± 7.0 (23)
4	276.1 ± 13.2 (10)	278.5 ± 17.8 (7)
Moulted	304.5 ± 7.4 (24)	274.6 ± 9.2 (28)
	Adults	
Unmoulted		308.6 ± 9.6 (5)
Moulting	292.3 ± 10.9 (17)	340.5 ± 41.0 (10)

a. mean ± standard error (sample size) in mg

10.5 Variations in the concentration of lipid in the liver of juvenile Willow Warblers during/after the moult(mornings)

Although no significant changes in total liver weights could be found, differences in the mean weights of total and lean liver weights of juvenile birds suggest that there might be changes in liver lipid during the autumn. To test for these changes, lipid concentrations (mg lipid/100 mg liver fat-free dry weight) through

the moult were examined.

In 1970 the variances increased significantly in moult stage 4 and in moulted birds (1), so that all comparisons of means have been performed using a two-sample t-test. There were no significant differences between the means of moult stages 1,2 and 3 (2), and although the mean concentration had increased in moult stage 4, this increase was not significant (2). However, the mean for the sample of moulted birds was significantly larger than that for the sample of moulting birds in stage 3 ($.01 > p > .001$) (3), though no greater than the mean for moult stage 2 (4).

In 1971 the variance of the samples of moulting and unmoulted juveniles were homogeneous (Bartlett's test,5), and one-way analysis of variance performed on these five means (Table 10.5.1) showed that there was no significant effect due to the stage of moult ($p > .25$) (6); but the overall mean for these samples -13.70 $\pm$ 0.26 (SE) mg lipid/per 100 mg fat-free dry weight - was significantly smaller than the mean for the sample of moulted birds ($.05 > p > .02$) (7). Thus,in both years there is an increase in liver lipid concentration after completion of the moult, and in 1970 there was an increase in mean level by moult stage 4. Consequently, to examine this more closely the diurnal changes in liver composition were followed.

Table 10.5.1 Changes in mg liver lipid/100 mg fat-free dry weight of liver in juvenile Willow Warblers during and after the moult.

Moult stage	1970	1971
Unmoulted		13.80 ± 1.02 (8)
1	12.92 ± 0.74 (11)[a]	13.41 ± 0.78 (7)
2	12.96 ± 0.52 (11)	13.94 ± 0.41 (14)
3	11.87 ± 0.44 (21)	13.69 ± 0.39 (22)
4	13.17 ± 1.36 (10)	13.48 ± 0.84 (8)
Moulted	14.53 ± 0.83 (23)	14.75 ± 0.41 (23)

a. mean ± standard error (sample size)

10.6 Diurnal variations in liver fat-free wet weight of juvenile Willow Warblers (Figure 10a)

The fat-free (or lean) wet weight of the liver is a measure of liver size after the major energy source has been removed. This weight is used as a measure of cellular hyperplasia and/or hypertrophy; the weight of the other energy source, glycogen, was not subtracted as it seldom exceeded 1 mg in the whole liver.

In 1970 the variance for the samples from the three morning periods (Table 10.6.1) for both moulting and moulted birds are homogeneous (Bartlett's test, 1). A nested one-way analysis of variance applied to these six samples shows that there is a significant effect due to time of capture during the morning ($p < .01$), but not due to the state of the moult (2). Comparison of the means

by the Studentised Range Test shows that in 1970 there were no significant differences between the means of the morning subsamples of moulting birds. However, all the means of moulted birds for the three morning periods were significantly different from one another (at $p = .05$). So, the mean increase in liver lean wet weight for moulting birds was only 3.3 mg compared with 56.3 mg for moulted birds.

In 1971 two out of the three samples for the morning periods were skewed, and consequently the medians for all morning periods in 1971 were compared by the Mann-Whitney U-test (Table 10.6.1). None of the medians for moulting birds differed significantly from each other (3), but the medians for the two samples of moulted birds taken during the morning did differ, but not significantly.

Table 10.6.1 shows two independent estimates of liver lean wet weight for juveniles caught in the evening during the moult. The mean of sample of birds in moult stage 1 and 2 is significantly larger than the mean for birds in moult stage 3 ($.01 > p > .001$)(4), and both of these means are significantly larger than the mean (or median) for the last morning period in each year (5). The means of the two independent estimates of evening liver lean weight for moulted birds are not significantly different (Table 10.6.1), nor(6) are they significantly larger than the mean for the last morning period in 1970, but are larger than the means for 1971 (7).

Both of these means for evening-caught moulted birds are significantly smaller than the mean for evening-caught juveniles in moult stages 1 and 2 (8), but not significantly smaller than the mean for the sample in moult stage 3 (9).

Table 10.6.1 Diurnal variations in the liver fat-free wet weight of juvenile Willow Warblers during and after the moult.

	Morning (hours after sunrise)		
1970	0-2	2-4	4-6
Moulting	298.2±17.0 (8)[a]	284.7±7.1 (14)	301.5±14.0 (10)
Moulted	275.3±16.5 (11)	289.5±11.2 (13)	331.6±10.5 (10)
1971			
Moulting	285.0 (297-265) (14)[b]	286.7(300-278)(11)	285.0(355-272) (13)
Moulted	265.0 (288-245) (22)		290.0(316-244)(8)

	Evening
Moult stage 1/2	416.2±8.9 (10)
Moult stage 3	367.8±8.7 (7)
Moulted (1970)	345.8±10.3(15)
Moulted (1971)	335.5±16.3(10)

a. mean ± standard error (sample size) b. median (95% confidence limits)(Sample size)In mg

Thus the cycle of lean liver wet weight changes in moulting birds is distinctly different from that found in moulted birds; in the former there is no morning increase in weight, but the evening peak in weight is much larger than in moulted birds, and in the latter there is a distinct increase in weight by six hours after sunrise, but this is no different from the weights of the liver found in the evening.

10.7 Diurnal variations in the total water of the liver of juvenile Willow Warblers (Figure 10a)

The total water content of the liver is the component due to intracellular water and the extracellular blood. Since the volume of blood in chick liver is 231 µl/gm wet wt of tissue (Stearner 1958), the weight of blood in one gm of wet tissue would be 241 mg. As the water content of a 300 mg liver is about 200 mg (66%), the percentage of water weight due to extracellular blood would be 36%. Thus 64% of the weight of water in the liver estimated by drying the tissue is intracellular in origin.

Of the three morning samples of moulting birds in 1970, the sample for 2-4 hours was skewed. The median for the sample captured 4-6 hours after sunrise was not significantly larger than the median for this sample (Mann-Whitney U-test, $p=.08$)(1), but the mean for the 4-6 hours sample was significantly larger than the mean for the sample of birds caught in the first two hours after dawn ($.02>p>.01$) (2) (Table 10.7.1). All the morning samples of moulting birds in 1971 had skewed frequency distributions; comparison of the medians by Mann-Whitney U-test revealed no significant differences (3,4). A diurnal increase in liver total water had definitely appeared by the evening. Of the two independent estimates of total liver water in evening-caught moulting juveniles, the mean for birds in moult stages 1 and 2 was significantly larger than that for birds in moult stage 3 ($.01>p>.005$) (5). Both of these estimates of liver total water were significantly greater than the mean, or median level at 4-6 hours after sunrise ($p<.002$ in all cases)(6) (Table 10.7.1).

All the samples of moulted birds from the morning in 1970 were skewed, and consequently a Kruskal-Wallis one-way analysis of variance was performed on the three samples to determine whether they were all drawn from one distribution with a common median. They were not ($.01 > p > .001$) (7). The median for 4-6 hours after sunrise is significantly larger than the median for 0-2 hours (Table 10.7.1, $p=.003$)(8), and is larger than the median for 2-4 hours also ($p=.022$)(9). In 1971 the median for 3-5 hours after sunrise was larger than that for 0-2 hours, but this difference was not statistically significant (10). Probably, therefore, the increase in liver total water occurs late in the morning; the evidence from 1970 would also support this view. The median of the sample of evening-caught moulted birds was significantly larger than the median for the last morning periods in 1970 and 1971 ($p=.004$, and $p < .05$ respectively) (11), but is significantly smaller than the medians for evening-caught birds during the post-juvenile moult (12).

The differences between moulting and moulted birds in the diurnal change in liver total water, seems to depend primarily on the time at which the increases occur. In moulting birds the increase in total water weight during the first six hours of daylight is 27 mg in 1970 and 1.5 mg in 1971; in both years the increase for moulted birds is larger, 32 mg in 1970 and 10 mg in 1971. The increases in 1971 are smaller, presumably because the increase is measured over a shorter period of time. However, the size of the evening peak in liver water weight is greatest in moulting

birds; in fact examination of the means for the three evening samples strongly suggests that there is a progressive decrease in the evening weight of this component of the liver during the autumn.

Table 10.7.1. Diurnal variations in the total liver water weight of the juvenile Willow Warblers during and after the moult.

Morning		
	1970	
Hours after sunrise	Moulting	Moulted
0-2	182.5 (198-167) (10)[a]	186.4 (196.9-179.5) (22)
2-4	192.5 (207.2-184.5)(20)	196.3 (221.5-182.3) (23)
4-6	210.0 (230.2-199.6)(17)	217.5 (233.5-207.4) (26)
	1971	
0-2	191.0 (198.3-173.7)(14)	182.5 (197.5-167.0) (21)
2-3	190.5 (202.3-185.7)(14)	
3-5	192.5 (241.7-185.5)(14)	192.5 (212.7-140.1) (8)
Evening		
Moult stages 1/2	280.0 (319.7-275.3)(9)	
Moult stage 3	250.0 (295.9-247.0)(7)	
Moulted		230.0 (250.5-227.8)(27)

a. median (95% confidence limits)(sample size).In mg.

Direct references in the literature to the total water content of the liver are few. Extraction of figures from the published data of Dol'nik and Blyumental (1967) for caged juvenile Chaffinches during the autumn show a depression of the evening peak of liver water from c,620 mg in moulting birds to c, 450 mg in premigratory

Figure 10.a . Diurnal variations in the liver composition of juvenile Willow Warblers during and after the moult.

Ordinate - mg weight

Abscissa - the period of capture during the day; 0-2,2-4, and 4-6 are hours after sunrise; E - evening.

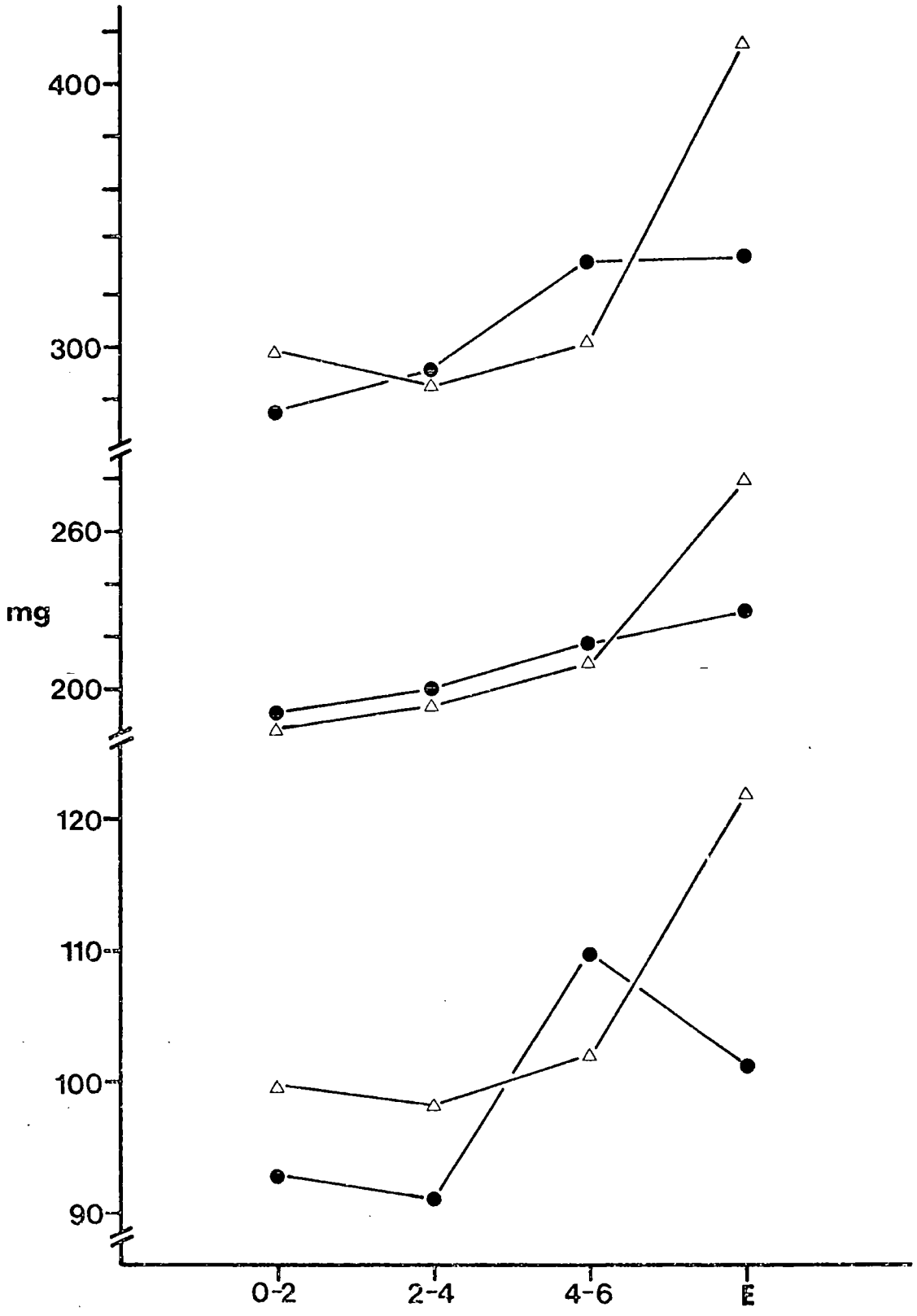
Top panel - mean total liver fat-free wet weights.

Middle panel - mean total water weights .

Bottom panel - mean total liver fat-free dry weights.

Closed circles - moulted birds.

Open triangles - moulting birds.


400
300
260
200
120
110
100
90
mg
0-2
2-4
4-6
E

birds. In the same species the morning level of liver water is also reduced from c,445 mg to c, 420 mg, but it is not clear whether this is significant. Overall they found a flattening out of the daily rhythm of liver water weight as the birds approach the migratory period, as found also in this study. Naik (1963) also found a decrease in percentage water in the liver in premigratory birds compared with non-migratory individuals shot in the morning, but this could have been due to an increase in some other liver component.

10.8 Diurnal variations in the water concentration in the liver of juvenile Willow Warblers

In 1970 all the samples examined from the morning had skewed frequency distributions, and consequently all analyses are by Mann-Whitney U-tests. As can be seen from Table 10.8.1 there are very small differences between medians for different times during the morning whether moulted or moulting birds were concerned. This is confirmed by significance tests (1). The ratio of mg water/ 100 mg fat-free dry weight is consistently larger in moulted birds, but this is not significant (2). In 1971, the frequency distributions of the samples were normal, and there were no significant differences between the means for moulting birds during the morning (3), nor were there any significant differences for moulted birds (4) (Table 10.8.1).

This ratio was larger in the evening. The median ratio for juveniles caught in the evening in moult stages 1 and 2 was not significantly different from the median ratio for birds in moult stage 3 caught at the same time of day (Table 10.8.1)(5); but both these medians were larger than those for the last morning

period in both years for moulting birds (6). The median level for moulted birds caught in the evening was, no larger than the ratio for moulting birds (7). This median ratio for moulted birds is significantly larger than that for the last morning period of moulted birds(8).

Table 10.8.1 Diurnal variations in the water concentration (mg water/100 mg fat-free dry weight of liver) in the liver of Willow Warblers during and after the post-juvenile moult.

Morning		
	1970	
Hours after sunrise	Moulting	Moulted
0-2	207.3 (213.9-206) (7)[a]	211.0 (215.2-203.8) (11)
2-4	208.5 (210.2-206.4)(14)	213.3 (224.3-207.7) (12)
4-6	208.7 (214.3-207.2)(10)	215.5 (317.3-206.4) (10)
	1971	
0-2	211.3±1.4 (15)[b]	213.4±1.4 (21)
2-3	209.8±0.6 (10)	
3-5	210.9±1.0 (11)	213.6±2.2 (8)
Evening		
Moult stages 1/2	237.3 (259.1-232.9) (9)	
Moult stage 3	255.0 (261.6-230.6) (7)	
Moulted		242.0 (255.4-234.4)(23)

a. median (95% confidence limits) (sample size) b; mean±standard error (sample size)

Since there were consistent (though not significant) differences between the values of this ratio for moulted and moulting birds, the relationship between fat-free dry weight and liver water was explored further.

10.9 The interrelation between liver water and liver fat-free dry weight during the morning

A direct plot of liver water against liver fat-free dry weight was not possible as liver water was not normally distributed. The fourth root was used to normalize the data on liver water. For both 1970 and 1971 a linear regression model was not an adequate fit to the data for moulting birds, and an analysis of variance showed that by adding a quadratic term a significant decrease in the residual mean square was achieved. Thus a curvilinear model gives the best fit for birds in moult (Table 10.9.1). By comparison, a plot of the transformed liver water against liver fat-free dry weight for moulted birds was adequately described by a linear model. This was the case in both years (Table 10.9.1). The slopes, which are the ratio of liver water (transformed) to liver fat-free dry weight, were, of course, significantly different (Table 10.9.1).

As a linear regression model adequately describes the relation between transformed liver water and fat-free dry weight, the ratio of these two parameters for moulted birds is constant, and as can be seen from Table 10.9.1 the slopes are very similar between the two years. In the case of moulting birds the curvilinear relationship means that this ratio decreases as liver fat-free dry weight increases. The reason that this is not reflected in a decrease in mean values of water concentration during the morning in moulting birds, is no doubt due to the small changes in liver fat-free dry weight (Chapter 10.11).

An alternative way to analyse the data is to plot the mg water/100mg fat-free dry weight ratio against the fat-free dry weight of the liver. A horizontal line would result when the rate of increase of

liver water paralleled the rate of increase of liver fat-free dry weight (when these parameters are, say, plotted against time). If, however, the plot has a negative slope, the amount of liver water per 100 mg of liver fat-free dry weight would be less at higher liver fat-free dry weights, and would indicate that the rate of increase of liver water (against time) was less than the rate for liver fat-free dry weight. Table 10.9.2 presents the results of such analysis for the 1970 birds. In this year the fat-free dry weight is plotted again liver water as the former is normally distributed; and as might be expected moulted birds show a regression line with no significant slope, whereas moulting birds do have a significant negative slope. A similar analysis for 1971 produces significantly negative slopes in both cases; this is presumably due to the lack of birds with high fat-free dry weights of the liver in this sample, due the the sampling regimen.

The important point is that in both 1970 and 1971 the weight of liver water for a given weight of fat-free dry liver was greater for moulted birds than for juveniles in the post-juvenile moult; this applies within the range of 80 to 140 mg liver fat-free dry weight, the weights of most livers. Thus the increase in liver weight is not due solely to increases in the weight of liver water and liver fat-free dry weight, but also to an increase in the ratio between these two.

Table 10.9.1. Regressions equations of $\sqrt[4]{\text{liver water in gm}}$ x 10^{-3}(y) against liver fat-free dry weight (gm x 10^{1}) (x).

		r	n
Moulting 1970	$y=3.39 - 0.082\ x + 0.461\ x^2$	0.999	31
Moulting 1971	$y=2.48 + 1.797\ x - 0.476\ x^2$	0.999	36
Moulted 1970	$y=2.94 + 0.773\ x + 0.088\ x^2$	0.999	33
Moulted 1971	$y=2.97 + 0.740\ x + 0.084\ x^2$	0.999	29

For the difference between the slopes 1970 $F=30$ $p<.001$; for the difference between the slopes 1971 $F=64$ $p<.001$.

Table 10.9.2 The regression of liver fat-free dry weight (mg,y) on liver water (mg) /100 mg fat-free dry weight (x) for morning-caught birds in 1970.

Moulting	$y=-1.59\ x + 427.6$	$SE_b = 0.467$	$t= 3.41$ $.01>p>.001$
Moulted	$y=-0.13\ x + 122.4$	$SE_b = 0.284$	$t= 0.45$ $.7>p>.6$

where, p-probability that the regression coefficient is significantly different from zero.

10.10 Discussion of the diurnal changes in the ratio of liver water to liver fat-free dry weight.

As was shown in the previous sections the mean levels for this ratio are higher for moulted birds than for moulting birds. These differences are not significant, but are the result of the different relationship between these two variables in samples of moulting and moulted birds. The curvilinear relation between liver water and liver fat-free dry weight means that for the range of liver weight values found in morning-caught birds, moulting birds have lower values of $\frac{mg}{}$ water/100 mg fat-free dry weight than

do moulted birds. The diurnal increases in liver lean weight found in moulted birds (Chapter 10.6) are partly a product of the increase in total water (Chapter 10.7) and partly due to increases in total fat-free dry weight (Chapter 10.11), but these two parameters do not increase in parallel. The origin of the extra water per 100 mg fat-free dry weight is not known. Possibly it is due to an increase in the blood volume of the liver, perhaps in response lipid synthesis. However, it may be due to variations in intracellular water, for, as was shown for the pectoralis muscles, such changes can occur.

10.11 Diurnal variations in liver fat-free dry weight of juvenile Willow Warblers during and after moult (Figure 10a)

The liver fat-free dry weight is that component of the dried liver from which lipid has been extracted; it closely approximates to the protein content of the liver, although this weight has not been corrected for the weight of tissue glycogen since this amounts to less than 1 mg.

In 1970 the variances of all six samples of Willow Warblers captured during the first six hours of daylight (Table 10.11.1) were homogeneous (Bartlett's test,1). Consequently a nested one-way analysis of variance was performed on the six means (Table 10.11.1). There was no significant effect due to the stage of moult, but a significant effect due to the time of capture during the morning ($.025 > p > .01$) (2). Comparison of the means, by the Studentised Range Test, for the three samples of moulting juveniles revealed that at $p = .05$ none of these means were significantly different. In

1971 the variances for the three samples of moulting birds caught during the morning are not homogeneous, and a comparison of the medians of the samples for 0-2 hours after sunrise (this sample has skew frequency distribution) and 4-6 hours, shows that the latter median is significantly larger than the former ($p=.05$) (3). However, the medians (for 0-2 hours after sunrise) and the means for 1971 do not differ significantly from those for the same morning periods in 1970 (4). Of the two independent estimates of liver fat-free dry weight of evening-caught birds, those juveniles in moult stages 1 and 2 had a significantly larger mean than the sample of birds in moult stage 3 ($p<.001$) (5). The mean of sample of birds in moult stages 1 and 2 was significantly larger than the mean for the 4-6 hours period after sunrise in both years ($p<.001$)(6). The mean for the birds caught in the evening in moult stage 3 does not differ significantly from the means for 0-2 or 4-6 hours after sunrise in 1970 (7,8).

The significant effect, due to time of capture, found in the nested analysis of variance conducted on the samples of juveniles captured in 1970, was due to mean differences between the samples of moulted birds. Comparison of the means reveals that, at $p = .05$, the mean for the sample of birds caught 4-6 hours after sunrise was significantly larger than the mean for the 0-2 hours after sunrise sample (Table 10.11.1). The mean for the sample of birds caught at 2-4 hours after sunrise did not differ significantly from either of these means. There was no significant difference between the two means for moulted birds in 1971 (Table 10.11.1), but this is to be expected since there is no

significant difference between the means for these two periods in 1970 either.

Table 10.11.1 Diurnal variations in liver fat-free dry weight of juvenile Willow Warblers during and after the moult.

Morning	1970	
Hours after sunrise	Moulting	Moulted
0-2	99.14±8.40 (7)[a]	92.73± 2.83 (11)
2-4	98.31±2.94 (15)	90.92± 3.72 (12)
4-6	102.20±3.98 (10)	109.10± 3.47 (10)
	1971	
0-2	88.7 ±2.7 (15)	88.8± 3.8 (22)
2-4	91.3± 1.3 (16)	
4-6	101.7 ±6.2 (6)	88.5± 3.7 (9)
Evening		
Moult stages 1/2	122.0± 1.9 (10)	
Moult stage 3	106.2± 2.6 (7)	
Moulted		101.3± 2.6 (24)

a. mean ± standard error (samplesize) in mg.

The mean of the sample of moulted birds caught in the evening is not significantly different from the mean for 4-6 hours after sunrise (9)(Table 10.11.1). However, this mean is significantly larger than the mean for the 2-4 hour period in 1970 (10). The mean weight for the sample of evening-caught juveniles after the moult is complete is significantly smaller than the mean weight for the sample of birds in moult stages 1 and 2 ($p < .001$) (11), but is not significantly different from the mean for birds in moult

stage 3 ($.4 > p > .3$) (12).

There are two main differences between the diurnal cycle of liver fat-free dry weight in moulting and moulted birds. First, the morning increase in premigratory birds is greater than in moulting birds (16 mg compared with 4.1 mg in 1970), and secondly the size of the evening peak in weight is reduced compared to birds during the post-juvenile moult. The weight of the fat-free dried liver, therefore, follows much the same pattern of diurnal change, as does the total water in liver.

These results differ from figures for Eastern Baltic Willow Warblers (Dol'nik and Blyumental 1967) in which, although there is an increase in the daily mean level of fat-free dry weight of the liver between moult and the autumn migration, the amplitude of daily weight variations also increases, from 10 mg per day to 50 mg. The corresponding figures from this study are 33 mg for the moult and 11 mg for premigration. My findings parallel the situation in East Baltic Chaffinches (Dol'nik and Blyumental 1967) in which the autumn migration sees the daily amplitude of variation in liver fat-free dry weight cut by two-thirds; this is also the case in the Willow Warblers examined in this study. However, over the premigratory period they demonstrated a overall decrease in mean weight of the dry fat-extracted livers of Chaffinches, but there is no such decrease in my Willow Warblers.

10.12 Diurnal variations in the total liver fat of juvenile Willow Warblers (Figure 10.6)

In 1970 the samples of moulting juveniles taken during the morning period had homogeneous variances (Bartlett's test,1). A one-way analysis applied to these three means (Table 10.12.1) showed that there was no significant effect due to the time of capture ($p > .25$) (2). Variances again were homogeneous for the three morning samples of moulting birds taken in 1971 (Bartlett's test,3), and one-way analysis of variance showed that the means didnot differ significantly ($p > .25$) (4). The means of two independent samples of liver fat in evening-caught juveniles were found not to differ significantly (5),and when combined this joint mean was significantly larger than the mean for the last morning period in either 1970 or 1971 ($p < .001$) (6,7).

In contrast all the samples of moulted birds had skewed frequency distributions, and when the medians for the three morning samples were compared by the Mann-Whitney U-test, all were found to be significantly different from each other (Table 10.12.1) (8). In 1971 only two period were sampled, and although the medians are different, they are not significantly so ($p=.23$) (9), presumably because the time period covered was shorter; the median value for 3-5 hours after sunrise in 1971 is similar to the median for 2-4 hours in 1970. Again independent samples of liver lipid levels in evening-caught juvenile Willow Warblers were available; the means did not differ ($.1 > p > .05$) (10). The mean of this combined sample was significantly larger than either the mean for 4-6 hours after sunrise in 1970, or the median for 3-5 hours in 1971 ($p < .00006$) (11). There is no difference in the medians between the two evening samples (12).

Table 10.12.1 Diurnal variations in the total liver lipid of juvenile Willow Warblers during and after the moult.

Morning					
	1970				
Hours after sunrise	Moulting		Moulted		
0-2	11.38 ± 0.60	(8)[a]	10.42	(12.47-9.39)	(11)[b]
2-4	12.26 ± 0.59	(14)	13.25	(15,17-11.42)	(13)
4-6	12.38 ± 0.99	(10)	17.00	(21.97-13.53)	(10)
	1971				
0-2	12.35 ± 0.57	(14)	12.30	(13.55-11.38)	(22)
2-3	12.60 ± 0.42	(11)			
3-5	13.56 ± 0.93	(13)	13.75	(14.75-10.36)	(8)
Evening	33.77 ± 1.46	(17)	31.00	(37.30-27.54)	(25)

a. mean ± standard error (sample size) b. median (95% confidence limits) (sample size) In mg.

In moulted birds, therefore during the morning (and in moulted birds only) the rate of lipid accretion in the liver exceeds the rate of lipid loss. It is not possible to establish from this data whether this lipid excess is dietary in origin or is synthesised de novo. However, as this period is characterized by increased food intake, and deposition of lipid in the adipose tissue, some de novo sythesis seems likely. In both moulting and moulted birds there is a period of lipid accretion in the liver during the afternoon; and this does not differ in magnitude in the two categories as the evening levels are the same. However, the enforced overnight fast reduced liver lipid to the same levels in both moulting and moulted birds. Thus any lipid synthesised in the liver during the premigratory period must be stored solely in the adipose tissue.

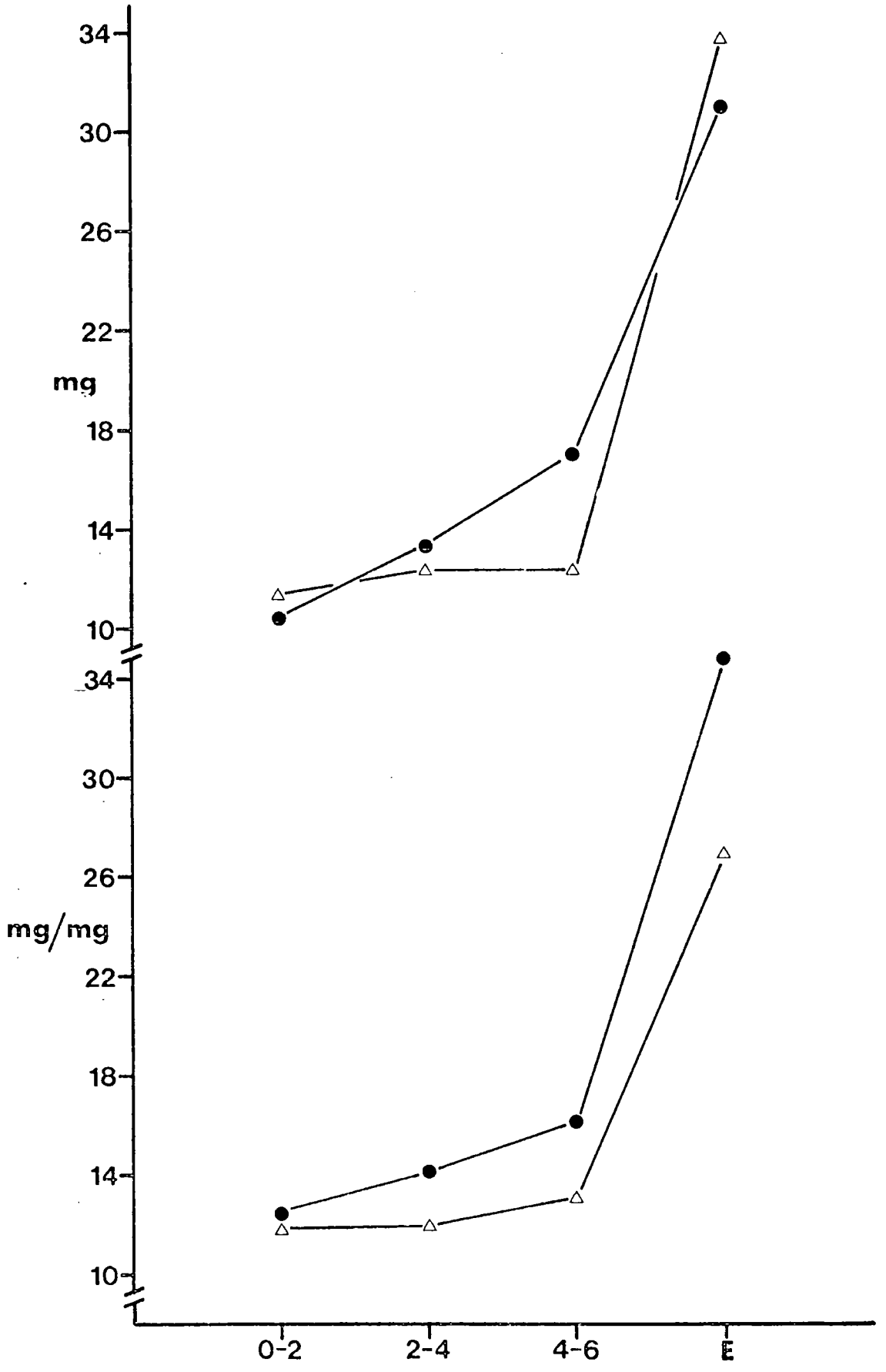
Figure 10.b . Diurnal variations in the total lipid (upper panel) and lipid concentration (lower panel) of the liver of juvenile Willow Warblers during and after the moult.

Ordinate - mg total liver lipid (upper), and mg lipid per 100mg fat-free dry weight (lower).

Abscissa is the period sampled during the day; 0-2, 2-4 and 4-6 are hours after sunrise, E - evening.

Open triangles are mean values for moulting birds, closed circles mean values for moulted juveniles.

The evening level for moulting juveniles is from the sample of birds in moult stages 1/2, the evening level for lipid concentration of moulted juveniles is from 1971; all morning mean values are from 1970.


34
30
26
22
mg
18
14
10
34
30
26
mg/mg
22
18
14
10
0-2
2-4
4-6
E

10.13 Diurnal variations in lipid concentration in liver of juvenile Willow Warblers (Figure 10.B)

The measure of lipid concentration adopted is the lipid index of other authors, mg lipid/100 mg fat-free dry weight. Often this index has been used simply as a correction for size, but implicit in many uses is that it is an approximation to the amount of lipid per cell.

In 1970 the variance of the three morning samples of moulting juveniles were homogeneous (Bartlett's test,1); consequently a one-way analysis of variance was conducted on the data (Table 10.13.1) to determine whether there were any significant effects due to time of capture. There was no such effect ($p > .25$) (2). In contrast an analysis of variance of the means for the samples of moulted birds showed that the mean of the sample for 4-6 hours after sunrise, was significantly larger than the mean of the sample captured at 0-2 hours after sunrise (Table 10.13.1) ($.05 > p > .025$)(3). Thus the mean increase in lipid concentration during the first six hours after sunrise was 1.08 mg/100mg fat-free dry weight for moulting birds, whilst the increase for moulted birds was four times greater (4.33 mg/100 mg fat-free dry weight).

In 1971 the variance of the all morning samples of moulting and moulted juveniles were homogeneous (4); a nested one-way analysis of variance found no statistical effect due to the time of capture or state of the moult ($.25 > p > .1$) (5). There was, therefore, no difference between the means of moulting birds whatever the time of capture. (Table 10.13.1). The mean levels for moulted birds were higher than for moulting juveniles (though not significantly),

and the inability of the test to detect any morning increase must be due partly to the shorter total time over which birds were captured in 1971 (5 hours), and partly to the fact that the moulted birds were collected at the beginning of the premigratory period.

Table 10.13.1 Diurnal variations in mg lipid /100 mg fat-free dry weight of livers of juvenile Willow Warblers during/after the moult.

Hours after sunrise	Moulting	Moulted
Morning		
	1970	
0-2	11.79±0.76 (7)[a]	12.06±0.73 (11)
2-4	12.03±0.57(15)	14.12±1.19 (12)
4-6	12.87±0.49(10)	16.39±1.44 (10)
	1971	
0-2	13.97±0.59(15)	14.32±0.47 (22)
2-3	13.96±0.37(10)	
3-5	13.65±0.40(11)	14.65±0.80 (8)
Evening		
Moult stages 1/2	26.85± 1.24 (10)	
Moult stage 3	34.82± 2.72 (7)	
Moulted (1970)		28.02±2.14 (16)
Moulted (1971)		34.78±2.85 (8)

a. mean ± standard error (sample size)

By evening in both moulted and moulting birds the level of lipid concentration in the liver has increased markedly. The means of the two independent samples of moulting juveniles caught in the evening are significantly larger than the means for the last morning period in either year ($p < .001$) (6), and the mean for the sample of birds in moult stages 1 and 2 is significantly smaller than the mean for juveniles in moult stage 3 ($.02 > p > .01$) (7) (Table 10.13.1).

The means of the samples of moulted birds caught in the evening are not significantly different from each other (8), but both are larger than the last morning sample for moulted birds in either year (9). Both these evening means are larger than the mean concentration for evening caught juveniles in moult stages 1/2, the sample from 1971 significantly so ($.05 > p > .01$) (10) (Table 10.13.1).

10.14 The interrelation of liver lipid and liver fat-free dry weight in juvenile Willow Warblers caught in the morning

By inspection it appears that as fat-free dry weight increases so does liver lipid, though not in the same proportion; this section analyses this relationship in more detail.

As both liver lipid weight and liver fat-free dry weight are normally distributed, a regression model can be fitted to a plot of these two variables and an analysis of variance performed. The linearity of the plot was tested by fitting a curvilinear model to the data; no significant improvement was found. The regression equations for 1970 and 1971 are presented in Table 10.14.1.

An analysis of covariance could detect no difference in the magnitude of the slopes in either year, contary to expectations. Nor was there any significant difference between the adjusted means for the 1971 data, although the sample from 1970 nearly attained significance at $p=.05(1)$, suggesting that the weight of liver lipid in moulted birds with small fat-free dry weights of the liver might be slightly greater than the amounts found in moulting birds. Of course, all the slopes were significantly different from zero (Table 10.14.1). Examination of the correlation coefficients in Table 10.14.1 shows that the percentage of variation explained by the regression was always greater for moulted birds. In 1970 it was 51.3% for moulted birds compared with 26.4% for moulting birds, and in 1971 the corresponding figures were 45.8% and 34.2%. Thus although there are no significant differences in the slopes, in 1970, when there was a fair number of large livers, this slope is larger; and in both years the percentage of variation associated with the regression was greater for moulted birds.

Table 10.14.1 Regression analyses of a plot of liver lipid (mg) against liver fat-free dry weight (mg).

Moulting		SE_b	t	df	p	r
1970	y=0.094x + 3.04	0.029	3.24	1,30	.01>p>.001	0.52
1971	y=0.136x + 0.36	0.032	4.26	1,34	p<.001	0.59
Moulted						
1970	y=0.169x - 2.50	0.029	5.83	1,31	p<.001	0.72
1971	y=0.107x + 3.22	0.022	4.95	1,28	p<.001	0.68

y is liver lipid; x is liver fat-free dry weight;

10.15 Discussion of the diurnal variations of liver lipid in juvenile Willow Warblers

Whether liver lipid expressed as a total weight for the liver or as concentration, there is greater increase with time during the first six hours after dawn in moulted birds (Figure 10.b). In 1970 this amounted to a total increase in lipid weight of 6.6 mg compared with only 1 mg for moulting birds. However, although the mean concentration of lipid in the liver (expressed as a lipid index) did increase much more in moulted juveniles than in birds during the moult, it was not possible to establish from regression analyses that these slopes of liver lipid against liver fat-free dry weight were in fact different. But the fact that moulted birds always had a larger percentage of the variation in their liver lipid weights associated with variations in liver fat-free dry weight suggests that this is the case.

The changes in liver size mask changes in evening liver lipid concentrations. There is, in fact, an increase in this parameter

for juveniles captured in the evening between the earliest moult stages and moulted birds (Figures 10.b), but the larger size of the livers found in birds at the start of the post-juvenile moult produce the same total lipid weight for these two categories. It seems, therefore, that not only is the rate of accretion of lipid by the liver greater in moulted birds during the first six hours of daylight, but this is also greater during the afternoon. In summary, the greater lipid content of the livers of moulted birds is due both to the increase in lipid/100 mg fat-free dry weight (lipid index) and to increases in liver fat-free dry weight.

Dol'nik and Blyumental (1967) compared the liver lipid levels of Willow Warblers in Estonia during the moult and during autumn migration. Birds caught during the migratoryperiod had twice as much liver lipid (20mg) as moulting birds (10 mg). Also the amplitude of daily change in lipid levels for migrating warblers was 25 mg compared with only 10 mg in moulting birds. The absolute value of liver lipid in moulting Willow Warblers examined in my study agree with the figures from these Russian populations, although the daily amplitude of change in lipid weight was twice their value (20mg). They did not discover, however, that the dawn value of liver lipid weight does not vary from moulting to premigratory birds, since they did not examine feral birds during the premigratory period. My results show that all the lipid accreted by the liver is either utilised overnight or transferred elsewhere. In captive birds, Dol'nik and Blyumental (1967)found very little variation in liver lipid of juvenile Chaffinches during the premigratory period. Published

figures hint at a slight decrease in the evening lipid level, and a slight increase in the morning level, but that is all.

During the spring migratory period of Rosy Pastors, Naik (1963) found an increase in liver lipid (as percent of liver dry weight) in the early morning. As shown above, this does not agree with the situation in Willow Warblers. Farner et.al. (1961) also found that captive White-crowned Sparrows in a migratory condition hadmore lipid in the liver at dawn, than birds kept on winter daylengths. Possibly the reason the liver lipid did not become depleted was that the birds in these experiments were kept at temperatures within the thermoneutral zone. These migratory White-crowned Sparrows did not show an enhanced increase in liver lipid during the first six hours after 'dawn'; the events described for the Willow Warblers in this study seem to be the first report of differences in the diurnal cycle of liver lipid between moulting and premigratory birds of the same species.

Table 10.15.1 summarises the published levels of mean daily liver lipid for long distance passerine migrants. It illustrates the fact, that the increase in liver lipid found in migratory finches (and the starling) is much larger (between non-migrants and migrants) than for the smaller warblers. In finches it is about double, in warblers less than this. The smallest increase is found in my study of Willow Warblers, but this is because the comparison is not with the migratory period.

Table 10.15.1 Mean liver lipid (mg/gm dry weight) in various long distance migrants.

White-crowned Sparrow		mg/gm dry wt	Reference
Captive[b]	8 hour photoperiod	168	Farner, Oksche,
	16 hour photoperiod	293	Kamemoto, King and
	20 hour photoperiod	306	Cheyney. (1961)
Wild, afternoon	Winter	130	King, Barker and
	Spring premigration	259	Farner (1963)
	Spring migration	242	
	Autumn migration	156	
Chaffinch			
Captive[b]	Moult	151	Dol'nik and
	Autumn migration	297	Blyumental (1967)
Wild[b]	Moult	192	
	Autumn migration	286	
Willow Warbler			
Wild, Kurische Nehrung[b]	Moult	130	Dol'nik and
	Autumn migration	167	Blyumental (1967)
Wild, Karelia[b]	Moult	118	Dol'nik and
	Autumn migration	190	Blyumental (1967)
Wild, Co. Durham[a]	Moult	114	This study
	Autumn premigration	141	
Garden Warbler			
Captive[b]	Moult	239	Dol'nik 1966
	Autumn migration	364	

Table 10.15.1 continued

Starling		mg/gm dry wt	Reference
Captive[b]	Moult	97	Dol'nik 1966
	Autumn migration	178	

a. based on the maximum mean value of total liver lipid for the morning period. b. daily means.

10.16 The diurnal variations in liver glycogen concentration (mg/gm wet weight) of juvenile Willow Warblers

As the sample sizes were smaller than for the other parameters measured the data for the two years were combined. The variances for the four samples of liver glycogen concentration taken during the morning (Table 10.16.1) were homogeneous (Bartlett's test,1), and a nested one-way analysis of variance showed that although there was no significant effect due to the state of moult (2), the effect due to time of capture was significant ($.05 > p > .025$) (3). Comparison of the four means by the Studentised Range Test revealed that the mean for the sample of moulting birds captured 3-5 hours after sunrise was significantly smaller than the mean for the first two hour period (at $p=.05$). There was no significant difference between the means of the two samples of moulted birds.

In the moulting juveniles the glycogen concentration increases again in the evening (Table 10.16.2). The mean glycogen concentration for birds in moult stages 1 and 2 was significantly larger than the means for both morning periods (4); and the mean concentration for birds in moult stage 1/2 caught in the evening was significantly larger than the mean for the last morning period (5). As shown in Table 10.16.2 the mean concentrations of glycogen

in the liver for moulting birds caught in the evening are larger than the means for evening-caught moulted birds. The sample of birds in moult stage 1/2 has a mean significantly larger than the mean for second sample of moulted birds (1971b) ($p<.001$) (6), and is also significantly larger than the sample of birds from moult stage 3 ($.005>p>.001$) (7). The sample of birds in moult stage 3 does not differ in mean values from the moulted birds sample from 1971(b).

Table 10.16.1 Diurnal variations in the liver glycogen concentration (mg/gm wet weight) of juvenile Willow Warblers during after after the moult.

Hours after sunrise	Moulting	Moulted
0-2	3.23±0.15 (14)[a]	3.40±0.08 (21)
3-5	2.89±0.10 (12)	3.25±0.23 (9)

a. mean ± standard error (sample size)

Table 10.16.2 Evening values of liver glycogen in relation to the state of moult in juvenile Willow Warblers.

Moult stage	mg/gm wet weight	Total weight mg
1/2	7.94±1.24 (9)[a]	3.55±0.53 (9)
3	4.11±0.53 (5)	1.66±0.20 (5)
Moulted,a (30/7)[d]	3.56±0.31 (5)	1.36±0.14 (5)
Moulted,b (6/8)[d]	3.08±0.26 (5)	1.21±0.16 (5)

a. mean ±standard error (sample size) d. date of capture

10.17 Diurnal variations in total liver glycogen of juvenile Willow Warblers

Since total liver glycogen is a function of both liver size and glycogen concentration, the distribution of values of total content might be expected to differ from the preceding section. An analysis of variance of the values of total liver glycogen was impossible as the variances were heterogeneous (Bartlett's test,1). Therefore, t-tests have been used to compare means throughout. There were no significant differences between the mean for moulting birds listed in Table 10.17.1 (2). Thus there seems to be no increases or decrease in total liver glycogen during the first five hours after dawn in moulting juveniles. The decrease in glycogen concentration found in the last of the morning periods (section 10.16) must be the 1 in 20 non-significant result expected. However, moulted birds show a decrease in total liver glycogen during the morning, but the mean for the 3-5 hours after sunrise period is not significantly smaller (3) than the mean for 0-2 hours.

Again there is an increase in liver glycogen by the evening (Table 10.16.2). The evening sample of juveniles in moult stage 3 is significantly larger than the means for 1-2 or 4-5 hours ($p < .001$) (4,5); the sample mean for the birds in moult

stages 1/2 is also significantly larger than the means for these two morning periods ($p < .001$) (6,7). As for glycogen concentrations, the mean for the sample of birds in moult stage 3, caught in the evening, is significantly larger than the mean for moulted birds (b) ($p < .001$) (8), as is the mean for juveniles in moult stages 1/2 ($p < .01$) (9). This mean for moulted birds caught in the evening (b) is not significantly different from the mean value for the sample of moulted juveniles from the first morning period (10).

Table 10.17.1 Diurnal variations in the total glycogen weight of the liver of juvenile Willow Warblers during and after the moult.

Hours after sunrise	Moulting	Hours after sunrise	Moulted
1-2	0.95±.04 (12)[a]	0-2	1.05±.06 (20)
2-3	0.92±.04 (13)		
3-4	0.82±.05 (6)	3-5	0.94±.08 (9)
4-5	0.93±.06 (6)		

a. mean ± standard error (sample size) in mg

10.18 Discussion of diurnal changes in liver glycogen during and after the moult

During the autumn in juvenile Willow Warblers the diurnal cycle of liver glycogen (whether expressed as total or concentration) discernable in moulting birds disappears. This is due to a progressive reduction in the size of the evening peak of liver glycogen. During the premigratory period, therefore, there is practically no diurnal variation in liver glycogen. Farner et.al.

(1961) found a similar difference in the diurnal cycle of liver glycogen in White-crowned Sparrows. Birds kept on an eight hour photoperiod showed a strong evening peak, whereas birds transferred to 20 hours showed no such evening peak,(the latter birds were however in a migratory state). Where Willow Warblers differ from White-crowned Sparrows is that there is no overall increase in liver glycogen during the premigratory period as found in the sparrows (Farner, Barker and King 1963). Dol'nik and Blyumental (1967) claim that in the Willow Warbler too there is an overall increase in liver glycogen between moulting birds and birds during the autumn migration. However, since the measure of liver glycogen they used was the variation in weight of the dry non-fat liver, and/as changes in this parameter exceed by over an order of magnitude the daily variations for glycogen found in my study, the weight variations they noted are probably a better indicator of changes in protein weight.

Naik (1963) found an increase in liver glycogen in Rosy Pastors at the end of the premigratory period; these birds were collected at dawn. No such increase was found in Willow Warblers. However, the situation Naik studied is probably closer to that studies on White-crowned Sparrows, as in both cases the birds had already undergone lipid deposition before the samples were taken.

10.19 Summary

Both total and lean liver weights of juvenile Willow Warblers showed no significant changes during or after the moult. However, lipid levels (mg/100 mg lean dry weight) were greater in moulted birds. For both total water and fat-free dry weight (= protein), the amplitude of daily variation was greater in moulting than moulted birds, but the latter category reach their maximum daily weight by six hours after dawn; moulting birds attained peak weights in the evening. Liver water levels (mg/100mg fat-free dry weight) were greater in moulted birds. Lipid weights in the liver increased in both groups in the afternoon, although the magnitude of this increase, on a per 100 mg fat-free dry weight basis, was greater in moulted birds, as was the increase during the morning. Liver glycogen showed diurnal variation only in moulting birds, and not in moulted, due to the disappearance of an evening peak in weight.

Chapter 11. Blood composition of Willow Warblers

11.1 Introduction

Since a number of investigators have shown that plasma glucose and plasma free fatty acids (FFA) are good indicators of changes involving carbohydrate and lipid metabolism in birds (Heald et.al. 1965, Langslow et.al. 1970), these two parameters have been measured throughout the moult and into the premigratory period in Willow Warblers.

11.2 Methods

Blood samples were either removed from unanaesthetised birds by cardiac puncture or by decapitation. When samples were obtained by cardiac puncture the syringe used was wetted with a little heparinized saline; blood samples were transferred to a centrifuge tube kept on melting ice. If decapitation was used the tube was wetted with a little heparinized saline. The same syringe, thoroughly washed was used for all sampling so that the dead volume of saline within the syringe was constant. Thus the values of plasma metabolites are slightly below the true in vivo values but by a constant amount.

The blood sample was spun for 1 minute at $4^{\circ}C$ at 2000g to separate the plasma, which was drawn off by a cooled Pasteur pipette to a tube coated with sodium fluoride (to halt glycolysis). The sample was stored at $-20^{\circ}C$ until analysis. The assays for plasma glucose and plasma free fatty acids are given in the section on General Methods (Chapter 2).

11.3 Blood glucose - summary of previous studies

Most of the information on blood glucose variations in migrant passerines comes from the studies made in Lithuania by Dol'nik and

his collective. Unfortunately, all these measurements are of total blood glucose rather than plasma glucose as used in this study. As pointed out by Bell (1957) there is practically no glucose in the erythrocytes of birds and so variations in erythrocyte volume of the blood can cause apparent changes in circulating glucose, even though the plasma level is constant. Extensive data on chickens presented by Bell (1971) shows the difference, in mg glucose per 100ml of blood (mg%), between total blood values and plasma values to be about 60 to 80 mg%. For an approximate conversion of Dol'nik's values to mg% of plasma his total blood levels should be increased by this amount.

Dol'nik and Blyumental (1967) found that the mean total blood glucose of moulting juvenile Chaffinches in captivity was greater than the mean level of the same birds during the autumn migration. Further studies of caged juveniles of this species (Dol'nik and Blyumental 1967) showed no consistent trend in blood glucose levels during the premigratory period. However, within the migratory period a sample of fat caged juvenile Chaffinches had a higher mean total blood glucose level than a sample of thin birds. Also the fat birds had little diurnal variation in blood glucose whereas the thin juveniles showed a diurnal decrease. The mean daily level of total blood glucose of these fat juveniles was 218 mg%, compared to the 147mg% of the thin birds (Dol'nik 1966). In the wild during the migratory period, feeding flocks of Chaffinches that have stopped en route contain fat birds with higher total blood glucose levels (mean 145 mg%) than thin birds (mean 116 mg%) (Dol'nik 1967). Although these flocks are not premigratory they are undergoing lipid deposition, and it could be that the reduced difference in mean levels between fat and thin birds is a reflection of this. When migrating

the fat birds in these flocks have increased blood glucose values (mean 210 mg%), while the glucose levels of thin migrants remain much the same (mean 114mg%).

Dol'nik (1968) concedes that diurnal variations in blood glucose could be due to diurnal changes in feeding activity but suggests that it is unlikely. Instead he proposes that the level of blood glucose is internally regulated to enhance appetite in both pre-migratory and migratory individuals, and further suggests that the low level of glycogen reserves found in Chaffinches in migratory periods assists this function by making less glucose available to maintain blood glucose levels. Low blood glucose during the migratory period is, he maintains, inhibitory to migratory flight, as fat birds have a morning peak of total blood glucose when they are migrating, and a lower level in the afternoon when they are eating (when levels might be expected to be elevated). Whether such generalisations can reasonably be proposed from data on total blood glucose levels will be explored in a later section.

Bergman (1950) in a study of individual Blackbirds, Fieldfares and Redwings in autumn, where the same bird was repeatedly sampled for total blood glucose, found a diurnal increase in this parameter irrespective of migratory condition; he concluded that there was no link between total blood glucose values and the migratory state of the bird. However, since repeated blood sampling can decrease the erythrocyte volume of the blood, and so increase total blood glucose levels even though plasma glucose may not alter, this might well account for lack of differences.

11.4 Variations in plasma glucose of Willow Warblers during the autumn

In both years examined the variances of the samples of morning caught juvenile Willow Warblers were homogeneous (Bartlett's test, 1,7).

In 1970 a nested one-way analysis of variance was used to test whether there was any significant effect due to stage of moult or time of capture. For this analysis birds in moult stage 2,3 and 4 were grouped together as 'moulting' and the means for the three morning periods compared with the corresponding means for moulted birds (Table 11.4.1). There was no effect due to the time of capture during the morning ($p > .25$) (2), but differences between the means could be attributed to the stage of the moult ($.01 > p > .005$) (3). The mean plasma glucose levels for moulted birds were consistently lower during the three morning periods; and the overall morning mean for moulting birds was 249 $\pm$ 9 (SE)mg% compared with 227 $\pm$ 12 (SE) mg% for the moulted juveniles. Table 11.4.1 shows that the mean increase in plasma glucose levels for moulted birds is much larger than for moulting juveniles, mainly because of the lower levels during the first two morning periods. As Table 11.4.2. shows, even when the moult stages are separated the mean level of plasma glucose for moulted birds is still the smallest of the four groups (Figure 11.4.a).

There are no significant diurnal changes in mean plasma glucose between morning and evening samples (in 1970) in either moulting or moulted birds (4), nor is there any significant difference between these means (5). Adults in moult have a larger mean plasma glucose level than moulting juveniles, though not significantly so (6).

Table 11.4.1 Diurnal variations in the plasma glucose (mg%) of juvenile Willow Warblers during and after the moult.

Morning	1970	
Hours after sunrise	Moulting	Moulted
0-2	246 ±11 (11)[a]	210 ± 19 (9)
2-4	246 ±15 (13)	223 ± 24 (8)
4-6	259 ±24 (7)	249 ± 22 (8)
Evening	234 ±11 (8)	226 ± 8 (13)
	1971	
0-2	236 ±13 (16)	243 ± 21 (9)
2-4	236 ±11 (19)	213 ± 45 (4)
4-6	247 ±13 (7)	

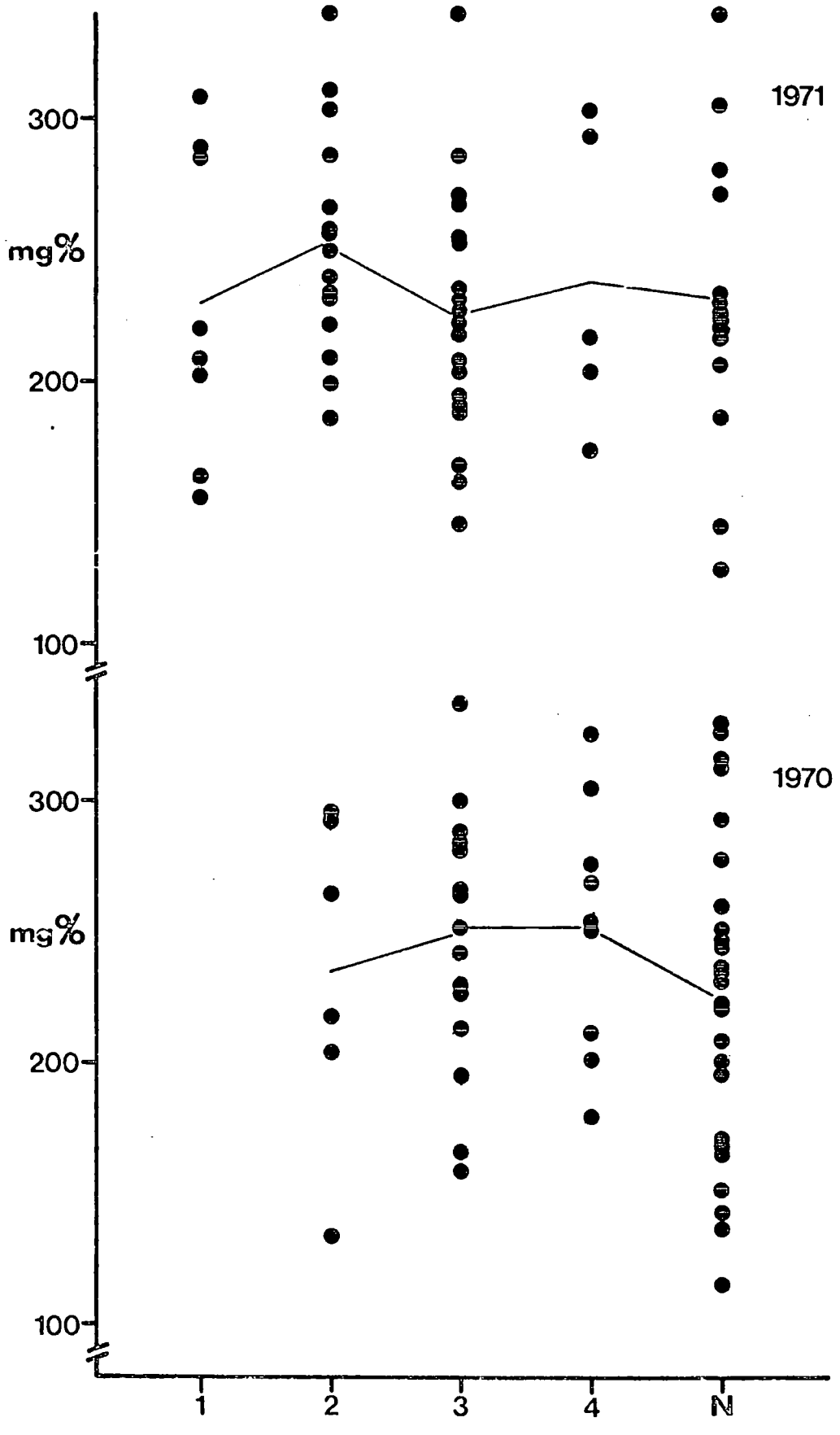
a. mean ± standard error (sample size)

Table 11.4.2 Mean morning levels of plasma glucose (mg%) of Willow Warblers during and after the moult.

Juveniles		
Moult stage	1970	1971
Unmoulted/1		229 ± 21 (8)
2	235 ± 26 (6)[a]	253 ± 11 (16)
3	252 ± 13 (16)	225 ± 10 (21)
4	252 ± 16 (9)	238 ± 25 (5)
Moulted	227 ± 12 (25)	233 ± 18 (14)
Adults		
Moulting	271 ± 15 (13)	

a. mean ± standard error (sample size)

Figure 11.4.a . Variations in the plasma glucose levels of Willow Warblers during the post-juvenile moult. Ordinate is the plasma glucose level in mg%. Abscissa the stage of moult; 1,2,3,4 - moult stages; N - moulted birds. The solid lines join mean values for the moult stages. Upper panel -1971, lower panel - 1970.


1971
300
mg%
200
100
1970
300
mg%
200
100
1
2
3
4
N

Less clear cut differences are present in the samples of juveniles caught in 1971. A nested one-way analysis of variance performed on the five samples or morning-caught birds, showed no significant effect due to stage of moult or time of capture (Table 11.4.1.) (8). Plasma glucose levels are higher for moulted birds in 1971, but this is not suprising since the birds were collected early in the pre-migratory period, however the lower values during the moult in this year are unexpected. Possibly there are interyear differences in the absolute values of plasma glucose depending on the source of nutrition.

In summary, there are apparently no diurnal variations in mean plasma glucose levels during the moult or the premigratory period; however, in one year (1970) there is a decrease in plasma glucose between moulted birds and birds in moult. Since there is insufficient data from the start of the moult, it is difficult to decide from these results whether this is a return to normal (pre-moult) from high levels during the moult, or a decrease specific to the pre-migratory period. The data from 1971 does suggest, as lower plasma glucose levels are found during the post-juvenile moult in 1971, that there is no increase in plasma glucose during the moult, and that sampling conditions are responsible for the lack of a reduction in this parameter in the premigratory sample in this year.

11.5 Plasma glucose - discussion

Nutrition does affect the levels of plasma glucose in the fowl, but to a variable extent. Fasts of 24 hours can have no effect on plasma glucose levels (Bell 1971), or it can effect a reduction (Langslow et.al. 1970) which can continue for up to 72 hours, at which

time there is an increase in gluconeogenesis and plasma glucose levels may rise (Hazelwood and Lorenz 1959, Langslow et.al.1970). Passerine birds in temperate latitudes would seldom experience such prolonged fasts, the maximum they might be expected to face would be the length of the night, (in this study about 6 to 9 hours). Thus, one would expect little reduction in plasma glucose levels overnight; indeed in Willow Warblers in this study there is none. Dol'nik (1967,1968) also found little reduction in total blood glucose levels during the night in Chaffinches in the autumn.

Since short-term fasts cannot explain the variations in plasma glucose found in this study, the possibility that feeding activity may influence these changes must be considered. Administration of large amounts of glucose either orally or intracardiacally results in large increases in plasma glucose levels in the chicken (Langslow et.al.1970), but whether food intake affects plasma glucose levels of feral birds is not known. The food intake of juvenile Willow Warblers, as measured by the dry weight of the stomach contents, does not vary much during the morning in the period of the post-juvenile moult and nor do the mean plasma glucose levels. In moulted birds a significant increase in the dry weight of the stomach contents corresponds with a small increase in mean plasma glucose levels. However, the large plasma glucose values of over 300mg% (Figure 11.4.a), must result from the effects of nutrition (since we have seen that they are not caused by fasting). But in general the effect of changes in feeding activity on this parameter is probably not great.

As in the case of juvenile Chaffinches (Dol'nik and Blyumental 1967) the highest mean blood glucose levels are found during the moult, but

in 1971 this was for only one moult stage. As explained in the previous section, there are probably interyear differences in nutrition which account for these differences. However, the possibility that the high plasma glucose levels found in 1970 during the moult are associated with increased gluconeogenesis for thermoregulation cannot be discounted. As to the suggestion by Dol'nik (1968) that low blood sugar of premigratory birds will stimulate appetite, my data do not permit any conclusions to be drawn. However, in view of the arguments presented in later sections the reverse of this relationship seems more likely; increased appetite and the resulting lipid deposition causes a decrease in plasma glucose levels.

11.6 Plasma free fatty acid (FFA) levels in birds

Non-esterified fatty acid (FFA) levels in the plasma of birds are an important indicator of metabolic changes. Nutritional status can affect the levels, as starvation for 7 hours or longer results in a rise in plasma FFA (Langslow et.al.1970), and the administration of oral or intracardiac glucose can depress this level (Langslow et.al. 1970); although lower doses of glucose administered this way may have no effect (Heald et.al.1965). Thus in general there is an inverse relationship between plasma glucose levels and plasma FFA.

However, as well as being influenced by nutritional state the plasma free fatty acids are under hormonal control, primarily by glucagon and insulin. Glucagon injections raise plasma FFA in chickens (Langslow et.al.1970), and infusions of this hormone produce the same effect in geese and ducks (Grande and Prigge 1970, Oya et.al 1971) as well as in carnivorous owls (Grande 1970a). These changes are, of course, accompanied by hyperglycaemia. Insulin injections too are effective in increasing plasma FFA in chickens (Langslow et.al.

1970, Heald 1965), but not in owls (Grande 1970). In ducks insulin does increase plasma FFA (Samols et.al.1969) and as pancreactomised individuals given insulin are unable to respond in this manner (Mialhe 1969), this confirms the suggestion of Heald et.al.(1965) that insulin produces its effect by increasing the release of glucagonfrom the pancreas. This indirect action of insulin on FFA levels is also supported by the inability of insulin to affect avian adipose tissue (Langslow and Hales 1969). This contrasts with the situation in the mammal where glucagon decreases plasma FFA probably mediated by the anti-lipolytic effect of insulin (Samols et.al.1969).

The major source of plasma FFA is the adipose tissue, and not suprisingly glucagon has a potent lipolytic effect on adipose tissue in vitro at very low concentrations both in chicken (Langslow and Hales 1969) and the pigeon (Goodridge and Ball 1965). This effect appears to be similar to the situation in mammals mediated by cyclic 3',5' -AMP, as both theophylline and dibutyryl 3',5' -(cyclic) -AMP increase lipolysis in chicken adipose tissue in vitro (Langslow and Hales 1969). Catecholamines, which are potent lipolytic agents in mammalian adipose tissue, stimulate lipolysis only slightly in birds and then only at high doses (Goodridge and Ball 1965,Langslow and Hales 1969) and are probably not important physiologically.
All these hormonal effects on adipose tissue have been demonstrated in White-crowned Sparrows (Goodridge 1964), and although the lipolytic side of migratory obesity is not understood, it does seem possible that glucagon, as a lipolytic agent, might well play a part in the production or the maintenance of migratory obesity.

Before considering the evidence from Willow Warblers during the autumn we will examine the only type of avian obesity which is now understood completely. Hypophysectomy in the fowl produces marked obesity with reduced food intake (Gibson and Nalbandov 1966b), but in spite of this large increase in lipid weight in the bird, plasma FFA differs little between control and experimental birds. Nor do the hypophysectomized birds respond to fasting by elevation of the plasma FFA levels (Gibson and Nalbandov 1966a). As shown by Gibson and Nalbandov (1966b) this is due to reduced lipid mobilisation from the adipose tissue, and is mediated by a reduction in the levels of cyclic 3',5' -AMP in the tissue (Chandrabose and Bensadoun 1971a). Hypophysectomy has no effect on the incorporation of acetate-1-C^{14} or glucose-U-C^{14} into liver lipids, but does reduce the activity of NADP-linked malic enzyme, an enzyme involved in fatty acid synthesis, (Chandrabose and Bensadoun 1971b); but in this case this effect can be reproduced by thyroidectomy, and so seems secondary. The plasma FFA levels of hypophysectomized birds can, however, be elevated by injection of chicken pituitary powder, and this is probably a direct effect as other endocrine organs (thyroid, testes) are atrophied after hypophysectomy (Gibson and Nalbandov 1966a). Pituitary fractions responsible for this effect might be ACTH or growth hormone (chicken) since both of these increase lipolysis in chicken adipose tissue (Langslow and Hales 1969). A further alternative exists therefore, that migratory obesity may be similar to hypophysectomy-induced obesity and probably due to the lack of a pituitary lipid mobilising factor.

11.7 Variations in plasma free fatty acid levels of juvenile Willow Warblers during and after the moult

In both years examined the variances of the samples of morning-caught juveniles were homogeneous (Bartlett's test,1).

For 1970 a nested one-way analysis of variance for the three morning periods for both moulted and moulting birds, showed that there were no significant effects due either to the time of capture or the stage of the moult (Table 11.7.1) (2). A single comparison t-test between the mean for moult stages 1 and 2 combined in 1970 and the mean for the sample of moulted birds in this year, showed that these were significantly different (Table 11.7.2). But as the analysis of variance showed no effect this difference must be treated with reserve. An increase in plasma FFA was undeniably present in 1970 between the morning samples and the evening samples for moult stage 4 and moulted birds; ($.01 > p > .005$ for moult stage 4, $.05 > p > .01$ for moulted birds) (4,5); as predicted this increase is accompanied by a decrease in plasma glucose.

Similarly in 1971 a nested one-way analysis of variance performed on the morning samples listed in Table 11.7.1, showed that there was no effect due to time of capture or moult stage (6).

For individual birds there was no correlation between the level of plasma FFA and the size of the adipose fat reserves (as measured by residual carcase lipid weight) within any sample. For example, using Kendall's rank correlation coefficient, the sample of moulted birds caught in the morning during 1971 had a coefficient whose probability of being different from zero was only 0.43, and two samples of birds in moult

stage 3 from different times during the morning had probabilities of .14 and .09.

Table 11.7.1. Diurnal variations in plasma FFA levels of juvenile Willow Warblers during the moult and the premigratory period.

	1970			
	hours after sunrise			
Moult stage	0-2	2-4	4-6	evening
1/2	1.33±.13 (5)[a]	1.26±.10 (6)	0.68±.14 (2)	
3	1.55±.05 (2)	1.31±.09 (5)	1.41±.12 (4)	
4	1.61±.09 (3)	1.51±.04 (5)		2.13±.36 (6)
Moulted	1.54±.10 (10)	1.61±.18 (6)	2.02±.36(6)	2.09±.21(13)
	1971			
1	1.12±.12 (6)	1.41±.11 (2)	1.51±.13 (2)	
2	1.20±.18 (4)	1.24±.23 (4)	1.61±.12 (4)	
3	1.32±.14 (9)	1.45±.16(10)	1.47±.34 (2)	
Moulted	1.38±.09(13)	1.44±.20 (7)		

a. mean in mM FFA± standard error (sample size)

Table 11.7.2 Variations in the morning levels of FFA (mM) of juvenile Willow Warblers during and after the moult.

Moult stage	1970	1971
1/2	1.20±.09 (13)[a]	
1		1.25±.09 (10)
2		1.35±.11 (12)
3	1.39±.06 (11)	1.39±.10 (21)
4	1.56±.04 (9)	
Moulted	1.69±.12 (22)	1.40±.09 (20)

a. mean ± standard error (sample size)

The lack of any variation in mean plasma FFA levels of juvenile Willow Warblers between the moult and the premigratory period would seem to suggest a possible reduction in the rate of lipolysis at the adipose tissue, since during the period the size of the lipid deposits increases enormously; such a large increase in lipid should produce an increase in plasma FFA values if the lipolysis rates at the tissue remain constant.

11.8 Variations in plasma FFA levels in Willow Warblers - discussion

In both years examined there is very little variation in the mean levels of plasma FFA of morning-caught juvenile Willow Warblers during or after the moult, so that on a total body lipid weight basis the mean plasma FFA levels decrease. Further, there is no correlation within samples between the weight of adipose lipid of an individual and the plasma FFA value. However, on a daily basis there is a significant increase in absolute plasma FFA mean values between morning and evening in birds in moult stage 4 and moulted birds; but on a lipid weight basis there is still a decrease. Whether this absolute diurnal increase in plasma FFA is due to the greater adipose lipid levels in evening-caught birds or a mobilisation in response to a specific stimulus in the evening is not known.

Since no change in mean plasma FFA levels can be demonstrated between moult and the premigratory period in spite of large increases in body lipid, it seems reasonable to suggest that a reduction in adipose tissue lipolysis rates is one of the adaptations found in premigratory fattening in passerine birds. At least it seems probable enough to make a direct demonstration worthwhile.

If we accept that there is a reduction in lipolysis at the adipose tissue in premigratory birds, we return to the possibility raised in the introduction. In this one respect premigratory birds would resemble hypophysectomized chickens, though not in any other (caloric intake for instance). In hypophysectomized chickens the lack of lipid mobilisation is probably due to a lack of pituitary lipid mobilizing factor (Gibson and Nalbandov 1966a), and this might well be the case in migratory birds. Superficially, migratory obesity does have some features in common with obesity caused by hypophysectomy, but if data were available probably similar events would be found in obesity caused by hypothalamic lesions, a condition which shares with migratory obesity an increase in caloric intake (Kuenzel and Helms 1970).

11.9 Summary

Plasma glucose levels show no significant diurnal variation. A decrease in mean (morning) plasma glucose level between moulting and premigratory birds was demonstrable in one of two years. Mean plasma FFA levels did not rise significantly between the moulting and premigratory periods, suggesting that the rate of lipolysis of the increased quantities of adipose tissue carried by premigratory birds was reduced.

Chapter 12 Body composition and flight ranges of migrating Warblers

12.1 The body compositions of migrating Willow Warblers

A sample of 24 Willow Warblers killed at Bardsey Lighthouse, North Wales, on the night of the 6/7.9.69 was analysed for the major body components of lipid, water and lean dry weight. The mean values are presented in Table 12.1.1. The juveniles differ from the adult birds in having a higher mean lipid weight, greater total body water, greater lean dry weight, and consequently a higher mean body weight. The absolute mean weight of body lipid in these migrating birds is about the same as the maximum found in evening-caught juvenile Willow Warblers on the breeding grounds in Co.Durham. However, the weight found in the Durham pre-migratory birds are at the low end of the range of lipid weights found in this sample of migrants, even though, presumably, the latter were not at the start of their journey. The lipid index of these birds at the start of their migratory journey would have been greater, but it is impossible to say by how much; although presumably they originated from North-West England or Southern Scotland.

As Table 12.1.2 illustrates there are no correlations between any of the parameters measured and total lipid weight in either juveniles or adults. There are no possible migratory adaptations as found in Grasshopper Warblers as shown in Chapter 12.8.

Table 12.1.1. The body composition of Willow Warblers killed at Bardsey Lighthouse on 6/7.9.69.

	Adults	Juveniles
Live weight	8.66 ± 0.11 [a]	9.24 ± 0.11
Total lipid wt	1.01 ± 0.06	1.20 ± 0.09
Lean weight	7.63 ± 0.14	8.01 ± 0.13
Lipid index	11.67 ± 0.76	13.01 ± 0.93
Total water wt	5.39 ± 0.14	5.79 ± 0.10
Total lean dry	1.53 ± 0.06	1.58 ± 0.05

a. mean (gms) standard error; lipid index - gms of lipid per gm body weight. Adult sample size 8; Juvenile sample size 16.

Table 12.1.2. The interrelation of the body components of Willow Warblers killed at Bardsey Lighthouse on 6/7.9.69.

	b	t	p
Juveniles (n = 16)			
Lean weight vs Total lipid	-0.477	1.40	.2>p>.1
Total water wt vs Total lipid	-0.386	1.37	.2>p>.1
Lean dry wt vs Total lipd	-0.065	0.46	p>.5
Adults (n = 8)			
Lean weight vs Total lipid	-1.09	1.29	.4>p>.2
Total water vs Total lipid	-0.98	1.17	.4>p>.2
Lean dry wt vs Total lipid	-0.09	0.23	p>.5

p-probability that regression coefficient is significantly different from zero.In gms.

12.2 The body composition of migrating Grasshopper Warblers

For comparison a sample of Grasshopper Warblers killed on the same night as the Willow Warblers was analysed for lipid, water and lean dry weight; and in addition to further examine the suggestion of

muscle hypertrophy of Fry et.al.(1972) the weights and composition of the pectoralis muscles were examined. Table 12.2.1 illustrates that, as in the case of the Willow Warblers the mean values of all the parameters examined, except pectoralis weight, are greater for the juvenile birds. Compared with juvenile Willow Warblers the lipid index is greater for juvenile Grasshopper Warblers. This might be expected if the destination was similar as the larger Grasshopper Warbler would expend more energy in flight due to its greater weight.

However, unlike the Willow Warblers, as Table 12.2.2 illustrates, there are correlations between the different parameters. Only in the case of the juveniles are these correlations significant, although the lack of correlation for adult birds may be due to the small sample available.

Table 12.2.2. shows there is a positive correlation between the lean weight of the bird and the total body lipid, and that this is due to a positive correlation between the weight of the total body water and the weight of the total body lipid. It is not due to an increase in the lean dry weight of the carcase with total body lipid. As noted by Fry et.al.(1972) for the Yellow Wagtail there is a positive correlation between the wet weight of the pectoralis muscle and the weight of body lipid. However, in Grasshopper Warblers this correlation is due to variations in the water fraction of the pectoralis muscle and not due to change in the weight of the lean dry fraction, so that the percentage of water in the muscle varies with the weight of body lipid. Nor is there any correlation between the lean weight of the body minus the lean weight of the pectoralis muscle, and the lean weight of this muscle. Not suprisingly there is a positive correlation between

the weight of water in the pectoralis muscle and the total weight of water in the body, minus the contribution from the pectoralis muscles. A small part of the pectoralis muscle wet weight increase with body lipid is due to a positive correlation between the weight of lipid in the pectoralis muscles and the total weight of lipid in the body.

Table 12.2.1. The body composition of Grasshopper Warblers killed at Bardsey Lighthouse on 6/7.9.69.

	Adults	Juveniles
Live weight	13.91 ± 0.25[a]	14.08 ± 0.27
Total lipid weight	1.80 ± 0.11	2.24 ± 0.15
Lean weight	12.04 ± 0.20	11.82 ± 0.16
Lipid index	12.94 ± 0.63	15.66 ± 0.77
Total water weight	8.32 ± 0.19	8.43 ± 0.16
Total lean dry weight	2.73 ± 0.06	2.66 ± 0.06
Pectoralis muscle wet weight	1.54 ± 0.05	1.45 ± 0.03

a- mean (gms)±standard error; Lipid index - gm lipid per gm wet weight. Adult sample size 8, juvenile sample size 21.

So though like Yellow Wagtails, Grasshopper Warblers have a positive correlation between pectoralis wet weight and total body lipid, the composition of the muscle is not constant (Fry et al.1972), and the weight changes are due to a larger water fraction in the birds with greater lipid weights. The significance of these changes in the water fraction of the body will be discussed later.

Table 12.2.2 The interrelations of body components of Grasshopper Warblers killed at Bardsey Lighthouse on 6/7.9.69.

	b	t	p	n
Juveniles				
Lean weight vs Total lipid wt	0.600	2.86	.05>p>.01	21
Total water wt vs Total lipid wt	0.635	3.26	.005>p>.001	21
Lean dry wt vs Total lipid wt	-0.003	0.03	p>.5	21
Pectoralis muscle wet vs lipid wt.	0.091	2.51	.025>p>.01	21
Pectoralis muscle water wt vs total lipid weight	0.062	2.35	.05>p>.025	21
Pectoralis lean dry weight vs total lipid weight	0.015	1.49	.2>p>.1	16
Pectoralis muscle water wt vs residual body water wt	0.063	2.38	.05>p>.025	21
Pectoralis lean dry wt vs residual lean dry body wt	0.029	2.04	.1>p>.05	16
Pectoralis total lipid (mg) vs total lipid wt	12.72	5.80	p<.001	16
Adults				
Lean weight vs total lipid wt	0.926	1.49	.2>p>.1	8
Total water wt vs total lipid wt	0.913	1.63	.2>p>.1	8
Lean dry body wt vs total lipid weight	-0.065	0.28	p>.5	8
Pectoralis wet wt vs total lipid wt	0.112	0.63	p>.5	8

p-probability that the regression coefficient is significantly different from zero; all variables are in gms except where specified.

12.3 Flight ranges of migratory birds

The flight range is defined as the distance which an individual bird could fly in still air if all the extractable body lipid were used as an energy source for that flight. Odum (1960b) assumed that 0.5 gm of lipid was not metabolizable, but in the species examined in this chapter there is no evidence that this is the case. In Willow Warblers the weight of body lipid during the post-juvenile moult is about 0.2 gm, and it might be argued that this represents the non-metabolizable portion of the extractable lipid. But as Rogers and Odum (1966) have shown that immediate postmigrants have no lipid, or indeed have used body protein (probably from the breast muscles) at the end of the journey, all extractable lipid has been regarded as available for metabolism in subsequent calculations.

The calculation of flight ranges is based primarily on the formulae given by Pennycuick(1969), who provides an extensive theoretical framework from which flight performance of individual birds can be estimated. However, as there is some disagreement as to the precision of these estimates; for comparison estimates of flight ranges have also been calculated from the formulae given by Tucker (1971) and the method of Odum (1960b). In the sections immediately following reference to any of these three names will refer to these papers. The International System of units has been used throughout the following calculations.

Three basic assumptions are involved in the estimation of still air flight ranges by the method of Pennycuick.

a. the bird is considered to be flying at maximum range speed, that is the speed at which the distance travelled per unit work done is maximised. This is not the airspeed at which the energetic cost of flight is least.

b. the efficiency of conversion of chemical energy (from lipid) to mechanical work is 20%.

c. the power needed to overcome the profile drag is twice the power needed to overcome the parasite drag and induced drag combined, (this follows Pennycuick who measured this value for the pigeon). There is no evidence as to the value of this ratio in small birds, and alternative ratios and their effect on estimated flight ranges will be considered later.

The range is calculated from Pennycuick's equation (48)

$$\text{Range (in meters)} = (K/g).(L/D)\text{eff}. \log_e(W1/W2)$$

The constants in this equation are;

K - 7.6×10^6 joules/kg of lipid. This is the mechanical energy available from the extractable lipid using Johnston's (1970) value of 3.80×10^7 joules/kg for the energy content of lipid in migratory birds.

g - accelaration due to gravity, 9.81 m sec^{-2}

The variables are:

(L/D)eff - this is a dimensionless quantity, the effective lift:drag ratio. As shown by Pennycuick the ultimate value of the lift:drag ratio, that obtainable by a perfect bird, is equal to $\sqrt{S_d/A}$, where S_d is the disk area and is equal to $1/4.\pi.b^2$ (b being the wingspan in meters); and A is the equivalent flat plate area. A is calculated from Pennycuick's formula (6) as $A=.00155(W/4)^{2/3}m^2$, where W is the weight of the bird in Newtons. The weight used in these calculations is the mean weight for the sample, (of adults or juveniles).

As shown by Pennycuick the effective lift:drag ratio will increase as lipid is utilised, due to a decrease in A. For Willow Warblers this increase is only about 4%, and consequently an average value of W and hence A and (L/D)eff has been used. The maximum lift:drag ratio attainable by a real bird is at the maximum range speed, and with a profile power ratio of two it is 0.42 (the ultimate lift:drag ratio). It is this value (L/D)maximum, which is the (L/D)eff used in the formula.

W1 and W2 are the starting and finishing weights of the bird on its migratory flight. W2 is calculated by subtracting the weight of extractable lipid from the starting weight.

Tucker has developed a method of estimating flight ranges of migratory birds based on starting weight and percentage of body lipid. It is based on estimates of the cost of flying in both insects and birds by indirect calorimetry (Tucker 1970), and a least squares regression fitted to this data enables the prediction of the energetic cost of flying for any bird. The equation for the prediction of still air range is:

$$\text{Range (meters)} = (\%\text{lipid in body}).(7.46).10^{4}.m^{0.227}$$

where m is the initial mass of the birds in kilograms.

The third method of estimating flight ranges of migratory birds is due to Odum and involves the estimation or measurement of the standard metabolic rate of the bird. In the following sections this has been estimated from the equation relating body weight and standard metabolic rate in passerine birds (Lasiewski and Dawson 1967). Odum

assumed that the energy requirements of migratory flight were six times the standard metabolic rate; this relation has been used in the following sections. However, LeFebvre (1964) estimated that this ratio was from 6 to 10, and Tucker (1968) found that in the budgerigar this ratio was 12.8. Observing that Pennycuick's (1969) theory does not fit too well with measurements by indirect calorimetry in the budgerigar and laughing gull, Tucker (1973) attempted to modify this theory to fit the observed data. Basically Pennycuick's (1969) theory agrees fairly well with known performances of pigeon-sized birds at medium airspeeds; but Tucker suggests that it underestimates the power consumption of small birds and overestimates the energy consumption of large ones. The underestimation of the power consumption of small birds is, he suggests, due in part to a low estimate of induced and parasite power, and partly due to Pennycuick's neglect of the cost of basal metabolism, ventilation and heart pumping. With a larger proportion of the total power consumed being accounted for by the processes just mentioned, the ratio of the power needed to overcome profile drag to the power needed to meet other forms of drag and energy consumption is nearer one than two. Whether the species studied by Tucker (1968,1972) are typical remains to be seen, if not they can hardly be used to improve the theory of Pennycuick (1969).

12.4 Flight times of migratory birds

The flight time is defined as the time it would take a migratory bird to cover the estimated still-air range. As the airspread of a bird varies with weight$^{\frac{1}{2}}$, the maximum range speed of the bird will decrease as the lipid is utilised. This is taken into account in the formula for flight time developed from Pennycuick's (1969) formulae (48) and (13); for the derivation of the following formula see Appendix 3.

$$t(\text{flight time, secs}) = 2K''/a.\ w_o^{\frac{1}{2}}\ (1 - e^{\text{range}/2K''})$$

where,

$K'' = K/g.(L/D)eff$

and $a = 1/\ p^{\frac{1}{2}}A^{\frac{1}{4}}S_d^{\frac{1}{4}}$, where p is the air density at 0.915 Km above sea level, $1.12 \times 10^{-6} Kg/cm^3$

and w_o - starting weight

No other method of estimating flight times has been attempted.

12.5 Flight ranges of migrating Willow Warblers

The estimated still-air flight ranges of juvenile and adult Willow Warblers killed at Bardsey Lighthouse on the night of 6/7.9.69 arepresented in Figure 12.A All these ranges are calculated by Pennycuick's method. In both adults and juveniles the mode is 500-600 km, in spite of the lower lipid index of the adults; but this is compensated for by the lower weights of all components of the adult Willow Warbler carcase. As Figure 12.5.a shows, the scatter of these ranges, assuming a southerly route from Bardsey, would fall about a mean (large open circle Figure 12.5.a) in Brittany. I have placed these termini along the west coast of France simply because this is the area from which most ringed birds have been recovered; of course estimated flight ranges give no information as to the direction of migration. As shown by Spencer (1972) 16 Willow Warblers were recovered in the Loire region of France up to 1970 compared with only 5 from the north coast of Brittany. There seems therefore to be a discrepancy between the estimated flight ranges of these birds and their known halting places. This raises the question of the accuracy of these estimates.

As explained in the previous sections flight ranges calculated by Tucker's (1971) method are smaller than those calculated from the theory of Pennycuick (1969). Using the mean values of starting weight (at Bardsey) and percentage lipid in the body for juvenile birds the mean range estimated from Tucker's method is 332 km, compared with 602 km calculated by Pennycuick's method. Thus by the former calculations most birds would terminate their journey in the English Channel. Tucker (1971) does, however, admit that his estimates cannot account for known migratory performances, and estimates that his formula might be out by a factor of 1.5 for individual birds.

Estimates based on Odum's (1960b) method do however agree with Tucker's estimates. Lasiewski and Dawson's (1967) equation relating standard metabolic rate and body weight gives a standard metabolic rate for a juvenile Willow Warbler of the mean weight found in this sample as 7.57×10^2 joules/hr. Thus the energy consumption of flight would be, according to Odum, 45.4×10^2 joules/hr, which with mean lipid reserves of 4.58×10^4 joules, a flight of 10.1 hours could be managed. At the maximum range speed calculated from Pennycuick's (1969) formula (13) of 6.9 m/sec the mean range would be 252 km: even at 10 m/sec, an airspeed considered more realistic by some (Odum 1960b, Tucker 1971), the range would still be only 364 km. At the moment it is impossible to decide which estimate is correct; but two alternatives are plainly present, either the birds terminate their migratory flight in southern England, or the Loire region of France. If, however, the profile power ratio of Willow Warblers is less than two

Figure 12.A . Estimated still-air ranges of Willow Warblers (upper two histograms) and Grasshopper Warblers (lower two histograms) calculated from the method of Pennycuick (1969). The ordinate is the number of birds; ads - adults, juvs - juveniles.
The figures on which the histograms are based are given in Appendix 2.

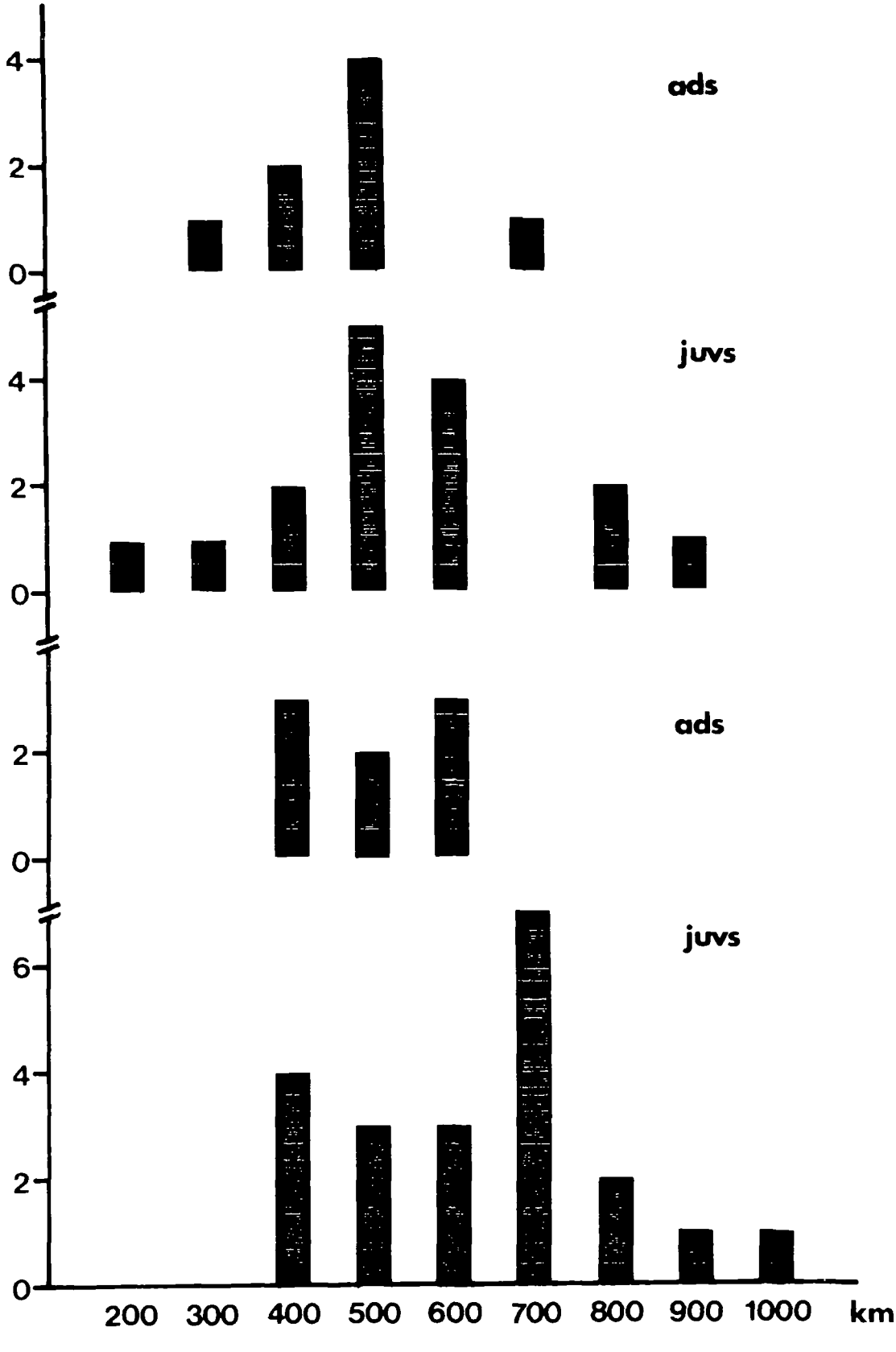
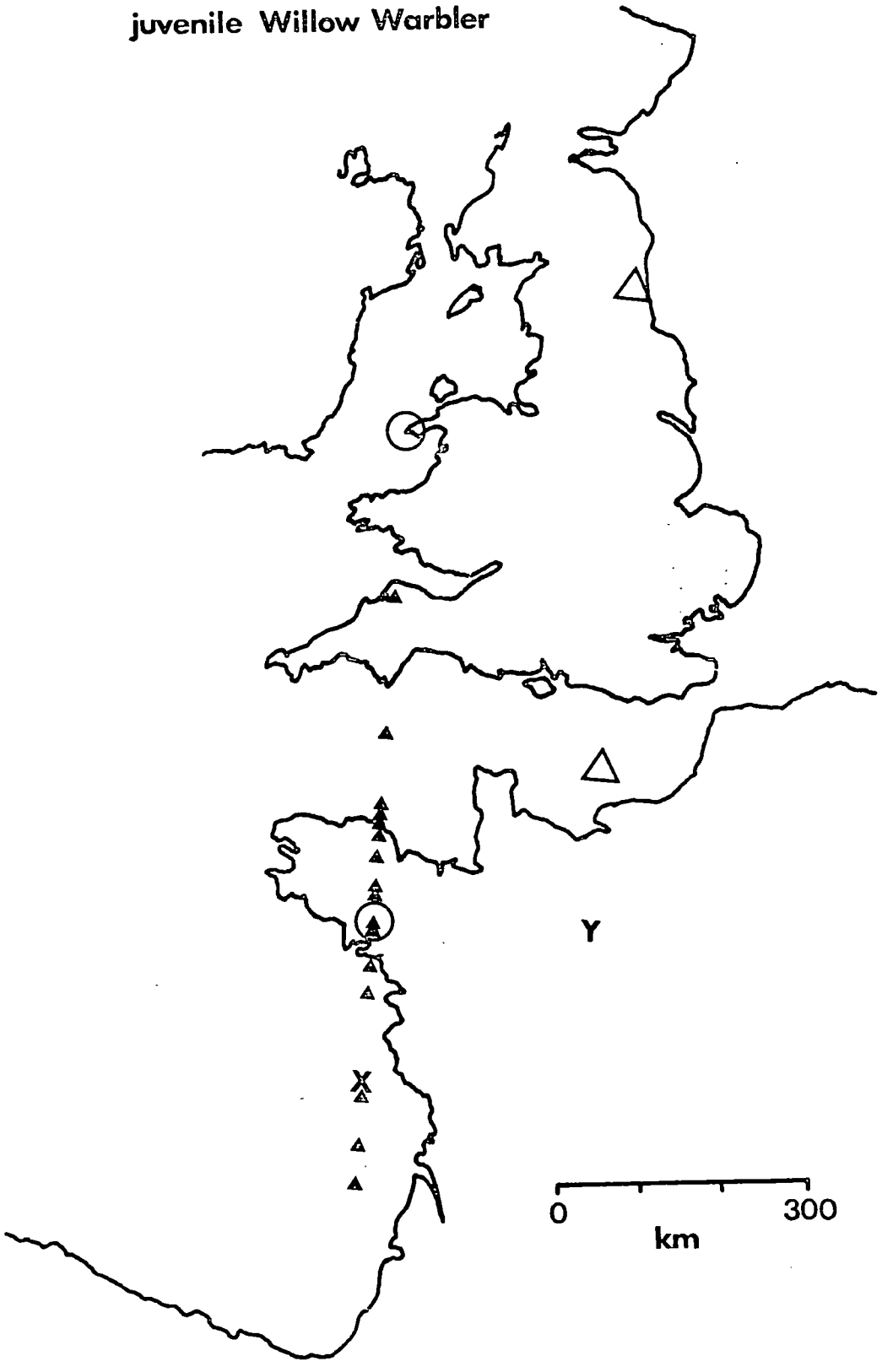

ads
juvs
ads
juvs
4
2
0
4
2
0
2
0
6
4
2
0
200
300
400
500
600
700
800
900
1000
km

Figure 12.5.a . The estimated geographical termini of the migratory flights of juvenile Willow Warblers killed at Bardsey Lighthouse on 6/7.9.69. Ranges are calculated by the method of Pennycuick (1969). The large open circle in Wales marks Bardsey Island, and the same symbol in France is the mean range of the sample assuming a profile power ratio of two; the X marks the mean range if the profile power ratio is one. The large open triangles mark the breeding grounds in Co. Durham and the mean still-air range if a profile power ratio of two is assumed; Y is the mean range if a profile power ratio of one is assumed.


juvenile Willow Warbler
Y
0
300
km

all birds would easily reach the Loire. The X in Figure 12.5.a. marks the mean range of the sample of juvenile Willow Warblers if the profile power ratio is one. It is possible, on the other hand that birds migrate with lipid loads insufficient for the journey contemplated and rely on tailwinds as, in these, birds may well decrease their airspeed, so increasing their range (Tucker and Schmidt-Koenig 1971, Bellrose 1967, Schnell 1965). However, they do not always set out with tailwinds.

Juvenile Willow Warblers departing from their breeding grounds in Co.Durham have a little less lipid than the migrating sample killed at Bardsey, and the birds in that sample were probably not at the beginning of their journey. The fattest birds caught in the evening in Co.Durham had 1 to 1.4 gms of lipid, and even assuming a profile power ratio of one the mean range would be about 800 km. As Figure 12.5.a. shows (Y) this would barely place them in northern France assuming a southerly exit from the country. As shown by Spencer (1972) and Lack and Eastwood (1962), the autumn exodus is in a south-south easterly direction in Britain, and with a profile power ratio of two this would place most of these birds along the south coast of England. Ranges estimated by the methods of Tucker (1971) or Odum (1960b) would also place these birds in southern England. There seems little doubt therefore that juvenile Willow Warblers migrating from northern England in the autumn only fly to southern England, where presumably they fatten up again.

12.6 Flight ranges of migrating Grasshopper Warblers

The estimated still-air flight ranges of juvenile and adult Grasshopper Warblers killed at Bardsey Lighthouse on the night of 6/7.9.69 were calculated by Pennycuick's (1969) formulae for comparison with the sample of Willow Warblers. As shown in Figure 12.a the frequency distribution of flight ranges for the juvenile birds is bimodal; with

modes at 400-500 km and 700-800 km. Whilst adults have ranges between 400 and 700 km with no peak at 700-800 km. The maximum ranges of juvenile Grasshopper Warblers estimated in this way are 1000 to 1100 km, and there are none below 400 km. Thus in spite of being larger birds the greater lipid index ensures that the still air ranges are greater, such that most birds would easily reach the Loire area of France (Figure 12.6.a) assuming that they head for the western seaboard of France. Ringing recoveries are no help in deciding the probable destination of these birds as only 8 had been recovered up to 1970 (Spencer 1972).

Estimates of the flight range using the method of Tucker (1971) and using mean values from the sample of juveniles for starting weight and lipid content produce again a smaller range compared with Pennycuick's method; 445 km against 681 km. Odum's (1960) method produces an estimate of mean flight range closer to the estimate from Tucker's method than Pennycuick's. The standard metabolic rate for a juvenile Grasshopper Warbler of the mean weightof the sample is 1.05×10^3 joules/hr (from Lasiewski and Dawson 1967). As the energy cost of flight is taken to be six times this the mean range of this hypothetical juvenile, travelling at maximum range speed (6.9 m/sec calculated from Pennycuick's (1969) formula (13)), is 340 km; even at 10 m/sec, a speed closer to that used by Odum (1960b) in his studies the range is still only 490 km. Thus as in the case of the migrating Willow Warblers Odum's estimates agree more closely with Tucker's than Pennycuick's.

12.7 Flight times of migrating Willow Warblers

As shown in Figure 12 B the mode of flight times for both adult and juvenile birds is 20-24 hours, with a maximum of 32-34 hours in two juveniles. However, if Odum's method of calculation of the flight ranges are used the flight time would be only 10 hours. Thus

Figure 12.B . Estimated still-air flight times of Willow Warblers (upper two histograms) and Grasshopper Warblers (lower two histograms) calculated from the formula derived in Appendix 3. The ordinate is the number of birds; ads -adults, juvs - juveniles. The figures on which these histograms are based are given in Appendix 2.

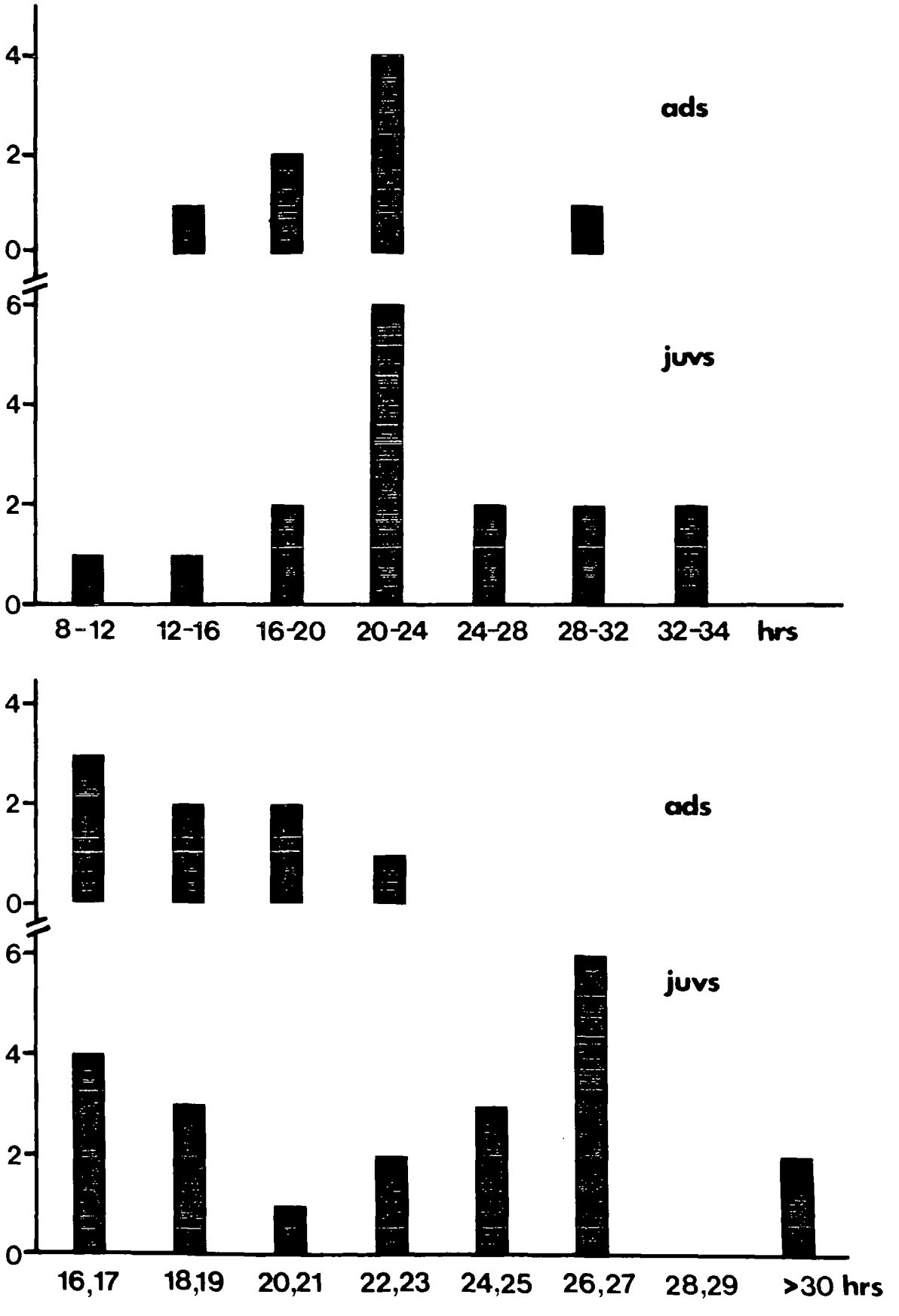

4
2
0
ads
6
4
2
0
juvs
8-12
12-16
16-20
20-24
24-28
28-32
32-34
hrs
4
2
0
ads
6
4
2
0
juvs
16,17
18,19
20,21
22,23
24,25
26,27
28,29
>30 hrs

Figure 12.6.a . The estimated geographical termini of the migratory flights of juvenile Grasshopper Warblers killed at Bardsey Lighthouse on 6/7.9.69. Ranges are calculated by the method of Pennycuick (1969). The large open circle in Wales marks Bardsey Island, and the same symbol in France is the mean range of the sample assuming a profile power ratio of two; the X marks the mean range if a profile power ratio of one is assumed.

juvenile Grasshopper Warbler

according to the estimates the birds would finish their journey the evening of the following day, whereas on Odum's estimates, they could fly only until the morning. It does seem strange that the arrival times should be in the evening, though of course the birds do not necessarily fly to exhaustion of their lipid reserves.

It is worth pointing out that the flight times would be much longer if the increase in body lipid in these birds was not reflected in an increase in body weight; heavier birds fly faster, but of course have a smaller range. As shown in Chapter 12.1 there are no correlations between the body components of this sample of migrating Willow Warblers, so that body weight increases solely reflect the amount of lipid deposited.

12.8 Flight times of migrating Grasshopper Warblers

As might be expected the flight times of juveniles show a bimodality as do the flight ranges (Figure 12.B); with modes at 16 to 17 hours and 26 to 27 hours. The adults show a range of values from 16 to 23 hours. What is remarkable is that these birds which have much larger ranges than the sample of Willow Warblers have much the same flight times. In fact the maximum flight range calculated for the Grasshopper Warblers is 1030 km with a flight time of 32 hours, while the largest range of a Willow Warbler is 927 km, with a flight time of 35 hours. Part of this reduction in flight time is due to Grasshopper Warblers being larger birds anyway, but most is due to the positive correlation between body water and total lipid weight. If there was no such correlation there would be only minor gains in range (as heavier birds consume more power), but considerable increases in flight times as shown below:

Juveniles	Mean range	Mean flight time
Lean weight remains constant at mean weight for sample	757 km	30 hrs
Lean weight is correlated with total lipid weight	681 km	24 hrs
Difference	76 km	6 hrs

Thus there would be a gain in range of only about 11%, but an increase in flight time of a quarter.

Whether these estimates based on Pennycuick's (1969) theory are correct matters little, for irrespective of the magnitude of the saving, a lipid correlated increase in lean body weight will reduce the time needed for completion of the still-air range.

As noted for Willow Warblers the flight times calculated by Pennycuick's (1969) theory, require the bird to fly the full still-air range; this would result in arrival times either the following evening or the morning after that. However, such an estimate requires information on the time at which the samples were captured; I have none. Such calculations do show that for the birds to reap benefit of still-air range and the time savings outlined, they would have to fly non-stop, that is day and night. Again there is no information on this point.

12.9 The state of moult of migrating Warblers

A number of authors have suggested that moult and migration are temporally distinct processes, and that juveniles born to late broods of migratory species accelerate their moult accordingly (Baggott 1970, Gwinner 1970, Snow 1969). A few juvenile Willow Warblers, and most of the adults still had feathers incompletely

grown in these samples of migrating birds. However, most of the Grasshopper Warblers showed some incompletely grown feathers. All of the eight adults examined had the tail coverts and ventral tract containing incompletely grown feathers, and five had some of the tertials not fully grown. Nine out of the twenty-one juveniles examined had some incompletely grown feathers on the head, breast or belly. Thus, it would seem that in this species anyway moult is not terminated before migration, and the smaller ranges found for the adults sample are associated with a greater degree of moult.

Since total body water is elevated in passerine birds during the moult (Evans 1969, Newton 1968),it is possible that the correlation between total body lipid and total body water seen in this species is an extension of this phenomenon found during the moult.

12.10 Summary

Flight ranges and times are estimated for samples of migrating Willow and Grasshopper Warblers killed at Bardsey Lighthouse,North Wales. Grasshopper Warblers have longer ranges than Willow Warblers (as they have a greater lipid index), but both species have approximately the same flight times. This is due to a lipid-correlated increase in total body water in Grasshopper Warblers, which reduces the time needed for completion of the estimated range.

Chapter 13 Changes in appetite and body composition of Bramblings given injections of prolactin or stimulatory photoperiods.

13.1 Introduction

Subcutaneous or intramuscular injections of mammalian prolactin can produce fattening in migratory birds which is similar in magnitude to that found in the wild (Meier and Davis 1967, Meier and Farner 1964), but it has not been established that this is a true migratory obesity (which requires both hyperphagia and lipid deposition), rather than the form of obesity which can be produced by hypophysectomy in chickens (Gibson and Nalbandov 1966) in which lipid mobilization is impaired, and food intake not altered. The following experiments were designed to distinguish these possibilities, to compare photoperiodically-induced fattening and hormone-induced fattening, and to examine the role of the liver in these.

In these experiments gross energy intake has been measured rather than net energy intake, as the former gives a better measure of appetite. Variations in the extent of lipid deposition have been examined by measuring weights of fat 'pads', total body lipid, and total body weight. In addition changes in liver composition have also been examined.

13.2 Methods

Bramblings were captured from feeding flocks during February 1972 and held two to a cage on an eight-hour photoperiod (8L:16D) (lights on at 0830 GMT) at outdoor temperatures. All birds were provided with food and water ad libitum throughout their captivity. The seed mixture supplied consisted principally of millet, rape, linseed

hemp, and niger; the last three being the seeds usually consumed. For the experiments the birds were transferred from the stock room to individual cages in another room. The photoperiod in this room was adjustable and the temperature was maintained in the birds' thermoneutral zone, as a thermostat prevented the air temperature dropping below 20°C; the room temperature was usually within 2 to 4°C above this minimum. Birds were acclimatized to the new conditions in the experimental room for one week before measurements were commenced.

Cages were designed to retain all spilled food and excreta, and the floor was covered with foil that was changed daily. Cages were emptied of their contents at the end of each photoperiod, and at the same time birds were weighed to the nearest 0.5 gm. The birds were weighed at this time, rather than at the start of the photoperiod, as this gives a better measure of the effectiveness of a treatment in producing weight changes on a particular day of the experiment. However, this method does mean that the body weight includes the weight of any undigested food in the stomach; but only if the feeding pattern of control and experimental birds is different will this be of importance.

Excreta was separated from spilled food by hand sorting and the spilled seed weighed to 0.1 gm. This weight, subtracted from the weight of seed supplied the previous day gave a measure of the food intake.

In the experiments involving the administration of exogenous hormone, ovine prolactin (Sigma) was injected at 7.5 or 10 IU per day dissolved

in 40 µl of a solution consisting of 3.6% 0.1 N NaOH in 0.9% saline. Controls were given 40 µl of this saline solution without any of the hormone. The injections were subcutaneous on the leg.

Birds were killed two hours after the start of the photoperiod on the day after the last treatment.

In the experiment involving a simulated photoperiod of 14 hours (8L:6D:1L:9D) (as a light-pulse) the food and excreta were collected at the end of the first eight-hour light period; on four occasions the amount of seed eaten during the one hour light pulse was measured, but this amounted at most to 0.5 gm, and usually was negligible.

13.3. Analysis of data

The data for food intake are presented both as running means for two days at a time, and as cumulative weight of seed eaten day by day. In the latter case a least squares regression line has been calculated for the cumulative weights prior to the start of the treatments. The extrapolation of this line into the treatment period provides a prediction of food intake for any of the days during this period. The data on body weights have been presented for each day of the experiments. All statistical comparisons are made by the Mann-Whitney two sample-test, and the probabilities cited are two-tailed.

13.4 Testicular condition. (Table 13.4.1)

Testes size, mean seminiferous tubule diameters and histological state were examined, firstly to check that the controls were remaining in a photosensitive state over the long period of captivity, and secondly to determine whether prolactin had any effect. The former

was particularly important since exogenous gonadotropins are known to synergise with prolactin to produce lipid deposition (Meier and Farner 1964). The mean tubule diameters were calculated from a sample of about 10 tubules which were circular in cross-section; where possible serial sections from widely separated parts of the testes were used.

Table 13.4.1 Testes size and seminiferous tubule diameter of male Bramblings

Bird	Gonad diameter (mm)	Tubule diameter (μ)
Experiment 1		
Controls		
1C	0.60	48.9 ± 3.9[a] (11)
Experimentals		
1E	0.70	53.4 ± 1.9 (16)
1F	0.74	54.5 ± 5.2 (11)
1H	0.62	62.8 ± 4.8 (12)
1I	0.56	48.1 ± 1.7 (10)
Experiment 2		
Controls		
2B	1.20	62.4 ± 5.4 (13)
Experimentals		
2H	0.96	50.9 ± 5.2 (17)
2I	0.72	45.7 ± 4.5 (18)
Experiment 3		
Controls		
3A	1.52	54.2 ± 3.4 (10)
3B	1.20	58.1 ± 7.1 (10)
3L	0.88	48.1 ± 7.2 (6)
3J	1.04	75.4 ± 5.8 (10)

Table 13.4.1 continued

Bird	Gonad diameter (mm)	Tubule diameter (μ)
Experimentals, ad libitum food		
3D	1.44	43.8 ± 6.1 (10)
3E	0.80	44.2 ± 4.1 (8)
3F	0.80	42.3 ± 3.6 (7)
3G	1.60	42.3 ± 4.7 (5)
Experimentals, food restricted		
3C	1.12	75.6 ± 9.6 (9)
3H	0.96	-
3I	0.88	-
3K	1.00	63.3 ± 6.1 (11)
Experiment 4		
4C	3.4	363.0 ± 36.9 (10)
4D	1.6	92.7 ± 12.8 (11)
4E	2.5	244.9 ± 32.4 (12)

a. mean ± standard deviation (sample size)

13.5 Experiment 1: The effect of prolactin injections four hours after the start of the photoperiod.

Although mammalian prolactin administered to migratory birds can produce lipid deposition of comparable magnitude to that found in photoperiodically-induced fattening (Meier and Farner 1964), whether this response is expressed depends on the time of injection of the hormone within the daily photoperiod. Meier and Davis (1967) found that prolactin administered early in the photoperiod (16L:8D) to photorefractory birds produced a decrease in mean body weight relative

to the controls, whereas injections late in the photoperiod produced an increase. These weight changes were due,at least in part, to changes in body lipid weight, though body compòsiton was not determined quantitatively. Subsequently, Meier and Martin (1971) have proposed that this diurnal response is due to temporal synergism with corticosterone.

My first experiment was designed (together with the next one) to test whether such an effect was detectable in photosensitive Bramblings, and to establish what carcase components might be responsible for the weight changes. Additionally, food intake was monitored to elucidate what role appetite played in any effect.

Birds were kept on 8L:16D, and the experiment was started on 10/3/72. Injections of prolactin at 7.5 IU per day lasted for 10 days.

13.6 Experiment 1 - food intake

As shown in Figure 13.6. a the running means of seed consumed by control birds were unaffected by injections of saline solution. This is confirmed by reference to Figure 13.6.b, which shows that the observed cumulative weights of seed eaten by the control birds during the period of saline injections did not differ much from the weights predicted: in fact, if anything the observed weights are less than predicted. That saline injections had no effect on food consumption is further confirmed by bird 1B, whose food intake was decreasing before saline injections began, but these injections did not prevent the weight of food eaten recovering subsequently to previous levels.

In contrast those birds injected with ovine prolactin showed an increase in the running means of seed eaten during the injection period (Figure 13.6.A.). This is seen more clearly in Figure 13.6.C

where in four out of five birds the cumulative weights of seed eaten exceeded the predicted amounts by the end of the experiment. However, in these birds the increase in food intake is not detectable until 3 to 4 days after the first hormone injection. Bird IF is anomalous in that food intake was erratic and decreasing before injections began (Figure 13.6 A), but again food intake did not increase until the fourth day of prolactin injections: this supports the observation that prolactin injected subcutaneously is ineffective in increasing appetite until the third or fourth day of hormone administration. The experiment was not continued long enough to determine whether the food intake of this bird may eventually have reached levels found in the other experimental birds.

13.7 Experiment 1 - body weight

Nearly all the birds showed a slight decrease in body weight when the injections began (Figure 13.7 A), but in all cases except 1F this loss was regained within 3 to 6 days; however, fluctuations were small usually less than a gram. Bird 1F which was heavier than others at the start of the experiment showed a marked reduction in body weight after the start of the prolactin injections, levelling off to a weight nearer the rest of the experimental group.

13.8 Experiment 1 - testicular condition

All the males in this experiment, whether given prolactin injections or not, had similar testes sizes (Table 13.4.1), and similar seminiferous tubule diameters. All tubules showed only primary spermatogonia in a single layer next to the outside of the tubule. When compared with mean tubule diameters of a large sample of control birds from experiment 3 the tubule diameters of the hormone-treated birds in this experiment were not significantly different ($p=.48$). All birds had

Figure 13.6.a . The running means of the weight of food eaten (in gms) by Bramblings in Experiment 1.
Ordinate - gms of food eaten.
Abscissa - the days of the experiment.
Open triangles - birds given saline injections four hours after the start of the photoperiod.
Closed triangles - birds given prolactin injections four hours after the start of the photoperiod.
Vertical arrows indicate the point at which injections were initiated.

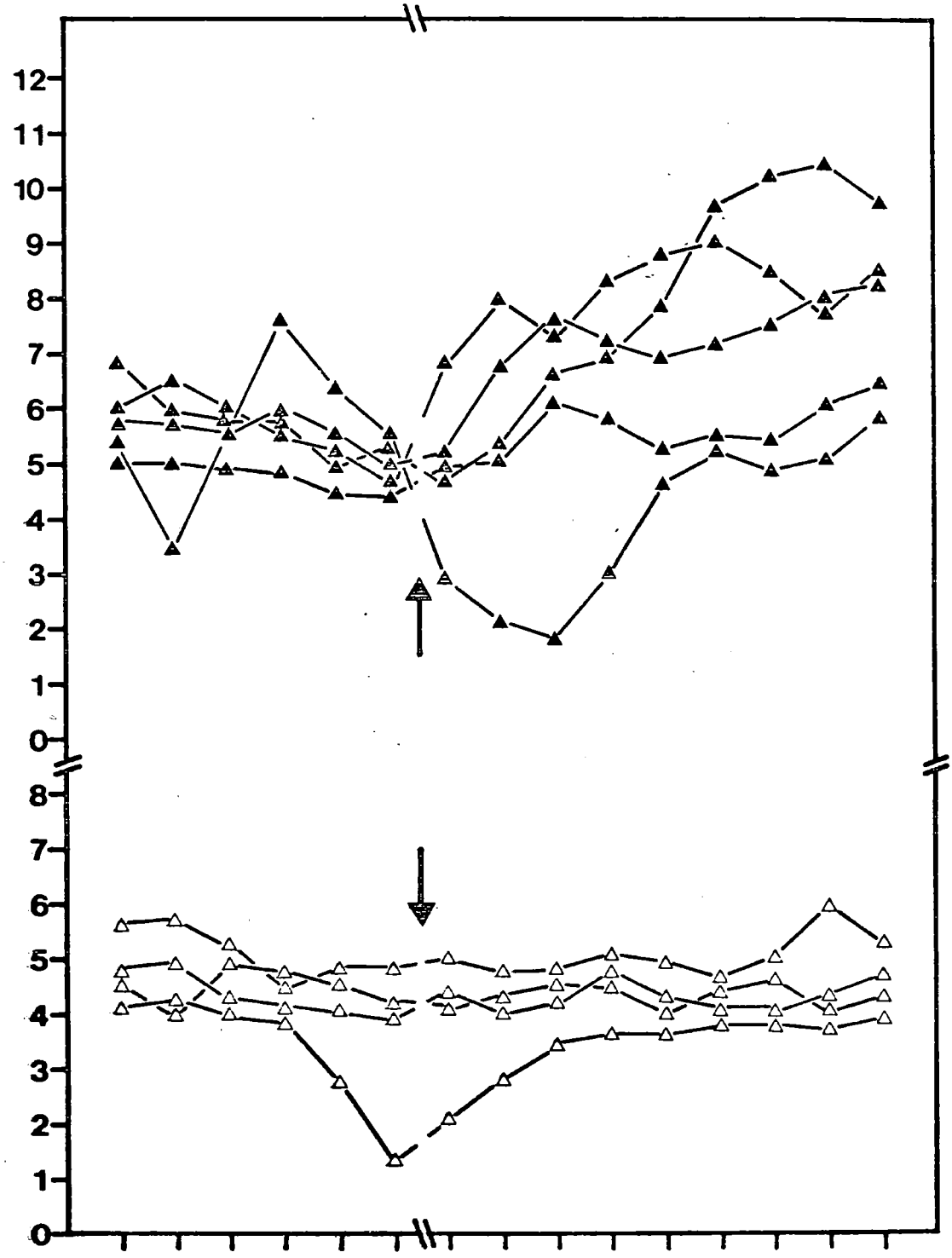

12
11
10
9
8
7
6
5
4
3
2
1
0
8
7
6
5
4
3
2
1
0

Figure 13.6.b . The cumulative weight of food eaten by control birds in Experiment 1.
Abscissa - days of the experiment.
Ordinate - the zero marks correspond to zero weight of seed eaten at zero time; the scale of gms of seed eaten is given separately. The open triangles indicate the point at which control injections were initiated. The solid lines are the least squares regression lines fitted to the pre-injection cumulative food weights and extrapolated to the injection period.
From top to bottom the birds are D,C,B and A.

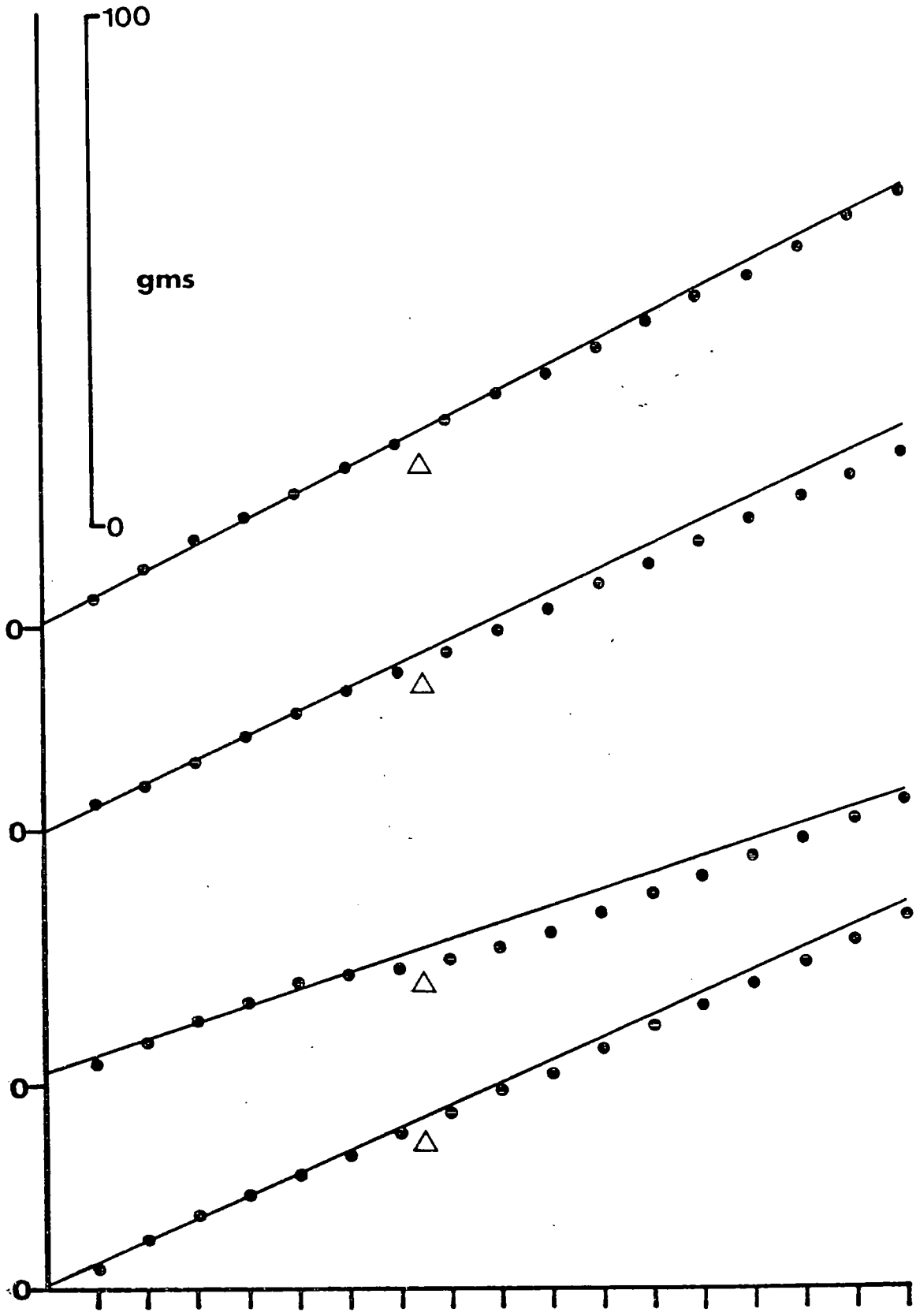

100
gms
0
0
0
0

Figure 13.6.c . The cumulative weight of food eaten by experimental birds in Experiment 1.
Abscissa - days of the experiment.
Ordinate - the zero marks correspond to zero weight of seed eaten at zero time; the scale of gms of seed is given separately. The open triangles indicate the point at which prolactin injections were initiated. The solid lines are the least squares regression lines fitted to the pre-injection cumulative food weights and extrapolated to the injection period.
From top to bottom the birds are H,G,F,I, and E.

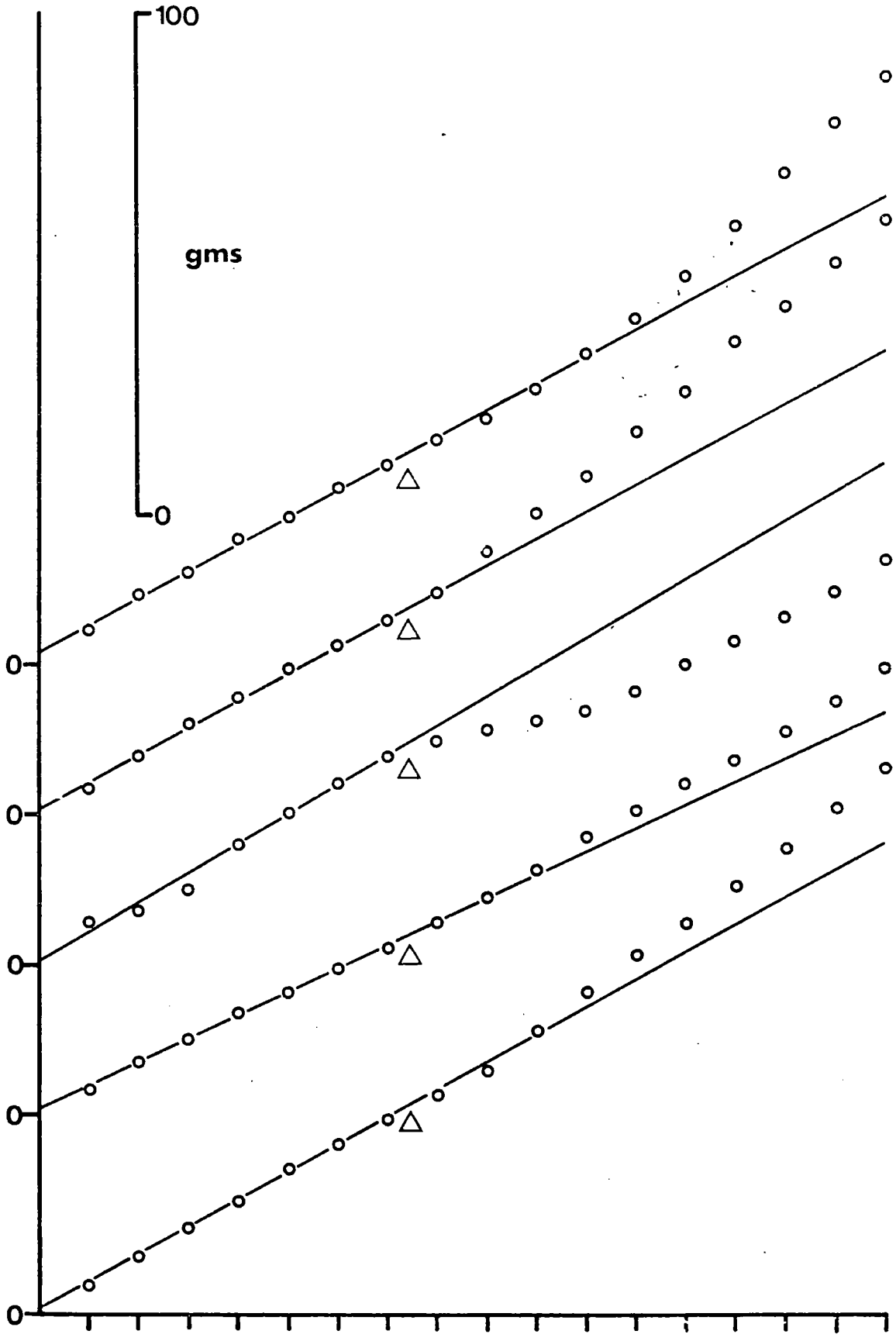
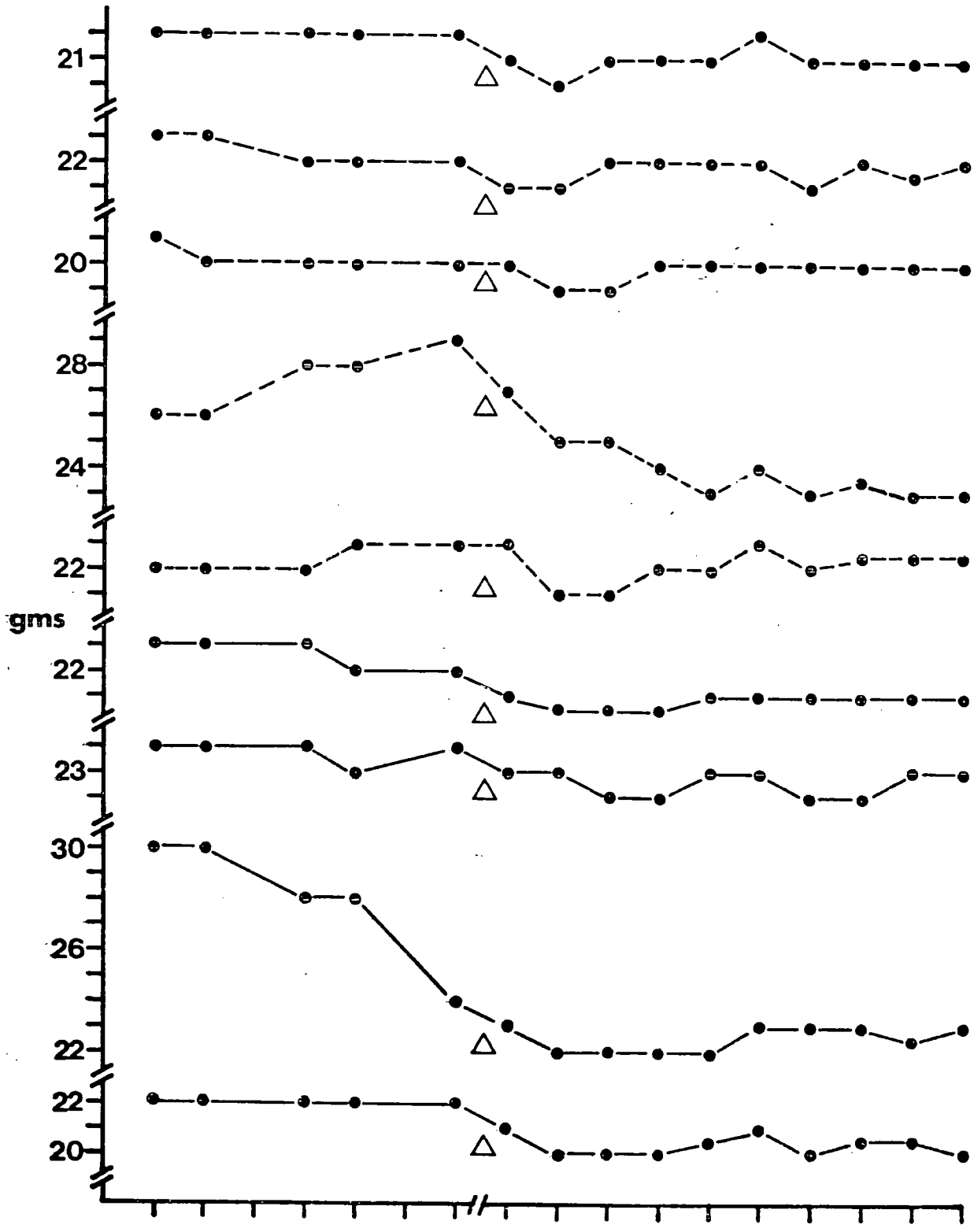

100
gms
0
0
0
0
0

Figure 13.7.a . Variations in the body weights of control birds (solid line) and experimental birds (broken line) in Experiment 1. The open triangles indicate the point at which injections commenced.
Ordinate - gms total body weight.
Abscissa - days of the experiment.
From top to bottom the birds are I,H,G,F and E; D,C,B and A.


21
22
20
28
24
22
gms
22
23
30
26
22
22
20

fibroblastic Leydig cells.

13.9 Experiment 2: The effect of prolactin injections at the end of the photoperiod and at two different dose rates

Since Meier and Davis (1967) have shown that prolactin injections late in the photoperiod are most effective in promoting weight gains in photorefractory birds, this experiment was undertaken to complement experiment 1 and to determine if there is a similar effect in photosensitive Bramblings. Two different dose rates (7.5 and 10 IU) were given and the effects on food intake, body weight and composition monitored.

The experiment was started on 5/4/72, and the birds were kept on 8L:16D. The prolactin injections lasted for eight days, and were given in the last half-hour of the photoperiod.

13.10 Experiment 2 - food intakes

As shown in Figure 13.10.a the running means of seed consumed by control birds were unaffected by saline injections. Confirmation is provided in Figure 13.10.b which shows that the observed cumulative weights of seed consumed did not differ from the predicted weights over the period of the saline injections. By contrast all birds given 7.5IU of ovine prolactin in saline each day showed an increase in the running means of seed eaten during the injection period (Figure 3.10 1), and all cumulative weights of seed are in excess of the weights predicted by the regression equations (Figure 13.10.b, 2F,G,E). In these birds the increase in food intake was detectable in the first day after the start of the prolactin injections (Figure 13.10.b). The running means of seed consumed for the two birds given 10 IU per day (2H,I) were also higher than the pre-injection levels, but as Figure 13.10.b illustrates the higher dose did not produce any markedly greater

additional food intake.

13.11 Experiment 2 - body weight

Saline injections in the four control birds did not produce any increase in body weight (Figure 13.11.a). In three birds (2 A,B,C,) small fluctuations were produced, but these were always reductions compared to pre-injection weights. A female, 2D, showed large decreases in weight during the injection period, but usually these were followed by recoveries to pre-injection weights.

Of the three birds given 7.5 IU prolactin, two showed increases in body weight (2F male, and 2G female). Whereas 2E (male) showed no appreciable change in weight (Figure 13.11a). However, even in the former two birds the weight increases were not large (c. 1 gm). Injection of 10 IU of prolactin produced much greater increases in body weight over pre-injection levels; 1.5 gms in bird 2H and 3 gms in 2I. To examine more fully the changes in body weight in response to 10 IU of prolactin this part of the experiment was repeated as part of experiment 3.

13.12 Experiment 2 - testicular condition

The two males given 10 IU of prolactin per day had smaller testes than the only male in the control group (Table 13.4.1). They also had smaller tubule diameters. These birds also had smaller tubule diameters than the control group in experiment 3, though not significantly so. The control male in experiment 2 had spermatogonia in more than one layer, whereas the two experimental males had many fewer of these outer spermatogonia. In all three males the Leydig cells were fibroblastic.

Figure 13.10. a. The running means of weight of food eaten by Bramblings in Experiment 2.
Ordinate - gms of seed.
Abscissa - days of the experiment.
The birds given prolactin injections are in the upper panel , 7.5 IU per day (closed circles) and 10 IU per day (open squares).
Controls are in the lower panel.
The vertical arrows mark the point at which injections were commenced.

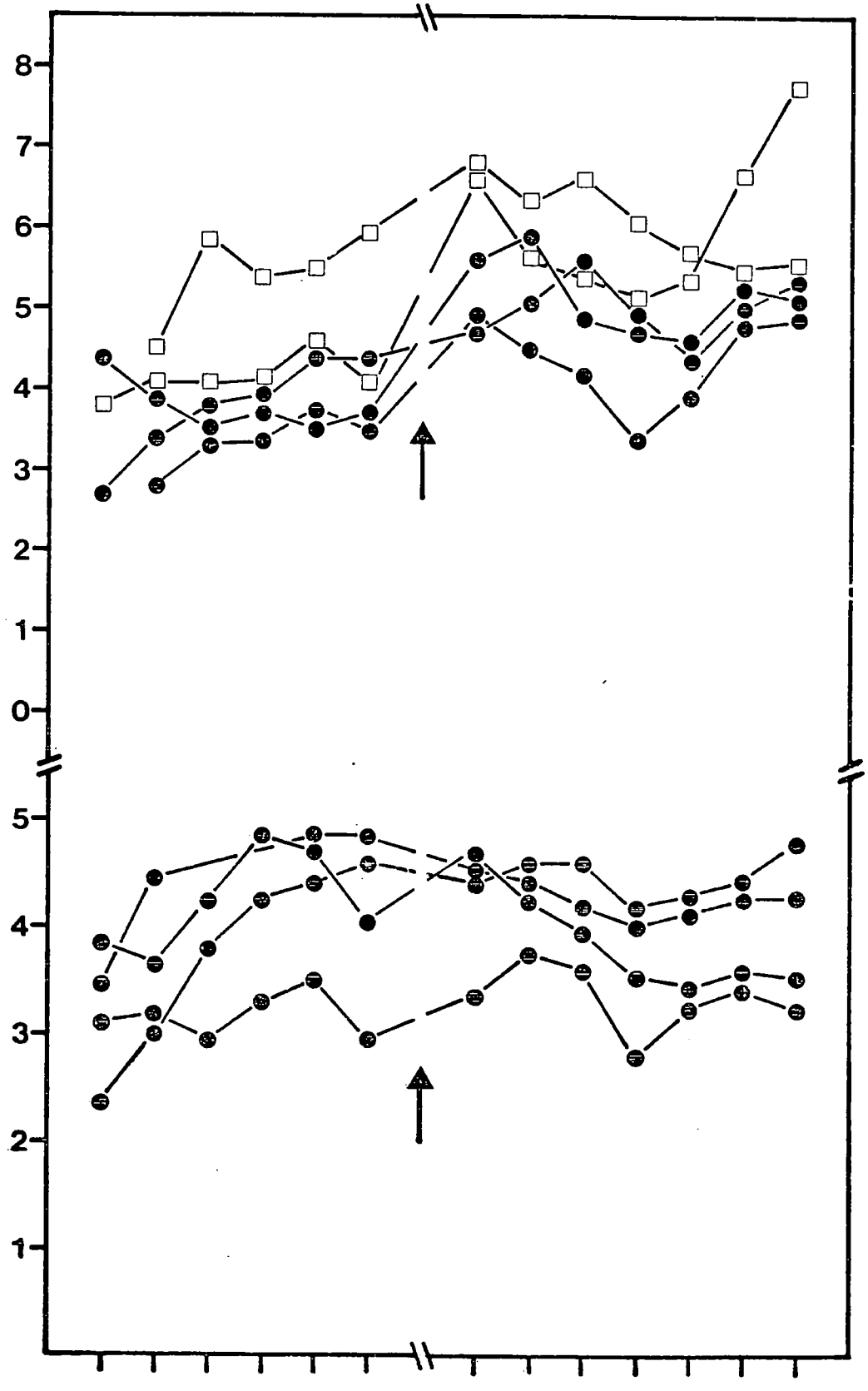

8
7
6
5
4
3
2
1
0
5
4
3
2
1

Figure 13.10.b . The cumulative weights of seed eaten by Bramblings in Experiment 2.

Ordinate - the zero marks on the ordinate mark zero food consumed at zero time, the scale is given separately.

Abscissa - days of the experiment.

Birds given prolactin injections are open circles; birds given saline injections closed circles. The open triangles mark the point at which injections were initiated.

The solid lines are the least squares regression lines fitted to the pre-injection cumulative weights and extrapolated to the post-injection period.

From top to bottom the birds are I,H,F,E,G,D,A,B,C.

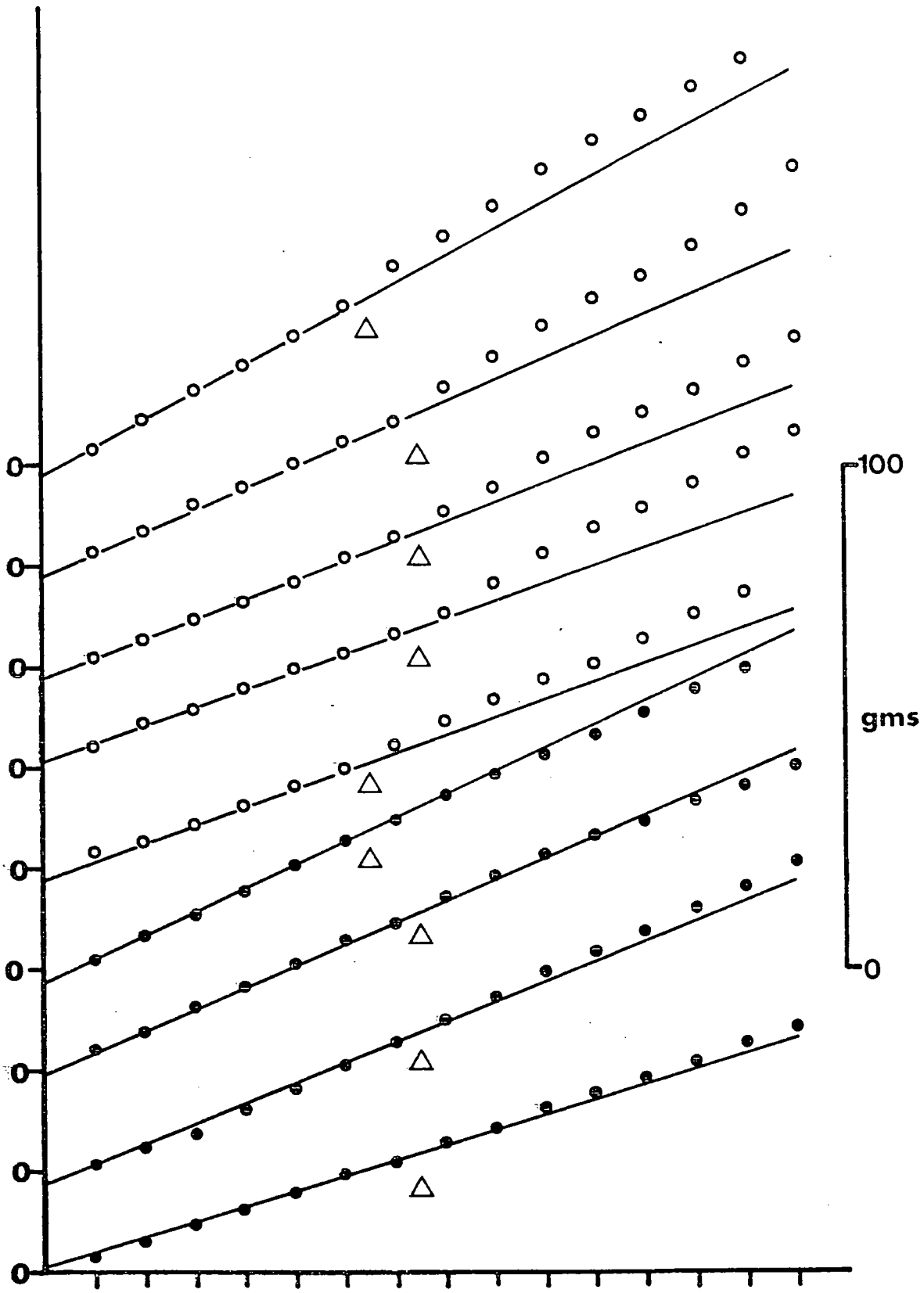
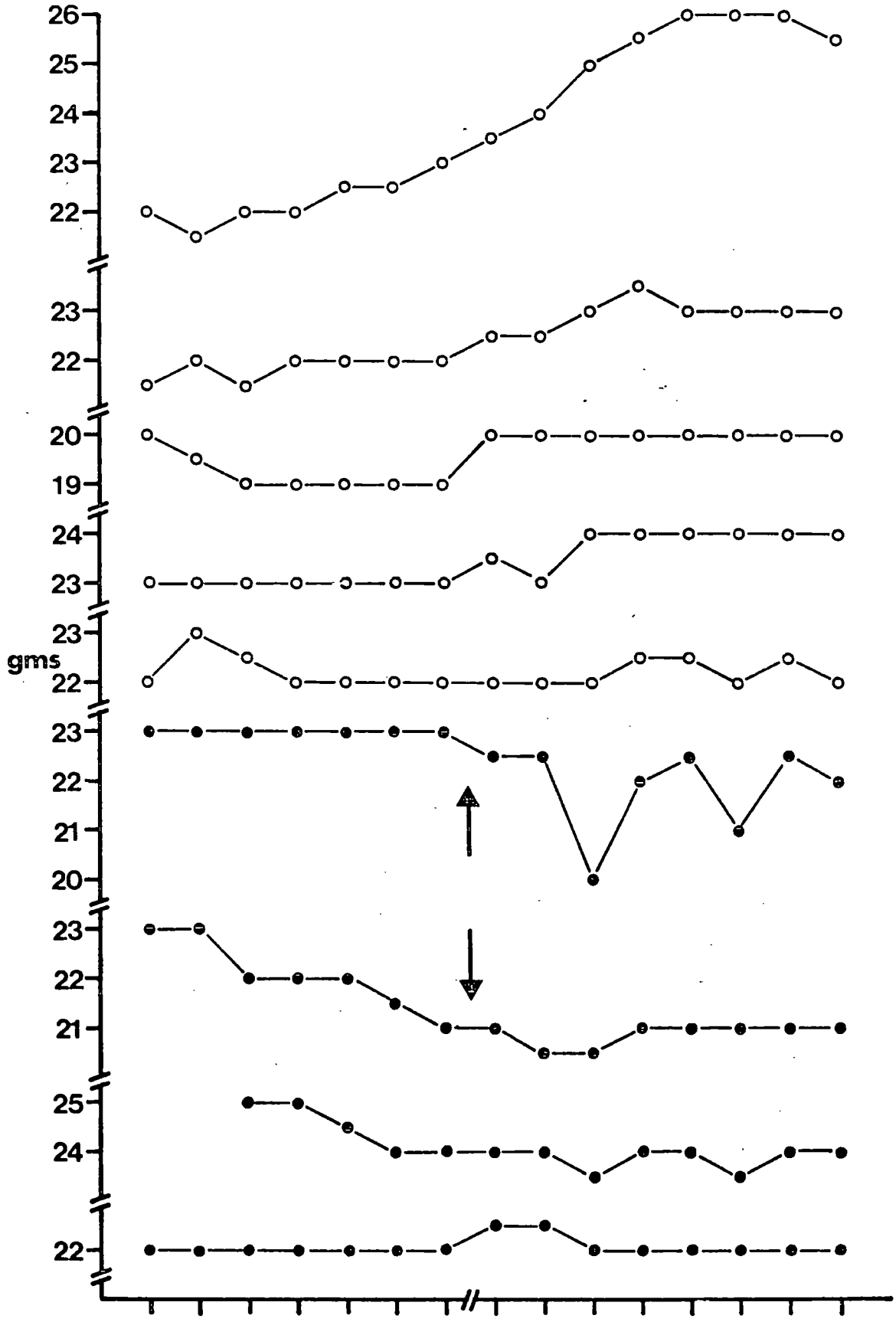

0
0
0
0
0
0
0
100
gms
0

Figure 13.11. a . Variations in the body weight of birds given control (closed circles) or prolactin (open circles) injections in Experiment 2. The vertical arrows indicate the time at which injections commenced.
Ordinate - gms total body weight.
Abscissa - the days of the experiment.
Birds from top to bottom are I,H,G,F,E,D,C,B, and A.


26
25
24
23
22
23
22
20
19
24
23
23
22
gms
23
22
21
20
23
22
21
25
24
22

13.13 Experiment 3: The effect of prolactin injections at the end of the photoperiod on male Bramblings given ad libitum vs restricted amounts of food.

As shown in the previous experiment prolactin injections given at the end of the photoperiod produce an increase in appetite which is not markedly dose dependent, but increases in body weight that are. In this experiment 10 IU of prolactin per day were given at the end of the photoperiod to birds provided with food ad libitum to determine the magnitude of the weight increases, and to birds provided with only 4 gms of food per day to determine if body weight increases can occur without hyperphagia. Additionally, this experiment was continued for only 4 or 5 days to determine the magnitude of the effect on body composition at a shorter time after the start of the prolactin injections, than the 8 days used in experiment 2.

Birds were kept on 8L:16D and the experiment started on 26/4/72. Prolactin and control injections were given in the last half-hour of the photoperiod.

13.14 Experiment 3 - food intake

Saline injections did not affect the running means of seed consumed by the control birds (Figure 13.14.a). As Figure 13.14.b shows, the observed cumulative weights of seed eaten by the control birds did not differ from the predicted weights except for bird 3J, whose food consumption was highly erratic, as was its body weight. In the group supplied with only 4 gms of seed a day after prolactin injections began the running means of food intake before hormone injections began were constant, except in one case (Figure 13.14.a). During the period of prolactin injections these birds ate all of the 4gms of seed. Birds given 10 IU of ovine prolactin per day and food ad libitum showed an increase in the running means of seed consumed

(Figure 13.14.a), during the injection period. This is confirmed by reference to Figure 13.4.b, which shows that the observed cumulative weights of seed eaten significantly exceeded predictions. As in experiment 2 the response was detectable by the first day after the start of hormone injections.

13.15 Experiment 3 - body weight

As Figure 13.15.a shows, the weights of three of the control birds deviate little from pre-injection levels, but 3J does show erratic changes in body weight, which subsequently return to former pre-injection levels. Birds given 10 IU per day and food ad libitum show increases in body weight of about 2 to 4 gms during the period of the injections, and are still at this weight at the end of the experiment (5 days). Thus the quantitative response as measured by body weight was greater for birds given 10 IU per day than those given 7.5 IU per day in both experiments 2 and 3. Birds given 10 IU of prolactin per day but with eating only 4 gms of seed a day, did show an increase in weight, but this was smaller and had disappeared by the fourth day after the start of prolactin administration.

13.16 Experiment 3 - testicular condition

There are no differences in testes size between the groups of birds, but birds given 10 IU of prolactin and food ad libitum had smaller mean seminiferous tubule diameters than the control group, ($p=.028$) (Table 13.4.1). All the control birds had a single layer of primary spermatogonia with three birds having a few above this layer. The injected birds with food ad libitum had a single layer of spermatogonia only, whereas the birds given 10 IU of prolactin but only 4 gms of seed a day were much more like the control birds, and also had larger tubule diameters than the ad libitum group.

Figure 13.14.a . The running means of food eaten by Bramblings in Experiment 3. Ordinate - gms of seed eaten; abscissa - day of experiment. The large vertical arrows mark the start of the prolactin or control injections.
Open triangles - birds given prolactin injections and food ad libitum .
Closed triangles - birds given prolactin injections and only 4 gms of food per day.
Open squares - birds given saline injections and food ad libitum .
Control bird J has been omitted from this figure.

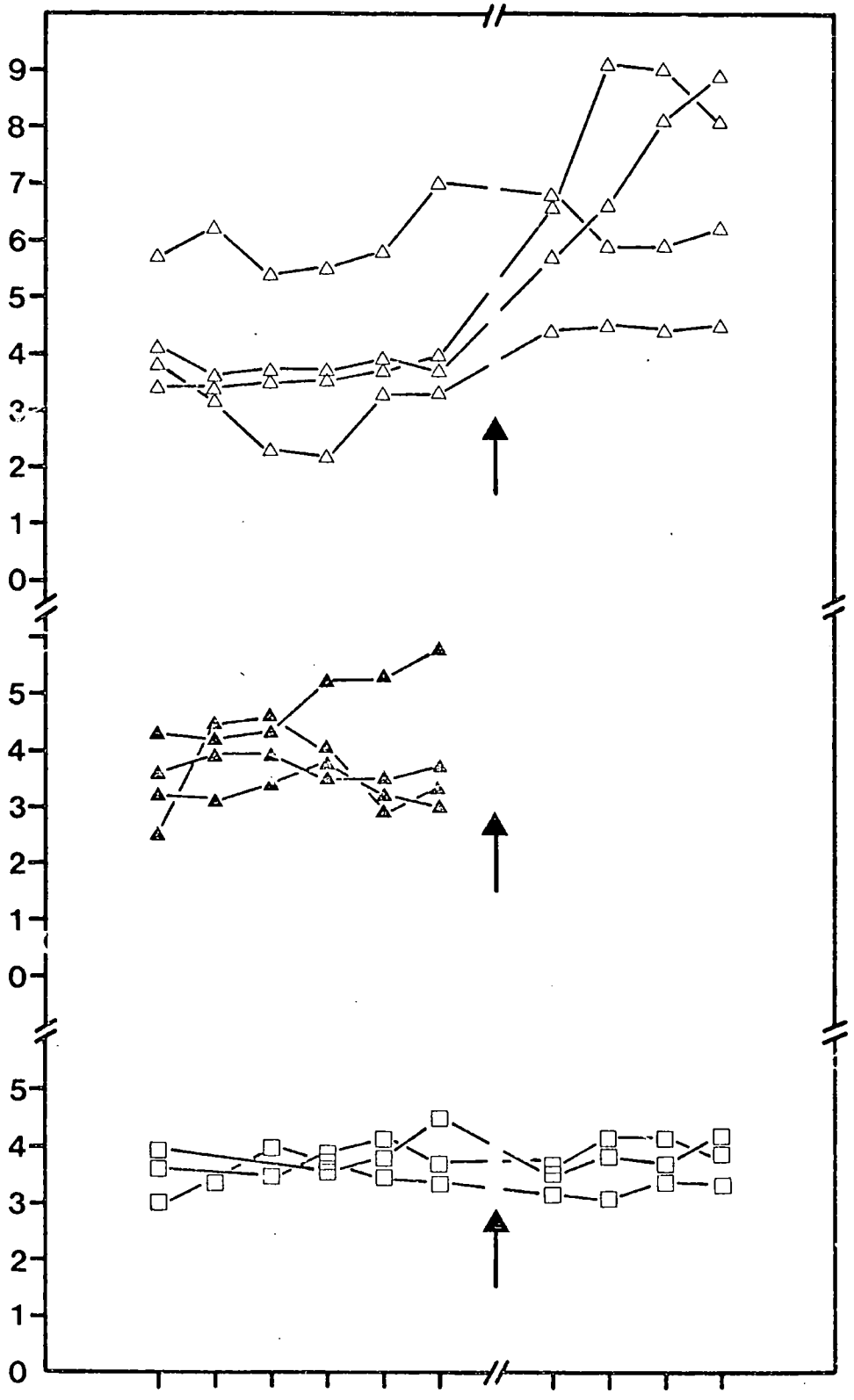

9
8
7
6
5
4
3
2
1
0
5
4
3
2
1
0
5
4
3
2
1
0

Figure 13.14.b . The cumulative weights of food eaten by Bramblings in Experiment 3. Open circles are birds given prolactin injections and ad libitum food, closed circles are birds given prolactin injections and restricted amounts of food.
Abscissa - days of the experiment
Ordinate - the zero marks on the ordinate are zero food eaten at zero time, the scale is given separately.
The open triangles indicate the point at which injections began.
From top to bottom the birds are E,G,D,F,J,L,B,A.

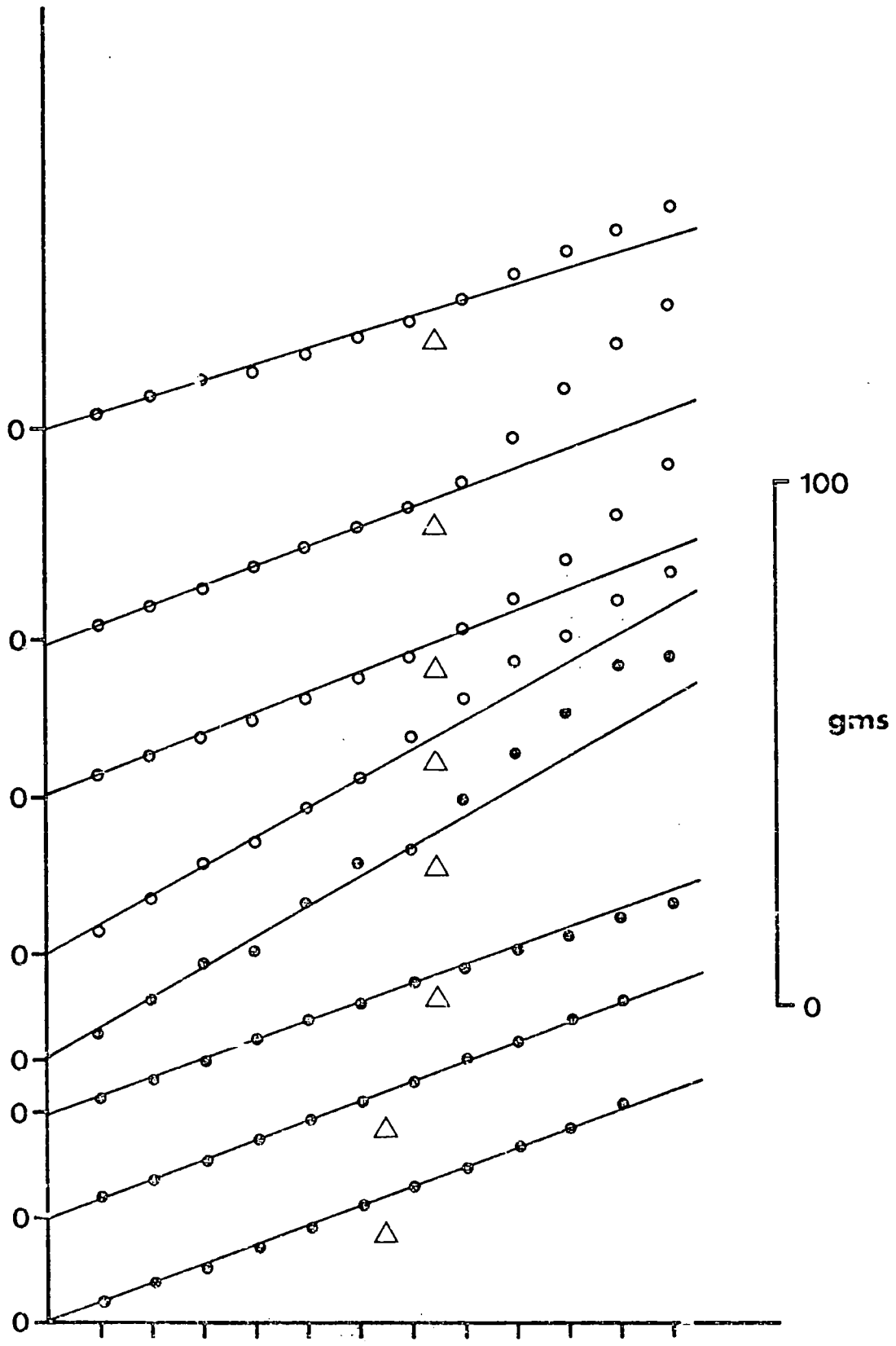
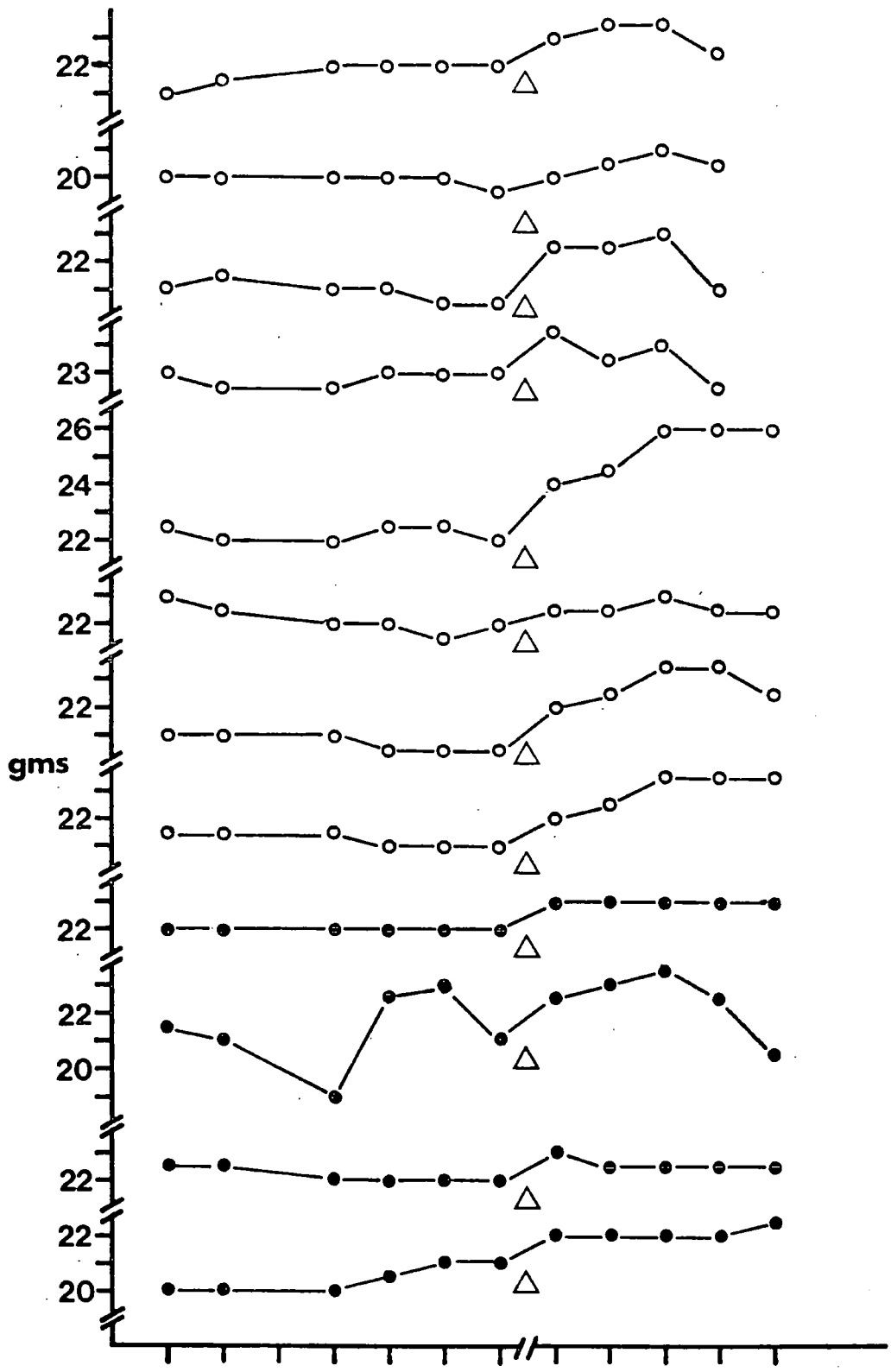

0
0
0
0
0
0
0
100
gms
0

Figure 13.15.a . Variations in the body weights of birds given saline injections (closed circles), or prolactin injections with restricted food (top four birds, open circles) or food ad libitum (middle four birds, open circles). The open triangles indicate the point at which injections commenced in Experiment 3.
Ordinate - gms total body weight.
Abscissa - days of the experiment.
From top to bottom the birds are K,I,H,C, G,F,D,E,L,J,B, and A.


22
20
22
23
26
24
22
22
22
gms
22
22
22
22
20
22
22
20

13.17 Experiment 4: The effect of a one hour light 'pulse' 14 hours after the start of an eight hour photoperiod

This experiment was designed to test whether a longer photoperiod would produce changes in body composition similar to those produced by prolactin injections. Farner (1959) has already shown that skeleton light schedules can induce fattening in White-crowned Sparrows; this technique has been adopted here to minimize any effects of a lengthened feeding time. The skeleton light schedule used is based on the light-pulse technique of Follett and Sharp (1969), except in this case the total photoperiod was not kept constant. Instead a light-pulse of one hour was added to the original eight hour photoperiod on which the birds had been kept. The light pulse was given at 14 hours (8L:6D:1L:9D) after the start of the photoperiod, as Lofts and Marshall (1960) have shown that a photoperiod of 14½ hrs is effective in producing premigratory fat deposition in photosensitive Bramblings.

The experiment was started on 26/4/72 and the light pulse was given for 17 days; this experiment was run for longer than the others to give all birds a chance to respond.

13.18 Experiment 4 - food intake

As Figure 13.18.a shows, the running means of food intake of four out of six of the birds increased during the period of the skeleton light schedule, but the amounts of seed consumed varied rather widely from day to day. Figure 13.18.B. confirms again that the general level of appetite was increased during the treatment period, as the observed cumulative weights of seed eaten exceeded the weights predicted. In two birds (4D,C) the response was immediate, but in the other two (4 A,B), it occured somewhat later. In two birds

Figure 13.18.a . The running means of the weight of food consumed by Bramblings in Experiment 4.
The vertical arrow marks the point at which the light pulse was first administered.
Ordinate - gms of seed consumed.
Abscissa - the days of the experiment.

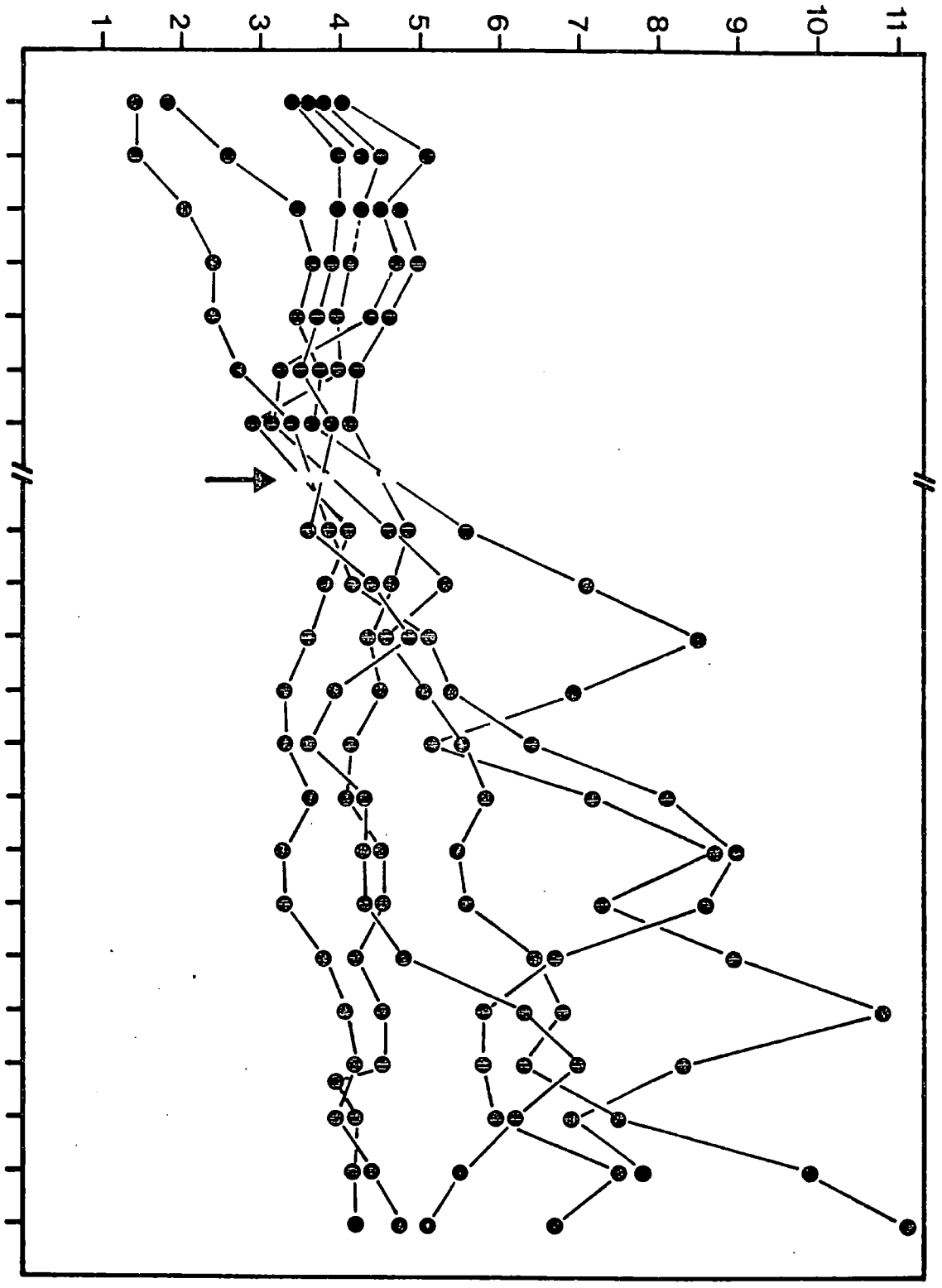

1
2
3
4
5
6
7
8
9
10
11

Figure 13.18.b . The cumulative weights of seed consumed by Bramblings in Experiment 4.
Ordinate - the zero marks zero seed consumed at zero time, the scale is given separately.
Abscissa - the days of the experiment.
The solid lines are the least squares regression lines fitted to the pre-injection food weights and extrapolated to the post-injection period.
The open triangles mark the point at which the light pulse was first administered.
From top to bottom the birds are D,C,A,B,E and F.

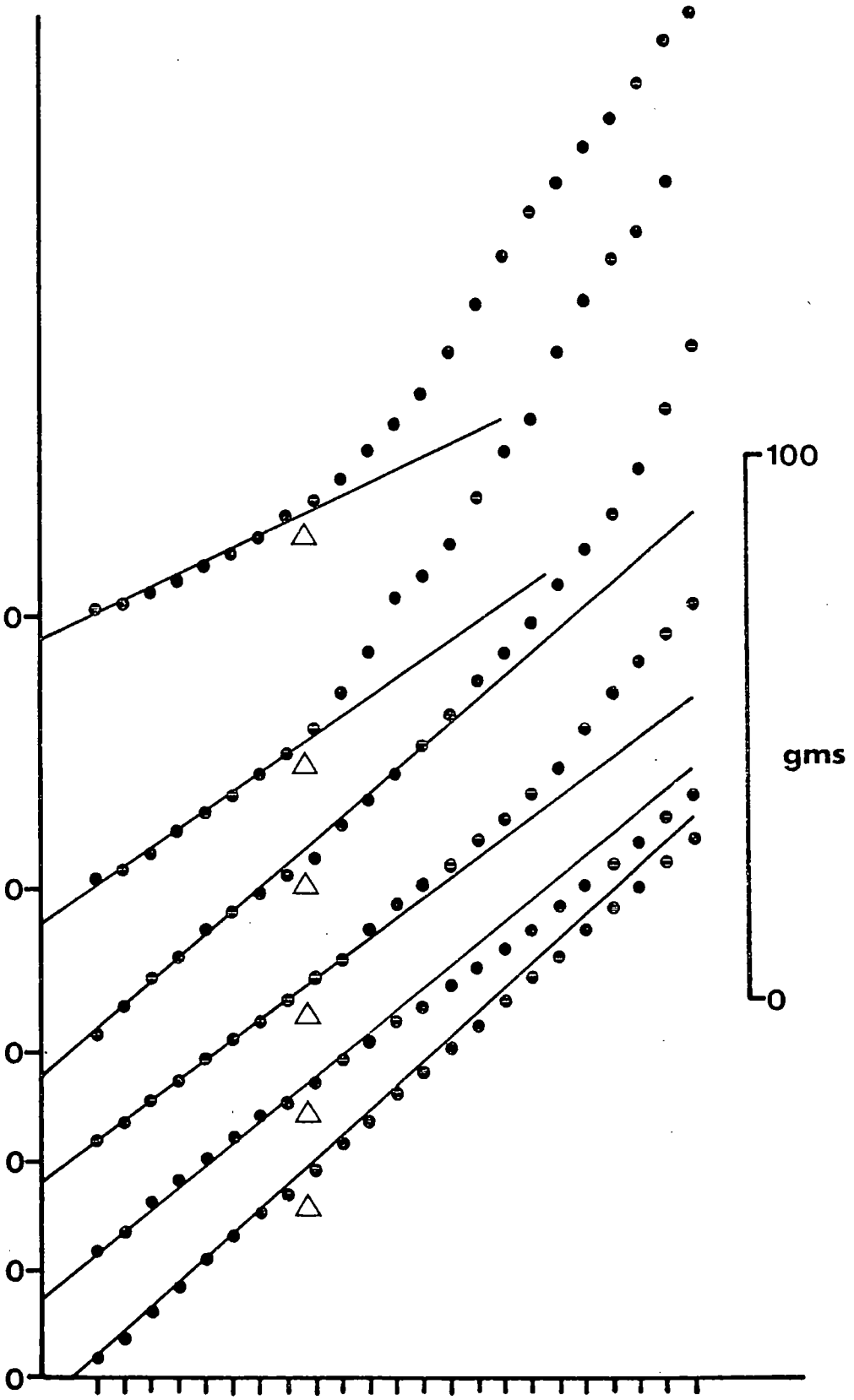
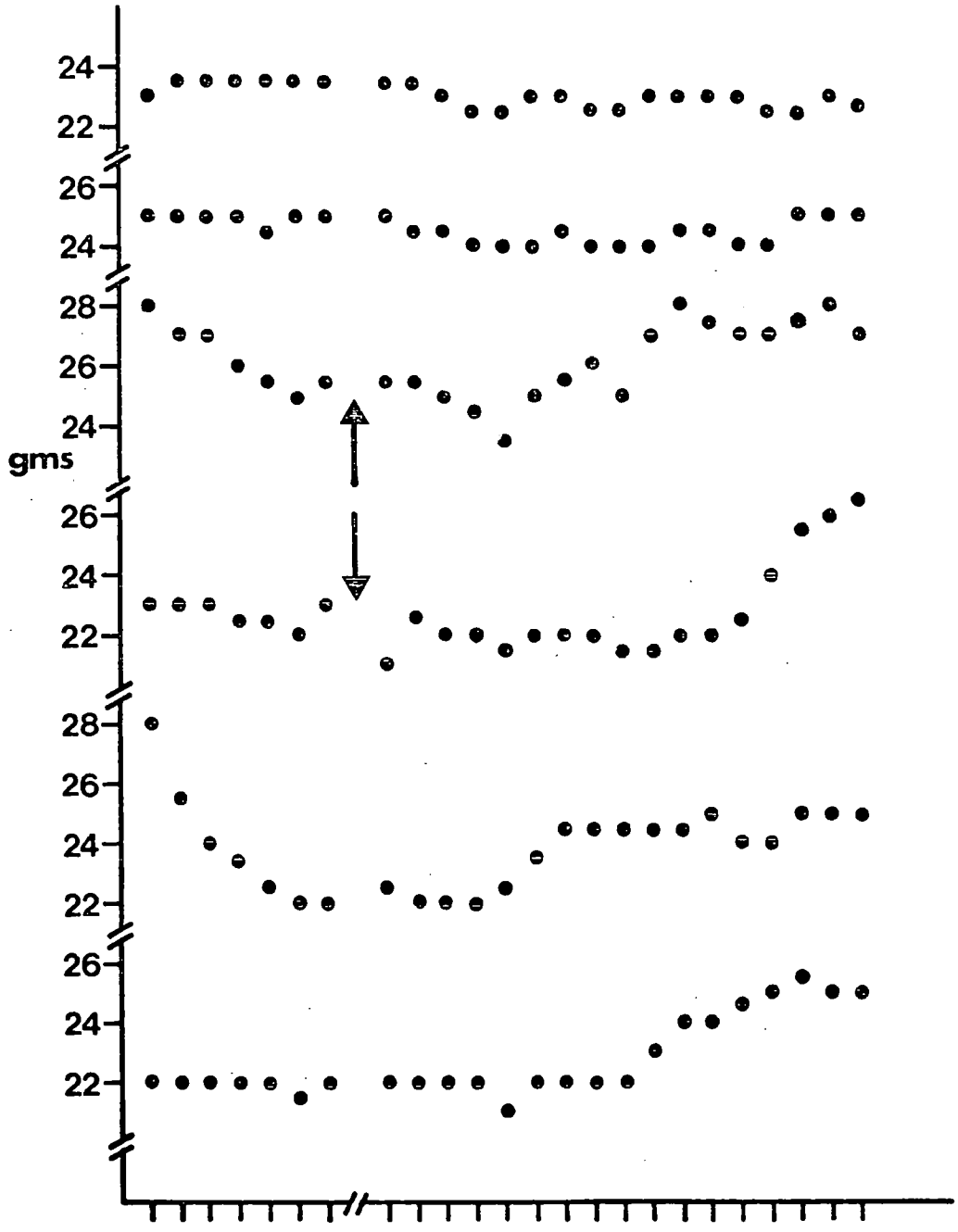

100
gms
0
0
0
0
0

Figure 13.19.a . Variations in body weight of Bramblings in experiment 4. The vertical arrows mark the point at which the light pulse was first administered.

Ordinate - body weight in gms.

Abscissa - days of the experiment.

From top to bottom the birds are F,E,D,C,B and A.


24
22
26
24
28
26
24
gms
26
24
22
28
26
24
22
26
24
22

(E,F) there was no response at all, with the observed cumulative weights of seed eaten during the treatment period being less than predicted.

13.19 Experiment 4 - body weight

The two birds which showed no increase in food intake (4E male, 4F female) showed no change in body weight, but all the other birds showed increases in weight of about 4 gms (Figure 13.19.a). These weight increases are larger than those found in photosensitive Bramblings treated with 10 IU of prolactin per day.

13.20 Experiment 4 - testicular condition

The three males involved in this experiment all show enlarged testes (Table 13.4.1) and larger mean seminiferous tuble diameters than control birds in other experiments. Even 4E, which was one of the birds that showed no increase in food consumption or body weight, showed a gonadal response. Both E and C had spermatogonia undergoing meiosis, and the Leydig cells were enlarged compared with control birds in other experiments. 4D had fibroblastic Leydig cells, and not so many spermatogonia, although meiosis was still visible.

13.21 Experiment 5: The effect of long photoperiods (15L:9D) on body composition of female Bramblings

This experiment was run as a parallel experiment to experiment 4, by using a full photoperiod of 15 hours. The primary aim was to examine organ weights after photostimulation. The experiment was run for eight days. Body weights were larger (max 28 gms) than the female control birds from the other experiments.

13.22 Body composition - adipose tissue

Reference to Table 13.22.1 shows that in most cases increases in body weight were accompanied by increase in adipose tissue weight, although as the birds were killed two hours after the start of the photoperiod these weights are not of the same magnitude as the body weight changes in the experiments, due to diurnal weight changes and variations in the weights of other body components. In experiment 1 prolactin injections early in the photperiod were ineffective in increasing adipose tissue weight (Table 13.22.1), and this is confirmed by measurements on total body lipid (Table 13.22.1) In experiment 2 prolactin injections at the end of the photoperiod produced increases in adipose tissue weight, such that four birds (F,G,H,I), irrespective of whether they received 7.5 IU or 10 IU of prolactin per day, have abdominal fad pad weights greater than all four controls (Table 13.22.1). Total body lipid measurements confirm this (Table 13.22.1). The results from the third experiment are less conclusive, as two of the controls had large abdominal fat pads, but some of the prolactin injected birds had heavier abdominal fat pads than control birds from other experiments; this even applied to the birds given a limited amount of food.

Comparison of the two 'longday' experiments show that the cervical fat pad weights of the birds given a light-pulse (experiment 4) were nearly significantly less than pad weights of the birds given full 15 hour photoperiod ($p=.064$). However, these light pulse (experiment 4) birds included two which did not respond; if these are excluded there is not much difference in cervical fat pad weights between birds in experiments 4 and 5.

Table 13.22.1. Adipose fat levels in Bramblings

Experiment 1	1A	1B	1C	1D	
Controls					
Abdominal fat pad wt(mg)	12	34	18	31	
Fat % dry wt carcase	12	14	10	23	
Experimentals	1E	1F	1G	1H	1I
Fat as % carcase dry wt	13	18	11	21	12
Abdominal fat pad wt (mg)	13	31	1	36	10
Experiment 2					
Controls	2A	2B	2C	2D	
Abdominal fat pad wt (mg)	12	21	tr	tr	
Fat % of dry carcase wt	15	16	13	13	
Experimentals					
Abdominal fat pad wt (mg)	2E	2F	2G	2H	2I
	14	13	68	58	324
Fat % of dry carcase wt		21	24	25	48
Experiment 3					
Controls	3A	3B	3J	3L	
Abdominal fat pad wt (mg)	234	76	tr	tr	
Experimentals - ad lib	3D	3E	3F	3G	
Abdominal fat pad wt (mg)	52	113	21	137	
Experimentals - rest	3C	3H	3I	3K	
Abdominal fat pad wt (mg)	44	20	50	95	

Experiment 4	Cervical (mg)	Experiment 5	Cervical (mg)
4A	406	5A	504
4B	264	5B	375
4C	326	5C	648
4D	416	5D	452
4E	135	5E	566
4F	trace	5F	257

Note: tr - trace

13.23 Body composition - pectoralis muscle wet weights

None of the experimental groups had significantly larger or smaller wet muscle weights than the controls of the same sex, so that hormone injections or photomanipulation had no effect on pectoralis muscle wet weights. Only the female Bramblings given a full 15 hour photoperiod had muscle wet weights significantly greater than the controls ($p=.008$).

Table 13.23.1 M.pectoralis wet weights (gms) of Bramblings

	Female		Male	
Controls	1A	3.673	3A	4.463
	1D	3.963	3B	4.585
	2A	3.859	3J	4.676
	2C	3.816	3L	4.744
	2D	4.156	2B	4.511
			1B	4.673
			1C	4.571
Experiment 1			1E	4.590
			1F	4.419
			1H	4.382
			1I	4.150
Experiment 2				
	2E	4.153	2H	4.190
	2F	4.263	2I	4.548
	2G	3.594		

	Ad lib		Restricted	
Experiment 3	3D	4.316	3C	4.271
	3E	4.018	3H	4.565
	3F	4.152	3I	4.049
	3G	4.605	3K	4.451

Table 13.23.1 continued

Experiment 4	Female	Male
	4B 4.160	4C 4.261
	4F 4.357	4D 4.543
	4A 3.761	4E 4.707

Experiment 5

5A	4.362
5B	4.044
5C	4.452
5D	5.031
5E	4.567
5F	4.380

All controls have been grouped by sex due to the difference in muscle size between the sexes; all comparisons are, therefore, between experimental and control groups of the same sex.

13.24 Body composition - liver

In the comparisons used hereafter controls have been grouped together by sex for the statistical tests as liver weights of males are larger than those of females. The experimental group from each of the treatments has been compared with the control group of the same sex.

There is no significant difference in either liver wet weights or fat free dry weights between controls and experimentals in experiment 1. Thus prolactin early in the photoperiod has no effect on liver size ($p > .5$). In experiment 2 the males injected with prolactin at the end of the photoperiod do not have significantly larger wet liver weights than the control group ($p = .36$), but the females might do ($p = .06$) (Table 13.24.1); this applies to fat-free dry weight also.

The birds given prolactin injections, but only 4 gms of seed a day in experiment 4, and killed after only 4 days on injections have significantly larger liver wet weights and fat-free dry weights than the control group (p=.012) (Table 13.24.1), whereas those birds given prolactin and food ad libitum do not (p=.16). But, when these two sub-samples are combined, the birds given prolactin injections in experiment 3 do have significantly larger liver wet weights and fat-free dry weights than the control group (p=.02) (Table 13.24.1). The males in experiment 4 have significantly smaller livers than the control group (p=.056) but this group does include one bird that did not respond to the light pulse; if excluded there is no significant difference, as also found in the case of the females in this experiment. The wet liver weights of the birds given 8 days of a full 15 hour photoperiod were significantly heavier than the corresponding female control group (p=.008), and were about the same size as the livers in females given 10 IU of prolactin at the end of the photoperiod. The amount of lipid did not vary (either total or as concentration) in these experiments (1-4); nor did the concentration of water in the liver.

Table 13.24.1 Liver composition of Brambling in experiments 1 to 5.

Controls	Bird	Wet wt mg	FFDW mg	Total fat (mg)	Fat/100mg	Water/100mg
Females	1A	435	105	19	18.2	296
	1D	581	581	23	15.2	270
	2A	462	142	17	12.0	214
	2D	495	121	20	18.2	282
	2C	491	135	22	14.8	250

Table 13.24.1 continued

Controls	Bird	Wet wt mg	FFDW mg	Total fat(mg)	Fat/100mg	Water/100mg
Males	1B	601	146	21	14.4	297
	1C	519	139	21	15.1	259
	2B	667	175	18	10.3	272
	3A	531	145	20	13.8	252
	3B	574	157	18	11.5	254
	3J	614	171	21	12.3	220
	3L	837	224	19	8.5	244
Experiment 1						
Female	1G	534	-	-	-	-
Male	1E	561	145	20	13.8	278
	1F	522	141	25	17.7	253
	1H	672	179	20	11.2	264
	1I	592	141	17	12.1	301
Experiment 2						
Female	2E	535	139	12	8.6	269
	2F	678	229	28	12.2	184
	2G	626	187	22	11.8	223
Male	2H	614	156	22	14.1	280
	2I	668	198	17	8.6	228
Experiment 3						
Ad libitum food						
Male	3D	714	194	17	8.8	264
	3E	634	176	22	12.5	248
	3F	634	169	20	11.8	263
	3G	696	191	20	10.5	254

Table 13.24.1 continued

Experiment 3	Bird	Wet wt mg	FFDW mg	Total fat(mg)	Fat/100mg	Water/100mg
Food restricted						
Male	3C	707	198	20	10.1	246
	3H	857	227	22	9.7	258
	3I	817	239	18	8.2	262
	3K	880	251	23	9.2	241
Experiment 4						
Female	4A	562	156	20	12.8	248
	4B	607	166	19	11.4	254
	4F	362	101	19	18.8	240
Experiment 4						
Male	4D	451	163	17	10.4	167
	4E	448	104	22	21.2	315
Experiment 5						
Female	5A	616				
	5B	694				
	5C	554				
	5D	939				
	5E	742				
	5F	826				

13.25 Discussion

All the birds used were kept on 8L:16D from late February until late May-early June in order to keep them photosensitive. Examination of the histology of the testes of control birds suggests that there were no long term alterations in the hormones of the pituitary-gonadal axis, as the testes remained in a regressed state. In view of the demonstration of seasonal rhythms in birds kept in constant conditions (Gwinner 1969, King 1968), some change might have been expected. This is of some importance as Meier and Farner (1964) have shown that gonadotropins can synergise with prolactin in the production of lipid deposition in migratory sparrows, and Stetson and Erickson (1972) have demonstrated that surgical castration before the start of photostimulation in the White-crowned Sparrow can abolish the fattening response; although this may be due to gonodal steriods affecting prolactin output (Weise 1967). Also in the Brambling, Schildmacher and Steubing (1952) have produced small increases in body weight with testosterone injections. In the Bramblings in these experiments, the responses are not due, therefore, to changing blood titers of gonadal hormones or gonadotropins acting synergistically with prolactin or on their own. I have no information on the remaining endocrine systems.

The only changes in testes condition appear to be a reduction in seminiferous tubule mean diameters in birds given exogenous prolactin. This is presumably a symptom of the anti-gonadal response/prolactin, as reported in other avian species (Lofts and Marshall 1956); interestingly this occurs despite the Brambling being a migrant, as according to Meier and Dusseau (1968) the anti-gonadal action of prolactin is expressed only in resident species. Meier, Martin, and MacGregor (1971) have

presented evidence from photosensitive White-throated Sparrows placed on continuous light that the time of maximum fattening response to exogenous prolactin coincides with the time of maximum gonadal response to this hormone. In these experiments the prolactin injections were given relative to an injection of corticosterone. In the Bramblings used in my experiments those birds responding to injections of ovine prolactin (by increases in body weight and lipid) showed no increase in testes size or change in histological conditions; however, it is not certain that the prolactin injections produced the maximum possible fattening, since only two times during the 24 hours were selected for injections.

Since the changes seen in the experimental birds can not be attributed to changes in the pituitary-gonadal axis alone, the effects are due to prolactin acting alone or synergistically with non-gonadal hormones. Meier and Davis (1967) found that the extent of the body weight increases and lipid deposition in photorefractory White-throated Sparrows given mammalian prolactin depended on the time of injection of the hormone during the photoperiod. Hormone injections early in the photoperiod decreased body weight and fat reserves (a catabolic effect, Meier 1969), but injections later in the photoperiod increased body and lipid weight. It has been suggested that these diurnal differences in response to prolactin are due to its interaction with corticosterone, thus Meier and Martin (1971) found that prolactin injections given at 4 or 12 hours after the administration of corticosterone produced an increase in body lipid, but that injections at 8 or 20 hours after corticosterone depressed the lipid levels. In photosensitive birds of the same species placed in continuous light the maximum lipid deposition occured at 12 hours after the corticosterone injection and the minimum lipid increase at 8 hours after the

injection of the steriod (Meier,Martin, MacGregor 1971). In the photosensitive Bramblings in my experiments only injections given eight hours after the start of the photoperiod were effective in producing lipid weight increases, whereas injections of prolactin four hours after the start of the photoperiod had no effect on body or lipid weight. Prolactin injections did not have a catabolic effect.

Interpretation of my findings on the basis of the hypotheses of Meier and his co-workers requires information on the diurnal timing of the peak level of plasma corticosterone in the Brambling. In spring the peak level in White-throated Sparrows occurs early in the 12 hour photoperiod (Dusseau and Meier 1971), while in the pigeon it is just before the start of the 12 hour photoperiod (Joseph and Meier 1973). If these results are applicable to the Brambling, then the time at which prolactin produces fattening in this species would be about 4 or 12 hours after the peak of plasma corticosterone; this seems therefore to be tentative confirmation of Meier's hypothesis. However, the maximum fat response in the pigeon occurs when prolactin and corticosterone injections coincide (Meier, Trobec, Joseph and John 1971), so that the relation between photoperiod and time of sensitivity to prolactin in the pigeon differs from that in the sparrow.

However, what is clear from the first two experiments is that the stimulation of appetite by prolactin is independent of the time of injection during the photoperiod (Figure 14.5.A); and incidentally this is the first proof that prolactin-induced fattening in

migratory birds is mediated by an increase in food intake, so that this obesity is of the migratory type and not of the type produced by hypophysectomy (Gibson and Nalbandov 1966) in which obesity is caused by impairment of lipid mobilisation. The ability of the bird to synthesise and/or store lipid is, however, dependent on the time of prolactin injection, as in experiment 1 there is no lipid deposition even though food intake is increased. Since the liver is the major site of lipid synthesis in birds (Goodridge and Ball 1967 a,b, O'Hea and Leveille 1968), and an enlarged liver (wet weight) is indicative of increased lipid synthesis by this organ (Goodridge and Ball 1967 b,c, Lepovsky 1973), it is a reasonable assumption that the component which is dependent on the time of prolactin injections is the capacity of the liver to synthesise lipid. Birds injected with 7.5 IU of prolactin at the beginning of the photoperiod (experiment 1) show no increase in body lipid and no increase in liver size. Goodridge and Ball (1967c) have shown that the activities of liver enzymes involved in lipid synthesis are not increased by prolactin in the pigeon if the bird is starved, suggesting tht the increase in enzyme activities seen in fed pigeons given exogenous prolactin is secondary to the increase in food intake that prolactin produces. However, the data from experiment 1 suggests that prolactin acts directly on the lipid synthesising system of the liver (Figure 14.5.A),and this is confirmed by experiment 3 in which there is an increase in liver size, even though food intake remains constant. Possibly there might be some impairment of digestion in the experimental birds from the first experiment, but this is unlikely as Soulairac (1947) has shown that the rate of absorption of food

across the small intestine is linked to the food intake. If this is the case then the results of the first two experiments also suggest that the enlargement of the liver is not a response to increased metabolite load as suggested by Lepovsky (1973) for the chicken.

The second and third experiments together illustrate that the extent of body weight increase and lipid deposition in response to exogenous prolactin is dose-dependent (not linear);as 10 IU of prolactin produces much larger body weight increases than 7.5 IU of prolactin per day when administered at the same time of day. However, all of the body weight increases seen in these birds cannot be due to body lipid as liver weight also increases in prolactin-treated birds. The contribution of liver to total body weight will be greater than suggested by the morning weights of the livers quoted in Table 13.24.1, since birds have a diurnal increase in liver weight (Fisher and Bartlett 1957) and my birds were weighed at the end of the photoperiod. In the birds given prolactin injections at the end of the photoperiod, but which consumed only 4 gms of seed per day, the weight increases of 1 to 2 gms found must in a great part be due to weight increases of the liver (amounting to about 300 to 400 mg) although some lipid deposition seems to have taken place also. Bates, Miller and Garrison (1962) have demonstrated that in hypophysectomised pigeons the liver wet weight is dose dependent in the case of prolactin injections, and examination of the liver weights from experiments 2 and 3 suggests that this is the case in the Brambling also, although the birds in experiment 3 were, of course, killed after only five instead of the eight days used in experiment 2.

In the experiments involving manipulations of the photoperiod experiment 4 clearly demonstrates that a skeleton light schedule can produce hyperphagia while providing only a small (12½%)increase in feeding time, as shown by Farner (1959) for the White-crowned Sparrow. The increases in both food intake and body weight were much greater for birds in experiment 4 than for the birds in experiments 2 and 3 given 10 IU of prolactin per day. As experiment 4 was continued for very much longer than the hormone injections experiments the data on liver weights are not comparable. However, it did show that by the end the liver weights had been reduced relative to the controls; presumably they had declined to these low levels during the 'static' phase of weight gain (Kuenzel and Helms 1968), after a weight increase in the 'dynamic' phase. A full 15 hour photoperiod given for eight days increased body weights and produced liver weights similar to those found in prolactin-treated birds (10 IU per day), suggesting that the causative factor may be the same. The body weight increases and adipose fat pad weights were very similar between birds given a skeleton light schedule and those given a full 15 hour photoperiod. This suggests that both are equally effective in producing premigratory lipid deposition, however two birds failed to respond to the skeleton light schedule.

13.26 Summary

Injections of mammalian prolactin in photosensitive Bramblings produced a stimulation of appetite which was independent of the time of hormone injection within the photoperiod, whilst lipid deposition (which was dose-dependent) was not. The increase in

body and liver weights (indicative of lipid synthesis) in birds injected with prolactin, but not allowed to increase their food intake, provided confirmation that prolactin acts directly on the liver in the fattening response of migratory birds.

Changes in adipose lipid and body weight, which were produced by a skeleton light schedule (8L:6D:1L:9D), were comparable to the effects on photosensitive Bramblings given a full 15 hour daily photoperiod. The latter group had enlarged livers of similar weights to those found in prolactin-treated Bramblings in which lipid deposition was produced.

Chapter 14. General discussion

14.1 Are moulted Willow Warblers premigratory?

Before discussing the possible premigratory metabolic changes found in Willow Warblers, one must first establish that the samples of moulted birds truly are premigratory, a fact that has been tacitly assumed so far. Previous studies on a variety of passerine species have shown that there are two main characteristic of the premigratory state: hyperphagia (King 1961a, 1961b for the White-crowned Sparrow; Dol'nik 1970, Koch and de Bont 1952, for the Chaffinch; Helms 1968, Odum and Major 1956, for the White-throated Sparrow; Odum 1960a for the Savannah Sparrow; and Rautenberg 1957, for the Brambling),and lipid deposition (King 1963, King and Farner 1959, King,Barker and Farner 1963, for the White-crowned Sparrow; Helms 1968, Odum and Perkinson 1951, for the White-throated Sparrow; Dol'nik and Blyumental 1967, for the Chaffinch and other european passerines; Odum 1960a for the Savannah Sparrow). A number of other characteristics of the premigratory state have also been reported, for example, increases in pectoralis muscle glycogen and lipid (King,Barker and Farner 1963 for the White-crowned Sparrow; George and Chandra-Bose 1967, Vallyathan 1963, and Vallyathan and George 1964, for the Rosy Pastor),and increases in liver lipid (King,Barker and Farner 1963 for White-crowned sparrow; Dol'nik and Blyumental 1967, for Garden Warbler, Willow Warbler and Chaffinch).

In the wild Willow Warblers captured in this study the two primary characteristics of the premigratory state were observed;

hyperphagia (inferred from the temporal pattern of the weight of the stomach contents, Chapter 5) and lipid deposition (using residual carcase lipid as a measure of adipose tissue lipid, Chapter 7). Whether other migratory characteristics were also present will be discussed below.

14.2 Physiological changes during the moult of juvenile Willow Warblers

As in other species of passerine studied (Dol'nik and Blyumental 1967, Evans 1969, Helms 1968, King and Farner 1959) low levels of carcase lipid were found during the moult (Chapter 7.4), compared to pre-moult and post-moult amounts. A corresponding decrease in total body weight also occurs (Chapter 4.3). However, this reduction in weight in the middle of moult is not due entirely to a decrease in body lipid. As shown in Chapter 9.6 there is a decrease in the total weight of the pectoralis muscles, which is primarily a reflection of changes in the weight of the fat-free dry material (= protein)(Chapter 9.9)and its associated water. The decrease in total muscle weights varied in the two years examined, but in 1970 it was quite substantial; it also appeared to involve the supracoracoideus. Part of the drop in body weight was due to a reduction in the weight of the residual carcase fat-free dry weight (Chapter 8.3) and, as suggested earlier, may well have been due to weight decreases of other skeletal muscles. This weight decrease of the flight muscles is unexpected, for it presumably reduces the thermoregulatory abilities of the bird, since heat production is only by muscle shivering (principally the M. pectoralis) (Hart 1962, Hart and Pohl 1963). This raises the

question of the thermal relations of juvenile Willow Warblers during their moult.

The standard metabolic rate of a 8 gm Willow Warbler is, according to the equation of Lasiewski and Dawson (1967), 670 J/hr. The mean overnight decrease in residual carcase lipid for juveniles at the peak of moult (stage 3) during 1971 was 245 mg (Chapter 7.4); assuming an energy content of the lipid of 3.8×10^7 J/kg (Johnston 1970), the total energy cost of the dark period was 9.28×10^3 J. Since at this time of year the night lasts about 7 hours, the basal energy cost over this time would be only 4.68×10^3 J, the difference of 4.6×10^3 J being the apparent overnight cost of thermoregulation. Unfortunately, there is no way of independently estimating the cost of overnight thermoregulation; Willow Warblers in autumn frequently experience temperatures approaching 0°C, but it is not known for how long, nor is the metabolic rate at these lower temperatures known. Consequently it is impossible to decide whether the overnight energy cost, estimated from the difference between morning and evening lipid levels, meets the actual costs of thermoregulation plus basal metabolic rate or whether some other source of energy is needed, such as muscle protein.

However, the overnight decrease in lipid levels found in moulted birds in 1971 was smaller than the mean decrease for those juveniles in moult stage 3. This comparison has been chosen, since in 1971 (but not 1970) the moulted birds were collected only from the beginning of the premigratory period, when the sample is more homogeneous than later in this period (when there is a higher

proportion of fat birds in evening samples, since many are migrating away from the area, and so will not be in the samples the following morning). The smaller overnight difference in lipid levels in moulted rather than moulting birds in 1971 means that the total energy cost overnight was smaller, only 8.85×10^3J, even though the night was longer (9hours). The basal energy cost for a nine hour night is 6.02×10^3J, so that the apparent cost of thermoregulation was 2.83×10^3J, even though the period of exposure to cold was longer. Since between moult stage 3 and the premigratory phase there is a mean increase in the protein content of the pectoralis muscle of morning-caught juveniles, it seems unlikely that the lower apparent cost of thermoregulation is produced by using greater quantities of muscle protein for thermogenesis (in this case the total cost of thermoregulation would probably be the same in both samples). Therefore, there must be a real increase in the insulative capacity of plumage between birds in the middle of the moult and birds that have completed moult. Indeed, as Chapter 3.5 illustrates, there is an increase in plumage dry weight over this period.

Although the data suggest that the reduced costs of thermoregulation in moulted birds are not due to the use of muscle protein for thermogenesis, they give no clue as to the cause of reductions in muscle protein weight in moulting birds. The increase in muscle protein weights between moulting and moulted juveniles could be due either directly to a cessation of feather growth (i.e. muscle proteins are no longer used for overnight feather growth as suggested by Ward (1969) and Newton (1968)), or indirectly to the increase in insulation by the plumage resulting in less or no

muscle protein being used as fuel for thermogenesis.

Independent evidence in favour of the latter view comes from the data on pectoralis energy reserves. The level of lipid provides no useful information since it is at a constant low level (Chapter 9.16) throughout the post-juvenile moult, and seems to follow the total amount of lipid in the body in a general way. The levels of muscle glycogen, whether expressed as a concentration or as a total, show a marked decrease during the moult (Chapters 9.20, 9.21); and as shown previously this is probably due to the low air temperatures over the pectoralis muscles during the moult (Chapter 9.23). On balance, therefore, since there does seem to be increased thermogenesis in the pectoralis muscles during the moult, it seems likely that the muscle proteins are being used for this purpose, rather than for feather growth. But why the remaining lipid of the body is not metabolised, when the system adopted here results in a decrease in the thermogenic capacity of the pectoralis muscles, I do not know.

Since the changes in pectoralis muscle composition seem to be mainly concerned with thermoregulation in the Willow Warbler, this species may differ from the Bullfinch (Newton 1968), in which it was suggested (without proof) that muscle proteins are used overnight for feather growth. This is a possibility because of the larger size of the bird, which makes thermal stresses less important; indeed there is little influence of the moult on lipid levels in this species. But Newton cites raised blood temperatures during the moult as evidence of increased thermogenesis,

so his speculation on the role of muscle protein as an aid to feather growth may be correct only in part. An experimental approach to his problem seems to be the only way to resolve it.

14.3 Premigratory changes in juvenile Willow Warblers

As mentioned in Chapter 14.1 a number of premigratory or migratory adaptations have been proposed in addition to lipid deposition mediated by hyperphagia. George and his co-workers have suggested that there is a switch in pectoralis muscle metabolism in favour of carbohydrate catabolism, and lipid synthesis (Vallyathan and George 1964). Undoubtedly, there is an increase in lipid synthesis in the pectoralis muscle in the Rosy Pastor during the premigratory (or more likely the migratory) period (George and Chandra-Bose 1967), since the capacity of the muscles to oxidise lipid is reduced (George and Iype 1964, George and Vallyathan 1964), as is the lipase activity (George and Vallathan 1964b). They cite the increase in muscle glycogen levels found between mid-winter and spring migration as evidence of this switch (Naik 1963, George and Chandra-Bose 1967, Vallyathan and George 1964), as well as a decrease in the utilisation of lipid during the day. In Willow Warblers, there is no evidence of such a switch from measurements of the morning levels of pectoralis glycogen. Indeed the increase found between moulting and moulted birds is a recovery from a mid-moult decrease, as the pre-moult and post-moult levels of muscle glycogen do not differ. Thus the situation appears to be different to that in the Rosy Pastor, in which there is no moult before the spring migration. It could be argued, however, that the metabolic condition of these muscles

before and after moult are the same; in this case the premigratory period and the immediate post-fledging period could both be times of muscle lipid synthesis. Measurements of muscle lipid in unmoulted birds indicate that this is not the case.

In Chapter 9.11 I have already examined the arguments of Fry et.al. (1972) for premigratory pectoralis muscle 'hypertrophy', and cast doubt on them. I need only add that they can be rejected outright only in the Willow Warbler, for as shown in Chapter 12.2 there is some 'hypertrophy' in Grasshopper Warblers, though it is not of the same nature as proposed by Fry et. al. (1972).

As pointed out in Chapter 11 the data on plasma glucose levels is eqivocal, due to the absence of any data from unmoulted juveniles. However, I interpret it as suggesting a real decrease in plasma glucose levels in premigratory birds. Such a decrease has been noted before (Dol'nik 1967) for the Chaffinch, and is of some importance in the interpretation of the hormonal control of fattening. Goodridge and Ball (1967b) found that in pigeons treated with ovine prolactin, there was a reduction in blood glucose and a decrease in the plasma half life of glucose, for the rate of synthesis of lipid from this substrate had increased to such an extent in the experimental birds that the glucose was withdrawn from the plasma more rapidly than in the controls. I would suggest that the decrease seen in Willow Warblers is due to such an effect, namely an increased rate of lipogenesis, since we have already seen (Chapter 10.13) that in premigratory birds the morning (when plasma glucose levels were measured) is a period of increased lipid accretion in the liver.

It has been inferred from the data on morning levels of plasma FFA that in premigratory birds there is a reduction in the lipolysis rates at the adipose tissue (Chapter 11.11). Such an adaptation has not been reported before, since most attention has been focussed on the lipogenic aspect of premigratory fattening; though, of course, the total increase in body lipid seen in migratory birds is the result of the difference in rates of lipogenesis and lipolysis. As plasma FFA levels depend on the rate of removal of FFA from the plasma as well as on the lipolysis rates at the adipose tissue, an alternative explanation is possible of the constancy of plasma FFA values in the face of increased weight of adipose tissue. Perhaps the rate of fatty acid removal from the plasma has increased, i.e. the rate of lipid utilization has increased during the premigratory period. From what we know of premigratory fattening in birds this is highly unlikely and the idea will not be developed further. Assuming a reduction in lipolysis rates it is not known whether this results from substrate feedback on the adipose tissue lipase or is mediated by the reduction of a lipolytic hormone, such as ACTH (Gibson and Nalbandov 1966a, Heald, McLachlan, and Rookledge 1965, Langslow and Hales 1969) or other pituitary hormone. Glucagon is a possible candidate (Langslow and Hales 1969), for Goodridge (1973) has recently shown that in the chick it inhibits liver lipogenesis.

The liver composition of premigratory and moulting Willow Warblers has been examined closely since this is the major lipogenic organ of birds (Goodridge and Ball 1967a). As shown in Chapters 10.3 and 10.4 there are no changes in fat-free wet weight or total wet weight of the liver during the post-juvenile moult; nor are there any changes in liver lipid concentrations until the premigratory period. Therefore, a comparison of moulting and moulted birds

seems reasonable.

Premigratory birds show an enhanced increase of liver fat-free wet weight during the morning compared to moulted birds, and this is due to increases in total water weight and total fat-free dry weight (=protein), accompanying this is an increase in the ratio of the two. This morning increase in liver weight is accompanied by a large increase in liver lipid concentration, and hence a large increase in the total weight of lipid in the liver. However, the liver lipid levels return to the same mean values each morning in both moulting and premigratory birds, so that if this increase in liver lipid represents de novo synthesis (as seems likely Chapter 10.12), all the lipid synthesised must be stored in the adipose tissue. Over the autumn there is a gradual flattening out of the diurnal increase in total liver weight, so that in premigratory birds there is little difference in mean weights between morning and evening samples, whereas in early stages of moult the difference might be as much as 100 mg. The reduction in the size of the evening peak weight also applies to liver glycogen, although in this case there is no difference between moulted and moulting birds in the morning. However, the peak evening level for liver lipid concentration is greater for moulted birds than for birds in moult; so not only is liver lipid accretion greater during the morning in premigratory individuals (compared to moulting birds), it is also greater during the afternoon also. Probably both this and the reduction in evening liver glycogen levels found in premigratory birds reflect an increased rate of lipid synthesis during the afternoon (Merkel 1958). Of course, moulting birds also showed an increase in liver lipid between morning and evening, but premigratory birds start this build

up earlier in the day, and it is of a greater magnitude. Oakeson (1953) has also found an increase in liver size during the premigratory period, in White-crowned Sparrows; however in her study the birds were caught at dawn. In my study there were no differences in liver size between moulting and moulted birds in samples caught at dawn, although the subsequent increase in the next six hours was much larger in the latter category.

The only other detailed study of liver composition during the premigratory period is the work of Dol'nik and Blyumental (1967) on the Chaffinch. As pointed out in Chapter 10.18, I do not accept their method of measurement of liver glycogen, but if we take their data at face value, that liver fat-free dry weight is precisely that, then there is a fair amount of agreement. As in Willow Warblers, they found that during the premigratory period in the Chaffinch there was a progressive decline of the evening level of fat-free dry weight of the liver (with date). However, unlike Willow Warblers premigratory birds did not have a larger gain in liver weight during the morning than moulting birds. As mentioned above they also found low blood sugar levels in fattening birds. Dol'nik (1967) suggested that the low levels of liver carbohydrate keep blood sugar levels low, and that carbohydrate is utilised preferentially to lipid during the premigratory period. Since he was not measuring carbohydrate this is untenable; more likely, low blood sugar and low evening levels of liver glycogen are the effects, rather than the cause, of lipid synthesis (as pointed out above). Farner et.al. (1961) also found a decrease in evening levels of liver glycogen in

in White-crowned Sparrows put on stimulatory 20-hour photoperiods. They suggested that this was due to increased glycolysis having a fat-sparing role. The data from the Willow Warblers does not permit a decision on this question. As shown by Morton (1967) premigratory birds do not eat much in the last few hours before dusk, and at this time glycogen could well be used preferentially to lipid. However, as shown earlier, in the morning liver glycogen levels are no lower for moulting than for premigratory birds. As the morning is a period of (apparently) liver lipid synthesis for premigratory birds the constancy of the glycogen levels indicates that they are apparently used neither for glycolysis or lipogenesis. The nature of the susbtrate for oxidation in the evening birds will have to await further studies.

14.4 The weight budget of migrating warblers

As shown in Chapter 9.12 a considerable percentage of the variations of total body weight of juvenile Willow Warblers during the premigratory period is associated with variation in the wet weights of the pectoralis muscles. In moulted birds this amounts to 68%, but in the middle of the moult it is only 9% (due to the weight reductions of the pectoralis muscles). The contribution of residual carcase fat-free dry weight to the variations in total body weight of moulted birds is only 11%; it is only important in moulting juveniles during the middle of the moult (60%) when the pectoralis muscles are reduced in size. Since variations in pectoralis wet weights have such a large influence on total body weight variations, this would be a good component of the total carcase to increase, if an increase in total body weight was required, in the premigratory period.

A potentially important source of variation in total body weight was not accounted for in this study (due to blood sampling), namely total body water weight. In Willow Warblers this is not important, since the total water weight of migrating Willow Warblers (Chapter 12.1) was not correlated with total body lipid weight. Of course, the total body water may vary in weight during the moult of juvenile Willow Warblers, as it does in the Lesser Redpoll(Evans 1969) or the Bullfinch (Newton 1968); but this will not be important from the point of view of flight ranges or times (Chapter 12.8) unless correlated with total body lipid weight.

In the Grasshopper Warbler (Chapter 12.2) both of these fractions, total body water and pectoralis muscle weight, are correlated with total lipid weight. This gives rise to an apparent hypertrophy of the pectoralis muscles, in that fatter birds have heavier pectoralis muscles. In fact this 'hypertrophy' is not of the same nature as that described by Fry et.al.(1972). Changes in the pectoralis weights of Grasshopper Warblers are due solely to changes in the weight of the water fraction; it is simply another manifestation of the increase in total water weight with the increase in weight of total body lipid. From a theoretical standpoint, if migratory birds are to increase their body weights in order to decrease flight times (Chapter 12.8) changes in weight of the water fraction will do it most effectively.

14.5 The hormonalcontrol of lipid deposition

Prolactin has, perhaps, the greatest number of multiple actions of any hormone known: 82 in 1971 (Nicoll and Bern,1972). These can be broadly grouped into actions related to water and electrolyte balance, growth, integumentary structures, reproduction,and synergism with steriods (Bern and Nicoll 1968, Nicoll and Bern 1972). Premigratory lipid deposition in birds belongs to the last category, since effects of prolactin injections can be enhanced by adrenal steriods (Meier and Martin 1971) or gonadal steriods (Stetson and Erickson 1972). However, in none of these investigations has the role of appetite been explored.

Prolactin injections can increase appetite in lizards (Licht and Hoyer 1968, Licht and Jones 1967) and in the non-migratory hypophysectomised pigeon (Bates, Miller and Garrison 1962). In the studies on migratory passerines (Meier and Farner 1964) this has been assumed. As shown in Chapter 13, appetite (as measured by food intake) is increased by prolactin injections in Bramblings, but this hyperphagia is not dependent on the time of injections of prolactin, nor does its magnitude depend on the dose administered. On the other hand, only those injections of prolactin given at the end of the photoperiod were able to produce fattening; and this fattening was dose-dependent. Prolactin fattened Bramblings showed increased body weight, increased adipose lipid weight and/or total lipid weight, and enlarged livers. Bramblings given prolactin injections four hours after the start of the photoperiod showed none of these symptoms. As shown in Experiment 3 (Chapter 12),Bramblings given 10 IU of prolactin at the end of the photoperiod, but only allowed to eat 4 gms of seed (no increase in appetite permitted) showed increases in body weight, adipose lipid (in some birds) and

liver size. Thus prolactin can cause increases in liver size (indicative of increased capability for lipid synthesis) independent of an increase in appetite. The reverse of this is found in Experiment 1; appetite can increase without a concomitant increase in liver size (lipid synthesis). Long photoperiods (15hrs)(without prolactin administration) produce larger increases in body weight, and about the same increase in liver size.

Two alternative hypotheses of the control of lipid depositon in migratory birds have been proposed; the first suggested by Meier and Martin (1971) proposes that prolactin acts synergistically with corticosterone to produce either weight increases or decreases depending on the temporal relationship of release of these two hormones, (see Figure 14.5.a). The second hypothesis, proposed by Stetson and Erickson (1972) is that gonadal steriods must be present for the expression of the fattening effect of endogenous prolactin (Figure 14.5.A). Figure 14.5.A shows a third alternative based on the results of the Brambling experiments: appetite is enhanced by secretion of endogenous prolactin, but whether lipid deposition occurs or not depends on the time of release; prolactin released during the latter half of the photoperiod is able to produce lipid deposition simply because there is substrate available at this time. I would argue that the synergistic effect with corticosterone found in White-throated Sparrows, is a reflection of the gluconeogenic effect of the steriod; it is simply making available substrate for lipid synthesis, since there is no increase in appetite in the photorefractory birds used in the experiments. Thus prolactin can act directly on the lipid synthesising capabilities of the liver,

Figure 14.5.a . Alternative schemes for the regulation of lipid deposition in migratory birds.

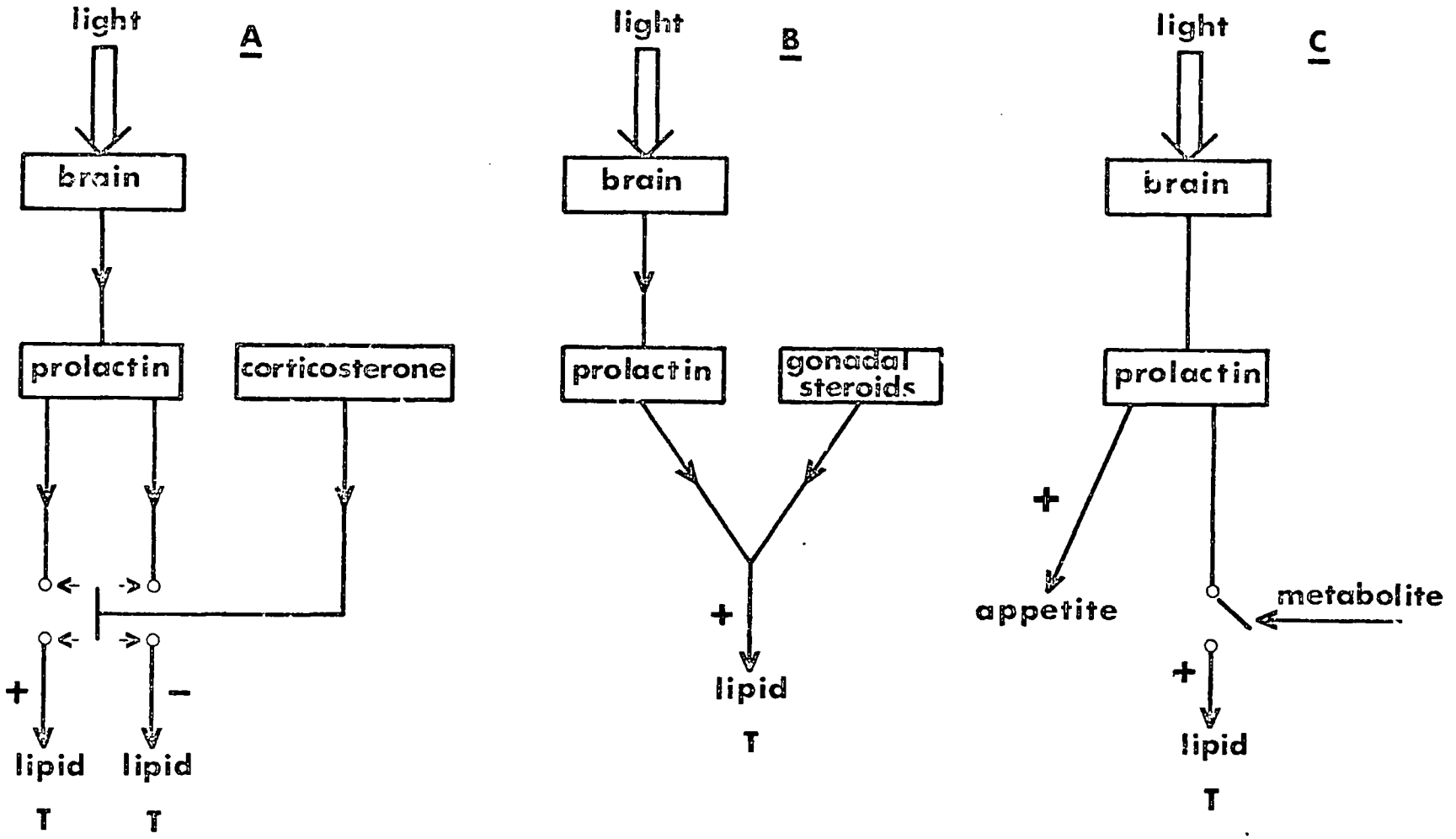
A - proposed by Meier and Martin (1971).

B - proposed by Stetson and Erickson (1972).

C - proposed in this study.

+ indicates an increase in lipid deposition or appetite, - a decrease in these parameters.

T indicates that lipid deposition depends upon the time of release of the hormone.


light
A
brain
prolactin
corticosterone
+
−
lipid
T
lipid
T
light
B
brain
prolactin
gonadal steroids
+
lipid
T
light
C
brain
prolactin
+
appetite
metabolite
+
lipid
T

but this only leads to lipid deposition when appropriate metabolites are provided. The best test of this hypothesis would be the measurement of the *in vitro* effect of prolactin on lipid synthesis in hepatocytes. As shown earlier (Chapter 13.25) the levels of gonadal steriods or gonadotropins are low in the birds used in my experiments. Its is possible that both or either of these could regulate the release of prolactin (Weise 1967, Stetson and Erickson 1972) but I have no evidence on this; however, Schildmacher and Steubing (1952) did produce small increases in body weight with photosensitive Bramblings with injections of testosterone. It is, however, unlikely that such a mechanism would be important in the autumnal premigratory period as the testes are regressed; yet in some species autumn lipid deposition is faster and more extensive than in the spring.

Fimally, the mechanisms of lipid deposition in the feral Willow Warblers examined in this study are not clear. There is an increase in liver size during the day in premigratory birds, but it is temporary. Each day the liver returns to its former dawn weight. However, this could be due to the thermal environment of these birds, as liver proteins as well as lipid may be needed for overnight thermogenesis. On balance, however, it does seem that prolactin is probably involved, as lipid synthesis is accompanied by more rapid increase in liver wet weight in premigratory birds, compared to moulting birds. As shown above, this is probably not a result of increased appetite but is produced independently.

References

Amadon, D. (1966). Avian plumages and molts. Condor 68:263 .

Baggott, G.K. (1970). The timing of the moults of the Pied Wagtail. Bird Study 17:45.

Bates, R.W., Miller, R.A. and Garrison, M.M. (1962). Evidence in the hypophysectomized pigeon of a synergism among prolactin, growth hormone, thyroxine and prednisone upon the weight of the body, digestive tract, kidney and fat stores. Endocrinology 71:345.

Bell, D.J. (1957). The distribution of glucose between the plasma water and the erythrocyte water in hens blood. Q. Jl exp. Physiol. 42:410 .

Bell, D.J. (1971). Plasma glucose. In, Physiology and Biochemistry of the Domestic Fowl. Eds. D.J.Bell and B.M.Freeman. Academic Press: London, New York. p 913.

Bellrose, F. (1967). Radar in orientation research. Proc. XIV Int. orn. Congr. :281.

Bergman, G. (1950). Zur Zugphysiologie der Drosseln (Turdus pilaris L., T. merula L., und T. musicus L.). Über der Blutzuckerspiegel, Körpertemperatur und Einwirkung von Adrenalin. Soc. Scient. Fenn., Comm. Biol. 10:1 .

Bern, H.A. and Nicoll, C.S. (1968). The comparative endocrinology of prolactin. Recent Progr. Horm. Res. 24:681 .

Berthold, P., Gwinner, E. and Klein, H. (1971). Circannuale Periodik bei Grasmücken (Sylvia) . Experientia 27:399 .

Chandra-Bose, D.A. and George, J.C. (1967). Effects of cold stress on the glycogen content of the pectoralis and the liver of the pigeon. Pavo 5:9 .

Chandrabose, K.A. and Bensadoun, A. (1971a). Lipolytic activity of isolated fat cells from intact and hypophysectomized cockerels, Gallus domesticus . Int. J. Biochem. 2:585 .

Chandrabose, K.A. and Bensadoun, A. (1971b). Effect of hypophysectomy on liver enzymes in the chicken, Gallus domesticus : replacement therapy with growth hormone and thyroxine. Comp. Biochem. Physiol. 39B: 55 .

Chandrabose, K.A., Bensadoun, A. and Clifford, R. (1971). Diurnal rhythms of liver enzymes in the chicken, Gallus domesticus . Int. J. Biochem. 2:581 .

Child, G.I. (1969). A study of nonfat weights in migrating Swainson's Thrushes (Hylocichla ustulata). Auk 86:327.

Curry-Lindahl, K. (1963). Molt, body weights, gonadal development and migration in Motacilla flava . Proc. XIII Int. orn. Congr. :960.

De Oya, M., Prigge, W.F., Swenson, D.E. and Grande, F. (1971). The role of glucagon on fatty liver production in birds. Am. J. Physiol. 221:25 .

Dole, V.P. and Meinertz, H. (1960). Microdetermination of long-chain fatty acids in plasma and tissues. J. biol. Chem. 235:2595 .

Dol'nik, V.R. (1966). Izmeninii scothosheniya zhirovogo i ugtevodnogo obmena kak endogennyi stimul k migratsii in ptits. (Changes in the ratio of fat and carbohydrate metabolism as an endogenous stimulus to migration in birds). Zool. Zh. 45:897 .

Dol'nik, V.R. (1967). Ovzaimosoyazi zhirovykh reservov perdetnykh ptits i migratsii. (On the relationship between fat deposition in migratory birds and migration). Trudy. zool. Inst., Leningr. 40:96 .

Dol'nik, V.R. (1968). Rol' zhirovykh depo v regulyatsii metabolizma i povedeniya ptits vo vremya migratsii (The role of fat deposits in metabolism regulation in bird behaviour during migration). Zool. Zh. 47:1205 .

Dol'nik, V.R. (1970a). Fat metabolism and bird migration. In, La photoregulation de la reproduction chez les oiseaux et les mammiferes. Eds. J.Benoit and I. Assenmacher. Colloques int.Cent. natn. Rech. scient. No. 172:351 .

Dol'nik, V.R. (1970b). Water storage by migratory fat deposition in Passer domesticus bactrianus - the arid zone migrant. International Studies on Sparrows 4:74.

Dol'nik, V.R. and Blyumental, T. (1967). Autumnal premigratory and migratory periods in the Chaffinch (Fringilla coelebs coelebs) and some other temperate-zone passerine birds. Condor 69:435 .

Dusseau, J,W. and Meier, A.H. (1971). Diurnal and seasonal variations of plasma adrenal steroid hormone in the White-throated Sparrow, Zonotrichia albicollis . Gen. comp. Endocr. 16:399.

Dwight, J. (1900). The sequence of plumages and moults of the passerine birds of New York. Annals N.Y. Acad. Sci. 13:73 .

Evans, P.R. (1966). Autumn movements, moult and measurements of the Lesser Redpoll Carduelis flammea cabaret. Ibis 108:183 .

Evans, P.R. (1969). Ecological aspects of migration and pre-migratory fat deposition in the Lesser Redpoll Carduelis flammea cabaret . Condor 71:316 .

Farner, D.S. (1959). Photoperiodic control of annual gonadal cycles in birds. In, R.B. Withrow (ed), Photoperiodism and related phenomena in plants and animals. Amer. Ass. Advance Sci., Washington, D.C. p 717.

Farner, D.S., Oksche, A., Kamemoto, F.I., King, J.R. and Cheyney, H.E. (1961). A comparison of the effect of long daily photoperiods on the pattern of energy storage in migratory and non-migratory finches. Comp. Biochem. Physiol. 2:125 .

Fisher, H.I. and Bartlett, L.M. (1957). Diurnal cycles in liver weights in birds. Condor 59:364 .

Folch, J., Lees, M. and Sloane Stanley, G.H.S. (1957). A simple method for isolation and purification of total lipides from animal tissues. J. biol. Chem. 226:497 .

Follett, B.K. and Sharp, P.J. (1969). Circadian rhythmicity in photoperiodically induced gonadotropin release and gonadal growth in the quail. Nature, Lond. 223:968 .

Fry, C.H., Ash, J.S. and Ferguson-Lees, I.J. (1970). Spring weights of some palaearctic migrants at Lake Chad. Ibis 112:58 .

Fry, C.H., Ferguson-Lees, I.J. and Dowsett, R.J. (1972). Flight muscle hypertrophy and ecophysiological variation of Yellow Wagtail Motacilla flava races at Lake Chad. J. Zool., Lond. 167:293 .

George, J.C. and Berger, A.J. (1966). Avian myology. Academic Press: New York, London.

George, J.C. and Chandra-Bose, D.A. (1967). Diurnal changes in glycogen and fat levels in the pectoralis of the migratory starling, Sturnus roseus. Pavo 5:1 .

George, J.C. and Iype, P.T. (1964). Fatty acid oxidation by breast muscle homogenates of a migratory and a non-migratory starling. Pavo 2:84 .

George, J.C., Susheela, A.K. and Vallyathan, N.V. (1964). Histochemical evidence for biochemical differentiation and regional specialization in the breast muscle of the House Sparrow. Pavo 2:115 .

George, J.C. and Vallyathan, N.V. (1964a). Capacity for fatty acid oxidation by breast muscle of the starling (Sturnus roseus) in the pre- and post-migratory periods. Can. J. physiol. Pharmacol. 42:447 .

George, J.C. and Vallyathan, N.V. (1964b). Lipase and succinic dehydrogenase activity of the particulate fractions of the breast muscle homogenate of the migratory starling Sturnus roseus in the pre-migratory and post-migratory periods. J. cell. comp. Physiol. 63:381 .

Gibson, W.R. and Nalbandov, A.V. (1966a) Lipid mobilization in obese hypophysectomized cockerels. Am.J. Physiol. 211:1345 .

Gibson, W.R. and Nalbandov, A.V. (1966b). Lipolysis and lipogenesis in liver and adipose tissue of hypophysectomized cockerels. Am. J. Physiol. 211:1352 .

Goldspink, G. (1962). Biochemical and physiological changes associated with the postnatal development of the biceps brachii. Comp. Biochem. Physiol. 7:157 .

Goldspink, G. (1970). The proliferation of myofibrils during muscle fibre growth. J. Cell. Sci. 6:593 .

Goodridge, A.G. (1964). The effect of insulin, glucagon and prolactin on lipid synthesis and related metabolic activity in migratory and non-migratory finches. Comp. Biochem. Physiol. 13:1 .

Goodridge, A.G. (1973). Regulation of fatty acid synthesis in isolated hepatocytes prepared from the livers of neonatal chicks. J. biol. Chem. 248:1924 .

Goodridge, A.G. and Ball, E.G. (1965). Studies on the metabolism of adipose tissue, XVIII. In vitro effects of insulin, epinephrine, and glucagon on lipolysis and glycolysis in pigeon adipose tissue. Comp. Biochem. Physiol. 16:367 .

Goodridge, A.G. and Ball, E.G. (1967a). Lipogenesis in the pigeon: in vivo studies. Am. J. Physiol. 213:245 .

Goodridge, A.G. and Ball, E.G. (1967b). The effect of prolactin on lipogenesis in the pigeon. *In vivo* studies. Biochemistry 6:1676 .

Goodridge, A.G. and Ball, E.G. (1967c). The effect of prolactin on lipogenesis in the pigeon. *In vitro* studies. Biochemistry 6:2335 .

Grande, F. (1970). Effects of glucagon and insulin on plasma free fatty acids and blood sugar in owls. Proc. Soc. exp. Biol. Med. 133:540 .

Grande, F. and Prigge, W.F. (1970). Glucagon infusion, plasma FFA and triglycerides, blood sugar, and liver lipids in birds. Am. J. Physiol. 218:1406 .

Greenewalt, C.H. (1960). Dimensional relationships for flying animals. Greenville, Delaware (privately printed).

Grinyer, I. and George, J.C. (1969). An electron microscopic study of the pigeon breast muscle. Can. J. Zool. 47:517.

Groebbels, F. (1932). Der Vogel, Bd. I. Berlin: Gebr. Borntraeger.

Gwinner, E. (1968). Circannuale Periodik als Grundlage des Jahreszeitlichen Funktionwandels bei Zugvögeln. Untersuchungen am Fitis (*Phylloscopus trochilus*) und am Waldlaubsänger (*P. sibilatrix*). J. Orn., Lpz. 109:70 .

Gwinner, E. (1969). Untersuchungen zur Jahresperiodik von Laubsängern. Die Entwicklung des Gefieders, des Gewichts und der Zugunruhe bei Jungvögeln der Arten *Phylloscopus bonelli*, *Ph. sibilatrix*, *Ph. trochilus* und *Ph. collybita* . J. Orn., Lpz. 110:1 .

Gwinner, E. (1970). Vergleichende Untersuchung der Jugendentwicklung eines ausgeprägten Zugvogels, *Sylvia borin*, und eines weniger ausgeprägten Zugvogels, *S. atricapilla*. Die Vogelwarte 25:297 .

Harman, J.W. (1956). The cytochondria of cardiac and skeletal muscle. Intern. Rev. Cytol. 5:89 .

Hart, J.S. (1962). Seasonal acclimatization in four species of small wild birds. Physiol. Zoöl. 35:224 .

Hart, J.S. and Pohl, H. (1963). Metabolism and shivering in evening grosbeaks (Hesperiphona vespertina) during acclimation to cold in the laboratory. Proc. XVI Int. orn. Congr. 2:61 .

Hartman, F.A. (1961). Locomotor mechanisms of birds. Smithsonian Inst. Misc. Collections 143:1 .

Hazelwood, R.L. and Lorenz, F.W. (1959). Effects of fasting and insulin on carbohydrate metabolism of the domestic fowl. Am. J. Physiol. 197:47 .

Heald, P.J., McLachlan, P.M. and Rookledge, K.A. (1965). The effects of insulin, glucagon and adrenocorticotrophic hormone on plasma glucose and free fatty acids of the domestic fowl. J. Endocr. 33:83 .

Helms, C.W. (1968). Food, fat and feathers. Am. Zoologist 8:151 .

Hissa, R. and Palokangas, R. (1970). Thermoregulation in the titmouse (Parus major L.). Comp. Biochem. Physiol. 33:941 .

Johnston, D.W. (1968). Body characteristics of Palm Warblers following an overwater flight. Auk 85:13 .

Johnston, D.W. (1970). Caloric density of avian adipose tissue. Comp. Biochem. Physiol. 34:827 .

Joseph, M.M. and Meier, A.H. (1973). Daily rhythms of plasma corticosterone in the common pigeon, Columba livia. Gen. comp. Endocr. 20: 326.

Kemp, A. and Kits van Heijningen, J.M. (1954). A colorimetric micro-method for the determination of glycogen in tissues. Biochem. J. 56:646 .

King, J.R. (1961a). The bioenergetics of vernal premigratory fat deposition in the White-crowned Sparrow. Condor 63:128 .

King, J.R. (1961b). On the regulation of vernal premigratory fattening in the White-crowned Sparrow. Physiol. Zoöl. 34:145 .

King, J.R. (1963). Autumnal migratory-fat deposition in the White-crowned Sparrow. Proc. XIII Int. orn. Congr. :940 .

King, J.R., Barker, S. and Farner, D.S. (1963). A comparison of energy reserves during the autumnal and vernal migratory periods in the White-crowned Sparrow, Zonotrichia leucophrys gambelii. Ecology 44:513 .

King, J.R. and Farner, D.S. (1959). Premigratory changes in body weight and fat in wild and captive White-crowned Sparrows. Condor 61:315 .

King, J.R. and Farner, D.S. (1963). The relationship of fat deposition to Zugunruhe and migration. Condor 65:200 .

King, J.R. and Farner, D.S. (1965). Studies on fat deposition in migrating birds. Ann. N.Y. Acad. Sci. 131:422 .

King, J.R., Farner, D.S. and Morton, M.L. (1965). The lipid reserves of the White-crowned Sparrow on the breeding grounds in central Alaska. Auk 82:236 .

Koch, H.J. and de Bont, A.F. (1952). Standard metabolic rate, weight changes and food consumption of Fringilla c. coelebs L., during sexual maturation. Ann. Soc. zool. Belg. 82:143.

Kuenzel, W.J. and Helms, C.W. (1970). Hyperphagia, polydipsia and other effects of hypothalamic lesions in the White-throated Sparrow, Zonotrichia albicollis. Condor 72:66 .

Kuroda, N. (1961). A note on the pectoral muscles of birds. Auk 78:261 .

Lack, D. and Eastwood, E. (1962). Radar films of migration over eastern England. Brit. Birds 55:388 .

Langslow, D.R., Butler, E.J., Hales, C.N. and Pearson, A.W. (1970). The reponse of plasma insulin, glucose and non-esterified

fatty acids to various hormones, nutrients and drugs in the domestic fowl. J. Endocr. 46:243 .

Langslow, D.R.and Hales, C.N. (1969). Lipolysis in chicken adipose tissue in vitro. J. Endocr. 43:285 .

Laurell, S. and Tibbling, G. (1967). Colorimetric micro-determination of free fatty acids in plasma. Clin. Chim. Acta. 16:57 .

Lasiewski, R.C. and Dawson, W.R. (1967). A re-examination of the relation between standard metabolic rate and body weight in birds. Condor 69:13 .

Lasiewski, R.C., Galey, F.R. and Vasquez, C. (1965). Morphology and physiology of the pectoral muscles of hummingbirds. Nature 206:404 .

LeFebvre, E.A. (1964). The use of D_2O^{18} for measuring metabolism in Columba livia at rest and in flight. Auk 81:403 .

Lepovsky, S. (1973). Hypothalamic-adipose tissue interrelationships. Fedn. Proc. Fedn. Am. Socs exp. Biol. 32:1705 .

Licht, P. and Hoyer, H. (1968). Somatotropic effects of exogenous prolactin and growth hormone in juvenile lizards (Lacerta s. sicula). Gen. comp. Endocr. 11:338 .

Licht, P. and Jones, R.E. (1967). Effects of exogenous prolactin on reproduction and growth in adult males of the lizard Anolis carolinensis . Gen. comp. Endocr. 8:228 .

Lofts, B. and Marshall, A.J. (1960). The experimental regulation of Zugunruhe and the sexual cycle in the Brambling Fringilla montifringilla. Ibis 102:209 .

Lofts, B., Marshall, A.J. and Wolfson, A. (1963). The experimental demonstration of pre-migration activity in the absence of fat deposition in birds. Ibis 105:99 .

Meier, A.H. (1969). Diurnal variations of metabolic response to prolactin in lower vertebrates. Gen. comp. Endocr. Suppl. 2:55 .

Meier, A.H. and Davis, K.B. (1967). Diurnal variations of the fattening reponse to prolactin in the White-throated Sparrow, Zonotrichia albicollis. Gen. comp. Endocr. 8:110.

Meier, A.H. and Dusseau, J.W. (1968). Prolactin and the photoperiodic gonadal response in several avian species. Physiol. Zool. 41:95 .

Meier, A.H. and Farner, D.S. (1964). A possible endocrine basis for premigratory fattening in the White-crowned Sparrow, Zonotrichia leucophrys gambelii (Nuttall). Gen. comp. Endocr. 4:584 .

Meier, A.H. and Martin, D.D. (1971). Temporal synergism of corticosterone and prolactin controlling fat storage in the White-throated Sparrow, Zonotrichia albicollis. Gen. Comp. Endocr. 17:311 .

Meier, A.H., Martin, D.D. and MacGregor, R. (1971). Temporal synergism of corticosterone and prolactin controlling gonadal growth in sparrows. Science, N.Y. 173:240 .

Meier, A.H., Trobec, T.N., Joseph, M.M. and John, T.M. (1971). Temporal synergism of prolactin and adrenal steroids in the regulation of fat stores. Proc. Soc. exp. Biol. Med. 137:408 .

Mendel, B., Kemp, A. and Myers, D.K. (1954). A colorimetric micro-method for the determination of glucose. Biochem. J. 56:639 .

Merkel, F.W. (1958). Untersuchungen über tages- und jahresperiodische Änderungen in Energieaushalt gekäftiger Zugvögel. Z. vergl. Physiol. 41:154 .

Mialhe, P. (1969). Some aspects of the regulation of carbohydrate metabolism in birds. Proceedings of 3rd International Conference on Endocrinology. p 158 .

Miller, A.H. (1928). The molts of the Loggerhead Shrike, Lanius ludovicianus, Linnaeus. Univ. Calif. Publ. Zool. 30:393 .

Morton, M.L. (1967). Diurnal feeding patterns in White-crowned Sparrows, Zonotrichia leucophrys gambelii. Condor 69:491 .

Naik, D.V. (1963). Seasonal variation in the metabolites of the liver of the Rosy Pastor, Sturnus roseus (Linnaeus). Pavo 1:44 .

Nakamura, T. (1962). The change of body lipid amount and some chemical observations in migratory Eastern Great Reed-Warbler in the breeding ground. Misc. Rep. Yamashina Inst. Orn. Zool. 3:51 .

Newton, I. (1968). The temperatures, weights and body composition of molting Bullfinches. Condor 70:323 .

Newton, I. (1969). Moults and weights of captive Redpolls Carduelis flammea. J. Orn., Lpz. 110:53 .

Nicoll, C.S. and Bern, H.A. (1972). On the actions of prolactin among the vertebrates: is there a common denominator? In, Lactogenic Hormones. Eds, G.E.W. Wolstenholme and J.Knight. Churchill Livingstone:London, Edinburgh. p 299.

Oakeson, B.N. (1953). Cyclic changes in liver and spleen weights in migratory White-crowned Sparrows. Condor 55:3 .

Oakeson, B.B. (1956). Liver and spleen weights in non-migratory White-crowned Sparrows. Condor 58:45 .

Odum, E.P. (1960a). Premigratory hyperphagia in birds. Am. J. clin. Nutr. 8:621 .

Odum, E.P. (1960b). Lipid deposition in nocturnal migrant birds. Proc. XII Intern. orn. Congr. :563 .

Odum, E.P., Connell, C.E. and Stoddard, H.L. (1961). Flight energy and estimated flight ranges of some migratory birds. Auk 78:515.

Odum, E.P. and Major, J.C. (1956). The effect of diet on photoperiod-induced lipid deposition in the White-throated Sparrow. Condor 58:222 .

Odum, E.P. and Perkinson, J.D. (1951). Relation of lipid metabolism to migration in birds: seasonal variations in body lipids of the migratory White-throated Sparrow. Physiol. Zoöl. 24:216 .

O'Hea, E.K. and Leveille, G.A. (1968). Lipogenesis in isolated adipose tissue of the domestic chick (Gallus domesticus). Comp. Biochem. Physiol. 26:111 .

Pearson, D.J. (1973). Moult of some palaearctic warblers wintering in Uganda. Bird Study 20:24 .

Pennycuick, C.J. (1969). The mechanics of bird migration. Ibis 111:525 .

Pohl, H. (1971). Seasonal variation in metabolic functions of Bramblings. Ibis 113:185 .

Rautenberg, W. (1957). Vergleichende Untersuchungen über den Energiehaushalt des Bergfinken (Fringilla montifringilla L.) und des Haussperlings (Passer domesticus). J. Orn., Lpz. 98:36 .

Rogers, D.T. and Odum, E.P. (1966). A study of autumnal postmigrant weights and vernal fattening of North American migrants in the tropics. Wilson Bull. 78:415 .

Rowan, W. (1925). Relation of light to bird migration and developmental changes. Nature, Lond. 115:494 .

Salt, W.R. (1963). The composition of the pectoralis muscles of some passerine birds. Can. J. Zool. 41:1185 .

Samols, E., Tyler, J.M., Marks, V. and Mialhe, P. (1969). The physiological role of glucagon in different species. Proceedings of 3rd International Conference on Endocrinology. p 206.

Schildmacher, H. and Steubing, L. (1952). Untersuchungen zur hormonalen Regulierung des Fettwerdens der Zugvögel im Frühjahr. Biol. Zbl. 71:17 .

Schnell, G.D. (1965). Recording the flight-speed of birds by Doppler radar. Living Bird 4:79 .

Siegel, S. (1956). Nonparametric statistics for the behavioral sciences. McGraw-Hill: New York.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. Iowa State University Press.

Snow, D.W. (1967). The moult of British Thrushes and Chats. Bird Study 16:115 .

Sokal, R.R. and Rohlf, F.J. (1969). Biometry. The principles and practice of statistics in biological research. Freeman: San Francisco.

Soulairac, A. (1946). Influence of the adrenals on intestinal absorption of glucose. Comp. Rend. Soc. Biol. 140:253 .

Spencer, R. (1972). Report on bird-ringing for 1970. Bird Study 19: suppl.

Stearner, S.P., Sanderson, M.H., Christian, E.J. and Brues, A.M. (1958). Evidence of circulatory failure in the early radiation repsonse of the chicken. Am. J. Physiol. 192:620 .

Stetson, M.H. (1971). Neuroendocrine control of photoperiodically induced fat deposition in White-crowned Sparrows. J. exp. Zool. 176:409 .

Stetson, M.H. and Erickson, J.E. (1972). Hormonal control of photoperiodically induced fat deposition in White-crowned Sparrows. Gen. comp. Endocr. 19:355 .

Tucker, V.A. (1968). Respiratory exchange and evaporative water loss in the flying budgerigar. J. exp. Biol. 48:67 .

Tucker, V.A. (1970). Energetic cost of locomotion in animals. Comp. Biochem. Physiol. 34:841 .

Tucker, V.A.(1971). Flight energetics in birds. Am. Zoologist 11:115 .

Tucker, V.A. (1972). Metabolism during flight in the laughing gull,

Larus atricilla. Am. J. Physiol. 222:237 .

Tucker, V.A. (1973). Bird metabolism during flight: evaluation of a theory. J. exp. Biol. 58:689 .

Tucker, V.A. and Schmidt-Koenig, K. (1971). Flight speeds of birds in relation to energetics and wind directions. Auk 88:97 .

Vallyathan, N.V. (1963). On the lipid content and lipase activity in the breast muscle of Sturnus roseus (Linnaeus). Pavo 1:106 .

Vallyathan, N.V. and George, J.C. (1964). Glycogen content and phosphorylase activity in the breast muscle of the migratory starling, Sturnus roseus (Linnaeus). Pavo 2:55 .

Ward, P. (1969). The annual cycle of the Yellow-vented Bulbul Pycnonotus goiavier in a humid equatorial enviroment. J. Zool., Lond. 157:25 .

Weise, C.M. (1967). Castration and spring migration in the White-throated Sparrow. Condor 69:49 .

Wolfson, A. (1945). The role of the pituitary, fat deposition and body weight in bird migration. Condor 47:95 .

Wolfson, A. (1954). Production of repeated gonadal, fat and molt cycles within one year in the Junco and White-crowned Sparrow by manipulation of daylength. J. exp. Zool. 125:353.

Wolfson, A. (1960). Role of light and darkness in the regulation of the annual stimulus for spring migration and reproductive cycles. Proc. XII Int. orn. Congr. :758 .

Yarborough, C.G. and Johnston, D.W. (1965). Lipid deposition in wintering and premigratory Myrtle Warblers. Wilson Bull. 77:175 .

Appendix 1. Details of significance tests.

These are classified as to section, and the numbers refer to the numbers in parenthesis within the body of the text. Where an equal variance t-test is performed (variances not significantly different) the degrees of freedom are given; where an unequal variance t-test is used (variances significantly different) the sample sizes are given. df - degrees of freedom; U - Mann-Whitney U; n - sample size; p - probability than the null hypothesis is correct.

3.5

(1) $\chi^2 = 2.92$, 4 df , $.75 > p > .5$

(2) F = 3.98 4,94 df

(3) $\chi^2 = 5.67$, 5 df, $.5 > p > .25$

(4) F = 17.9 5,103 df

(5) t = 3.0 54 df

3.6

(1) $SE_b = 0.0026$, t=3.99 , 5df, $.025 > p > .01$

4.3

(1) $\chi^2 = 7.7$, 4 df, $.25 > p > .1$

(2) F = 1.01 4,66 df

(3) F = 2.1 2.17 df

5.3

(1) U = 57.5 n - 20,13

(2) U = 36 n - 20,7

(3) U = 47 n - 13,7

(4) U = 53 n - 11,8

(5) U = 33 n - 7.14

7.3

(1) all $p > .1$

(2) U = 18 n - 7,12

(3) U = 31 n - 9,9

(4) U = 15 n - 7,9

(5) z = 3.1 n - 26,11

Medians of the morning periods referred to in tests 2 to 5.

Hours after sunrise	Moulting	Moulted
0-2	0.19	0.32
2-4	0.22	0.35
4-6	0.25	0.52

(6) z = 2.1 n- 21,15

(7) z = 1.5 n - 9,27 p=.14

(8) U = 37 n - 8,19

(9) U = 28 n - 7,19

(10) z = 4 n - 7,24

(11) z = 2.6 n - 7,27

(12) z = 1.7 n - 10,24

8.3

(1) χ^2 = 1.2, 4 df, .9>p>.75

(2) χ^2 = 4.1, 5 df, .75>p>.5

(3) F = 1.2 4,73 df

(4) t = 1.9 96 df

(5) F = 0.6 5,85 df

(6) t = 2.5 105 df

9.2

(1) moult stage 1 .5>p>.4; moult stage 2 .4>p>.2; moult stage 3 .4>p>.2; moult stage 4 .4>p>.2.

(2) t = 2.7 60df

9.4

(1) t = 8.9 41 df

9.6

(1) χ^2 = 2.7 , 4 df, .75>p>.5

(2) F = 7.1 4,101 df

(3) χ^2 = 1.1, 5 df, .98>p>.95

(4) F = 6.8 5,71 df

9.7

(1) χ^2 = 8 , 3df , .05> p >.025

(2) F = 1.7 3,42 df, .25> p >.1

9.8

(1) χ^2 = 2.4 , 4df, .75> p >.5

(2) F = 3.8 4,55df

(3) χ^2 = 0.82, 5 df, .99 >p >.98

(4) F = 0.7 5,46 df

9.9

(1) χ^2 = 1.2 , 4df , .9 >p >.75

(2) F = 4.1 4,54 df

(3) χ^2 = 0.68, 5 df, .98 >p >.95

(4) F = 1.6 5,36 df

9.10

(1) χ^2 = 2.6, 3 df, .5 p >.25

(2) F = 1.9 3,42 df

(3) t = 1.2 n - 46, 22

(4) t = 2.0 n - 10,22

(5) t = 3.7 17 df

(6) t = 2.2 n - 9,9

(7) t = 5.5 13 df

9.15

(1) χ^2 = 5.6, 3df , .25 >p >.1

(2) F = 2.5 3,42 df

(3) t = 3.4 n - 46, 13

(4) χ^2 = .11, 4df, p>.99

(5) F = 1.3 4,28 df

(6) t = 2.7 n - 33,9

9.16

(1) χ^2 = .95, 3 df, .9 >p >.75

(2) F = 3.2 3,42 df

(3) t = 3.4 n - 46,22

(4) χ^2 = .08 , 4 df, p>.99

(5) F = 0.47 4,28 df

(6) t = 2.5 n - 33,9 df

(7) t = 3.0 16 df

(8) t = 1.9 26 df

(9) t = .08 20 df

(10) t = 1.7 14 df

9.20

(1) H = 12.2 4 df

9.21

(1) H = 10.9 4 df

10.3

(1) χ^2 = 4.7, 4 df, .5>p>.25

(2) F = 2.3 4,132 df

(3) χ^2 = 10.1 , 5 df, .1>p>.05

(4) F = 1.7 5,85 df

10.4

(1) χ^2 = 2.8 4 df, .75>p>.5

(2) F = 1.9 4,70 df

(3) χ^2 = 7.9 5 df, .25>p>.1

(4) F = 1.6 5, 77 df

10.5

(1) F = 4.22 p<.05

(2) F = 1.43 2,40 df p>.2; mean for moult stage 4 vs overall mean for moult stages 1,2,3 t = 0.55 n10,43 , p .05

(3) t = 2.84 n21,23

(4) t = 1.61 n - 11,23 .2>p>.1

(5) χ^2 = 4.6 , 4 df, .3>p>.2

(6) F = 0.1 4,54 df

(7) t = 2.2 80 df

10.6

(1) $\chi^2 = 7.7$, 5 df, $.25 > p > .1$

(2) F = 3.8 4,60 df

(3) 0-2 vs 3-5 moulting U = 62 $p > .1$; 0-2 vs 2-3 moulting $p > .1$;
2-3 vs 3-5 moulting U = 70 $p > .1$; 0-2 vs 3-5 moulted
U = 72 $p > .05$.

(4) t = 3.8, 15 df

(5) 1/2 evening vs 4-6 , 1970 t = 6.3 $p < .001$; 3 evening vs
4-6, 1970 t = 3.6 $.01 > p > .001$.
1971 U = 0 in all cases, 1/2 vs 3-5 $p < .002$, 3 vs 3-5 p= 0

(6) t = .63 23 df $p > .5$

(7) 1970 evening moulted vs 4-6, t = .89 23 df $.4 > p > .2$;
1970 evening moulted vs 3-5, 1971 U = 15 $.02 > p > .002$;
1971 evening moulted vs 3-5, 1971 U = 9 p = .051

(8) 1970 t = 4.8 23 df $p < .001$: 1971 t = 4.8 18 df $p < .001$

(9) 1971 t = 1.8 15 df, $.1 > p > .05$; 1970, t = 1.4 20 df, p = .2

10.7

(1) z = 1.8 p =.08

(2) t = 2.6 25 df

(3) 0-2 vs 2-3 z = 1.1 p = .26

(4) 2-3 vs 3-5 z = 0.1 p = .9

(5) t = 3.2 14 df

(6) U = 0 in all cases $p < .002$

(7) H = 13.1 2 df

(8) z = 2.9 n - 22,26

(9) z = 2.3 n - 26,23

(10) z = .5 n - 21,8

(11) 1970 z = 2.9 n - 27,26

(12) 1/2 evening vs moulted z = 3.7 $p < .0002$
3 evening vs moulted z = 2.1 $p < .02$

10.8

(1) 0-2 vs 4-6 moulting U = 39 $p > .1$

0-2 vs 4-6 moulted U = 31 .1> p> .05

(2) 4-6 moulting vs 4-6 moulted U = 45 n - 10,10 p>.1

(3) 0-2 vs 2-3 t = .98 n - 15,11

2-3 vs 3-5 t = .9 n - 10,11

(4) t = .06 27df p>.1

(5) U = 23 p = .31, n - 9,7

(6) 1970, 4-6 vs 1/2 U = 0 p<.002; 1970, 4-6 vs 3 U = 0 p<.002

(7) Evening 1/2 vs moulted evening U = 90 z = .09

Evening 3 vs moulted evening U = 74 z = .29

(8) U = 1 p<.002 , 1970.

10.11

(1) χ^2 = 7.8 , 5 df, .25>p>.1

(2) F = 3.25 4,59 df

(3) U = 20 n - 6,15

(4) 0-2 moulting 1970 vs 0-2 1971 U = 42 n - 7,15

2-4 moulting 1970 vs 2-4 1971 t = 1.7 29df .1>p>.05

4-6 moulting 1970 vs 4-6 1971 t = .5 14 df .7>p>.6

(5) t = 5.4 15 df

(6) t = 4.8 n - 10,10 1970; 1971, 1/2 vs 4-6 am. t = 3.1 n - 6,10

.02>p>.01

(7) t = 1.5 n - 7,10 .2>p>.1

(8) t = 1.7 n - 7,10 .2>p>.1

(9) evening moulted vs 4-6 1970 t = 1.7 32 df

(10) evening moulted vs 2-4 1970 t = 2.3 34df .05>p>.02

(11) t = 6.7 32df

(12) t = 0.99 29df

10.12

(1) χ^2 = 3.4 2df, .25>p>.1

(2) F = .35 2,29 df

(3) χ^2 = 4.5 , 2df, .2>p>.1

(4) F = .8 2,35df

(7) 1971 t = 11.7 n - 17,13

(5) t = 1 15df, .4>p>.2

(6) 1970 t = 12.1 n - 17,10

(8) 0-2 vs 2-4 U = 40 .05>p>.02; 2-4 vs 4-6 U = 29 .05>p>.02
0-2 vs 4-6 U = 19 .02>p>.002

(9) z = 1.2 n - 22,8

(10) t = 1.9 23df

(11) z = 4.2 n - 25,10 1970; 1971 p>.05

(12) z = 1.1 n - 17,25

10.13

(1) χ^2 = 1.23 , 2df, .75>p>.5

(2) F = .77 2,29 df p>.25

(3) χ^2 = 3.6 2df .25 p>.1
F = 3.4 2,30 df

(4) χ^2 = 5.9 4df .1>p>.05

(5) F = 1.7 4,61df

(6) 1/2 vs 4-6 , 1970 t= 9.1 18 df; 1/2 vs 3-5 , 1971 t = 10.1
n - 10,10; 3 vs 4-6 , 1970 t = 7.6 n - 7,10; 3 vs 3-5, 1971
t = 7.7 n - 7,11.

(7) t = 2.8 15df .02>p>.01

(8) t = 1.9 22df .1>p>.05

(9) 1970 evening vs 4-6 1970 t = 5.5 n - 16,10; 1970 evening vs
3-5 , 1971 t = 1.9 n - 16,18 .1>p>.05; 1971 evening vs
4-6 1970 t = 6.8 n - 10,8; 1971 evening vs 3-5 , 1971
t = 2.7 n - 8,8 .05>p>.02

(10) t = 2.6 16df .05>p>.01

10.14

(1) F = 3.9 1,60df

10.16

(1) χ^2 = 7.2 3df, p>.05

(2) F = 2.2 1,2df

(3) F = 3.8 2,52df

(4) 1/2 evening vs 0-2 t = 2.6 .025>p>.01 21df

1/2 vs 3-5 t = 3.1 , 29df, .005> p> .001

(5) t = 4.2 n - 5,12, .005 >p >.001

(6) t = 6.9 n - 5,9

(7) t = 3.6 12df

10.17

(1) χ^2 = 126 , 3df, p >.005

(2) 1-2 vs 2-3 t = .5 23df, p >.5

1-2 vs 3-4 t = 1.8 16df .1>p> .05

(3) 3-5 vs 0-2 t = 1.05 27df p=.3

(4) t = 26.2 n - 12,5

(5) t = 29.1 9df

(6) t = 32 19df

(7) t = 24 12df

(8) t = 17.8 8df

(9) t = 3.2 12df

(10) t = 1.12 23df .3>p>.2

11.4

(1) χ^2 = 4.35 , 5 df, p = .5

(2) F = 0.58 4,50df

(3) F = 22.1 1,4df

(4) 4-6 vs evening moulting t = .98 .4>p>.2 ,1970

4-6 vs evening moulted t = 1.16 19df .4>p>.2 , 1970

Moulted 1970 vs evening moulted t = .89 36df, p>.5

Moulted 1971 vs evening moulted t = .04 25df p>.5

(5) t = .59 19df p>.5

(6) t = 1.28 77df .4>p>.2

(8) F = .29 3,54df

11.7

(1) χ^2 = 12.8 10df , .25>p>.1 , 1970; χ^2 = 10.2 , 10 df p> .25

(2) F = 1.33 8,43df p>.75 time of capture,

F = 2.4 3,8df p>.1 moult.

(3) t = 2.78 33df .05>p>.01

(4) $t = 4.35$ n - 9,6

(5) $t = 2.78$ 33df

(6) F (time) $= .12$ 8,57df $p > .25$

F (moult) $= .43$ 3,8df $p > .25$

Appendix 2. Flight ranges and times of the samples of migrating warblers killed at Bardsey Lighthouse on 6/7. 9. 69.

Willow Warbler - Juveniles

Body weight	Flight range(km)	Flight time(hrs)
8.74	447	19
9.80	530	22
8.47	667	27
9.25	575	22
8.56	462	19
9.28	207	8
10.09	879	32
8.89	579	23
9.28	545	21
9.51	605	23
8.46	695	23
9.24	820	31
9.06	616	24
9.20	381	15
9.52	927	35
10.02	568	21

Willow Warbler - adults

8.38	484	19
8.68	518	20
8.09	727	29
8.55	387	15
9.13	459	18
8.90	514	20
9.02	598	23
8.35	558	22

Grasshopper Warbler - juveniles

Body weight	Flight range(km)	Flight time(hrs)
15.19	984	32
13.80	540	19
17.41	1030	32
14.66	753	26
14.93	808	27
13.11	776	28
13.17	618	24
15.46	763	25
14.91	825	28
13.01	477	18
14.17	786	27
13.07	601	22
12.17	425	16
13.60	759	27
13.79	540	19
15.82	724	24
14.37	536	19
13.75	481	17
12.81	453	17
14.01	747	26
12.50	671	25

Grasshopper Warbler - adults

14.51	480	17
13.96	540	19
13.38	491	18
13.58	601	22
13.06	543	20
14.44	672	23
15.02	632	21
12.76	435	16

Appendix 3. Derivation of the equation for the calculation of the flight time of migratory birds.

From Pennycuick's (1969) formula (48),

$$y = K''.\ \log_e\ (w_0/w) \qquad (1)$$

where y is the range covered by the bird if its weight decreases from an original weight w_0 to weight w. K" is K/g.(L/D)eff. Then from (1) by rearrangement,

$$y/K'' = \log_e\ (w_0/w)$$

so
$$w = w_0 \cdot e^{-y/K''} \qquad (2)$$

From Pennycuick's (1969) equation (13)

$$V = dy/dt = a.\ w^{\frac{1}{2}} \qquad (3)$$

where, V is the maximum range speed, the weight of the bird at any instant is w, and a = $1/p^{\frac{1}{2}}\ A^{\frac{1}{4}}\ S_d^{\frac{1}{4}}$; p is the air density, A is the equivalent plate area, and S_d disk area.

Substituting (2) in (3),

$$dy/dt = a.\ w_0^{\frac{1}{2}} .\ e^{-y/2K''}$$

so
$$dy = a.\ w_0^{\frac{1}{2}}.\ e^{-y/2K''}.dt$$

and
$$dy/e^{-y/2K''} = a.\ w_0^{\frac{1}{2}}.dt$$

Integrating,

$$\int_0^Y dy/e^{-y/2K''} = a.\ w_0^{\frac{1}{2}} \int_0^T dt$$

where Y is the flight range, and T the flight time.

therefore

$$2K''(e^{-0/2K''}) - 2K''(e^{-Y/2K''}) = a.\ w_0^{\frac{1}{2}}.\ T$$

so that,

$$2K''(1 - e^{-Y/2K''}) = a.\ w_0^{\frac{1}{2}}.\ T$$

therefore flight time for flight range Y is,

$$T = 2K''/a.\ w_0^{\frac{1}{2}}\ (1 - e^{-Y/2K''})$$

If the International System of Units is used this gives the flight time in seconds.

Appendix 4. The food consumption and body weights of Bramblings in experiment 1 to 4.

For each bird denoted by the code letter, the four columns, left to right,are food eaten per day (gms), cumulative weight of food eaten (gms), running mean (gms), and the body weight (gms).

Experiment 1 - controls.

	A				B				C				D			
1	4.3	4.2		22	4.0	4.0		30	5.1	5.1		24	5.6	5.6		23
2	5.2	9.5	4.8	22	4.2	8.2	4.1	30	3.8	8.9	4.5	24	5.5	11.1	5.6	23
3	4.6	14.1	4.9	-	4.3	12.5	4.3	-	4.5	13.4	4.2	-	5.9	17.0	5.7	-
4	4.0	18.1	4.3	22	3.7	16.2	4.0	28	5.2	18.6	4.9	24	4.6	21.6	5.3	23
5	4.2	22.3	4.1	22	3.9	20.1	3.8	28	4.3	22.9	4.8	23	4.3	25.9	4.5	22
6	3.7	26.2	4.1	22	1.6	21.7	2.8	28	4.7	27.6	4.5	-	5.3	31.2	4.8	-
7	3.9	30.1	3.9	22	1.1	22.8	1.4	24	3.6	31.2	4.2	24	4.3	35.5	4.8	22
8	4.1	34.2		21	1.6	24.4		23	3.8	35		23	4.7	40.2		21
9	4.6	38.8	4.4	20	2.6	27.0	2.1	22	4.4	39.4	4.1	23	5.3	45.5	5.0	20
10	3.3	42.1	4.0	20	3.0	30.0	2.8	22	3.8	43.2	4.1	22	4.2	49.7	4.8	20
11	5.0	47.1	4.2	20	3.8	33.8	3.4	22	4.8	48.0	4.3	22	5.1	54.8	4.7	20
12	4.5	51.6	4.8	20.5	3.5	37.3	3.7	22	4.4	52.4	4.6	23	5.0	59.8	5.1	21
13	4.1	55.7	4.3	21	3.7	41.0	3.6	23	4.1	56.5	4.3	23	4.8	64.6	4.9	21
14	4.0	59.7	4.1	20	3.9	44.9	3.8	23	4.6	61.1	4.4	22	3.8	68.4	4.3	21
15	4.1	63.8	4.1	20.5	3.7	48.6	3.8	23	4.6	65.7	4.6	22	6.2	74.6	5.0	21
16	4.5	68.3	4.3	20.5	3.7	52.3	3.7	22.5	3.9	69.6	4.3	23	5.7	80.3	6.0	21
17	4.9	73.2	4.7	20	4.1	56.4	3.9	23	4.7	74.3	4.3	23	5.0	85.3	5.4	21

Experiment 1 - experimentals

	E				F				G				H			
1	5.8	5.8		22	8.4	8.4		26	5.1	5.1		21	6.7	6.7	23	
2	5.7	11.5	5.8	22	2.3	10.7	5.4	26	6.8	11.9	6.8	20	7.0	13.7	6.9	23
3	5.6	17.1	5.7	-	4.6	15.3	3.5	-	6.1	18.0	6.5	-	4.8	18.5	5.9	-
4	5.5	22.6	5.6	22	8.5	23.8	6.5	28	5.3	23.3	5.7	20	6.7	25.2	5.8	22
5	6.5	29.1	6.0	23	6.7	30.5	7.6	28	5.7	29.0	5.5	20	4.5	29.7	5.6	22
6	4.7	33.8	5.6	-	6.0	36.5	6.4	-	4.8	33.8	5.3	-	5.8	35.5	5.2	-
7	5.2	39.0	5.0	23	5.1	41.6	5.6	29	5.2	39.0	5.0	20	4.6	40.1	5.2	22
8	4.7	43.7		23	3.1	44.7		27	5.6	44.6		20	4.6	44.7		21
9	5.6	49.3	5.2	21	2.7	47.4	2.9	25	8.1	52.7	6.9	19	4.7	49.4	4.7	21
10	7.9	57.2	6.8	21	1.4	48.8	2.1	25	7.8	60.5	8.0	19	6.0	55.4	5.4	22
11	7.5	64.7	7.7	22	2.2	51.0	1.8	24	7.4	67.9	7.6	20	7.2	62.6	6.6	22
12	7.0	71.7	7.3	22	3.8	54.8	3.0	23	9.2	77.1	8.3	20	6.7	69.3	7.0	22
13	6.8	78.5	6.9	23	5.4	60.2	4.6	24	8.3	85.4	8.8	20	9.0	78.3	7.9	22
14	7.5	86.0	7.2	22	5.6	65.2	5.2	23	9.7	95.1	9.0	20	10.3	88.6	9.7	21
15	7.5	93.5	7.5	22.5	4.7	69.9	4.9	23.5	7.2	102.3	8.5	20	10.1	98.7	10.2	22
16	8.7	102.2	8.1	22.5	5.4	75.3	5.1	23	8.5	110.8	7.9	20	10.7	109.4	10.4	21.5
17	7.8	110.0	8.3	22.5	6.2	81.5	5.8	23	8.3	119.1	8.4	20	8.8	118.2	9.8	22

Experiment 1 - experimentals

I

1	4.8	4.8		22
2	5.7	10.5	5.3	22
3	4.3	14.8	5.0	-
4	5.5	20.3	4.9	22
5	4.2	24.5	4.9	22
6	4.7	29.2	4.5	-
7	4.2	33.4	4.5	22
8	5.1	38.5		21
9	4.9	43.4	5.0	20
10	5.6	49.0	5.3	21
11	6.6	55.6	6.1	21
12	5.0	60.6	5.8	21
13	5.5	66.1	5.3	22
14	5.0	71.1	5.3	21
15	5.8	76.9	5.4	21
16	6.3	83.2	6.1	21
17	6.6	89.8	6.5	21

Experiment 2 -controls

	A				B				C				D			
1	3.9	3.9		22	1.8	1.8		-	3.0	3.0		23	2.0	2.0		23
2	3.8	7.7	3.9	22	2.9	4.7	2.4	-	3.2	6.2	3.1	23	4.9	6.9	3.5	23
3	3.5	11.2	3.7	22	3.1	7.8	3.0	25	2.9	9.1	3.1	22	4.0	10.9	4.5	23
4	5.0	16.2	4.3	22	4.5	12.3	3.8	25	3.0	12.1	3.0	22	-	-	-	23
5	4.7	20.9	2.9	22	4.0	16.3	4.3	24.5	3.6	15.7	3.3	22	4.5	15.4	-	23
6	4.7	25.6	4.7	22	4.8	21.1	4.4	24	3.4	19.1	3.5	21.5	4.9	20.3	4.7	23
7	3.4	29.0	4.1	22	4.4	25.5	4.6	24	2.5	21.6	3.0	21	4.8	25.1	4.9	23
8	5.1	34.1		22.5	4.5	30.0		24	3.7	25.3		22.5	4.4	29.5		22.5
9	4.3	38.4	4.7	22.5	4.3	34.3	4.4	24	3.0	28.3	3.4	20.5	4.6	34.1	4.5	22.5
10	4.2	42.6	4.3	22	4.9	39.2	4.6	23.5	4.5	32.8	3.8	20.5	4.2	38.3	4.4	20
11	3.7	46.3	4.0	22	4.3	43.5	4.6	24	2.7	35.5	3.6	21	4.2	42.5	4.2	22
12	3.4	49.7	3.6	22	4.1	47.6	4.2	24	2.9	38.4	2.8	21	3.9	46.4	4.1	22.5
13	3.5	53.2	3.5	22	4.5	52.1	4.3	23.5	3.6	42.0	3.3	21	4.6	51.0	4.3	21
14	3.7	56.9	3.6	22	4.4	56.5	4.5	24	3.5	45.5	3.6	22.5	4.4	55.4	4.5	22.5
15	3.4	60.3	3.6	22	5.2	61.7	4.8	24	3.0	48.5	3.3	21	4.2	59.6	4.3	22

Experiment 2 - experimentals

	E				F				G				H			
1	4.4	4.4		22	2.5	2.5		23				20	3.5	3.5		21.5
2	4.3	8.7	4.4	23	2.9	5.4	2.7	23	2.4	2.4		19.5	4.0	7.0	3.8	22
3	3.4	12.1	3.9	22.5	3.8	9.2	3.4	23	3.1	5.5	2.8	19	4.7	12.2	4.2	21.5
4	3.7	15.8	3.6	22	3.7	12.9	3.8	23	3.4	8.9	3.3	19	3.6	15.8	4.2	22
5	3.6	19.4	3.7	22	3.9	16.8	3.8	23	3.9	12.8	3.7	19	4.7	20.5	4.2	22
6	3.3	22.7	3.5	22	5.0	21.8	4.5	23	3.5	16.3	3.7	19	4.1	24.6	4.4	22
7	4.0	26.7	3.7	22	3.9	25.7	4.5	23	3.5	19.8	3.5	19	4.1	28.7	4.1	22
8	4.0	30.7		22	5.1	30.8		23.5	4.7	24.5		20	7.0	35.7		22.5
9	7.2	37.9	5.6	22	4.3	35.1	4.7	23	4.6	29.1	4.7	20	6.1	41.8	6.6	22.5
10	4.5	42.4	5.9	22	6.0	41.1	5.2	24	4.3	33.4	4.5	20	5.5	47.3	5.8	23
11	5.2	47.6	4.9	22.5	5.1	46.2	5.6	24	4.1	37.5	4.2	20	5.7	53.0	5.6	23.5
12	4.2	51.8	4.7	22.5	4.1	50.3	4.6	24	2.7	40.2	3.4	20	4.5	57.5	5.1	23
13	4.9	56.7	4.6	22	4.6	54.9	4.4	24	5.0	45.2	3.9	20	6.2	63.7	5.4	23
14	5.7	62.4	5.3	22.5	5.5	60.4	5.1	24	5.1	50.3	5.1	20	7.2	70.9	6.7	23
15	4.5	66.9	5.1	22	5.0	65.4	5.3	24	4.7	55.0	4.9	20	8.3	79.2	7.8	23

Experiment 2 - experimentals

	I			
1				22
2	3.2	3.2		21.5
3	5.8	9.0	4.5	22
4	5.9	14.9	5.9	22
5	4.9	19.8	5.4	22.5
6	6.1	25.9	5.5	22.5
7	5.8	31.7	6.0	23
8	7.5	39.2		23.5
9	5.9	45.1	6.7	24
10	6.8	51.9	6.4	25
11	6.4	58.3	6.6	25.5
12	5.8	64.1	6.1	26
13	5.6	69.7	5.7	26
14	5.5	75.2	5.6	26
15	5.6	80.8	5.6	25.5

Experiment 3 - controls

	A				B				J				L			
1	4.3	4.3		20	4.1	4.1		22.5	6.0	6.0		21.5	2.9	2.9		22
2	3.4	7.7	3.9	20	3.5	7.6	3.8	22.5	6.1	12.1	6.1	21	3.1	6.0	3.0	22
3									6.9	17.0	6.5		3.9	9.9	3.5	
4	3.1	10.8		20	3.5	11.1	3.5	22	2.4	21.4	4.7	19	3.8	13.7	3.9	22
5	4.0	14.8	3.6	20.5	3.8	14.9	3.7	22	8.8	30.2	5.6	22.5	3.6	17.3	3.7	22
6	3.6	18.4	3.8	21	4.0	18.9	3.9	22	8.0	38.2	8.4	23	3.2	20.5	3.4	22
7	4.5	22.9	4.5	21	3.3	22.2	3.7	22.	2.0	40.2	5.0	21	3.9	24.4	3.6	22
8	3.3	26.2		22	3.8	26.0		23	9.7	49.9		22.5	2.9	27.3		23
9	3.6	29.8	3.5	22	4.2	30.2	3.6	22.5	8.9	58.8	9.3	23	3.4	30.7	3.2	23
10	3.9	33.7	3.8	22	3.8	34.0	4.0	22.5	8.8	67.6	8.9	23.5	2.7	33.4	3.1	23
11	3.4	37.1	3.7	22	4.3	38.3	4.1	22.5	8.3	75.9	8.6	22.5	3.8	37.2	3.3	23
12	4.9	42.0	4.2	22.5	3.6	41.9	4.0	22.5	1.4	77.3	4.9	20.5	2.8	40.0	3.3	23

Experiment 3 - experimentals, food ad libitum

	D				F				G				E			
1	5.1	5.1		21.5	5.2	5.2		23	3.1	3.1		22.5	4.1	4.1		21
2	3.1	8.2	4.1	21.5	6.2	11.4	5.7	22.5	3.7	6.8	3.4	22	3.4	7.5	3.8	21
3	4.1	12.3	3.6		6.1	17.5	6.2		3.2	10.0	3.5		3.4	10.9	3.4	
4	3.2	15.5	3.7	21.5	4.6	22.1	5.4	22	3.7	13.7	3.5	22	1.1	12.0	2.3	21
5	4.2	19.7	3.7	21	6.4	28.5	5.5	22	3.6	17.3	3.7	22.5	3.2	15.2	2.2	20.5
6	3.6	23.3	3.9	21	5.1	33.6	5.8	21.5	3.7	21.0	3.7	22.5	3.4	18.6	3.3	20.5
7	3.8	27.1	3.7	21	8.8	42.4	7.0	22	4.0	25.0	3.9	22	3.1	21.7	3.3	20.5
8	5.7	32.8		22	7.2	49.6		22.5	4.9	29.9		24	4.1	25.8		22
9	5.6	38.4	5.7	22.5	6.4	56.0	6.8	22.5	8.6	38.5	6.8	24.5	4.7	30.5	4.4	22.5
10	7.6	46.0	6.6	23.5	5.1	61.1	5.8	23	9.5	48.0	9.1	26	4.2	34.7	4.5	23.5
11	8.5	54.5	8.1	23.5	6.6	67.7	5.9	22.5	8.5	56.5	9.0	26	4.6	39.3	4.4	23.5
12	9.2	63.7	8.9	23.5	5.7	73.4	6.2	22.5	7.7	64.2	8.1	26	4.3	43.6	4.5	22.5

Experiment 3 - experimentals, food restricted

	C				H				I				K			
1	3.9	3.9		23	3.4	3.4		21	4.9	4.9		20	1.7	1.7		21.5
2	3.3	7.2	3.6	22.5	3.0	6.4	3.2	21.5	3.7	8.6	4.3	20	3.3	5.0	2.5	21.5
3	4.5	11.7	3.9		3.0	9.4	3.1		4.0	12.6	3.9		4.8	9.8	4.1	
4	3.2	14.9	3.9	22.5	3.8	13.2	3.4	21	4.6	17.2	4.3	20	3.7	13.5	4.3	22
5	3.8	18.7	3.5	23	3.6	16.8	3.7	21	5.8	23.0	5.2	20	3.8	17.3	3.8	22
6	3.1	21.8	3.5	23	2.7	19.5	3.2	20.5	4.8	27.8	5.3	20	2.6	19.9	3.2	22
7	4.3	26.1	3.7	23	3.3	22.8	3.0	20.5	6.7	34.5	5.8	19.5	3.5	23.4	3.1	22
8				24.5				22.5				20				23
9				23.5				22.5				20.5				23.5
10				24				23				21				23.5
11				22.5				21				20.5				22.5

Experiment 4

	A				B				C			
1	3.1	3.1		22	1.7	1.7		28	2.9	2.9		23
2	3.7	6.8	3.4	22	1.0	2.7	1.4	25.5	5.2	8.1	4.1	23
3	4.2	11.0	4.0	22	1.7	4.4	1.4	24	5.0	13.1	5.1	23
4	3.7	14.7	4.0	22	2.4	6.8	2.1	23.5	4.0	17.1	4.5	22.5
5	4.0	18.7	3.9	22	2.4	9.2	2.4	22.5	5.4	22.5	4.7	22.5
6	3.4	22.1	3.7		2.5	11.7	2.5		3.3	25.8	4.4	
7	3.5	25.6	3.5	21.5	2.8	14.5	2.7	22	3.5	29.3	3.4	22
8	4.2	29.8	3.9	22	4.0	18.5	3.4	22.5	3.3	32.6	3.4	23
9	4.0	33.8		22	3.3	21.8		22.5	3.0	35.6		21
10	3.1	36.9	3.6	22	3.6	25.4	3.5	22	6.2	41.8	4.6	22.5
11	5.6	42.5	4.4	22	4.8	30.2	4.2	22	4.4	46.2	5.3	22
12	4.8	47.3	5.2	22	5.2	35.4	5.0	22	4.9	51.1	4.7	22
13	3.0	50.3	3.9	21	5.6	4.0	5.4	22.5	5.2	56.3	5.1	21.5
14	4.1	54.4	3.6	22	7.1	48.1	6.4	23.5	5.9	62.2	5.6	22
15	4.4	58.8	4.3	22	9.1	57.2	8.1	24.5	5.8	68.0	5.9	22
16	4.1	62.9	4.3	22	8.8	66.0	9.0	24.5	5.1	73.1	5.5	22
17	4.5	67.4	4.5	22	8.4	74.4	8.6	24.5	6.1	79.2	5.6	21.5
18	5.0	72.4	4.8	23	5.3	79.7	6.9	24.5	7.2	86.4	6.7	21.5
19	7.5	79.9	6.3	24	6.3	86.0	5.8	24.5	6.4	92.8	6.8	22
20	6.4	86.3	7.0	24	5.3	91.3	5.8	25	6.1	98.9	6.3	22
21	6.0	92.3	6.2	24.5	7.1	98.4	6.2	24	8.7	107.6	7.4	22.5
22	5.0	97.3	5.5	25	7.8	106.2	7.5	24	11.0	118.6	9.9	24
23	5.2	102.5	5.1	25.5	5.6	111.8	6.7	25	11.4	130.0	11.2	25.5
24				25				25				26
25				25				25				26.5

Experiment 4

	D				E				F			
1	1.2	1.2		28	3.5	3.5		25	3.5	3.5		23
2	2.3	3.5	1.8	27	4.2	7.7	3.9	25	4.1	7.6	3.8	23.5
3	2.9	6.4	2.6	27	4.7	12.4	4.5	25	4.5	12.1	4.3	23.5
4	3.8	10.2	3.4	26	4.3	16.7	4.5	25	4.9	17.0	4.7	23.5
5	3.8	14.0	3.8	25.5	3.5	20.2	3.9	24.5	5.0	22.0	5.0	23.5
6	3.1	17.1	3.5		3.9	24.1	3.7		4.1	26.1	4.6.	
7	4.0	21.1	3.6	25	4.0	28.1	4.0	25	4.0	30.1	4.1	23.5
8	3.9	25.0	4.0	25.5	2.6	30.7	3.3	25	3.6	33.7	3.8	23.5
9	4.7	29.7		25.5	3.8	34.5		25	4.6	38.3		23.5
10	6.5	36.2	5.6	25.5	3.8	38.3	3.8	24.5	4.9	43.2	4.8	23.5
11	7.6	43.8	7.1	25	3.7	42.0	3.8	24.5	4.0	47.2	4.5	23
12	9.4	53.2	8.5	24.5	3.4	45.4	3.6	24	5.1	52.3	4.6	22.5
13	4.4	57.6	6.9	23.5	3.2	48.6	3.3	24	3.9	56.2	4.5	22.5
14	5.9	63.5	5.2	25	3.5	52.1	3.4	24	4.4	60.6	4.2	23
15	8.5	72.0	7.2	25.5	3.6	55.7	3.6	24.5	4.1	64.7	4.3	23
16	8.8	80.8	8.7	26	3.1	58.8	3.4	24	4.5	69.2	4.3	23
17	5.7	86.5	7.3	25	3.7	62.5	3.4	24	4.4	73.6	4.5	22.5
18	12.2	98.7	9.0	27	3.8	66.3	3.8	24	4.0	77.6	4.2	23
19	9.4	108.1	10.8	28	4.3	70.6	4.1	24.5	5.1	82.7	4.6	23
20	7.1	115.2	8.3	27.5	4.0	74.6	4.2	24.5	4.0	86.7	4.6	23
21	6.7	121.9	6.9	27	3.8	78.4	3.9	24	4.2	90.9	4.1	23
22	8.8	130.7	7.8	27	4.9	83.3	4.4	24	4.2	95.1	4.2	22.5
23				27.5	4.4	87.7	4.7	25	4.2	99.3	4.2	22.5
24				28				25				23
25				27				26				22.5

Appendix 5. The Latin names of species mentioned in the text.

Blackbird	Turdus merula
Budgerigar	Melopsittacus undulatus
Bullfinch	Pyrrhula pyrrhula
Brambling	Fringilla montifringilla
Chaffinch	Fringilla coelebs
Chicken	Gallus domesticus
Duck	Anas sp.
Eastern Great Reed Warbler	Acrocephalus arundinaceus orientalis
Fieldfare	Turdus pilaris
Garden Warbler	Sylvia borin
Goose	Anser sp.
Grasshopper Warbler	Locustella naevia
Great Tit	Parus major
Greenfinch	Carduelis chloris
House Sparrow	Passer domesticus
Lesser Redpoll	Carduelis flammea cabaret
Laughing Gull	Larus atricilla
Myrtle Warbler	Dendroica coronata
Oregon Junco	Junco oreganus
Great Horned Owl	Bubo virginianus
Pigeon	Columba livia
Redwing	Turdus musicus
Robin	Erithecula rubecula
Rosy Pastor	Sturnus roseus
Starling	Sturnus vulgaris
Savannah Sparrow	Passerculus sandwichensis
Siskin	Spinus spinus
Tree Sparrow	Passer montanus
White-crowned Sparrow	Zonotrichia leucophrys gambelii
White-throated Sparrow	Zonotrichia albicollis

Willow Warbler	Phylloscopus trochilus
Yellow-vented Bulbul	Pycnonotus goiavier
Yellow Wagtail	Motacilla flava