EFFECT OF AGE, SEX, AND LEVEL OF FAT DEPOSITION ON MAJOR BODY COMPONENTS IN SOME WOOD WARBLERS

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Studies of lipid deposition in migratory birds (Odum and Perkinson, 1951; Odum and Connell, 1956; Odum, 1958, 1959; Connell *et al.*, 1960) indicate that while the amount of fat tissue varies over a wide range, the fat-free weight and nonfat dry weight remain relatively constant within a species. As defined in this study "nonfat dry weight" is the total weight minus fat and water, while "fat-free weight" is the total weight minus only the fat. In a detailed study of the Savannah Sparrow (*Passerculus sandwichensis*), these weights were found to be even less variable when birds of the same wing length were compared (Connell *et al.*, 1960). The standard (flattened) wing length measurement, as made on the folded wing, is an indicator of the size of the bird's framework and hence is a convenient measurement to which the weight of body components may be compared.

A statistical study of the body composition of specimens of migratory birds from the family Parulidae was undertaken with three objectives: (1) to discover if the stage in migration and level of fat deposition affect total water content or nonfat dry weight of the body, (2) to discover if nonfat dry weight is affected by age or sex of the bird, and (3) to establish wing length and fat-free weight relationships that might be used to estimate the amount of fat in a living bird.

Materials and Methods

All specimens studied were obtained from the Tall Timbers Research Station where they had been killed in nocturnal migration by colliding with a television tower just north of Tallahassee, Leon County, Florida. Mr. H. L. Stoddard, Sr., or his associates at Tall Timbers collected the birds, from 1956 to 1962, at dawn following the nights they were killed in the spring and fall migrations. All specimens were sealed in plastic bags and stored in a freezer. We used only specimens collected from 1960 to 1962 in calculations of fat-free weights, because older birds may have become dehydrated in the freezer despite the careful storage. A comparison of the water content of 1960 and 1962 birds showed no appreciable difference. Thus, it is safe to assume that the 1960 birds had not dehydrated enough to affect the results of water content analyses.

Sufficient numbers of specimens of five species were available for intensive study: Parula Warbler (*Parula americana*), Prothonotary Warbler (*Protonotaria citrea*), Chestnut-sided Warbler (*Dendroica pensylvanica*), Palm Warbler (*Dendroica palmarum*), and American Redstart (*Setophaga ruticilla*). Two subspecies, *D. p. palmarum* and *D. p. hypochrysea*, could be distinguished among the Palm Warblers. Geographical races are but weakly developed or have not been described in the other species.

The Palm Warbler winters partly in the southeastern United States. The other species winter in Central and South America, with the exception of a few Parula Warblers which winter in southern Florida.

After the specimens were weighed, the age, sex and wing length were determined. The sex of each specimen was determined by inspection of the gonads. Ages in fall specimens were designated as mature or immature by the degree of ossification of the skull. Ossification was complete in all spring birds. Wing length was measured from the proximal end of the carpometacarpus to the tip of the longest primary as the wing lay flat on a ruler.

The birds were placed in a vacuum oven and dried at 40° C after their breast muscles had been macerated to permit faster drying. After the dry weight had been determined, the specimens were blended in a Waring blender with 95 per cent ethanol and placed on a steam bath until the mixture boiled. The blended residue was separated from the dissolved fat by use of a fine-mesh screen. The residue was put through subsequent extractions with petroleum ether until the remaining fat was removed. The residue was dried in an oven at 80° C, and the nonfat dry weight was obtained. A good estimate of body water is possible from the data thus obtained. Total dry weight minus nonfat dry weight gave the estimated fat weight. Since digestive tracts of migrating birds are generally empty, it was not necessary to correct for non-assimilated material in the body.

Additional checks revealed two errors involved in the extraction procedure. An average of 0.11 g of nonfat dry material was found to be lost through the strainer. A gain in moisture of approximately 0.09 g occurred at the final weighing which was done after the residue had reached an equilibrium with the air. Although the two errors nearly cancel each other, 0.02 g was added to the final nonfat dry weight as a correction factor.

Analysis of variation in nonfat dry weight, due to sex and age, was accomplished with a standard **F** test procedure. The method of partial correlations was used for testing the effect of fat levels on nonfat dry weight and water content. The values obtained from these two procedures were converted to confidence statements by referring to F tables and r tables (Steel and Torrie, 1960). In this study the conventional 95 per cent confidence level was accepted as the minimum acceptable significance. The analysis of least squares was used to obtain the regression equations relating wing length to fat-free weight. Several types of equations were tried but the one which best fitted the data was of the general form $y = a + bx + cx^2$. When presented in the form of a graph, "y" measures vertical distance, "a" indicates the point of the intercept of the line on the "Y" axis, "b" and "c" indicate the slope of the line.

RESULTS

The analyses of the effect of fat, age, and sex on nonfat dry weight and water content of the body are summarized in Table 1. The values in the table are the levels of confidence, in per cent, for stating that the indicated factors are affecting each other. Thus, in the case of the Parula Warbler, it can be said that there is a 70 per cent probability that the amount of fat affects the nonfat dry weight and less than 50 per cent probability that it affects water content of the body.

Effect of age and sex.—Nonfat dry weight was affected by age only in the redstarts and Parula Warblers and was affected by sex only in the

TABLE 1

CONFIDENCE LEVELS (IN PERCENTAGES) FOR POSITIVE CORRELATION BETWEEN THE INDICATED COMPONENTS[1]

Components	*Parula Warbler*	*Palm Warbler*	*American Redstart*	*Prothonotary Warbler*	*Chestnut-sided Warbler*
Fat versus nonfat dry weight	70 (75)	99 (54)	50 (30)	50 (71)	50 (48)
Fat versus water content	50 (75)	70 (54)	70 (30)	50 (71)	—
Age versus nonfat dry weight	99 (64)	50 (18)	95 (43)	—	50 (48)
Sex versus nonfat dry weight	50 (64)	50 (18)	50 (43)	95 (43)	50 (48)

[1] The number of specimens in each case is enclosed in parentheses.

Prothonotary Warblers. In order to obtain an estimate of the magnitude of the effect in these species, individuals of the same wing length, season, and fat level were compared. The nonfat dry weights of 7 Prothonotary Warblers of each sex had means of 3.61 ± .10 (S.E.) g for males and 3.56 ± .10 g for females; 20 redstarts from each age class had means of 2.09 ± .04 g for immature and 2.12 ± .03 g for mature birds; 12 immature Parula Warblers had a mean of 1.99 ± .04 g while 12 mature birds had a mean of 1.95 ± .05 g. Since differences due to age and sex were very small even in the three species showing greatest differences in the analysis of variance procedure (Table 1), the effects of these factors were ignored in the analysis of the effects of fat levels. It should be reemphasized that this does not mean that sex and age have no effect on size; males, for example, average larger than females in these species of parulids. What the analysis does show is that individuals of the same wing length do not differ appreciably as a result of age or sex.

Effect of fat level.—Table 1 indicates also that fat levels can be considered as having little effect on the nonfat dry weight and water content, with the exception of the Palm Warblers where nonfat dry weight was affected by fat level. However, since all the fat Palm Warblers belonged to the western race while the majority of the lean birds were of the eastern race, it is likely that racial differences in body size (i.e., nonfat weight) may account for positive correlation in this species in contrast to the situation in the other four species. The number of available specimens was too small to test this possibility statistically.

A direct comparison of small groups of fat and lean specimens of the same species and wing length also pointed to the relative constancy of the nonfat body components. For example, eight Parula Warblers having fat indices (g fat/g nonfat dry weight) greater than 1.0 did not differ significantly in mean water or nonfat weight from a group of 8 individuals of the same wing length having fat indices less than 0.5. The actual differences in means and the standard errors of difference were 0.20 ± 0.15 g for water and 0.10 ± 0.06 g for nonfat dry weight. Likewise seven fat redstarts did not differ significantly from seven lean redstarts, similarly paired, in regard to mean water or nonfat content (differences 0.06 ± 0.17 g and 0.06 ± 0.06 g).

DISCUSSION

According to Stevenson (1957), American Redstarts, Prothonotary Warblers, Parula Warblers, and Chestnut-sided Warblers are trans-Gulf migrants in the spring. There is no reason to believe that they are not also trans-Gulf migrants in the fall. Lowery (1945) stated that trans-Gulf migrants in spring usually fly inland for several hundred miles. Since Tall Timbers Research Station lies within 60 miles of the Gulf, it was assumed that the tower casualties in the spring had not yet made their first stop, while fall casualties had made their last stop before crossing the Gulf. The latter assumption seems to be valid on the basis of the consistently high fat content of the fall casualties. Odum *et al.* (1961) have estimated that birds with such high fat content have sufficient energy for nonstop flights across the Gulf. Of course, it cannot be stated with absolute certainty that the spring migrants had not stopped, or that fall migrants would not stop, before crossing the Gulf.

The fact that the effect of fat levels on nonfat dry weight and water content seems to be negligible is important because it indicates a remarkable homeostasis of water content in birds even during long flights. That is, water lost in respiration and evaporation (heat loss) is replaced by metabolic water from oxidation of fat fuel so that total water content of the body remains constant even though the total weight of the bird is declining as the fat stores are depleted. Nisbet *et al.* (1963) have discussed the feasibility of metabolic water being an adequate replacement for evaporative water losses during migratory flights. Our data, of course, indicate that water produced in fat metabolism is adequate to maintain water balance. On the other hand catabolized nonfat dry weight could not be replaced since migrating birds would be in negative nitrogen balance. As indicated in more detail elsewhere (Odum *et al.*, 1964) such losses seem negligible as long as the fat level is high and strenuous flying conditions are not encountered. All specimens extracted in the present study had a fat index of 0.3 or greater, and were in evident good physical condition, as is the case with practically all birds killed at the Tallahassee tower. We do see a few specimens with very low fat indices that have lost appreciable nonfat dry weight. Apparently, about 0.2 g fat/g nonfat is tissue fat and can be utilized only at the expense of burning nonfat components as well. The water index (g water/g nonfat) of such depleted birds may actually be higher than that of normal migrants. Thus, contrary to the suggestion of Yapp (1962), fuel, not dehydration, is the limiting factor in long non-stop flights as long as weather conditions and winds remain favorable for easy flight.

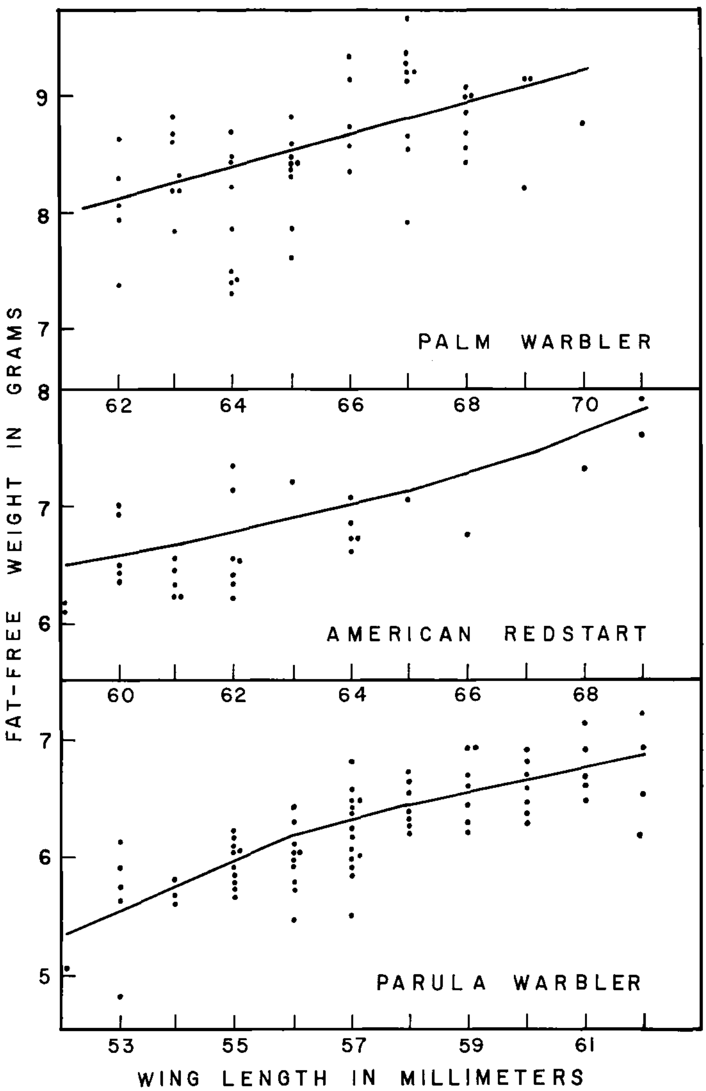

Figure 1. Scattergraphs and calculated regression lines relating wing length to fat-free weight. Regression equations: Palm Warbler $y = 6.5750 + .1196\ (x-50) + .0007\ (x-50)^2$; American Redstart $y = 6.4101 - .0506\ (x-50) + .0065\ (x-50)^2$; Parula Warbler $y = 4.8175 + .2686\ (x-50) - .0081\ (x-50)^2$.

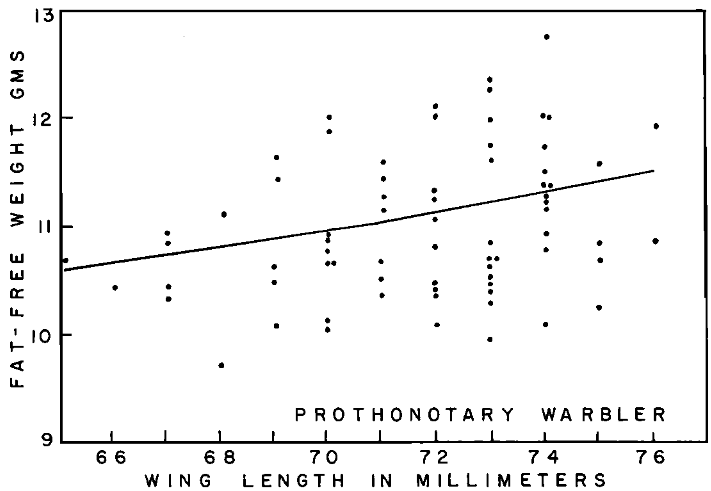

Figure 2. Scattergraph and a calculated regression line relating wing length to fat-free weight. Regression equation: Prothonotary Warbler $y = 9.9110 + .0259\ (x - 50) + .0014\ (x - 50)^2$.

Also of consequence is that weight losses or gains in the migrant bird can be considered as gains or losses in dry fat. The latter finding has an important bearing on laboratory studies that deal with bioenergetics of photoperiod-induced lipid deposition. Since adipose tissue contains water, it has generally been assumed that the caloric equivalent of changes in body weight during periods of fat deposition or depletion is something less than

TABLE 2

AVERAGE NONFAT DRY AND FAT-FREE WEIGHTS OF WING LENGTH CATEGORIES

Species	*Wing length (mm)*	*Nonfat dry weight (g) (Mean and S.E.)*[1]	*Fat-free weight (g) (Mean and S.E.)*[1]
American Redstart	59–62	2.13 ± 0.01 (53)	6.60 ± 0.08 (19)
	63–69	2.25 ± 0.02 (44)	7.08 ± 0.11 (12)
Palm Warbler	61–65	2.74 ± 0.02 (57)	8.27 ± 0.09 (29)
	66–70	2.96 ± 0.02 (42)	8.92 ± 0.09 (26)
Prothonotary Warbler	65–70	3.56 ± 0.04 (22)	10.85 ± 0.12 (22)
	71–73	3.75 ± 0.04 (31)	11.10 ± 0.12 (31)
	74–76	3.88 ± 0.05 (19)	11.39 ± 0.15 (19)
Parula Warbler	52–55	1.92 ± 0.02 (34)	5.76 ± 0.10 (20)
	56–57	2.02 ± 0.02 (28)	6.19 ± 0.07 (24)
	58–59	2.15 ± 0.02 (24)	6.57 ± 0.08 (16)
	60–62	2.22 ± 0.02 (15)	6.73 ± 0.09 (15)
Chestnut-sided Warbler[2]	59–63	2.59 ± 0.02 (42)	7.93 ± 0.06 (42)
	64–67	2.73 ± 0.02 (31)	8.83 ± 0.08 (31)

[1] Sample size in parentheses.

[2] Fat-free weights may be a little lower than those of fresh birds because of weight loss while frozen.

TABLE 3

FAT-FREE WEIGHTS OF SPECIMENS COMPARED WITH ESTIMATES (TABLE 2) AND THE REGRESSION EQUATIONS (FIGURES 1 AND 2) WITH PER CENT ERRORS OF THE ESTIMATES

Species	*Wing length (mm)*	*Total weight (g)*	*Fat-free weights (g) and per cent errors*				
			Actual	*From table 2*	*Per cent error*	*From regression*	*Per cent error*
Parula Warbler	55	6.67	5.81	5.76	0.86	5.96	2.58
Parula Warbler	58	9.30	6.44	6.57	2.02	6.45	0.16
Palm Warbler	65	11.60	8.84	8.27	6.45	8.53	3.51
Palm Warbler	67	11.22	9.23	8.92	3.36	8.81	4.55
Chestnut-sided Warbler	62	11.60	8.15	7.93	2.70		
Chestnut-sided Warbler	65	9.63	8.16	8.33	2.08		
American Redstart	61	10.06	6.70	6.60	1.49	6.64	0.90
American Redstart	61	8.41	6.94	6.60	4.90	6.64	4.32
Prothonotary Warbler	71	18.77	11.56	11.10	3.98	11.07	4.24
					3.09[1]		2.89[1]

[1] Mean per cent error.

the caloric value of pure fat. In man, a gain in weight due to fat deposition has been estimated to be equivalent to 6 kcal/g by Keys and Brozek (1953) and 7.8 kcal/g by Wishnofsky (1958). King (1961), in his study of bioenergetics of fat deposition in the White-crowned Sparrow (*Zonotrichia leucophrys*), used a value of 7.0 kcal/g based on average water content of subcutaneous fat deposits in that species. Our present analysis indicates that the caloric equivalent of weight changes in migrating birds should be at least 9 kcal/g. For further support of the high caloric value of weight loss, see Odum *et al.* (1964).

Regression equations were obtained relating fat-free weight to wing length without consideration of age, sex, or fat level as shown in Figures 1 and 2. The regression lines are significant, with values of $P = .05$ or less in all cases. The fat-free weights in Table 2 are based on wing length categories which gave the lowest standard errors of the means. Table 3 shows the results of predicting the fat-free weights of specimens by using the regression equations and Table 2. The mean per cent errors indicate that both methods are satisfactory as rough estimates. Furthermore the grouping of wing lengths is nearly as effective as the more involved regression analysis. We are preparing tables similar to Table 2 for other species so that fat content of living birds or intact specimens may be estimated on the basis of two measurements, total weight and wing length.

ACKNOWLEDGMENTS

The birds were made available and identified to subspecies by H. L. Stoddard, Sr., and R. A. Norris, of the Tall Timbers Research Station at Tallahassee, Florida. Statistical analyses were carried out under the direction of P. V. Rao, of the University of Georgia Department of Experimental Statistics. We are indebted to Mrs. Shirley Marshall for assistance in laboratory analyses.

This study was supported by grants from the National Science Foundation (No. G-9955) and the National Institutes of Health (No. H-4844) to the University of Georgia.

SUMMARY

1. Specimens of five species of migrating birds of the family Parulidae, killed during spring and fall nocturnal migration at a Gulf coast television tower, were analyzed for nonfat dry weight, fat weight, and water content.

2. The effects of age and sex on the nonfat dry weights of specimens were negligible within a wing length category.

3. Fat levels had no significant effects on water content and also had little effect on the nonfat dry weight, except in the Palm Warbler. However, since Palm Warblers form strong races, the significance may be attributed to racial differences in wing length and fat-free weight relationships rather than the effect of fat levels. Therefore, the caloric equivalent of weight gained or lost during premigratory and migratory periods is at least 9.0 kcal/g.

4. Regression equations for wing length and fat-free weight relationships were determined. These equations and a table using wing length categories were used to predict the fat-free weights of several intact specimens. The per cent errors reveal that the table is nearly as good for prediction as the regression equations, and both are adequate for rough approximations. Both methods gave an average error of approximately 3 per cent.

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