PHYSIOLOGICAL CONDITION AND SEX DETERMINATION OF EASTERN MEADOWLARKS IN NORTHCENTRAL OKLAHOMA

By

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INTRODUCTION

This thesis is comprised of 3 manuscripts written in formats suitable for submission to international scientific journals. Each manuscript is complete without supporting materials. The manuscript, "The effects of tissue preparation on ether extractable lipids" (Chapter II), was written in the format of the WILDLIFE SOCIETY BULLETIN. Chapter III, "Sexing eastern meadowlarks in northcentral Oklahoma" was written in the format of the JOURNAL OF FIELD ORNITHOLOGY. Chapter IV, the principle manuscript of this thesis, "Seasonal changes in lipid content of eastern meadowlarks in northcentral Oklahoma" was written in the format of the AUK.

CHAPTER II

THE EFFECTS OF TISSUE PREPARATION ON ETHER EXTRACTABLE LIPIDS[1]

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INTRODUCTION

Recent avian research has shown that lipid levels provide an important index to physiological condition (Evans and Smith 1975, Woodall 1978, Bailey 1979, Iverson 1981). Lipid reserves are important to organisms as a source of energy and contain over twice the potential energy of proteins or carbohydrates per gram (Whittow 1965, Ricklefs 1974). The lipid levels of birds vary throughout the year in response to migration, reproduction, habitat quality, and weather (Raveling 1979, Iverson 1981, Krapu 1981, McLandress and Raveling 1981). Because lipid reserves are important in determining an organism's physiological

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[1] Contributed by the Oklahoma Cooperative Wildlife Research Unit: Oklahoma State University, Oklahoma Department of Wildlife Conservation, U.S. Fish and Wildlife Service, and Wildlife Management Institute Cooperating.

condition, methods used in estimating these reserves should be standardized to obtain comparable results between studies. While portions of the lipid extraction procedure have been standardized by using either a Goldfisch or Soxhlet fat extractor (Kerr et al. 1982), other steps in the extraction process, i.e. drying temperature, drying time, extraction time, and blending, have not been standardized.

The purpose of this study was to determine the most time-efficient method of preparing tissue samples for lipid extraction on a Goldfisch fat extractor. This paper reports the effects of drying temperature, drying time, extraction time, and sample reblending on the amount of ether extractable lipids.

METHODS

Effects of Drying Temperature on Drying Rate and Extractable Lipids

Tissue samples were obtained from 6 western meadowlarks (Sturnella neglecta) collected in Payne County, Oklahoma during the fall of 1982. Each bird was plucked, feet removed above the tarsus, ingesta removed from the gizzard and intestine, and the carcass frozen. Each frozen carcass was ground 3 times in a meat chopper. The carcasses were combined and homogenized together (Sample A) in a food processor for 2.5 min. Seventy-two 3-4 g aliquots were removed from Sample A and divided into 4 18-aliquots groups.

To determine sample drying rates at different temperatures, one 18-aliquot group was placed in a forced air oven at 60 C and another at 100 C. Over a 48 hour period each group was removed from the oven at approximately 4 hour intervals, cooled for 30 min in a dessicator, weighed to the nearest 0.01 g on a Mettler H10T balance, and replaced

in the oven. The recorded drying time was the cumulative time spent in the oven up to a total of 48 hours.

We calculated percentage weight loss of each aliquot at each weighing period by:

$$\% \text{ Weight Loss} = \frac{\text{Wet Sample Weight} - \text{Dry Sample Weight}}{\text{Wet Sample Weight}} \times 100$$

To determine the effects of drying temperature on extractable lipids, we dried the remaining 2 18-aliquot groups of Sample A at 60 C and at 100 C in a forced air oven. Each group was removed from the oven after 48 hours, allowed to cool in dessicators, weighed, and lipids were extracted on a Goldfisch fat extractor. Samples were refluxed for a minimum of 10 hours.

We calculated the percentage lipids removed from each aliquot by:

$$\% \text{ Lipid} = \frac{\text{Extractable Lipid Weight}}{\text{Dry Sample Weight}} \times 100$$

ANOVA was used to test for significant differences between raw percentages and arcsin square root converted percentages of extractable lipids in samples dried at 60 C and 100 C (SAS Institute, INC 1982). To reject the null hypothesis, $P < 0.05$ was required.

Effects of Reblending on Extractable Lipids

The control sample (blended once) for this experiment was the 18-aliquot group removed from Sample A and dried at 100 C as described above. The homogenate remaining after removing Sample A was frozen and later thawed and blended a 2nd and 3rd time for 2.5 min. A subsample (18-aliquots) was removed from the homogenate after each reblending. All 3 18-aliquot groups were dried for 48 hours at 100 C.

After 48 hours the 3 18-aliquot groups were removed from the oven,

cooled in dessicators, weighed, and lipids removed on a Goldfisch fat extractor. ANOVA was used to determine whether significant differences in percentage fat occurred between the 3 18-aliquot groups.

Effects of Extraction Time on Extractable Lipids

Thirteen eastern meadowlarks (Sturnella magna) collected during the winter of 1982-83 in northcentral Oklahoma were prepared as explained for Sample A. Eighty-four 3-4 g tissue aliquots of the homogenate were placed in numbered glass extraction thimbles. A random number table (Snedecor and Cochran 1967) was used to divide the 84 aliquots into 7 12-aliquot groups. The homogenate was placed on ice throughout the procedure and the samples were randomized to eliminate any bias related to the melting and settling of lipids during the sample preparation process.

The 7 12-aliquot groups were dried for 18 hours at 100 C in a forced air oven, allowed to cool in dessicators, and then weighed; lipids were then extracted on a Goldfisch fat extractor. Each of the 7 groups was refluxed on the extractor for a different period of time: 1, 2, 4, 6, 8, 12, and 16 hours. The percentage fat was calculated for each sample based on its dry weight.

Effects of Length of Drying Time on Extractable Lipids

Fifteen eastern meadowlarks collected during the winter of 1983-84 in northcentral Oklahoma were prepared as described for Sample A. The homogenate was placed on ice and 100 3-4 g tissue aliquots were placed in numbered glass thimbles. The 100 aliquots were randomly divided into 7 12-aliquot and 2 8-aliquot groups.

The 7 12-aliquot groups were dried at 100 C for 0, 3, 6, 9, 12, 15, and 18 hours, respectively. The 2 8-aliquot groups were dried at 100 C for 31 and 48 hours, respectively. After drying, aliquot groups were placed in dessicators, weighed and lipids were extracted on a Goldfisch fat extractor. Within 1.5 hours after removal from the oven, lipid extraction was started. This procedure was followed to reduce the possible loss of additional moisture. Percentage fat was calculated for each aliquot based on its weight before extraction.

Extractor Conditions

We used a Labconco Goldfisch fat extractor, model no. 35001* in which about 20 ml of technical grade petroleum ether (30-60 C b.p.) was used to extract lipids from each tissue sample. The ether was boiled on heaters preset to maintain distilled water at a temperature of 84 C and recondensed on a water cooled manifold cone. The water flow rate through the manifold assembly was approximately 14 liter/minute and the water temperature was maintained at 17-22 C. This temperature range did not affect the ether condensation rate of approximately 5-6 drops/second.

RESULTS

Effects of Drying Temperature on Drying Rate and Extractable Lipids

Average weight loss was significantly greater (t = 3.8913, df = 19, $P < 0.0010$) at the 100 C drying temperature throughout the 48 hour drying cycle (Fig. 1). The cumulative percent weight loss changed very

* Mention of a product does not constitute government endorsement.

little after 18 hours at 100 C and 32 hours at 60 C (Table 1). At the end of the 48 hour drying cycle, a 1% difference in percentage weight loss existed between samples dried at 60 C and the 100 C, 63.5% and 64.5%, respectively. There was no significant difference in average percentage extractable lipid levels (t = 0.0110, df = 31, P = 0.9913) between aliquots dried at 60 C and 100 C (Table 2).

Effects of Reblending on Extractable Lipids

The average percentage lipids in sample sets blended 1 and 2 times was not significantly different (t = 1.2378, df = 33, P = 0.2245), but there was a 3.2% difference in the percentage fat obtained (Table 3). Percentage fat values between the subsets blended 2 and 3 times were not significantly different at the 0.05 level (t = 1.9691, df = 34, P = 0.0591). However, a 5.1% decrease in percentage fat was obtained. Samples blended 3 times had significantly less (8.3%) extractable lipids than samples blended once (t = 4.1191, df = 33, P = 0.0032).

Effects of Extraction Time on Extractable Lipids

There was a significant difference (F = 42.72, df = 4, 35, P = 0.0010) in percentage extractable fat obtained between 1 hour and 8 hours extraction (Fig. 2). However, there was no significant difference (F = 0.6500, df = 2, P = 0.5271) in percentage fat values obtained at 8, 12, and 16 hour intervals.

Effects of Length of Drying Time on Extractable Lipids

After the samples were dried at least 9 hours at 100 C the average percentage lipids removed from the samples were not significantly

different (F = 1.5300, df = 5, 77, P = 0.1924) and varied less than 0.60%. A linear regression line (Y = 11.49 + x0.51$\ln x$) was fitted to the lipid values which had an R^2 value of 0.95 (Fig. 3).

DISCUSSION

Lipid extraction is a time consuming procedure and it is desirable to reduce processing time to a minimum, yet retain the methodology's lipid estimate accuracy. We found a 14 hour reduction in drying time was achieved by increasing the drying temperature from 60 C to 100 C and samples dried at 60 C and 100 C reached a constant weight at 32 hours and 18 hours, respectively. However, the samples dried at 60 C had a dry weight 1% higher than the samples dried at 100 C. Kerr et al. (1982) observed a similar trend and attributed the lower weights in samples dried at 100 C to a possible change in protein, water, or lipid composition due to increased heat. Dowgiallo (1975) and Blem (1976) reported that some volatile fatty acids may be lost from tissues dried at high temperatures. To offset these losses Blem (1976) recommended tissues be freeze-dried or oven-dried at low temperatures. Kerr et al. (1982) found no significant difference between extractable lipids obtained from tissues that were freeze-dried or oven-dried at 100 C but warned that oven-drying temperatures that might cook the sample should be avoided because changes in lipid or protein content could occur. We found that the percentage lipids did not differ between samples dried at 60 C and 100 C and there does not appear to be any advantage to drying tissue samples at temperatures lower than 100 C. Also, the mean percentage lipids of 3-4 g samples dried for 9 hours at 100 C differed little from samples dried for longer periods of time. However, since

the cumulative weight loss of samples did not reach 99% until after 13 hours at 100 C, we recommend that 3-4 g samples be dried for at least 12-13 hours at 100 C before lipids are extracted.

A second method of reducing the time required in the extraction process is to reflux tissue on the Goldfisch fat extractor for the minimum time necessary to remove all ether soluble lipids. We found that essentially all extractable lipids were removed from 3-4 g samples after 8 hours, and refluxing tissue longer than 8 hours resulted in no significant increase in percentage extractable fat. However, to allow for diffences in sample porosity and lipid content (Sawicka-Kapusta 1975), we recommend refluxing a 3-4 g sample for at least 10 hours.

Lipid content variance between samples from the same homogenate can be high if the homogenate is not mixed sufficiently. To offset these differences, Dowgiallo (1975) recommended that an average of 3 samples from the same homogenate for hard to blend materials be used to estimate percentage lipids. However, this approach does not entirely solve the problem and large variances can still occur. When the variance between samples is large it is often necessary to reblend the homogenate and remove more samples. Yet, we observed that the process of blending the homogenate for a 2nd or 3rd time can reduce the lipid content of the tissue. The reduction in percentage lipids due to reblending could be due to several factors. First, blending a tissue sample causes a thin layer of lipids to adhere to the mixing container. To retain these lipids the mixing container can be cooled before each sample is blended but this procedure is not feasible when doing a large number of consecutive samples. Second, blending a small quantity of tissue results in high losses because of the high surface to volume ratio

between the tissue and mixing container. Third, most blenders cannot be slowed down to a speed where the homogenate is not thrown onto the mixer sides. Adherance to the blender sides would increase the surface to volume ratio, thus increasing lipid loss.

Another factor that can affect the amount of lipids lost during reblending is the tissue's total lipid content. For example, tissue from a meadowlark collected during the winter would probably be affected more by reblending than one collected in the summer because winter meadowlarks have a greater lipid content than do summer meadowlarks (Kemmerer, unpubl. data).

The most practical way to eliminate the bias associated with reblending small tissue samples is to blend the sample thoroughly and to initially collect one or more extra tissue samples during sample preparation. These extra samples can be used for any additional analysis and no tissue reblending will be necessary.

SUMMARY

Lipid extraction is a time consuming procedure in which a reduction of sample process time without decreased accuracy is an advantage. We found no significant difference in percentage lipids extracted from tissues dried at 60 C and 100 C and recommend that a 3-4 g tissue sample be dried for 12-13 hours at 100 C before lipid extraction. Dried tissue samples (3-4 g) should be refluxed for a minimum of 10 hours on a Goldfisch fat extractor to remove all extractable lipids. The percentage lipid content is reduced by reblending a homogenate a 2nd or 3rd time. Therefore, if variance between samples is a problem we

recommend removing extra aliquots after the first blending to be used for additional analysis.

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Table 1. Average cumulative percentage weight loss of 18-aliquot subsets of meadowlark tissue dried during a 48 hour drying period at 60 C and 100 C drying temperatures.

Hours dried	Temperature	
	60 C	100 C
	Cumulative % Loss	Cumulative % Loss
6	50.49	95.36
9	89.07	97.65
13	95.29	98.80
18	97.76	99.30
23	98.80	99.48
27	99.27	99.64
32	99.53	99.73
37	99.75	99.83
42	99.91	99.88
48	100.00	100.00

Table 2. The mean percentage lipids extracted from 3-4 gram samples of meadowlark tissue dried at 60 C and 100 C for 48 hours.

Drying Temperature (C)	N	Mean % Lipids	SD
60	18	15.14	1.2054
100	17[a]	15.13	0.9705

[a] One aliquot was omitted because total sample set variance was reduced from 6.2% to 0.97%.

Table 3. The effects of reblending on percentage lipids extracted from 3-4 gram samples of meadowlark tissue.

No. of Blendings	Drying Temperature (C)	N	Mean % Fat	SD	Arcsin Conversion
1	100	17[a]	15.13	0.9705	0.15
2	100	18	14.63	1.3906	0.15
3	100	18	13.88	0.8181	0.14

[a] One aliquot was omitted because the total sample variance was reduced from 6.2% to 0.97%.

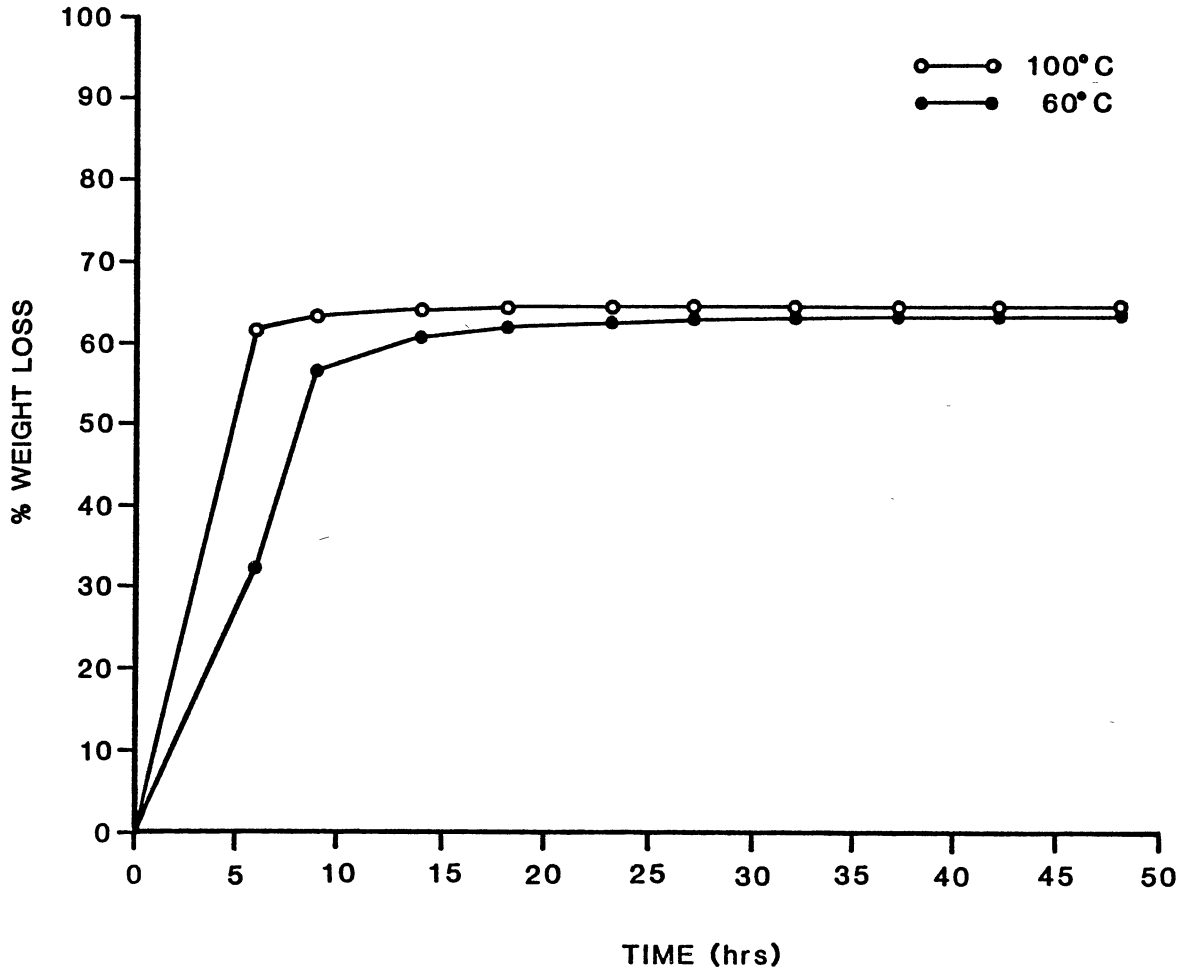

Figure 1. Percentage weight loss in 3-4 gram aliquots at 60 C and 100 C drying temperatures over a 48 hour period.

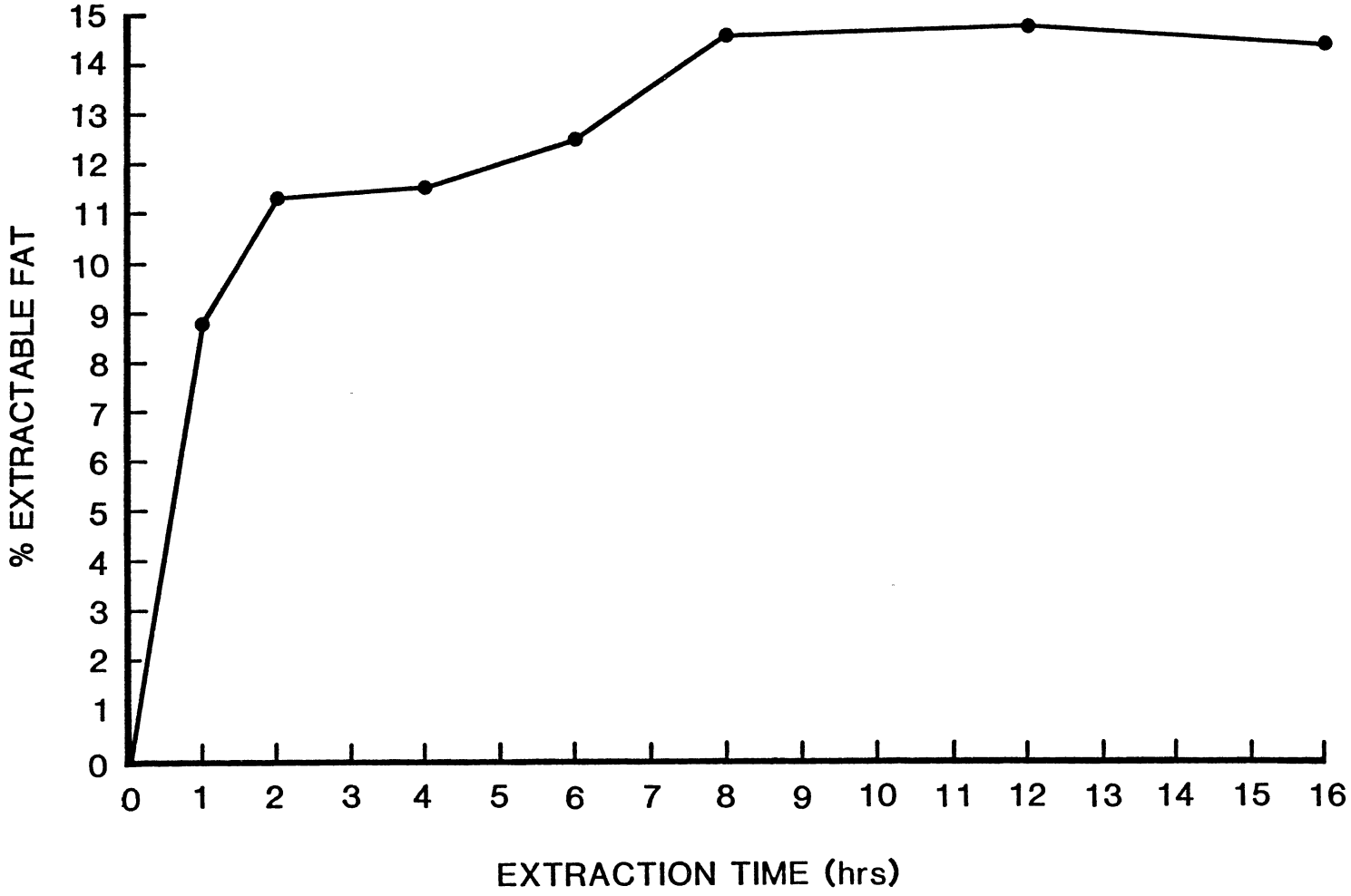

Figure 2. Changes in percentage ether extractable fat in 3-4 gram aliquots with increasing extraction time.

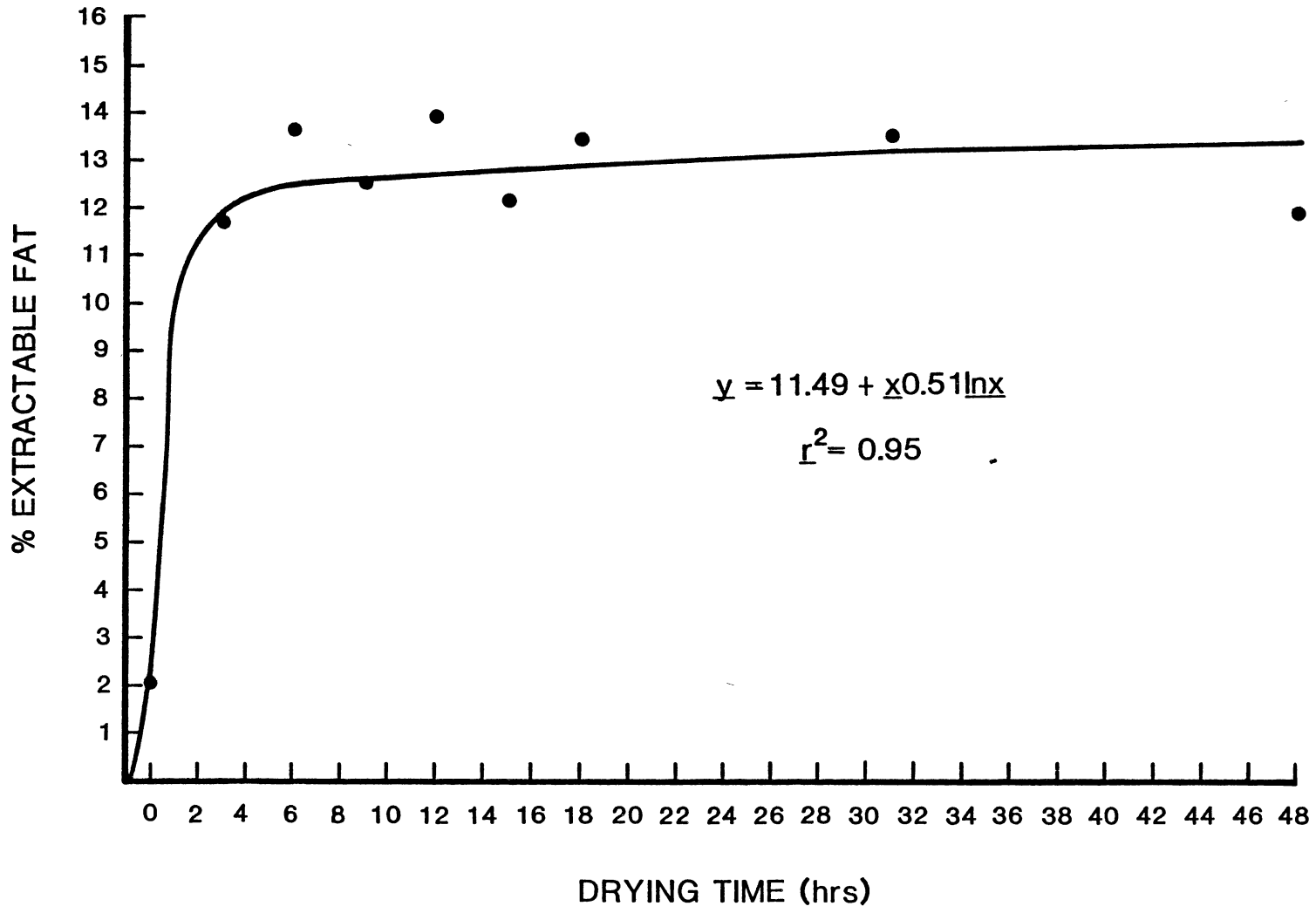

Figure 3. Changes in percentage ether soluble fat in 3-4 gram aliquots with increasing drying time at 100 C.

CHAPTER III

SEXING EASTERN MEADOWLARKS IN NORTHCENTRAL OKLAHOMA

BY MICHAEL R. KEMMERER AND LARRY G. TALENT

INTRODUCTION

Sexing Eastern Meadowlarks (Sturnella magna) is often difficult for the inexperienced researcher and bird bander, although the sexes are reported to be morphologically dimorphic (Baird et al. 1874, Goss 1891, Ridgeway 1896, Ridgeway 1902, Lanyon 1962, Chapman 1966, Dickerman and Phillips 1970). In addition to being morphologically smaller, the plumage of female meadowlarks is reported to differ from that of males by being slightly duller in color and having a more restricted black area on the throat (Ridgeway 1896, Dwight 1900, Ridgeway 1902). In northcentral Oklahoma, Eastern Meadowlarks vary in size and color and sexing meadowlarks in this region of the state can be difficult. Nice (1931) listed S. m. argutula as a permanent resident in eastern Oklahoma and S. m. magna as a summer resident of central and western Oklahoma. Sutton (1967) observed that Eastern Meadowlarks breeding in northcentral Oklahoma were small enough to be the southern race S. m. argutula but their coloration resembled the northeastern race S. m. magna. This observation lead Sutton (1967) to tentatively identify the race breeding in northcentral Oklahoma as S. m. hoopesi, although previously the northern range of this race was described as being in Texas (Ridgeway

1902). During the fall and spring migration season, possibly all 3 of the aforementioned races of Eastern Meadowlark may be present in northcentral Oklahoma. Therefore, there is a need for an accurate method to sex Eastern Meadowlarks in field studies during all months of the year.

The purposes of this paper are to describe sexual differences in morphological measurements and bill color of Eastern Meadowlarks in northcentral Oklahoma and to provide a mathematical equation that will identify the sex of Eastern Meadowlarks found in northcentral Oklahoma throughout the year.

METHODS

We collected 457 adult and 91 juvenile Eastern Meadowlarks from August 1982 through September 1983 in northcentral Oklahoma tallgrass prairies (Fig. 1). An additional 58 specimens were collected from the same area during the 1984 breeding season. Morphological measurements taken from all birds collected in both years included field weight (included feathers and ingesta), total length, tail length, flattened wing chord (Fig. 2), culmen (from the base), anterior end of the nostril to the tip of the bill, bill width and height at the nostril, tarsal length (Fig. 2), middle toe and hallux without claw, and feathered and unfeathered keel (keel apex to keel base). All lengths were measured to the nearest 0.5 mm with a stainless steel ruler, except feathered and unfeathered keel which were measured to the nearest 0.1 mm with vernier calipers. Field weight was measured to the nearest 0.1 g on an Ohaus triple-beam balance soon after the birds were collected. All birds were dissected and sexed by gonadal examination. Age was determined by the presence (juvenile) or absence (adult) of the bursa of

Fabricus. Juvenile birds in juvenal plumage are referred to as immatures, whereas juvenile birds from September-November were called subadults because a bursa was present but externally they resembled adults.

An unpaired *t*-test was used to test for significant differences in morphological measurements between males and females (SAS Institute, INC. 1982). We used stepwise discrimination (SAS Institute, INC. 1982) to determine which measurements were most important in differentiating the sexes of 180 breeding Eastern Meadowlarks collected in 1983. The measurements obtained in the stepwise discrimination procedure were used in multiple linear regression to develop a sex prediction equation. These measurements were used in various combinations within the equation to obtain the best R^2 value, percentage of birds sexed correctly, and the fewest number of birds scored near 0.5, the division between males and females. Birds receiving score values > 0.5 and < 0.5 were classified as females and males, respectively. Subsequently, we applied the equation to all adult and juvenile birds, of known sex, collected from August 1982 through September 1983 and to the 58 adult meadowlarks collected during the 1984 breeding season to test the overall and monthly accuracy of the equation. Eighteen adult birds collected in 1982-83 were omitted from this portion of the study because one or more morphological measurements were not taken from each bird due to damage to a morphological structure by collection procedures.

The 58 breeding Eastern Meadowlarks collected during 1984 were used to determine sexual differences in bill color between males and females. Bill color was recorded immediately after collection by matching bill colors to color plates in the Naturalist's Color Guide (Smithe 1975,

1981) in direct sunlight in the field. To statistically test differences between the upper mandible coloration of males and females, values were assigned in relation to the total percentage of dull black [sepia 119, blackish neutral gray 82, or Vandyke brown 221 (Smithe 1975, 1981)] on the upper mandible from the posterior edge of the nostril forward to the bill tip. An upper mandible that was entirely dull black recieved a value of 100.0, whereas an upper mandible devoid of dull black coloration was given a 0.0 value. Most males received values of 85.0-95.0, whereas females received values of 30.0-60.0. An unpaired *t*-test was used to determine whether significant differences in bill color existed between sexes.

RESULTS AND DISCUSSION

Thirteen morphological measurements of breeding males were significantly larger ($P < 0.0001$) than those of adult females (Table 1). Although male Eastern Meadowlarks were generally larger than females, there was some overlap between small males and large females (Table 1) and, therefore, no single measurement could be used to sex all birds. Field weight, flattened wing chord, unfeathered keel, and tarsal length were the only measurements that contributed significantly in separating the sexes. Using 3 of these measurements, i.e. field weight, flattened wing chord, and tarsal length, (unfeathered keel was deleted because it did not increase the accuracy of the sexing equation) we developed the following sex determination equation:

$$\text{Scored Sex Value} = 6.7367\ (\text{Tarsal Length} / \text{Field Weight}) + 0.0015\ (\text{Flattened Wing Chord}) - 0.0618\ (\text{Tarsal Length}),$$

where males and females are indicated by scored sex values of < 0.5 and

> 0.5, respectively. This equation correctly sexed 97.8% and 96.2% of known adult males and females, respectively, during the 1982-1983 field season (Table 2), and 100% of the 58 adult Eastern Meadowlarks collected during the 1984 breeding season. Achieving a higher percentage of correct sex identificantion with the equation throughout the year, for each sex, was not possible because the population contained a few small males and large females that overlapped in morphological measurements.

The sexing equation was less effective at sexing juveniles. Overall, only 68.5% and 100.0% of juvenile males and females, respectively, of known sex, were correctly sexed (Table 3). Juvenile birds were divided into 2 age classes for further analysis: immatures (in juvenal plumage) and subadults (in adult plumage but the bursa of Fabricus was present). The sexing equation correctly sexed 100% of the immature females but only 28.9% of the immature males. The incorrectly sexed immature males were classified as females because of their overall smaller morphological size; immature males were significantly smaller morphologically than subadult males ($\underline{t}$ = -6.3951, df = 47, $\underline{P}$ = 0.0001). Subadult males and females were sexed correctly 94.3% and 100.0%, respectively, using the equation. Subadult males were sexed with a greater degree of accuracy than immatures because subadults had attained adult plumage and their skeletal development was near completion; morphologically, subadults were not significantly smaller than adults ($\underline{t}$ = 0.0071, df = 76, $\underline{P}$ = 0.9943). When subadults and adults were combined, 97.8% and 96.9% of the males and females, respectively, were correctly sexed with the equation.

Because a small percentage of adult males and females could not be distinguished on the basis of morphological measurements during the

breeding season, another character was needed to sex adult birds correctly. We noted during the 1983 breeding season that bill color varied between sexes but no data were taken. Therefore, we collected 58 adult Eastern Meadowlarks (27 males, 31 females) during the 1984 breeding season and found there was a significant difference in bill color between the sexes (t = 31.4796, df = 56, $P < 0.0001$). We accurately sexed 100% of the males and females using bill color as the only sexing character.

The primary difference in bill color between breeding male and female Eastern Meadowlarks in northcentral Oklahoma is the coloration of the upper mandible (Fig. 2). The color of the anterior 3/4 of the upper mandible of typical breeding males is dull black [sepia 119, blackish neutral gray 82, or Vandyke brown 221 (Smithe 1975, 1981)] and the posterior 1/4 of the upper mandible is bluish gray [Pratt's Payne gray 88 (Smithe 1981)].

The color of the anterior 1/4 to 1/3 of the upper mandible of typical females is also dull black [sepia 119, blackish neutral gray 82, or Vandyke brown 221 (Smithe 1975, 1981)]. However, the coloration of the posterior 1/4 of the upper mandible length is ivory and the remaining middle portion of the upper mandible length is an intergradation region between dull black and ivory (Fig. 3). The coloration of this region does vary in length among individuals with some having the ivory color extending farther anteriorly and others having dull black extending slightly more than 1/3 of the upper mandible length posteriorly.

During the peak of breeding season other characters can also be used to help verify sex of Eastern Meadowlarks, i.e., incubating females

have a brood patch present on the breast, laying females often have a distended abdomen and enlarged cloaca, and males may have a cloacal protuberance.

Although our sex determination equation will accurately sex most adult and subadult Eastern Meadowlarks in northcentral Oklahoma, it will probably have to be modified if used in other areas. However, the equation is inaccurate for sexing immature males probably due to juvenal plumage and incomplete skeletal development. Because of this problem we do not recommend using the sex determination equation on any juvenile Eastern Meadowlark before the post-juvenal molt. A more useful and consistent method of sexing breeding adult Eastern Meadowlarks may be to observe sexual differences in bill color. However, the reliability of bill color as a criteria of sexing adult Eastern Meadowlarks needs to be determined throughout the range of the species.

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Table 1. Morphological traits and statistical differences between morphological traits of adult male and female Eastern Meadowlarks in northcentral Oklahoma.

		Sex					
		Male			Female		
Measurements	t-value	N	Mean ± SD	Range	N	Mean ± SD	Range
Field Weight	30.25*	100	113.9 ± 7.14	97.1 - 133.6	80	84.2 ± 5.67	69.7 - 97.1
Total Length	19.92*	100	240.3 ± 6.81	223.0 - 255.0	78	220.0 ± 6.57	199.0 - 236.0
Tail Length	16.64*	100	75.9 ± 2.98	66.0 - 86.0	80	67.2 ± 2.98	61.0 - 74.0
Flattened Wing Chord	25.18*	100	118.9 ± 3.21	109.0 - 128.0	80	107.2 ± 3.02	100.0 - 114.0
Culmen	6.54*	100	32.0 ± 1.53	22.0 - 34.5	78	28.7 ± 1.65	22.0 - 32.0
Bill Width	2.54*	100	8.2 ± 0.45	6.5 - 9.0	80	7.2 ± 0.47	6.0 - 8.5
Bill Height	5.67*	99	8.5 ± 0.45	7.5 - 9.5	79	7.7 ± 0.41	7.0 - 8.0
Nostril to Tip	12.89*	99	20.8 ± 1.08	19.0 - 24.0	78	18.7 ± 1.03	16.0 - 21.0
Tarsal Length	14.54*	97	43.7 ± 1.57	40.0 - 49.0	76	40.5 ± 1.33	37.0 - 43.5
Hallux Length	8.93*	97	19.6 ± 0.97	19.0 - 22.0	79	18.3 ± 1.00	16.0 - 20.5
Middle Toe Length	8.61*	98	25.0 ± 1.65	21.5 - 28.0	78	23.3 ± 1.32	20.0 - 29.0
Feathered Keel	26.93*	96	39.5 ± 1.53	36.4 - 44.3	77	33.2 ± 1.65	25.3 - 36.1
Unfeathered Keel	26.04*	96	40.0 ± 1.53	36.6 - 45.3	77	33.3 ± 1.56	27.4 - 37.2

*Indicates measurements were significantly different between males and females at a significance level of $P = 0.0001$.

Table 2. The proportion of adult Eastern Meadowlarks correctly sexed using the morphological sexing equation by month.

Months	Sex Classes	
	Males	Females
August 1982	11/12[a] (91.7)[b]	14/14[a] (100.0)[b]
September 1982	15/20 (75.0)	13/13 (100.0)
October 1982	21/21 (100.0)	11/12 (91.7)
November 1982	17/17 (100.0)	2/4 (50.0)
December 1982	14/14 (100.0	0/0
January 1983	32/32 (100.0)	0/0
February 1983	12/12 (100.0)	1/1 (100.0)
March 1983	23/23 (100.0)	0/0
April 1983	20/20 (100.0)	12/12 (100.0)
May 1983	20/20 (100.0)	19/20 (95.0)
June 1983	25/25 (100.0)	15/16 (93.8)
July 1983	32/32 (100.0)	28/28 (100.0)
August 1983	16/16 (100.0)	18/19 (94.7)
September 1983	16/16 (100.0)	20/20 (100.0)
Overall	274/280 (97.8)	153/159 (96.2)

[a] The number of birds sexed correctly / the actual number of birds for that month.

[b] The percentage of birds sexed correctly by the sexing equation.

Table 3. The percentage of juvenile Eastern Meadowlarks correctly sexed using the morphological sexing equation by month.

Months	Sex	
	Male	Female
August 1982	2/8[a] (25.0)[b]	0/0[a]
September 1982	9/13 (76.9)	1/1 (100.0)[b]
October 1982	13/15 (86.9)	2/2 (100.0)
November 1982	13/13 (100.0)	0/0
December 1982	7/7 (100.0)	1/1 (100.0)
June 1983	0/2 (0.0)	0/0
July 1983	0/4 (0.0)	1/1 (100.0)
August 1983	4/7 (57.1)	7/7 (100.0)
September 1983	2/4 (50.0)	6/6 (100.0)
Overall	50/73 (68.5)	18/18 (100.0)

[a] The number of birds sexed correctly / the actual number of birds for that month.

[b] The percentage of birds sexed correctly by the sexing equation.

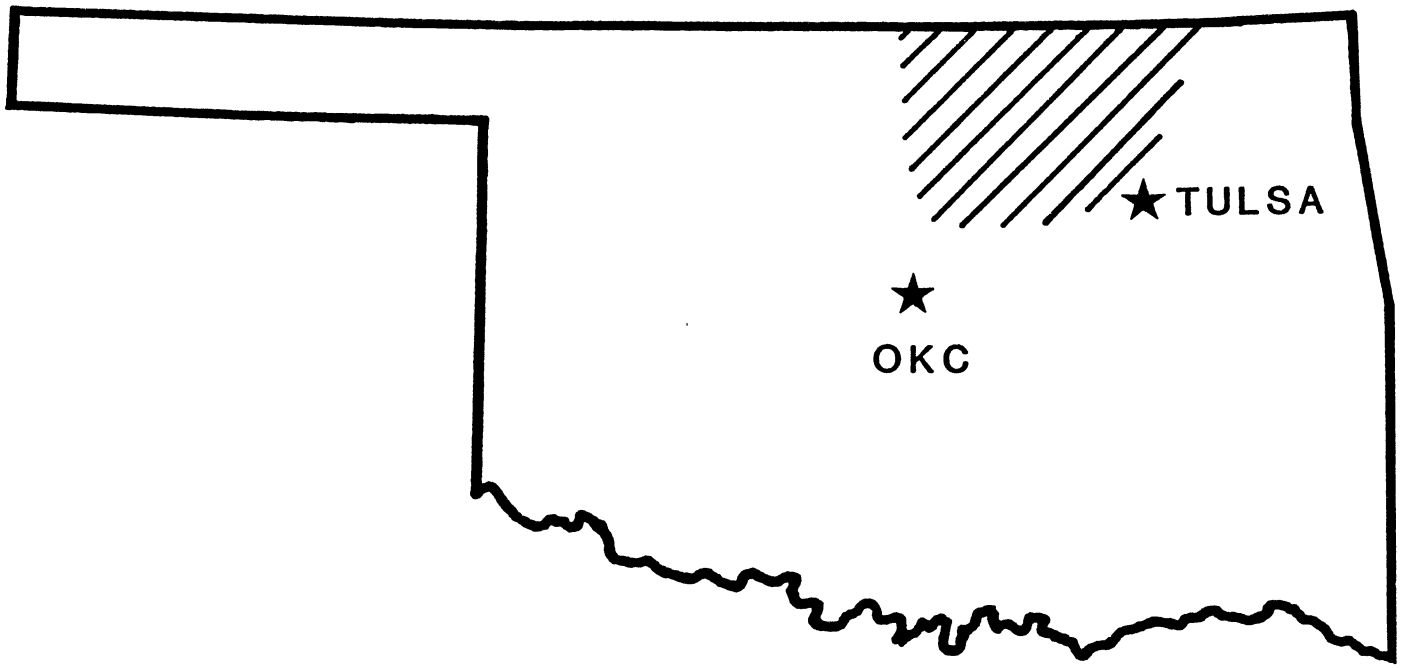

Figure 1. Map of the study area in northcentral Oklahoma.

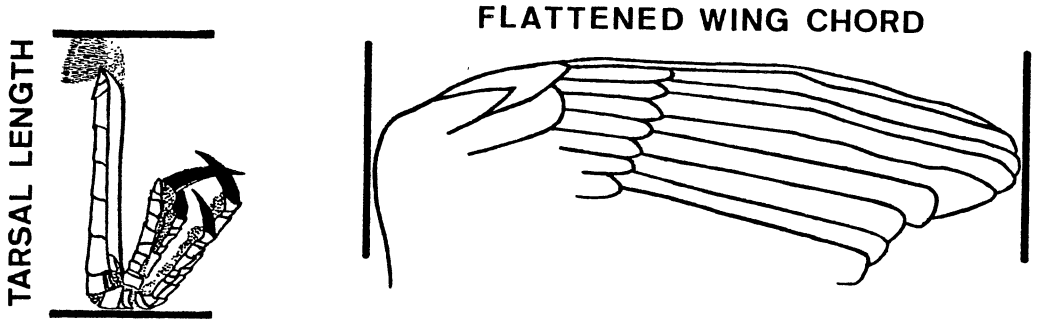

Figure 2. Example of how flattened wing chord and tarsal lengths were measured.

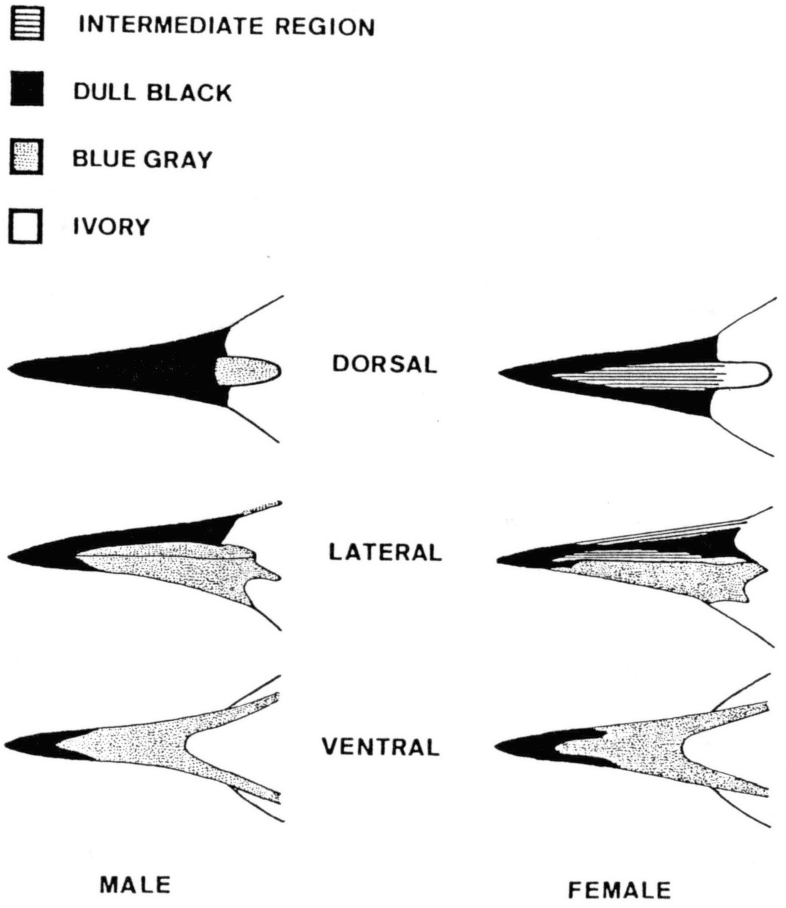

Figure 3. Differences in bill coloration between breeding adult male and female Eastern Meadowlarks in northcentral Oklahoma.

Chapter IV

SEASONAL CHANGES IN BODY COMPOSITION OF ADULT EASTERN MEADOWLARKS IN NORTHCENTRAL OKLAHOMA

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INTRODUCTION

Many birds store energy reserves in the body for use during periods of stress, e.g. inclement weather, defending territories, and reproduction. Most energy is stored as lipids with the remainder stored as protein or carbohydrates (Whittow 1965). Lipids hold over twice the potential energy per gram as carbohydrates or protein (Farner et al. 1961, Whittow 1965, Ricklefs 1974). Carbohydrates and proteins are utilized at different rates, depending on the season, and are not utilized as energy reserves to the same extent as lipids (Jones and Ward 1976, Raveling 1979).

Lipid deposition and seasonal factors affecting energy reserves have been studied extensively for some waterfowl species (Barry 1962, Harvey 1971, Ryder 1970, MacInnes et al. 1974, Milne 1976, Raveling and Lunsden 1977, Krapu 1981). In addition, seasonal lipid cycles have been

documented for several passerine species including White-crowned Sparrows (Zonotrichia leucophyrys gambelii) (King and Farner 1965), House Sparrows (Passer domesticus) (Blem 1976), Yellow-rumped Warblers (Dendroica coronata) (West and Meng 1968), and others. However, no studies have documented the annual and seasonal changes of lipid and protein reserves for a grassland inhabiting species in regard to environmental and physiological changes. Therefore, the purpose of this paper is to describe the seasonal changes in body composition of Eastern Meadowlarks (Sturnella magna) in northcentral Oklahoma in relation to environmental and physiological factors.

STUDY AREA

All Eastern Meadowlarks were collected in tallgrass prairie habitat in northcentral Oklahoma (Fig. 1). This study area is characterized by rolling plains of tallgrass prairie interspersed with post oak-blackjack oak forest (Duck and Fletcher 1943). The principal plant species in the collection areas were little bluestem (Schizochyrium scoparium), big bluestem (Andropogon gerardi), Indian grass (Sorghastrum nutans), ragweed (Ambrosia spp.), annual broomweed (Guteirezia dracunculoides), and silver bluestem (Bothriochloa saccharoides).

The climate of northcentral Oklahoma is influenced by the Gulf air stream during the summer and Continental air masses during the winter. A combination of Gulf, Continental, and Pacific pressure systems influence the spring and fall climate. The prevailing climate is temperate with pronounced seasonal variation in both temperature and precipitation. Annual rainfall in the study area varies from 102.6 cm

to 813.0 cm, and snowfall averages 20.0 cm with a mean of 3.8 days with measurable snowfall (Myers 1982). Relative humidity varies from about 50% at noon in July, to about 60% at noon in January with considerable variation between these periods (Myers 1982). The temperature varies from a mean of -0.5 C in January to a mean of 28.9 C in August. The mean number of days with a minimum temperature of 0 C or less is approximately 96 (Myers 1982), which results in a growing season of 200-210 days (Environmental Data Service 1976).

METHODS AND MATERIALS

Ten to thirty adult Eastern Meadowlarks were collected at two-week intervals from August 1982 to September 1983. Collected birds were placed on ice in the field to prevent decomposition and taken to the laboratory. At the laboratory, each carcass was weighed to the nearest 0.1 g (including ingesta and feathers) and frozen at -29 C. Subsequently, the carcasses were thawed, externally measured, and molt intensity was scored. Molt intensity was ranked by summing the scores of individual feather tracts. Each feather tract was given a score ranging from one to four; one, two, three, and four indicated that 0-25%, 26-50%, 51-75%, and 76-100% of the feathers were molting, respectively. The birds were then plucked and dissected. The gizzard, right pectoral muscle mass (pectoralis and supracoracoideus) and the right leg muscle mass were weighed to the nearest 0.1 g. The length and width of the testes and ovaries were measured to the nearest 0.1 mm with vernier calipers. Subsequently, testes, ovaries and oviducts were removed and weighed to the nearest 0.001 g on a Mettler analytical balance. The contents of the intestines were removed and discarded and

gizzard contents were placed in 70.0% isopropyl alcohol for later identification. The carcasses were then refrozen.

Lipid extraction on carcasses and eggs were as follows: Each frozen carcass, minus the tarsi and feathers, was ground 3 times in a meat grinder. The ground carcass was then homogenized in a blender for 2 min. Three 3-4 g tissue aliquots were removed and dried in a forced air oven for at least 10 hrs. The dried tissue aliquots were removed from the oven and allowed to cool for 30 min. in a desiccator before being weighed to the nearest 0.001 g on a Mettler analytical balance. In addition, lipid analysis was conducted on eight Eastern Meadowlark eggs collected in the 1984 breeding season. Each egg, minus the shell, was placed in a thimble and a wet weight was obtained. The eggs were then placed in a forced air oven and dried for at least 10 hrs. Eggs were reweighed to obtain dry weight and were then placed on a Goldfisch fat extractor for lipid extraction.

Ether extractable lipids were removed from the dried tissue aliquots and eggs using technical grade petroleum either (30-60 C b.p.) and a 10 hr refluxing time on a Goldfisch fat extractor. The ether refluxing rate was 5-6 drops per second.

The percent lipids [(g of extracted lipids/aliquot wet weight) X 100] was determined on the basis of wet weight because the potential for desiccation was similar for each carcass and curves based on wet weight and dry weight were highly correlated ($r = 0.97$, $P = 0.0001$). The percent water content of each aliquot [(aliquot wet weight - aliquot dry weight)/aliquot wet weight) X 100] was calculated and the percent non-ether extractable residue [(100 - % water) - % lipids (Iverson 1981)] was used to estimate protein content (Raveling 1979). The

percent lipid, protein, and water of each homogenized bird were estimated by averaging the values obtained from the 3 tissue aliquots from each bird.

The annual cycle of eastern meadowlarks in northcentral Oklahoma was divided into 4 seasons as follows:

(a) Fall (August through November). Both the molt cycle, which occurred from late July through September, and migration were included under fall because molting meadowlarks migrate in northcentral Oklahoma.

(b) Winter (December through March). Most females had moved out of the study area prior to winter, leaving only adult males. Males, consisting of both resident breeding birds and migrant birds, were present on the study area.

(c) Spring (April). April was the month migrating females returned to the study area.

(d) Breeding Season (May through July). Courtship, nest building, incubation, and fledging of young occurred during this season.

All statistical analysis incorporated the use of the Statistical Analysis System (SAS Institute, Inc. 1982). ANOVA (General Linear Model procedure), modified Duncan's Multiple Range Test (Kramer 1956) and unpaired *t*-tests were used to test the significance of seasonal differences within and between sexes. Simple correlation was used to test relationships between testes weight, ovary width, temperature, field weight; and percent lipid, percent protein, and percent water.

RESULTS AND DISCUSSION

There were generally significant ($P < 0.05$) seasonal differences in

lipid levels, protein levels and water content of males and females (Table 1)(Appendix). The single exception to this trend was that female protein levels were not significantly different among seasons (F = 0.00, df = 2, 99, P = 0.9994).

FALL

During fall, no significant difference was observed between sexes in percent lipids, percent protein, and percent water ($P > 0.05$). In general, lipid levels of both sexes gradually increased during the fall season (Table 2).

During the fall migration period, molting Eastern Meadowlarks were collected from late July through September on the study area. Males began molting in late July to early August, followed by females in early to mid-August. Prior to the molt cycle, in July, we observed a higher water content in females and a slightly lower protein content in both males and females (Fig. 2). Once molt was initiated, however, there was no significant difference ($P > 0.05$) in percent lipids, percent protein, or percent water in relation to intensity of molt.

King et al. (1965) found that premigratory White-crowned Sparrows had relatively low levels of protein and suggested the decrease in protein was an adaptation for reducing excess weight in preparation for migration. However, the high water content and decreased protein levels observed in Lesser Redpolls (Carduelis flammea cabaret: Evans 1969) and non-migratory Bullfinches (Pyrrula pyrrula: Newton 1968) during molt were thought to be an adaptation to molting rather than migration. Newton (1968) suggested that increased water content of molting birds was the result of increased blood volume during the molt process as an adaptation to nourish the growing feathers.

Eastern Meadowlarks apparently do not gain water or lose protein due to molt and, therefore, the loss of protein observed in Eastern Meadowlarks prior to molt is possibly an adaptation for migration as King et al. (1965) suggested occurs in White-crowned Sparrows.

Birds normally increase lipid deposits during fall in preparation for migration and winter (Odum and Perkinson 1951, King 1963, King and Farner 1965, Reinecke et al. 1982). Although meadowlarks are reported to migrate in the fall (Saunders 1932, Lanyon 1957, Bent 1958), the increase in lipid levels does not resemble the lipid cycle observed in most other migratory species. Most migratory passerines apparently have lipid peaks in the fall and spring (King and Farner 1965, Blem 1976). The lipid cycle of Eastern Meadowlarks resembles the lipid cycle of a non-migratory species, such as the House Sparrow, which gradually increase lipid levels in the fall, peaks once during the winter and decreases to the lowest lipid levels during the summer (Blem 1976).

Odum (1960) proposed 3 patterns of migratory obesity: "(1) short range migrants which attain moderate obesity but begin migration before peak deposition; (2) short range migrants which begin migration after peak deposition; (3) and long range migrants which become extremely obese just prior to long flights." Meadowlarks in northcentral Oklahoma appear to exhibit the migratory obesity pattern of a short range migrant which migrates before peak lipid deposition (Fig. 2). Meadowlarks were detected migrating in the study area as early as August and September (Kemmerer, unpubl. data). It may not be necessary for birds to increase lipid levels greatly at this time of year because food is abundant.

The interpretation of the fall lipid cycle of Eastern Meadowlarks is complicated because meadowlarks migrate while they are still molting.

However, because we detected no significant changes in water, protein, or lipid content of Eastern Meadowlarks that could be related to molt intensity, the influence of molt on body composition may be relatively unimportant.

WINTER

Although male Eastern Meadowlarks were common on the study area during the winter, females were not present. Males had significantly higher lipid levels and lower water content during the winter than during any other season (Table 1). Males showed a significant inverse relationship between percent moisture and percent lipids ($r = -0.81$, $\underline{P} = 0.0001$) during winter, a relationship that has also been observed in the body composition changes of other avian species (Helms et al. 1967, Peterson and Ellarson 1979, Wishart 1979, Iverson 1981).

Adult male lipid levels reached a peak of 14.5% (19.4 g) in January and then decreased significantly to 3.1% (3.9 g) in March. During the same period of time, significant increases were observed in percent protein and percent water ($\underline{P} < 0.05$)(Appendix).

One factor that has been suggested to be involved in the decrease in lipids was snowfall. Two periods of snowfall occurred during our study, one on 31 January and the other on 3 February 1983, with three to four inches of snow accumulation each time. There was no significant difference between the percent lipids of males collected on 30 January and 12 February (Fig. 3). Therefore, snow apparently had no significant affect on the lipid decrease that occurred in late winter.

Mean ambient temperature was inversely correlated with lipid levels and body weight ($r = -0.53$, $\underline{P} = 0.0001$), and ($r = -0.54$, $\underline{P} = 0.0001$), respectively, and the drop in body weight was correlated with the drop

in lipid levels ($r = 0.57$, $P = 0.0001$). Inverse relationships between body weight or lipid reserves and temperature have been discussed by a number of authors (Baldwin and Kendiegh 1932, Odum and Perkinson 1951, King and Farner 1966, Helms 1968).

The data indicate that the reproductive cycle was the primary factor responsible for the drop in lipid levels in late winter (Fig. 3). Field observations showed that on 12 February 1983 males had started singing for the first time during the winter and appeared to be forming territories. By 26 February 1983, a majority of the males were singing and had formed and were defending territories. A significant drop in the lipid levels between these 2 dates (Fig. 3) suggested that increased territorial displays and defense required a larger amount of energy than male Eastern Meadowlarks were able to obtain from their immediate food sources. Corresponding to the increase in territoriality, the food habits of adult males changed from a diet consisting of about 75% high energy seeds in January and early February to a diet of approximately 100% animal matter at the end of February (Kémmerer unpubl. data). The behavioral changes as a result of the reproductive cycle and the dietary change apparently were the principal factors responsible for the decrease of lipid levels. There was also a high inverse correlation between increased testes weight and the reduction in body lipids ($r = -0.70$, $P = 0.0001$). We do not think the increase in testes size was related to the decrease in body lipids because King (1972) reported the energy requirement for seasonal growth of the testes is a negligible fraction of the energy budget of adult male birds.

SPRING

During the spring, females returned to the study area with

significantly higher levels of lipids (2.9% or 2.1 g) than males (2.1% or 2.3 g) (t = 2.42, df = 32, P = 0.0218). However, males and females did not differ significantly in percent water or percent protein ($P > 0.05$) (Appendix).

Nothing is known about the lipid levels of wintering female Eastern Meadowlarks, but they are apparently able to accumulate sufficient lipids to supplement the energy requirement necessary for migration and still have a reserve left when they arrive on the breeding grounds. The value of arriving on the breeding grounds with high lipid reserves has been demonstrated for several species of birds, especially waterfowl. Waterfowl need high lipid reserves when they reach the breeding grounds in order to help insure egg hatchability (Barry 1962, Ryder 1970, Harvey 1971, MacInnes et al. 1974, Milne 1976, Raveling and Lumsden 1977, Krapu 1981). In addition, Mallard hens (Anas platyrhynchus) apparently use lipid reserves as a source of energy that permits them to feed on high protein but low energy aquatic invertebrates that are necessary for egg formation (Krapu 1981).

BREEDING SEASON

During the breeding season, percent lipids, percent protein, or percent water were also not significantly different ($P > 0.05$) between sexes.

Lipid levels did decrease during parts of the breeding cycle (Fig. 2) and a slight correlation existed between the decrease in lipid levels and an increase in reproductive organ size of females (r = -0.35, P = 0.0429). Although the drop in female lipid reserves might be attributed to a high protein animal matter diet, a simultaneous decrease also occurred in protein levels. Back calculations from the date

juveniles appeared in the samples to when eggs were being laid, indicated the peak of the egg laying period corresponded to the last of May, the period when the lipid and protein levels decreased and reached their lowest point, i.e. 1.1% (0.8 g), 22.3% (15.9 g), respectively, and when ovarian widths first peaked (Fig. 4).

Egg production requires relatively large amounts of energy, approximately 13-16% above the daily energy requirements in passeriforms (King 1972). Therefore, females often use body stores of lipids for the production of eggs. The average percentage lipids found in eight meadowlark eggs was 5.3% based on wet weight or 0.26 g of lipids per egg (Table 3). Based on these data, female meadowlarks do contain enough lipids (2.3 g or 3.2%) when they arrive on the breeding grounds to produce an average clutch of 4-5 eggs (1.0-1.3 g lipids) on a gram per gram basis and still have an average of 1.1 g of lipids remaining. At the peak of the egg laying period, females contained about 0.8 g of lipids which strongly suggests body fat deposits were the principal source of lipids for the first clutch. Therefore, female meadowlarks apparently obtain the majority of their maintenance energy from their diet, and utilize stored lipids for production of the first clutch.

Female meadowlarks lost 22.5% (4.6 g) of their stored protein during the egg laying period and much of that protein appeared to be used to produce eggs. Assuming the remaining portion of an egg after drying and lipid removal was protein, an average meadowlark egg contained 12.3% (0.6 g) protein (Table 3). On a gram to gram basis, the protein loss of females during the egg laying period (4.6 g) was greater than the amount of protein required to lay an average 4-5 egg clutch (2.5-3.1 g). Several protein sources were measured to determine

where protein catabolism might have occurred. The major areas of protein loss in females were the gizzard and the pelvic and pectoral muscles (Table 4). The summation of weight loss from the gizzard and pelvic and pectoral muscles of females accounted for 3.6 g of the total 4.8 g protein lost during the egg laying period in May. The remainder of the protein that was lost during the egg laying period may have come from unmeasured body muscles. Some of the catabolized protein may have been redistributed to the reproductive organs. During the incubation period, female gizzard and pelvic and pectoral muscle weights increased as protein values increased to a second peak on 18 June and then decreased to a second low on 30 July corresponding to a similar protein decrease.

Our data suggest that females may obtain protein from the gizzard, and pelvic and pectoral muscles for the production of the first clutch but protein for second clutches, and renesting attempts apparently comes from the diet. Eastern Meadowlarks appear to use protein from the body muscles for egg production as have been discussed for Red-billed Quelea (Quelea quelea) (Kendall et al. 1973, Jones and Ward 1976), and for Common Eiders (Somateria mollissima) (Korschgen 1977).

Lipid reserves of female Eastern Meadowlarks increased during the incubation period between the end of May and the end of June suggesting that incubating was less energetically stressful than egg production. However, as young began to hatch and fledge during June and July, lipid and protein levels decreased, indicating that care of young and possibly renesting was an energy costly process.

Saunders (1932) reported that meadowlarks continued to feed their young for at least 2 weeks after they fledged. Females that layed a

second clutch after the first brood fledged, often started laying as early as 3-4 days after the first brood fledged. Therefore, the energy demand on double-brooding females may be quite high in the nesting season. However, most Eastern Meadowlarks probably do not produce two broods because Roseberry and Klimstra (1970) reported that nest mortality may exceed 70%. Therefore, there was probably a high level of renesting throughout the nesting period. The extra energy demand of brood-rearing and renesting would explain the low levels of protein and lipids found late in the breeding season and the prolonged period of time they stayed low.

Male lipid levels were constant throughout the breeding season at a similar level as that reached in March after territories were formed. However, protein levels decreased from a maximum of 27.5% (30.0 g) on 9 April to a low of 21.2% (21.8 g) on 21 May. Male meadowlarks actively defend territories during courtship, mating, and egg laying, but the intensity of territorial defense apparently decreases once incubation is initiated (Saunders 1932). Therefore, the date male protein levels reached their lowest point corresponds closely to the date we calculated to be the peak of the laying period and presumably males were actually defending territories. Subsequent to the peak of egg laying, protein levels of males increased until 18 June when protein content was 26.4% (24.9 g). Presumably, males relaxed territorial defense as incubation proceeded. The relaxation in territorial defense apparently decreased energetic output and allowed more time to feed. Subsequent to the incubation period, however, protein levels decreased and reached a low value of 18.6% (20.2 g) on 2 July which corresponds to the period when Saunders (1932) reported males are actively feeding the young. After

brood rearing ceased, a rapid increase in protein levels occurred prior to molt in males, as was also observed in females.

Significant decreases ($P < 0.05$) occurred in gizzard weight, right pectoral muscles, and right pelvic muscles in males. These muscle weight changes closely paralleled the changes observed in the overall protein content throughout the breeding season and possibly were catabolized to meet energy needs.

Males appear to use lipid reserves as an energy source for defending territories and courtship activities. Once lipid reserves are depleted, protein is catabolized to meet energetic demands.

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Table 1. Seasonal variation in percent lipids, protein, and water of Eastern Meadowlarks collected from August 1982 to July 1983 in northcentral Oklahoma. Means underscored by lines at the same level are not significantly different ($P > 0.05$, Modified Duncan's Multiple Range Test). Mean (SD)/sample size.

	Fall	Winter	Spring	Breeding Season
LIPIDS				
Males	3.8 (2.3)	9.2 (5.1)	2.1 (0.9)	2.0 (1.2)
	66	86	20	52
Females	3.8 (2.0)		2.9 (0.9)	2.1 (1.0)
	39		13	50
PROTEIN				
Males	26.3 (2.5)	24.8 (2.6)	27.0 (2.2)	26.3 (3.1)
	66	86	20	52
Females	26.0 (2.5)		26.0 (2.0)	26.0 (3.2)
	39		13	50

Table 1. Continued.

	Fall	Winter	Spring	Breeding Season
WATER				
Males	69.6 (1.9)	66.0 (4.4)	71.1 (2.5)	73.9 (5.0)
	66	86	20	52
Females	70.2 (2.4)		70.7 (1.2)	75.2 (4.6)
	39		13	50

Table 2. Monthly mean body weight, percent lipids, percent protein, and percent water of adult male (M) and female (F) Eastern Meadowlarks collected in northcentral Oklahoma.

Month (YR)	Sex	N	% Lipids	% Protein	% Water	Body Weight
August 82	M	9	3.0(1.1)[a]	26.7(2.7)[a]	70.5(2.3)[a]	106.5(17.6)[a]
	F	10	2.7(0.5)	25.6(1.3)	71.7(1.2)	87.2(9.3)
September 82	M	20	2.9(1.2)	26.1(1.3)	70.7(1.3)	106.1(15.8)
	F	13	2.9(0.7)	25.6(2.5)	71.4(2.2)	89.3(10.6)
October 82	M	21	2.9(1.8)	27.3(1.3)	69.7(1.3)	115.5(10.9)
	F	12	5.2(3.0)	26.6(1.2)	68.2(2.1)	92.6(11.1)
November 82	M	17	6.8(2.5)	25.4(1.3)	67.6(1.6)	123.9(9.7)
	F	4	5.3(0.9)	26.0(1.0)	68.7(0.5)	101.1(13.7)
December 82	M	15	10.7(2.4)	24.6(0.8)	64.7(1.9)	124.4(6.6)
	F	0				
January 83	M	32	14.5(2.5)	23.7(0.7)	61.9(2.2)	133.7(8.9)
	F	0				
February 83	M	14	7.4(3.4)	24.8(2.1)	67.8(3.5)	124.1(8.2)
	F	0				
March 83	M	26	3.1(1.9)	26.3(2.3)	70.6(1.9)	124.4(8.9)
	F	0				
April 83	M	20	2.1(0.9)	26.7(2.3)	71.2(2.5)	121.1(6.7)
	F	13	2.9(0.9)	26.4(1.3)	70.7(1.2)	84.8(4.3)
May 83	M	20	2.0(1.5)	23.3(5.9)	74.7(6.4)	115.3(4.9)
	F	20	1.6(0.9)	23.6(4.5)	74.9(4.7)	84.9(5.0)
June 83	M	16	1.6(0.9)	24.5(4.9)	73.9(5.1)	109.2(4.1)
	F	10	2.6(0.7)	24.3(3.6)	73.1(4.1)	87.9(5.3)

Table 2. Continued.

Month (YR)	Sex	N	% Lipids	% Protein	% Water	Body Weight
July 83	M	18	2.0(0.7)	24.3(3.7)	73.7(3.9)	110.6(1.5)
	F	21	1.8(0.7)	21.9(4.2)	76.3(4.6)	82.4(6.8)
August 83	M	6	1.8(0.9)	23.9(3.4)	74.4(3.5)	113.5(5.1)
	F	9	2.4(0.9)	24.5(2.9)	73.1(3.5)	82.8(5.2)
September 83	M	16	3.8(0.8)	25.8(1.0)	70.5(0.9)	118.5(6.4)
	F	21	3.3(0.9)	25.9(1.0)	70.9(0.8)	87.5(4.9)

[a] Value (Standard Deviation)

Table 3. Lipid, protein, and water content, based on wet weight, of eight Eastern Meadowlark eggs collected during the 1984 breeding season in northcentral Oklahoma.

		Composition					
		Lipid		Protein		Water	
No.	Egg Weight(g)	grams	%	grams	%	grams	%
1	5.1811	0.2604	5.0	0.5553	10.7	4.3654	84.3
2	5.6224	0.2739	4.9	0.5860	10.4	4.7625	84.7
3	4.2328	0.2895	6.8	0.5004	11.8	3.4429	81.3
4	4.7639	0.2521	5.3	0.4850	10.2	4.0268	84.5
5	5.0606	0.2577	5.1	0.6869	13.6	4.1560	81.3
6	4.1052	0.2558	6.2	0.7101	17.3	3.1393	76.5
7	6.7351	0.3001	4.5	0.7979	11.8	5.6371	83.7
8	4.1906	0.1848	4.4	0.5872	14.0	3.4186	81.6
$\bar{X}$	4.9865	0.2593	5.3	0.6136	12.3	4.1186	82.2

Table 4. Mean weights of gizzard, pelvic muscle, and pectoral muscle of adult Eastern Meadowlarks collected during the 1983 breeding season in northcentral Oklahoma. Mean ± SD (sample size).

Date	Sex	Gizzard	Pelvic Muscle	Pectoral Muscle
9 April	M	3.3 ± 0.4 (9)	7.5 ± 0.5 (9)	12.1 ± 1.5 (9)
	F	2.1 ± 0.5 (7)	4.1 ± 0.3 (7)	7.9 ± 0.6 (7)
23 April	M	2.8 ± 0.6 (11)	7.5 ± 0.7 (11)	12.0 ± 0.7 (11)
	F	2.2 ± 0.3 (6)	4.8 ± 0.5 (6)	8.2 ± 0.4 (6)
7 May	M	2.1 ± 0.6 (10)	7.6 ± 0.4 (10)	11.2 ± 0.8 (10)
	F	1.7 ± 0.3 (10)	4.7 ± 0.2 (10)	8.6 ± 0.6 (10)
21 May	M	2.3 ± 0.3 (10)	7.5 ± 0.6 (10)	10.9 ± 0.5 (10)
	F	1.7 ± 0.3 (10)	4.0 ± 0.5 (10)	7.2 ± 0.6 (10)
4 June	M	2.3 ± 0.3 (11)	7.3 ± 0.9 (11)	10.6 ± 0.7 (11)
	F	1.7 ± 0.2 (10)	4.7 ± 0.4 (10)	7.5 ± 0.7 (10)
18 June	M	1.8 ± 0.3 (14)	6.5 ± 0.6 (13)	9.6 ± 1.0 (13)
	F	1.7 ± 0.3 (7)	4.9 ± 0.7 (7)	7.3 ± 0.5 (7)
2 July	M	1.8 ± 0.4 (12)	6.4 ± 0.9 (10)	9.2 ± 0.9 (12)
	F	1.5 ± 0.4 (11)	4.5 ± 0.5 (11)	7.2 ± 0.6 (11)
16 July	M	2.4 ± 0.3 (14)	7.0 ± 0.7 (14)	10.1 ± 0.7 (14)
	F	1.7 ± 0.1 (9)	4.3 ± 0.8 (9)	6.7 ± 0.9 (9)
30 July	M	2.2 ± 0.3 (7)	6.9 ± 0.7 (9)	10.3 ± 0.8 (9)
	F	1.7 ± 0.3 (8)	4.3 ± 0.8 (9)	6.8 ± 0.9 (9)
13 August	M	2.1 ± 0.2 (10)	6.4 ± 0.7 (10)	10.0 ± 1.0 (10)
	F	1.5 ± 0.4 (13)	4.4 ± 0.7 (13)	7.0 ± 0.7 (13)
27 August	M	2.9 ± 0.5 (5)	7.2 ± 0.8 (5)	10.9 ± 0.4 (5)
	F	2.1 ± 0.4 (8)	4.4 ± 0.6 (8)	7.8 ± 0.9 (7)

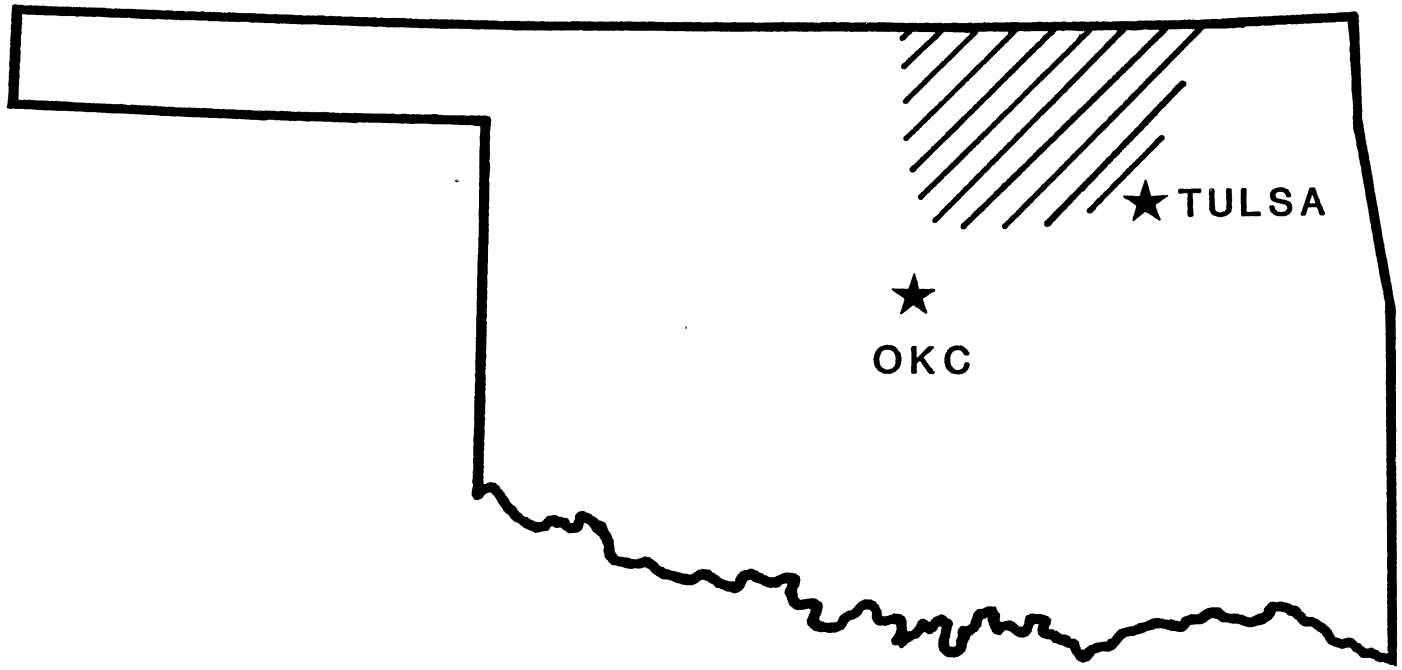

Figure 1. Map of the study area in northcentral Oklahoma.

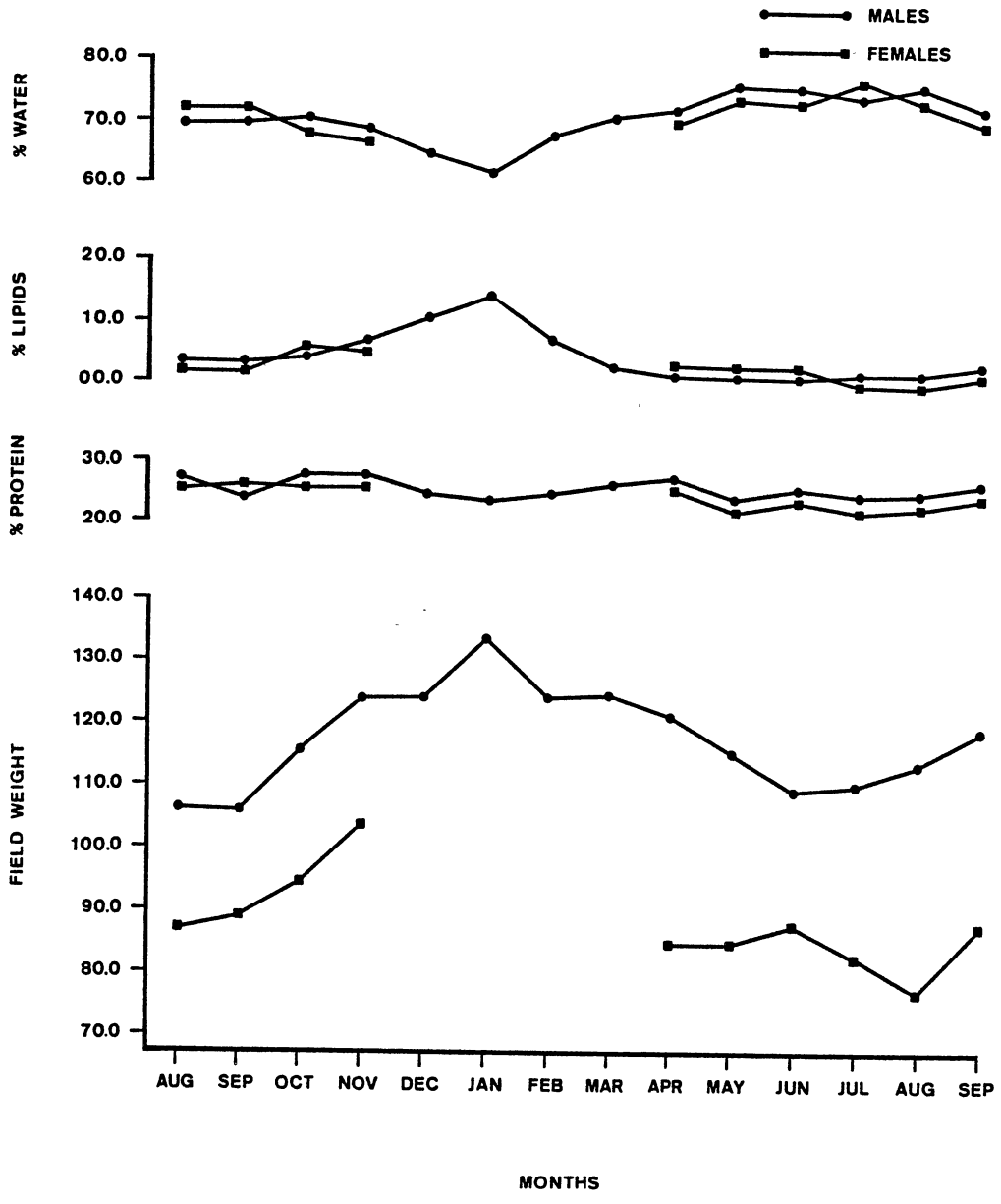

Figure 2. Annual variation in percent lipids, percent protein, percent water, and field weight (g) of adult male and female Eastern Meadowlarks in northcentral Oklahoma.

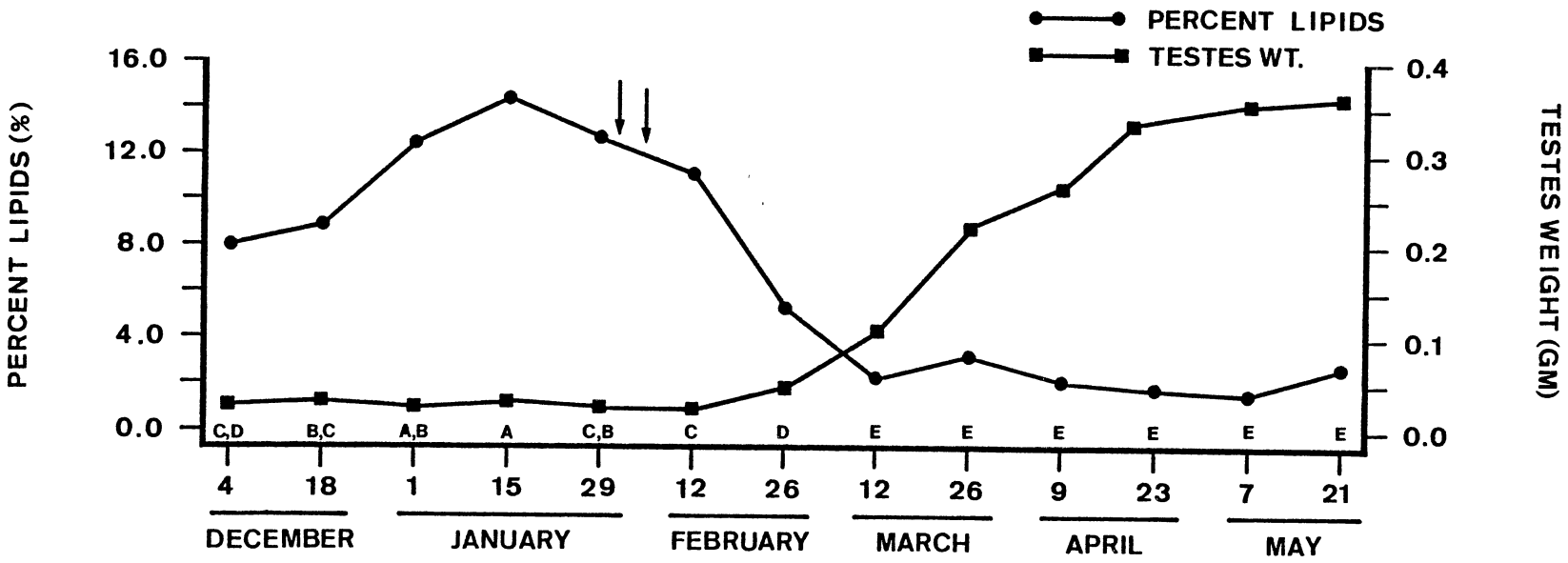

Figure 3. Changes in mean percent lipids in relation to testes weight of adult male Eastern Meadowlarks. Arrows indicate dates snowfall occurred with accumulation on the ground. Letters designate sets of lipid means not significantly different from each other (1-way ANOVA and Modified Duncan's Multiple Range Test, $\underline{P} > 0.05$).

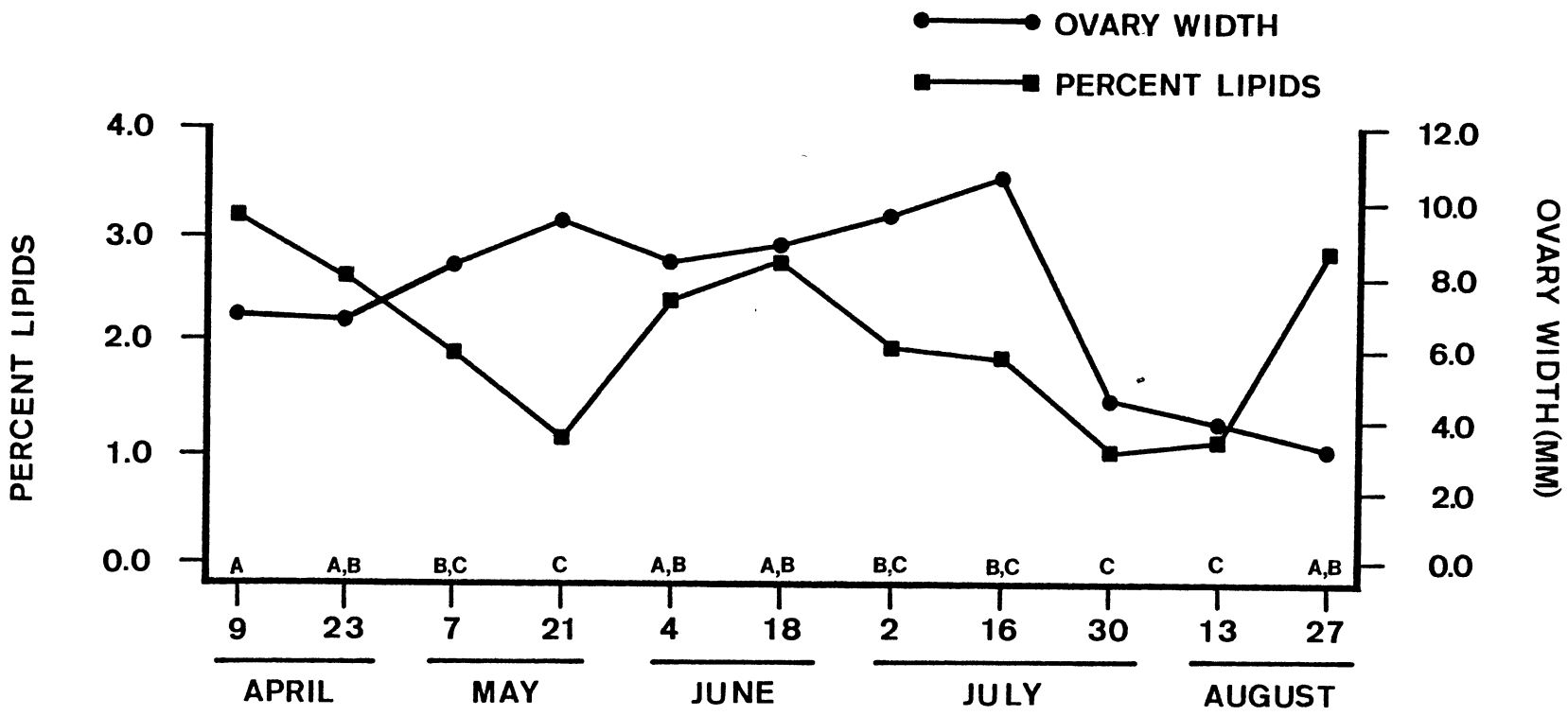

Figure 4. Changes in mean percent lipids in relation to ovary width of adult female Eastern Meadowlarks. Letters designate sets of lipid means not significantly significantly different from each other (1-way ANOVA and Modified Duncan's Multiple Range Test, $\underline{P} > 0.05$).

APPENDIX

DETAILED STATISTICAL ANALYSIS OF SEASONAL CHANGES IN BODY COMPOSITION OF ADULT EASTERN MEADOWLARKS IN NORTHCENTRAL OKLAHOMA

There were significant seasonal differences in lipid levels of males (F = 56.36, df = 3, 220, P = 0.0001) and females (F = 15.17, df = 2, 99, P = 0.0001) (Table 1). There was also significant seasonal variation in protein levels of males (F = 7.02, df = 3, 220, P = 0.0002), but female protein levels were not significantly different among seasons (F = 0.00, df = 2, 99, P = 0.9994). In addition, water content was significantly different among seasons for both males (F = 48.33, df = 3, 220, P = 0.0001) and females (F = 23.12, df = 2, 99, P = 0.0001).

FALL

During fall, no significant difference was observed between sexes in percent lipids (t = 0.10, df = 103, P = 0.9035). Males and females had an average of 3.8% (3.9 g) and 3.8% (3.0 g) lipids, respectively. Percent protein showed no significant difference between sexes [(males: 26.3% or 25.6 g; females: 26.0% or 20.3 g) (t = 0.50, df = 103, P = 0.6186)] during the fall season. Males and females, also showed no significant difference in percent water (t = 1.57, df = 103, P = 0.1190)

with males and females having 69.6% (67.7 g) and 70.2% (54.7 g) water, respectively. In general, lipid levels of both sexes gradually increased during the fall season (Table 2).

During the fall migration period, molting Eastern Meadowlarks were collected from late July through September on the study area. Males began molting in late July to early August, followed by females in early to mid-August. Prior to the molt cycle, in July, we observed a higher water content in females and a slightly lower protein content in both males and females (Fig. 2). Once molt was initiated, however, there was no significant difference in percent lipids (males: $F = 0.67$, df = 23, 65, $P = 0.8542$; females: $F = 0.22$, df = 22, 63, $P = 1.0000$), percent protein (males: $F = 0.64$, df = 20, 65, $P = 0.8824$; females: $F = 1.20$, df = 22, 63, $P = 0.2806$), or percent water (males: $F = 0.66$, df = 23, 65, $P = 0.8688$; females: $F = 0.84$, df = 22, 63, $P = 0.6674$) in relation to intensity of molt.

WINTER

Adult male lipid levels reached a peak of 14.5% (19.4 g) in January and then decreased significantly ($F = 153.3$, df = 2, 69, $P = 0.0001$) to 3.1% (3.9 g) in March. During the same period of time, significant increases were observed in percent protein [23.7% (31.7 g) to 26.3% (32.7 g)] ($F = 16.83$, df = 2, 69, $P = 0.0001$) and percent water [61.9% (82.8 g) to 70.6% (87.8 g)] ($F = 97.67$, df = 2, 69, $P = 0.0001$).

SPRING

During the spring, females returned to the study area with significantly higher levels of lipids (2.9% or 2.1 g) than males (2.1% or 2.3 g) ($t = 2.42$, df = 32, $P = 0.0218$). However, males and females

did not differ significantly in percent water (t = 0.65, df = 32, P = 0.5239); males and females had 71.1% (76.5 g) and 70.7% (52.4 g) water, respectively. In addition, the percent protein of males and females, 27.0% (29.0 g) and 26.0% (19.3 g), respectively, was not significantly different (t = 1.29, df = 32, P = 0.2062).

BREEDING SEASON

During the breeding season, percent lipids were also not significantly different (t = 0.14, df = 100, P = 0.8953) between sexes with males and females having 2.0% (2.0 g) and 2.1% (1.5 g), respectively. In addition, no significant difference was observed in percent protein (t = 0.44, df = 100, P = 0.6646) between males (26.3% or 25.9 g) and females (26.0% or 19.2 g), or percent water [males: 73.9% (72.9 g); females 75.2% (55.4 g)], (t = 1.31, df = 100, P = 0.1941).

VITA 2

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