DIURNAL CYCLES IN LIVER WEIGHTS IN BIRDS

By HARVEY I. FISHER and LAWRENCE M. BARTLETT

While gathering data for a study of changes in organ weights during various phases of the reproductive cycle of Red-winged Blackbirds (*Agelaius phoeniceus*), we were first struck by a difference in the color of the liver of birds collected in the morning compared with others collected in the evening. We thought differences in constituents might be causing the color variations, and this prompted us to compare liver weights. The great overnight drop in liver weight led to a preliminary study to determine the factors involved in the daily changes noted.

Further, we were unable to find in the literature any reference to diurnal cycles of weight or constituents of the liver in normal, wild animals except in Odum and Perkinson (1951:217) who stated that birds were collected "in the late afternoon, so as to minimize daily variations in lipid content." Since nothing further about "daily variations" was mentioned in their paper we assume they were exercising normal care in obtaining a homogeneous sample based on their knowledge of the literature describing diurnal cycles of fat, glycogen, and protein in laboratory animals.

Another factor emphasizing the importance of information on a possible diurnal cycle in liver weight is that in virtually none of the multitude of papers on the liver is there any mention of the time of day when the animals were killed; the papers by Odum and Perkinson (1951) and Oakeson (1953, 1956) are exceptions. If there is a diurnal cycle in liver weight and in fat, protein, or carbohydrate constitutents of the liver, data presented without precise information on time of collection of the specimens is of no value for comparative purposes.

MATERIALS AND METHODS

Collections.—While netting the Red-wings we also took a number of Starlings (*Sturnus vulgaris*); data from these are included. The specimens used in this study were either shot or captured at their roosts in a Japanese "Mist Net" between February 16 and March 27, 1956, in the vicinity of Carbondale, Jackson County, Illinois. The major part of the collection was made between February 27 and March 21. Time of kill is given in the tables. No significant differences were found between the mean body or liver weights of birds shot and birds netted. Furthermore there appeared to be no difference in these average weights between 12 female Red-wings held in cages outdoors (30°F.) and 14 female Red-wings held in cages indoors (70°F.). In addition there was no difference between birds netted and killed in the morning as compared with those netted in the evening and held over, without food, to be killed the following morning. In Red-wings there was no significant difference in the data between adult and first-year males. Therefore, the data from the various collecting and handling procedures have been combined for the statistical treatment of the differences between sexes and times of day.

One group of 27 female Red-wings (table 2), held for 12 or more hours, was provided with scratch food and water, but when they were killed we found no significant evidence that they had fed. The Starlings (table 3) were held without food and water.

A total of 45 male and 72 female Red-wings and 20 male and 11 female Starlings were used in this study.

Weights.—Body weights were obtained from a triple-beam balance accurate to 0.1 gram; liver weights were measured on a Roller-Smith torsion balance, to the nearest milligram. The livers were removed as soon as possible after death. In most instances this was within one-half hour, but all specimens were worked up within one and one-half hours. As each liver was removed, it was freed of any extraneous tissue and blotted on paper towels before being weighed. Each liver was then divided. One part was weighed, placed in ethanol-ether (3:1) and stored in the refrigerator for later quantitative analysis of the lipids. The other part was placed in a dry shell vial, corked, and quick-frozen for glycogen analysis.

Chemical methods.—During the analysis of fats a short circuit in the refrigerator in which the samples were stored ignited the ether, causing an explosion and fire which destroyed 37 of the 80 samples of fat. The pieces of liver for glycogen analysis were stored in the freezing unit of the same refrigerator, but 52 of the original 78 samples appeared to be unharmed. Nevertheless, a few "odd" results in our data may be attributed to this accident. Although we cannot attach too much significance to the individual results, we feel that the trends shown justify the inclusion of the data on fat and glycogen analysis, pending a more complete study.

The analysis of fats was carried out according to Bloor's (1928) titrimetric method. We used a factor of 3.7 in the calculations to arrive at our estimate of total fat.

Since the separation and purification of the glycogen was a combination of several procedures (Consolazio, Johnson, and Marek, 1951; Snell and Snell, 1953; and Wagtendonk, Simonsen, and Hackett, 1946) we shall describe the steps briefly. The liver sample was placed in a 15-ml. centrifuge tube with sufficient 35 per cent KOH to cover it. This was heated in a boiling water bath for 30 minutes, with occasional shaking, until the sample was completely digested. After cooling, 95 per cent ethanol was added, the solution mixed vigorously with a platinum wire, and then immersed in hot water to bring the alcohol just to its boiling point. It was then centrifuged while warm, the supernatant decanted, and the residue dissolved in 5 ml. of warm water. One drop of Lugol's solution was added, and the color read at a wavelength of 470 in a colorimeter against a reagent blank. The amount of glycogen was determined in the usual manner from a calibration curve.

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RESULTS

Changes in weight.—Figure 1 and tables 1 to 3 summarize the body and liver weights and the ratio of liver to body weight. In comparing any two means, a "t" test was used to determine the probabilities discussed in the text.

Although the body weight of male Red-wings decreases slightly during the night, both actual and relative liver weights decrease more rapidly. Data in table 1 show that the liver weight in male Red-wings decreases by one-third during a 12-hour night period. When the ratio of liver to body weight is considered, the overnight decrease is nearly as great (30 per cent).

Data in tables 1 and 2 show that both the relative and actual overnight decreases in body weight are greater in females than in males—10.7 versus 4.5 per cent and 5.2 versus 3.2 grams, respectively. Males show a greater loss in liver weight overnight than do females (33 versus 22 per cent). When calculated as per cent of body weight, liver weight in the males drops 30 per cent; in females this relative loss in weight is about 12 per cent. Part of this apparently lesser loss in females may be attributed to their greater loss in body weight.

Since we have no data for males killed after being held in captivity for more than 12 hours, we have no basis for distinguishing sexual differences in the changes in body

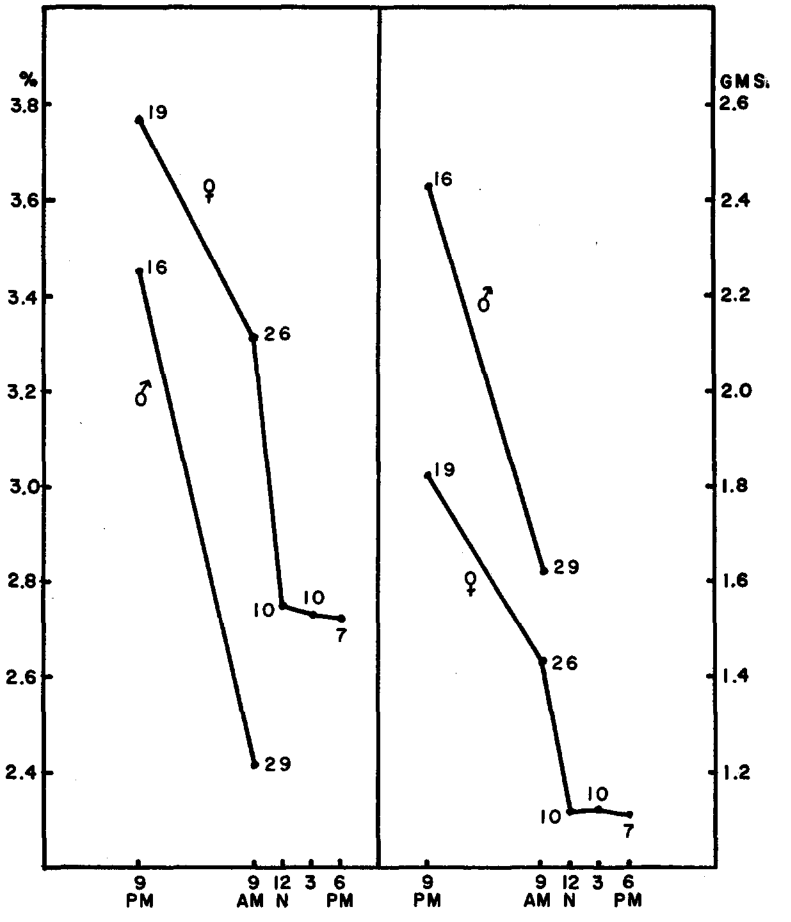

Fig. 1. Liver weights of Red-winged Blackbirds. Left, weights expressed as per cent of body weight. Right, weight in grams.

or liver weight beyond the overnight period. Although females (table 2) showed no significant loss in body weight after 9 a.m., even though they had not fed, the actual liver weight between 9 a.m. and noon decreased by 19 per cent and the relative liver weight went down 17 per cent. After noon there was no further significant change in either actual or relative liver weights.

Although we had only a small sample of Starlings (table 3), it would appear that the body weight of male Starlings decreases more (15 per cent) in the overnight period than it does in females (9 per cent). This is the reverse of the situation we found in the Red-wings (tables 1 and 2). The data on male Starlings, as on female Red-wings, after a 12-hour period in captivity do not indicate any loss in body weight during that time.

There was nearly a 40 per cent decrease in liver weight in male Starlings between the time they were captured in the evening and late the following morning; females showed only a 25 per cent decrease during the same period. The ratio of liver weight to body weight decreased 29 per cent for males and 18 per cent for females in this period. In these respects, the data from the two species are comparable.

Table 1

Body and Liver Weights of Male Red-winged Blackbirds

	Number specimens	Mean	Range	Standard deviation	Coefficient of variation
		Body weight in grams			
5–8 p.m.	16	70.4±1.28	63.0–77.4	4.96	7.0
6–10 a.m.	29	67.2±0.76	58.6–78.9	4.00	6.0
		Liver weight in grams			
5–8 p.m.	16	2.424±0.110	2.010–3.110	0.348	14.4
6–10 a.m.	29	1.621±0.035	1.354–1.962	0.183	11.3
		Liver weight as per cent of body weight			
5–8 p.m.	16	3.45±0.14	2.63–4.19	0.448	13.0
6–10 a.m.	29	2.42±0.05	1.97–2.83	0.262	10.8

Table 2

Body and Liver Weights of Female Red-winged Blackbirds

	Number specimens	Mean	Range	Standard deviation	Coefficient of variation
		Body weight in grams			
9 p.m.	19	48.5±0.95	41.8–55.5	4.02	8.3
9 a.m.	26	43.3±0.78	36.8–52.0	3.91	9.0
12 noon	10	42.1±1.05	38.2–46.1	3.16	7.5
3 p.m.	10	42.6±0.58	40.0–44.6	1.73	4.1
6 p.m.	7	41.6±1.16	38.5–47.2	2.84	6.8
		Liver weight in grams			
9 p.m.	19	1.823±0.052	1.450–2.390	0.222	12.2
9 a.m.	26	1.424±0.033	1.120–1.782	0.164	11.5
12 noon	10	1.155±0.039	0.981–1.436	0.118	10.2
3 p.m.	10	1.162±0.064	0.822–1.461	0.191	16.4
6 p.m.	7	1.130±0.068	0.925–1.319	0.167	14.8
		Liver weight as per cent of body weight			
9 p.m.	19	3.77±0.09	3.00–4.34	0.394	10.5
9 a.m.	26	3.31±0.09	2.47–3.96	0.449	13.6
12 noon	10	2.75±0.07	2.51–3.11	0.214	7.8
3 p.m.	10	2.73±0.14	2.06–3.30	0.412	15.1
6 p.m.	7	2.72±0.14	2.32–3.26	0.333	12.2

Changes in fat and glycogen.—Data in table 4 show that the fat content dropped overnight in Red-wings and Starlings. It increased again during the following afternoon in the male Starlings and female Red-wings. It may be recalled that the Starlings were held without food or water whereas the Red-wings had access to both but apparently did not eat.

Data in table 5 indicate that the glycogen content of the liver also dropped overnight. However, the drop was undoubtedly not as great as indicated by the averages since the three specimens possessing extreme glycogen supplies (11.5, 20.2, and 14.6 mg./gm., maxima) were all killed in the evening. These "extreme" results tend to raise the average value of the evening group of birds and thus increase the apparent overnight drop. Whereas the fat content appeared to make a recovery during the afternoon, the data suggest that there was little or no glycogen recovery during this period.

Table 3

Body and Liver Weights of Starlings Killed at Different Times of Day

	Males				Females			
	No. spec.	Mean	Range	Standard deviation	No. spec.	Mean	Range	Standard deviation
			Body weight in grams					
First p.m.	5	88.3±2.18	84.3–94.0	4.36	7	78.8±0.83	76.1–80.7	2.03
Morning	8	75.2±0.74	62.8–82.3	1.95	3	71.6±2.33	68.4–75.0	3.30
Second p.m.*	7	73.2±2.18	67.5–83.6	5.34				
			Liver weight in grams					
First p.m.	5	3.55±0.310	2.64–4.21	0.620	7	3.29±0.149	2.95–3.98	0.366
Morning	8	2.17±0.090	1.81–2.54	0.239	3	2.46±0.198	2.13–2.64	0.281
Second p.m.*	7	2.09±0.133	1.83–2.73	0.326				
			Liver weight as per cent of body weight					
First p.m.	5	4.04±0.390	3.07–4.94	0.779	7	4.18±0.165	3.67–4.93	0.402
Morning	8	2.89±0.114	2.58–3.44	0.301	3	3.43±0.221	3.11–3.69	0.313
Second p.m.*	7	2.86±0.171	2.33–3.63	0.419				

* Held in captivity for 24 hours.

DISCUSSION

Decreases in weight, fat, water, glycogen and protein have been shown by Higgins, Berkson, and Flock (1932, 1933) in rats deprived of food and water for 24 hours, but nowhere have we been able to find reference to a normal diurnal change in liver weight of any animal. This seems strange in view of the diurnal fluctuations in liver fat and glycogen which have been demonstrated in various laboratory animals. One would expect some change in gross weight of the organ as a result of changes in fat and glycogen content. However, one would certainly not expect, from anything in the available literature, that an overnight drop of about 30 per cent would occur. The magnitude of the fluctuation, then, was one of the most striking aspects of our study.

In our data the apparently greater drop in liver weight shown by the Starlings is probably not real. Most of the morning Starlings were sacrified later in the morning (about 11 a.m.) than the Red-wings. If we graph the drop in liver weight of the Red-wings we find that, by 11 a.m., the decrease was comparable to that shown by the Starlings. Therefore, it seems justifiable to assume that the degree of loss in liver weight may be comparable in the two species.

Weekly differences.—Seasonal differences in organ weights have been demonstrated for many species of birds. Since our collections were made at a time when at least some individuals of the species were in migration, and since Odum and Perkinson (1951) have demonstrated that fat deposition may occur over a very short period of time prior to the spring migration in the White-throated Sparrow (*Zonotrichia albicollis*) we were led to compare, at weekly intervals, the liver weights of female Red-wings during the three-week period over which most of our collections were made (table 6). Although there was little if any statistically significant weekly difference in mean liver weight of birds collected at the end of the day, there seemed to be a highly significant difference in birds collected in the morning. The morning values of 1.23 and 1.31 (first and second weeks) are not significantly different, but 1.31 and 1.56 (third week) are different (1 in 1000) and 1.23 and 1.56 are different (1 in 10,000). Thus, there was exhibited a trend of lesser overnight decrease in liver weight during the period of the study. Despite this trend, the differences between evening and morning weights remained significant (table 6). It may also be worth noting that the coefficient of variation is generally greater for evening than for morning liver weights. These data suggest the possibility that the female Red-wings were using stored materials in the liver at a greater rate during the nights of the week of February 27-March 4 than was the case between March 12 and 18.

Fat depletion overnight.—Our data are not sufficient to say with certainty whether the loss of fat from the liver could account fully for the loss in weight of the liver between 9 p.m. and 9 a.m. However, the four female Red-wings (table 4) sacrified at 9 p.m. and the five sacrified at 9 a.m. suggest this possibility. The average total amount of fat "lost" was 243 mg. while the average liver weight "lost" was only 190 mg. This suggests, ignoring possible quantitative errors in the chemical procedures, that some other constituent may be increasing while the fat is being depleted, but not at a rate which prevents a pronounced drop in liver weight. In spite of the fact that these nine birds did not show as great a drop in liver weight (1.77 to 1.58 gms.) as would be indicated by our other data, it would appear that changes in the fat content may account for the major, if not entire, observed drop in liver weight.

Table 4

Fat Content of Liver

Time	Sex	Number specimens	Av. mg./gm. fresh weight	Range
		Red-wings		
p.m.	♀	4	228	155–379
a.m.	♀	5	99	31–174
3 p.m.	♀	2	174	144–204
6 p.m.	♀	2	258	169–347
		Starlings		
9–12 p.m.	♂	3	187	110–234
	♀	6	262	65–439
Noon	♂	6	108	68–168
9–12 p.m.	♂	5	212	12–367

Comparison with other species.—Riddle (1928) working with the Ring Dove (*Streptopelia risoria*) found the liver weight greater in the female than in the male at all four seasons of the year, but it should be noted that in this species the female is the heavier sex. Oakeson (1953, 1956) found in the non-migratory race of the White-crowned Sparrow (*Zonotrichia leucophrys nuttalli*) that the liver weight of the female was greater than that of the male only in the spring of the year. Stegeman (1954) studied the body weight and weight of various internal organs in a group of 455 Starlings captured on a single night, chloroformed, kept in a cold room overnight, and weighed in groups of 50 on the following day. Stegeman gave the liver weights as male (4.110 ± 0.04) and female (3.823 ± 0.04); comparable data of ours are 3.55 ± 0.31 and 3.29 ± 0.15, respectively. In terms of body weight, he gave the liver as 5.0 per cent in both sexes, and we calculated it as about 4.0 per cent. The higher weights of livers of Stegeman's Starlings probably reflected the coagulated blood contained in them as a result of his method. The greater variability in the liver weights of our birds resulted perhaps from some differential retention of blood in fresh specimens and quite probably from the small size of our sample.

Whereas our data tend to be in agreement with Stegeman's statement that the liver weight of the male Starling is greater than that of the female, this is true only for birds collected in the evening. In others collected in the morning, our data indicate that the liver weight of the male is insignificantly less than that of the female.

When liver weight is considered as per cent of body weight, the female is "heavier" in the morning in both Starlings and Red-wings, whereas there appears to be no difference between sexes in the evening. However, during the night the male Red-wing loses only 5 per cent of its body weight and the female 10 per cent. In Starlings the male's body weight drops 15 per cent and the female's less than 9 per cent.

It may well be that the greater decrease in the female Red-wing's body weight accounts for all of the relatively larger liver in that sex. However, there is the possibility that the physiological events occurring in the liver during the period of sleep are different, at least in magnitude, between the sexes. Clavert (1944) has shown that administration of estrogen increases liver fat and protein as well as the number and size of the liver cells. The possible sex difference indicated in our work might, therefore, be attributed to the influence of estrogen on fat and protein content. Our data are not yet sufficient to say with certainty that there is any difference in the fat content of the liver between sexes at different times of the day.

Odum and Perkinson (1951) suggested that total lipids in the female White-throated Sparrows may be greater than in the male, especially in the premigratory period, when total lipids are considered as per cent of body weight. In their midwinter period (January-February) which is most comparable perhaps to the period of ours of late February to mid-March, they reported that 10.4 per cent of the fresh weight of the liver is lipid material in birds collected in "late afternoon." Because no definite time of collection was stated, it is impossible to know whether their data are comparable to our 3 p.m. kill (table 4) or to our 6 p.m. kill, but in any case our observed values seem to be considerably higher. Their data cannot be compared to our "p.m." data secured from birds killed, for the most part, between 9 p.m. and midnight.

The data of Musacchia (1953) cannot be used for comparison, partly because of seasonal differences, but primarily because there is no indication of the time of kill of the birds. There is, of course, no evidence to indicate any diurnal cycle or sexual difference in the livers of the species he studied, but perhaps only because this aspect has not been investigated.

In any event, it appears that the avian species thus far studied, regardless of the time of day, have a higher lipid content in the liver than do the familiar laboratory mammals (3 to 5 per cent).

Glycogen content of the liver in Starlings and Red-wings dropped between 9 p.m. and 9 a.m. and then maintained its level for at least 9 to 12 hours under starvation conditions. Fat content has just been shown to increase after a low at 9 a.m. Thus our data

Table 5

Glycogen Content of Liver

Time	Sex	Number specimens	Av. mg. per gm.*		Range
		Red-wings			
9 p.m.	♀	4	4.98		1.47–11.50
9 a.m.	♀	10	2.34		0.94– 6.71
Noon	♀	3	2.65		1.72– 4.13
3 p.m.	♀	3	1.17		0.75– 1.92
6 p.m.	♀	6	2.18		0.77– 4.86
		Starlings			
10 p.m.	♂	2	12.85	9.64	5.50–20.20
	♀	3	7.51		3.85–14.63
Noon	♂	6	2.27	2.28	1.35– 5.32
	♀	2	2.29		2.02– 2.56
10 p.m.	♂	6	2.68		1.63– 6.36

* Mg./gm. fresh liver weight in Red-wings and mg./gm. frozen liver weight in Starlings.

possess limited agreement with those of Petrén (1939) who found a maximal glycogen content in guinea pig livers between 11 a.m. and 3 p.m. and a minimal level between 5 and 9 a.m. and with those of Marble, Grafflin, and Smith (1940) who found no differences in glycogen, fat or water content in guinea pig livers between 9 a.m. and 3 p.m.

MacLachlan *et al.* (1942), Ota (1940) and Chevillard, Hamon, and Mayer (1937) reported accumulation of fat during fasting in several species of rodents. Ohlsson and Blix (1934, *in* Bloor, 1943) reported that in rats a high-fat phase coincides with a low-glycogen phase and vice versa. Our data for evening birds do not support this thesis; data on birds held under fasting conditions do corroborate the reverse relationship.

These comparisons with mammals may be specious. Liver metabolism is highly variable and in some studies (Kilborn, 1939) livers of birds have been shown to differ from mammals in that the type of fat stored in the bird's liver differs according to the type of fat in the diet.

Table 6

Comparison of Morning and Evening Liver Weights of Female Red-winged Blackbirds by Weeks

	Feb. 27–Mar. 4		March 5–11		March 12–18	
	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.
Sample size	9	17	5	5	6	7
Liver weight						
Mean	1.87±0.09	1.23±0.04	1.66±0.08	1.31±0.03	1.83±0.10	1.56±0.05
Range	1.46 – 2.39	1.00 – 1.52	1.45 – 1.86	1.20 – 1.36	1.68 – 2.10	1.43 – 1.78
Standard deviation	0.27	0.15	0.16	0.06	0.21	0.12
Coefficient variation	14	12	10	5	12	8

It does appear that fat moves into the liver from various fat depots during fasting, and we may presume that the 12-hour overnight period is one of fasting. Since glycogen is high in the evening, the transport of fat to the liver for conversion to glycogen and energy is not rapid during the first 12 hours of fasting and both the fat and the glycogen show overnight decreases. As fasting, or insufficient intake of food compared to energy requirements, goes on during the next day, glycogen reaches minimal levels and fat is moved to the liver more rapidly than it is converted to glycogen. Hence, fat levels in the liver go up in the afternoon—perhaps partly from fat depots and partly from food ingested during the day. If the high metabolic rate of birds permits rapid acquisition of glycogen from daily food and rapid conversion of fat to glycogen, it is possible that glycogen levels in birds may be lower than in mammals.

SUMMARY

A pronounced diurnal fluctuation in liver weight has been demonstrated in Red-winged Blackbirds and Starlings.

Based on small samples it appears that both fat and glycogen stores in the liver decrease overnight and that the decrease in fat content is primarily responsible for the observed loss in liver weight. The fat and glycogen content of the liver is highest sometime in the early evening, and, for Red-wings and Starlings, seems to exceed any previously published values, probably because previously most work has been done on birds collected in the mornings when these constituents are near their minimum in the diurnal cycle.

Comparison of evening and morning liver weights of Red-wings and Starlings indicates that the male's liver weight drops more rapidly than that of the female.

During the three-week period from February 27 to March 18, the morning liver weight of female Red-wings increased, apparently as the result of a smaller overnight drop in weight. This suggests the possibility that, as spring comes on, a smaller amount

of stored food from the liver is necessary to supply the metabolic needs of the roosting bird.

Comparison with data on liver weights obtained by other workers shows the need for consistency in methods of gathering the data in terms of time of day and handling procedures if interspecific comparisons are to be valid.

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