NUTRIENT RESERVES AND THE ENERGETICS OF REPRODUCTION IN AMERICAN COOTS

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ABSTRACT.—To investigate the bioenergetics of reproduction in American Coots (*Fulica americana*) we collected 108 males and 93 females at Delta Marsh, in southern Manitoba, in 1981. Prenesting and nesting birds were analyzed for fat, protein, and ash content in the nonreproductive tissue. For females, these values were compared to the nutrient requirements of the reproductive tissue during egg-laying. We suggest that feeding conditions before arrival can affect subsequent reproductive output. Fat reserves may function as a threshold that influences the initiation of breeding; protein reserves and time of arrival influence the timing of a nesting attempt. We infer that territory quality is important and that it can result in the termination of laying before nutrient reserves are depleted. By distributing costs of clutch formation before (through nutrient storage) and after (through increased biparental care of eggs and young) clutch formation, the required energy intake concurrent with egg-laying is substantially reduced in female coots. *Received 23 January 1984, accepted 18 August 1984.*

To understand avian breeding strategies, it is necessary to know the temporal distribution of costs and how nutrients are obtained to meet them (Drobney 1980). Because of the disparity in gamete size, female birds have much greater nutrient demands during reproduction than do males. The male, however, can influence how a female's demands are met (e.g. through nest building and territorial defense), thereby allowing her to spend more time feeding. Recent research has suggested that there is a great interspecific variation in how females and males obtain nutrients for reproduction: most arctic-nesting geese rely almost exclusively on endogenous reserves (Ankney and MacInnes 1978, Raveling 1979, but see Ankney 1984), prairie-nesting ducks (Krapu 1981), Wood Ducks (*Aix sponsa*, Drobney 1980), and Red-billed Queleas (*Quelea quelea*, Jones and Ward 1976) utilize both endogenous and exogenous nutrients, and Brown-headed Cowbirds (*Molothrus ater*, Ankney and Scott 1980) rely on exogenous nutrients. As pointed out by Drent and Daan (1980), however, much more research is needed to evaluate the relative importance of endogenous and exogenous nutrients in affecting clutch size and the timing of breeding in birds.

American Coots (*Fulica americana*, hereafter called coots) are ideal for such research because they show great variation in clutch size (4–17 eggs, Fredrickson 1977) and timing of nesting (Kiel 1954). Also, both sexes incubate (Sooter 1941), and the age of a nesting coot can readily be determined (Crawford 1978). Thus, we undertook to 1) document the timing of acquisition of reserves, 2) describe the energetic cost associated with the formation of a clutch of eggs by coots, 3) determine how much female coots rely on endogenous nutrients for clutch formation, 4) provide an unequivocal approach to assessing the importance of nutrient reserves for clutch formation, 5) describe the dynamics of male body composition during the nesting season, 6) investigate the effects of age on levels of endogenous nutrients, and 7) integrate these results with knowledge about the breeding biology of coots.

METHODS

We collected 108 male and 93 female coots on the Delta Marsh, Manitoba (50°11′N, 98°19′W) from 17 April to 9 July 1981. Coots were assigned to various categories of the reproductive cycle:

Arriving (17 April to 11 May).—Collected from flocks of three or more birds before the first known date of nest initiation.

Paired (29 April to 9 June).—Birds that were with one member of the opposite sex.

Laying (11 May to 9 June).—Males shot flushing from a nest that contained an egg deposited less than 24 h previously, or those that had an incomplete brood patch. Females were separated into subcategories after inspection of their ovaries: *Prelaying* [females with vascularized and yolky follicles (>5 mm), but with no postovulatory follicles (POF)]; *Laying*

(ovary with developing follicles and at least one POF); and *Postlaying* (females with no developing follicles but with at least one POF).

Hatching (4–12 June).—Coots that were trapped on the nest at the end of incubation. Most such nests contained pipped eggs or hatchlings.

We used tarsal color, as described by Gullion (1952) and refined by Crawford (1978), to assign coots to one of three age classes (1-, 2-, or >2-yr-olds).

Carcass analysis.—Birds were weighed, plucked, and reweighed to the nearest 1.0 g on the day of collection. The ovary and oviduct were removed from females and the testes from males. Carcasses (minus the reproductive organs) were double-bagged, labeled, and frozen.

Carcasses were later thawed and the liver, gizzard, and intestines, with caeca attached, were removed. These organs were cleaned of adhering fat and weighed to the nearest 0.1 g. The weight of the digestive tract contents was determined as the difference in weight between the cleaned and uncleaned organs making up the tract. Body weight reported here is the fresh weight of a coot minus the weight of the contents of its digestive tract.

Breast and leg muscles, as defined by Ankney and MacInnes (1978), were excised (left side only) and weighed. All organs described and the rest of the carcass including head, wings, and feet were dried to constant weight (0.1 g) at 80–100°C (see Kerr et al. 1982). The dried leg and breast muscles were each passed twice through a Wiley mill. The remaining dried organs and carcass were ground together at least 3 times using a hand-operated meat grinder. The leg and breast muscles and about 15 g of the dried carcass were analyzed separately for lipid content by extraction with petroleum ether in a Soxhlet apparatus. Values for lean dry weight (LDW) of breast and leg muscle were doubled and are presented herein.

Dry body weight was the sum of the weights of all dried components excluding the ovary, oviduct, and feathers. Fat reserve was the sum of breast fat, leg fat, and carcass fat and refers to the fat present in adipose cells of the nonreproductive tissue. Fat reserve was subtracted from dry body weight to yield the LDW of the nonreproductive tissue. Our definition of "nutrient reserve" is the measure of a fraction (fat, protein, or mineral) of the whole bird (excluding feathers, ovary, oviduct, and testes) that may respond to nutritional demands of egg synthesis or other changes in energy balance. Conversely, we refer to the eggs, follicles, oviduct, and testes as reproductive tissue.

LDW can be used to index protein, but this relationship may be confounded by variation in ash (i.e. mineral, Ricklefs 1974: 161) content. The skeleton is the major source of ash (Robbins 1983: 211). In female birds variation in ash may result from the daily cycling of medullary bone, or a continual decline in

TABLE 1. Ages and numbers of female and male coots from arriving (ARR) and paired (PRD) samples.

	Age	ARR	PRD
Females[a]	1	7	12
	2	7	9
	>2	13	3
Males[b]	1	13	8
	2	16	8
	>2	12	9

[a] $\chi^2 = 7.67$; $P < 0.025$.
[b] $\chi^2 = 0.43$; $P < 0.50$.

cortical bone that can function as a mineral reserve for eggshell synthesis (Taylor and Moore 1954). Medullary bone is not present in males (Simkiss 1975: 307) so changes in LDW must parallel changes in protein. Thus, the following procedure was applied only to females. A sample of about 15 g from each dried, ground carcass was placed in a muffle furnace for 6 h at 550°C. The residue was used to calculate the ash content for each bird that, when subtracted from body LDW, yielded the ash-free LDW, an index of protein.

Reproductive organ analysis.—Both testes were weighed to the nearest 0.01 g and discarded. The ovaries and oviducts were weighed and, except for those removed from prelaying and laying females, discarded. Large yolky ovarian follicles were removed from ovaries of prelaying and laying females, weighed, placed in a drying oven with the weighed oviduct, and dried to constant weight at 90°C. Lipids were extracted from developing follicles with petroleum ether in a Goldfisch apparatus. Dry follicle weight minus lipid weight yielded the LDW of each follicle. Carbohydrates and minerals comprise only 2% of chicken egg yolks (Romanoff and Romanoff 1949), so we hereafter refer to the LDW of developing follicles and egg yolks as yolk protein.

From 17 nests we removed fresh eggs, weighed them, and heated them in a 70°C water bath to ease separation of yolk from albumen (after Lack 1968). Eggshell membranes were peeled away from shells and added to the egg white. These membranes consist mainly of protein with a minute amount of carbohydrate (Sturkie 1976: 318); because deposition of both egg white and membrane around the yolk is complete in 4–6 h (Sturkie 1976: 318), we refer to them collectively as albumen. The shell, yolk, and albumen were weighed separately and dried to constant weight at 80–90°C. Eggshell thickness was the mean of 5 measurements (to the nearest 0.02 mm) made with calipers on each egg. Ether-extractable lipids were removed from dried yolks in a Goldfisch apparatus. In this way, yolk lipid, yolk protein, albumen, shell weight, and shell thickness were determined for 98 coot eggs.

The exclusion of badly damaged organs or egg

TABLE 2. Changes in body composition and dried organ weights (g) of male coots during the nesting season. ARR = arriving, PRD = paired, LAY = laying, HTC = hatch. Values are $\bar{x} \pm 1$ SE, with sample sizes in parentheses.

| Variable | Prenesting | | | Egg-laying | | | Incubation |
	ARR	P^a	PRD	P	LAY	P	HTC
Body weight	575.2 ± 10.2 (41)	NS	595.9 ± 11.3 (25)	NS	611.6 ± 7.6 (24)	NS	584.3 ± 13.7 (11)
Fat	39.5 ± 4.8 (40)	NS	32.4 ± 4.1 (22)	NS	32.5 ± 3.3 (22)	NS	27.1 ± 3.8 (11)
Protein	126.9 ± 1.9 (40)	NS	130.5 ± 3.0 (22)	*	139.4 ± 2.1 (22)	NS	141.4 ± 3.7 (11)
Breast LDW	16.4 ± 0.4 (41)	NS	16.7 ± 0.6 (22)	NS	16.2 ± 0.4 (24)	NS	15.6 ± 0.5 (11)
Leg LDW	18.8 ± 0.4 (41)	**	21.8 ± 0.8 (24)	**	24.4 ± 0.4 (24)	NS	24.5 ± 1.0 (11)
Liver	4.07 ± 0.12 (41)	NS	4.02 ± 0.20 (25)	NS	4.20 ± 0.11 (24)	**	5.08 ± 0.27 (11)
Gizzard	10.46 ± 0.31 (41)	NS	9.66 ± 0.39 (25)	NS	9.66 ± 0.35 (24)	NS	9.30 ± 0.46 (11)
Intestine	4.56 ± 0.23 (40)	NS	4.79 ± 0.34 (25)	NS	5.08 ± 0.39 (24)	NS	5.77 ± 0.31 (11)

^{a} P = significance level of t-test between means in adjacent columns. * $P < 0.05$; ** $P < 0.01$; NS indicates $P > 0.05$.

components from analyses led to variation in sample sizes.

RESULTS

ARRIVAL CHRONOLOGY

Age and sex of individual coots were determined after collection; we assumed that our samples were unbiased and represented the proportion of different-aged coots present in the population of arriving and paired birds (see Methods).

Female age ratios were not independent of prenesting status (Table 1); 48% of the arriving, but only 12.5% of the paired female sample were >2-yr-olds. This indicates that when arriving coots were collected, a large proportion of the younger females had not yet reached the breeding grounds. Male age ratios did not change from arrival to pairing (Table 1).

MALE BODY COMPOSITION

Male coots arrived on Delta Marsh with high levels of fat. No segment of the nesting season caused a change in male fat reserves (Table 2), but their fat reserves were lower at the end of incubation than at arrival ($t = 2.02$, df = 49, $P < 0.05$).

Endogenous protein in males increased during the laying period (Table 2). Thus, unlike fat, protein was accumulated on the breeding grounds.

Male leg muscle increased in weight during prenesting and egg-laying (Table 2). During incubation, liver weight increased to a maximum, temporally corresponding to maximum protein levels. No changes were detected in any of the other organs.

There was no consistent relationship between fat reserves and age in male coots (two-way ANOVA, $P > 0.50$). However, oldest males consistently had the highest protein levels in all reproductive categories (Fig. 1), partly because they were structurally largest (Alisauskas unpubl. data). Male protein was correlated with testes weight ($r = 0.465$, df = 100, $P < 0.001$).

NUTRIENT RESERVES OF PRENESTING FEMALES

Female arrivals had relatively larger fat reserves (accounting for 26.6% of dry body weight) than males (21.8%; t-test on arcsine transformed percentages, $t = 2.45$, df = 64, $P < 0.02$). Absolute fat and mineral reserves of females did not change during prenesting (Table 3).

Protein was accumulated after arrival, with hypertrophy of leg muscle accounting for 31.5%

of the increase (Table 3). Liver weight increased significantly, but weights of the other digestive organs and of flight muscles did not change. Increases in ovary weight of prenesting females were positively correlated with endogenous protein ($r = 0.428$, df = 43, $P < 0.01$), but not with fat reserves ($r = 0.107$, df = 46, $P > 0.05$) or minerals ($r = 0.172$, df = 43, $P > 0.05$).

Fat and protein reserves were not related to the age of prenesting female coots (two-way ANOVA, $P > 0.5$ and $P = 0.3$, respectively). Ovarian recrudescence was most advanced in the oldest females at arrival, but age-related differences were not significant among paired birds (Table 4), possibly because the sample size of older birds was small.

IMPORTANCE OF NUTRIENT RESERVES FOR
CLUTCH FORMATION

We investigated the relative importance of endogenous vs. exogenous nutrients in egg production. First, we determined the fat, protein, and mineral fractions of the reproductive tissue (ovary, oviduct, and eggs) for female coots in various stages of rapid follicular development (prelaying, laying) and regression (immediate postlaying). We then compared the nutrients present in the reproductive tissue of individual females with their nutrient reserves (i.e. in their somatic tissue).

We defined "reproductive fat" as the sum of fat present in the ovary and total fat present in eggs already laid (as evidenced by the number of postovulatory follicles). "Reproductive protein" was the sum of the protein present in the ovary, the oviduct, and the total albumen and yolk protein of eggs laid (including oviducal eggs).

Significant variation in yolk lipids, yolk proteins, and albumen were a result of, and inversely related to, laying sequence ($r^2 = 39.8\%$, $P < 0.001$; $r^2 = 11.5\%$, $P < 0.001$; and $r^2 = 8.8\%$, $P < 0.01$, respectively). Dry eggshell weights declined with laying sequence ($r^2 = 7.8\%$, $P < 0.01$), but laying sequence had no effect on shell thickness ($r^2 = 2.8\%$, $P > 0.05$). We used the mean value of each component, relative to egg sequence (Fig. 2), to estimate nutrient commitments to eggs.

Effect of clutch formation on nutrient reserves.— We used one-tailed tests of significance for regression analysis of nutrient reserves (Y) on
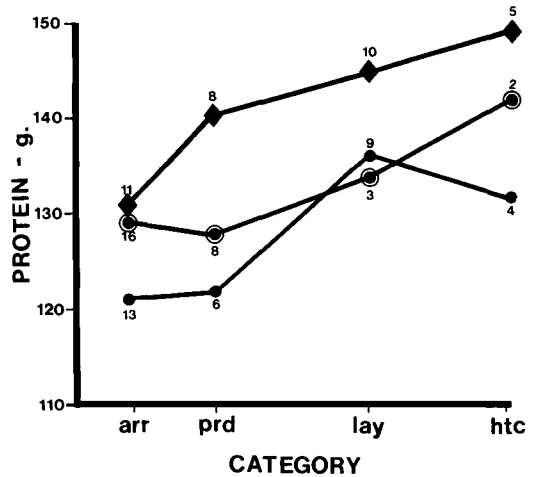

Fig. 1. Mean protein reserves (g) of breeding male coots in relation to age. Numbers beside points indicate sample sizes; closed circles are 1-yr-olds, double circles are 2-yr-olds, and diamonds are >2-yr-olds.

nutrients committed to reproduction (X) because, a priori, if female coots used nutrient reserves to form eggs, then clutch formation would cause a decline in those reserves (i.e. we expected the slope to be negative). Regression analysis is useful because the slope provides an estimate, with measures of variance, of the proportion of the clutch formed with endogenous nutrients. The 95% confidence interval of the intercept gives a measure of the minimum amount of reserve that an individual bird possesses before egg formation starts.

Figure 3 illustrates changes in reserves with respect to nutrients committed to the reproductive organs. Seven prelaying, 8 laying, and 6 postlaying females that had completed laying within a day or two were used in the analyses. The status of 2 of the prelaying females was questionable, as we suspect that they would not have initiated a laying cycle. Although they were classified according to our a priori definition of a prelayer (see Methods), their nutrient commitment to follicular growth was relatively slight, and there was no guarantee that they would have laid. We provide the results of the analyses both with and without these 2 individuals in the sample (Fig. 3, Table 5). In all cases except one, the results were significant ($P < 0.05$), whether or not these females were included. However, we think that the analyses

TABLE 3. Changes in body composition and dried organ weights (g) of prenesting female coots. ARR = arriving, PRD = paired. Values are $\bar{x} \pm 1$ SE, with sample sizes in parentheses.

Variable	ARR	P^a	PRD
Body weight	459.7 ± 9.1 (24)	*	498.0 ± 11.9 (24)
Fat	39.2 ± 5.1 (26)	NS	33.5 ± 5.1 (24)
Ash	14.9 ± 0.4 (25)	NS	15.8 ± 0.6 (21)
Protein	83.1 ± 1.9 (25)	**	90.2 ± 1.6 (21)
Breast LDW	13.6 ± 0.5 (26)	NS	14.1 ± 0.4 (24)
Leg LDW	14.6 ± 0.4 (27)	***	16.9 ± 0.4 (24)
Liver	3.37 ± 0.12 (27)	**	4.19 ± 0.22 (24)
Gizzard	8.19 ± 0.27 (27)	NS	8.37 ± 0.38 (24)
Intestine	3.90 ± 0.22 (27)	NS	4.20 ± 0.19 (24)

[a] P = significance level of t-test between means in adjacent columns. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS indicates $P > 0.05$.

that excluded these birds provided a better representation of nutrient-reserve use by female coots.

Female coots used endogenous fat and protein for clutch formation (Fig. 3, Table 5). For every gram of reproductive fat produced, fat reserves, on average, declined 0.85 g. The lower confidence limit of the intercept (or the average amount of fat reserve before any allocation to rapid ovarian growth) was 27.5 g; thus, we conclude that this value corresponds to a threshold above which 97.5% of females would initiate a laying cycle. Endogenous protein was less important as a reserve, providing only 28% of the egg protein requirements. Ash content did not vary as a result of eggshell synthesis.

Of the organs investigated that made up the endogenous protein fraction, only breast and leg muscle showed a consistent negative relationship with reproductive protein commitment (Table 5); the decline in LDW of these organs accounted for half the decline in total body protein. Regression analysis showed that variation in gizzard weight was unaffected by reproductive protein allocations. The results for intestinal weight were equivocal.

It is noteworthy that some birds had completed laying even though they possessed more

fat and protein reserves than birds that were still laying when collected (Fig. 3).

Energy budget of egg-laying females.—To construct an energy budget, several physiological variables must be measured: the daily laying rate, the follicular growth rate, the number of days required to complete rapid follicular growth, the oviducal growth rate, the amounts of fat and protein in the ovary, and the albumen content of an egg.

Removal of eggs from nests during this study substantiated Sooter's (1941) assertion that coots lay 1 egg/day. With this laying rate, the difference in weight between any two consecutively developing ovarian follicles is the growth of the larger follicle over 24 h (Ricklefs 1974). We thereby quantified daily nutrient allocation associated with producing the yolk fat and protein.

Coots that had at least one POF never had >7 developing follicles. Thus, it takes 7 days for a follicle in the rapid-growth stage to attain maturity, i.e. from the first noticeable increase in ovum size (day -8) until ovulation (day -1). The predicted length of the rapid-growth phase, as a function of egg mass, was 7.3 days [Walsberg's (1983: 175) equation].

Oviduct weights of prelaying females were

TABLE 4. Comparisons (one-way ANOVA) of ovary wet weights (g) of female coots by age within breeding status during prenesting. ARR = arriving, PRD = paired. Values are $\bar{x} \pm 1$ SE, with sample sizes in parentheses.

Breed-ing status	Age			P^a
	1	2	>2	
ARR	0.559 ± 0.065 (7)	0.624 ± 0.065 (7)	0.835 ± 0.062 (11)	*
PRD	1.057 ± 0.160 (11)	1.184 ± 0.216 (9)	1.450 ± 0.165 (3)	NS

[a] * $P < 0.05$; NS indicates $P > 0.05$.
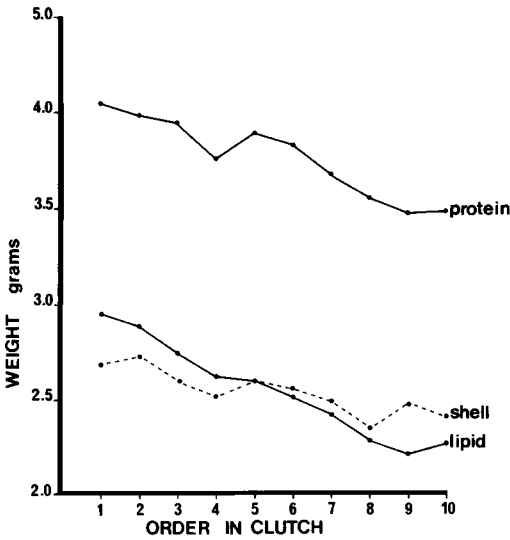
Fig. 2. Decline in mean nutrients (g) in coot eggs with respect to laying sequence.
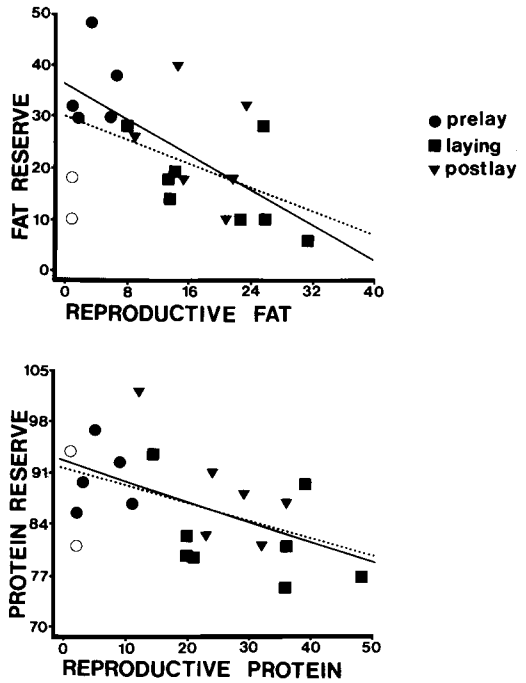

Fig. 3. Decline of fat and protein reserves (g) related to increase in allocation of fat and protein (g), respectively, to "reproductive tissue" in female coots during clutch formation. Open circles represent females 168 and 206 (see text). Dotted and solid lines are fitted using least-squares regression with and without females 168 and 206, respectively.

placed in categories of days-from-ovulation (based on the weight of the heaviest follicle in the hierarchy). The relationship between oviduct dry weight (Y) and day from ovulation (X), based on data for days -8, -7, -5, -3, -1, was described by the equation

$$\log_{10}Y = 0.858 + 0.14X$$
$$(r^2 = 99.6\%, P < 0.05), \qquad (1)$$

which we used to estimate dry oviduct weights for days -6, -4, and -2.

The daily production of each nutrient in the ovary, the oviduct, and the first 8 eggs was used to estimate the cost of producing an 8-egg clutch. To determine the relative importance of reserves in terms of female energy expenditures during clutch formation, 1 g of stored fat was assumed to equal 37.67 kJ (Ricklefs 1974: 160). For protein, an energy equivalent of 23.86 kJ/g was used (Kleiber 1961). Body water and ash were not considered in the energy budget because they have no available chemical energy (Robbins 1983: 213). Because the conversion of unprocessed material to egg nutrients is not 100% efficient, the total cost of producing an egg is greater than the energy content in the egg. Brody (1945) gave 77% as the production efficiency for the synthesis of eggs. However, this value applies to the conversion of dietary nutrients to egg nutrients. Costs of biosynthesis associated with protein accumulation and

the conversion of digestible carbohydrates to fat at the time of storage should not be incorporated into costs that a female incurs at the time of egg-laying. Efficiency values for the conversion of nutrient reserves to egg components are not available but are undoubtedly greater than 77%; because we lacked an empirical alternative, we assumed 100% efficiency, but those portions of the egg requirements met exogenously were divided by 0.77 to incorporate the cost of biosynthesis concurrent with clutch formation. To account for the difference in conversion efficiency of stored vs. dietary nutrients, the appropriate correction between the amount of nutrients in the clutch and a female's expenditures in providing them would be:

$$C = E[RP_R^{-1} + (1 - R)P_D^{-1}], \qquad (2)$$

where C = cost to female (kJ) in producing 1 g of egg nutrient, E = energy equivalent of egg

TABLE 5. Summary of regression analysis of *Y* (nutrient reserves) on *X* (nutrient commitment to clutch formation). Analyses with *n* = 19 do not include females 168 and 206 (see text and Fig. 3).

Y	X	n	Intercept[a]	Slope[a]	r^2	P[b]
Fat reserve	Reproductive fat	21	30.2 ± 8.4	-0.55 ± 0.52	20.9	**
		19	36.1 ± 8.6	-0.85 ± 0.51	42.5	**
Protein reserve	Reproductive protein	21	91.3 ± 5.0	-0.24 ± 0.20	24.3	*
		19	92.6 ± 5.6	-0.28 ± 0.22	30.2	**
Fat reserve and protein reserve	Reproductive fat and reproductive protein	21	121.4 ± 11.7	-0.36 ± 0.29	26.6	**
		19	128.6 ± 12.7	-0.51 ± 0.30	42.9	**
Ash	Shell	21	16.6 ± 1.1	0.003 ± 0.08	0	NS
		19	16.4 ± 1.3	0.010 ± 0.09	0.3	NS
Breast LDW	Reproductive protein	21	13.5 ± 1.5	-0.07 ± 0.062	23.0	*
		19	13.3 ± 1.8	-0.07 ± 0.07	19.0	*
Leg LDW	Reproductive protein	21	17.9 ± 1.4	-0.06 ± 0.058	19.5	*
		19	18.2 ± 1.7	-0.08 ± 0.065	22.2	*
Gizzard weight	Reproductive protein	21	7.74 ± 1.0	-0.031 ± 0.041	11.9	NS
		19	1.39 ± 1.2	-0.020 ± 0.046	4.6	NS
Intestine weight	Reproductive protein	21	4.47 ± 0.7	-0.020 ± 0.029	9.4	NS
		19	4.84 ± 0.8	-0.031 ± 0.010	21.7	*

[a] $\pm 95\%$ confidence interval.
[b] Probability that $r = 0$: * $P < 0.05$; ** $P < 0.01$, NS indicates $P > 0.05$.

nutrient (kJ/g), R = proportion of egg nutrients supplied by female reserves, $(1 - R)$ = proportion of egg nutrients supplied directly by the diet, P_R = efficiency for converting nutrient reserves to egg nutrients = 1.00, and P_D = efficiency for converting dietary nutrients to egg nutrients = 0.77. For example, for each gram of egg lipid produced, the female would expend $37.67(0.85 + 0.15/0.77) = 39.36$ kJ. For egg-protein synthesis, C is equivalent to 28.99 kJ/g. Equation 2 would apply to any strategy of nutrient reserve use, but values of C for fat and protein would be species-specific.

Daily energy costs of providing each egg nutrient were estimated by multiplying the actual nutrient allocation by the corrected conversion from Eq. 2. We modeled the energy budget for an 8-egg clutch for two reasons. First, although there was a significant inverse relationship between the size of a clutch and its initiation date ($r = -0.679$, df = 60, $P < 0.001$; Fig. 4), the removal of eggs involved 17 clutches that began between 12 and 26 May. The mean clutch size of all nests on the study area begun during that time was 8.3. Second, laying females had, on average, 4.9 developing follicles and 3.3 post-ovulatory follicles, i.e. the mean potential clutch size of birds in this first wave of nesting (see Fig. 4) was 8.2 eggs.

The temporal distribution of energy expen-

ditures by females producing 8 eggs spans 15 days (Fig. 5). Maximum costs would be incurred by females only during days 0 and 1. The average weight of laying females was about 500 g. Basal metabolic rate (BMR) was estimated as 185 kJ/day, using the equation of Aschoff and Pohl (1970) for resting nonpasserines. Thus, maximum cost of egg formation (about 223 kJ/day) corresponds to about 121% of the BMR of laying females. Coots laying clutches smaller than the number of days needed for follicle growth (i.e. 7) would not reach this maximum. These results empirically support King's (1973) model for the temporal distribution of energy expenditure for egg synthesis by birds in general.

The estimated total cost of production over the 15 days was 1,830 kJ. The greatest demands would be for yolk synthesis, with yolk lipids requiring the largest commitment (43.7%) from the energy budget. Albumen would account for 27.1%, yolk proteins 21.9%, and the oviduct 7.3% of a female's total expenses as she produced an 8-egg clutch.

Effect of declining egg weight.—We reestimated costs assuming all eggs were equal in energy content to the first egg. This increased the total estimated requirements for clutch formation by 9.0% (Fig. 5). The greatest increases would be 15.8% for yolk proteins and 12.0% for yolk lip-
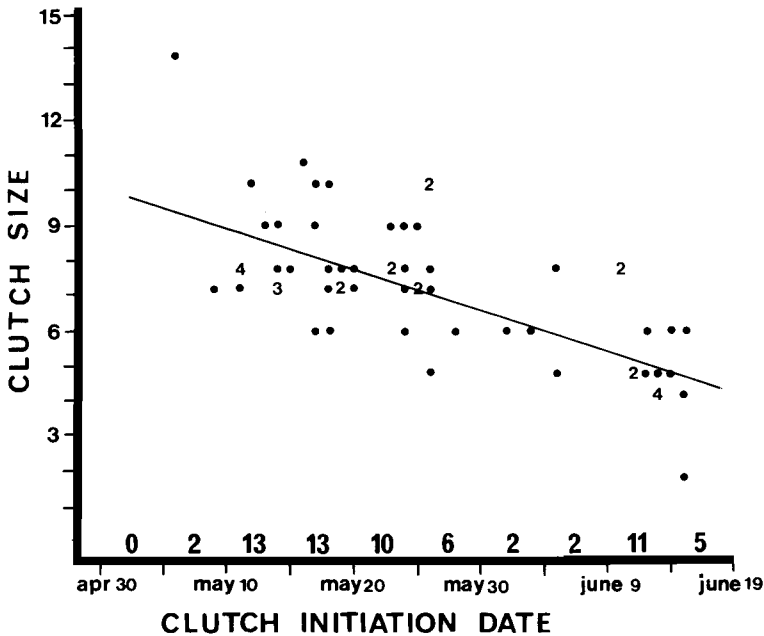
Fig. 4. Relationship between clutch initiation date and clutch size in coots; numbers above the x-axis are nests initiated per 5-day interval.

ids. Albumen allocation varied little from first to last egg and increased the estimated cost by only 1.2%. Costs in producing the oviduct would not change because a minimum size is presumably required to accommodate the first egg, which is the largest, in all cases. Thus, the sequential decline in egg size reduced the estimated expenditures over the 15 days.

Because most egg lipids are supplied endogenously, the amount of egg lipid produced presumably depends on the fat reserves that females possessed immediately before rapid ovarian growth. First eggs of a clutch had an average of 2.95 g of yolk lipid. A clutch of 8 consecutively smaller eggs contained 21.0 g of lipid. Hypothetically, if females allocated the same amount of fat reserves to subsequent eggs as they did to the first egg, there would only be enough stored fat for 7 instead of 8 eggs (21.0 g/2.95 g = 7.1).

NUTRIENT RESERVES OF INCUBATING FEMALES

There was no change in body weight, fat reserves, or protein reserves of incubating fe-

males. Of the organs investigated, only intestine weights increased (df = 21, $P < 0.001$).

DISCUSSION

MALE NUTRIENT RESERVES

Oldest males had the highest levels of endogenous protein before and during the nesting season (Fig. 1). Ryan and Dinsmore (1980) found that the oldest male coots were the most aggressive. Ankney (1977a) suggested that large muscles and physical strength of male Snow Geese (Chen caerulescens) were important for defending the female during nesting. Thus, for a species such as the coot where nest sites appear to be a limiting factor (see Sugden 1979, Crawford 1980) and must be acquired and maintained by intraspecific aggression, older males should be most successful because of greater physical strength and aggressive behavior. Greater male age also may be partially responsible for the increased reproductive success of older females documented by Crawford (1980), who found that pairing by coots usually involved birds of similar age.
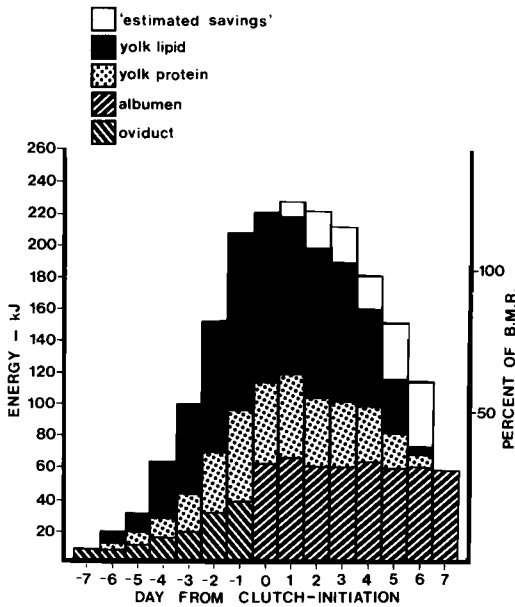
Fig. 5. Cumulative daily expenditures of female coots producing a clutch of 8 eggs expressed in kJ and as a daily percentage of daily basal metabolic rate (BMR). "Estimated savings" are the daily differences in cost for clutches composed of eggs with sequentially declining nutrient contents and a hypothetical case where eggs receive nutrient allocations equal to that of the first egg.

FEMALE NUTRIENT RESERVES

Fat and protein.—The critical level of fat reserves, above which most females would nest, was 27.5 g in 1981. Moreover, most (85%) of the lipids required for egg production came from reserves. This suggests that the size of a female's fat reserves influences whether she will nest (see Drent and Daan 1980) and her clutch size, as demonstrated in Snow Geese (Ankney and MacInnes 1978).

Coots did not store fat after arrival on the breeding grounds. This is similar to what occurs in Mallards (*Anas platyrhynchos*, Krapu 1981) and Snow Geese (Ankney and MacInnes 1978); in these species, weather conditions on the wintering grounds or during spring migration affect subsequent recruitment (Heitmeyer and Fredrickson 1981, Boyd et al. 1982). Thus, feeding conditions on the wintering grounds and/or during spring migration influence the ability of female coots to store fat and consequently to reproduce.

Krapu and Doty (1979) showed that among prenesting female Mallards, adults had consis-

tently larger fat reserves than did yearlings, and the adults laid earlier and had larger first clutches. Although Crawford (1980) reported that older female coots laid larger clutches and typically nested earliest, we found no consistent relationship between fat reserves and age in prenesting females. We suggest that earlier nesting by older females occurs because they arrive earlier than 1- and 2-yr-old females. Clutch-initiation dates were bimodally distributed in 1981 (Fig. 4), which is common in the nesting phenology of coots (see Kiel 1954, Fredrickson 1970, Sugden 1979, Crawford 1980). Territoriality and the distribution of nesting cover are the main factors influencing the dispersion of coot nests (Sugden 1979). The delayed breeding of some individuals is related to the availability of nesting habitat associated with the new growth of emergent vegetation (Sugden 1979, Crawford 1980); younger females are primarily responsible for the second wave of nesting. This suggests that there is an advantage in arriving early, and among females, >2-yr-olds arrived earliest (Table 1).

Ovarian size in prenesting coots was positively correlated with age (Table 4) and with protein reserves. In those species storing nutrients for reproduction, the size of their protein reserves may proximately control the timing of a breeding attempt (see Ashmole 1971, Jones and Ward 1976, Drent and Daan 1980). Ovary weight was correlated with the levels of both fat and protein reserves in Snow Geese during spring migration (Wypkema and Ankney 1979). We suggest that in coots, fat reserves allow the female to "evaluate" whether she possesses the minimum required for a breeding attempt; if this is satisfied, then the rate of acquisition of protein reserves, in addition to time of arrival, influence the timing of breeding. Nesting coots show high levels of inter- and intraspecific aggression against intruders on their territories (Gullion 1953). Given such spacing behavior, pairs with females that have the largest protein reserves at arrival or that acquire protein most rapidly after arrival probably nest earliest.

Minerals.—Coots did not store long-term mineral reserves. Ash content did not change through laying (Table 5), indicating that there was no depletion of cortical bone, i.e. long-term mineral reserve (see Simkiss 1967: 173, Ankney and Scott 1980). Taylor and Moore (1954) showed that weight of cortical bone declined

but that of medullary bone remained constant when laying hens were fed a low-calcium diet. Coots presumably met all of their calcium requirements without storing or using minerals in the cortical bone.

On the Delta Marsh, sources of dietary calcium appeared to be abundant (see Alisauskas 1982). Calcium deficiency is known to cause eggshell thinning in laying hens (Taylor and Moore 1954), but in laying coots, although shell weight declined with egg sequence (i.e. as the eggs decreased in size), shell thickness did not. Therefore, the calcium requirements of eggshell formation were not greater than what females could acquire daily from their territories.

Control of clutch size.—While levels of endogenous fat probably control the initiation of rapid ovarian growth, termination may result before fat or protein reserves are depleted. Most of our postlaying females retained levels of fat and protein greater than birds that still had not completed laying (Fig. 3). This suggests that exogenous nutrients, and therefore territory quality, may regulate clutch size. Coots rarely wander far from their territories before hatch (Ward 1953). Thus most protein and all minerals for egg synthesis must be acquired from areas established as territories before nesting. Given that a female has sufficient fat reserves to start a clutch, the number of eggs laid may be correlated with some empirical measure of territory quality (i.e. the availability of materials amenable for conversion to egg proteins and eggshells). Age-related differences in foraging efficiency among individual females, or the physique of their mates (see above), might somewhat confound such a relationship. Clearly, selection and defense of territories are important steps toward acquiring sufficient exogenous proteins and minerals. This may explain the aggressive nature of nesting coots.

Reduction of daily expenditures for egg-laying.—To determine the increase in daily energy intake necessary to meet the added costs of egg production, it is appropriate to relate reproductive costs to the daily energy expenditure (DEE) of nonbreeding females. A nonbreeding 500-g coot (the weight of the average laying female) would require 568 kJ of energy/day (from Walsberg 1983: 193). Given a conversion efficiency of 77%, this bird would have to ingest 738 kJ/day to balance DEE. On day 0 of clutch formation (see Fig. 5), when energy demands for eggs are maximal, females must pro-

vide 3.88 g of protein, of which 72% (2.79 g) must be exogenous. To do this, at a conversion efficiency of 77%, the female must consume 3.62 g (86.6 kJ) on the same day. At the same conversion efficiency, she also must consume 22 kJ of material to provide 0.43 g of egg lipid. Therefore, to meet the costs of egg production, laying females would have to increase their daily energy intake by 109 kJ per 738 kJ, or by 14.8% above what is required for DEE. If females relied entirely on exogenous nutrients for clutch formation, the required intake on day 0 would be 261 kJ, or 35.4% more energy relative to the DEE of a nonbreeding female. Therefore, storage of reserves before nesting reduced the energy intake required on day 0 of clutch formation from 35% to 15% above that needed concurrently for DEE. We emphasize that our calculations provide only rough estimates of the energy cost of egg formation because precise estimates of conversion efficiencies are unavailable. Clearly, there is a need for research to determine the specific production efficiencies of converting endogenous and exogenous fat and proteins (and carbohydrates) into egg nutrients.

Decreases in egg size with laying sequence also reduced energy requirements (Fig. 5) during the second half of the laying cycle. Egg lipids showed the most consistent decline. Reduced lipid allocation began immediately after the first egg, before reserves declined to basal levels, and allowed an extra egg to be produced. Thus, if all young survive, such as when food is abundant, parental fitness would be increased by $\frac{8}{7}$, or 1.14 times. From this result, we suggest that studies on energy budgets in birds should question the assumption of constant egg weight relative to laying sequence, as this may affect estimates of energy expenditure for an entire clutch.

Incubation.—Maximum weights of the liver of males, and of the gizzard and intestine of females at this time, strongly suggest that feeding is important to coots during incubation. Intestine weight and length in birds are known to be sensitive to changes in diet quality (Pendergast and Boag 1973, Miller 1976). The diet of coots at this time is most likely vegetation with high levels of fiber, leading to the increased mass of digestive organs that we observed. Anorexia resulting in weight loss during incubation was suggested for Lesser Snow Geese (Ankney 1977b), and demonstrated in

Red Junglefowl (*Gallus gallus spadicus*, Sherry et al. 1980) to functionally alter energy balance. Coots remain active during incubation (Ryan and Dinsmore 1979), and continued feeding resulted in high levels of protein at hatch in 1981. Because both sexes incubate, female coots are released from having to rely on body reserves as an energy source during incubation. Moreover, this allows females to use most of their fat reserves for egg formation. Thus, incubation does not affect body weight of female coots as it does in many waterfowl species (see Gatti 1983). An exception to this may be found in the hole-nesting Wood Duck, which can incubate a clutch without significant weight loss and without male assistance (Drobney 1980).

LITERATURE CITED

ALISAUSKAS, R. T. 1982. Nutrient reserves and the bioenergetics of reproduction in American Coots. Unpublished M.Sc. thesis, London, Ontario, The University of Western Ontario.

ANKNEY, C. D. 1977a. The use of nutrient reserves by breeding male Lesser Snow Geese *Chen caerulescens caerulescens*. Canadian J. Zool. 55: 1984–1987.

———. 1977b. Feeding and digestive organ size in breeding Lesser Snow Geese. Auk 94: 275–282.

———. 1984. Nutrient reserve dynamics of breeding and molting Brant. Auk 101: 361–370.

———, & C. D. MACINNES. 1978. Nutrient reserves and reproductive performance of female Lesser Snow Geese. Auk 95: 459–471.

———, & D. M. SCOTT. 1980. Changes in nutrient reserves and diet of breeding Brown-headed Cowbirds. Auk 97: 684–696.

ASCHOFF, J., & H. POHL. 1970. Rhythmic variations in energy metabolism. Fed. Proc. 29: 1541–1552.

ASHMOLE, N. P. 1971. Seabird ecology and the marine environment. Pp. 223–286 *in* Avian biology, vol. 1 (D. S. Farner and J. R. King, Eds.). New York and London, Academic Press.

BOYD, H., G. E. J. SMITH, & F. G. COOCH. 1982. The Lesser Snow Geese of the eastern Canadian Arctic. Occ. Pap., Canadian Wildl. Serv., No. 46.

BRODY, S. 1945. Bioenergetics and growth. New York, Reinholt Publ. Corp.

CRAWFORD, R. D. 1978. Tarsal color of American Coots in relation to age. Wilson Bull. 90: 536–543.

———. 1980. Effects of age on reproduction of American Coots. J. Wildl. Mgmt. 44: 183–189.

DRENT, R. H., & S. DAAN. 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68: 225–252.

DROBNEY, R. D. 1980. Reproductive bioenergetics of Wood Ducks. Auk 97: 480–490.

FREDRICKSON, L. H. 1970. Breeding biology of American Coots in Iowa. Wilson Bull. 82: 445–457.

———. 1977. American Coot (*Fulica americana*). Pp. 123–147 *in* Management of migratory shore and upland game birds in North America (G. C. Sanderson, Ed.). Washington, D.C., Intern. Assoc. of Fish and Wildl. Agencies.

GATTI, R. C. 1983. Incubation weight loss in the Mallard. Canadian J. Zool. 61: 565–569.

GULLION, G. W. 1952. Sex and age determination in the American Coot. J. Wildl. Mgmt. 16: 191–197.

———. 1953. Territorial behavior of the American Coot. Condor 55: 169–186.

HEITMEYER, M. E., & L. H. FREDRICKSON. 1981. Do wetland conditions in the Mississippi Delta hardwoods influence Mallard recruitment? Trans. 46th North Amer. Wildl. and Nat. Res. Conf.: 44–57.

JONES, P. J., & P. WARD. 1976. The level of reserve protein as the proximate factor controlling the timing of breeding and clutch-size in the Red-billed Quelea *Quelea quelea*. Ibis 118: 547–574.

KEER, D. C., C. D. ANKNEY, & J. S. MILLAR. 1982. The effect of drying temperature on extraction of petroleum ether soluble fats of small birds and mammals. Canadian J. Zool. 60: 470–472.

KIEL, W. H. 1954. Nesting studies of the coots in southwestern Manitoba. J. Wildl. Mgmt. 19: 189–198.

KING, J. R. 1973. Energetics of reproduction in birds. Pp. 78–107 *in* Breeding biology of birds (D. S. Farner, Ed.). Washington, D.C., Natl. Acad. Sci.

KLEIBER, M. 1961. The fire of life. New York, J. Wiley and Sons.

KRAPU, G. L. 1981. The role of nutrient reserves in Mallard reproduction. Auk 98: 29–38.

———, & E. A. DOTY. 1979. Age-related aspects of Mallard reproduction. Wildfowl 30: 35–39.

LACK, D. 1968. Ecological adaptations for breeding in birds. London, Methuen.

MILLER, K. J. 1976. Gut morphology of Mallards in relation to diet. J. Wildl. Mgmt. 39: 168–173.

PENDERGAST, B. A., & D. A. BOAG. 1973. Seasonal changes in the internal anatomy of Spruce Grouse in Alberta. Auk 90: 307–317.

RAVELING, D. G. 1979. The annual cycle of body composition of Canada Geese with special reference to control of reproduction. Auk 96: 234–252.

RICKLEFS, R. E. 1974. Energetics of reproduction in birds. Pp. 152–292 in Avian energetics (R. A. Paynter, Ed.). Cambridge, Massachusetts, Nuttall Ornithol. Club.

ROBBINS, C. T. 1983. Wildlife feeding and nutrition. New York, Academic Press.

ROMANOFF, A. L., & A. J. ROMANOFF. 1949. The avian egg. New York, J. Wiley and Sons.

RYAN, M. R., & J. J. DINSMORE. 1979. A quantitative study of the behavior of breeding American Coots. Auk 96: 704–713.

———, & ———. 1980. The behavioral ecology of breeding American Coots in relation to age. Condor 82: 320–327.

SHERRY, D. F., N. MROSOVSKY, & J. A. HOGAN. 1980. Weight loss and anorexia during incubation in birds. J. Comp. Physiol. Psychol. 94: 89–98.

SIMKISS, K. 1967. Calcium in reproductive physiology. London and New York, Chapman and Hall.

———. 1975. Calcium metabolism and avian reproduction. Pp. 307–337 in Avian physiology (M. Peaker, Ed.). Symp. Zool. Soc. London No. 35, New York, Academic Press.

SOOTER, C. A. 1941. Ecology and management of the American Coot Fulica americana americana Gmelin. Unpublished Ph.D. dissertation, Ames, Iowa, Iowa State University.

STURKIE, P. D. 1976. Reproduction in the female and egg production. Pp. 303–330 in Avian physiology (P. D. Sturkie, Ed.). New York, Springer-Verlag.

SUGDEN, L. G. 1979. Habitat use by nesting American Coots in Saskatchewan parklands. Wilson Bull. 91: 599–607.

TAYLOR, T. G., & J. H. MOORE. 1954. Skeletal depletion in hens laying on a low calcium diet. Brit. J. Nutr. 8: 112–124.

WALSBERG, G. 1983. Avian ecological energetics. Pp. 161–220 in Avian biology, vol. 7 (D. S. Farner, J. R. King, and K. C. Parkes, Eds.). New York and London, Academic Press.

WARD, P. 1953. The American Coot as a game bird. Trans. North Amer. Wildl. Conf. 18: 322–329.

WYPKEMA, R. C. P., & C. D. ANKNEY. 1979. Nutrient reserve dynamics of Lesser Snow Geese staging at James Bay, Ontario. Canadian J. Zool. 57: 213–219.

Kemp, systematics of hornbills (Bucerotidae) and birds of prey (Falconiformes); Bruce Lyon, ecological correlates of nest dispersion in the Sabine's Gull (*Xema sabini*); Mara McDonald, the evolution and biology of Hispaniolan Palm-Tanagers (genus *Phaenicophilus*); Mary V. McDonald, observational and experimental study on the function of vocalizations in Seaside Sparrows; Kevin J. McGowan, development of social behaviors in young Florida Scrub Jays (*Aphelocoma c. coerulescens*); Brian A. Millsap, geographic variation and taxonomy of the Gray Hawk (*Buteo nitidus*); Gerhard Nikolaus, avifauna and distribution atlas of the Sudan: migratory birds; Jay Pitocchelli, speciation in the genus *Oporornis*; Bruce H. Pugesek, age-specific reproductive tactics; Gretchen Rasch, ecology of the Stitchbird (*Notiomystis cincta*) on Little Barrier Island; Walter V. Reid, reproductive effort and the cost of reproduction in the Glaucous-winged Gull; John D. Reynolds, philopatry and breeding site fidelity in the Red-necked Phalarope; Brian J. Sevick, use of space by Red-tailed Hawks in southern Alberta; Michael Shelton, partial migration in the Iceland Snow Bunting (*Plectrophenax nivalis insulae*); Julia I. Smith, hybridization in Golden-fronted and Red-bellied woodpeckers; Linda K. Southern, relationship of sex to breeding area fidelity in the Ring-billed Gull (*Larus delawarensis*); Cynthia Anne Staicer, song and the behavioral ecology of a resident wood warbler (*Dendroica adelaidae*); Mark A. Stern, site fidelity and mate fidelity in colonial nesting Black Terns; Kimberly Sullivan, energetic constraints on the development of time-budgeting in Yellow-eyed Juncos; William J. Sydeman, cooperative breeding in the Pygmy Nuthatch (*Sitta pygmaea*); Gregory J. Transue, mate replacement in Herring and Great Black-backed gulls (*Larus argentatus*, *L. marinus*); Katherine A. Voss-Roberts, parental attentiveness and embryonic tolerance of thermal stress in Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*); Ian G. Warkentin, wintering ecology of the Richardson's Merlin in Saskatoon, Saskatchewan; David Westmoreland, effects of clutch size on the length of the nesting cycle and reproductive success in Mourning Doves; David French Westneat, parental care and alternative mating strategies in the Indigo Bunting; Nathaniel T. Wheelwright, consequences of eating fruits, an experimental study of the American Robin; Rory Paul Wilson, foraging behavior of Jackass Penguins (*Spheniscus demersus*) at different islands; Licia Wolf, biparental care in the monogamous Dark-eyed Junco: an experimental test of the adaptive hypothesis; Marina Wong, plant phenology, foliage-arthropod abundance, and the trophic organization of the birds in Panamanian forest understory: a comparison with Malaysian dipterocarp forest.