COSTS AND RATES OF EGG FORMATION IN RUDDY DUCKS[1]

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Abstract. We analyzed composition of rapidly developing ovarian follicles and eggs of Ruddy Ducks nesting in southern Manitoba in 1988. For ducks with at least one ovulated follicle, there was a significant correlation between amount of fat in the largest developing follicle and time of day that ducks were shot. If ovulations occur, on average, 24 hr apart, then this correlation indicates a high degree of synchrony of ovulation in this population. We used regression analysis to estimate that 95% of ovulations occurred between early evening (18:17 CST) and mid morning (08:45 CST). These results support observations by Siegfried (1976) and Tome (1984) that the interval between eggs is 24 hr in wild Ruddy Ducks. We never found more than six rapidly developing follicles per female, and given that eggs are laid daily, duration of rapid follicle growth is 5–6 days. This conflicts with an estimate of 11 days for follicle maturation made by Gray (1980) who counted pairs of light and dark rings in egg yolks. We explain this incongruity by proposing that egg-laying Ruddy Ducks may show a diphasic, and perhaps crepuscular, foraging schedule whereby two sets of rings are deposited/24 hr (Dobbs et al. 1976). Maximum daily cost (584 kJ) of egg production, scaled against basal metabolic rate (BMR, 208 kJ), is among the highest (280% of BMR) recorded for waterfowl. However, Ruddy Ducks that lay seven eggs, i.e., the modal clutch size, would incur maximum costs for only one day.

Key words: Egg composition; energy budgets; laying rates; ovarian follicles; Oxyura ja-maicensis; Ruddy Ducks.

INTRODUCTION

Daily energy equivalents of nutrients produced by egg-laying waterfowl are among the highest of any birds (Ricklefs 1974, King 1973, Alisauskas and Ankney 1992). To calculate maximum daily costs of egg production for a species, the following data are required: (1) chemical composition (and thus energy density) of eggs, (2) number of days required for maturation of rapidly developing follicles, (3) time interval between laying of successive eggs, and (4) number of eggs laid per clutch (King 1973). Egg composition is determined by collecting and separating eggs into shell, yolk, and albumen, with further separation of yolk into lipid and lean dry mass fractions. Determination of the number of days of yolk formation requires knowledge about mass of each follicle in a size hierarchy, and the interval between laying of successive eggs (see also Grau 1976). Egg interval, or laying rate, can be measured by direct observation or with event recorders (Cooper 1978). Often direct observation involves daily nest checks, but this may lead

to erroneous conclusions by underestimating egg interval (see Alisauskas and Ankney 1992:38–39). Moreover, nest visits by researchers induce nesting females to abandon clutches, particularly during egg laying (Gloutney et al. 1993). Clutch size is measured by counting eggs in nests when visits are made, but this can be inaccurate if intraspecific nest parasitism or partial clutch predation occurs. Herein, we describe a method, using inferences drawn from data collected solely from dead specimens, that supports conclusions made by others (Siegfried 1976, Tome 1984) of within-clutch one-day egg intervals in Ruddy Ducks. Although unnecessary in this study, direct observation of egg interval, or laying rate, could provide independent support for inferred egg intervals in other species.

METHODS

Thirty-one female Ruddy Ducks were shot under permit on wetlands within 50 km of Minnedosa, Manitoba in 1988. Collection times varied from 16:15 to 22:05. Ovaries, oviducts and oviducal eggs were excised through the dorsal portion of the ribcage. After postovulatory follicles were

counted (N_{POF}), reproductive tissue was placed in 10% formalin. The rest of each bird was then labelled, sealed in plastic bags, and frozen until other dissections were done, during which age was determined based on the presence (yearling) or absence (adult) of a bursa of Fabricius (Hochbaum 1942). To determine egg composition, single unincubated eggs from each of 12 nests were collected, weighed (0.01 g), boiled and frozen for storage. Thawed eggs were reboiled, and shell, yolk and albumen (including shell membranes) weighed separately. Albumen was dried to constant mass and reweighed, yielding dry albumen mass ($L_{albumen}$). Fat was removed from each yolk (F_{yolk}) using petroleum ether in a modified Soxhlet apparatus (Dobush et al. 1985), leaving the residue which, when weighed, gave lean dry mass of the yolk (L_{yolk}).

After removing preserved ovaries and oviducts from storage containers, each developing follicle was removed from the rest of the ovary. Follicles and oviducts were weighed (0.01 g) separately, dried to constant mass, and reweighed (0.01 g) to yield dry follicle mass and oviduct mass (L_{ovid}). Fat from each follicle (F_{fol}) and rest of each ovary (F_{ovary}) was removed using petroleum ether as a solvent in a modified Soxhlet apparatus. Remaining residue of each follicle (L_{fol}) and remainder of the ovary (L_{ovary}) consisted of lean dry mass. These variables were used in the construction of energy budgets.

Occasionally, developing follicles were damaged during collection, and yolk escaped into the body cavity so that follicle components could not be determined accurately using fat extraction only. As it was necessary to know mass of all developing follicles for estimating nutrients committed to clutch formation, we estimated fat and LDM of damaged follicles. To do this, we pooled data from all females in various stages of RFG and used all pairs of undamaged sequential follicles to derive the relations of component masses. To estimate fat (F_{fol}) and lean dry mass (L_{fol}), from those of the next smallest follicle (F_{fol-1}) and (L_{fol-1}) respectively, we used:

$$F_{fol} = 0.381 + 2.365(F_{fol-1}) - 0.166(F_{fol-1}^2),$$

$$df = 125, r^2 = 0.953, P < 0.001$$

$$L_{fol} = 0.255 + 2.411(L_{fol-1}) - 0.248(L_{fol-1}^2),$$

$$df = 125, r^2 = 0.957, P < 0.001.$$

When there were no developing follicles smaller than the damaged one, we used fat, F_{fol+1}, and

lean dry mass, L_{fol+1}, of the next largest follicle to estimate component masses, F_{fol} and L_{fol}, respectively, of damaged follicles using:

$$F_{fol} = -0.003 + 0.192(F_{fol+1}) + 0.054(F_{fol+1}^2),$$

$$df = 125, r^2 = 0.960, P < 0.001$$

$$L_{fol} = 0.007 + 0.227(L_{fol+1}) + 0.069(L_{fol+1}^2),$$

$$df = 125, r^2 = 0.959, P < 0.001.$$

After we established bivariate relations using linear regression of fat in the largest follicle on time of day that ducks were shot, we estimated the average time of day for ovulation using mean fat content in egg yolks, and the prediction techniques of Sokal and Rohlf (1981:498).

RESULTS AND DISCUSSION

EGG INTERVAL

Fat content of the largest ovum in a series of rapidly developing follicles was related to collection time (Fig. 1). Time of day for ovulation (X) was predicted using its relation to fat in the largest follicle (Y): $X = 3.20Y - 3.84$ (Fig. 1). Thus, assuming that ovulation occurs at the time when maximum size is reached, and with average yolk fat equal to 9.18 g (Table 1, range: 7.86–10.51 g), the predicted hour for ovulation in this sample of ducks on average was about 01:30 hr CST. Although this estimate varies considerably, the correlation suggests a significant degree of synchrony that is possible only if Ruddy Ducks in this population ovulate an average of 24 hr apart. Ninety-five percent CI (cf Sokal and Rohlf 1981:498) for this estimate (01:30 hr) ranged about 14.5 hr (from 18:17 hr [earlier] to 08:45 hr the following morning). Thus, it is unlikely that any ovulations occur in the early afternoon. This strongly suggests that the average interval between ovulations was one day; if it had been 1.5 days (Cramp and Simmons 1977), the correlation in Figure 1 would not be significant. Note also that a correlation between fat in the largest follicle and time of day would not exist in species with individuals that ovulate asynchronously to time of day or to each other, even if ovulation intervals for individuals had been one day.

To examine another source of variation, we used egg fat content for a subset ($n = 12$) of the ducks in Figure 1 that contained unbroken oviducal eggs, and used these to test the significance of any effect on individual variation in fat content of the largest developing follicle. A general
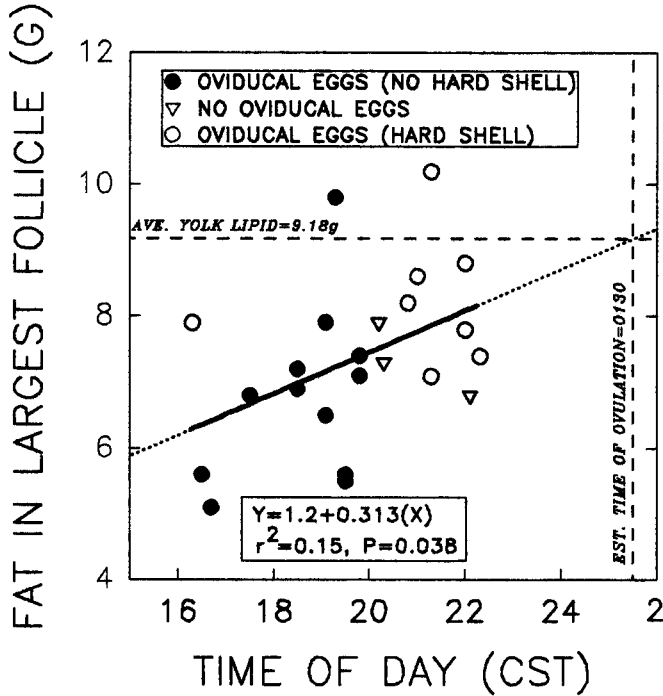
FIGURE 1.   Diurnal pattern of lipid content in the largest rapidly developing ovarian follicle of Ruddy Ducks. Data include only birds with >0 ovulated follicles (which all had >5 g lipid in the largest ovum).

linear model (SAS 1990) showed that after accounting for variation in time of day, fat in the largest developing follicles were significantly related to fat in oviducal eggs of the same individuals ($F = 5.9$, df = 1, 10, $P = 0.038$). Therefore, variation in fat content (Fig. 1) is inflated as it includes interfemale variation in mass of mature follicles, and the 95% CI reported above is confounded somewhat by this additional variation.

We never encountered more than a single ovum in any oviduct which indicates that duration of ovum passage through oviducts, during which albumen and shell are secreted, was <24 hr. Oviducal eggs with hard shells are nearing completion, and seven of eight ducks that contained hard-shelled oviducal eggs were also collected after 20:00 (Fig. 1). Ducks with at least one developing follicle, a large ovulated follicle, but no oviducal eggs, had likely laid an egg recently and would shortly ovulate again. Only three of 23 ducks with post-ovulatory follicles (Fig. 1) were without oviducal eggs but this depended on the time of day: three of 10 ducks shot after 20:00 hr, whereas all of 13 shot before 20:00, had oviducal eggs, further suggesting that laying begins about 20:00 hr. This is also evidence that days

are not normally skipped because, if they were, then at least half of the females in this sample would not be expected to have an egg in the oviduct. Thus laying times are likely strongly correlated with ovulation times, although laying time precedes ovulation time by an unknown interval.

It is important to know egg-laying rates when constructing energy budgets as small errors in estimating this variable will result in large errors in estimates of maximum daily egg energy. Con-

TABLE 1.   Composition (g) of 12 Ruddy Duck eggs, each from a different nest.

Egg component	Mean	Standard deviation	Coefficient of variation (%)
Fresh mass	68.92	4.24	6.15
Shell dry	6.80	0.59	8.68
Albumen protein	4.59	0.34	7.41
Yolk lipid	9.18	0.80	8.71
Yolk protein	4.43	0.30	6.77
Yolk mineral	0.35	0.04	11.43
Energy content (kJ)[a]	575	37.5	6.52

[a] Estimated as lipid × 39.5 + (albumen protein + yolk lean dry mass) × 23.6; yolk lean dry mass = yolk protein + yolk mineral, and was used instead of yolk protein in calculating energy content so that it be consistent with determinations in most other studies which use yolk lean dry mass.
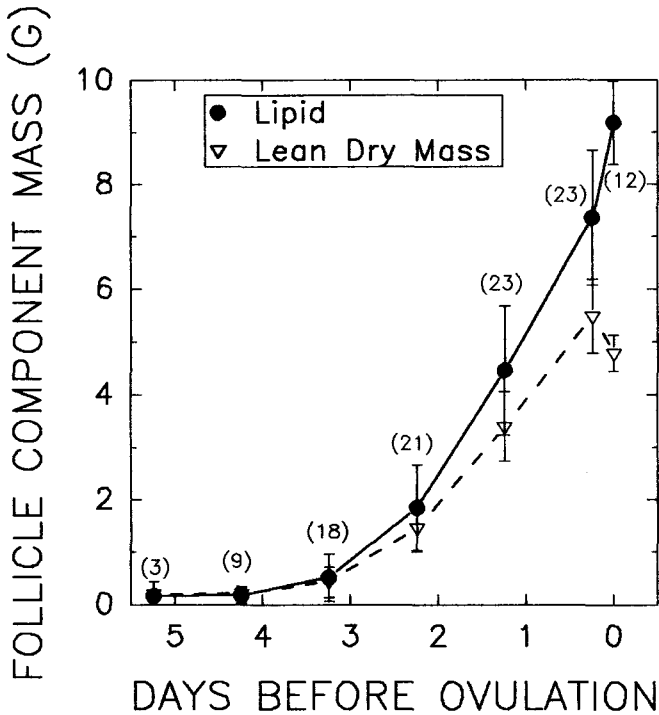
FIGURE 2.   Development patterns of rapidly growing ovarian follicles in Ruddy Ducks with at least one ovulated follicle. Data for day 0 are egg composition data. Shown are yolk lipid and yolk lean dry mass. Note that the time interval between estimates for largest follicles and eggs is 5.8 hr and is based on the estimated number of hours between the average time of day that birds were collected and the projected average time of ovulation (see Fig. 1 and text).

clusions about one day egg intervals in Ruddy Ducks by Siegfried (1976), Gray (1980), and Tome (1984) were based on daily visits to nests rather than constant monitoring (see Alisauskas and Ankney 1992). Kear (in Cramp and Simmons 1977:693) observed that captive Ruddy Ducks laid eggs 1.5 days apart. Our findings support conclusions of those who studied *wild* Ruddy Ducks.

FOLLICLE GROWTH RATE

With one day egg intervals and never more than 5–6 rapidly developing follicles present in any female, about 5–6 days are required for a rapidly developing follicle to mature (Fig. 2). Mean time of collection of this sample was 19:45 which was 5 hr, 45 min before estimated time of ovulation. Maximum lean dry mass of follicles was attained before ovulation whereas fat continued to be supplied until ovulation. Lean dry mass of egg yolks was somewhat less than the average found in the largest follicles (Fig. 2, Table 1), a difference ex-

plained by separation of the ovum from the surrounding follicle at ovulation.

Avian egg yolk typically is structured in concentric rings composed of heterogeneous material. Differences in the staining properties of these bands enhance their visibility, and allow them to be counted (Grau 1976). From staining egg yolks of Ruddy Ducks, and counting number of resulting rings using Grau's (1976) method, Gray (1980) concluded that 11 days were required for growth by ovarian follicles before ovulation. Based on examination of ovaries, and an inferred egg interval of one day (Fig. 1), we concluded that the phase of rapid follicular development was only 5–6 days. A pair of light and dark rings in egg yolks have been assumed to represent one day of ovum growth (Grau 1976, Roudybush et al. 1979). Instead, each pair may represent one discrete, high volume meal in (e.g., crepuscular) animals with distinct foraging bouts followed by an interval of no feeding. Incubating dabbling ducks show crepuscular incubation recesses (Gloutney et al. 1993), but diurnal patterns in
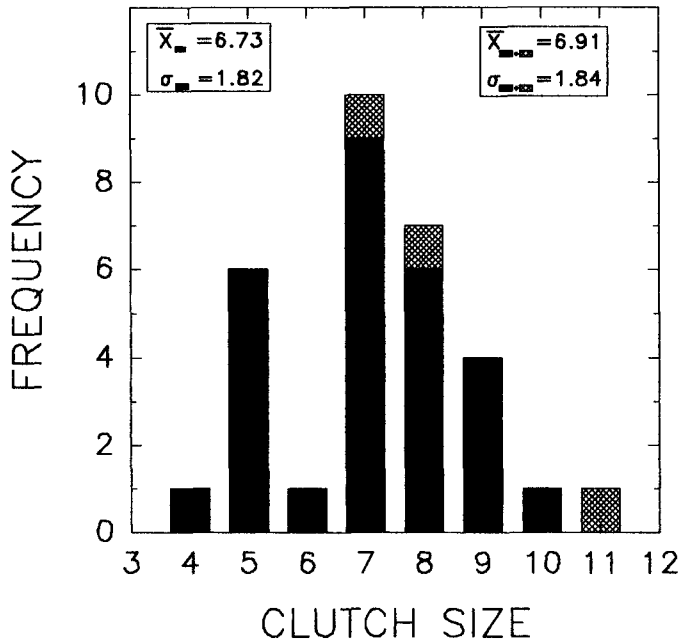
FIGURE 3.   Clutch size of Ruddy Ducks as determined from inspection of ruptured and developing ovarian follicles. Clutch size = No. developing follicles + No. ovulated follicles for birds with ≤5 developing follicles (solid) and those with six (crosshatched). Sample includes all ducks ($n = 23$) shown in Fig. 1 and $n = 8$ with no developing follicles.

feeding intensity are unknown for individually marked egg-laying ducks. Possibly, egg-laying Ruddy Ducks show a diphasic feeding schedule, resulting in diphasic pattern of nutrient deposition in ova. Such a pattern has been demonstrated by Dobbs et al. (1976) in Japanese Quail (*Coturnix japonica*) using Grau's (1976) method: two dark bands were produced per day after feeding intake of experimental birds was restricted to two 4 hr periods per day, as compared with a single dark band per day in control birds.

Tome (1991) and Gray (1980) quantified activity budgets of breeding female Ruddy Ducks from Manitoba and California, respectively, but neither presented information on diurnal feeding patterns of egg-laying females. In Manitoba, foraging bouts of Ruddy Ducks during breeding lasted 20–30 min (Tome, pers. comm.), and the frequency of such bouts may show two pulses/ day. The effect of such a diphasic pattern could produce two sets of rings per 24 hr (Dobbs et al. 1976), and so Gray's estimate may be biased; possibly, follicle maturation times of Ruddy Ducks in California are twice what they are in Manitoba, but we suspect that such intraspecific variation is unlikely. Conclusions about devel-

opment time of ova, as determined from stained egg yolks laid by wild birds, should be supported with information about daily feeding schedules, or with methods presented herein.

CLUTCH SIZE

Clutch size was calculated by adding number of developing and ovulated follicles. If only females with five or fewer developing follicles are considered, average clutch size was 6.73 eggs (Fig. 3). Only three of 31 females with ≥1 ovulated follicle had >5 developing follicles and thus their potential clutch sizes could not be known. To assess potential bias in estimating average clutch size by including or excluding such females in the calculation of average clutch size using this method, we assumed that these three would have laid 11 eggs (the potential clutch size of one of these was 11 = 6 developing follicles + 5 ovulated follicles, Fig. 3). Assuming such an unlikely situation, then average clutch size for all 31 females would equal 7.42 and the mode, 7. Thus, the influence of the absence of data from these three ducks in biasing average clutch size downward was very small. Inclusion of these three ducks resulted in an average clutch size of 6.91.
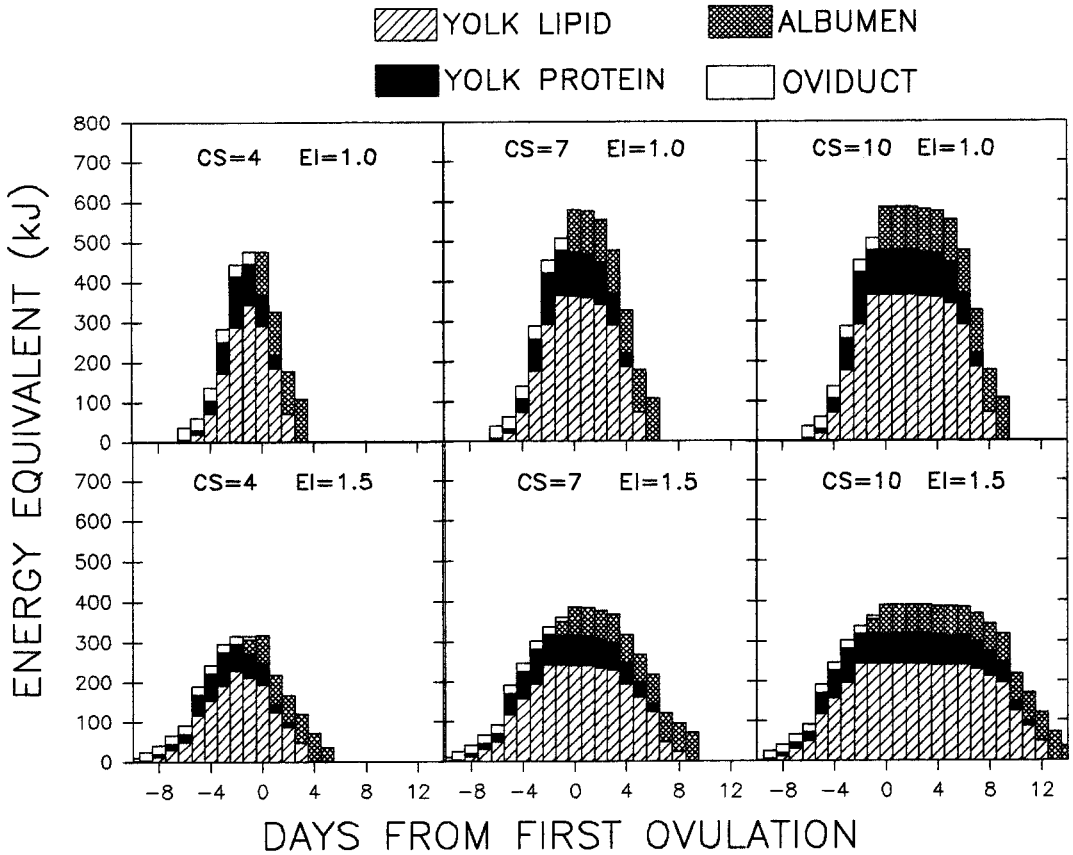
FIGURE 4.   Estimated energy budgets of Ruddy Ducks using clutch sizes (CS) of four, seven, and 10 eggs, and egg intervals (EI) of 1.0 (upper panels) and 1.5 days (lower panels). Energy equivalents of 23.6 and 39.5 kJ/g of protein and fat, respectively.

In any of the three cases, modal clutch size was seven eggs. This compares with clutch sizes of 7.7 (Siegfried 1976) and 7.6 (Tome 1984) as determined by checking nests in Manitoba. No differences were found in clutch sizes of adults and yearlings ($F_{(1,26)} = 0.12$, $P = 0.731$).

ENERGY EQUIVALENTS OF CLUTCH NUTRIENTS

Stiff-tailed ducks (Tribe Oxyurini) show the highest daily egg energy costs as a proportion of basal metabolic rate (BMR) among waterfowl for which data are available (Tome 1984, Alisauskas and Ankney 1992), because of their very large eggs relative to body mass (Lack 1968). Temporal distribution of egg-laying costs were calculated assuming equal rates of development of all follicles in each female (i.e., the averages in Fig. 2) for clutches of four, seven and 10 eggs (Fig. 4). Duration of the clutch production cycle,

from initial development of first ova to deposition of the last egg, required 10, 13, and 16 days, respectively, for ducks that average one day between eggs, and 16, 20, and 24 days, respectively, if Ruddy Ducks hypothetically laid eggs 1.5 days apart. Maximum daily costs of egg production (kJ/day) for clutches of one to 10 eggs increase until a plateau is reached at seven eggs (Fig. 5). For modal clutch size of seven, maximum costs of egg production (584 kJ/day) are incurred for only one day (Day 0, Fig. 4). Thus, laying interval has a large influence on the timing and extent of energy or nutrient costs during egg laying.

Basal metabolic rate is frequently used as a physiological standard against which to compare energy costs associated with egg production (Alisauskas and Ankney 1992). With daily laying and a modal clutch size of seven exceeding the growth phase of 5–6 days of each follicle, maximum daily cost of egg production by Ruddy Ducks is most
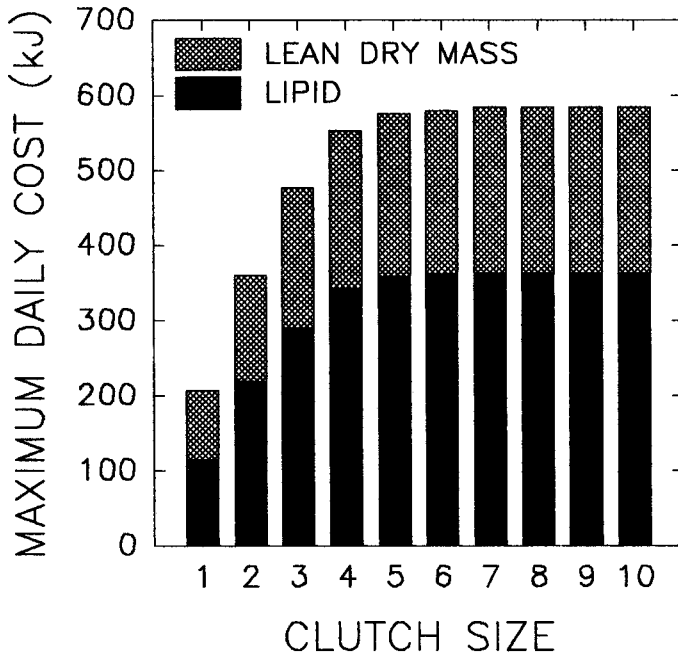
FIGURE 5.   Maximum daily energy expenditure as a function of clutch size (CS) in Ruddy Ducks.

often equivalent to that of an egg (about 580 kJ). BMR was predicted (208 kJ/day) from body mass (587 g, Alisauskas and Ankney, unpubl. data) of non-breeding Ruddy Ducks using Aschoff and Pohl's (1970) equation for non-passerines at rest. Thus maximum daily costs of egg production, scaled against BMR, are among the highest of all waterfowl (Alisauskas and Ankney 1992). The ability of these small ducks to produce clutches of ≥6 large eggs at intervals of one day is remarkable. Adaptations for satisfying high daily nutrient demands of egg-laying Ruddy Ducks include use of fat, protein and mineral reserves; the proximate link between nutrient reserves and reproduction in Ruddy Ducks is critical in that minimum levels of nutrient reserves are a prerequisite for breeding attempts (Alisauskas and Ankney, unpubl. data).

LITERATURE CITED

ALISAUSKAS, R. T., AND C. D. ANKNEY. 1992. The cost of egg laying and its relation to nutrient reserves in waterfowl, p. 30–61. In B.D.J. Batt et al. [eds.], The ecology and management of breeding waterfowl. Univ. of Minnesota Press, Minneapolis.

ASCHOFF, J., AND H. POHL. 1970. Rhythmic variations in energy metabolism. Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29:1541–1552.

COOPER, J. A. 1978. The history and breeding biology of the Canada Geese in Marshy Point, Manitoba. Wildl. Monogr. 61:1–87.

CRAMP, S., AND K.E.L. SIMMONS. 1977. Handbook of the birds of Europe, the Middle East and North Africa, Vol. I Ostrich to Ducks. Oxford Univ. Press, Oxford, England.

DOBBS, J. C., C. R. GRAU, T. ROUDYBUSH, AND J. WATHEN. 1976. Yolk ring structure of quail subjected to food deprivation and refeeding. Poultry Sci. 55:2028–2029.

DOBUSH, G. R., C. D. ANKNEY, AND D. G. KREMENTZ. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of Snow Geese. Can. J. Zool. 63:1917–1920.

GLOUTNEY, M. L., R. G. CLARK, A. D. AFTON, AND G. J. HUFF. 1993. Timing of nest searches for upland nesting waterfowl. J. Wildl. Manage. 57:597–601.

GRAU, C. R. 1976. Ring structure of avian egg yolk. Poultry Sci. 55:1418–1422.

GRAY, B. J. 1980. Reproduction, energetics and so-

cial structure of the Ruddy Duck. Ph.D.diss. Univ. of California, Davis, CA.

HOCHBAUM, H. A. 1942. Sex and age determination of waterfowl by cloacal examination. Trans. N. Amer. Wildl. Conf. 7:299–307.

KING, J. R. 1973. Energetics of reproduction in birds, p. 78–117. In D. S. Farner [ed.], Breeding biology of birds. National Academy of Sciences, Washington, DC.

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

RICKLEFS, R. E. 1974. Energetics of reproduction in birds, p. 152–292. In R. A. Paynter [ed.], Avian energetics. Publ. Nuttall Ornith. Club No. 15.

ROUDYBUSH, T. E., C. R. GRAU, M. R. PETERSEN, D. G. AINLEY, K. V. HIRSCH, A. P. GILMAN, AND S. M. PATTERN. 1979. Yolk formation in some charadriiform birds. Condor 81:293–298.

SAS INSTITUTE. 1990. SAS/STAT User's Guide, version 6, 4th ed. SAS Institute, Cary, NC.

SIEGFRIED, W. R. 1976. Social organization in Ruddy and Maccoa Ducks. Auk 93:560–570.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. W. H. Freeman and Co., New York.

TOME, M. W. 1984. Changes in nutrient reserves and organ size of female Ruddy Ducks breeding in Manitoba. Auk 101:830–837.

TOME, M. W. 1991. Diurnal activity budget of female Ruddy Ducks breeding in Manitoba. Wils. Bull. 103:183–189.