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Significance of social parameters on differential nutrient investment in guira cuckoo, *Guira guira*, eggs

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We examined how different facets of guira cuckoo sociality influence reproductive investment in eggs (mass, volume, albumen, yolk, shell weight and thickness). The highly variable egg size in this species suggests the possibility of reproductive manipulation by females. Our data support the hypothesis that investment in eggs and clutches reflects a social context. The lowest egg ejection probabilities occurred in very small communal clutches where there were fewer laying females. Eggs retained in the nest had larger yolks than those ejected, a possible mechanism for minimizing energy expenditure in eggs with high chances of failure. Results showed that eggs laid in smaller communal clutches had, on average, less investment in all nutrient measures, except yolk, compared with eggs from larger communal clutches. It may be important for eggs to be of high quality in larger communal clutches, where competition between hatchlings will most likely be intense. Larger guira cuckoo eggs in this study produced heavier chicks. We found that chicks that were smaller and lighter at hatching were victimized by infanticide significantly more than chicks that were heavier and bigger at hatching.

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In most bird species studied to date, females lay eggs that may vary greatly in size both within and between clutches. Whether this variability reflects an adaptive response to the environment or is an intrinsic characteristic of the bird has been investigated recently in a number of studies (Wiggins 1990; Jover et al. 1993; Williams 1994; Amundsen et al. 1996; Flint & Grand 1996; Erikstad et al. 1998). Egg size variation has been assessed with regard to female quality (e.g. age, body size, experience), clutch size, timing of breeding, and position of the egg in the laying sequence of the clutch (Wiggins 1990; Croxall et al. 1992; Flint & Grand 1996; Perrins 1996). In these studies, strong support has been found for a positive relationship between egg size and maternal age. However, maternal age may reflect other factors, more difficult to measure, such as the individual's ability to acquire resources in its habitat or the amount of body nutrient reserves. Lack (1967) suggested that, among waterfowl, the average amount of food for the female at the time of laying may have influenced the evolution of clutch and egg sizes. Arnold & Rohwer (1991) pointed out that this hypothesis is flawed and argued that if a bird can obtain enough daily resources to produce a clutch of a certain size, then it could produce eggs indefinitely by continuous foraging. They suggested that clutch size is not limited by the daily ingestion of food, but more probably by the stored nutrients of females, presumably acquired prior to the onset of laying.

The fitness consequences of egg size have been evaluated in some species, especially waterfowl. Large eggs may enhance hatching success, and normally yield larger chicks with higher survival rates than small eggs (reviewed in Williams 1994). However, most of the studies associating egg size with hatchling rate of survival were non-experimental, and the effect of parental care posthatch may have obscured the final effects of egg size per se.

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A study of the common eider, *Somateria mollissima*, side-stepped this problem by hatching eggs and then measuring the growth of chicks in the laboratory (Erikstad et al. 1998). The results not only agreed with the premise that egg size influences hatchling size and growth, but also indicated that females in this species vary the allocation of nutrients to eggs according to their position in the clutch. This is consistent with the adaptive hypothesis that nutrient allocation to eggs may be influenced by the probability that the egg will hatch and the chick will survive (Quinn & Morris 1986; Williams et al. 1993).

The influence of specific egg components on the size and quality of chicks has been examined in fewer studies, and there has been some discussion about the relative importance of each. Parsons (1970) suggested that a considerable part of hatchling variation in mass can be attributed to differences in the quantity of yolk. On the other hand, approximately 67% of the protein content of the egg is contained in the albumen (Romanoff & Romanoff 1949). Enhanced quantities of protein could influence embryonic tissue development and result in larger nestlings (Finkler et al. 1998). Other studies have also suggested that water content is the crucial component positively correlated with size and subsequent success of hatchlings (Simkiss 1980; Arnold et al. 1991). Sotherland et al. (1990), for instance, showed that intraspecific increases in albumen in the egg (which is more than 90% water in most species: Sotherland & Rahn 1987) are associated with similar rates of increases in hatchling mass. Experimental manipulations of albumen (primarily water) and yolk content in chicken eggs by Finkler et al. (1998) indicated that there is a positive relationship between albumen content of the egg and tibiotarsus length of the hatchling. Leg length may be an important aspect of hatchling success, given its role in movement. Albumen, as the primary source of water in the egg, may be the major determining factor in hatchling size (Finkler et al. 1998). However, the manipulation of the amount of yolk in eggs did not influence the assimilation of yolk into tissue, but affected only the residual amount left in the yolk sac at the end of incubation.

One of the least studied aspects of reproductive strategies of birds is the adaptive response of individuals to changing social and mating circumstances, in the form of variations in clutch and egg characteristics. Some forced-mating experiments have found that females not allowed to mate with their chosen partner produced fewer and lower-quality offspring than those produced by females that chose their mates (Bluhm 1985; Yamamoto et al. 1989). Recently, Cunningham & Russell (2000) showed that female mallards, *Anas platyrhynchos*, lay larger eggs after mating with preferred males and smaller ones when mating with less preferred males, yielding offspring with decreased body condition in the latter case. Fewer studies have examined the relation between a female's investment in clutch size and/or egg mass and her social status. This is due to the fact that almost all studies of such characteristics, to date, have concentrated either on birds that nest in isolation, or have ignored effects of social circumstances on reproductive investment in eggs. One study that attempted to address this issue considered egg size variation relative to mating status in the polygynously breeding northern lapwing, *Vanellus vanellus* (Grønstøl 1997). Monogamous and primary females in this study had similar egg sizes whereas eggs of secondary females were smaller. However, the lower egg volume of secondary females was attributed to their laying later in the season. Grønstøl (1997) suggested that earlier breeding monogamous and primary females may have been of higher quality, thus not only producing larger eggs due to intrinsic factors, but also benefiting from settling on territories at earlier dates. It is clear that secondary females, whether due to intrinsic quality differences or to their lower status leading them to settle later in the territories, produced smaller eggs. One could argue in this case, therefore, that the lower status of females was the underlying cause of their producing small eggs, although the mechanism, genetic or environmental, was not elucidated in the study.

Guira cuckoos are among the very few birds that breed communally (Brown 1987), with as many as seven females in a group using a joint nest (Cariello et al. 2002). Although group members join in several cooperative ventures, competition is exercised by the ejection of eggs from the nest and through infanticide (Macedo & Melo 1999). Females may have their eggs subjected to different ejection rates, depending on factors such as the number of cobreeding females and whether their egg laying is concentrated early or late during the group's laying bout (Macedo 1992). Some females can also space out their eggs through the laying sequence of the group (Cariello et al. 2002; Macedo et al. 2004). In the case of guira cuckoos, egg ejection exposes individuals to energetic expenditures that may be quite significant, because eggs in this species are relatively large (25 g on average) and each egg represents approximately 16% of an adult female's weight (Macedo 1992). However, any strategy adopted by females to reduce investment in egg nutrients to avoid energetic losses could also result in smaller hatchlings and possibly compromise their quality and survival.

Eggs vary widely in size, both within nests as well as in the population in general. Guira cuckoo eggs from our study population can vary remarkably in mass, from 15.8 to 32.3 g with a mean ± SD of 24.57 ± 2.94 g ($N = 509$ eggs; R. H. Macedo, unpublished data). Such extreme variability provides an ideal situation to examine how females invest in their eggs relative to a number of social conditions. Here we examine these issues by addressing the following questions.

(1) What are the interrelated associations between the position of an egg in the laying sequence within the communal clutch, its probability of ejection and the number of breeding females?

(2) How are social circumstances, such as the level of egg-ejection pressure and communal clutch size, related to nutrient investment in eggs?

(3) How do individual female levels of nutrient investment in their clutches vary, taking into consideration the female order of entering the laying sequence?

(4) What are the interrelated associations of egg mass and hatch order with hatchling size and short-term survival and fledging of chicks?

METHODS

Study Area and Field Methods

We conducted fieldwork in the semiurban area around Brasilia, Brazil (15°47′S, 47°56′W, altitude 1158 m), which has a highly seasonal climate with a rainy season from September to March. The study area lies within the Brazilian central plateau and is dominated by savannah vegetation denominated 'cerrado', largely altered in our field site, and patchily distributed among cultivated plots and gardens. Reproduction of the guira cuckoo is restricted mostly to the rainy season. At the beginning of each reproductive season, we searched for old nests, which are commonly reused by groups, and placed nylon fishing nets beneath them to collect ejected eggs. Once reproduction was detected, we visited nests daily to monitor egg laying and ejection, and counted adult birds around the nest during each visit, to estimate group size from repeated maximum counts. From July to December in 1998 and 1999, we collected data on fresh eggs and developed the egg maternity protocol (see below). From August 2000 to March 2001, we collected information concerning the relation between egg and hatchling sizes and their subsequent survival.

Egg Contents and Determination of Maternity (1998–1999)

In the 1998–1999 seasons, eggs were collected directly from nests or, if ejected, from the fishing nets. We substituted eggs taken from the nests with dummy eggs, which were hand-painted chicken and pigeon eggs, or guira cuckoo eggs collected outside the field site. The birds readily accepted the dummy eggs and continued normal ejecting and laying of eggs and, eventually, incubated the clutches of dummy eggs (Macedo et al. 2004). We took the real eggs to the laboratory and measured several aspects associated with nutrient investment: maximum length (L) and width (W) with digital callipers (precision 0.1 mm), and weight with a digital scale (precision 0.1 g). We used Hoyt's (1979) formula to calculate egg volume: $(0.51) \times L \times W^2$. After photographing the eggs, we opened them and separated the yolk and albumen, which were weighed separately, and then froze both yolk and albumen at −20°C. The eggshells were weighed fresh and stored at room temperature. With precision callipers (precision 0.01 mm), we took three measures of thickness of each eggshell where there was no superimposed calcareous speckling and three additional measures where speckling occurred. For analyses, we used the mean thickness of eggshells measured where there was no overlaid speckling, because this measure was less variable. The nutrient investment measures analysed in this study were egg weight and volume, yolk mass, albumen mass, eggshell weight and eggshell thickness. The yolk samples were further used to determine maternity in a protein electrophoresis analysis, described in detail elsewhere (Cariello et al. 2002). Briefly, yolk samples were digested by trypsin, incubated and heated to 94°C for 30 s. Samples were diluted with a loading buffer, then heated

again, and subsequently run on 12% denaturing polyacrylamide gels according to Laemmli's (1970) protocol. Proteins were then visualized by silver staining (Morrissey 1981). Identical banding patterns obtained from eggs within a nest indicated that the same female laid them, and different patterns of bands indicated that different females laid them.

Egg Mass, Hatchling Size and Chick Survival (2000–2001)

In the 2000–2001 season, we only substituted eggs with dummy eggs (in this case made with plaster and hand painted) at the end of incubation. As eggs were laid, we measured their length and width with digital callipers (precision 0.1 mm), and weighed them with a digital scale (precision 0.1 g) while in the field, returning them to the nest immediately. We substituted eggs in the nest with dummy eggs on the 10th day of incubation (incubation period in this species is approximately 12 days). In each nest we left one real egg from the clutch, to provide the cues during hatching (e.g. vocalization of the chick within the egg and at hatching) that may elicit normal brooding and feeding behaviour of adults. In almost all cases, the birds continued incubating the clutches of dummy eggs while the real eggs were hatched in a commercial incubator in the laboratory. Soon after hatching (from 0 to 6 h), we weighed chicks with a digital balance measured them with digital callipers (bill length, left wing length, left tarsus), and marked them with coloured plastic straws cut to fit their tarsi. This procedure was also carried out for the chicks that hatched from the single eggs left in each nest. Chicks that hatched in the incubator were returned to the nest within 12 h after hatching (some hatched at night), in the same order that they hatched. We monitored nests to assess the survival of marked chicks after returning all chicks to nests, and also registered fledging dates for surviving chicks.

Data Analysis

In all analyses of eggs, we only used those communal clutches where at least some of the eggs were not ejected. We did this because nesting bouts ending in desertion due to the ejection of all eggs presented significantly smaller communal clutch sizes compared with those that suffered only partial ejection (Pacheco 2002). In some analyses, we compared eggs laid first versus those laid last, and these refer to the very first and last eggs laid in communal clutches. In other types of comparisons between eggs from different-sized communal clutches, we calculated a sequence index for each egg by dividing its position by the total number of eggs laid in that communal clutch. For example, an egg laid in position 2 in a clutch of five eggs would have a sequence index of 0.40, whereas an egg in the same position in a clutch of three eggs would have a sequence index of 0.67. Thus, the higher the index (independent of communal clutch size), the closer the egg was laid towards the end of the laying sequence in that nesting bout. The probability of ejection for eggs in

distinct positions in the communal clutches was calculated by dividing the number of eggs ejected by total eggs laid in each position in all clutches monitored. The relation between sequence index and probability of ejection was analysed using a cubic spline. This nonparametric analysis involves no a priori assumptions concerning the shape of the curve, and any shape up to a straight line can be fitted to the data (Schluter 1988; Ritchie 1996). Before fitting splines, a random error of 2.5% was introduced to the egg sequence category to avoid ties, and the smoothing factor was held at −5.

We tested all data for normality with the Kolmogorov–Smirnov test, and used nonparametric analyses (Spearman rank correlation, Kruskal–Wallis test, Mann–Whitney U test) for data that were not normally distributed (variables: group size, communal clutch size, ejection probability) and for which data transformation did not result in normality. We used parametric analyses (Pearson correlation, t test) for all other variables that were normally distributed. We analysed the survival of chicks to the 10th day relative to their order of hatching with a Fisher's exact test. The order of hatching was classified as either first or second day, as this was the extent of asynchrony in hatching verified during the study. We used SYSTAT software version 9.0 (SPSS 1999) for all analyses. All tests were two-tailed and conducted at the 0.05 significance level.

RESULTS

Egg Position in Laying Sequence, Probability of Ejection and Communal Clutch Size

Figure 1 shows the shape of the spline fitted to the egg ejection probability according to position of eggs in the communal clutch. Eggs laid at the beginning of the nesting bout suffered higher rates of ejection than did those laid later, independent of the communal clutch size. There was, however, a secondary peak of egg ejection for later eggs, laid at approximately the 75th percentile of the communal clutch. A comparison of the ejection probabilities of eggs laid first ($\overline{X} \pm \mathrm{SE} = 0.496 \pm 0.213$) versus eggs laid last ($\overline{X} \pm \mathrm{SE} = 0.316 \pm 0.162$) in all nesting bouts revealed a significantly higher ejection rate for those laid first (Mann–Whitney U test: $U = 866$, $N_1 = 28$, $N_2 = 40$, $P < 0.001$).

To assess the probability of ejection and nutrient investment levels in communal clutches of different sizes, we divided them into categories: A = very small (1–3 eggs), B = small (4–6 eggs), C = average (7–9 eggs), D = large (10–12 eggs) and E = very large (>12 eggs). These categories differed significantly in their mean probabilities of egg ejection (Fig. 2; Kruskal–Wallis test: $H_4 = 11.903$, $P = 0.02$). This difference was due to the lower ejection probability associated with eggs from very small clutches, in comparison with other categories (Kruskal–Wallis one-way analyses of variance conducted for all category combinations, with the only significant difference being between A versus any other category, and no significant differences between other categories).
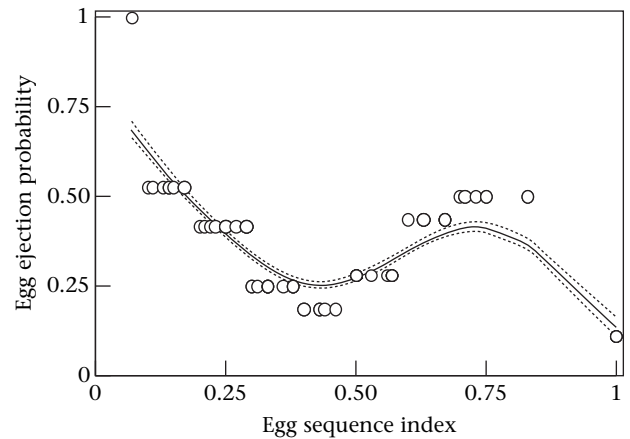

Figure 1. The relationship between egg ejection probability and egg sequence index. The curve shown is fitted to the cubic spline data points (solid line) and ± 1 SE from 1000 bootstrapped replicates (dashed lines). An open circle represents the egg ejection probability according to the position of the egg in the communal clutch.

Social Circumstances and Egg Nutrients

Two social factors that possibly affect female nutrient investment in eggs are (1) the level of egg-ejection pressure within their group and (2) communal clutch size, which is highly correlated with the number of laying females in the nesting bout (Pearson correlation: $r_{14} = 0.98$, $P < 0.001$; Cariello et al. 2002). This factor should naturally increase the genetic diversity within the brood and also the number of hatchlings, which can lead to increased levels of competition among nestlings. Because we found that the position of an egg in the laying sequence was associated with its probability of ejection, we first examined whether there was a correlation with nutrient investment. However, no such relationship was found between the position of eggs in the laying sequence and any of the investment measures (Spearman rank correlations: all NS). Similarly, eggs laid first and eggs laid last showed no statistical differences in nutrient
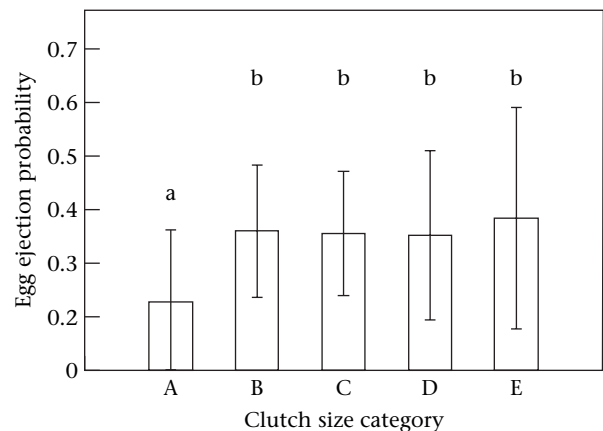

Figure 2. Distribution of egg ejection probability ($\pm$ SE) among clutch-size categories (A–E; see text for details). Clutch-size categories having significant differences ($P < 0.05$) in mean ejection probability are indicated by different letters above the bars.

investment (t tests for all nutrient variables; all NS). In a related line of inquiry, we asked whether eggs ejected from the nest differed in content from those retained. We found that ejected eggs had significantly smaller yolks ($t_{120} = 1.98$, $P = 0.05$) and heavier shells ($t_{142} = -2.349$, $P = 0.02$) that were also thicker ($t_{147} = -2.131$, $P = 0.04$; Fig. 3). The other investment measures did not differ between ejected and retained eggs.

With the exception of yolk, all nutrient measures were intercorrelated (Pearson correlation: $P < 0.001$). Yolk, however, was an exception, and was unrelated to the amount of albumin ($r_{155} = 0.00$, $P = 0.58$), to shell weight ($r_{160} = 0.20$, $P = 0.55$) and to shell thickness ($r_{159} = 0.17$, $P = 0.74$). With the exception of yolk ($r_{160} = 0.00$, $P = 0.94$), all nutrient measures showed a positive and significant correlation with increasing communal clutch sizes (Fig. 4; using raw data values and not clutch-size category means: mass: $r_{178} = 0.35$, $P < 0.001$; volume: $r_{187} = 0.32$, $P < 0.001$; albumin: $r_{155} = 0.33$, $P < 0.001$; shell weight: $r_{183} = 0.36$, $P < 0.001$; shell thickness: $r_{189} = 0.27$, $P < 0.001$). A statistical interpretation of these results is that, although the small correlation coefficients indicate weak associations, they are still statistically significant and indicative
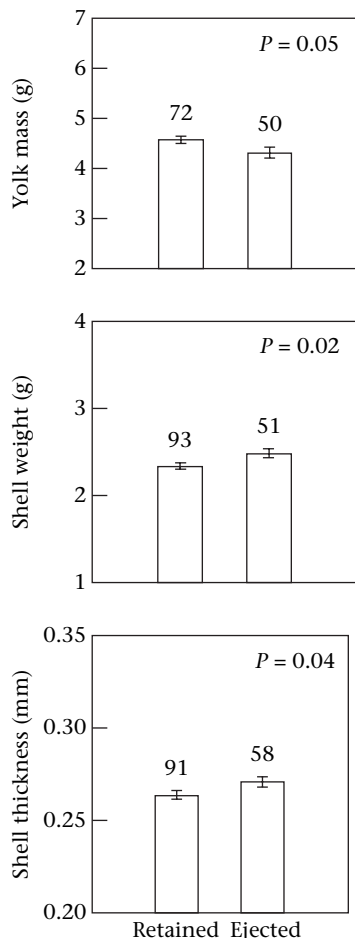
of a genuine pattern due to the large sample sizes used, and are unlikely to have occurred due to spurious correlations resulting from chance sampling errors (Fowler & Cohen 1990). Thus, in groups with more females, and therefore, larger communal clutches, individuals lay eggs that are, on average, larger in almost all nutritional aspects, with the exception of yolk.

Individual Female Clutches and Nutrient Investment

For these analyses, we used the maternal identifications provided by the yolk protein electrophoresis protocol. We pooled communal clutches of all sizes and, to maximize chances of detecting opposing trends, we compared only females that were first to enter the egg laying sequence of their groups with females that were last. First-laying females averaged slightly larger individual clutches than did last-laying females, and also suffered a slightly higher rate of ejection of their eggs, although these differences were not significant (Table 1). However, these measures of the average individual clutch size for such females in each nesting bout, together with average egg values, allowed an estimate of total nutrient investment of a female in her clutch in the two categories (first versus last; Table 2). Females that laid first in their groups showed lower investments per egg for all nutrient measures, including significantly lower investments for total mass, volume and yolk mass. When the mean clutch sizes for first and last females (from Table 1) are multiplied by the average egg nutrient values, the total clutch investment estimate is obtained. For all nutrient measures, this total clutch value was higher, although not significantly, for first-laying females. Thus, first-laying females laid significantly smaller eggs with less yolk than did last-laying females. But because first-laying females laid slightly larger clutches, the total clutch investments in nutrients of first- and last-laying females were not statistically distinguishable.

Egg Mass, Hatchling Size and Chick Survival

Larger eggs produced heavier chicks at hatching, with an increment of 1 cm^3 in the egg volume resulting in an increase of 0.35 g in the mass of the chick (Fig. 5). Except for wing length, morphometric measures of chicks were not correlated with egg volume (tarsus: $r_{38} = 0.22$, $P = 0.12$; bill: $r_{38} = 0.30$, $P = 0.40$; wing: $r_{38} = 0.35$, $P = 0.02$). A comparison of chicks that died due to infanticide (either directly observed or inferred from strong circumstantial evidence; for descriptions of infanticide and types of evidence see Macedo & Melo 1999) with those that eventually fledged showed that, at hatching, the latter were significantly heavier and also larger in two of the three size characters measured (Table 3). Additionally, hatching order influenced chick survival: chicks in the same nest that hatched first versus second had greater chances of survival until the 10th day (Fisher's exact test: $N = 33$, $P = 0.04$).
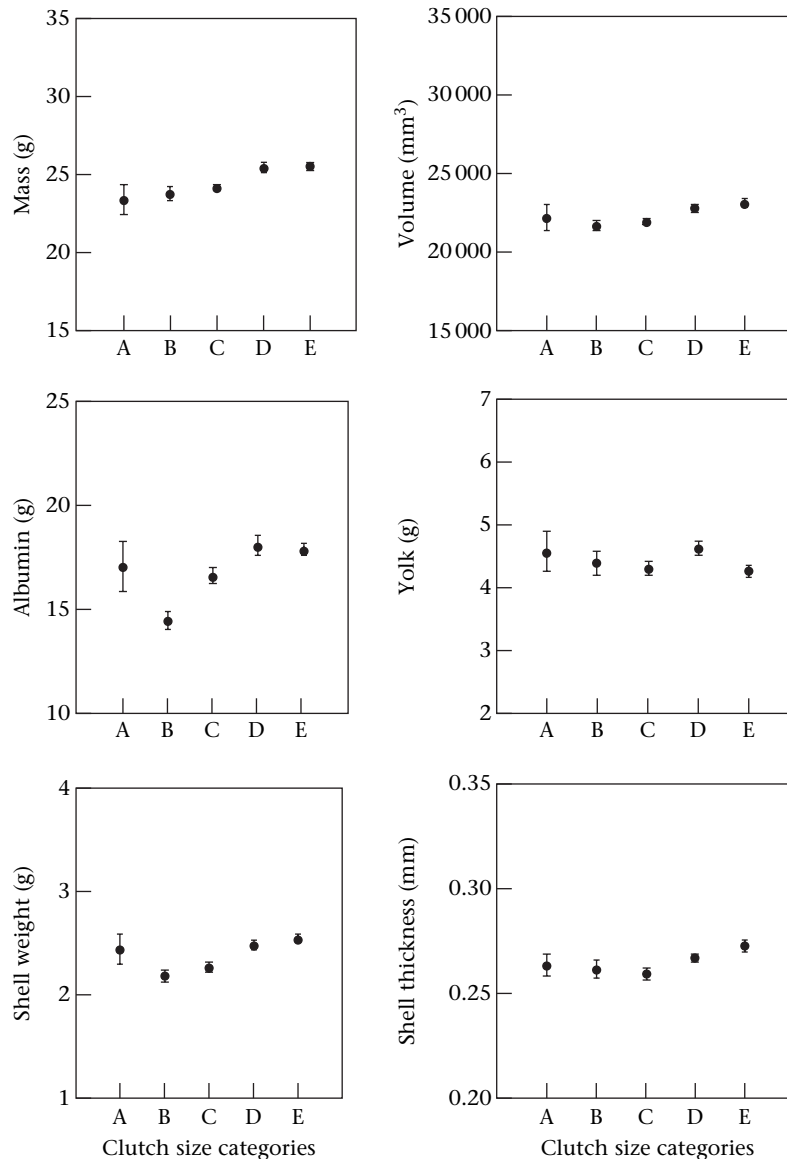

Figure 3. Mean ± SE egg nutrient measures that differed in ejected and retained eggs. Numbers above bars are sample sizes.

Figure 4. Egg nutrient investment measures (±SE) relative to different-sized communal clutch categories: A = very small (1–3 eggs), B = small (4–6 eggs), C = average (7–9 eggs), D = large (10–12 eggs) and E = very large (>12 eggs).

DISCUSSION

In this study, we examined three social configurations reflecting varying levels of risk of egg loss. First, there was the possibility that the position of the egg in a communal clutch could be associated with a certain probability of

ejection. Second, larger communal clutch sizes, produced by larger numbers of females in a group, most likely signify intense levels of competition between chicks due to limitations of food and space. And finally, the position occupied by a female in entering the laying sequence of her group could potentially influence egg loss. Given these circumstances, we investigated how female guira cuckoos invest in their eggs, and whether differential egg investment may have an impact upon chick size and, possibly, survival.

We found that eggs laid early in the sequence of laying had the highest chances of ejection, independent of clutch size. There was also a high probability of ejection associated with eggs laid towards the end of communal clutches, although well below that of first eggs. An egg laid first had approximately a 50% chance of being ejected, and an egg laid last, about 32%. Thus, the position of any

Table 1. Clutch characteristics for female guira cuckoos that entered the laying sequence first and last for all clutch sizes

Female order	Eggs laid (±SD; N)	Eggs ejected (±SD; N)
First	2.11 (±1.02; 18)	1.57 (±0.94; 14)
Last	1.80 (±1.08; 15)	1.29 (±0.49; 7)
Mann–Whitney U	163.0	54.5
P	0.28	0.62

Table 2. Comparison (*t* test) between mean investment per egg and per clutch between first- and last-laying guira cuckoo females

Nutrient	First female*		Last female†		P	
	Egg ($\pm$SD)	Clutch ($\pm$SD)	Egg ($\pm$SD)	Clutch ($\pm$SD)	Egg	Clutch
Mass (g)	23.10 ($\pm$2.03)	57.75 ($\pm$18.67)	24.63 ($\pm$2.78)	49.06 ($\pm$32.45)	0.04	0.47
Volume (mm³)	21 176.32 ($\pm$1649.74)	52 940.79 ($\pm$16 605.05)	22 546.22 ($\pm$2387.19)	44 898.65 ($\pm$29 648.82)	0.03	0.46
Yolk (g)	4.10 ($\pm$0.63)	10.24 ($\pm$2.61)	4.70 ($\pm$0.71)	9.44 ($\pm$5.36)	0.01	0.67
Albumin (g)	15.78 ($\pm$1.80)	39.437 ($\pm$13.81)	16.91 ($\pm$2.79)	33.47 ($\pm$23.56)	0.12	0.50
Shell weight (g)	2.33 ($\pm$0.25)	5.81 ($\pm$2.02)	2.41 ($\pm$0.32)	4.84 ($\pm$3.16)	0.31	0.42
Shell thickness (mm)	0.26 ($\pm$0.01)	0.65 ($\pm$0.22)	0.27 ($\pm$0.02)	0.53 ($\pm$0.34)	0.12	0.37

*$N = 20$ for eggs and $N = 8$ for clutches.
†$N = 23$ for eggs except for albumin and shell thickness, where $N = 22$; $N = 11$ for clutches.

individual egg within the laying sequence is associated with differential ejection risks.

Another risk factor we examined concerned the size of the communal clutch and its possible association with the probability of ejection. In this case, eggs from very small communal clutches (1–3 eggs) suffered little risk of ejection, whereas eggs belonging to all other categories of larger clutch sizes had uniformly higher risks. This seems to be a logical result, given that a completed communal clutch of one to three eggs belongs to one or two females only. Ejection levels become higher as more females join the laying bout and compete for nest space for their eggs and, eventually, survival of their chicks in what can be crowded conditions.

Given the reported levels of ejection risk and the potential for energetic losses, our expectations were that females might adjust nutrient investment accordingly, by lowering investment when eggs had high probabilities of being destroyed. We found that, contrary to our first set of expectations, there was no adjustment of nutrient allocation to eggs according to their position in the laying sequence of communal clutches, even when comparing the very first with the very last laid eggs in all clutches. But a comparison of eggs that were in fact ejected, whatever their positions in the laying sequence, with those that were retained in the nest showed clear differences in terms

of nutrient investment. Eggs retained in the nest had larger yolks compared with those that were ejected, and the latter had more robust shells. These findings indicate that females possibly have some clues concerning the ultimate fate of their eggs. It is not likely that egg discrimination is based upon egg shape or eggshell speckling patterns, given the variable shapes and patterns produced by individual females (Cariello et al. 2004) and also the high acceptance of dummy eggs. Females may possibly use a combination of factors, such as their own position in the laying sequence, together with the relative position of an egg in the communal clutch, to gauge the risk of losing an egg.

Investment in yolk is possibly the most energetically costly part of egg production for females, and yolk formation also takes several days, compared with the single day to add albumen and shell after ovulation (Burley & Vadhera 1989). Conversely, the high water content of the albumen (Sotherland & Rahn 1987) should render investment in this component relatively cheap compared with yolk. Females laying eggs that have a high probability of ejection may skimp on the yolk content. Skimping on yolk in these cases may be possible with little cost in fitness to females because yolk content may not be the most important egg component affecting hatchling size (Finkler et al. 1998) and, possibly, its subsequent survival.

The significance of more robust shells in eggs that were ejected is more puzzling. Hardier shells may withstand more manipulation without breaking. Guira cuckoos eject eggs by rolling them out of the nest with their bills or by picking them up using their bills as pincers, and dropping them outside the nest. Occasionally, instead of ejecting eggs, individuals bury them with leaves, as do anis, *Crotophaga* spp. (Vehrencamp 1977; Loflin 1983), and effectively prevent their incubation. But frequently, eggs are covered and later uncovered. Thus, eggs targeted for ejection may be substantially manipulated, and sturdier shells could result in the ultimate survival of the egg, should ejection/burial not be successfully performed.

Females potentially can use different reproductive tactics, either by producing eggs of constant size (i.e. fixed at large or small size), or by varying egg size according to external social and environmental conditions. This creates a potential for a trade-off between clutch and egg sizes for species where clutch size is variable (Flint & Grand 1996); for example, females laying a large clutch could do so by
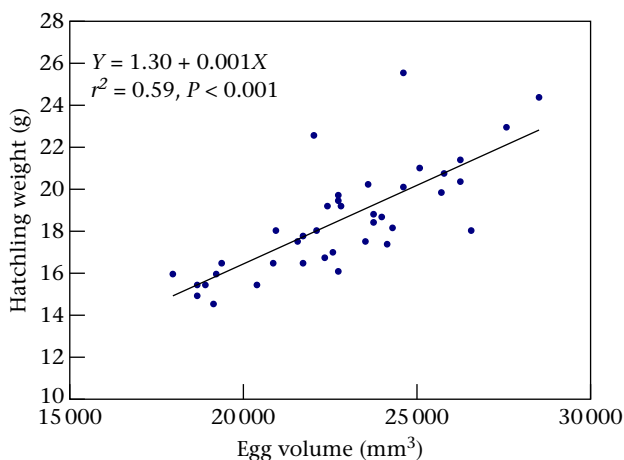

Figure 5. The relationship between egg size and hatchling body mass of guira cuckoo chicks ($N = 40$).

$Y = 1.30 + 0.001X$
$r^2 = 0.59, P < 0.001$

Table 3. Comparison of weight and morphometric characteristics at hatching of guira cuckoo chicks that fledged or died through infanticide

Chick destiny	Mean±SD (N)			
	Mass (g)	Tarsus (mm)	Bill length (mm)	Wing length (mm)
Fledged	25.70±12.23 (15)	18.98±5.12 (16)	12.47±1.38 (16)	22.00±1.27 (12)
Infanticide	18.47±3.12 (14)	15.73±0.89 (12)	11.53±0.63 (12)	24.08±4.87 (16)
t	−2.15	−2.17	−2.19	−1.43
df	27	26	26	26
P	0.04	0.04	0.04	0.16

producing smaller eggs, on average, than females laying a small clutch.

We found that females that entered the laying sequence in the very first position, compared with those that entered last, tended to lay more eggs per individual clutch and also suffered a slight tendency to have more of their eggs ejected. The eggs produced by first-laying females had significantly less mass, volume and yolk. This tendency was repeated for the other nutrient measures, although no statistically significant differences were found. First-laying females thus appear to decrease energy investment on a per-egg basis. However, their total investment on a per-clutch basis was equivalent to that of last-laying females. Considering that first-laying females suffered higher ejection losses because they initiated the laying sequence, this tactic may decrease energetic losses by distributing overall investment over a larger number of eggs laid over a longer period. This longer laying period could extend into the stage of nesting when the chances of ejection are much lower. In another communal breeder, the acorn woodpecker, *Melanerpes formicivorus*, joint-nesting females frequently lay runt eggs as the first egg in their clutches. Koenig et al. (1995) proposed, as one possible explanation, that runt eggs could minimize the energetic losses due to egg destruction. However, their data failed to support this hypothesis. Instead, they suggested that this strategy allows a female that lays a first runt egg to destroy more normal eggs laid simultaneously by her cobreeders. Egg burying, a competitive outcome of joint-nesting groove-billed and smooth-billed anis, *C. sulcirostris* and *C. ani*, respectively (Davis 1942; Sick 2001), is inflicted overwhelmingly on early-laid eggs in the communal clutch (Vehrencamp 1977; Loflin 1983). As in guira cuckoos, early-laying groove-billed females laid more eggs than last-laying females, and this appears to be a strategy to minimize the effects of egg ejection/burial on the size of the final clutch that is incubated.

If size at hatching is important in competitive abilities of nestlings to secure food, or to escape infanticide, females should enhance investment in the albumen content of the egg. Increasing albumen in eggs may be relatively less costly than increasing yolk content, and also of greater value in producing larger hatchlings. We found significant differences relative to the investment in eggs between different-sized clutches for all nutrient measures except yolk. Thus, most of the increases in mass and volume can be ascribed to greater quantities of albumen, because yolk content did not vary between clutch-size categories. In general, smaller communal clutches had the

lowest investment rates in eggs. A possible interpretation of this result is that in large communal clutches, where the offspring of many females hatch, it might be important for eggs to be of higher quality. Larger eggs in this species, as verified for other birds, do indeed yield heavier chicks. Despite the small sample size in this analysis, as we only used chicks that we actually saw being killed or in cases that presented overwhelming evidences of infanticide, our results are consistent with the above assumption: chicks victimized by infanticide were lighter and smaller at hatching than those that survived.

Most chick mortality is caused by infanticide, which is practiced typically during the first 5 days posthatch (Macedo & Melo 1999). We speculate that heavier chicks hatching from high investment eggs may have better competitive abilities (e.g. begging and grasping of prey items brought by adults) that allow faster growth. This could not only shorten their period of vulnerability to infanticide, but also make it harder for an infanticidal adult to handle and/or carry them from the nest. Similarly, chicks that hatched on the first day had greater chances of survival. Again, being initially larger than nestmates could result in faster growth and may give a slight advantage to a chick when an adult is intending infanticide. In several studies, egg size has been shown to have a positive effect upon chick size in the early developmental phase (Amundsen & Stokland 1990; Reid & Boersma 1990; Magrath 1992; Smith et al. 1995), whereas a strong parental effect occurs in the later development of the nestling. Thus, it seems that both egg size and parental quality affect nestling growth in different degrees and at distinct times. In the case of guira cuckoos, posthatch effects of parental investment upon chick survival would not cause differential growth among nestlings in a communal brood, because all chicks in the nest are subjected to the same 'parental' conditions, and there is no discriminative feeding by adults among nestlings (Macedo 1994).

The large variation in egg size in this species suggests that females can manipulate reproductive investment, and that this may enhance individual fitness. Our results are consistent with the expectations of how females should invest, given certain social conditions. We found that in groups where more reproductive females produce larger communal clutches, eggs are on average larger, due to a higher amount of albumen, not yolk. This is in agreement with recent studies showing that albumen strongly influences hatchling size, which in our study had important consequences for chick survival. We also found that ejected eggs had smaller yolks; yolk is a costly

component of eggs, but one that may not severely affect hatchling size. Finally, there appear to be differences in egg nutrient investment tactics depending on the female's position in the laying sequence. Although further studies are needed to allow conclusive generalizations, our results suggest a large degree of flexibility of females in modulating their investments in eggs according to subtle variations in the social environment.

Acknowledgments

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