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Nutritional quality of prebreeding diet influences breeding performance of the Florida scrub-jay

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Abstract Food supplementation studies of breeding birds have traditionally concentrated on energetic constraints on breeding performance. It is only recently that the nutritional quality of the prebreeding diet has also been considered influential. We examined the importance of specific nutrients in the prebreeding diet of the Florida scrub-jay (*Aphelocoma coerulescens*). Birds were provided with one of two supplements (rich in protein and fat or rich in fat only) prior to breeding in 2000 and 2001 and their breeding performance, in relation to unsupplemented (control) birds, was examined. Birds receiving both supplements significantly advanced laying in both years, and increased clutch size in 2000 but not in 2001. Laying date explained variation in clutch size in birds on dietary supplements. Egg mass and volume declined with laying order, irrespective of dietary treatment, but birds on the high fat, high protein diet laid heavier third eggs than controls and this was independent of laying date. Laboratory analysis of 14 abandoned and unhatched eggs revealed that as egg mass increased so did the absolute amount of protein and water while fat content remained relatively fixed. Using these relationships between the masses of egg components and fresh egg mass, we calculated that heavier third eggs laid by birds on high fat and high protein, compared with those laid by controls, contained more water that may be fundamental to chick growth and survival. This is the first demonstration for an avian species that nutritional quality of prebreeding diet can simultaneously influence laying date, clutch size, and egg size and composition.

Keywords *Aphelocoma coerulescens* · Egg size · Energy · Nutrients · Reproduction

Introduction

Reproduction in organisms is a nutrient-demanding process and most animals accumulate endogenous nutrient reserves in preparation for the production of offspring. In birds, food availability during the prelaying period can have a marked influence on egg production (review in Martin 1987). Supplemental food often advances the date of laying (e.g. Perrins and McCleery 1989) and less frequently increases clutch size (reviews in Arcese and Smith 1988; Boutin 1989) compared with individuals in the population that do not have access to supplemental food. Most food supplementation studies have fed nutritionally complete diets (i.e. include macronutrients – proteins, fats, carbohydrate, water - and micronutrients – essential amino acids, vitamins and ions) to birds and have focused on the absolute energetic content of food. Few studies have examined the relative importance of different macronutrients (by definition the major dietary components; Selman and Houston 1996) and those that have, have produced equivocal results. Bolton et al. (1992) provided lesser black-backed gulls (*Larus fuscus*) with two different foods (animal fat and fish) that were of equivalent energy content (i.e. isocaloric) and found that clutch size was increased by elevated protein only when natural food availability was low. Nager et al. (1997) provided great tits (*Parus major*) with foods of two different protein contents, but neither treatment influenced time of laying, clutch size or egg size, and supplemented tits bred no more successfully than unsupplemented ones. Ramsay and Houston (1997) supplemented blue tits (*Parus caeruleus*) with animal fat or cooked egg and found that both diets advanced the date of laying, but did not increase clutch size compared with

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Table 1 Nutrient and caloric composition of the two diets used to food-supplement Florida scrub-jays (*Aphelocoma coerulescens*) in 2000 and 2001

	High fat, high protein		High fat, low protein	
	mg g^{-1} food	kcal g^{-1} food	mg g^{-1} food	kcal g^{-1} food
Fat	197.0	1.78	197.5	1.78
Protein	323.5	1.30	33.0	0.14
Carbohydrate	347.0	1.39	650.5	2.61
Total calories (kcal g^{-1} food)	4.47		4.53	

unsupplemented birds. The timing and duration of food supplementation in relation to the reproductive cycle also is important if manipulations of diet quality are to have detectable effects on measures of breeding performance and these have varied greatly among studies. Relatively few studies have supplemented birds over a protracted period prior to the onset of egg-laying.

In addition to affecting the date of laying and the number of eggs that are laid, food availability also can affect egg size and quality. Ramsay and Houston (1997) found that egg volume was larger in blue tits fed with cooked eggs compared with those laid by unsupplemented birds. Furthermore, zebra finches (*Taeniopygia guttata*) fed a high quality diet (24% protein) briefly before pairing laid heavier eggs than birds on a low quality diet (12.9% protein; Selman and Houston 1996). Larger eggs may increase survival of offspring (e.g. Nisbet 1978; Bolton 1991; Magrath 1992, Blomqvist et al. 1997; Amat et al. 2001; but see Williams 1994). Because non-destructive techniques for the measurement of egg composition have only recently been developed (e.g. Sinervo and Huey 1990), few studies have examined the effects of variation in egg composition on chick growth and survival. Nisbet (1978) suggested that the protein component of the albumen may be crucial for embryonic tissue development. In contrast, Parsons (1970) suggested that yolk reserves may be fundamental to long-term survival of chicks while others have suggested that water content of the egg is the primary determinant of hatchling mass (e.g. Tullett and Burton 1982).

In the present study, we provided Florida scrub-jays with food supplements by establishing feeders centrally within known-established territories (Schoech 1996) 6–8 weeks prior to breeding, sufficiently early in the year to influence the timing of laying (see above). Furthermore, we provided birds with isocaloric dietary supplements rich in either fat or in fat and protein to test the importance of nutrient, as opposed to energetic, content of prebreeding diet to their breeding performance. We examined the effects of dietary supplementation on laying date, clutch size and the morphometric properties of eggs. We also estimated egg composition from fresh egg mass. The Florida scrub-jay is particularly well-suited to this experimental approach. Birds regularly visit feeders with minimal training, they take supplemental foods readily and, perhaps most importantly, they tolerate daily visits of experimenters to nests without abandoning nesting attempts. Florida scrub-jays have been provided with food supplements in previous studies. Schoech (1996) provisioned birds with dried dog food, peanuts and mealworms (*Tenebrio molitor*) and found that supplemented birds produced larger clutches and laid earlier than unsupplemented birds. In addition, Florida scrub-jays in suburban habitats with year-round access to anthropogenic food lay earlier and larger clutches than jays in natural habitats without access to human foods (Bowman et al., submitted). Based on these findings, we supplemented birds with different isocaloric diets of known nutritional content and compared their reproductive output with that of unsupplemented birds.

Materials and methods

Study population and general methods

We studied a population of Florida scrub-jays at Archbold Biological Station, Highlands County, Florida (27°10'N, 81°21'W, elevation 38–68 m) (for more details on this site see Schoech et al. 1991; Mumme 1992; Schoech et al. 1996). This population is adjacent to one studied by Woolfenden and colleagues for over 30 years (Woolfenden and Fitzpatrick 1977, 1984, 1990). All birds were ringed with unique colour combinations to facilitate individual identification. Sex, age, breeding status and group membership were known for each bird (for methods see Schoech et al. 1996 and references therein). Initiation of nest-building for each group of birds was determined from direct observation of the breeding pair and territories were searched systematically for nests. Nest locations were marked with coloured flagging tape. Nests were monitored throughout the nesting period.

Food supplementation

In 2000 and 2001, birds were provided with food supplements (Table 1) from the middle of January and supplementation continued at each territory until the breeding female had started laying. In 2000, territories were randomly assigned to either supplemented groups or a control (unsupplemented) group. Territories in the former group received one of two supplemental diets, either a high fat, high protein diet (HFHP) or a high fat, low protein diet (HFLP). The food supplements were isocaloric (Table 1). In 2001, territories were again randomly assigned a dietary treatment but treatments were reassigned if territories were allocated the same diet as the previous year. Supplemental foods were cylindrical pellets (10 mm long × 5 mm diameter) dispensed from bird feeders (The Absolute Squirrel Proof Feeder model HA-7526) placed on poles at the centre of each breeding territory. Feeders were mounted on poles to prevent access by terrestrial vertebrates, e.g. raccoons (*Procyon lotor*), and the arm on which birds perch while feeding was calibrated to prevent birds above 100 g in body mass from gaining access to food pellets. Florida scrub-jays are virtually always lighter than 100 g (Schoech 1996; Woolfenden and Fitzpatrick 1996). Feeders were replenished every 2 days with two handfuls (ca. 100 g) of pellets and, therefore, birds were provided with ad libitum food. We observed that birds fed exclusively at the feeder in their territory and excluded birds from neighbouring territories. The following numbers of breeding females were supplemented in 2000 and 2001, respectively: HFHP, 12 and 9; HFLP, 12 and 9; controls, 32 and 23.

Egg size

After birds had lined nests with fibres, clutch initiation was determined by checking nests daily at midday. Each egg was marked on the day that it was laid with small dots of indelible ink placed on its blunt end. The number of dots applied to each egg corresponded to the position of the egg in the laying order. Since the modal clutch size of the Florida scrub-jay is three (Woolfenden and Fitzpatrick 1996), all eggs in the clutch were measured on the third day of egg-laying when most birds had completed their clutches. Nests were checked daily until laying ceased and subsequent eggs in larger clutches were marked and measured on the day that they were laid. Eggs were weighed (to 0.05 g) with a 10-g Pesola spring balance and length and width were measured (to 0.05 mm) with calipers. Egg volume was determined from the following equation of Hoyt (1979):

$$\text{egg volume}\ \left(\text{cm}^3\right) = 0.51 \times \text{egg length (cm)} \times \text{egg width}^2\ \left(\text{cm}^2\right) \tag{1}$$

Egg composition

Ideally, we would have collected freshly laid eggs for egg composition analysis but the Florida scrub-jay is a threatened species. Thus, we collected only eggs that remained in abandoned nests or eggs that had failed to hatch. Nests were considered abandoned by the absence of birds in the vicinity of the nest, by a lack of nest sanitation determined by placing leaves in the nest, and by cold, unincubated eggs. We usually collected eggs within 1–2 days post-abandonment. Most eggs within a clutch hatch on the same day (Woolfenden and Fitzpatrick 1996) but we waited at least 48 h after the first egg hatched prior to collecting unhatched eggs. Eggs were immediately wrapped tightly in Parafilm and frozen until laboratory processing. In total, we collected and processed 14 Florida scrub-jay eggs. None of the eggs showed any signs of development nor were they addled when they were examined in the laboratory. They were weighed and then broken in self-contained extraction filters in which they dried at 50°C for 4 days to constant mass. Each egg was then reweighed and mass lost during this period represented the water component of the egg. Lipids were extracted with petroleum ether in a Soxhlet apparatus for 48 h. Each egg was then re-dried for 6 h, allowed to cool to room temperature and re-weighed to give a lipid-free mass. The mass of the lipids extracted from the egg was calculated by subtracting the post-extraction mass from the pre-extraction mass. The post-extraction mass of each egg represented the combined masses of carbohydrate, mineral and protein fractions, hereafter referred to as the 'protein' fraction since protein makes up the majority of the remaining fraction of the egg contents once water and lipid are removed (Romanoff and Romanoff 1949).

Statistical analyses

All statistical tests were performed using SAS (SAS Institute 1999). Only first nesting attempts were considered. Laying dates were analysed using two-way ANOVA (PROC GLM; SAS Institute 1999) with dietary treatment (unsupplemented, HFHP and HFLP) and year (2000 and 2001) as the two factors. We further examined inter-group differences in laying date by calculating coefficients of variation and by testing for the homogeneity of variance within years using the Scheffé-Box test (Sokal and Rohlf 1995). Sample sizes differ between analyses of laying dates and clutch sizes because at some nests eggs were depredated prior to clutch completion.

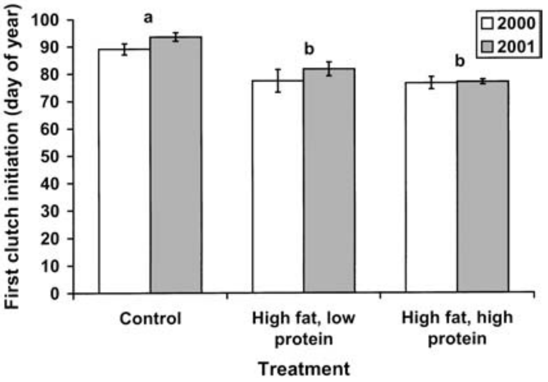

Fig. 1 Means and standard errors of first clutch initiation dates (1 January = day 1) of supplemented and unsupplemented (control) Florida scrub-jays (*Aphelocoma coerulescens*) nesting in two successive years. Data from 2000 and 2001 were pooled (see text). Significant differences across years among treatment groups are indicated by different *lowercase letters*

To test for differences in clutch size at different times of the breeding season, we used analysis of covariance (ANCOVA; PROC GLM; SAS Institute 1999) with dietary treatment and year as factors and laying date as the covariate. Egg size data were compared for females laying three or more eggs. Because relatively few clutches contained more than three eggs, data were analysed using a separate repeated-measures ANOVA (PROC GLM; SAS Institute 1999) for each size measure of the first three eggs laid in a clutch. We were interested in how egg size varied between successive eggs of a clutch and we, therefore, used polynomial analysis (von Ende 2001; also referred to as trend analysis, Winer et al. 1991) to construct independent contrasts between eggs in clutches and to detect significant first-order (linear) or second-order (quadratic) trends in data. In this way, predominantly quadratic responses (except where stated otherwise) of egg size across laying order were examined between dietary treatments and years. We used ANCOVA with laying date as the covariate to examine if the time of laying had a significant influence on any of the egg-size measures.

From examination of the composition of the collected eggs, we tested how reliably we could predict masses of individual egg components (water, protein and fat) from fresh egg mass. We regressed $\log_{10}$ (egg mass + 1) on $\log_{10}$ (fresh egg mass + 1) using the logarithmic transformation to reduce heteroscedasticity and adding one to all masses to avoid negative characteristics of calculated logarithmic values (Sokal and Rohlf 1995). For each of the above regressions, we examined whether the 95% confidence interval (CI) of the slope (b) of the regression line included 1.0. If this was the case, the mass of the egg component increased isometrically with fresh egg mass (i.e. eggs of different masses contained equivalent proportions of the egg component, Carey 1996). If b was significantly more or less than 1.0, the egg component comprised progressively larger or smaller proportions respectively of the fresh egg contents as egg mass increased.

Results

Laying date

Laying dates, expressed as day of year (1 January = day 1), differed significantly across dietary treatments for the 2 years of the study (two-way ANOVA, overall model, $F_{5,90}$=11.34, P=0.0001) (Fig. 1), but did not differ between years ($F_{1,90}$=2.39, P=0.13). Laying date differed

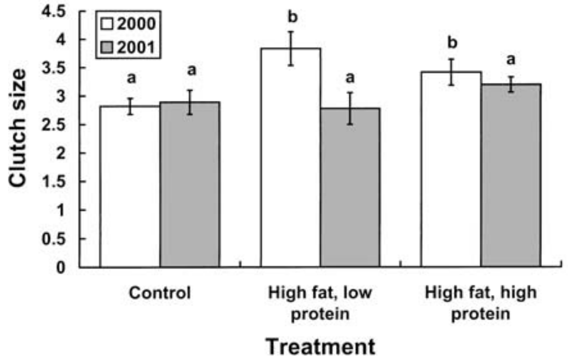

Fig. 2 Means and standard errors of first clutch sizes of supplemented and unsupplemented (control) Florida scrub-jays nesting in two successive years. Data were analysed separately for each year (see text). Significant differences within years among treatment groups are indicated by different *lowercase letters*

significantly among dietary treatments ($F_{2,90}$=26.39, P=0.0001) with birds on HFLP laying 11.7 days earlier (79.29±2.29 days, n=21) and those on HFHP laying 14.1 days earlier (76.86±1.05 days, n=21) than controls (90.94±1.25 days, n=54) (Duncan's multiple range test, P<0.05). Laying date did not differ within the two supplemented treatments (Duncan's multiple range test, P>0.05). The year × treatment interaction was not significant ($F_{2,90}$=0.41, P=0.67).

In 2000, we found that laying dates were equally spread for control birds and for those on HFLP (coefficients of variation, 9.76% and 11.44% respectively), but less spread for birds on the HFHP supplement (4.14%, Scheffé-Box test, $F_{2,11}$=9,29, P<0. 005). In contrast, in 2001, we found that laying dates were equally spread for the three dietary treatments (control; 10.15%; HFLP; 15.21%; HFHP; 8.61%; Scheffé-Box test, $F_{2,8}$=0.32, P>0.50).

Clutch size

Clutch sizes differed significantly between dietary treatments for the 2 years of the study (two-way ANOVA, overall model, $F_{5,88}$=3.35, P=0.008) (Fig. 2). Clutch size differed significantly between 2000 and 2001 ($F_{1,88}$=3.94, P=0.05) and between dietary treatments ($F_{2,88}$=3.04, P=0.05). The year × treatment interaction was significant ($F_{2,88}$=3.37, P=0.04) and, therefore, we compared clutch sizes between treatments for each year separately. Birds on both supplemental diets laid more eggs than controls in 2000 (Duncan's multiple range test, P<0.05 for both diets) but not in 2001 (Duncan's multiple range test, P>0.05 for both diets). Clutch sizes of birds on different supplemented treatments appeared to vary by year, but the differences were not significant (Duncan's multiple range test, P>0.05) (Fig. 2).

Laying date explained much of the variation in clutch size (ANCOVA, $F_{1,87}$=8.28, P=0.005) and when this effect was removed, clutch size did not differ significantly either between years ($F_{1,87}$=2.41, P=0.12) or between dietary treatments ($F_{2,87}$=0.20, P=0.82). In 2000, clutch size was significantly negatively correlated with laying date for unsupplemented birds (Pearson product-moment correlation, r_{33}=–0.52, P=0.002) and those on HFLP (r_{12}=–0.66, P=0.02) but not for birds on HFHP (r_{12}= –0.19, P=0.40). In 2001, no significant correlation existed between clutch size and laying date for birds on any dietary treatment (Pearson product-moment correlations, unsupplemented: r_{18}=–0.20, P=0.43; HFLP: r_9=0.51, P=0.16; HFHP: r_{10}=–0.11, P=0.76).

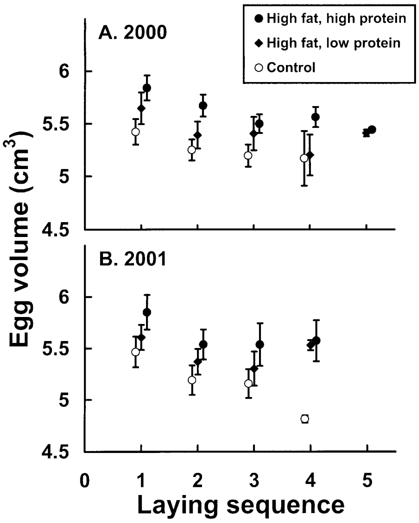

Fig. 3 Means and standard errors of egg volumes across laying order in first clutches of supplemented and unsupplemented (control) Florida scrub-jays nesting in **A** 2000 and **B** 200 1. Data are separated slightly for clarity

Egg size

Egg volume declined significantly with laying order (repeated-measures ANOVA, $F_{1,51}$=7.82, P=0.007) (Fig. 3), but did not differ significantly between dietary treatments ($F_{1,51}$=0.48, P=0.62) or between years ($F_{1,51}$=0.66, P=0.42). Egg volume was not significantly influenced by the laying date (ANCOVA, $F_{1,50}$=0.86, P=0.36).

Egg mass declined significantly with laying order (repeated-measures ANOVA, $F_{1,51}$=18.98, P=0.0001) (Fig. 4). Eggs did not vary in mass between 2000 and 2001 ($F_{1,51}$=0.00, P=0.95) and the year × treatment interaction was not significant ($F_{2,51}$=0.77, P=0.47). Egg mass varied significantly between dietary treatments for the 2 years combined ($F_{2,51}$=5.87, P=0.005). Birds on HFLP laid eggs of similar mass to those of controls, but birds on HFHP laid heavier third eggs than those laid by controls (Duncan's multiple range test, P<0.05). Laying date did not account for significant variation in egg mass (ANCOVA, $F_{1,50}$=0.39, P=0.53).

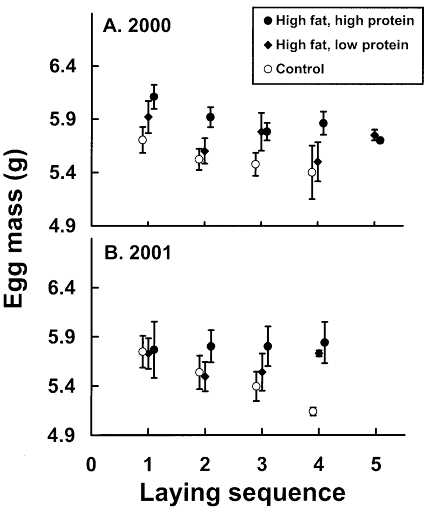

Fig. 4 Means and standard errors of egg masses across laying order in first clutches of supplemented and unsupplemented (control) Florida scrub-jays nesting in **A** 2000 and **B** 2001. Data are separated slightly for clarity

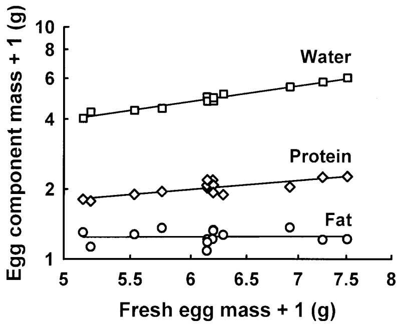

Fig. 5 The relationship of egg component mass + 1 to fresh egg mass + 1 plotted on log scales for three egg components. Results are from an analysis of composition of Florida scrub-jay eggs collected after either abandonment or failure to hatch. Linear regression equations for best-fit lines are provided in the text

Egg composition

The mass of the fat component of eggs was not significantly related to fresh egg mass (linear regression, $F_{1,12}$=0.02, R^2=0.001, P=0.90). However, fresh egg mass was significantly related to the masses of the water (linear regression, $F_{1,12}$=333.22, R^2=0.97, P<0.0001) and of the protein fractions (linear regression, $F_{1,12}$=24.02, R^2=0.67, P<0.001) (Fig. 5). For water, the 95% CI for the slope *b* included 1.0 (0.89–1.13) and, therefore, water made up an equivalent proportion of the egg contents for eggs of different masses. For protein, the 95% CI for the slope *b* did not include 1.0 (0.32–0.84) and, therefore, the protein fraction constituted a progressively smaller proportion of egg contents as egg mass increased. From these analyses, we used the following regression equations to predict the water:

$$\log_{10}[\text{water content (g)} + 1] = 1.01 \times \log_{10}[\text{egg mass (g)} + 1] - 0.12 \quad (2)$$

and protein:

$$\log_{10}[\text{protein content (g)} + 1] = 0.58 \times \log_{10}[\text{egg mass (g)} + 1] - 0.15 \quad (3)$$

contents of eggs laid by supplemented and control birds. Third eggs laid by birds receiving HFHP food supplements contained an extra 0.27 g of water (4.40±0.08 g vs 4.13±0.08 g; 75.97 vs 71.31% of fresh egg mass) and 0.06 g of protein (1.14±0.02 g vs 1.08±0.02 g; 19.68 vs 19.87% of fresh egg mass) compared with those laid by controls.

Discussion

We have shown that providing two isocaloric dietary supplements (HFLP and HFHP) to Florida scrub-jays early in the breeding season for two consecutive years resulted in advancement of laying, an increase in clutch size and maintenance of egg mass across laying order compared with birds that were not supplemented.

Our study does not simply add one more species to a long list of those in which food supplementation affects breeding performance (see reviews in Boutin 1989; Meijer and Drent 1999). Previously, only a single study has found simultaneous, significant positive effects of food supplementation on laying date, clutch size and egg mass (see Table 3 in Christians 2002). Compared with unsupplemented control birds, Högstedt (1981) found that magpies (*Pica pica*) supplemented with fish prior to breeding laid 3.5 days earlier (P=0.04) and produced 0.62 more eggs (*P*C0.06) that were 0.33 g heavier (*P*C0.02). We found significant positive effects of food supplementation on these three reproductive parameters of Florida scrub-jays, albeit for only 1 of the 2 years for clutch size. We believe that our conclusive results were obtained because of the protracted availability of supplements prior to the onset of laying, and because of the frequent and exclusive use of feeders by resident birds on supplemented territories.

Advancement of laying

Our finding that food supplementation markedly advanced laying supports the hypothesis that resource availability plays a key role in a female's decision of when to breed. This concurs with a food-supplementation study in 1993 when Florida scrub-jays laid 16 days earlier than unsupplemented jays (Schoech 1996) and this occurred in a year when the breeding season was exceptionally early. Timing of laying can be influenced by a number of endogenous (e.g. genetics, female body condition) and exogenous (e.g. photoperiod, ambient temperature) factors (see Ramsay and Houston 1997 and references therein), but food availability may be one of the most important influences on the timing of breeding. This is supported by the widespread advancement in laying date for the many studies that provide supplemental food during the prelaying period (see Table 2 in Meijer and Drent 1999), regardless of the relative quality, specific timing of supplementation or duration for which food was provided. Supplemental food may act at the proximate level by providing extra energy that augments endogenous reserves and, therefore, improves prebreeding condition. Furthermore, supplemental food may contain micronutrients (e.g. calcium; Reynolds and Perrins 2003) and macronutrients (e.g. protein; Houston et al. 1995) required for egg formation that may, at times, be limited in natural habitats. In addition, food may function at the ultimate level, acting as a cue for the breeding female, stimulating her to breed when she perceives food to be plentiful (but see Grieco et al. 2002), and thereby maximizing the likelihood that young are present when resources are abundant (the 'cue hypothesis'; Källander and Karlsson 1993).

In our study, birds were supplemented at least 7 weeks prior to the onset of breeding and timing of laying was advanced to a greater extent than for many other species that have been supplemented. For most supplemented species timing of laying has been advanced only by a maximum of 8 days (Yom-Tov 1974; von Brömsson and Jansson 1980; Poole 1985; Hochachka and Boag 1987; Clamens and Isenmann 1989; Hörnfeldt and Eklund 1990; Källander and Karlsson 1993; Nilsson and Svensson 1993; Arnold 1994; Soler and Soler 1996). In only a few studies has laying been advanced to the extent of our work (Smith et al. 1980; Ewald and Rohwer 1982; Davies and Lundberg 1985; Meijer et al. 1988; Kelly and van Horne 1997).

The duration for which supplemental foods are available before breeding may determine the extent of advancement of laying in birds. Meijer and Drent (1999) reviewed food supplementation studies and found that of five food-supplemented studies that did not show a significant advancement in laying, three provided supplemental food for only 2 weeks. In these cases, supplemental food may have had minimal effect on the timing of nest-building and clutch initiation. The remaining studies were conducted on colonial breeding gulls, the lesser black-backed gull and the glaucous-winged gull (*Larus glaucescens*), and synchronization of breeding activities appears to override the effects of supplemental food on timing of laying in colony members.

Nutritional quality of supplementary food also might influence the degree to which laying is advanced. Two of our findings offer some support for this expectation: first, when data are combined for both years, jays on HFHP tended to lay earlier than birds on HFLP (14.1 vs 11. 7 days advancement over controls); second, in 2000, jays supplemented with the HFHP showed little variation in clutch initiation date (see Fig. 1 and coefficient of variation data above). In contrast, laying date was more variable in unsupplemented birds and those on HFLP. This trend in reduced variation in laying date of birds on HFHP did not hold for 2001; however, inter-year variation in any number of environmental variables might explain the between-year lack of consistency in the dietary treatment effect (see discussion below).

Increased clutch size

Our overall finding that food-supplemented Florida scrub-jays laid larger clutches than unsupplemented controls agrees with previous research on this species (Schoech 1996; Bowman et al. submitted). Relatively few of the many food supplementation studies on birds have documented a significant increase in clutch size (Arcese and Smith 1988; Carlson 1989; Hörnfeldt and Eklund 1990; Hiom et al. 1991; Nilsson 1991; Arnold 1994; Soler and Soler 1996) and fewer still have found a significant increase in clutch size with an accompanying significant advancement in the timing of laying (Arcese and Smith 1988; Hörnfeldt and Eklund 1990; Arnold 1994; Soler and Soler 1996). Given the wide-spread across-species trend for clutch size to decline with laying date (see Daan et al. 1988), including in the Florida scrub-jay (Schoech 1996; Bowman et al., submitted), it is likely that the degree of advancement in the timing of laying that resulted from the prolonged period that supplemental food was available in this and the previous study (Schoech 1996), rather than some unique quality of Florida scrub-jays, explains our findings.

Clutch size increased in 2000 in supplemented birds, but not in 2001. However, the degree of advancement of laying date was similar in both years (Fig. 1). Although we lack data, we suspect that female scrub-jays may have been in better prebreeding condition in 2000 than in 2001 because of differences in rainfall and, potentially, in natural food availability (e.g. acorns, arthropods) between the 2 years. Low rainfall prior to the jays' breeding season may delay the emergence of insects and herpetological fauna (Pianka 1988; Wingfield and Kenagy 1991). Considerable data support the hypothesis that a shortage of food for omnivorous jays, when they are recrudescing reproductive tissues and engaged in courtship behaviour, may limit their nutrient intake and, therefore, delay breeding (Wingfield 1980, 1983; Wingfield and Kenagy 1991; Hahn et al. 1995). Schoech (1996) attributed an exceptionally late breeding season in 1992 to low rainfall (and reduced food availability) in the 3-month period (November to January) preceding the breeding season. Furthermore, Woolfenden and Fitzpatrick (1984) found that mean annual first-clutch size is highly correlated with total rainfall in the preceding summer (June to September inclusive). We examined rainfall records and found that rainfall was markedly higher during both of these periods prior to the 2000 prebreeding season compared to that of 2001 (November–January; 11.68 and 2.24 cm respectively; June–September; 100.25 and 47.52 cm respectively).

Although we found that unsupplemented birds laid earlier in 2000 than in 2001 (median lay dates; 30 March and 4 April respectively), suggesting that food availability was higher in 2000 than in 2001, this result was not statistically significant. Furthermore, with increased food availability in 2000, we would have predicted a smaller increase in clutch size and less advancement in laying in supplemented compared with unsupplemented birds in 2000 compared with 2001. While food availability may play a fundamental role in determining clutch size of scrub-jays in some years, it was not the primary determinant of clutch size of birds in our study in 2000 and 2001. The differences in clutch size between years and between dietary treatments were only marginally significant (P=0.05) and were not significant when we controlled for laying date. Therefore, we conclude that increased clutch size resulting from food supplementation is probably mediated through the advancement of laying rather than via absolute food availability per se.

Egg size and composition

Egg mass and volume tended to decline with laying order, but birds supplemented with HFHP laid significantly heavier third eggs than those laid by controls (Fig. 4). However, the mass of third eggs did not differ between the two dietary supplements. Few studies have found an increase in egg mass as a result of food supplementation (see Table 3 in Christians 2002). Heavier third eggs laid by birds on HFHP contained more water and protein (carbohydrate, mineral and protein; see methods), but not more fat than third eggs laid by control birds. Supplemented birds that laid more than three eggs maintained egg mass for fourth- and fifth-laid eggs as compared with these eggs laid by controls (Fig. 4). However, because too few birds laid clutches greater than three, we were unable to examine statistically these perceived differences. Unlike clutch size (see above), egg size (i.e. mass) was independent of laying date.

In many avian species, egg size declines with laying order (e.g. Parsons 1970; Bolton 1991; Reynolds 2001; but see Leblanc 1987). Eggs of greater mass (Nisbet 1978; Stokland and Amundsen 1988) and volume (Parsons 1970) usually result in larger hatchlings. Furthermore, egg size also influences survival of offspring, particularly in precocial species where chicks rely upon body reserves for the first few days post-hatching (Amat et al. 2001; Anderson and Alisauskas 2002). In contrast, although heavier eggs may produce larger and heavier nestlings in altricial birds, Magrath (1992) found no clear relationship between egg mass and survival of chicks to two weeks post-fledging at nutritional independence. However, we had insufficient data to test whether heavier Florida scrub-jay eggs produced larger and heavier nestlings that survived better than nestlings from lighter eggs.

Our data suggest that the additional resources available in the HFHP supplement enable females to maintain the same egg mass throughout the laying sequence. Specifically, birds on HFHP allocate more water and protein, but not fat, to third eggs and this may promote long-term survival of chicks that hatch from these eggs. Traditionally, supplementation studies have focused on the energetic content of supplemental food but, using isocaloric dietary supplements, we have shown that foods rich in both protein and fat enable Florida scrub-jays to lay heavier eggs. The effect of the HFLP supplement on egg mass (and volume) was less pronounced and not significant. Interestingly, Williams (1996) found that lipid supplementation resulted in no effect on egg size in zebra finches but birds on protein supplements laid eggs that were 10–12% larger than unsupplemented birds.

Florida scrub-jays appear to respond to food supplementation through investment in the quality, rather than the quantity, of eggs that they produce. Egg size, and therefore nutrient expenditure per egg, appears to be a more plastic reproductive response to food supplementation than is clutch size. Our results are similar to those of Clifford and Anderson (2001) who found that food-supplemented Nazca boobies (*Sula granti*) laid significantly larger second (insurance) eggs than did control birds, even in years when food availability was high. Increases in clutch size require further mobilization of nutrients (Monaghan et al. 1995) and sometimes protraction of the incubation period (Smith 1989), demands that ultimately may depress offspring fitness (see review by Monaghan and Nager 1997) and adult physiological condition (Monaghan et al. 1998). The nutritional, energetic and temporal demands of egg production are far from trivial, particularly in years of poor natural food availability, and so investment in egg quality, rather than egg quantity, may be a less costly means to invest in offspring when food is abundant.

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