Evolutionary Ecology Research, 1999, **1**: 753–768

Variation in egg mass in the Pied Flycatcher, *Ficedula hypoleuca*: An experimental test of the brood survival and brood reduction hypotheses

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ABSTRACT

Variation in egg mass is a characteristic feature among birds and varies both between and within species. It has been suggested that, in altricial birds that hatch their eggs asynchronously, two tactics regarding intra-clutch variation in egg mass could have evolved. Which of these two strategies is used depends on factors important for nestling survival – availability of food and nest predation. If when laying eggs parents can predict availability of food during nestling rearing, and if low food supplies are predicted, they should adopt a 'brood reduction strategy'; that is, invest less in the last eggs laid to minimize any wasted energy if all young cannot be raised. In contrast, if prospects are good for raising all young, parents should instead adopt a 'brood survival strategy'; that is, invest relatively more in the last eggs laid, as the chances of raising the last chicks to be hatched are better during good years. However, if nest predation is more important for nestling survival than food availability, and parents begin incubating before clutch completion to reduce nest predation (on eggs), then parents should also use a 'brood survival strategy'. I studied variation in egg mass in a population of Pied Flycatchers and found that there was a significant increase in egg mass with laying order. In an experiment in which last eggs to be laid were swapped between nests to increase or reduce the egg mass of the last egg laid, there was a positive effect of egg size on hatching mass and on nestling growth rate during the early nestling phase. Thus, nestlings hatching asynchronously and 'originating' from an experimentally larger egg, were compensated in their later hatching by a higher elevation of the slope of the growth curve compared with their siblings during the first part of the nestling phase. I also found that egg mass was positively correlated with wet yolk and wet albumen, as well as with energy content of the yolk and total energy content of the egg. Thus, Pied Flycatchers appear to use a 'brood survival strategy'; that is, they lay a large last egg, which contains more nutrients, to compensate for the late hatching of the last egg. It has also been shown for this species that, in a population breeding in natural holes, predation during the egg stage is the most important mortality factor.

Keywords: brood reduction, *Ficedula hypoleuca*, hatching asynchrony, variation in egg mass.

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INTRODUCTION

Asynchronous hatching, which occurs in many bird species, has been suggested to have evolved to adjust the brood size to the prevailing conditions during rearing of nestlings (Lack, 1954, 1968; Magrath, 1989). As females begin to incubate their eggs before a clutch is completed, the last eggs generally hatch several days after the first. In many passerine bird species, the hatching of eggs is semi-asynchronous (Mock and Schwagmeyer, 1990), such that the last eggs generally hatch 1–2 days after the other eggs in the clutch. Thus, a size hierarchy is formed within the brood and the nestlings that hatch later are smaller than their siblings at any given time (Lack, 1954; O'Connor, 1979). This size hierarchy has important consequences, as smaller siblings have a lower competitive ability relative to their siblings (Bengtsson and Rydén, 1983; Mock, 1984; Gottlander, 1987), which often leads to starvation and death (Mock, 1984; Lessells and Avery, 1989; Amundsen and Slagsvold, 1991).

Howe (1976) noted an increase in egg size with laying order in the Boat-tailed Grackle and suggested that this might be a counter-adaptation against the brood reduction mechanism, as an increase in the size of the last eggs in the sequence will result in a reduced size hierarchy among the nestlings, which is the opposite of what the brood reduction hypothesis predicts. Clark and Wilson (1981) proposed that the increase in egg size with laying order may be an adaptation enhancing the competitive ability and survival probability of the last nestlings to hatch. Slagsvold *et al.* (1984), in their review of variation in egg size in birds, discussed the possible importance of variation in size of the final egg for growth and survival of the last nestling to hatch. They argued that birds laying a relatively small last egg were adopting a 'brood reduction strategy', whereas those laying a relatively large last egg were using a 'brood survival strategy'. Thus, while some species adopt the former strategy to *widen* the differences in size created by hatching asynchrony, others adopt the latter strategy to *reduce* the risk of death for the last nestling to hatch.

Thus, different patterns of egg laying behaviour and energy allocation among the eggs in a clutch can be seen in different species, and egg size has been shown to vary considerably both among (Lessells *et al.*, 1989; Williams, 1994) and within species (Slagsvold *et al.*, 1984; Magrath, 1992). Intraspecific variation is primarily due to differences among females in the population (e.g. Ojanen *et al.*, 1978; van Noordwijk *et al.*, 1980; Bancroft, 1984; Greig-Smith *et al.*, 1988; Wiggins, 1990). However, egg size has also been found to vary within clutches (Slagsvold *et al.*, 1984; Krementz and Ankney, 1986; Amundsen and Stokland, 1990). In some species of gulls and terns, and birds of prey, egg size typically decreases with laying order (Lack, 1968; Parsons, 1970, 1975; Rahn *et al.*, 1975), whereas an increase in egg size with laying order has been reported for many passerines (Holcomb, 1969; Ojanen *et al.*, 1981; Slagsvold *et al.*, 1984; Wiggins, 1990; present study).

Several studies have shown that egg size has a positive effect on subsequent nestling growth (Schifferli, 1973; Parsons, 1975; Williams, 1980; Järvinen and Ylimaunu, 1984; Amundsen and Stokland, 1990; Grant, 1991), fledging weight (Howe, 1976; Birkhead and Nettleship, 1982; Furness, 1983) and survival rate of young (Davis, 1975; Howe, 1976; Thomas, 1983), whereas others have found no such relationships (O'Connor, 1979; Moss *et al.*, 1981; Bancroft, 1985; Greig-Smith *et al.*, 1988). It is important to examine what effects these different patterns might have on nestling growth and survival of asynchronously hatched young (Parsons, 1975; Stokland and Amundsen, 1988). Little is known about the relationship between egg size and hatching asynchrony (but see Parsons, 1970, 1975; Bancroft, 1985; Leblanc, 1987), and only a few studies have tackled this problem experimentally (see Parsons, 1970, 1975).

The aims of this study were to determine if a larger last egg in a clutch could compensate that chick for hatching 1 day later than its siblings, and if a larger last egg decreases the sibling size hierarchy within the brood. I also wished to determine if relationships exist between egg mass, egg composition and energy content of the egg, and if egg energy content is related to laying sequence.

I studied a nestbox-breeding population of the Pied Flycatcher, which is normally semi-asynchronous (Slagsvold, 1986; Hillström and Olsson, 1994). The size of the last egg in the sequence was manipulated so that it was smaller, larger or the same size (control) as the original last egg in the clutch, to determine whether this would lead to a decrease or increase in growth rate for those nestlings. This manipulation was combined with another designed to ensure that the last egg in the sequence in all broods would hatch 1 day later than the others. In an earlier study of this population of the Pied Flycatcher, it was shown that the last chick to hatch experienced a higher rate of mortality during poor conditions, whereas no relationship existed between rate of mortality and hatching order during good conditions (Hillström and Olsson, 1994).

METHODS

The study area was a deciduous woodland close to Uppsala, central Sweden (59°50′N, 17°40′E), and the experiments were performed from mid-May to late June 1989. Nest boxes were inspected daily to record nest building, date of egg laying, incubation and hatching of the young, which were weighed on the day of hatching and subsequently every second day until fledging. All pairs were monogamous, as all parents were seen feeding young in all nests (Hillström, 1995). Eggs were removed as they were laid, replaced by dummy eggs (eggs began to be removed after the third egg had been laid), weighed to the nearest 0.001 g in the laboratory, and subsequently stored in a refrigerator at 12°C until they were returned to the nests. After all eggs had been laid, all but the last were returned to the nest; the last egg was returned 1 day after the others to ensure it hatched 1 day later. As a result, all clutches became asynchronous to an equal degree.

Three different treatments were assigned at random to clutches. In two treatments, the last egg was either larger or smaller than the original last egg of the clutch and was returned to the nest 1 day after the others. Similarly, in the control condition, the original last egg was returned to the nest 1 day after the others. Eggs that were designed to be larger or smaller than the original last egg were switched between broods, such that one clutch received a larger egg and the other clutch a smaller egg than the original last egg. All eggs introduced in the experimental treatments had also been laid as final eggs by their own parents. Thus, after the manipulation, all last eggs in the treatments with larger or smaller eggs came from a parent other than the one incubating the eggs.

As the main aim of the experiment was to increase or decrease the size of the last egg laid in relation to the mean of the clutch, a larger or smaller egg than the mean of the clutch was retrieved from the clutches available in the population. Thus, for practical reasons, the experimental manipulation of last eggs was ‘non-randomized’ in the sense that an egg either larger or smaller in size was chosen among the ones available in the population. There was also a significant difference in clutch size between treatments – that is, between broods receiving a larger and a smaller last egg (see Results). However, this should lead to a conservative interpretation of the results, as eggs that were 'donated' in the larger egg treatment also had significantly larger clutches compared with the recipient of those eggs (i.e. the reduced treatment); therefore, the test should be a conservative test of the hypothesis of increased nestling growth in relation to egg mass. A simple criterion was to make the last egg 10–20% larger or smaller than the mean of the clutch in the experimental broods. In experimental broods which received larger last eggs, the last egg was on average 21.65% larger than the mean of the original clutch; in broods which received smaller eggs, the last egg was on average 9.92% smaller than the mean of the clutch.

After the experimental manipulation, the nests were inspected daily commencing on the expected hatching date, and nestlings were individually marked with Tippex© (a correcting fluid) on their claws; at an appropriate age, they were given colour rings. As I had standardized the hatching pattern by removing all eggs in all clutches, returning all eggs to the nest at the same time after the female had started to incubate – except for the last egg, which was returned 1 day later – the last egg consequently hatched 1 day later than the others. Nestlings were weighed from the day of hatching (with spring scales to the nearest 0.1 g) once a day until all eggs had hatched, and then every second day until fledging. Wing length (ninth primary) was measured with a ruler (to the nearest 1 mm) and tarsus length with a dial caliper (to the nearest 0.1 mm) when the young were 13 days old, that is just prior to fledging. Parents were caught and measured either during the incubation phase (females), or on one of the first days after hatching. Clutches which had an unusually high hatch failure, or which did not hatch according to the manipulated pattern, were excluded from any further analysis on nestling growth.

To determine if the mass of the eggs was related to albumen or yolk mass and to the energy content of the eggs, I collected all eggs in eight Pied Flycatcher clutches in an adjacent breeding area. For these eight clutches, mean (± S.D.) clutch size was 6.38 ± 0.74 and mean egg mass was 1.74 ± 0.08 g. Yolk is rich in fat and energy and thus important for survival and nestling growth after hatching, while albumen is rich in protein, which is an important resource for nestling growth. These eggs were removed as they were laid and were replaced by dummy eggs. The females were captured for measurement (and then released) either during egg laying or after the start of incubation of the dummy eggs. The real eggs were stored in a refrigerator at 12°C (to keep them fresh) for a maximum of 7 days before analysing their composition and energy content ($\text{kcal} \cdot \text{g}^{-1}$ albumen and yolk). The procedure for analysing the eggs was as follows. Eggs were weighed on a balance (to an accuracy of 0.001 g) and then boiled to separate yolk and albumen. They were then weighed again and the contents separated into albumen and yolk, which were weighed separately and then dried in an exiccator with silica gel (bluegel) with under-pressure (95%) for 3–4 days before analysing their energy content. After their dry weights had been determined, energy content was analysed for the two components separately using a 'Scanacon CP500' calorimeter.

Analysis of variance was performed using PROC GLM, Type III-SS (SAS Inc., 1990). A mixed model was used to estimate the effects of laying sequence on variation in egg mass. Pearson correlations were used to determine the relationship between egg size and nestling growth, and Spearman Rank correlations to analyse the yolk, albumen and energy content of eggs. Two-tailed tests were used in all instances.

RESULTS

Variation in egg mass within and between clutches

To determine if there was an increase in egg mass with laying sequence in clutches, a mixed model was used with egg mass as the dependent variable, laying sequence as a fixed factor and clutch size as a random factor (Fry, 1992). This showed that there was a significant effect of egg mass on laying sequence ($F_{7,212} = 5.83$, $P = 0.001$), but no covariance effect of clutch size ($Z = 1.17$, $P = 0.24$) (Fig. 1). Thus, typically, there was an increase in egg mass with laying sequence in clutches of six, seven or eight eggs, but not in clutches of five eggs, which also had the largest mean egg mass per clutch (Fig. 1). Also, in a larger study of intra-clutch variation in egg mass over 5 years in the same species (performed in deciduous forests located at the same latitude as the present study), there was a significant increase in egg mass with laying sequence (L. Hillström, unpublished data). In the present study, there was no significant correlation between egg mass and date of laying in clutches of six eggs ($r = 0.30$, $P = 0.091$, $n = 13$) or seven eggs ($r = -0.22$, $P = 0.36$).

In all clutches before experimental manipulation, the last egg was the largest in 22 of 34 (65%) clutches, but not the largest in 12 of 34 (35%) clutches. As the experiment was designed so that last eggs laid should hatch asynchronously, 1 day later than the others, the mass of the last egg laid was compared with the mean mass of the other eggs in the clutch. The last egg laid was on average 5.19 ± 4.12% larger than the mean mass of the clutch in clutches of six (1.78 ± 0.10 g, $n = 13$) and seven eggs (1.67 ± 0.10 g, $n = 17$), which accounted for most clutches in this study and which also are the most common clutch sizes in Pied Flycatchers in the study area (Lundberg and Alatalo, 1992). The difference in mass between the last egg laid and mean clutch egg mass was largest in clutches of six eggs (6.6 ± 2.91%) and next largest in clutches of seven eggs (4.04 ± 4.72%). In both clutches of five (1.96 ± 0.10 g, $n = 2$) and eight (1.67 ± 0.02 g, $n = 2$) eggs, the last egg laid was in one case smaller (–0.5% and −1.8%) and in one case larger (1.0% and 3.0%) than the mean clutch egg size, respectively.

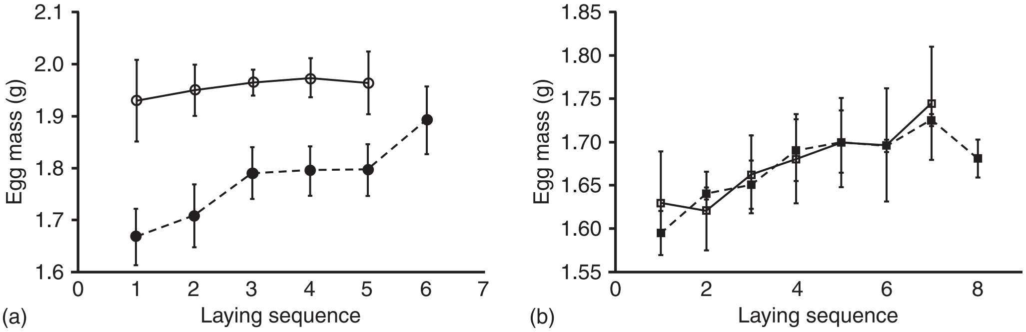

Fig. 1. Egg mass as a function of laying sequence in Pied Flycatcher clutches of five and six eggs (a) and of seven and eight eggs (b) (mean ± S.E.). Clutch size: ——○——, $n = 5$; – – ● – –, $n = 6$; ——□——, $n = 7$; – – ■ – –, $n = 8$. See text for statistics.

Correlation between egg mass and nestling mass hierarchy

There was a significant positive correlation between egg mass and mean nestling mass at hatching ($r = 0.650$, $P = 0.0045$, $n = 24$), and this relationship was still significant when the nestlings were 2 days old ($r = 0.544$, $P = 0.012$, $n = 21$) (Fig. 2). I also regressed the coefficient of variation of nestling mass on mean egg mass, which showed there was a significant effect of egg mass on sibling hierarchy within broods at a nestling age of 2 days ($F = 8.48$, $P = 0.009$, $r^2 = 0.32$); that is, an increase in egg mass resulted in a decrease in sibling hierarchy within broods. At the end of the brooding phase (i.e. when the nestlings reached 4 days of age), there was no significant correlation between mean egg mass and mean nestling mass ($r = 0.225$, $P = 0.341$, $n = 20$).

Composition and energy content of eggs

Comparisons were made of mean egg mass and the different constituents of eggs, as well as the energy content of albumen and yolk, for those clutches in which the difference in egg mass between the largest and smallest egg was at least 0.15 g (range 0.15–0.45 g), or there was an average 15.4% difference from mean egg size. For these clutches, there was a significant positive correlation between egg mass and wet albumen mass ($r = 0.91$, $P = 0.011$, $n = 6$), wet yolk mass ($r = 0.96$, $P = 0.003$, $n = 6$) and dry yolk mass ($r = 0.91$, $P = 0.013$, $n = 6$), but no correlation with dry albumen mass ($r = 0.44$, $P = 0.38$, $n = 6$). The energy content of eggs was calculated by multiplying the dry mass of albumen and dry mass of yolk with the energy content of the egg (in $\text{kcal} \cdot \text{g}^{-1}$).

There was a significant positive correlation between mean egg mass and the energy content of the yolk ($r = 0.83$, $P = 0.042$; Fig. 3a), and between mean egg mass and the total energy content of the egg ($r = 0.96$, $P = 0.003$; Fig. 3b); there was no correlation between mean egg mass and the energy content of albumen ($r = 0.40$, $P = 0.44$). Using all eight clutches, the correlation between laying sequence and the total energy content of the egg was nearly significant ($r = 0.27$, $P = 0.057$, $n = 8$). In summary, this small sample of clutches of eggs from the same population of Pied Flycatchers has shown that a larger egg mass results in more (wet and dry) yolk and more (wet) albumen for the embryo, and more energy for the larger eggs at the end of the laying sequence.

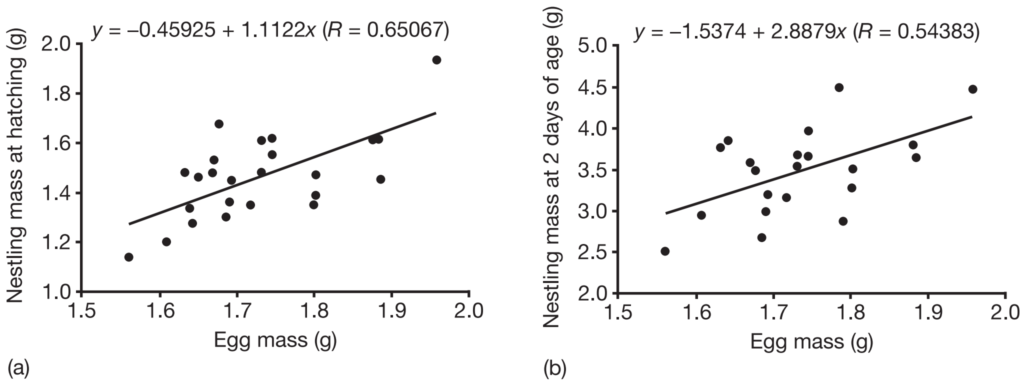

Fig. 2. Correlation between mean egg mass and mean nestling mass at hatching (a) and at 2 days of age (b).

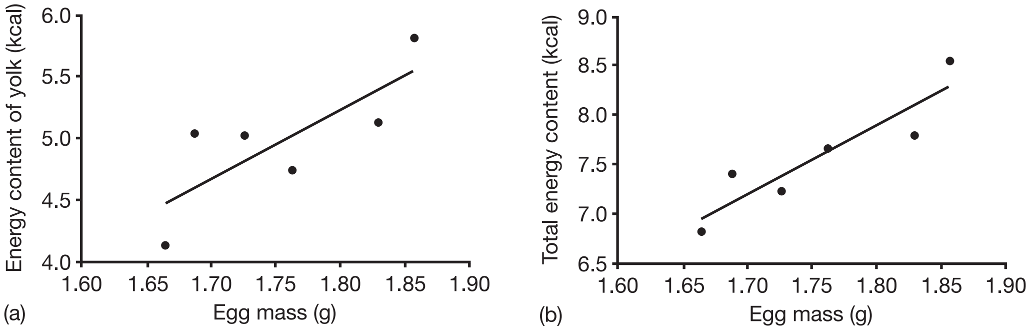

Fig. 3. Energy content of the yolk (a) and total energy content of the egg (b) as a function of egg mass in six Pied Flycatcher clutches. See text for statistics.

Effects of hatching order on hatching mass and nestling growth

To determine differences in growth rate between nestlings hatching in a different order, analyses of variance were performed with hatching order (hatching last *vs* not hatching last) as the independent variable and age of nestlings as a covariate; mass of nestlings (from hatching to an age of 6 days) was the dependent variable. Analyses were performed separately for each treatment. There was a significant effect of age on nestling mass in all groups, as expected, but there was also a significant effect of hatching order on the growth curve (i.e. on the intercept of the growth curve) in broods with a larger egg substituted ($F = 8.29$, $P = 0.007$), where young hatching asynchronously had a significantly higher intercept than young hatching together (Table 1). However, there was no difference in the slopes of the growth curves in this treatment ($F = 0.19$, $P = 0.66$; i.e. the interaction term in the ANCOVA). Thus, nestlings grew at the same rate, but nestlings from larger eggs had a higher hatching mass, which they kept until brooding was over (Table 1). For the other treatments, there were no differences in the intercept or in the slopes of the growth curves (Table 1).

To estimate the relative effects of egg manipulation and hatching order on hatching mass, and on the relative difference in mass between siblings within a brood (i.e. sibling hierarchy), an analysis of variance was performed with treatment and hatching order as independent variables, nestling mass as the dependent variable and clutch size as a covariate. There were no significant differences in hatching mass of nestlings between treatments ($F = 0.55$, $P = 0.58$), but a trend was noted for a higher hatching mass of nestlings hatching

Table 1. Results of effects of hatching order on nestling growth from hatching to 6 days post-hatching

	Larger egg added			Control			Smaller egg added		
Source	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Model	2,31	531.1	0.0001	2,73	417.8	0.0001	2,66	572.1	0.0001
Age	1	1054	0.0001	1	831.4	0.0001	1	1141.6	0.0001
Hatching order	1	8.29	0.007	1	0.38	0.539	1	2.56	0.11
Age × hatching order	1	0.19	0.66	1	0.44	0.507	1	0.01	0.97

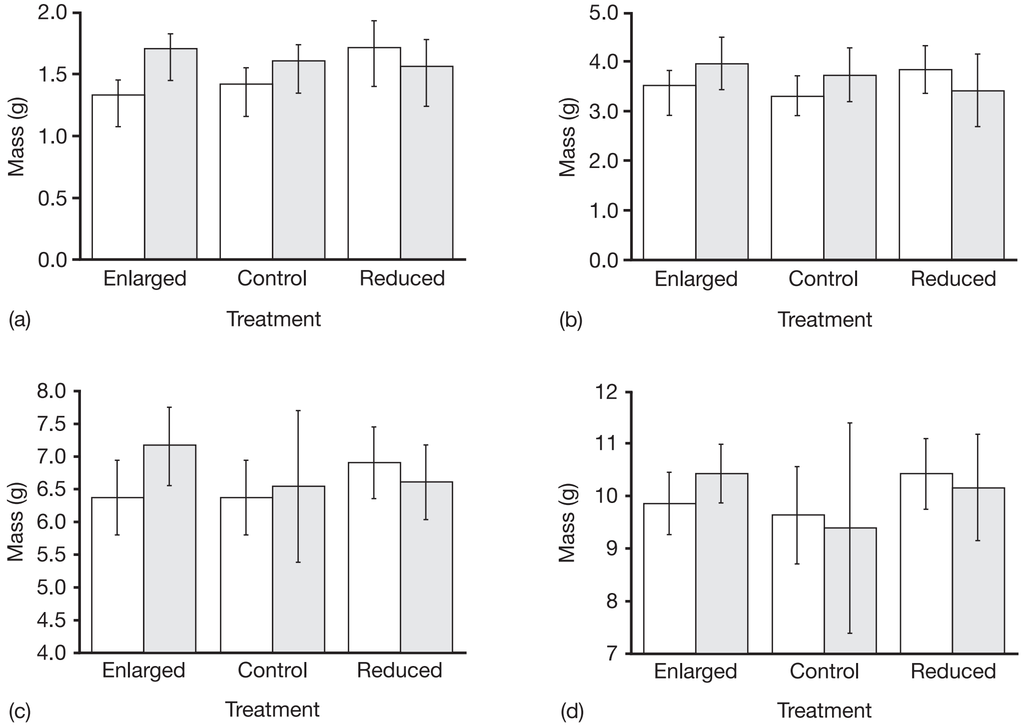

Fig. 4. Nestling mass in the different treatments: enlarged (*n* = 4), control (*n* = 13) and reduced (*n* = 9) egg treatments, at (a) hatching, (b) 2 days of age, (c) 4 days of age and (d) 6 days of age (mean ± S.E.). □, nestlings hatching first; ■, nestlings hatching asynchronously, 1 day after the others. See text for statistics.

asynchronously ($F = 3.37$, $P = 0.070$). Thus, these nestlings hatched from eggs that were laid last in the laying sequence and hatched asynchronously, and included eggs from all treatments. There was a significant interaction between treatment and hatching order ($F = 4.81$, $P = 0.013$), such that nestlings hatching asynchronously, both in broods with experimentally larger eggs and control broods (which naturally had larger last eggs in 7 of 12 clutches; i.e. after the manipulation had been made), were heavier at hatching than their nest-mates, whereas the reverse pattern was found for nestlings hatching asynchronously in the treatment where smaller eggs were substituted (Fig. 4). There was no effect of clutch size in this model ($F = 0.70$, $P = 0.41$, $n = 47$). Thus, eggs that were larger also produced heavier nestlings in broods with experimentally larger eggs. The last egg in the control broods also produced a nestling (young hatching last) that had a larger hatching mass than its siblings (young not hatching last) (Fig. 4).

When the nestlings were 2 days old, there was no effect of treatment ($F = 2.30$, $P = 0.11$) or of hatching order ($F = 1.11$, $P = 0.30$). However, there was a significant effect of clutch size ($F = 8.18$, $P = 0.007$), where clutches of eight eggs had the smallest young (3.09 ± 0.16 g), clutches of six and seven eggs had young of intermediate size (3.52 ± 0.60 and 3.50 ± 0.51 g, respectively), and clutches of five eggs had the heaviest young (4.14 ± 0.42 g). The interaction between treatment and hatching order was still significant ($F = 3.88$,

$P = 0.030$), with nestlings that hatched from larger eggs (young hatching last) again heavier than their nest-mates (young not hatching last). In control broods, nestlings hatching asynchronously (young hatching last) were also heavier than their nest-mates, whereas nestlings from experimentally smaller eggs (young hatching last) were still lighter than their siblings at age 2 days (Fig. 4b). Thus, both at hatching and again at 2 days of age, nestlings hatching last in broods where a larger egg was substituted – and also to some extent in control broods – were heavier than their siblings.

When the nestlings had reached 4 days of age (and brooding of young by females had almost ended), there were no significant differences in nestling mass between treatments ($F = 0.69$, $P = 0.51$) or between nestlings of different hatching order ($F = 0.64$, $P = 0.43$, $n = 45$); there was no interaction between treatment and hatching order ($F = 1.18$, $P = 0.32$). However, the mass of asynchronously hatched young at 4 days of age in broods where a larger egg was substituted were still heavier than their siblings (Fig. 4c). At 6 days of age, there was no difference in nestling mass between treatments or between hatching orders, although nestlings hatching from experimentally larger eggs were still heavier than their siblings (Fig. 4d).

Breeding success and condition of fledglings

There was a significant difference in clutch size between treatments, with the treatment where larger eggs were substituted resulting in significantly larger clutch sizes than the treatment with smaller eggs substituted. However, this should lead to a conservative estimate of nestling growth rate in the condition with experimentally larger eggs, as nestlings receive less food per nestling when brood size increases (e.g. Nur, 1987). There was no significant difference in brood size or number of fledged young between treatments, or in the timing of the first egg laid or the first egg to hatch (Table 2). Nor was there any significant difference in hatching success between treatments, although the experimental broods (with a smaller or larger egg substituted) seemed to suffer a reduction in hatching success compared with the control broods (Table 2).

There were no significant differences in fledging weight between treatments ($F = 1.66$, $P = 0.20$) or between nestlings of different hatching order ($F = 1.83$, $P = 0.18$; two-way ANOVA) (Fig. 5a). No significant effect of treatment on tarsus length ($F = 1.18$, $P = 0.32$) or wing length ($F = 1.71$, $P = 0.20$) was noted (Fig. 5b,c). However, there was a significant effect of hatching order on both tarsus length ($F = 7.54$, $P = 0.009$; two-way ANOVA) and wing length ($F = 5.62$, $P = 0.023$) (Fig. 5b,c), where nestlings hatching asynchronously had

Table 2. Breeding phenology and success of Pied Flycatchers in the three treatments (mean ± S.D.)

Variable	Larger egg added	Control	Smaller egg added	*F*	*P*
Date of first egg*	15.00 ± 4.54 (11)	16.14 ± 4.62 (14)	16.27 ± 4.54 (11)	0.27	0.768
Date of first hatch	37.00 ± 4.22 (11)	37.57 ± 4.03 (14)	37.27 ± 4.61 (11)	0.06	0.946
Clutch size	7.00 ± 0.63 (11)	6.50 ± 0.65 (14)	6.09 ± 0.54 (11)	6.07	0.006
Brood size	5.73 ± 1.68 (11)	6.21 ± 0.97 (14)	5.18 ± 0.98 (11)	2.16	0.13
Number of fledged young	4.27 ± 2.80 (11)	4.85 ± 1.86 (13)	4.09 ± 1.97 (11)	0.38	0.68

* 1 = 10 May. Figures in parentheses indicate sample size.

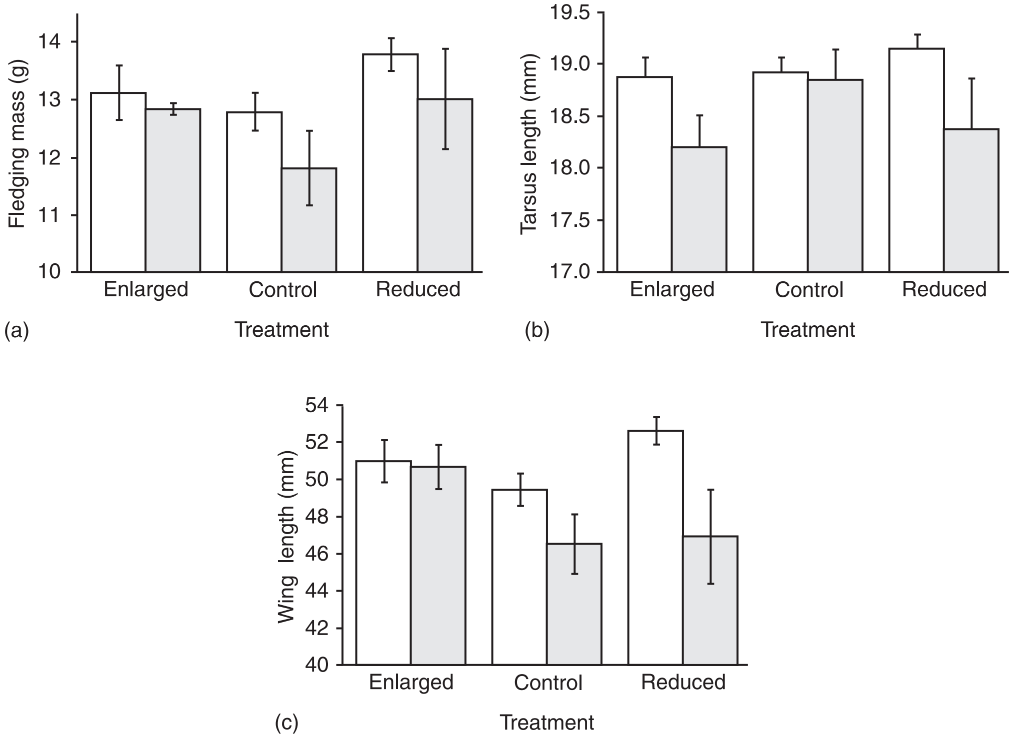

Fig. 5. (a) Mass, (b) tarsus length and (c) wing length (mean ± s.e.) of young at fledging. See text for statistics.

significantly shorter tarsi and shorter wing lengths than young not hatching last. There was also a trend for less of a difference in fledging weight and wing length between young hatching last and not hatching last in the treatment where a larger egg was substituted (Fig. 5a,c). When comparing the variance in fledging mass, tarsus length and wing length for young hatching last, there was a trend towards a higher variance in nestlings originating from experimentally smaller eggs than experimentally larger ones (Fig. 5a,b,c).

DISCUSSION

Intra-clutch variation in egg mass and hatching asynchrony

The main results in this study of the Pied Flycatcher were: (1) A significant variation in egg size within clutches, such that egg mass increased with laying sequence, although not for clutches of five eggs, where investment was large in all eggs in the clutch. (2) Egg mass had a significant positive effect on nestling growth during the early nestling phase, where an experimentally larger egg led to faster growth for nestlings hatching from those eggs. (3) Yolk, albumen and energy content of the egg were positively correlated with egg mass.

Slagsvold *et al.* (1984) noted that two profoundly different strategies can be discerned: a ‘brood survival strategy’, where egg mass should increase with laying sequence, and a 'brood reduction strategy', where egg mass decreases with laying order. Which of these strategies is favoured depends on the relative importance of predation and food for the survival of nestlings. However, a 'brood survival strategy' may also be adopted if, during laying, females can predict good food availability during the rearing of nestlings (Slagsvold *et al.*, 1984). This study has demonstrated that Pied Flycatchers adopt the 'brood survival strategy', and that females may adopt this strategy to increase the probability of survival of the nestlings emerging from the last eggs in a clutch to be laid.

Nest predation and egg mass variation in altricial birds

Asynchronous hatching results in a size hierarchy among siblings and the last nestling to hatch, 'the runt', is generally smaller than its siblings. In passerine and many non-passerine bird species, the runt often dies from starvation (e.g. Ricklefs, 1969; Clark and Wilson, 1981; Graves *et al.*, 1984; Amundsen and Stokland, 1988). Thus, in many species, the runt tends to have a lower probability of survival than its nest-mates. Clearly, this is the core of the 'brood reduction hypothesis', which suggests that sibling hierarchy is an adaptive trait to facilitate the trade-off between quantity and quality of young (i.e. reducing the brood when food conditions deteriorate; Lack, 1954). However, as Clark and Wilson (1981) suggested, if asynchronous hatching in some species is an adaptation to reduce the rate of predation on nests, then it may be argued that parents reducing the sibling hierarchy should enjoy an advantage compared with birds that do not. Thus, if the survival rate of nestlings hatching asynchronously is lower, and if this represents a decrease in parental fitness, then we should expect selection to favour an increased investment in the last eggs laid. It has also been proposed that reduced juvenile survival can select for increased egg size (Kolding and Fenchel, 1981; Sibly and Calow, 1986).

Variation in egg mass and nestling mass hierarchy

The increase in egg mass was generally associated with a reduction in the sibling hierarchy; that is, siblings from clutches where a larger egg was substituted were more similar in size than siblings in clutches where a smaller egg was substituted. Where nest-mates are more equal in size, brooding efficiency may be improved (Royama, 1966) and it may be less demanding of energy of parents (Bryant and Tatner, 1990), especially during cold weather (Haftorn and Reinertsen, 1985), when a larger body size should enhance their thermoregulatory properties and should allow nestlings to endure longer spells without parental brooding (Rhymer, 1988). Experimentally increasing the mass of the last egg in the sequence resulted in young hatching from those eggs with a larger hatching mass than their siblings, and this body mass advantage remained when more than half of the final mass had been attained; that is, in experimental broods where a larger egg was substituted, there was an elevation in the growth curve until 6 days post-hatching. In addition, nestlings hatching last in control broods also had a larger hatching mass than their nest-mates, although this advantage disappeared about 4 days after hatching. As the most critical period for nestling starvation and nestling mortality due to thermal exposure in cold weather is during the first few days after hatching (Graves *et al.*, 1984; Rhymer, 1988; Hillström and Olsson, 1994), a larger mass may be of critical importance during the early nestling period (i.e. before young reach homeothermy; Rhymer, 1988).

Composition and energy content of eggs

Analysis of egg composition and the energy content of eggs showed that there was a positive correlation between egg mass and wet and dry yolk mass, and also with wet albumen mass. The energy content of the yolk and total energy content of the egg were also positively correlated with egg mass. As the yolk is rich in energy, the increase in yolk mass with egg mass indicates a larger lipid reserve for young hatching from larger eggs and that growth rate is as fast as, or faster than, that for nestlings hatching from larger eggs (Williams, 1994). The positive correlation between egg mass and wet albumen mass indicates that nestlings hatching from those eggs could also have a faster growth rate, as albumen is an important nutrient for nestling growth during the early nestling phase. Thus, the results of this study support the hypothesis that chicks hatching from larger eggs are heavier at hatching, as a consequence of having more nutrient reserves (Williams, 1994). I have also shown that larger eggs contained more nutrients, as indicated by the positive correlation between egg mass and the energy content of yolk and the total content of the egg. In a study of the intra-clutch variation of Ring-billed Gulls, *Larus delawarensis*, Meathrel and Ryder (1987) showed that larger eggs contained more albumen. In larids, increased levels of albumen in eggs and the resultant larger hatching size have been shown to increase hatchling survival (Parsons, 1970; Lundberg and Väisinen, 1979).

The effects of egg size and hatching order on condition of fledglings

It has been reported that egg size is positively correlated with fledgling survival (Parsons, 1970) and fledging condition (Schifferli, 1973). I failed to show any effect of an increase or decrease in egg size on fledging weight, tarsus length or wing length. However, there was a significant difference between young of different hatching order, in that asynchronously hatching chicks had a shorter tarsus and wing than synchronously hatching young. In the treatment where a larger egg was substituted, however, the differences between last hatched young and their siblings in terms of fledging weight and wing length were less compared to the treatment where a smaller egg was substituted and the control condition. Also, comparing asynchronously hatching young between the two experimental treatments, there was a smaller variance in fledging condition for chicks from experimentally larger eggs than experimentally smaller ones.

In a study of the relative importance of egg size and parental quality and their influence on nestling growth in the Shag, *Phalacrocorax aristotelis*, Amundsen and Stokland (1990) showed that egg size is more influential than parental quality during the initial third of the nestling phase. In a cross-fostering experiment on the European Starling, *Sturnus vulgaris*, Smith *et al.* (1995) showed that the positive effect of egg size on nestling size diminished after less than 1 week. Thus, the relative importance of hatching asynchrony versus egg size for nestling growth, may have a greater impact on the fledgling's tarsus, supporting the view that nutrient reserves do not give rise to structurally larger hatchlings but that they get heavier. From the results of these studies, we may deduce that, when the brooding phase in altricial birds is coming to an end, egg size has less of an impact on the growth of the chicks, with other factors becoming more important; that is, the competition between siblings for food delivered by the parents is probably stronger after they have reached homeothermy (but see O'Connor, 1978; Mock and Parker, 1997). Selection on increased egg mass is possible if this has a positive effect on survival during the first part of the nestling phase, which is also the period when nestling mortality is at its highest in many altricial and semi-precocial birds (Ricklefs, 1969; Mock and Parker, 1997). Thus, there could be selection for increased egg mass with laying sequence, if this increases survival during the critical first part of the nestling phase.

Proximate and ultimate constraints on variation in egg mass

In clutches of five and eight eggs, the last egg was smaller in two of the clutches and only 1% and 3% larger than the mean for clutches of five and eight eggs, respectively. There was also an indication of a trade-off between egg mass and clutch size, such that mean egg mass was largest in clutches of five eggs and smallest in clutches of eight eggs, similar to that found in a larger sample of the same population (L. Hillström, unpublished data). In this study, all eggs in five-egg clutches were large, whereas all eggs in eight-egg clutches were small. Therefore, there may be constraints on how much energy can be invested in the final egg, which depends on clutch size and energy invested per egg for the other eggs in the clutch. Two other studies on Pied Flycatchers have also indicated that energy invested in the last egg can vary with environmental conditions (Ylimaunu and Järvinen, 1987; Slagsvold and Lifjeld, 1989).

Ylimaunu and Järvinen (1987) suggested that constraints on the energy of females may explain why they lay smaller last eggs in years of poor climatic conditions. In a between-year comparison of egg mass variation within clutches, they showed that in the year with poor climatic conditions, there was no increase in egg mass with laying sequence; in the other two years, however, there was an increase with laying order. Slagsvold and Lifjeld (1989) reported that Pied Flycatcher females invested less in the final egg during repeated nesting (i.e. where the first clutch was experimentally removed), suggesting that this was because of proximate energetic constraints acting on the female (i.e. females were unable to invest more in the last egg laid in repeated nesting attempts).

There are two explanations for this pattern of egg mass variation within clutches. First, an explanation based on energetic constraints on the female; for example, it is difficult to lay a larger last egg when food supplies are low (Ylimaunu and Järvinen, 1987; Slagsvold and Lifjeld, 1989). Second, if during egg laying females can predict that the availability of food will be less than average when rearing nestlings, less energy should be invested in the last egg laid; that is, use a ‘brood reduction strategy’ and invest more energy in the last egg laid if the availability of food is predicted to be good (Slagsvold *et al.*, 1984). However, the most likely scenario is that both explanations combine to induce constraints that are important for explaining variation in egg mass within clutches in altricial birds.

CONCLUSION

In conclusion, the present study has shown that the most important effect on hatching mass is egg mass, both for all clutches combined and within clutches, such that an increased egg size produces heavier nestlings. The positive effect of egg size was found to be strongest during the early part of the nestling phase. It is also in this phase that most mortality occurs in the Pied Flycatcher, as was shown in an earlier study of this species. The present results support the use of the ‘brood survival strategy’ by the Pied Flycatcher; that is, it reduces the size disadvantage of the last hatched nestling (to increase its chances of survival) by adding more nutrients to the last egg laid. Hatching asynchrony induces a sibling hierarchy, which may increase competition for food, but this competition is probably stronger in the middle or the late part of the nestling phase. This may explain why the effect of egg mass on nestling mass decreases when young reach the middle of the nestling phase and often diminishes further by the fledgling stage. Further studies on variation in egg mass in relation to clutch size and age or experience of females are needed to establish the options available to females in terms of energy allocation during egg laying. We also need to explore how the environment (e.g. availability of food during laying) may affect the decision of a female to allocate energy resources to the different eggs in the laying sequence.

ACKNOWLEDGEMENTS

I thank L. Holmquist for help in the field. I also thank the late I. Borg and M. Hultqvist for help with analysing the energy contents of eggs. Many valuable comments on earlier drafts of the manuscript were provided by A. Forsman, B.R. Grant, L. Gustafsson, A. Lundberg, J. Merilä, T. Slagsvold, S. Ulfstrand, D. Wiggins and an anonymous referee. I also wish to express special thanks to Rosemary Grant for inspiring and interesting discussions. I am grateful for financial support from the Sandviken Göranssons' Fund, Zoologiska Stiftelsen at the Department of Zoology, Uppsala University and from the Swedish Royal Academy of Science. The manuscript was completed while the author held a post-doctoral position at the Biological Institute of Donana, Seville, Spain.

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