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Costs of avian incubation: a synthesis of how fitness, energetics and behaviour impinge on the evolution of clutch size

Maaïke E. de Heij

INTRODUCTION

The number of eggs in a clutch can be seen as a 'behavioural decision' of the female bird or the pair. In most species the clutch size is not fixed and individuals have the option to stop laying early resulting in a small clutch or to lay more eggs resulting in a large clutch. Given such a set of options, the female or the pair has to 'decide' to lay a particular number of eggs. In this thesis I have been interested in the question how such clutch size 'decisions' affect selection on clutch size. To answer this question, I have taken an experimental approach (chapters 2, 3, 6 and 7). By changing the number of eggs I mimicked a change in the clutch size of parents in order to study its consequences. In addition I studied effects of natural variation in clutch size on selection (chapter 4). In this synthesis, I will highlight the main findings of this thesis in three different sections.

To begin with, I will highlight the findings that have increased our general knowledge on energetics and behaviour during the incubation phase itself. Within this section, I will put forward three topics that deserve more attention in future work. These topics are 1) the effect of ambient temperature on the energetic costs of incubation and why this is important, 2) the energetic costs of foraging during the incubation phase for the attending female, and 3) the role of the male during the incubation phase and its importance for the female.

In the second section, I discuss the causal effects of clutch size on energetics, behaviour and fitness using clutch size manipulations during the incubation phase. I discuss the behaviour and energy expenditure of the incubating females, several offspring fitness components within the same breeding attempt (i.e. short term) fitness consequences in terms of both offspring and parental local survival (i.e. long term). At the start of the study I expected that with increasing clutch size, an incubating female would need to change her behaviour, and consequently would increase her energy expenditure. The increase in expenditure would lead to a reduction in fitness, either via the offspring or via the parents themselves. In this section, I will summarize and integrate the actual findings.

Finally, I discuss the implications of our findings for selection on clutch size in our own and in other populations of the great tit, *Parus major*. In this last section, I will also present several points that deserve further consideration.

DAILY ENERGY EXPENDITURE DURING THE INCUBATION PHASE

Daily energy expenditure in perspective

The incubation phase has long been ignored in studies estimating selection on clutch size. The reason for this is that the act of rearing nestlings was thought to be energetically considerably more costly than incubating and laying eggs. Egg laying was assumed to be less costly than rearing nestlings, because females were

found to replace removed eggs seemingly without costs. Keeping eggs warm was thought to be energetically less costly, especially because the nests in which birds incubated the eggs would create a favourable environment (Walsberg and King 1978). The only empirical data available at that time were in disagreement with this theoretical expectation (Drent 1975). It was not until the 1990's that the controversy was settled by refined empirical results (Williams 1996; Ward 1996; Monaghan and Nager 1997; Tinbergen and Williams 2002).

The results in this study are at variance with King's idea that the energetic costs during the incubation phase are low. In chapter 6, my co-workers and I found that the metabolic rate during nocturnal incubation (MR_{inc}) was 2.0 times BMR (basal metabolic rate), while in chapter 7 I found that the daily energy expenditure during the incubation phase (DEE_{inc}) was 2.4 times BMR. In a review on the energetic costs of incubation, Tinbergen and Williams (2002) compare the existing body of data on daily energy expenditure for birds incubating eggs and for birds provisioning nestlings with food. They show that birds expend about the same amount of energy during both phases. Particularly, for small species of passerines, and for uniparental incubators the daily energetic cost of incubation is equivalent to that of females rearing nestlings.

The incubation phase may be energetically costly for several reasons. One potential reason is that birds have limited food at their disposal to balance the expenditure; food is generally assumed to peak later during the nestling phase (Perrins 1970; van Balen and Cave 1970; Nager and van Noordwijk 1995). For species with uniparental incubation, the low food availability may specifically be demanding for the attending sexes, since they have limited time – often only 25% of active day (Deeming 2002) – available for foraging (i.e. investment in self-maintenance) due to the requirement to incubate the eggs (i.e. investment in current reproduction).

Another reason why the incubation phase is energetically costly – and in my view a point of main importance – is the influence of ambient temperature. A comparison of the metabolic rate during nocturnal incubation (MR_{inc}) standardised to 8 °C with the metabolic rate of a resting bird at 8 °C ($MR_{8°C}$) and BMR for several passerine species showed that MR_{inc} was relatively high (chapter 6). This was especially so for the smaller passerines, which could be explained by their extra costs of thermoregulation. In line with this idea, ambient temperature had a strong impact on the energetic costs of incubation (chapter 6 and 7; Bryan and Bryant 1999; Weathers *et al.* 2002). In this study, a fall in ambient temperature of 10 °C resulted in a 43 % – 49 % increase in metabolic rate of incubating females during nocturnal incubation (chapter 6), while over a 24-h period, such a change in temperature resulted in about 50 % increase in daily energy expenditure (chapter 7). Accepting that lower temperature is causing high energy expenditure during the incubation phase, then in seasonal environments the high costs of incubation may be the rule due to the fact that the incubation phase precedes the

nestling phase, and ambient temperatures generally increase throughout the breeding season. Since the egg laying phase precedes incubation, and thus also the nestling phase, the same reasoning may hold for this reproductive phase.

To investigate the actual energetic costs throughout the reproductive phase, my aim was to compare the daily energy expenditure of female great tits during the three consecutive phases (the egg laying, the incubation and the nestling phase) in the same population to limit the confounding effects of uncontrolled factors. Because such a data set was not available for the great tits in the Lauwersmeer, I collated data for those populations in which the daily energy expenditure of female great tits was measured during at least two of the three reproductive phases. The data set is based on data from three populations; two Dutch populations; Hoge Veluwe and Lauwersmeer, and one Scottish population; Stirling (Fig. 9.1; for details about these studies see figure legend). This dataset may provide a first insight in the question whether the costs during the egg laying and the incubation phase are energetically high.

In support of the above reasoning that in a seasonal environment temperatures increases, figure 9.1 shows that the range of ambient temperatures during the three reproductive phases was only partly overlapping. Ambient temperatures were indeed lowest during the egg laying phase and mostly below 10 °C. During the incubation phase, ambient temperatures were intermediate and around 10 °C, while during the nestling phase ambient temperatures were highest and mostly above 10 °C. In the Scottish population, mean ambient temperatures during both the egg laying and the incubation phase were lower than in both the Dutch populations, due to the difference more northern location.

In all the three reproductive phases, a negative relation between daily energy expenditure and ambient temperature was found. The correlation between daily energy expenditure and ambient temperature was stronger in both the egg laying and the incubation phase than in the nestling phase. In the two earlier reproductive phases, the variation in ambient temperature was also largest during these two phases, which may also influence the energy expenditure (Pendlebury *et al.* 2004). The regression between daily energy expenditure and ambient temperature was not demonstrably different between the populations in the egg laying and the incubation phase. In the Scottish population, however, the DEE_{inc} of females tended to be more strongly related to the mean ambient temperature than those in the Lauwersmeer population (interaction temperature times population; $F_{1,33} = 3.3$, $p = 0.08$). Nevertheless, some of the females in the Scottish population experienced extremely low minimum ambient temperatures of around freezing point during incubation, and these females had extreme high DEE_{inc} , affecting the mean value of DEE_{inc} for the population. DEE_{inc} of female experiencing the same range of ambient temperatures during the incubation phase were overlapping. The relation between daily energy expenditure and ambient temperature was different between the populations in the nestling phase. For the sake of simplicity in the

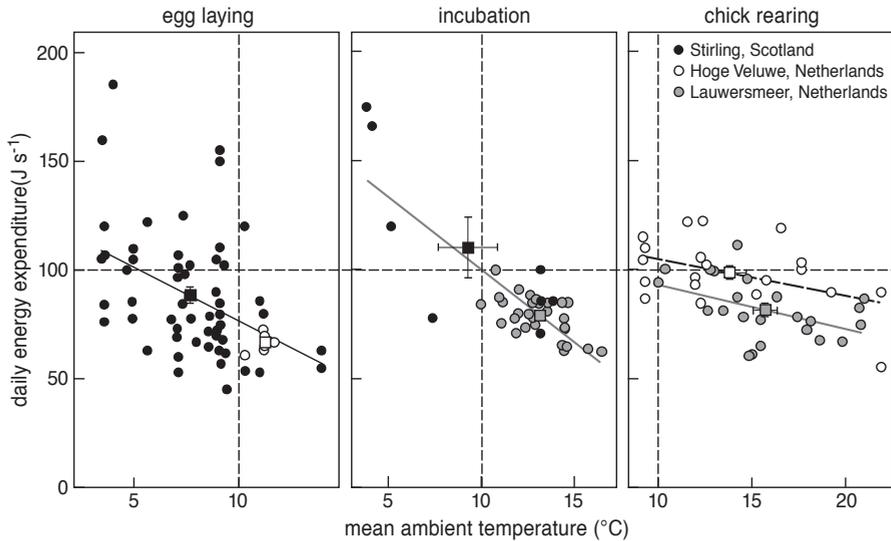


Figure 9.1. Daily energy expenditure (kJ day^{-1}) of female great tits from three populations during either the egg laying (DEE_{egg}), the incubation (DEE_{inc}) or the nestling phase ($\text{DEE}_{\text{nestling}}$) in relation to mean ambient temperature. Circles represent individual measurements, while squares represent the mean energy expenditure (\pm SE) for a specific population. For reference, a vertical line is given at 10°C and a horizontal line at an expenditure of 100 kJ day^{-1} . Data used were either original (Hoge Veluwe: egg laying phase, unpublished data P.Gienapp, M.E. Visser and G.H.Visser; nestling phase, Tinbergen and Dietz 1994; Lauwersmeer: incubation phase, chapter 7; nestling phase Wiersma and Tinbergen 2003, Sanz and Tinbergen 1999) or derived data (i.e. values were read from graphs; Scottish population; egg laying phase, Stevenson and Bryant 2000; incubation phase, Bryan and Bryant 1999). Data on daily energy expenditure in the Scottish population were given in relation to either the maximum ambient temperature or the minimum ambient temperature; I converted these values to mean ambient temperature using correlations between mean, minimum and maximum ambient temperatures obtained for the Lauwersmeer area. DEE_{egg} was negatively related to mean ambient temperature ($F_{1,60} = 12.0, p < 0.001$), and did not depend on the population in which the measurement took place ($F_{1,60} = 0.3, p = 0.60$). DEE_{inc} was negatively related to mean ambient temperature ($F_{1,34} = 42.7, p < 0.001$), and did not depend on the population in which the measurement took place ($F_{1,34} = 1.9, p = 0.17$). Note that for the females in the Scottish population the slope of DEE_{inc} in relation to the mean ambient temperature tended to be steeper than for those in the Lauwersmeer population (interaction temperature times population; $F_{1,33} = 3.3, p = 0.08$). $\text{DEE}_{\text{nestling}}$ was negatively related to mean ambient temperature ($F_{1,42} = 16.9, p < 0.001$), and differed between females of the two populations ($F_{1,42} = 9.6, p < 0.001$); females in the Hoge Veluwe population had higher $\text{DEE}_{\text{nestling}}$ than those in the Lauwersmeer populations.

analyses, I assumed that all individuals of the different reproductive phases were belonging to one and the same population.

Controlling for ambient temperatures ($F_{1,142} = 55.5$, $p < 0.001$), the daily energy expenditure of females did indeed depend on the reproductive phase. Females providing nestlings with food had higher daily expenditure than those incubating eggs ($F_{1,80} = 14.3$, $p < 0.001$, controlled for temperature), while females incubating eggs had higher daily expenditure than those laying eggs ($F_{1,97} = 16.5$, $p < 0.001$, controlled for temperature). At a mean ambient temperature of 10 °C, for instance, females spent more energy when rearing nestling than when incubating, while females spent more energy when incubating than during the egg laying phase. However, under the ambient regime prevailing locally, daily energy expenditure of females during the egg laying phase, the incubation phase and the nestling phase were roughly equivalent ($F_{2,143} = 0.4$, $p = 0.68$).

The analysis reveals that the nestling phase is not energetically more costly than either the egg laying or the incubation phase, and as a consequence both the egg laying phase and the incubation phase may be of importance for clutch size decisions. These latter phases are relatively demanding due to their timing in the season, and consequently lower food availability and lower ambient temperatures. Especially in populations with strong seasonality, the energetic costs of early reproduction may prevent early breeding due to low ambient temperature (Bryan and Bryant 1999). Ambient temperature early in the season thus likely puts a selection pressure towards later egg laying. In addition, the findings support the idea first formulated by West (1968) that birds behave in such a way that their daily energy expenditure is relatively constant over the annual cycle (referred to as the reallocation hypothesis).

Costs of foraging versus contact incubation

The measurements on daily energy expenditure (chapter 7) and nocturnal incubation (chapter 6) in combination with data on the time budget of incubating females made it possible to couple the behaviour of the female to her energy expenditure and provided an estimate of the energetic costs during the three main activities of an incubating female over a 24-h period; foraging, rewarming the clutch and contact incubation (chapter 8). During the incubating phase, one of the main activities of the female great tit is providing favourable developmental conditions for embryos (i.e. contact incubation). Yet, contact incubation turns out to be energetically the least demanding activity, while foraging was estimated to be energetically the most demanding activity during that phase (chapter 8). When the value of energy spent per time unit is extrapolated to the total amount of energy females spent during that particular activity, females spent on average 54% of their energy during the active day outside the nest-box on foraging activities and 46% of that inside the nest-box while incubating the clutch (including all activities in the nest-box).

There are two main reasons why foraging may be so energetically demanding. Firstly, foraging may be energetically costly, because in species with uniparental incubation – as in the great tit – the incubating bird has limited time available for foraging. During the absence of the incubating female, the eggs cool towards ambient temperatures, which in temperate zones are well below temperatures that are favourable for embryonic development. Unfavourable temperatures may slow down embryonic development or even be lethal (Webb 1987). This poses a constraint on the female; she has to trade-off incubation of the clutch – and thus the fitness of the offspring of the current clutch – with foraging – and thus self-maintenance and future clutches. At the same time, food availability is thought to be relatively low during the incubation phase and peaks later during the nestling phase (Perrins 1970; Nager and van Noordwijk 1995). Low food availability limits an individual's opportunity to balance its expenditure. The female may compensate for this low food availability by working relatively hard during her time away from the nest to obtain the required intake rate within a limited amount of time.

A second reason why foraging may be energetically demanding during the incubation phase is the high body mass of the female. Females are typically heavier during the egg laying and the incubation phase, and drop in weight when rearing nestlings. In our population, females weighed around 20 g at day 9 of the incubation period (12–13 days), while they weighed around 17 g when feeding nestlings of about 7 days old. Several studies have found that flight costs are related to body mass (Wiersma *et al.* 2005; Engel *et al.* 2006), therefore, the flight costs are probably higher during the incubation phase than during the nestling phase and this may add to the increased foraging costs.

The role of the male during the incubation phase

Calculations on the daily energy expenditure and the time available for foraging provided an estimate on the intake rate females need to obtain during foraging (chapter 8). This intake rate was twice that of a female during the nestling phase. This value is high, even inconceivably so, and the question arises how females are able to obtain such a high intake rate. One possible explanation is that the female is forced to change her foraging behaviour by foraging on different prey types. Such a change may appear in the condition of the female. Alternatively, the female could take more risk while foraging by spending less time on being vigilant (Lima and Dill 1990), which can be measured directly by a reduction in the probability to survive during the incubation phase. Also, the female could be assisted by her partner. Even though the male does not incubate the eggs, he can assist by providing food at the nest (Lyon and Montgomerie 1985; i.e. incubation feeding; Halupka 1994; Martin and Ghalambor 1999; Pearse *et al.* 2004).

A few studies have tried to determine the importance of the incubation feeding of the male for the female. Pearse *et al.* (2004) provided supplementary food in the nest-box of female wrens and found that females spent on average a longer

period on the nest, and consequently the total time the female spent on the nest during the day was longer. In an empirical study by Halupka (1994) on the relation between incubation feeding and nest attentiveness, a similar positive relation was found. Although this finding does not say directly something about the role of the male, it reveals the trade-off between foraging and contact incubation for the female, and thus the nutritional need of the female during the incubation phase. In the same experimental study, Pearse *et al.* (2004) found that the male reduces his feeding frequency to his partner, when she received supplementary food (Pearse *et al.* 2004). Thus, the male seemed to tune the amount of provisioning to the nutritional need of the female. Also at lower ambient temperature, and thus at higher energetic costs for the incubating female, the male increased its feeding frequency (Pearse *et al.* 2004). Lyon and Montgomerie (1985) removed the male at the start of the incubation period, and found that females spent more time away from the nest, when males were removed. As a consequence, eggs of widowed females had a prolonged developmental time and a reduced probability to hatch (Lyon and Montgomerie 1985). This finding suggests more strongly than the two previous studies that males indeed contribute to the intake rate of females, and thereby may influence her incubation behaviour.

In the population of great tits in the Lauwersmeer, I have tried to gain more insight in the role of the male during incubation. I expected the nutritional need of incubating females to increase with the clutch size they are incubating. Therefore, I examined the contribution of the male in relation to the manipulated clutch size the female was incubating. With use of video camera's that were located outside the nest-box, I recorded the incubation behaviour of the female and the frequency of incubation feeding of the male for those nests that participated in clutch size manipulations in the breeding season of 2002. The data did not reveal that the amount of incubation feeding of the male depended on the experimental treatment during the incubation phase. Nevertheless, there was large variation in the frequency of incubation feeding between males; some males were not feeding in the nest-box, while others came twice during the incubation bout of their partner. Based on the sound track on the video observations, males most often seem to use a specific call when they were in the vicinity of the nest-box. After this call, the female would leave the nest-box. Occasionally, the male was observed to feed the female outside the nest-box. More often, however, the male and the female flew away from the nest-box together. This led to the idea that the male leads the female to good foraging patches. By doing so, the intake rate of the female may increase because of she does not have to spend much time on searching for good foraging locations. At the same time, the male may assist the female even more by being on the look out for predators, so that the female can continue foraging and/or the male can continue feeding its partner. If this is indeed the task of the male during the incubation phase, the importance of the male for the female will be hard to capture in detail.

CLUTCH SIZE RELATED COSTS DURING THE INCUBATION PHASE

What can we conclude from the findings presented in this thesis in light of the original question whether selection on clutch size during the incubation phase is important for clutch size decisions? I will first present and discuss the findings of clutch size related costs in terms of energy and behaviour, then the costs in terms of fitness and thereafter I will integrate the different results.

Energy and behaviour

In chapter 6, I determined the energetic costs of incubation in relation to clutch size by measuring the oxygen consumption of incubating females at night, while they were incubating either a reduced, enlarged, or control clutch size. All experimental females received all treatments in consecutive nights. This set-up enabled me to study the effect of clutch size within individuals. This is important, because there is large variation between individuals. During nocturnal incubation, females spent energy at a significantly higher rate when incubating experimentally enlarged clutches compared to control or reduced clutches (chapter 6). The absolute difference, nevertheless, was relatively small.

In chapter 7, I used the doubly labelled water (DLW) – technique to measure the daily energy expenditure of females incubating either experimentally enlarged clutches or control clutches, and thus could compare the effect of clutch size manipulation between individuals. I could not detect a significant difference between females incubating enlarged or control clutches. The females of the two treatment groups also did not differ in their incubation behaviour (i.e. the number of recesses and time spent on the nest during the active day; chapter 7).

Two main explanations may account for this contradiction in findings between the two studies. One explanation is that there was an effect of clutch enlargement on the daily energy expenditure, but that I could not detect it. This could be due to large between-individual variation in behaviour, and thus expenditure or to measuring error (5 - 10% ; Tatner and Bryant 1989; Speakman 1997). To get some insight whether this between-individual variation could explain the difference between the studies, I performed a simple test. Of the data set on the energy spent during nocturnal incubation in the year 2004, I randomly chose for each individual one of the two observations; thus either the energy spent while incubating control or enlarged clutch. Subsequently, I analysed whether in this data set, the metabolic rate of nocturnal incubation was still depending on the experimental treatment, as in the previous test with within individual comparison (chapter 6). No effect of clutch enlargement on energy expenditure could be detected between females of the two treatment categories ($F_{1,15} = 2.1$, $p = 0.17$). This simple test suggests that between-individual variation may mask small effects of clutch size. In addition, a power test revealed that a much larger sample size was

needed to be able to find the small effect of clutch enlargement on DEE_{inc} as was found during nocturnal incubation (chapter 7).

An alternative explanation is that there was no effect of clutch enlargement on daily energy expenditure, because birds compensated their daily energy expenditure by lowering expenditure in another activity, such as self-maintenance (chapter 7). A reduction in the allocation of energy to processes of self-maintenance means less investment in repair (oxidative stress) or in immune function. Consequently, an individual becomes more vulnerable for diseases, and this may influence survival probability. Several studies reveal a trade-off between investment in reproduction and immune function (Hanssen *et al.* 2003; Hanssen *et al.* 2005).

Fitness consequences

In chapters 2 & 3, I manipulated clutch sizes for the duration of the incubation phase to determine the fitness consequences of incubating manipulated clutch sizes in the short and the long term (chapter 2 and 3). The measure of fitness has two components; the parental component – the probability that parents survived locally until the next year and were found reproducing again – and an offspring component – the probability of offspring to survive and recruit in the local breeding population. Like previous studies, I have determined the effect of clutch size manipulation on both parents and offspring. Nevertheless, I also have realised that effect on post-hatching performance could be achieved via the parents – as a result of a change in ability of parents to provide nestlings with food due to the experimental treatment during incubation – or via the eggs – as a result of detrimental effects due to developmental conditions experienced during incubation.

As explained in chapter 3, I used a rather specific manipulation scheme, which deviated from schemes used by previous studies. The specific manipulation scheme that I used, see chapter 3, has several advantages. First, the effects of clutch size manipulation on pre-hatching performance (i.e. developmental time and hatching probability) could be determined, while taking into account the parental origin of the egg, and hence the inherited egg quality (chapter 3). Second, the scheme enabled me to distinguish between effects via eggs and effects via parents. The effects of clutch size manipulation on post-hatching performance could be determined via the eggs by comparing the performance of offspring with known egg treatment (chapter 3). The effect of clutch size manipulation on offspring performance via the parents could be determined (chapter 2), because parents of different treatment category reared offspring with on average the same egg treatment (for further explanation see chapter 3).

I will first consider the effects found of clutch enlargement via the eggs and than those via the parents, before synthesizing them.

Effects via eggs

I could not detect any significant of egg treatment in terms of the probability of an egg to hatch, but eggs incubated in enlarged clutches had longer developmental time in one of the two years (chapter 3). This result indicates that the clutch size manipulation during incubation, and thereby potentially the developmental conditions of the embryos during incubation, could affect the performance of the offspring. Nevertheless, the effect of clutch size on developmental time was not a general pattern in both years, suggesting the influence of year-to-year variation in the environmental conditions.

In both years, offspring that hatched from eggs that experienced the enlarged treatment during incubation, had shorter tarsi during the nestling phase (chapter 3). This implies that the clutch size during incubation had consequences on the growth of offspring. Despite this effect on tarsus length, egg treatment did not affect the performance of offspring in terms of the probability to fledge and the probability to recruit. Although egg treatment had no detectable effect on these two fitness components, the effect on growth is expected to induce a difference in fitness. Birds with relatively larger tarsus length have been found to have better access to food, and to have more success in obtaining a breeding territory (Drent 1983), and mate preference in males is found to be related to tarsus length (Blakey 1994, Kempenaers *et al.* 1992, Verboven and Mateman 1997). Thus, it might be that if I had used a measure of reproductive success in the year after the clutch size manipulation (rather than merely the measure of recruitment probability), I might have detected an effect of egg treatment on the post-hatching performance of offspring.

Effects via parents

Offspring of first clutches did not differ in their probability to hatch, fledge or recruit with treatment (chapter 2), indicating that parents of the three treatment categories could care equally well for their offspring. Offspring of second clutches that were raised by parents that received enlarged first clutches during the incubation phase, on the contrary had a lower fledging probability than offspring reared by parents with either reduced or control first clutches during incubation (chapter 2). These findings suggest that parental ability to successfully rear a second clutch of nestlings was affected by their treatment during incubation of the first clutches. Parents that received experimentally enlarged clutches during the incubation phase had a reduction on the probability to survive locally to the subsequent breeding season in two out of three years (chapter 2). This finding implies that there is a cost of incubation for parents, but that these costs are not apparent every year. Note that clutch enlargement negatively affected the local survival probability of both female and male parents. Possibly, females can shift costs on their partners. They may do so either during the incubation phase itself, for instance, by demanding a larger contribution of incubation feeding of the male,

but also or during the nestling phase, for instance, by shifting a larger share of the required care for offspring to their partner.

The clutch size manipulations revealed some effects of clutch enlargement on fitness of either offspring or parents in the short term (i.e. within the current breeding attempt). In the long term, however, clutch size manipulations reveal a strong effect on the probability to survive to the subsequent breeding season of parents that received enlarged clutch during the incubation phase. The consequences of clutch enlargement were more apparent for the parents than for the offspring, which suggests that the trade-off between investment in current breeding attempt and self-maintenance of parents is more biased towards the offspring. Given that the great tit is a short lived species, such a balance is not surprising.

What is the mechanism that underlies the fitness costs of clutch enlargement during the incubation phase?

There is little data revealing the underlying mechanism explaining the reduced survival probability of parents that had to care for enlarged clutches during the incubation phase. The effect of egg treatment on the developmental time of eggs suggests that females were either not able or not willing to efficiently incubate enlarged clutches. Since females incubating experimentally enlarged clutches behaved similarly to females incubating control clutches, the most plausible scenario might be that it was the intensity of incubation (i.e. the amount of heat provided per egg within the clutch) that was different between the enlarged and control treatment groups. Clutch enlargement had little consequences within the season in terms of behaviour, energy or fitness components on eggs or chicks, but had fitness consequences on the parents in the subsequent breeding attempt within the same season and in the subsequent breeding season. This suggests that parents can bare the costs of incubating enlarged clutches after the actual breeding phase. To give an example of one possible scenario; if parents reduced their investment in immune function when rearing first clutches, this may have increased the chance on infections and this may thus have reduced their condition when breeding/ rearing second clutches (Siikamäki *et al.* 1997; Hanssen *et al.* 2005).

As an aside, I have made a first step to look into this idea. I have collected blood samples of some of the individuals (both males and females) involved in the clutch size manipulations during the breeding season of 2003. Blood smears of these samples indicate that great tits in the Lauwersmeer are infected with three types of blood parasites: Haemoproteus, Plasmodius, and Hepatozoon. The number of birds that were infected was relatively high (about 35 %). I could not detect a difference in the probability of being infected of birds from the three different treatment groups. However, I did find that females that had incubated enlarged clutches had a higher number of blood parasites (number of blood parasites in 10 000 red blood cells), and that if multiple infections occurred, this always concerned females from the 'enlarged' category (de Heij unpublished data). These

results support the idea that birds from the enlarged category may have invested less in immune defence, thereby running a higher risk of (multiple) infection. Yet, the year 2003 was the year in which no effect of clutch enlargement on the survival probability of parents was found. Further investment in this direction seems worthwhile.

CLUTCH SIZE DECISIONS IN GREAT TITS

The Lauwersmeer population

To estimate the overall fitness costs of caring for experimentally enlarged clutches from incubation onwards (i.e., during both the incubation and the chick rearing phase), the quantitative effects of both clutch size manipulations (i.e., during the incubation phase: chapter 2) and brood size manipulations (i.e., during the chick rearing phase: Tinbergen and Sanz 2004) were combined (for calculations: see chapter 2). These calculations revealed that the fitness consequences of incubation an enlarged clutch could be strong enough to change the positive selection on clutch size during the nestling phase into stabilising selection in the two years that there were clutch size related costs of incubation for the parents (Fig. 9.2, panel I). This means that in those years, clutch size decisions in the Lauwersmeer can be regarded as adaptive, i.e. the natural clutch size that maximize fitness. In the next section, I will present several points that deserve further consideration.

Year-to-year variation

In this thesis, several findings (chapter 2 and 3) indicate that temporal variation in selection pressure on clutch size in this population was considerable. The causes of these differences between the years remain unknown. Differences in environmental conditions between years (like the amount of precipitation, ambient temperature and food availability) are likely to play a role. Ambient temperature early in the season is expected to play an important role not only because low temperatures increase energy expenditure, but also reduce the food availability and hence the input of energy to balance the expenditure.

To avoid year-to-year variation, the costs of incubation and that of rearing nestlings should be measured in the same year. This can be achieved by using an experimental approach used by, for instance, Heaney and Monaghan (1995), and Visser and Lessells (2001) (see chapter 3 for a more detailed explanation). This approach has, however, also a disadvantage. To obtain a reliable estimate of the effect of the manipulation treatment, a certain number of nests per treatment category are needed and this requirement limits the number of categories possible. Most studies then only use two treatment categories per reproductive phase (control and enlarged clutches). As a result of omitting the third category (the reduced category in this example), the shape of the fitness curve over clutch size can not

be estimated. Thus, there is a trade-off between collecting knowledge on a more complete fitness curve and on avoiding possible confounding effects of year-to-year variation.

Costs of egg laying

In the calculations on overall fitness, the costs of egg laying was assumed to be low, and unrelated to clutch size (Fig. 9.2, panel I). Nevertheless, several studies reveal that there are fitness consequences related to laying extra eggs (Heaney and Monaghan 1995; Visser and Lessells 2001; Kalmbach *et al.* 2004). Visser and Lessells (2001), who performed a study on great tits in the Hoge Veluwe population have shown that the survival probability of female great tits that laid additional eggs was reduced in both years in which the experiment was performed (1998 and 1999). Depending on the costs of laying larger clutches, the inclusion of these costs in the calculations of the overall fitness for the Lauwersmeer population does not change the shape of the fitness curve over clutch size, but does affect the strength of stabilizing selection on clutch size (Fig.9.2, panel II). Few studies have tried to measure the benefits of egg laying less eggs than planned. If the costs of egg laying is linearly related to clutch size, laying smaller clutches should result in a fitness benefit for the parents. Depending on the strength of the fitness benefit, the selection on clutch size may change from stabilizing to negative selection (Fig.9.2, panel III). It is worthwhile to include estimates of the costs of egg laying in the study on clutch size decisions for the great tit population in the Lauwersmeer. Potentially, the costs of egg laying may also provide insight in the year-to-year variation in the selection on clutch size.

How to combine the costs of different reproductive phases?

When calculating the overall fitness for parents that took care of additional eggs from incubation onwards, the question arises how costs of the different reproductive phases interact. Costs of different reproductive phases may be independent of each other, so that the costs of the two reproductive phases can simply be added. Alternatively, the costs of different phases may interact with each other, in which case the costs of reproductive phases cannot be combined without empirical estimates. The latter scenario is actually quite likely, given that the allocation of resources to one phase will reduce the opportunity to invest resources in another phase. Thus, the costs of different reproductive phases may well interact with each other. As discussed in chapter 3, there are two general approaches to determine the costs of incubation. Either the costs of incubation are determined by comparing the performance of parents that incubated extra eggs and reared additional nestlings with that of parents rearing only additional nestlings (Heaney and Monaghan 1995; Visser and Lessells 2001) or the performance of parents that incubated extra eggs with those incubating control clutches (e.g. Heaney and Monaghan 1996; chapter 2). These two approaches may differ in the costs they

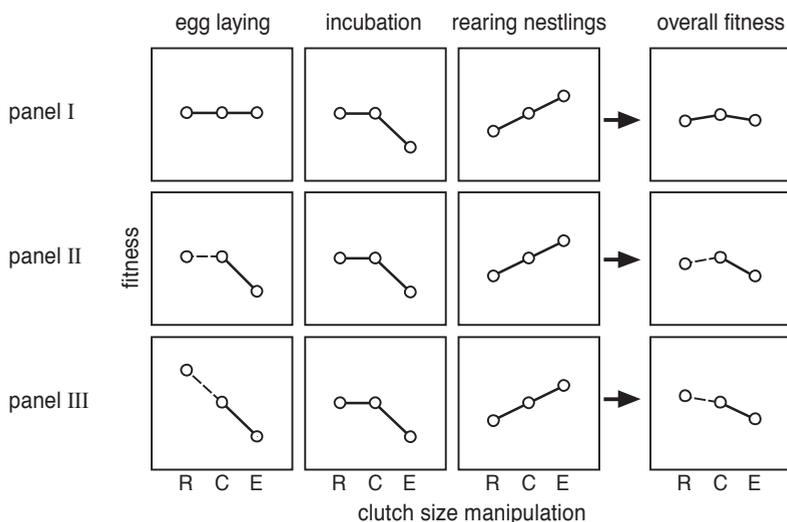


Figure 9.2. Estimated overall fitness for the great tit population in the Lauwersmeer based on the fitness consequences of three reproductive phases; the egg laying phase (Visser and Lessells 2001), the incubation phase (chapter 2) and the nestling phase (Tinbergen and Sanz 2004) in relation to clutch size manipulation (R, reduced; C, control; E, enlarged). In the first panel (I), overall fitness is determined on the basis of the costs of incubation and that of rearing nestlings; the costs of egg laying are assumed to be unrelated to clutch size (like in our study, see discussion chapter 2). In panel II, overall fitness is determined on basis of clutch size related costs during all the three reproductive phases; only a cost of laying larger clutches are assumed on basis of the work of Visser and Lessells 2001 for great tits in de Hoge Veluwe, the Netherlands. In panel III, overall fitness is determined on the basis of clutch size related costs during all three reproductive phases; the costs of egg laying are assumed to be negatively related to clutch size; the fitness benefits in relation to laying smaller clutches is purely deductive, and therefore the line is dotted.

measure. The former approach may identify the costs of incubation and that of the nestling phase in interaction with each other, while the latter determines the costs of incubation in isolation. The number of studies available is still too low to compare the performance of these two approaches in any detail. An experiment in which the two approaches are combined may reveal whether the costs of different reproductive phases are independent of each other additive or whether they interact. In such an experiment, at least three experimental categories are needed; parents of the first two categories would receive additional eggs at the start of the incubation period, of which parents in one of the categories would have to care for the additional eggs from incubation onwards, while those in the other category would only have to care for the additional eggs for the duration of the incubation phase. Parents in the third category would then receive additional chicks at the beginning of the nestling phase.

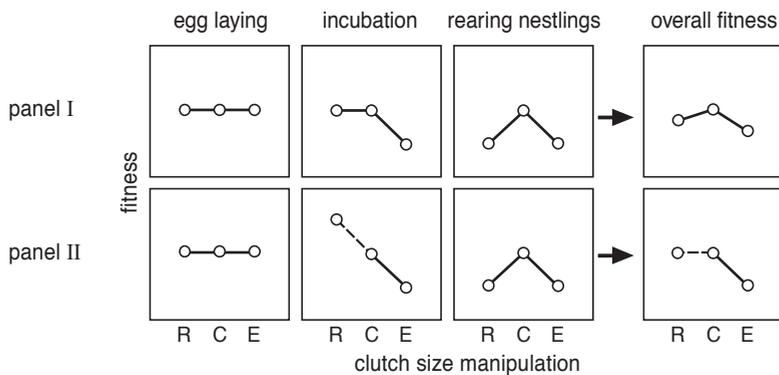


Figure 9.3. Estimated overall fitness based on the fitness consequences of three reproductive phases; the egg laying phase (Visser and Lessells 2001), the incubation phase (chapter 2) and the nestling phase (Tinbergen and Sanz 2004) in relation to clutch size manipulation (R, reduced; C, control; E, enlarged) for those populations of great tit in which brood size manipulations already revealed that birds lay the clutch size that maximise their fitness (for instance, the Hoge Veluwe). In the first panel (I), overall fitness is determined on the basis of the costs of incubation and that of rearing nestlings; the costs of egg laying are assumed to be unrelated to clutch size. In panel II, overall fitness is also determined on the basis of the costs of incubation and that of rearing nestlings only; in this panel the possible effect of a fitness benefit related to incubating reduced clutches is expressed.

Implications for other studies

Given that brood size manipulation in other populations (e.g. Perrins and Moss 1975; Pettifor *et al.* 1988; Lindén 1990; Tinbergen and Daan 1990; Pettifor *et al.* 2001) found that birds lay the clutch size that maximize their fitness, the findings in this study with regard to the strong effect of clutch enlargement on the reduction in survival probability of parents is not expected to effect the results of these studies (figure 9.3, panel I). Only if clutch reduction during the incubation phase results in a strong fitness benefit, the form of the fitness curve changes such that it would affect the clutch size maximizing fitness (figure 9.3, panel II). In this thesis, I did not find experimental evidence that parents with reduced clutches had fitness benefits; neither in terms of energy expenditure during nocturnal incubation nor in terms of fitness. Nevertheless, in the study on the fitness consequences related to clutch size manipulation, parents with reduced clutches were more likely to abandon their nest directly after manipulation (chapter 2), and therefore, extrapolation of these data should be done with caution, because the remainder group may not be a random selection of the population (chapter 2).

Conclusion

To conclude, the costs of incubation could be strong enough to change the positive selection detected on basis of brood size manipulations in the same population to

stabilizing selection. However, the costs of incubation were not apparent in all years. This discrepancy takes a long term experiment to judge the year effect in detail.

My findings for the Lauwersmeer population open the question whether costs and benefits originating from other reproductive phases will affect the conclusions from other studies that relied on brood size manipulations alone. One can imagine that in populations that experience strong seasonality, the fitness costs of egg laying may have a stronger impact on clutch size decisions than that of the nestling phase. Especially if laying and incubating smaller clutches increases parental survival substantially, the clutch size that maximizes fitness will change by shifting downwards.

The prime importance of ambient temperature in determining the energetic costs of reproduction poses the question how far we can unravel clutch size decisions separately from decisions on the timing of reproduction. Given seasonal temperature profiles, choosing a certain laying date implies certain energetic costs for all the three reproductive phases. Hence, clutch size and laying date decisions need to be studied in combination. Early breeding with a small clutch size may for instance be as costly as late breeding with a large clutch size. Obviously, combining the study of the decisions on both the timing and the intensity of reproduction in different reproductive phases would complicate matters; nevertheless, I believe that we should follow such an experimental approach if we really want know how selection shapes reproductive decisions.

