Energetic constraints on avian incubation: studies of three passerine species.

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Abstract.

Field studies were conducted with three species of passerine, in order to investigate the possibility that an energetic constraint limits reproduction during incubation. Swallows (*Hirundo rustica*), Dippers (*Cinclus cinclus*) and Great Tits (*Parus major*) were studied at sites in Central Scotland. All three species exhibit gynelateral intermittent incubation, so time and energy must be allocated between the conflicting demands of reproduction and self-maintenance.

An assessment of incubation ability in the Swallow was conducted by the manipulation of clutch size during incubation. There was evidence of a clutch size dependent cost, as the duration of the incubation period was prolonged for enlarged (15.6d) compared to reduced (14.8d) clutches. The proportion of eggs hatching successfully was also lower in enlarged (81%) than in reduced (92%) clutches, though enlarged clutches still produced the greatest number of hatched young. Clutch manipulation did not influence patterns of nest attendance, or female body condition. No effects of incubation effort were detected post-hatch on either parents or offspring.

The effects of clutch size on field metabolism during incubation were investigated in the Dipper. Clutch size was manipulated and energy use measured by means of the doubly labelled water technique. The results were combined with previous data collected for incubating Dippers. The field metabolic rate of 33 incubating females averaged 5.41 ± 1.34 cm^3 CO_2 g^{-1} h^{-1} , equivalent to a daily energy expenditure of 211.52 ± 51.25kJ $ind^{-1}d^{-1}$, *c*.3 times the basal metabolic rate. Clutch enlargement resulted in an increase in energy use to 4-6 times basal metabolism for some birds, but not for others. While the mean energy use did not differ between groups, the variation amongst birds was significantly greater for enlarged than control clutches. Energy use was also influenced by river flow rates, the duration of incubation sessions and behaviour during incubation recesses.

Manipulation of the energy budget of incubating Great Tits was achieved by the reduction of thermoregulatory demands. Treated nest boxes were supplied with additional heat during the hours of darkness, resulting in an elevation of nest air temperature of *c*.4°C above the corresponding temperature for a control group, lasting for a period of 9 hours. This produced an estimated energetic saving of 10kJ per night. Heated birds increased the duration of both the period of continuous incubation overnight and of incubation sessions throughout the following day, resulting in an additional 51 minutes per day spent incubating compared to the control group. The metabolic rate of 22 incubating Great Tits was 7.79 ± 2.43 cm^3 CO_2 g^{-1} h^{-1}, or 106.4 ± 32.2 kJ ind^{-1} d^{-1}, equivalent to *c*.3 times basal metabolism. Energy use escalated for control, but not for heated birds at low ambient temperatures.

The importance of reserve storage and utilisation, and of provisioning by the mate were evaluated in each species. A combined hypothesis was proposed to account for body condition during incubation, incorporating elements of programmed reserve utilisation, mass adjustment, maintenance of an insurance reserve and reproductive stress.

In summary, the study found evidence of an energetic constraint acting during incubation in these species. Energy use increased in a probabilistic manner with increasing clutch size, such that birds with large clutches increased their risk of being unable to incubate the entire clutch successfully. It was suggested that such a constraint could contribute to the determination of an upper limit for avian clutch size.

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Chapter 1:

Introduction.

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Introduction.

1.1 The evolution of avian clutch size.

One of the fundamental assumptions of life history theory is that individuals have access to only limited resources, which they must allocate between conflicting demands (Levins 1968, Sibly and Calow 1986). The advantages to be accrued from investment in one behaviour must be off-set against those foregone from an alternative tactic (Williams 1966). Natural selection will act on the heritable component of the variation in allocation schedules, to favour those individuals which maximise the contribution of their genotype to the next generation. Within certain physiological, mechanical and phylogentic constraints, 'optimal' behaviours might be expected to evolve, which represent the allocation of time and materials to activities in ways which maximise individual fitness (Stearns 1992).

Selection for these 'optimal' strategies during the breeding season may be expected to be strong, as production of offspring offers an opportunity of directly augmenting fitness, while high levels of energy usage at this time (Bryant and Tatner 1988a) could make mistakes costly. There has been much interest in the factors that determine the level of investment in reproduction, specifically, what forces constrain the evolution of escalating offspring production.

Lack (1947, 1954) stimulated much subsequent study on the evolution of avian clutch size, by his proposal that the most common clutch size in a population should be that which results in the production of the greatest number of offspring, i.e. that reproductive investment is constrained by parental ability to feed the young. However, several studies have shown that the highest number of young are commonly reared from nests with clutch sizes greater than the modal clutch size (Perrins 1964, Cavé 1968, Perrins and Moss 1975). Three hypotheses have been suggested to explain this paradox. While they may not be mutually exclusive, their significance may vary in natural populations.

(i) The 'year to year' hypothesis.

Firstly, the annual variation in conditions, and hence in selection pressures, could serve to maintain a variety of genotypes, represented by females with a range of clutch sizes (Lack 1954, Nur 1987).

(ii) The 'individual optimisation' hypothesis.

The 'individual optimisation' hypothesis (IOH) suggests that optimal clutch size varies between individuals on the basis of their own condition (Drent and Daan 1980) or the quality of their habitat (Högstedt 1980) and that birds are able to phenotypically adjust their clutch size in relation to their circumstances (Perrins and Moss 1975).

(iii) The 'trade-off' hypothesis.

A third hypothesis suggests that the variety of clutch sizes represent a range of reproductive strategies, all of which are equivalent in terms of fitness pay-offs, due to trade-offs experienced between current reproduction and other life history variables (Cody 1966, Williams 1966, Charnov and Krebs 1974, Calow 1979, Stearns 1989). For example, current fecundity may be offset against future fecundity, or against the condition, or the survival rates of parents (Stearns 1992). The number of offspring produced may be increased only at the expense of their survival or fecundity (Smith *et al.* 1989).

1.2 Evidence for a cost of reproduction,

Although many researchers have undertaken to investigate the relative importance of these hypotheses, the results have been controversial and open to differing interpretations (e.g. Nur 1984, Pettifor 1993a). Evidence suggests that the optimal clutch size does vary from year to year, (Perrins and Moss 1975, Järvinen and Väisänen 1984, Nager and van Noordwijk 1995), providing some support for the first hypothesis. Experimental manipulation of reproductive output, by manipulation of the number of young reared has shown, in certain studies, that females laid a clutch size which, consistent with the IOH, maximised their own reproductive success at the current breeding attempt (Högstedt 1980, Alatalo and Lundberg 1989, Pettifor *et al.* 1988, Pettifor 1993). However, in other studies, productivity could be increased by enlarging brood size (De Steven 1980, Slagsvold 1982, Nur 1984), with no apparent cost.

Evidence of a cost associated with reproduction has been found in many, but not all, studies that have investigated the problem (for reviews see Lessells 1991, Roff 1992, Stearns 1992). Examination of phenotypes in non-manipulated populations has revealed some evidence of a trade-off. For example, a negative correlation was found between current reproduction and survival in invertebrates (Maynard-Smith 1958, Snell and King 1977, Partridge and Farquhar 1981) and in birds (Bryant 1979, Tinbergen *et al.* 1985). Again, the evidence is conflicting, as other studies have found evidence of positive correlation between fecundity and survival (Högstedt 1980). Phenotypic correlations of this sort may be complicated by the existence of a third confounding variable. If birds are indeed able to phenotypically adjust their clutch size to suit their conditions (as proposed by the IOH), large clutch sizes will be found where conditions are good, and parameters such as survival or future reproductive success are also likely to be high.

Experimental manipulation of reproductive effort is more likely to establish whether a cost exists (Gustafsson and Sutherland 1988), because the link between reproductive effort and ability may be broken. Manipulative studies have found evidence that the level of current reproduction can have effects on future fecundity (Slagsvold 1984, Røskaft 1985, Lessells 1986, Lindén 1988, Smith *et al.* 1987, 1989, Tinbergen 1987, Hegner and Wingfield 1987, Gustafsson and Pärt 1990, Wernham 1993), on parental condition (Lessells 1986), on survival (Askenmo 1979, Nur 1984, Dijkstra *et al.* 1990, Thompson 1992) or on the survival and fecundity of offspring (Gustafson and Sutherland 1988, Smith *et al.* 1989). Again, other studies were able to find no evidence for a cost of reproduction, although there has been some suggestion that the statistical power of the tests used were frequently insufficient to detect small, but biologically meaningful, costs (Graves 1991, Roff 1992).

1.3 During which stage of the breeding cycle is reproduction constrained?

Ideally, manipulation of reproductive effort should incorporate the demands of each stage of offspring production and care throughout the breeding cycle. It is perhaps due to Lack's (1947) proposal, that the clutch size of altricial birds be constrained by the number of young which the parents can feed, that the majority of manipulations of reproductive effort have been performed by removal or addition of young just after hatch (Askenmo 1979, De Steven

1980, Slagsvold 1984, Finke *et al.* 1987, Hegner and Wingfield 1987, Lindén 1988, Pettifor 1993), thus neglecting the costs of egg production, incubation and post-fledging care.

Recent studies have suggested that the demands of egg production are not negligible (Bolton *et al.* 1993, Houston *et al.* 1995). A trade-off between egg production and fledging success has been demonstrated experimentally in the Lesser Black-Backed Gull (Monaghan *et al.* 1995). The period of post-fledging care has also been postulated to be demanding (Drent and Daan 1980), as parents may feed fledglings at a higher frequency than nestlings (Smith 1978, Moreno 1984). Where first and second broods overlap temporally, the time allocated to post-fledging care of the first brood, at the expense of egg production and incubation of a second clutch, may represent a trade-off between reproductive success early and later in the season (Hegner and Wingfield 1987, but see also Verhulst and Hut 1996).

1.4 The 'incubation ability' hypothesis.

Early workers recognised the possibility that the ability of a parent to successfully incubate the clutch could, theoretically, limit clutch size (Lack 1954, Klomp 1970). Although the energetic demands of incubation have been the subject of some controversy (Kendeigh 1973, Mertens 1977, Walsberg and King 1978, Vleck 1981, Biebach 1979, 1984, Haftorn and Reinertsen 1985, Colemann and Whittall 1988), the time constraints imposed by incubation behaviour may make demands on the rate of energy acquisition which are difficult to satisfy, thus making incubation an 'energetic-bottleneck' in the reproductive process (Yom Tov and Hilborn 1981). For species where incubation is undertaken by one sex alone, levels of nest attendance of up to 80% of the active day (Skutch 1962) restrict the time available for foraging. Reliance must be made upon the provision of food by the mate or on the programmed use of stored reserves (Moreno 1989b), or foraging rates must be increased to 4-8 times those of non-breeding birds (Walsberg and King 1978), in order to satisfy the energy budget.

Given these reasons to suspect that incubation may constitute a potential period of crisis or an 'energetic bottleneck' in the reproductive process (Yom Tov and Hilborn 1981), it is important that evidence for a reproductive constraint during incubation be evaluated.

Until recently, the energy required during normal patterns of incubation in the field has proved difficult to measure. Attempts to model the costs of incubation have been made by a combination of measurement and estimation of heat flows in the nest (Kendeigh 1973, Mertens 1977, Walsberg and King 1978). Techniques of gas analysis, using the nest box as a chamber calorimeter have enabled the study of the metabolic rate of birds whilst on the nest (Vleck 1981, Biebach 1981, 1984, Haftorn and Reinertsen 1985). These methods were restricted to estimate the demands of 'incubation' on the nest, and neglect the demands of 'incubation' in the broader sense of the word, which incorporates those activities off-nest which form a normal, indeed an essential, part of free-living behaviour at this time.

In order to include the demands of other associated behaviours, total energy usage during 'incubation' has been estimated by measures of food intake (El Wailly 1966, Colemann and Whittall 1988). Such studies are effectively restricted to laboratory situations. Recently, the doubly labelled water technique, which enables study of the energy use by free-living birds (Bryant and Tatner 1985) has been applied to incubating birds (for review see Weathers and Sullivan 1989, Tatner and Bryant 1993). Results to date suggest that the energetic demands of incubation do not differ systematically from those during nestling rearing, generally thought of as the 'high cost' stage in the breeding cycle.

1.5 This study: aims and approaches.

The central aim of this study was to investigate the possibility that birds are energetically constrained during incubation, and, as a subsidiary aim, to contribute to the body of data on free-living energy use by incubating birds, by the application of the doubly labelled water technique to Dippers and Great Tits. These aims were approached by manipulative studies on three passerine species, each study differing in both the method of manipulation and the parameters measured to monitor the effects of the manipulation.

In two studies (Chapters 3 and 4), the level of reproductive output was manipulated directly, via the experimental modification of clutch size in Swallows and Dippers. Chapter 3 describes an experiment in which a large scale approach to manipulation of clutch size during incubation was employed in the Swallow. Parameters of intra-seasonal reproductive success were measured in order to investigate the possibility that clutch-size dependent reproductive costs are associated with reproductive effort during incubation. Chapter 4 describes an experiment which was conducted in the shorter term, using Dippers as subjects. Again, the effort devoted to reproduction was manipulated by alteration of clutch size during incubation.

In this study, the doubly labelled water technique was applied to measure free-living energy expenditure over a 48 hour period. This was combined with techniques of automated nest monitoring and behavioural observations, in order to investigate the effect of clutch size on patterns of energy use and incubation behaviour.

The approach of the third study (Chapter 5) differed, in that it attempted to manipulate the supply of energy available for reproduction, rather than reproductive output *per se*. In this study, clutch size was not manipulated. Instead, the possibility that incubating Great Tits were constrained in their investment in incubation by energy acquisition was investigated, by manipulating the energy available for incubation in the energy budget. The response was monitored by recording patterns of energy allocation and behaviour.

Traditionally, manipulation of the supply of energy during reproduction has been attempted via supplementary feeding (Cavé 1968, Kjallander 1974, Yom Tov 1974, Smith *et al.* 1980, Högstedt 1981, Newton and Marquiss 1981, Dijkstra *et al.* 1982, Harper 1984, Davies and Lundberg 1985, Nilsson 1994). The manipulation of food availability could alter a bird's perception of current and future conditions, as well as simply the immediate availability of energy. This study employed a different technique, to 'liberate' energy from the birds' own budget, by the reduction of energy demanding processes which conflict with reproduction. Thermoregulatory demands were reduced for experimental birds by the manipulation of temperature in the nest box overnight. The subsequent energetic and behavioural response was monitored during the following day and compared with that of a control group of birds, whose nests were unheated but were otherwise exposed to identical conditions of current ambient temperature and food availability.

The energetics approach to these problems is based on an assumption that high levels of energy use correlate with reproductive costs (Bryant 1991). Levels of expenditure beyond some threshold level, representing a sustainable work rate, could incur physiological costs and increase the probability of mortality (Drent and Daan 1980, Bryant and Tatner 1985). If energy expenditure is high relative to energy availability, the probability of energy imbalance and subsequent deterioration in body condition will increase. The deployment of increasingly risk-prone modes of foraging may also contribute to an increase in the probability of mortality (Lima 1988). In these ways, high levels of energy use during breeding may translate, in a probabilistic manner, to fitness costs in terms of reduction in future fecundity or survival.

Mass loss, or deterioration in 'condition' during reproduction has been interpreted as being indicative of stress or as a cost of reproduction, (Ricklefs 1974, Bryant 1979, Nur 1984a). However, the possibility that mass loss during breeding may be adaptive must be addressed (Freed 1981, Norberg 1981) if the costs, as well as the benefits of reserve storage are to be taken into account (Witter and Cuthill 1993). During incubation, the costs and benefits of mass storage may differ from those at any other time of year, as incubating parents are sedentary and potentially insulated by nest material for a high proportion of the day. In some species, mass change during incubation may function as a transition between the high level of reserves necessary for egg production and the advantages of a low mass during the highly active stage of nestling rearing (Moreno 1989b). The three studies provided an opportunity to evaluate the factors which influence mass change in each of the three species.

Swallows, Dippers and Great Tits are all small (15 - 75g) passerines, whose pattern of incubation conforms to that described as GII (Williams 1992), or gynelateral intermittent incubation. In each species, the female incubates alone, receiving only limited aid from her mate in the form of a certain degree of provisioning. Each species differed in ways which suited each experiment. Differences in the extent of reliance on feeding by mates and the programmed reliance on stored reserves enabled investigation of the factors influencing the strategies of energy use and acquisition by incubating birds.

Chapter 2:

General Methods.

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2.1 Introduction.

This chapter describes the methodology of those techniques which were common to the three studies described in subsequent chapters. Fieldwork was carried out with incubating Swallows, Dippers and Great Tits during the breeding seasons of 1993 to 1995 inclusive, at sites in Central Scotland. The distribution of breeding dates of the three species enabled study of all three species during a single season. However, in order to focus effort more intensely, Swallows were studied in 1993 and 1994 only and Great Tits were studied in 1994 and 1995. Dippers were studied in all three seasons.

2.2 Capture and examination of individuals.

In each of the three species studied, incubation was performed by the female alone, so capture techniques were directed at incubating females, though males were sometimes caught incidentally. During breeding, Swallows, Dippers and Great Tits could be reliably sexed by the presence of a brood patch.

Incubating Swallows were caught either during the daytime, by use of a mist-net erected across the entrance to the building containing the nest, or by night, between midnight and dawn, by placing a hand-net over the nest and using the beam of a torch to dazzle the birds. Following night capture, females were either returned to the nest, or released outside the barn if natural light was adequate. Most captures were made just before dusk, when females were not readily flushed from the nest and males were commonly roosting within the barn. Combined use of a hand net to capture the female from the nest and a mist-net over the barn entrance at this time, generally resulted in capture of both members of the pair. Birds were then processed and released with sufficient natural light still available to enable their successful return to the nest. Records of temperature in the nest showed that females did resume incubation before nightfall following release at dusk.

Incubating female Dippers were caught by day from the nest, by placing a fisherman's landing net over the nest entrance.

Great Tits nested in boxes around the campus of Stirling University. Nests were attached to the supporting tree in such a manner that they could be brought down for examination of the nest contents by the use of a 1.5m pole. The majority of incubating Great Tits would remain on the nest while the box was removed from the tree and could then be lifted from their eggs. Occasionally, particularly wary birds were captured within the box by fitting a perspex trap door to the nest, which could be pulled across the entrance from a distance by means of a string, to trap an incubating female.

2.2.1 Structural size and mass of incubating birds.

Adult biometrics were recorded as follows:

1. **Wing length** (Swallows, Dippers and Great Tits): using 150mm stopped rule (nearest mm). Flattened maximum chord.
2. **Outer-tail length:** (Swallows, Great Tits): using 150mm unstopped rule (nearest mm). Pygostyle tip to the tip of the outermost tail feather. Both left and right tail feathers were measured and an average value used in calculations unless one feather was obviously broken, in which case the longer measurement only was taken.
3. **Inner tail length** (Swallows): 150 mm unstopped rule (nearest mm). Pygostyle tip to the tip of the innermost tail feather.

   Measures 4 to 8 were made using dial callipers (nearest 0.1mm):
4. **Keel length** (Swallows, Dippers, Great Tits): Anterior notch to posterior edge of sternum.
5. **Head to bill length** (Swallows, Dippers, Great Tits): Maximum length from back of skull to bill tip.
6. **Bill length** (Dippers): Tip of bill to the end of the feathering on the top surface of the upper mandible.
7. **Bill depth** (Dippers): Measured vertically at the anterior edge of the nares.
8. **Tarsus** (Swallows, Dippers, Great Tits): Length of tarso-metatarsus bone.

Mass was measured at each capture using a Pesola spring balance (50g for Swallows and Great Tits, to the nearest 0.1g, 100g balance for Dippers, to the nearest 0.1g). Where defecation occurred during handling, post-defecation mass was recorded and used in subsequent analyses.

2.2.1.1 Swallows.

Table 2.1 shows structural size measured for incubating female Swallows in 1993 and 1994. Each female was included only once in the analysis; where a bird was caught in both years, measures from a single observation were selected at random. Pearson correlation coefficients between each pair of measures are shown in Table 2.2.

Using stepwise multiple regression techniques, 12.9% of the variation in mass was explained by keel and wing length. Statistics for the regression are shown in Table 2.3. Principal components analysis was used to reduce the data to two factor scores, the first being interpreted as a measure of wing and tail feather length and the second as a measure of structural size. Regression of mass on these factor scores explained a similar amount of the variation (12.1%) to the multiple regression. The factor loading scores are presented in Table 2.4, and the relevant regression equation in Table 2.5. The first factor only was entered as significant.

2.2.1.2 Dippers.

Measures of the structural size of 29 incubating female Dippers, measured in 1993, 1994 and 1995 are presented in Table 2.6. One measure per female was included in the analysis. Pearson correlation coefficients between the variables are presented in Table 2.7.

A single value for the mass of each individual was calculated by taking the average of the mass recorded at the beginning and end of a 48 hour period, comprising the doubly labelled water sampling period. Where birds were sampled in more than one year, one such mean was selected at random. In a stepwise multiple regression, 25% of the variation in mass was explained by wing length alone (regression statistics in Table 2.7). Principal components analysis was used to reduce the data to two factor scores, whose factor loadings are shown in Table 2.8. Stepwise multiple regression included factor 2 only (Table 2.9). The regression was able to explain less of the variation in mass (21.8%) than the linear regression on wing length alone.

2.2.1.3 Great Tits.

The mean structural size of 26 female Great Tits captured during incubation in 1994 and 1995 is shown in Table 2.11. Each bird was entered only once. The data set was too small or too variable for correlations to be significant (Table 2.12). In a multiple regression of mass (averaged for first and second capture) on measures of structural size, only tail length was entered as significant, although this alone accounted for 60.1% of the variation in mass (Table 2.13). Principal components analysis extracted 3 factors, whose rotated factor loadings are shown in Table 2.14. Stepwise multiple regression techniques selected only the first factor as significant at the 5% level. This factor was tightly related to tail length. P.C.A. failed to explain any more of the variation in mass than tail length alone (28.1%).

2.2.2 Body condition of incubating birds.

Whilst the concept of body condition is an important one in many avian studies, its definition and quantification are often problematic (Piersma and Davidson 1991). Condition is commonly used to describe the size of the nutrient reserve, estimated by the quantity of protein or lipid reserves. However, the costs associated with avian fat storage (Witter and Cuthill 1993) can create situations where individuals storing high reserve levels may be less fit than leaner individuals (Witter *et al.* 1994). The strategies of reserve maintenance during breeding are discussed elsewhere (Chapters 4 and 6.).

Body condition in incubating Dippers and Great Tits was estimated by mass, or size-adjusted mass, where this accounted for a significant proportion of the variation. Estimates of body condition in incubating Swallows were provided by three measures; body mass and estimates of the protein and the lipid reserve. In Swallows, mass has been shown to be a reliable indicator of both total lipid and protein content (Thompson 1992), while size-adjusted measures only marginally improved the relationship.

Table 2.1 Mean structural size of incubating female Swallows, measured in 1993 and 1994.

Size measurement.	Mean (mm)	s.d.	n.
Wing	124.83	2.57	104
Keel	20.91	1.10	103
Tarsus	11.35	0.46	103
Head-bill	30.26	0.68	104
Outer tail	90.18	6.01	104
Inner tail	44.93	1.96	102

Table 2.2 Pearson correlation coefficients between measurements of structural size of incubating female Swallows. Values of the correlation coefficient are presented with sample sizes in brackets, and the significance of the correlation represented by p values.

	Wing	Keel	Tarsus	Head-bill	Outer tail
Keel	.090 (103) p = .364				
Tarsus	.204 (103) p = .039	.177 (102) p = .076			
Head-bill	.111 (104) p = .261	.289 (103) P = .003	.291 (103) p = .003		
Outer tail	.466 (104) p = .000	-.106 (103) p = .286	.120 (103) p = .226	-.014 (104) p = .886	
Inner tail	.436 (102) p = .000	-.017 (101) p = .864	.211 (101) p = .034	.047 (102) p = .642	.357 (102) p = .000

Table 2.3 **Stepwise multiple regression statistics for the relationship between mass of incubating female Swallows (g) and keel and wing length (mm). Variables not entered at the 5% level of significance were tarsus, outer tail, inner tail, head-bill.**

Variable	Cum. r^2	Regression coefficient	t	p	Beta
Keel	.076	.297	2.520	.014	.250
Wing	.129	.125	2.335	.022	.231
Constant		-.292	-.042	.966	

Table 2.4 **Factor loading scores of a principal components analysis of the structural size of incubating female Swallows.**

	PC1	**PC2**
Wing	.787	.170
Keel	-.120	.719
Tarsus	.304	.612
Head-bill	.015	.768
Outer tail	.784	-.124
Inner tail	.750	.065

Table 2.5 **Stepwise multiple regression of mass of incubating female Swallows with factor scores derived from P.C.A. of structural size. The first factor (PC1) was not entered as significant at the 5% level.**

Variable	Cum. r^2	Regression	T	p	Beta weight
PC2	.121	.464	3.532	.001	.347
Constant		21.514	161.538	.000	

Table 2.6 Structural size of incubating female Dippers, measured 1993 to 1995.

Measurement	Mean (mm)	s.d.	n
Wing	89.16	1.65	29
Keel	27.37	1.56	29
Tarsus	28.32	.84	29
Head-bill	46.28	.87	29
Bill length	15.77	.68	29
Bill depth	4.48	.12	29

Table 2.7 Pearson correlation coefficients between measures of structural size in incubating Dippers. Correlation coefficients and p values are presented for 29 females.

	Wing	Keel	Tarsus	Head-bill	Bill length
Keel	-.109 p = .574				
Tarsus	.228 p = .234	.304 p = .109			
Head-bill	.196 p = .309	.355 p = .059	.348 p = .065		
Bill length	-.101 p = .601	.420 p = .023	.334 p = .077	.403 p = .030	
Bill depth	.032 p = .868	.243 p = .203	.163 p = .399	.494 p = .006	.590 p = .006

Table 2.8 **Stepwise multiple regression of mass of incubating female Dippers (g) on measures of structural size (mm). Of wing, keel, tarsus, head-bill, bill length, bill depth, only wing length was entered as significant at the 5% level of significance.**

Variable	Cum. r^2	B	T	p	Beta weight
Wing	.248	.724	2.983	.006	.498
Constant		-1.794	-.083	.935	

Table 2.9 **Factor loading for rotated factors derived using principal components analysis of measures of structural size in female Dippers.**

	PC1	PC2
Wing	-.202	.891
Keel	.683	-.032
Tarsus	.411	.579
Head-bill	.644	.452
Bill length	.846	-.008
Bill depth	.739	.108

Table 2.10 **Stepwise multiple regression analysis of mass of incubating Dippers (g) against factor scores derived from structural measures by Principal Components Analysis. PC1 was not entered as significant at the 5% level.**

Variable	Cum. r^2	B	T	p	Beta weight
PC2	.218	1.119	2.742	.011	.467
Constant		62.726	156.430	.000	

Table 2.11 **Structural size of incubating female Great Tits in 1994 and 1995. Data represent the mean and standard deviation of data from 26 females.**

Measurement.	Mean (mm)	s.d.	n
Wing	73.28	1.46	26
Keel	17.67	1.78	26
Tarsus	20.68	.35	26
Head-bill	31.73	.52	26
Bill-length	11.21	.63	26
Outer tail	60.47	2.79	26

Table 2.12 **Pearson correlation coefficients between measures of structural size in incubating Great Tits. Correlation coefficients and p values are presented for 26 females.**

	Wing	Keel	Tarsus	Head-bill	Bill length
Keel	.166 p = .419				
Tarsus	-.124 p = .546	.130 p = .528			
Head-bill	.157 p = .444	.041 p = .842	-.324 p = .107		
Bill length	-.211 p = .302	.073 p = .724	.086 p = .677	.236 p = .247	.236 p = .247
Tail	.326 p = .104	-.007 p = .974	.026 p = .900	.008 p = .970	-.201 p = .325

Table 2.13 Regression of mass of incubating female Great Tits on measures of structural size. In a stepwise regression in which wing, keel, tarsus, head-bill, bill length and outer tail were independent variables, only outer tail was entered as significant at the 5% level.

Variable	Cum. r^2	Regression	T	p	Beta weight
Tail	.601	.286	4.910	.0002	.775
Constant		4.317	1.217	.241	

Table 2.14 Factor loading for rotated factors derived using principal components analysis of measures of structural size in female Great Tits.

	PC1	PC2	PC3
Wing	-.637	.274	.260
Keel	.121	-.047	.839
Tarsus	-.111	-.736	.398
Head-bill	-.053	..845	.201
Bill length	-.637	.258	.436
Tail	.721	-.029	.045

Table 2.15 Stepwise multiple regression analysis of mass of incubating Great Tits (g) against factor scores derived from structural measures by Principal Components Analysis. PC1 only was entered as significant at the 5% level.

Variable	Cum. r^2	B	T	p	Beta weight
PC1	.282	.571	2.505	.024	.531
Constant		21.698	107.849	.000	

The pectoral muscles comprise about half of the skeletal muscle mass in birds (Ward 1969) and are often used as an index of total protein content. The condition of this muscle has been related to breeding ability (Jones and Ward 1976, Ankney and McInnes 1978, Houston *et al.* 1983, Bolton *et al.* 1993). Various methods have been used to measure the size of the protein reserve using non-destructive techniques in live birds (Walsberg 1988, Bolton *et al.* 1991, Selman and Houston in press). In this study, ultrasonic sound was used to estimate thickness of the pectoralis muscle (Baldassare *et al.* 1980, Sears 1988, Newton 1993).

A portable Krautkramer ultrasonic flaw detector (model USK7) with an Alpha2 Aerotech 10MHz transducer probe was used. The probe emits an ultrasonic pulse, which is reflected on detecting a change in medium, in this case, where muscle meets the bone of the sternum. The time taken for the pulse to travel through the muscle is displayed on a cathode ray screen in arbitrary units. The equipment was checked prior to each reading by measuring the thickness of a Perspex block. Breast feathers were dampened with alcohol and brushed aside, whilst the probe was placed lightly on the left pectoralis muscle, normal to the surface and adjacent to the keel. The contact between probe and skin was moistened with alcohol. Three measurements of pectoralis thickness were made at the anterior (US1) and posterior (US3) ends of the muscle block and mid-way between the two (US2). Measurements were taken from the left pectoralis only to reduce handling time, as measurements taken from both left and right sides have been shown to be highly correlated (Ward 1992).

Three indices of the size of the pectoralis muscle were calculated from these measures (following Newton 1989):

(i) USMEAN: This is an average measure of pectoralis thickness, calculated as

Eqn 2.1.

$$\text{USMEAN} = \frac{(\text{US1} + \text{US2} + \text{US3})}{3}$$

(ii) USVOL: This represents the absolute volume of pectoralis muscle present.

Eqn 2.2.

$$\text{USVOL} = \frac{(\text{US1} + \text{US2} + \text{US3}) \times \text{keel length (mm)}}{1000}$$

(iii) USI: This is a size-adjusted index of muscle thickness per unit of keel length .

Eqn. 2.3.

$$\text{USI} = \frac{(\text{US1} + \text{US2} + \text{US3})}{\text{keel length (mm)}}$$

Estimates of body lipid content were made by assigning a rank score to the visible deposits of subcutaneous fat. Although this technique has been applied with variable accuracy (Krementz and Pendleton 1990, Scott *et al.* 1996), it has been used reliably in the Swallow ($r^2 = .749$, n = 11 non-laying female Swallows, Thompson 1992), the House Martin (Bryant and Wetserterp 1983) and the Sand Martin (Jones 1987). In this study, the deposits in both the tracheal/claviculo-coracoid (inter-clavicular) pit and at the posterior edge of the sternum were scored on a 5 point scale (5 being the greatest), to give an anterior and posterior scores (FS1 and FS2 respectively). These were summed to give an overall measure of lipid deposition.

2.2.3 Ringing and ageing individuals.

All individuals were equipped with a metal British Trust for Ornithology ring at capture, which displayed a unique identification number. Dippers were assigned a unique combination of three colour rings by which they could be identified without capture.

A minority of Swallows had been ringed in previous studies (Thompson 1992, Ward 1992) and could be aged from previous records. In other cases, age could be determined accurately only for those birds ringed as pulli in 1993 which returned to breed in 1994.

Dippers were generally ringed as pulli in the course of concurrent studies (Logie 1996). For unringed adults, distinction was made between first year breeders and older birds by the presence of white tips on the primary and greater coverts, and of a buff coloration under the tail coverts of yearlings (Galbraith and Broadley 1980, Galbraith *et al.* 1981, Svensson, 1970).

Very few Great Tits had been ringed in previous studies (Johnstone 1990). Distinction was made between first year birds and older individuals according to the coloration of the primary coverts (Svensson 1970).

2.3 Nesting parameters.

During the weeks preceding laying, nest and potential nest sites of each species were visited on a weekly basis in order to identify nest occupation. More regular inspections were made during nest building and completion to determine laying dates. Some nests were visited daily during laying, but where this was not possible or undesirable due to disturbance, the assumption was made that egg laying had proceeded at a rate of one egg per day, which is the general pattern in most small passerines (Perrins 1979). However, the occurrence of nest parasitism or laying interruptions would violate this assumption. Laying interruptions have been reported in this population of Swallows, at a frequency of 14 - 18% of nests (Ward 1992), associated with periods of poor weather. Laying interruptions in the Tits have been observed in 6.7% of nests (Dhondt *et al.* 1983). Again, these are thought to coincide with periods of energetic stress, their frequency being reduced by experimental alteration of temperature (Yom Tov and Wright 1993) and food availability (Nilsson and Svensson 1993). In a previous study (Ward 1992), no interruptions were recorded in the laying sequence at 11 Dipper nests which were visited daily. However, in this study, one interruption was recorded, where laying of the penultimate egg was suspended by one day. The date of suspension coincided with the peak rates of flow of a short flood in late March 1994, when flow rates reached 11.7 m^3s^{-1}, compared with a monthly average of 6 m^3s^{-1}. No other cases of interrupted laying were recorded for Dippers, although, in general, Dipper nests were not checked daily in this study.

Intra-specific nest parasitism has been reported amongst several species (Birkhead and Møller 1992). Egg dumping is apparent at sites where colonies are large and population density high (Møller 1987 (a) and (b)). Although Ward (1992) did not detect any evidence of egg dumping in this Swallow population (Ward 1992), current DNA fingerprinting suggests that egg-dumping does occur, albeit rarely (Bryant pers comm.).

Because of the difficulty in monitoring the transitional onset of incubation in the period between laying of the first egg and onset of full incubation, incubation was assumed to begin on the day of clutch completion in all cases. However, in all three species, females incubated the partially complete clutch to some extent during the night over the course of the laying period and some clutches were also found with eggs uncovered and warm during the day at this time.

In a sample of the Swallow nests inspected during laying, incomplete clutches were found to be warm on 12 of 30 occasions (40%). On one occasion, the first egg of the clutch was found warm on the day it was laid. However, in most cases, incubation during laying began after the laying of the third or fourth egg.

In 21 Dipper nests visited before clutch completion, 7 nests (33%) were found with warm eggs, 3 of which were found on the day the penultimate egg was laid, but on 4 occasions the first egg of the clutch was found to be warm. In one case, the female was flushed from the nest containing the first egg of the clutch at 12:00h. Tyler and Ormerod (1994) suggest that not all Dipper eggs are laid early in the morning, so in these cases, the nest checks, made around 12:00h, could have occurred shortly after laying whilst eggs still retained some warmth. Tyler and Ormerod (1994) interpret the somewhat asynchronous hatch (1 to 2 days) at some nests as evidence that incubation may commence with the laying of the penultimate egg.

Incubation in the Great Tit may begin before the clutch is complete, at least during the night. The amount of time devoted to incubation during the laying period is variable, and has been shown to vary inversely with clutch size (Haftorn 1981b). Use of the nest temperature monitor during the laying period in these species would provide more accurate information on the extent of incubation performed before clutch completion.

Where clutches were found during incubation and date of laying was unknown, the date of hatch (DOH) was used to estimate laying dates, under the assumptions that (i) eggs were laid daily from the date of laying the first egg (DOFE = date of first egg), (ii) the clutch size at finding was equal to the original clutch size (CS), (iii) incubation commenced on the date of clutch completion and (iv) incubation lasted for an incubation period of length IP days before the chicks hatched.

Eqn 2.4. $\mathrm{DOH} = \mathrm{DOFE} + (\mathrm{CS} - 1) + \mathrm{IP}$

In cases where date of hatch was also unknown, or where eggs did not hatch, the number of days since the start of incubation was estimated using the airspace diameter technique (Section 2.3.1, Ward 1992).

2.3.1 The use of egg airspace diameter to determine the stage of incubation.

Introduction. Many studies of avian reproduction require accurate information on dates of egg laying and of hatch. Whilst daily nest inspection is the most certain method of obtaining these data, the time investment required can limit the number of nests studied and the necessary level of disturbance to breeding birds may be unacceptable. Efforts have been made to develop techniques which estimate the age of eggs of a clutch, found complete and warm.

Eggs of all species lose approximately 16% of their initial mass during incubation (Drent 1975), due almost entirely to water loss (Drent 1970). The resultant decline in egg density through incubation has been used to estimate the progress of incubation. Estimates of density can be made by measurement of egg mass and calculation of egg volume (Furness and Furness 1979) or by the degree of floatation of eggs in water (Lundberg and Väisänen 1979).

Immediately after laying, the contents of an egg begin to cool and contract and an airspace begins to form at the blunt end of the egg. This increases in volume and hence in diameter as water is lost progressively through incubation. Measurement of the diameter of this airspace has been used to estimate the progress of incubation for Swallows and Dippers (Ward 1992). In the present study, the technique was tested with Swallows and Dippers and a new equation was created for Great Tits.

Methods. Eggs were placed over the beam of a torch in order to make the airspace clearly visible. Airspace diameter was measured using dial calipers to the nearest 0.1mm. Ward (1992) produced regression equations relating the airspace diameter of an individual egg to the number of days since clutch completion (assumed to be the start of incubation). In the present study, in order to reduce the variability arising from differences between airspaces within a clutch, according to order of laying (Ward 1992), a single mean airspace was calculated for each clutch by averaging the airspace diameter of all but sterile eggs.

Results.

(i) Dippers.

Measurements of mean clutch airspace diameter were taken from 14 Dipper nests of known laying date in 1993 and 1994, ranging from 6 to 14 days since clutch completion. Ward's (1992) equation for regression of airspace diameter on the number of days since clutch completion (Equation 2.5) was used to predict the number of days since the start of incubation, or 'DAYIN' (assuming DAYIN=1 on the date of clutch completion) for these clutches. The predicted number of days was accurate on only two of the fourteen occasions, and was otherwise consistently greater than the observed number of days since the start of incubation (Wilcoxon's signed rank test $z = -3.059$, $p = 0.002$), suggesting that there was a difference in measurement technique between observers.

Eqn 2.5. Regression of airspace diameter (mm) on days of incubation for Dipper eggs (from Ward 1992)

airspace = (.527 x DAYIN) + 8.678.

(ii) Swallows. The airspace diameter of Swallow eggs was measured throughout the incubation period in 1993 and 1994, at nests of known laying or hatching date. The date of airspace measurement was calculated by choosing a random value of 'DAYIN' between 1 and 16. Ward's (1992) equation for Swallow airspace diameter (Equation 2.6) was used to predict DAYIN. For 53 nests, the predicted values differed significantly from the observed values, (Wilcoxon's signed rank test, $z = -5.685$ $p < .001$), the predicted values generally exceeding the observed DAYIN.

Eqn 2.6. Regression of airspace diameter (mm) on days of incubation for Swallow eggs (from Ward 1992)

airspace = (.296 x DAYIN) + 6.09 .

In order to eliminate errors due to measurement technique, a new regression equation was created for Swallow airspace diameter which was specific to the author. Using the data gathered in 1993 and 1994, the mean clutch airspace diameter was plotted against either or 'DAYFROM', calculated as the number of days from the date of airspace measurement to the date of hatch (Figure 2.1 (i) and (ii)). Significant linear regressions were calculated for each relationship, but the greatest amount of variation was explained by fitting a cubic function in each case. Linear and cubic functions were described as follows:

For the regression of airspace diameter (mm) on DAYIN:

Eqn. 2.7. **(i) Linear:** $y = 6.697 + .390x$

$r^2 = .719$, $F_{(1,51)} = 130.448$ $p < .001$

(ii) Cubic: $y = 5.620 + .916x - .060x^2 + .002x$

$r^2 = .745$, $F_{(3,49)} = 47.738$, $p < .001$.

For the regression of airspace diameter (mm) on DAYFROM:

Eqn 2.8. **(i) Linear:** $y = 13.256 - .408x$

$r^2 = .763$, $F_{(1,47)} = 151.552$, $p < .001$

(ii) Cubic: $y = 12.971 - .370x + .004x^2 - .0004x^3$

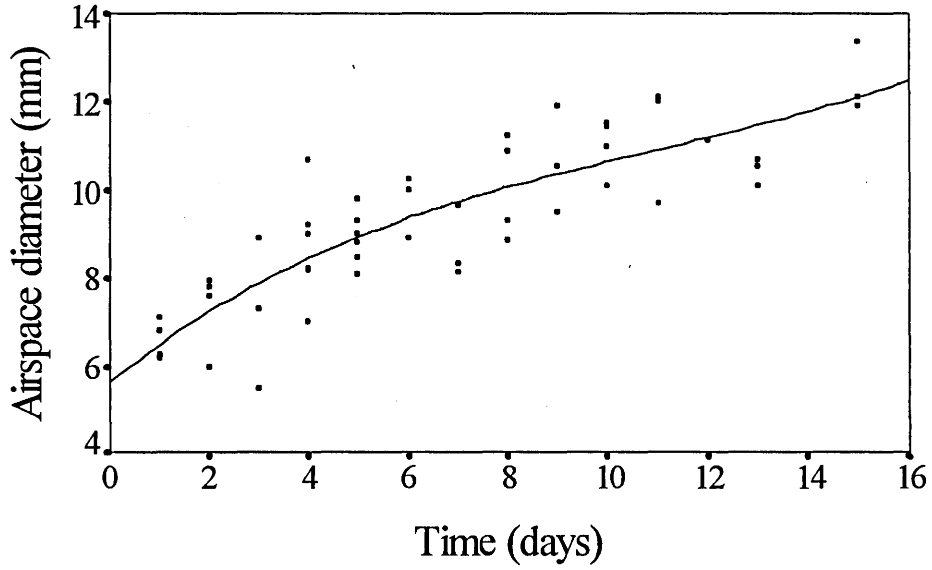
$r^2 = .767$, $F_{(3,45)} = 49.426$, $p < .001$

Theoretical reasons suggest that airspace diameter should describe a curvilinear increase with egg age. Firstly, a linear increase in airspace volume would translate to a curvilinear increase in airspace diameter, as the two are related by equations describing the volume of a sphere, to which the blunt end of the egg approximates. Secondly, Furness and Furness (1981) found that rate of decline in density of eggs of the Great and Arctic Skua increased as the incubation temperature of eggs rose during early incubation and increased again late in incubation, as metabolism of the embryo became more important. As the decline in density is inversely related to the volume of the air sac, a sigmoidal relationship between air space volume and DAYIN would be expected.

However, in the regressions of airspace on DAYIN or DAYFROM, 2nd or higher degree polynomials did not provide a significantly better fit to the data than linear regressions (for DAYIN, the improvement from linear to quadratic equation was close to significance, $0.05<p<0.10$; for DAYFROM, the quadratic term did not improve the description significantly, $p >0.5$). Because of this and the practical difficulties of solving for x with known values of y in cubic functions, linear equations were used to estimate the progress of Swallow incubation in the field. This was liable to overestimation of DAYIN in the first few days of incubation, and underestimation towards the end.

Confidence limits (95%) were calculated for these linear regressions, for predicted values of DAYIN or DAYFROM from a known clutch airspace, according to the techniques of inverse prediction outlined by Zar (1984). Confidence bands are at their narrowest at values of y around the mean, so these values were substituted to estimate the maximum accuracy of the regression. The 95% confidence intervals for predicted values of DAYIN or DAYFROM were found to extend over at least a 10 day interval, or 66% of the total incubation period.

(i) DAYIN



(ii) DAYFROM

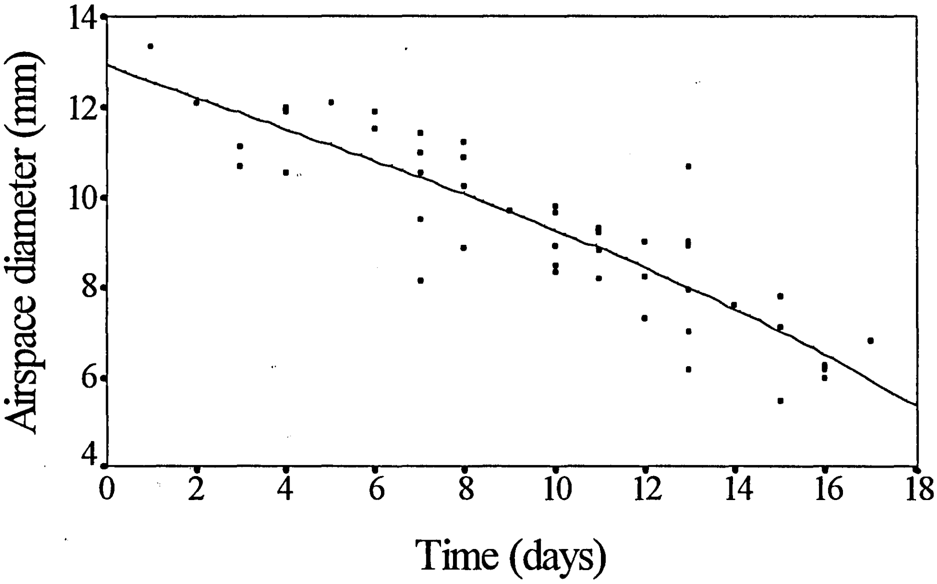


Figure 2.1 **Swallow airspace diameter (mm) in relation to the stage of incubation (i) DAYIN represents time since clutch completion, where DAYIN = 1 on the date of clutch completion (ii) DAYFROM, representing the number of days till hatch. Cubic functions describing the data are shown in each case (see Eqns 2.7 and 2.8).**

The accuracy of the predictions was tested, using airspace diameters measured from individual Swallow eggs (i.e. not clutch means) in 1994. Although these eggs were in the same nests that contributed to the data set on which the regressions were based, measurements from individual eggs were always made on different dates. Using the linear equations of Equations 2.7 and 2.8, predicted values of DAYIN and DAYFROM were obtained from the measured airspace diameter that did not differ significantly from the observed values (Wilcoxon's signed rank test, $p = .120$ for DAYIN and $p = .711$ for DAYFROM). The mean error in the prediction of DAYIN was 1.7 days, with 90% of the errors being within 3 days ($n = 39$ eggs from 39 nests). The mean error in predictions of DAYFROM was 1.3 days, with 90% erring within 2 days ($n = 35$ eggs from 35 nests).

(iii) Great Tits. For Great Tits, a regression of whole clutch airspace on 'DAYIN' was created, using data from 17 nests where the date of laying was known. The regression was significant, $r^2 = .699$, $p < .001$, (Equation 2.7), however 95% confidence limits for inverse prediction from this equation exceeded the complete duration of incubation. In this case, the data set was probably too small and too variable to provide a useful method of prediction. Further data collection would show whether the degree of variation in the proportion of incubation undertaken by the date of clutch completion in Great Tits makes this species unsuitable for the use of the airspace diameter technique.

Eqn 2.9. Airspace diameter (mm) in relation to days since incubation in the Great Tit, where DAYIN = 1 on the date of clutch completion.

$$\text{Airspace} = (0.212 \times \text{DAYIN}) + 7.250.$$

In summary, if information is not available concerning dates of laying or of hatching, it is concluded that measurement of airspace diameter provides a quick and non-intrusive method of estimating the stage of incubation. Although the 95% confidence limits suggest that the precision of the technique is limited, in practice, the linear regression for Swallow airspace diameter proved to be of a comparable precision to that obtained from measures of density decline (Furness and Furness 1981) and certainly of sufficient precision to be of use in the field. There is evidence to suggest that a non-linear model, ideally specific to both species and observer, would predict the stage of incubation more accurately than techniques relying on density decline, particularly for birds with small eggs, or where lack of shelter at the nest site makes weighing imprecise.

2.4 Use of nest temperature monitor to determine incubation schedules.

The pattern of time spent on and off the nest by incubating Swallows, Dippers and Great Tits was monitored using two identical nest temperature monitors, designed by Stirling University Microcomputer and Media Technology Group. Each monitor consisted of seven temperature sensitive thermistor probes, of diameter 1.5 mm, connected to a battery powered data-logger which prompted sampling and storage of the temperature at each probe once every minute. The data were stored in hexadecimal format to reduce memory requirements. The monitor was capable of storing 22.5h data at this rate of sampling. Data were downloaded in the field to a portable Psion organiser. This process took approximately 20 minutes, after which time the monitor could be reset, thus enabling almost continuous sampling. Data were then transferred to the University Mainframe, where the output was converted into temperature readings and time-budgets were analysed.

Both monitors was calibrated in a constant temperature chamber at the beginning of each season, to adjust for the slight drift that occurred from year to year. An example of the calibration of monitors A and B is presented in Appendix 1. Temperature and monitor output were best described by a $\log_e$- $\log_e$ relationship.

The precision of the monitor was limited by the fact that monitor output was in integer format. Because of the $\log_e$ - $\log_e$ relationship between temperature and monitor output, the temperature increase represented by 1 monitor unit differed according to the absolute temperature sampled. The temperature represented by one additional monitor unit is shown for a range of temperatures, expressed in degrees Celsius, in Appendix 1. The monitor was precise to within 1°C at all temperatures over 0°C.

Probes were arranged within and around the nest to sample temperatures close to the female, amongst and under eggs, in the nest cup and wall, and in shade outwith the nest in order to provide a record of ambient temperature. An example of the output of a selection of probes from the trace for an incubating Swallow, Dipper and Great Tit during the daytime are shown in Figure 2.2 (i) to (iii). In all three species, the female incubated for one continuous

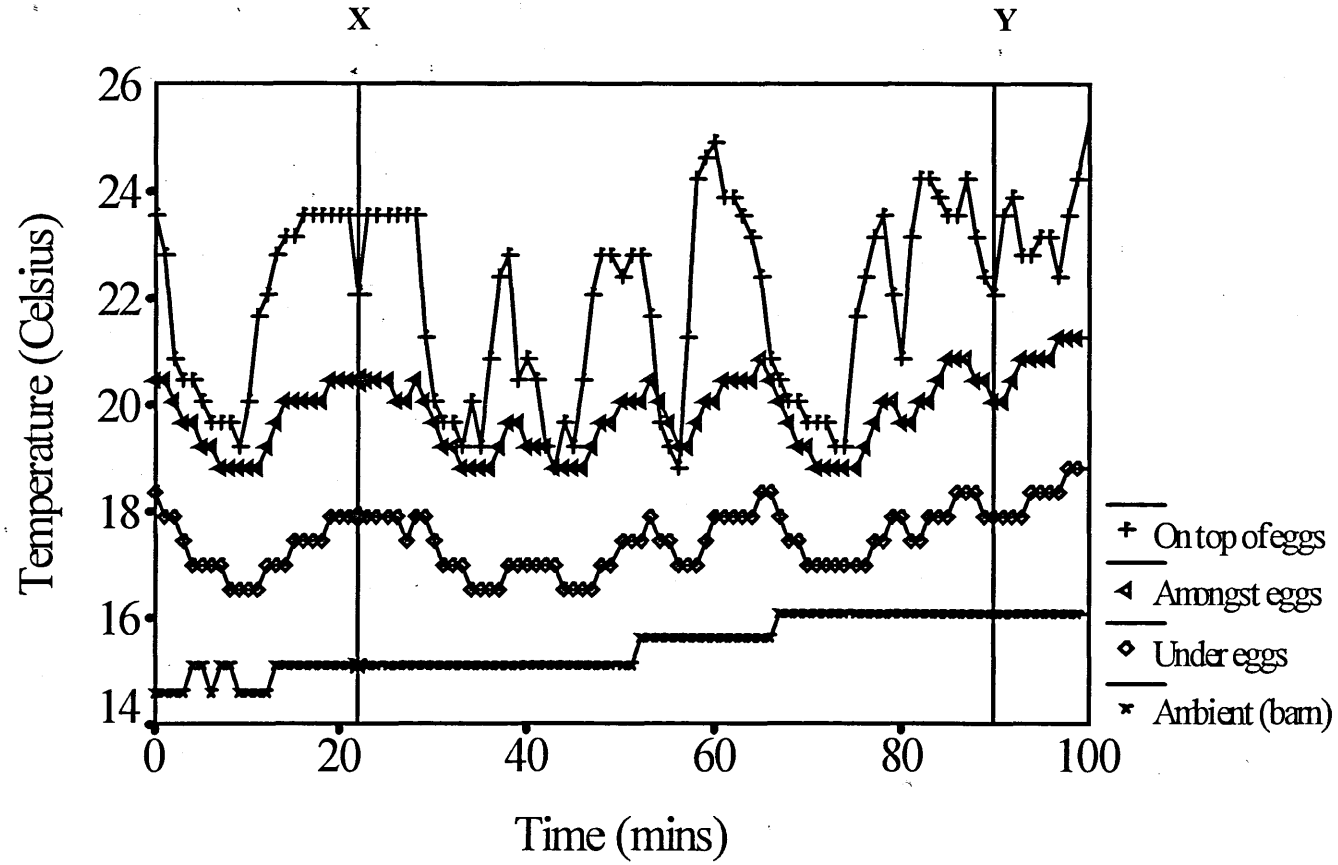


Figure 2.2. (i) Temperature profiles in and around the nest of an incubating swallow. Reference lines mark points X and Y, referred to in the text.

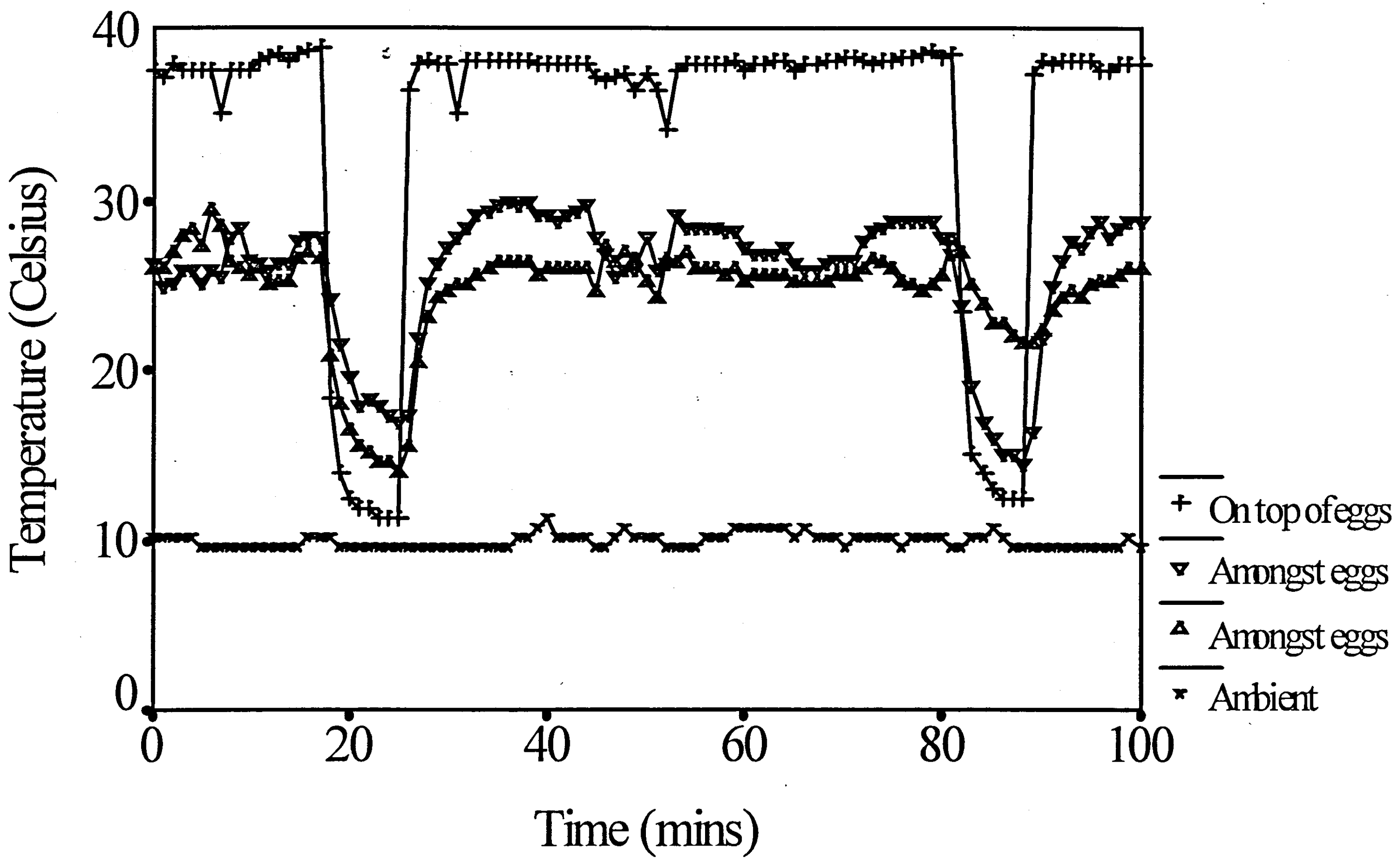


Figure 2.2. (ii) Temperature profiles in and around the nest of an incubating Dipper.

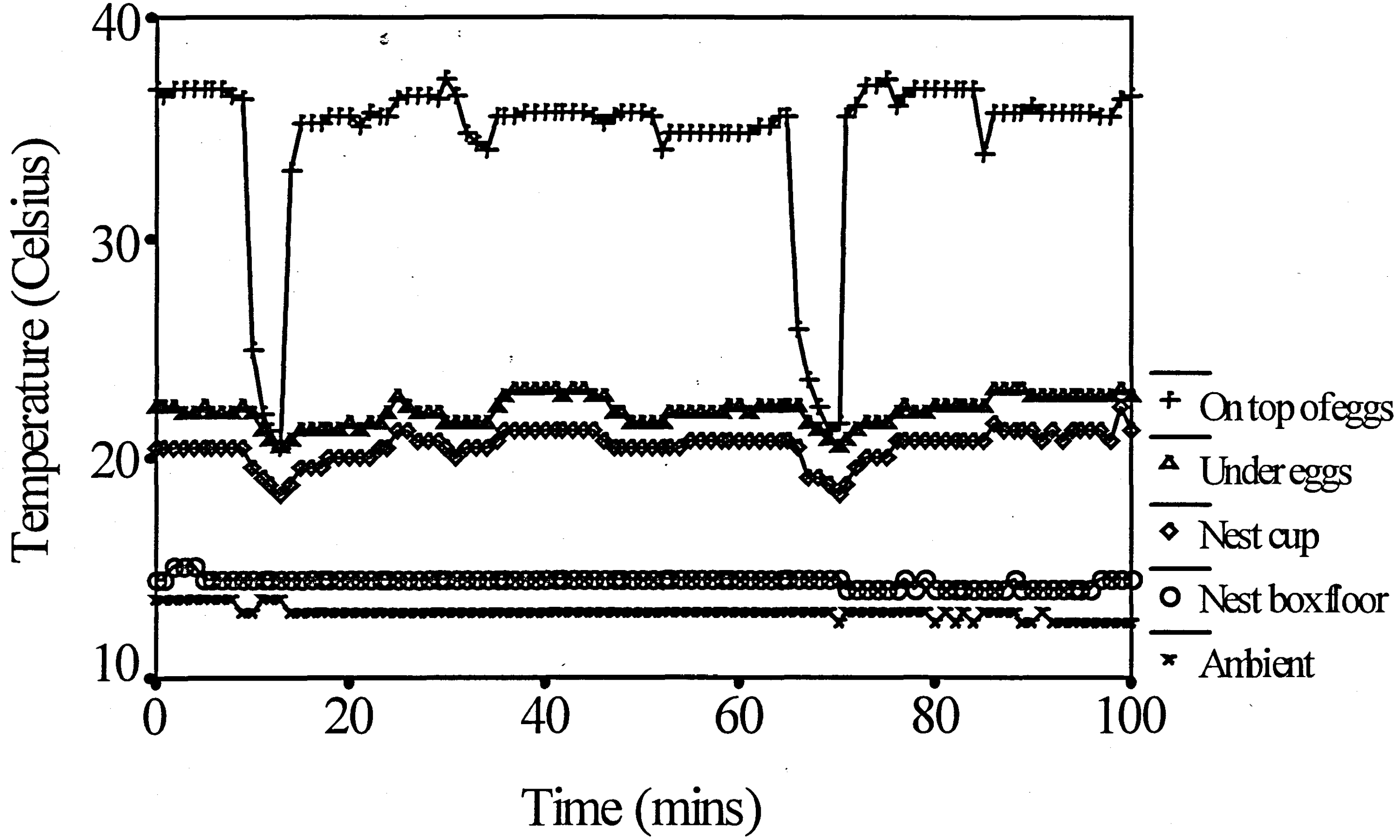


Figure 2.2. **(iii) Temperature profiles in and around the nest of an incubating Great Tit.**
Temperatures were sampled every minute by the probes of the nest temperature monitor, at various positions in and around the nest.

period during the hours of darkness (the 'night session'). The 'active' day was divided into periods of incubation (sessions), interspersed with periods away from the nest (recess periods), mostly spent feeding. Time spent incubating (sessions) and away from the nest (recesses) could, in most cases, be readily deduced from the rapid fall and subsequent rise in the temperature on top of the eggs as the female left the nest, then returned to resume incubation, but was always confirmed by consistent responses of temperatures amongst and below the eggs. Figure 2.2(i) shows two examples, marked X and Y, when the temperature on top of the eggs fell for a short period of time (1-2 minutes only) before rising again. In such situations, it was not always clear from the output of a single probe whether the female had left the nest or whether movements within the nest led to a loss of contact with the single probe. Output from other probes amongst and below the eggs confirmed that the female remained on the nest at X, while at Y she left the nest for a short recess of 2 minutes duration.

2.5 The doubly labelled water technique.

2.5.1 Theoretical basis of the technique.

Energy forms the basis for life. The balance between energy acquisition and expenditure underlies many behavioural and physiological adaptations. Differential survival and reproductive capabilities may be determined by the success of individuals in maintaining this balance. The doubly labelled water technique enables the study of energy expenditure by subjects in the wild, throughout various stages in their life-cycle.

The foundations for the technique were laid by Lifson *et al.* (1949), with their discovery that, if the body water of rats and mice were isotopically enriched with $H_2{}^{18}O$, the oxygen of expired carbon dioxide was in isotopic equilibrium with the oxygen in the body water. This paved the way for the development of techniques to measure the carbon dioxide production and hence metabolic rates, of subjects using water enriched with stable isotopes, (Lifson and M^{c}Clintock 1966).

The application of the doubly labelled water technique involves the simultaneous introduction into the body of deuterium and ^{18}O. Oxygen from body water finds its way via the tricarboxylic acid cycle into carbon dioxide. The action of carbonic anhydrase in the blood ensures that the oxygen in dissolved carbon dioxide comes into complete isotopic equilibrium with the oxygen in the body water (Speakman and Racey 1988). The concentration of ^{18}O is depleted according to both the rate of production of carbon dioxide and of water loss (evaporation, faeces and urine), whilst the rate of depletion of the deuterium label will vary according to water loss alone. The difference in fractional turnover rates of these isotopes can, therefore, be used to calculate the rate of carbon dioxide production and hence of metabolism.

The doubly labelled water technique involves the introduction of isotopes into the subject by injection or by oral administration. After a period allowing for isotopic equilibration, a blood sample is taken to establish initial levels of isotopic enrichment. The subject can then be released to resume normal activity for a period of time, normally 24 or 48 hours in small animals, in order to avoid diurnal bias. During this time, isotopic depletion occurs towards the level of background enrichment, so when a second capture is undertaken, a final blood sample can be used to calculate the rates of turnover of the two isotopes.

The following equations may be used to determine the rate of carbon dioxide production (see Lifson and M^{c}Clintock 1966 for derivation):

Eqn. 2.10. $$r_{CO_2} = \frac{N}{2.08}(K_O - K_D) - 0.015K_D N$$

where r_{CO2} is the rate of carbon dioxide production, expressed in mMol h^{-1}, N is the body water pool, expressed in mMol (Equation 2.12), K_o and K_D represent the fractional turnover rates of ^{18}O and D respectively, calculated using Equation 2.11. The denominator 2.08 is the product of a fractionation factor to account for fractionation between $H_2\,^{18}O$ (liquid) and $C^{18}O_2$ (gas), and a stoichiometric factor, as 1 mole of CO_2 contains the oxygen equivalent of 2 moles of water. The correction factor ($-0.015K_DN$) also accounts for the effects of isotopic fractionation, based on the assumption that evaporative water loss comprises half of the total loss of body water. Fractional turnover rates are calculated as:

Eqn. 2.11. $$K_O = \frac{\ln(^{18}O_i - ^{18}O_b) - \ln(^{18}O_f - ^{18}O_b)}{t}.$$

where K_o is the fractional turnover rate of ^{18}O, $^{18}O_i$ represents the initial concentration of the isotope in ppm, $^{18}O_f$ is the final concentration and $^{18}O_b$ represents the background concentration in the population. K_D is calculated using the same equation, substituting D for ^{18}O.

The size of the body water pool (N) may be calculated by the isotope dilution method (Schoeller *et al.* 1980) but difficulties may be experienced with small (<100g) animals (Bryant and Tatner 1989). In this study, an average figure for the proportion of body mass that is water was obtained from studies of carcass composition in the literature. For incubating Great Tits, body water averaged 66% of total body mass (Mertens 1987); for Dippers in Central Scotland, 63% of total mass was body water (Newton 1989). The relevant value was substituted for p in Equation 2.12.

Eqn. 2.12. $$N = (M \times p) \times \left(\frac{1000}{18}\right)$$

N is the volume of the body water in mM, M is mass in g, p is the mean proportional body water content . The constant 1000/18 converts g H_2O to mM.

The rate of carbon dioxide production can be expressed as an energetic equivalent by calculation using the relevant R.Q. value (Brody 1945) to represent the heat equivalent of $1 cm^3$ of CO_2. Rates of energy use are normally expressed as three variables:

(i) Average Daily Metabolic Rate (ADMR) represents the rate of carbon dioxide production per gram body mass per hour:

Eqn.2.13. $\text{ADMR} = (rCO_2 \times 22.4)/\text{body mass}$ in $cm^3 CO_2\, g^{-1} h^{-1}$.

The constant 22.4 converts mM CO_2 to cm^3.

(ii) Daily Energy Expenditure (D.E.E.) expresses the energy usage by an individual on a daily basis,

Eqn. 2.14. $\text{DEE} = (\text{ADMR} \times 24 \times \text{mass} \times q)/1000$ in $kJ\ indiv^{-1} d^{-1}$

The value 'q' represents the heat equivalent in kJ of $1 cm^3$ CO_2, and varies according to the R.Q. (respiratory quotient) of the diet. Calculation of the correct value of the respiratory quotient applicable to the current diet of subjects is rarely made in the field. In this study, an R.Q. of 0.75 was adopted for both Dippers and Great Tits, based on a diet of mixed lipid and protein, which equates to a value of 26.44 J released for every $1 cm^3$ CO_2 liberated (Brody 1945).The constant 24 converts hours to days.

(iii) Metabolic Intensity expresses Daily Energy Expenditure as a multiple of basal metabolic rate, a value which is ideally measured for the individual concerned, but is more generally estimated from allometric equations (Bryant and Tatner 1985). Metabolic intensity has no units.

Eqn. 2.15 M.I. = D.E.E./ B.M.R.

In this study, M.I. was expressed in terms of a multiple of the basal metabolic rate, calculated from the equations of Aschoff and Pohl (1979), and as a multiple of measured B.M.R., for local populations, where these were available.

2.5.2 Application of the doubly labelled water technique to incubating birds.

The doubly labelled water technique relies on several simplifying assumptions, detailed by Lifson and M^cClintock (1966). They are summarised briefly as:

(i) body water volume is constant
(ii) rates of flux of water and carbon dioxide production are constant
(iii) within the body, isotopes label water and carbon dioxide only
(iv) isotopes leave the body only as water and carbon dioxide
(v) the isotopic enrichments of water and carbon dioxide leaving the body are the same as those in the body water.
(vi) water or carbon dioxide does not enter the animal across the skin or lungs.

It is likely that, under normal conditions of free-existence, at least one of these assumptions will be violated to some extent. The significance of the violations encountered in a normal free-living situation has been evaluated (Nagy 1980, Nagy and Costa 1980). In general, the doubly labelled water technique is thought to be relatively robust under most conditions. Studies comparing the doubly labelled water technique with alternative methods of measuring energy use (gas analysis, intake-output) have shown that the mean absolute discrepancy between the two is generally less than 10% for vertebrates (see Bryant and Tatner 1989 for review).

Chapters 4 and 5 describe how the doubly labelled water technique was applied to incubating Dippers and Great Tits. The application of the technique to incubating birds may pose a special set of problems and these must be evaluated to determine the validity of the technique.

The first assumption states that body water, body mass and composition of body solids should remain constant throughout the study period. Both Dippers and Great Tits are known to lose mass throughout the course of incubation (see Section 4.4 and 5.6.). In the Great Tit, body water content has been shown to decrease both in absolute amount and also slightly in proportion to total body mass during incubation (Mertens 1987). The maximum deviation in percentage body water content was a 10% decrease in magnitude over the full course of incubation (Mertens 1987), equivalent to less than a 2% change in the size of the body water pool over a 48 hour study period. Modification of the equations to account for a linear change in mass or body water volume is possible (Lifson and M^{c}Clintock 1966), but the difference between results obtained by this method and that using the steady state equation differ by less than 5%, so long as the body water pool declines by less than 50%, or increase by less than 100% of its initial value (Nagy 1980). Because the observed variation in mass (and hence body water) was well within this range, the steady state equation was applied to all birds.

Violation of assumptions (ii) to (iv) should be no more likely to occur during incubation than at other stages in the life cycle. The significance of these violations was discussed by Nagy (1980).

The fifth assumption can be violated in one of two ways: incomplete mixing of isotopes within the subject and isotopic fractionation effects. The latter may be problematic in some elements of this study. Isotopic fractionation occurs as a liquid passes to a vapour stage, as the lighter isotopes escape to the gaseous phase more readily than heavier isotopes, leaving the liquid isotopically enriched relative to the vapour above it. For example, the physical fractionation factor of deuterium for evaporative water loss is 0.93, thus water lost from the body by evaporation will contain 93% of the deuterium represented in the body water, leaving the latter at a higher enrichment than if water loss were through a liquid route. In Equation 2.10, the denominator (2.08) and the final term ($-0.0015K_DN$) account for this fractionation by assuming that evaporative water loss forms 50% of the total water loss. Evaporative water loss is liable to be affected by temperature.

Chapter 5 describes an experiment in which the free-living energy expenditure of incubating Great Tits was measured for a group of birds whose nests were heated at night and a control group. It is possible that the treatment leads to an increase in evaporative water loss for heated birds compared to control birds, as evaporative water loss will vary with temperature although the temperature of the respiratory tract of homeotherms will be approximately constant irrespective of ambient temperature. An under-estimate of the proportion of water loss that is evaporative would lead to an over-estimate in the rate of carbon dioxide production.

The sixth assumption states that no entry of carbon dioxide or water (labelled or otherwise) should take place across the respiratory surfaces or the skin. Under humid conditions, or in situations where carbon dioxide can accumulate, entry via these routes may become significant. Enclosed environments, such as burrows or nests may provide such conditions.

Humidity in enclosed nests is normally at least equal to, and generally slightly above ambient humidity, (Withers 1977, Brown 1994) although for some species, the hygroscopic nature of the nest may act to reduce nest humidity (Bartholomew *et al.* 1976).

Exhaled carbon dioxide may accumulate in a confined nest during incubation (Ar and Piontkewitz 1992). Apart from invalidating the results of doubly labelled water studies, there are a number of reasons why it would be disadvantageous for this accumulation to take place. Elevated levels of carbon dioxide can produce an increase in minute volume and the rate (and hence cost) of respiration for adults, while a reduction in oxygen availability can be lethal to embryos (Tazawo *et al.* 1992). It has been suggested that the relatively short duration of incubation in the cavity-nesting Picidae is an adaptation to the problems of hypoxia and hypercapnia experienced by the embryos and incubating parent (Yom-Tov and Ar 1993). In most hole nesters, a number of processes serve to ventilate the nest chamber (White *et al.* 1978).

Diffusion of gases may occur through the nest material or through the nest entrance, the latter being due to the 'Bernoulli' effect. This results from the action of wind moving across the nest entrance, drawing air out of the box (White *et al.* 1978, Howe *et al.* 1987). Movement of the adult in and out of the nest, or even within the nest, will cause mixing of gases (Ar & Piontkewitz 1992). Temperature gradients between the box and ambient air can produce convective currents out of the box, again helping to reduce gas accumulation (Howe *et al.*. 1987, Howe and Kilgore 1987).

A combination of the above may be expected to reduce the concentrations of carbon dioxide and the humidity around the female within the nests of Dippers and Great Tits. Both nests are enclosed, save an entrance hole of 3.5cm for the Great Tit, 4.5-7 cm for the Dipper (Tyler and Ormerod 1994). Gas accumulation is more likely to be problematic for the Great Tit, as the wooden nest box wall is less permeable than the moss walls of a Dipper's nest. In addition, the entrance to a Great Tit's nest is situated some 10cm above the incubating female, while the incubating Dipper faces the hole directly, and exhaled gases have an easier path of escape.

In order to assess the likelihood of entry of carbon dioxide across the skin or lungs, a short experiment was conducted to monitor the accumulation of gases in a Great Tit's nest box.

2.5.3 Gaseous composition of air in the nest of a Great Tit.

Methods. An experiment was conducted to investigate the extent to which carbon dioxide accumulation occurred in the nests of incubating birds. Conditions were selected to represent the greatest likelihood of accumulation experienced by incubating birds during the doubly labelled water study.

Gas accumulation in the nest of an incubating Great Tit would be expected to be greatest during the night-time session (Howe *et al.* 1986), when the bird incubates continuously for the longest period of time. In order to monitor nest air composition throughout the night, a nest box was brought into the laboratory and modified such that the gases in the box could be sampled at short intervals. The box contained a complete nest. The entrance to this experimental nest box was covered with a wide mesh wire gauze to restrain the female within the box while allowing normal movement of air through the entrance hole. A small sampling pipe (diameter = 4mm) was inserted through a hole at the back of the box, originally designed for insertion of the probes of the nest temperature monitor. The open end of the pipe was covered with a fine mesh gauze to prevent entry of nest material, and was positioned approximately 2cm above the nest cup. The pipe was connected to a V.G. Quadruple mass spectrometer, running modified Mediflex software.

During July 1995, a brooding female was captured at 22:00 hours from the nest where she was roosting with her 10 day old chicks. The chicks were maintained within their own nest in an incubator at 25°C during the course of the experiment. The female was placed in the modified nest box and allowed to settle in complete darkness

Gaseous sampling commenced immediately. Under normal conditions of use, sampling draws air at a rate of 1litre min^{-1} from the box. This was inappropriate, as exhaled gases would be drawn from the box in a manner that did not represent the situation in the wild. Sampling was therefore conducted intermittently, for short periods only, the pipe being clamped and the mass spectrometer disconnected after each gas sample was taken.

During the first hour after entry, sampling was conducted for 2 minute periods, once every 15 minutes; subsequently, 5 minute samples were taken once every hour. This was continued until just before dawn, after which time the female was returned with her chicks to her own nest. Continuous sampling of nest air was carried out following the removal of the female to monitor the rate of gas dispersal from the box.

Results. Figure 2.3 shows laboratory temperature through the course of the experiment. Figures 2.4 and 2.5 show the fluctuations in levels of oxygen and carbon dioxide within the box, presented as a percentage of the total gas by volume (excluding water vapour). The bird entered the box at time = 0 and was removed 5.58 hours later. Removal entailed lifting the lid from the nest box, so conditions do not accurately replicate those observed during the normal departure of the female from the box. The period of continuous sampling after removal may also have encouraged faster depletion of gases than would occur normally. The results following departure therefore represent gas dispersal at rates that are likely to exceed those occurring under natural conditions. Means for 10 second periods at intervals of 5 minutes following departure are shown on the same graphs.

Oxygen concentration fell rapidly immediately after female entry to the box, to a minimum of 19.2%. Between the second and third hours after entry, oxygen concentrations rose again, presumably reflecting more settled behaviour and a probable switch to night time metabolism. Concentrations fell again 4 hours after entry of the female, at 03:30h, probably representing a switch to daytime levels of metabolism around dawn. Body temperature of incubating Blue Tits has been shown to rise markedly from about 03:00 hours until leaving the nest 1 to 1.5 hours later (Haftorn and Reinertsen 1982).

Carbon dioxide concentrations rose rapidly during the first hour in the box, reaching maximal levels of 0.4% one hour after entry. Concentrations then remained stable, at levels between 0.3 and 0.4%. Carbon dioxide concentration declined as the bird left the nest, at first rapidly as the lid was raised and air disturbed and then in a linear fashion, returning to ambient levels after 20 minutes.

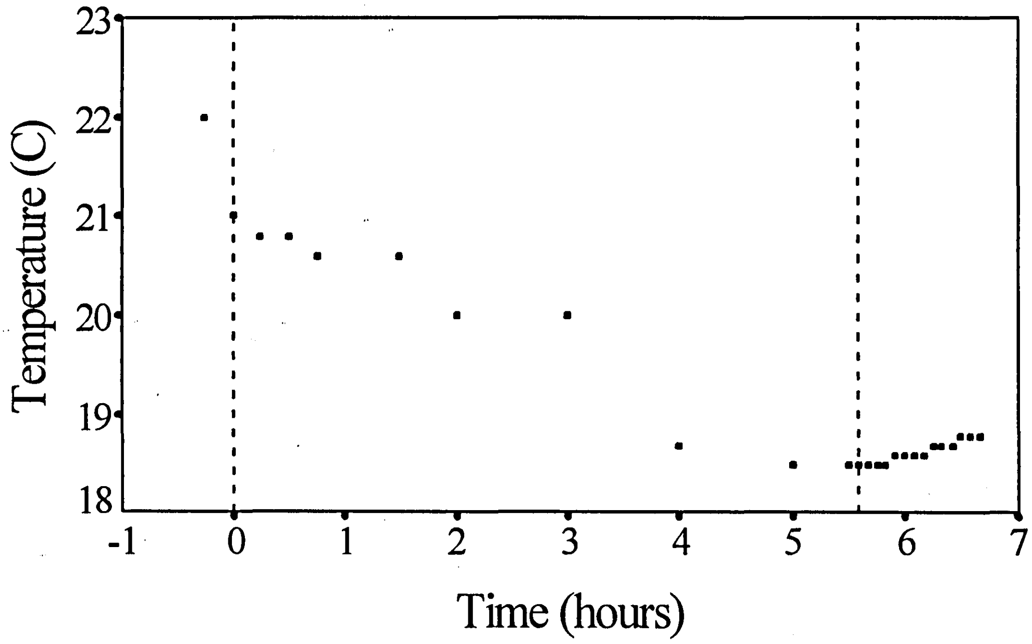


Figure 2.3 **Laboratory temperature during the course of the experimental sampling of gases in the nest of a Great Tit.**

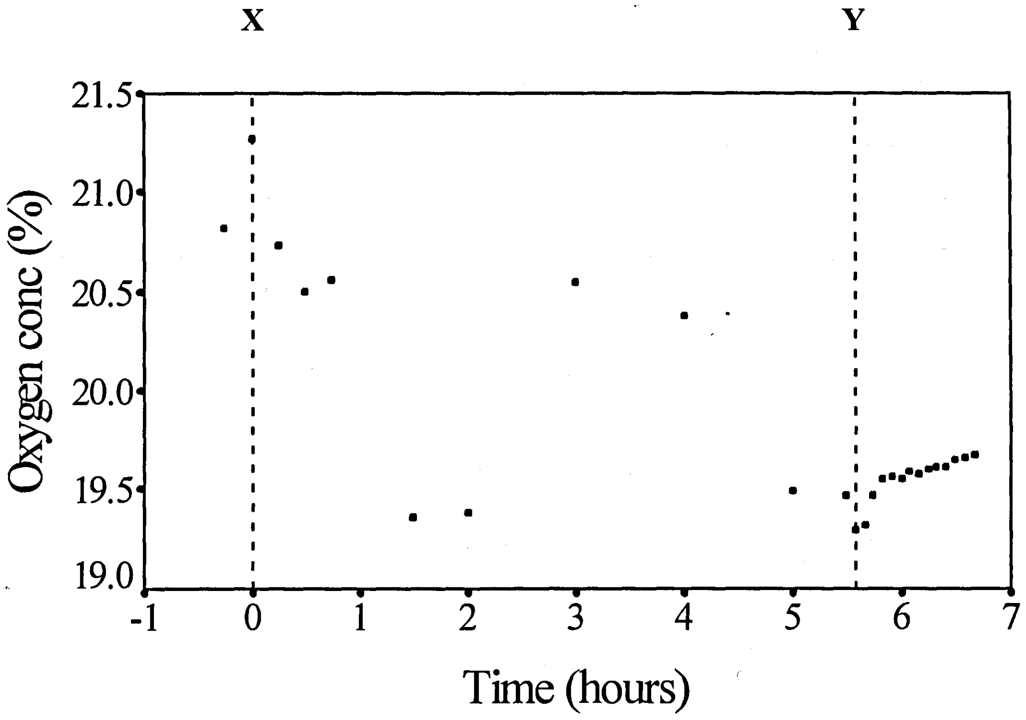


Figure 2.4 **Oxygen concentration (percentage by volume) inside a nest box containing a female Great Tit. The bird was introduced to the box at time=0 (point X) and removed at time = 5.58h, (point Y).**

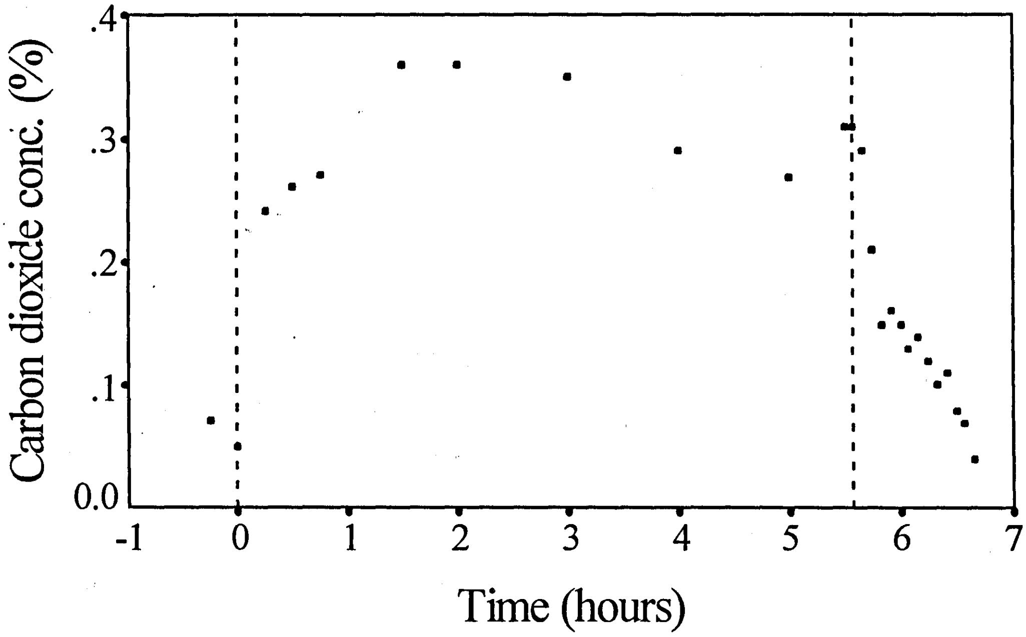


Figure 2.5 **The concentration of carbon dioxide (percentage by volume) sampled in the gases inside a nest box containing a female Great Tit.**

Discussion. While the oxygen concentration within the box varied, probably according to the level of metabolism, carbon dioxide accumulated to a plateau of 0.3 to 0.4% and further metabolism did not increase this figure. Carbon dioxide has a greater density than air, so it is likely that the sampled gases, towards the bottom of the box, were representative of the highest CO_2 concentrations to be found in the box. Concentrations built-up rapidly following entry of the bird, reaching 0.3% during the length of an average day-time incubation session (c. 30 minutes Section 5.4.4).

Following the female's departure from the nest box, dispersal of the accumulated gas was slow; even with the disturbance involved in extraction of the female, concentrations did not return to ambient until some 30 minutes following removal. Daytime recess periods are generally much shorter than this (mean = 8 minutes), so it is likely that elevated carbon dioxide concentrations persist until the following incubation session.

The experimental conditions differed from the situation of an incubating bird in a number of ways. (i) Metabolism of incubating birds is higher than that of roosting birds (Haftorn and Reinertsen 1985) and in the latter stages of incubation, metabolism of the embryos would also increase the production of respiratory gases. (ii) Temperatures in the laboratory (mean 19°C) were higher than those generally experienced during incubation (mean 10°C); again, metabolism would be higher at lower temperatures. (iii) Counteracting this, conditions inside the laboratory were still, compared with even windless conditions outside, so a greater degree of gas mixing would be expected under field conditions. (iv) Even small temperature differentials between nest air and ambient can have a dramatic effect on increasing the rate of gas dispersal (Howe and Kilgore 1987); birds may exploit the position and insulative nature of the box to maximise this temperature gradient (O'Connor 1978). On balance, laboratory conditions could, therefore, provide a reasonable representation of conditions in the wild.

The effect of entry of carbon dioxide or water vapour across lungs or skin on the assumptions of the doubly labelled water procedure will depend on the relative isotopic enrichments of body water and nest air (Lifson and McClintock 1966). In order to account for any error resulting from this source, accurate information on the isotopic composition of nest air would be necessary. In the absence of such data, it is only possible to speculate on the isotopic enrichment of gases in the box.

Large errors in doubly labelled water analysis can occur if the concentrations of isotopes in air exceed those in body water, or if unlabelled carbon dioxide or water vapour enter across the skin or lungs (Nagy 1980). Even so, Gettinger (1983) conducted doubly labelled water trials with mammals under conditions of high concentrations of unlabelled water vapour and found doubly labelled water results that were in close agreement with estimates of metabolism made from food intake. If the concentrations of D and ^{18}O in nest air were approximately equal to that of body water, the doubly labelled water technique should be free from error (Nagy 1980).

During the doubly labelled water protocol, birds were held off nest for around one hour during labelling. In this time, any accumulated (unlabelled) gases within the nest should have dispersed. Following release of the female and the subsequent resumption of incubation, carbon dioxide concentrations would most likely accumulate towards the 0.4% plateau observed experimentally. The specific activity of isotopes in the nest box gases would also increase relative to those in the body water of the bird. During the day, the ratio of the specific activity of isotopes in nest air to those in body water (k) would be likely to remain below unity, however, due to mixing of nest air with unlabelled gases by movements of the parent in and out of the box. During the night, k may approach or even exceed unity, if isotopic depletion were to occur at a faster rate in the bird than the rate at which gases were lost from the box. Errors with the doubly labelled water analysis should be small if k fluctuates in this way at values around unity.

In summary, carbon dioxide concentrations of up to 10 times ambient levels are likely to exist in the nest of the Great Tit during incubation. These values are consistent with data recorded in other natural enclosed nests (mean concentration of 0.37% in the nest of the Northern Flicker (Howe *et al.* 1987), 0.3% in the Cliff Swallow (Withers 1977)), but are substantially lower than those observed in burrows of both birds and mammals (c. 6% Boggs *et al.* 1984). No information on nest air humidity was recorded in this experiment. Further study on the isotopic activity of the gases in the nest box is needed to determine the extent of any error in the doubly labelled water calculations. However, under the conditions outlined above, it seems that birds will generally be exposed to gases deriving mostly from themselves, thus doubly labelled water results from studies of incubating birds are likely to be accurate.

2.5.4 Field protocol of the doubly labelled water technique.

In general, the field protocol described in Bryant and Tatner (1989) was followed. Incubating female Dippers and Great Tits were caught from the nest between 14:00 and 16:30h. Birds were weighed, abdominal feathers were brushed aside and the abdominal skin was swabbed with a little alcohol. A dose of 15µl of heavy water per gram body mass (0.524g of 99% D_2O in 10ml 14APE $H_2\,^{18}O$) was administered via intraperitoneal injection. An equilibration period of one hour was allowed, during which time the bird was measured then held in a cloth bag (Dippers were held over ice during periods of hot weather). Blood samples were taken by puncture of the femoral vein for all Dippers and most Great Tits and from the ventral side of the carpal wing joint for the remaining Great Tits. The blood was stored in glass capillaries, with 5µl in each tube. Eight to ten tubes were filled at each sampling. Capillaries were sealed as quickly as possible by an assistant, using a blow torch and were stored for a maximum period of six months until analysis.

The wound was sealed by the application of light pressure and smeared with a small quantity of antiseptic cream. Before release, any blood was cleaned from the feathers with alcohol. Dippers were marked with a narrow stripe of red, non-permanent marker pen across the white plumage feathers, to enable rapid identification for behavioural observations over the doubly labelled water study period.

In general 48 hours, occasionally 24 hours, elapsed before final capture. Birds were weighed and a final blood sample was collected before release. Blood samples were also obtained from non-labelled Dippers and Great Tits in the study population, in order to determine the background concentration of deuterium and oxygen-18. These birds were captured from the study populations, within 1 month of each study period in order to avoid variation due to habitat or seasonal differences (Tatner 1990).

2.5.5 Isotopic analyses.

The concentration of oxygen-18 and deuterium in the blood samples was measured using isotope ratio mass spectrometry, at the Stable Isotope Laboratory, Scottish Universities Reactor Research Centre at East Kilbride, in 1993 and 1994 by S.M. Bryan and in 1995 by J. Weir. Methods of preparation of samples for gas analysis are presented in full in Tatner and

Bryant (1989). For each blood sample taken, two replicate capillaries were processed for deuterium analysis and a further two for oxygen-18 analysis. Replicates were rejected if ppm values were outwith 5% of each other. In this case, a further capillary was then processed and the mean of the two closest replicates was used in subsequent analysis.

Hydrogen/deuterium was isolated from the blood by reduction of the water present over a uranium furnace at 800°C (Wong and Klein 1986) and the gas was collected on activated carbon prior to analysis by mass spectrometry. In order to avoid problems with the memory effect of the uranium furnace, sample processing was structured to begin with the low enrichment samples (background and final samples) and to work up towards the high isotopic enrichment. Standards of suitable concentration were processed prior to sample analysis, in order to 'condition' the furnace.

The analysis of ^{16}O:^{18}O ratios by mass spectrometry was performed with the oxygen in the form of carbon dioxide, as this gas is easily purified, is non-corrosive and has no memory effect during mass spectrometry. Conversion of the oxygen in the body water to carbon dioxide was achieved by reactions with guanadine hydrochloride and phosphoric acid (Boyer *et al.* 1961, Degen *et al.* 1985).

Gas samples were analysed using the auto-run procedure of a Sira 9 (D/H samples) and a Sira 2 and Sira 10 ($C^{18}O^{16}O/C^{16}O^{16}O$ samples) mass spectrometer. For both H/D and ^{18}O sampling, a series of international and laboratory standards were processed in order to derive calibration curves to convert the output of the mass spectrometer to isotopic concentrations in parts per million. Because of the problem of drift in the mass spectrometer readings, individual calibrations were produced for each series of analyses. The calibration equations are presented in Appendix 2 .

2.6 Determination of egg cooling rates in the Great Tit.

Introduction. In order to maintain eggs at incubation temperature, a parent bird must compensate for any heat lost from the eggs to the surrounding air and nest materials by supplying heat energy to the clutch via the brood patch. How much energy is required will be determined, to a large extent, by the rate at which the clutch cools within the nest. Accurate determination of egg cooling rate is, therefore, an essential part of the calculation of the energetic demands of incubation.

Egg cooling rates differ between species according to egg characters such as size, shape and shell thickness. Cooling rates for any species may be estimated by extrapolation from rates measured in species with eggs of a similar size: Ricklefs (1975) suggests the use of a regression equation of cooling rate on egg mass, derived from Kendeigh's (1973) measurements for 10 species. However, this equation describes the cooling rate of a single, isolated egg, outwith the microclimate of the nest. The degree of insulation provided by adjacent eggs and by the nest material will differ between species, potentially exerting a considerable influence on the rate of egg cooling .

In view of this potential error, measured values of egg cooling rates were preferred to extrapolation from allometric regressions. The cooling rates of Swallow eggs have been studied in the laboratory by Jones (1985) and those of Dipper eggs in the field by Ward (1992). The following study was undertaken in order to measure the cooling rate of Great Tit eggs, within a clutch of average size, in the microclimate of a fully lined Great Tit nest.

Methods. Addled or deserted eggs were collected from nest boxes during November 1995. Eight eggs were blown, cleaned by flushing with water, then filled by injection with albumen from a fresh hen's egg. One temperature sensitive thermistor probe was then inserted into each of six of the eggs. Joints were sealed using molten candle wax. These 'thermistor' eggs were placed, with the two remaining eggs in a fully lined Great Tit nest, within a wooden nest box. The box was placed in an incubator at constant temperature and left for half an hour whilst a steady nest air temperature was reached. Heat was then applied to the eggs by means of an overhead 100W bulb. As the first eggs reached a temperature of 35 °C, consistent with the mean egg temperature observed in a natural Great Tit nest during the 'active' day (Haftorn 1981a, 1983), the heat source was removed to simulate the departure of the incubating female from the nest. Egg temperatures were then recorded automatically once a minute, for a 15 minute period. This protocol was repeated at four different nest air temperatures to cover the range experienced during natural incubation.

Results. Figure 2.6 shows the decline in egg temperature for the six 'thermistor' eggs within the clutch at a nest air temperature of 5 °C. Similar patterns of exponential decay were found at temperatures of 10, 15 and 20 °C, with eggs cooling more rapidly and towards a lower asymptote at the lower temperatures. The decline in mean temperature for all 'thermistor' eggs across each of the four temperature treatments is shown in Figure 2.7. Temperatures are presented in relation to the initial mean egg temperature, in order to standardise results.

Although the cooling curves are typical of exponential decay, they approximate well to linear decline during the first ten minutes following removal of the heat source. Linear regressions ($p<0.001$ in each case) were therefore produced for each probe at each temperature for the initial 10 minute period. The slopes of these regressions were then used to calculate an average cooling rate in terms of ^{o} C loss per ^{o} C difference between egg temperature and nest air temperature (calculated midway through the 10 minute period (following Kendeigh 1963)) per hour. The range of values calculated for each probe is shown in Figure 2.8. Rates differed by a threefold order of magnitude according to position in the clutch.

As cooling rates differed significantly between probes (oneway ANOVA, $p<0.001$, $n=21$) the whole data set was combined by using the average of the mean values for each probe, in order to calculate a mean cooling rate of 1.987 $^{o}C\,^{o}C^{-1}\,h^{-1}$ ± 0.94.

Discussion. The measured value of egg cooling rate was considerably lower than that predicted using the mass-based formula derived from Kendeigh's (1973) data; 1.987 $^{o}C\,^{o}C^{-1}h^{-1}$ from this study compared with a calculated value of 5.366 $^{o}C\,^{o}C^{-1}h^{-1}$ for eggs of mass 1.72 g (mean Great Tit egg mass in this study). Such a marked difference in the rate of cooling has implications for calculations of the energetic demands of maintaining egg temperature.

Measurements of heat loss in eggs of other species provide support for the observed, lower value. Cooling rates measured in a similar fashion for Swallow eggs produced results consistent with those found in this study : 1.137 $^{o}C\,^{o}C^{-1}h^{-1}$ for 1.85g eggs in a clutch of 7 within a lined nest, (Jones 1985). Again, Kendeigh's equation would overestimate this, giving a rate of 5.208 $^{o}C\,^{o}C^{-1}h^{-1}$. The smaller eggs of the Coal Tit were found to lose heat at a slightly higher rate of 2.037 $^{o}C\,^{o}C^{-1}h^{-1}$ within a natural nest (Haftorn 1984).

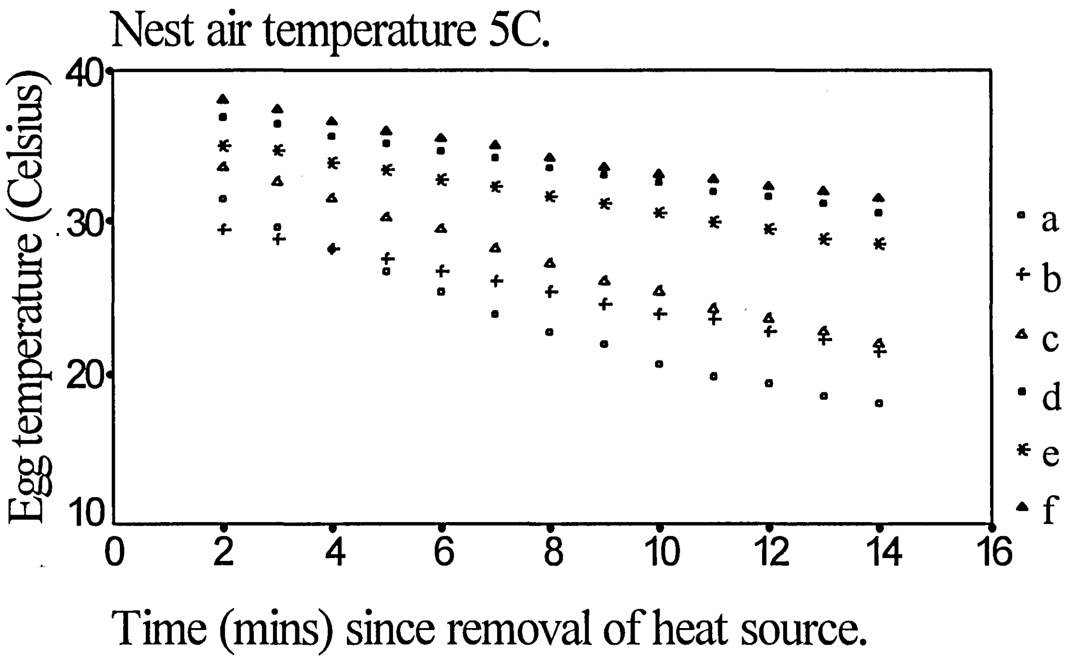


Figure 2.6 Egg temperature from 6 'thermistor' Great Tit eggs within an 8 egg clutch, following removal of heat source.

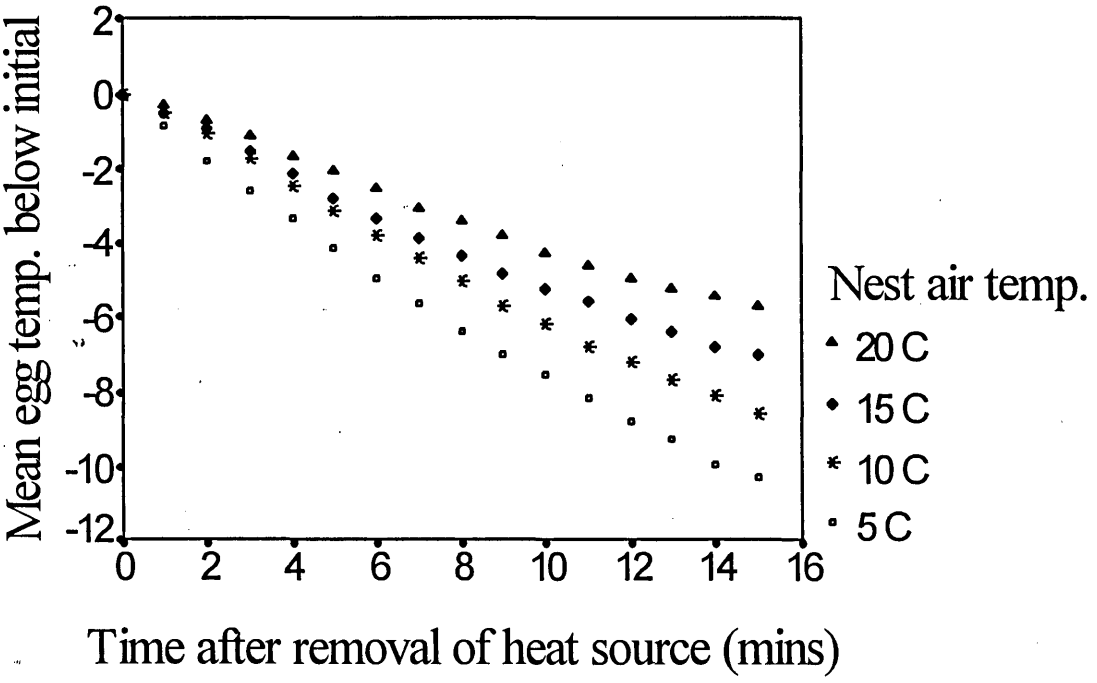


Figure 2.7 Decline in mean temperature for Great Tit 'thermistor' eggs at various nest temperatures (egg temperature expressed relative to initial at removal of heat source).

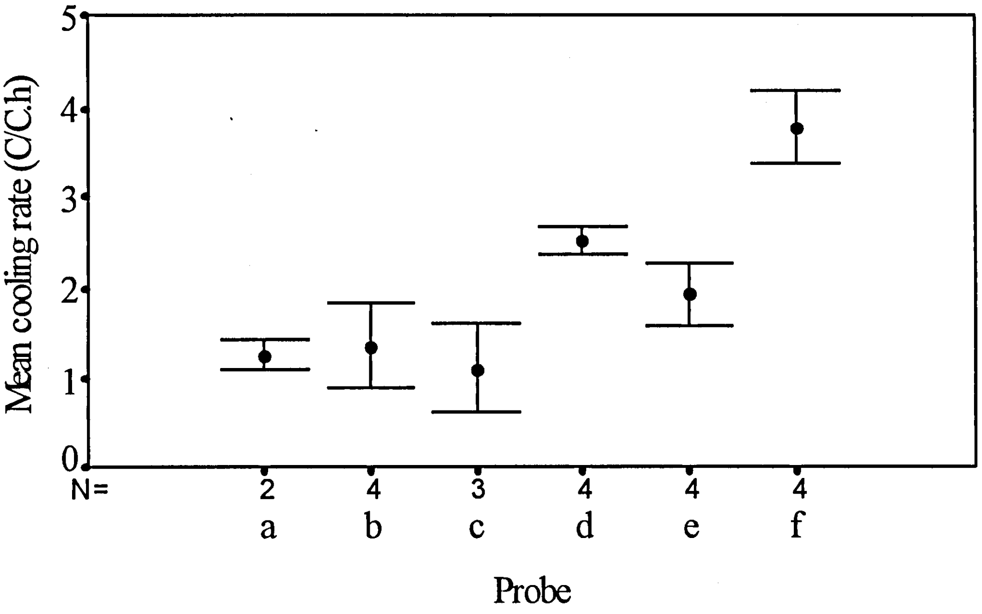


Figure 2.8 **Mean cooling rate for a range of positions of Great Tit 'thermistor' eggs placed at random within the clutch of 8 eggs (bars represent standard error of the mean).**

The wooden nest boxes used by Great Tits in the study area afforded protection from both wind and from direct solar radiation. Experimental conditions within the incubator probably approximate to those experienced in the field. The similarity of results, obtained both in other laboratory studies and in the wild, endorse the adoption of 1.987° C ° C $^{-1}$ h $^{-1}$ as a reliable estimate of Great Tit egg cooling rates within a natural situation.

2.7 Environmental data.

2.7.1 Weather.

A daily record of temperature maxima and minima (°C), of rainfall (mm), wind speed (knots) and cloud cover (oktas) was made at the Parkhead Meteorological Station, situated at Stirling University. The timing of sunrise and sunset were taken from records made at Glasgow in the appropriate year (Whitaker 1993, 1994, 1995). Sunrise and sunset were defined as the time when the true zenith distance of the sun's centre was 90° 50' (Whitaker 1993, 1994, 1995).

2.7.2 River flow rates.

Daily rates of flow (m^3s^{-1}) of the River Devon and the Allan Water were recorded by the Forth River Purification Board (now S.E.P.A.), at Castlehill Reservoir on the River Devon (NO 003030) and at Kinbuck on the Allan Water (NN 792053). In 1994, rates of flow at nine Dipper nest sites were also sampled using an impellor-type flow meter (Section 4.9).

2.7.3 Aerial insect availability.

Food availability to breeding Swallows was sampled by means of a 12.2m suction trap, situated at Stirling University, by the Parkhead Meterological Station, 0.5 to 10 km away from the study sites. This method of sampling is non-selective with respect to insect size and neutral in attraction and has been shown to provide a good indication of the availability of aerial insects (Waugh 1978). Although Swallows often feed closer to the ground than 12.2m, particularly in poor weather (the mean foraging height of Swallows was 7.4 ± 15.7 m (Waugh 1978)), the insect availability, sampled with a hand net at lower feeding stations, correlated well with suction trap volume (Turner 1982, Jones 1985). Although the suction trap volume cannot represent localised variation in insect abundance, it provides a measure of the availability of insects to Swallows over a wide area.

The trap drew air vertically downwards, avoiding directional effects and sampled at a constant rate throughout the day. Insects were trapped in a 1mm mesh gauze and were removed daily at 10:00h. The sample was preserved in 10:1 methanol : glycerol solution and the settled volume was estimated in a measuring cylinder (cm^3). No attempt was made to sort the samples, as taxa that were unavailable to breeding Swallows, including those that fly between dusk and dawn and those taxa not normally contributing to the diets of aerial feeding birds have been found to represent a small proportion of the total volume (Waugh 1979). Volumes were transformed using a logarithmic transformation ($Vt = \ln (V + 1)$, where V is volume of insects in cm^3), as the relationship between hirundine foraging rate and insect abundance is thought to be more likely to be logarithmic than linear in form (Bryant 1978).

2.8 Statistical analysis.

Statistical procedures followed Zar (1984) and were performed with the aid of the statistical package SPSS. Data were examined for normality by graphical methods and using the

Komologrov-Smirnov test. Non-parametric methods were applied in cases where the data deviated significantly from normality and were not improved by data transformation, or in cases where sample sizes were too small to warrant parametric techniques. Statistical significance was acceptable at the 5% level, i.e. $p<0.05$. Exact p values are presented where possible, but the following abbreviations are sometimes made for clarity:

$p<0.001$ ***;
$0.001<p<0.01$ **;
$0.01<p<0.05$ *,
$p>0.05$ n.s. (not significant).

Other statistical abbreviations used are shown in Table 2.16.

Means are presented ± standard deviation, with the sample size shown as a subscript, unless otherwise specified

Table 2.16 **Statistical abbreviations used in the text.**

Abbreviation	**Definition**
p	probability level
n	sample size
s.d.	standard deviation
s.e.	standard error
t	Student's t statistic
$F_{(x,y)}$	F statistic in analysis of variance, where x is the numerator and y the denominator degrees of freedom
r	correlation coefficient
r^2	coefficient of determination
χ^2	Chi-square statistic

2.9 Nomenclature. In general, species are referred to by their English names only. A full list of the scientific names of all animal species mentioned in the text is given in Appendix 3.

Chapter 3:

A cost of incubation in the Swallow.

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A cost of incubation in the Swallow.

3.1 Introduction.

A fundamental assumption of life-history theory is that organisms should allocate resources in ways which will maximise their fitness (Stearns 1976 for review). Limiting resources must be divided between conflicting demands and activities (formalised by the 'principle of allocation', Sibly and Calow 1986). When applied to iteroparous reproduction, this concept suggests a trade-off between current and future reproductive potential (Williams 1966, Charnov and Krebs 1974). It implies that increased reproductive effort at one breeding attempt would result in a reduction in residual reproductive potential. This could be manifest through a depression of parental condition, perhaps lowering the probability of survival (Bryant 1979, Tinbergen *et al.* 1985) or reducing future breeding performance (Røskaft 1985, Lessells 1986, Hegner and Wingfield 1987, Pettifor *et al.* 1988). The viability or the fertility of offspring could be reduced (Gustaffson and Sutherland 1988, Lindén and Møller 1988, Pettifor *et al.* 1988, Dijkstra *et al.* 1989, Thompson 1992). Such effects are thought to represent the costs of reproduction. They oppose the selective advantages accruing to higher reproductive investment. The resultant balance of selection pressure can result in the existence of an optimal level of investment, characterised by an optimal clutch size in birds.

Studies of reproductive costs have followed two basic approaches; correlative and manipulative (Roff 1992). Correlative studies have aimed to prove the existence, and to identify the nature, of costs for birds rearing natural clutches. The results have been prone to confusion by association with other variables. Natural clutch size may be a poor indicator of reproductive effort amongst individuals, as an individual may adjust clutch size to suit its own abilities and resources (Perrins and Moss 1975). Costs do not arise from effort *per se*, but from the ability to satisfy the demands imposed. If large clutch sizes are indicative of good resources, the correlation between clutch size and future reproductive success is more likely to be positive, than to show the negative relationship indicative of a trade-off. Certain correlative studies have shown that costs are associated with some aspects of reproductive effort (Bryant 1979, Klujver 1971), but the results have frequently been inconclusive and no general patterns have emerged.

By alteration of the number of young to be raised, techniques of experimental manipulation of reproductive effort have been used to break the link between a pair's ability to raise young and the reproductive effort demanded. Studies involving such manipulations have yielded more consistent evidence of reproductive costs (Reznick 1985). In some studies, trade-offs were found to act within the manipulated breeding attempt. For example, nestlings from enlarged broods were lighter and showed reduced survival (Linden and Møller 1988, Dijkstra 1989, Thompson 1992) or were less likely to be recruited to the breeding population than controls (Pettifor *et al.* 1988, Gustafsson and Sutherland 1988). Female Blue Tits rearing enlarged broods lost more mass than controls (Nur 1984 (a)). In other studies, reproductive costs were found to be paid during a second breeding attempt within the season. The probability of forming a second clutch may be reduced or its timing delayed (Pinowski 1977, McGillivray 1983, Slagsvold 1984, Roskaft 1985, Lessells 1986, Thompson 1992). Costs have even been found to be carried over to subsequent years: parents were less likely to survive or to return to breed in years following brood enlargement (Askenmo 1979, Nur 1984, Reid 1987, Wernham 1993), or their reproductive success in the season subsequent to manipulation was reduced (Røskaft 1985,Gustafsson and Sutherland 1988, Wernham 1993).

Ideally, direct manipulation of reproductive effort would involve all stages of offspring production; in birds, this should include egg laying, incubation and chick rearing, and arguably acquisition of a mate and territory and construction of the nest. Unfortunately, the costs associated with egg-formation and laying and with incubation have, until recently been excluded from such manipulations, the number of young generally being manipulated during the final stages of incubation or shortly after hatch (Murphy and Haukioja 1986, Gustafsson and Sutherland 1988, Pettifor *et al.* 1988, Partridge 1989, Barber and Evans 1995). In a recent study with Lesser Black-backed Gulls, Monaghan *et al.* (1995) were able to demonstrate a reduction in fledging success of chicks reared from nests where egg laying effort had been increased, while incubation and chick rearing effort were held constant. This suggests that reproductive costs are unlikely to be restricted to the chick rearing phase.

Studies involving experimental manipulation of clutch size during incubation have generally focused on the short term implications of the change, for example, by investigation of patterns of energy usage (Biebach 1981, Haftorn and Reinertsen 1985, Moreno and Sanz 1994). This approach was followed in a study of the energetics of incubation in the Dipper (Chapter 4).

Manipulative experiments which aim to evaluate the relationship between incubation effort and breeding success are less numerous than those dealing with the manipulation of brood size. However, the few studies where clutch size was manipulated during incubation have generally found evidence that reproductive costs are linked to incubation effort. The duration of incubation was found to be prolonged when clutches were enlarged in captive Bengalese Finches (Colemann and Whittall 1988) and in also in field situations in House Wrens (Baltz and Thompson 1988), Blue Tits (Smith 1989), Pied Flycatchers (Moreno and Carlson 1989, Siikamaki 1995) and Kentish Plovers (Székely *et al.* 1994). Hatching success was found to be reduced in enlarged clutches in Collared Flycatchers (Moreno *et al.* 1991) and in one study with the Pied Flycatchers (Siikamaki 1995).

The aim of this study was to manipulate clutch size, and hence reproductive effort, during incubation. By reversal of the treatment immediately prior to hatching, costs of brood rearing were to be held at levels associated with the natural, rather than the manipulated clutch, thus restricting the manipulation of reproductive effort to the incubation stage only. By monitoring a range of reproductive parameters, the study was designed to evaluate the existence of a short-term cost of incubation associated with clutch size, acting on either parent or offspring. The study was conducted with Swallows, which are generally double-brooded in the study area, so the experiment offered the opportunity to examine the intra-seasonal effects of incubation effort on parental ability to form a second clutch. While studies of reproductive effort during brood rearing frequently encompass an entire breeding season and may even extend to subsequent years, this study is unusual in looking for effects of incubation effort beyond the hatching of the manipulated clutch.

3.2 Methods.

3.2.1 Study species.

The Barn Swallow was chosen as an appropriate subject for this study because of the availability of nesting pairs throughout the study site and the ease of access to nests and of capture of adults. The prolonged breeding season enabled study of incubating birds from May to August. The birds are generally tolerant of handling during breeding.

The Swallow is an insectivorous passerine, feeding almost exclusively on flying insects which are generally taken by aerial pursuit, although other methods of capture have occasionally been recorded (Cramp 1988). The species is a summer migrant in Central Scotland (Cramp 1988), arriving to breed in late April and leaving between September and October for the wintering grounds in Africa, south of the Sahara, Arabia and the Indian subcontinent (Turner and Rose 1989).

The breeding biology of the species in this study area has been described in detail by Thompson (1992). Males generally arrive at the breeding grounds slightly earlier than females and pairs are established as the females arrive (Møller 1994), although a few birds apparently pair before arrival. Monogamy is the rule, though polygyny occurs rarely (Møller 1994). Extra-pair fertilisation, intra-specific brood parasitism and quasi-parasitism (a female participating in an EPC parasitizes the nest of the male she has mated with) have all been recorded in this population of the Barn Swallow (Bryant *et al.* unpublished). Copulation usually continues until late in the egg-laying sequence.

Nests are frequently located on rafters within barns, stables or outhouses. The nest is an open cup, constructed from mud pellets and straw (Turner and Rose 1989), and lined with hair and/or feathers. Nests may be constructed anew or old nests may be refurbished. Construction is performed mainly by the female (Cramp 1988) and takes 9 days for the mud nest, with between 2 and 10 additional days to complete the nest lining (Thompson 1992).

Clutch size varies between 2 and 7 in this population, with 85% of clutches consisting of 4 or 5 eggs (Thompson 1992). Most Swallows nesting in this area are double-brooded, with occasional pairs managing to raise three broods in one season. The mean clutch size for first broods (4.9 eggs) exceeds that for second broods (4.4 eggs); first broods also generally result in a greater number of fledged young (Thompson 1992). Eggs are normally laid daily, before 10:00h, although interruptions in the laying sequence of between 1 and 14 days have been recorded (Thompson 1992). Incubation usually commences with laying of the last or penultimate egg (Thompson 1992) and lasts for 14-16 days (rarely 11-19) (Turner and Rose 1989). Unlike the North American sub-species *Hirundo rustica erythrogaster* (Ball 1983), incubation is performed exclusively by the female, although instances of males covering eggs in the females' absence have been reported (Cramp 1988). The female alone develops a brood patch. For the majority of clutches, eggs hatch on the same day, though for some clutches, hatching extends over a period of up to three days (Thompson 1992). Chicks are fed in the nest for a period of 18-23 days (Turner and Rose 1989).

3.2.2 Study site.

Nests were studied at farms distributed within an area of mixed arable and grazing land around Stirling, Central Scotland (Figure 3.1). In this area, birds are loosely colonial, with 1-12, but usually only 2 or 3 nests occupied at each site. All sites were visited at 5 day intervals throughout the period when birds were arriving in 1993 to determine nest locations and dates of laying for first broods. In 1994, overlapping studies with Great Tits (Chapter 5) restricted the Swallow study to second broods only. These were easily located, as second broods were commonly initiated in a new, or refurbished, nest, within a few metres of that in which the first brood was raised. If clutches were discovered part-way through incubation, (eggs found warm), the number of days since laying was determined using the airspace diameter technique, outlined in Section 2.3.1.

3.2.3 Clutch manipulation.

Clutches were allocated to groups of three, such that dates of clutch completion within a group were within 2 ± 2 days (48 groups). The three clutches were then randomly assigned to one of three treatments: reduced ('R'), control ('C') or enlarged ('E'). Clutch manipulations were carried out on day 2.88 ± 1.93 $_{(95)}$ of incubation, where the date of clutch completion was day 1. On day 14.15 ± 1.98 $_{(97)}$ of incubation, the manipulation was reversed so that each female received her own eggs for hatching and subsequently raised her own chicks. The mean duration of the manipulation was 11± 2 days. Where eggs were lost during incubation due to hatching failure or to damage, the loss was not replaced. All eggs were marked with waterproof marker at the pointed end to enable identification of the donor nest.

The three treatments were as follows: two eggs were removed from the reduced group and added to the control nest. A further two eggs were removed from the control nest and added to the enlarged nest. All eggs were wrapped in cotton wool during transportation to avoid damage and to minimise heat loss.

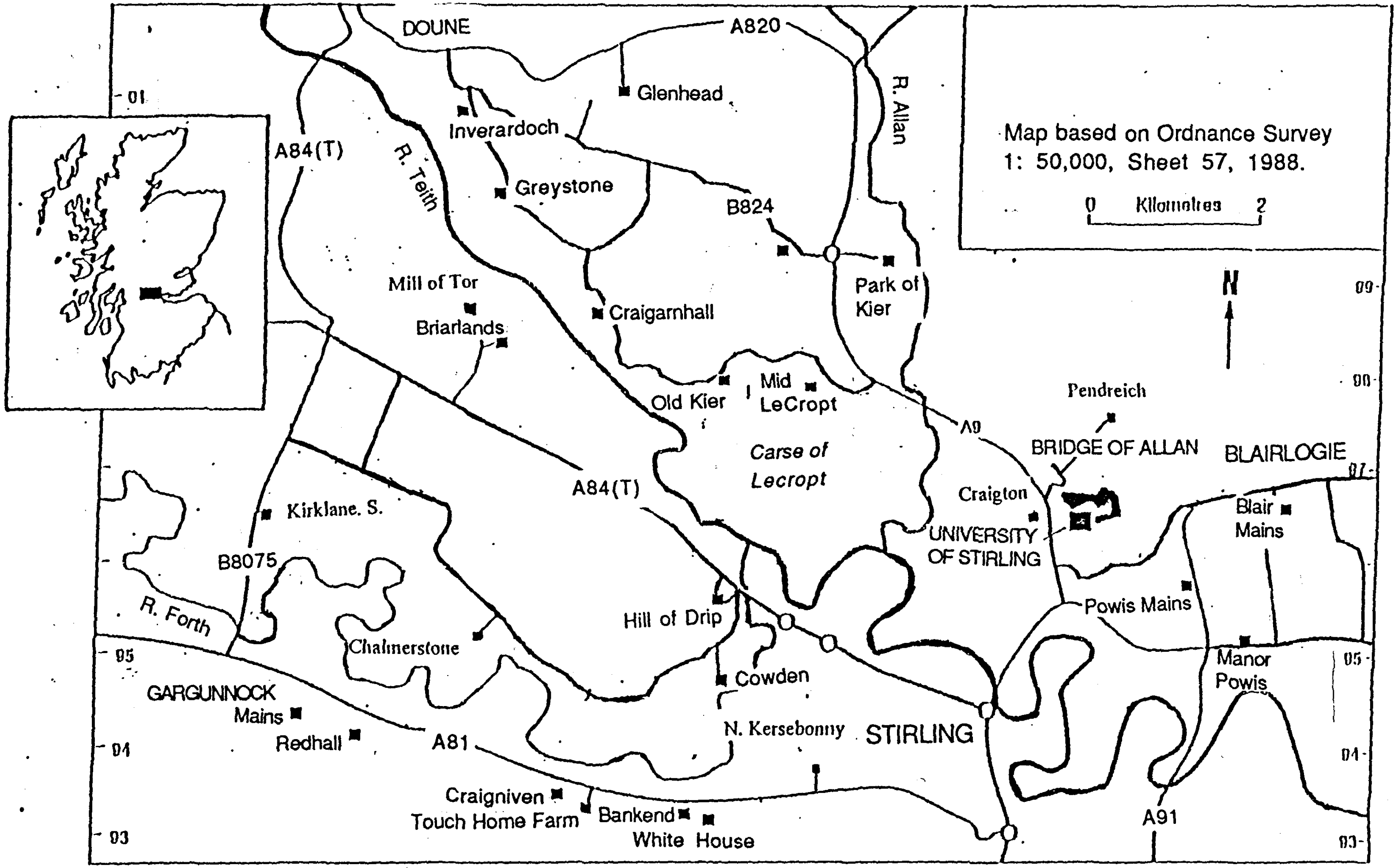


Figure 3.1 Swallow study sites in Central Scotland. The map shows the location of farms where Swallows were studied, roads and rivers.

Clutch size was equal to (natural clutch - 2) for 'R' birds, (natural clutch) for 'C', and (natural clutch +2) for 'E' birds. Birds were tolerant to clutch manipulation, with desertion immediately following clutch manipulation occurring in one instance only, where a clutch of 4 was reduced to 2 eggs. Thompson (1992) found a high risk of desertion if the manipulated clutch contained less than 3 eggs . For this reason, natural clutches of four eggs were not assigned to the 'R' group in 1993. However, this led to a bias in the clutch size between groups, which became a significant problem with second broods in 1993 (see Table 3.3). In 1994 clutches of 4 eggs were assigned to the reduced group, but only 1 egg was removed from the clutch. An additional egg was supplied to the C nest from a donor nest outwith the study to complete the manipulation. However, the problem still persisted to some extent in 1994.

If poor quality birds with smaller clutches were over-represented in the Enlarged treatment, this group may have been more susceptible to depressed reproductive success, regardless of clutch manipulation. Therefore, in order to guard against this possibility, trends of reproductive parameters across treatment groups were also examined within a subset of birds which had laid a clutch of 5 eggs.

The number of clutch manipulations performed is outlined in Table 3.1. In 1993, further controls were established by studying nests with no clutch manipulation, but nest checks, capture and handling were otherwise performed in a similar manner to the treated nests. In 1993, both the first and second broods of the same pair were included in the manipulation schedule. Attempts were made to reinforce any effect of the first clutch treatment by assigning the same manipulation to both first and second clutches. However this was not always practical; the resulting combination of treatments for birds with double manipulations within one season is outlined in Table 3.2.

Table 3.1 **Frequency of experimental clutch manipulations for Swallows in 1993 and 1994.**

		Reduced.	Control	Enlarged	No manipulation.
1993	1st brood / relays	14	7	14	39
	2nd brood	14	7	14	19
1994	2nd brood	18	15	19	-
Total		46	29	47	58

Table 3.2 **Treatment groups for double-brooded Swallows in 1993, for whom both first and second clutches were subject to manipulation.**

1st clutch manipulation	Reduced	Control	Enlarged
2nd clutch manipulation.			
Reduced	4	0	5
Control	1	0	1
Enlarged	1	2	4

Table 3.3 **Mean natural clutch size within each manipulation group. The difference between the groups was tested using oneway ANOVA.**

		Reduced	Control	Enlarged	p
1993	1st brood	$5.00 \pm 0.96_{(14)}$	$4.86 \pm 0.38_{(7)}$	$4.36 \pm 0.63_{(14)}$	0.081
	2nd brood	$5.07 \pm 0.47_{(14)}$	$4.29 \pm 0.49_{(14)}$	$4.43 \pm 0.51_{(14)}$	0.001
1994	2nd brood	$4.78 \pm 0.55_{(18)}$	$4.73 \pm 0.59_{(15)}$	$4.32 \pm 0.82_{(19)}$	0.083

3.2.4 Indicators of a reproductive cost.

The following parameters were measured to investigate the evidence for a short term cost of incubation.

3.2.4.1 Female mass and body condition.

Females were captured in the final days of incubation (day 14.15 ± 2.46) and measures of mass and structural size (Section 2.2.1) were recorded. Visible subcutaneous fat deposits were scored on a 5-point scale at two positions: the posterior edge of the sternum and the claviculo-coracoid (inter-clavicular) pit (Section 2.2.1). The thickness of the pectoralis muscle was estimated using an ultrasonic flaw detector (Section 2.2.1).

3.2.4.2 The duration of incubation.

This was calculated assuming that incubation began on the date of clutch completion. Although warm eggs were occasionally found prior to clutch completion (Section 2.3), incubation may not have been fully developed at this stage and may be more likely to occur at one part of the day than another. To avoid bias resulting from differences in the timing of nest checks, full incubation was assumed to commence on the date of clutch completion for every nest. Dates of hatch were determined by daily nest visits from day 14 of incubation onwards. Where hatching was asynchronous, the end of incubation was recorded as the date on which the majority of chicks hatched.

3.2.4.3 The rate of embryonic development.

From laying to hatch, eggs of all bird species lose up to 16% of their fresh weight at laying due to loss of water (Drent 1975). The subsequent decline in egg density has been used to determine the age of eggs (Lundberg and Vaïsänen 1979, Furness and Furness 1981). In this study, the utility of the rate of decline in egg density as a measure of embryonic development was investigated.

During 1993, a single egg, chosen at random from each of 11 nests was measured, (length and breadth to the nearest 0.1 mm using Vernier Calipers) and weighed daily between 11:00 and 14:00h to the nearest 0.01g using a 5g Pesola spring balance. During 1994, a single egg from each of 49 nests was measured in a similar manner, but the weighing frequency was reduced to a mean of 5 ± 2 measurements, spread evenly throughout the incubation period.

Egg volume can be calculated according to the formula $V = k\ l\ b^2$, where k is a constant specific to the species, l and b are length and breadth respectively (Hoyt 1979). Density (D) can be expressed in g cm^{-3} by calculating D = mass / V. The constant k was not calculated in this study, but was estimated from an average value of 0.507 (Hoyt 1979), derived from data for 26 species (egg mass range 6.7 to 1692 g). Hoyt's (1979) results suggest that the volume of any egg may be estimated from its linear dimensions with reasonable accuracy by the application of a single value of k. The calculated decline in egg density during incubation was compared across treatment groups.

3.2.4.4 Hatching success.

Hatching success was determined for each nest as the proportion of the eggs that were incubated by each female that were known to hatch. Thus, if a bird with an enlarged clutch incubated 7 eggs, her hatching success would be determined by the number of eggs hatching from her own clutch and that of the two eggs now returned to the donor nest. Efforts were made to ensure that infertile eggs were not included in the manipulations by candling to ensure some degree of chick development prior to egg transfer. In cases where clutches were deserted or eggs were damaged in the nest following reversal of the clutch manipulation, failure to hatch was apparently attributable to the laying bird, rather than to the incubating female. In such situations, hatching failure was not recorded for the incubating female.

Non-parametric analyses were performed as hatching success deviated significantly from a normal distribution (Komologrov-Smirnov test, $p< 0.001$).

3.2.4.5 Nest attendance.

Incubation schedules were determined for a sample of 18 second clutches during 1994 using two nest-temperature monitors (Section 2.4). The equipment was assembled gradually at the nest such that at least three probes were inside the nest cup amongst the eggs, an at least one probe sampled ambient temperature in the nest environs. Attendance were monitored simultaneously at pairs of one reduced and one enlarged clutch, several days after clutch manipulation. Twenty to twenty-four hours data were collected at each nest.

Analysis of the temperature profile of probes in the nest enabled identification of periods when the female left the nest for recess periods and subsequently recommenced incubation. Verification of these results was made by observing patterns of attendance during incubation at three sites for a total of 2 hours. The observed duration of session and recess periods was highly correlated to that derived from the nest temperature monitor (Pearson correlation coefficient $r = 0.950$, $n = 11$, $p < 0.001$). The small difference in timing resulted, in part, from the necessity of making observations outside the barn, so any time between nest departure and leaving the barn was incorrectly attributed to incubation sessions. In practice, analysis of results from the nest temperature monitor was probably at least as accurate as human observation and enabled simultaneous records to be made from two sites.

3.2.4.6 Nestling growth rates.

Any deterioration in the condition of the female through incubation could affect parental ability to feed nestlings (Lifjeld and Slagsvold 1986). The rate of chick growth was estimated by comparing measurements of wing length and mass made once for the whole clutch between day 2 and day 20 of nestling rearing. For consistency with the results of Thompson (1992), nestling age was expressed by assuming the date of hatch = day 0. Wing length increased linearly between d2 and day 20, while mass increases in a near linear fashion from day2 to 10, reaching a peak at day 13 (Thompson 1992).

The degree of asynchrony in hatch could affect age-specific measures of chick mass, as age was determined using the simplification that all chicks hatched on the same day. Suspicions that the degree of asynchrony was greater for clutches where eggs had been incubated in more than one nest (namely, those nests in the control and enlarged groups) led to the adoption of a two sample measurement technique, where chicks were measured at two intervals, usually d2 and d9, and the daily rate of change in mean wing length or mass compared across treatment groups.

3.2.4.7 Inter-brood interval.

Inter-brood interval was defined as the time interval between date of hatch of first clutch to date of laying of the first egg of the second clutch. This was calculated for those birds in 1993 whose first clutches were manipulated and whose second clutches were found. Nests were excluded from analysis if the first clutch or brood failed completely prior to fledging.

3.2.5 Environmental variation and food supply.

Daily information on weather was recorded at the Parkhead Meterological Station (Section 2.7). The availability of insect prey to Swallows was measured by the volume of insects caught in a 12.2.m suction trap (Section 2.7).

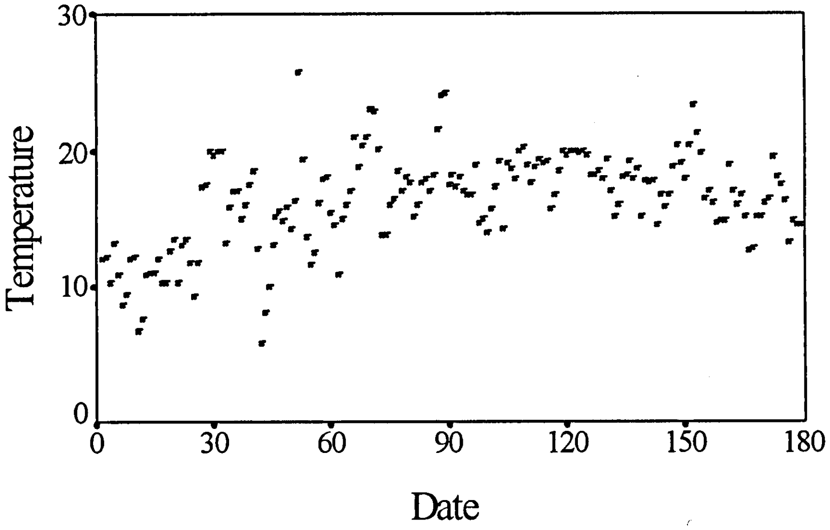
3.3 Results.

3.3.1 Seasonal variation in environmental conditions.

The seasonal variation in daily maximum and minimum temperature is shown for 1993 and 1994 in Figure 3.2. In both years, daily maximum temperatures rose significantly through the season to approximately day 120 and subsequently declined. In 1993, the slopes of both the seasonal increase and subsequent decline were lower and the data more variable than for the corresponding changes in 1994. The difference was not sufficient to attain significance in an analysis of covariance (for maximum or minimum temperature with date as a covariate, $p = 0.750$, $p = 0.111$ respectively).

Insect availability to Swallows was expressed as $Vt = \ln(v + 1)$, where v was the volume of insects caught in the suction trap in cm^3. The seasonal variation in this parameter is shown in Figure 3.3 for the two seasons. In 1993, Vt increased linearly throughout the season, while in 1994 the relationship did not attain significance. Analysis of covariance failed to detect any difference in the seasonal increase in Vt between years (main effect, $p = 0.187$). Values of Vt early in the season were higher in 1994 than 1993, but by early July this situation was reversed. Controlling for the effect of date, in 1993, Vt was positively correlated with maximum daily temperature, (partial correlation coefficient, $r = .261_{(176)}$, $p < 0.001$). In 1994, Vt was not significantly correlated with maximum temperature, but was negatively related to minimum temperature, (partial correlation $r = -.247_{(134)}$, $p = .004$). When data from both years were combined, the relationship between Vt and maximum daily temperature was best described by a negative quadratic function, shown in Figure 3.4.

(i) 1993.



(ii) 1994.

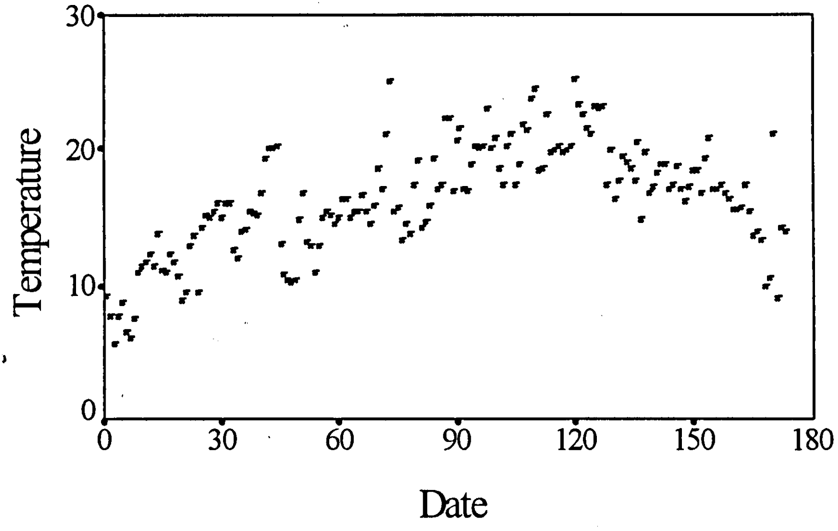
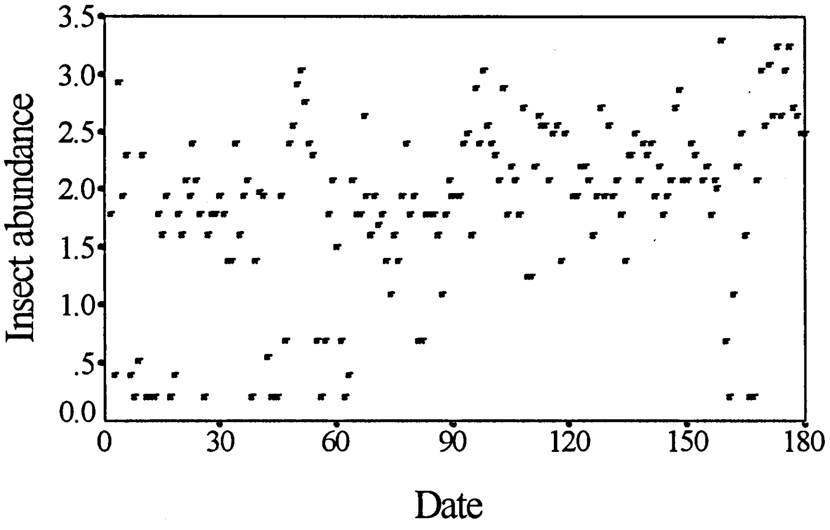


Figure 3.2 Seasonal variation in daily temperature maxima (°C) for (i) 1993 and (ii) 1994. Dates were coded using April 1st =1.

(i) 1993.



(ii) 1994.

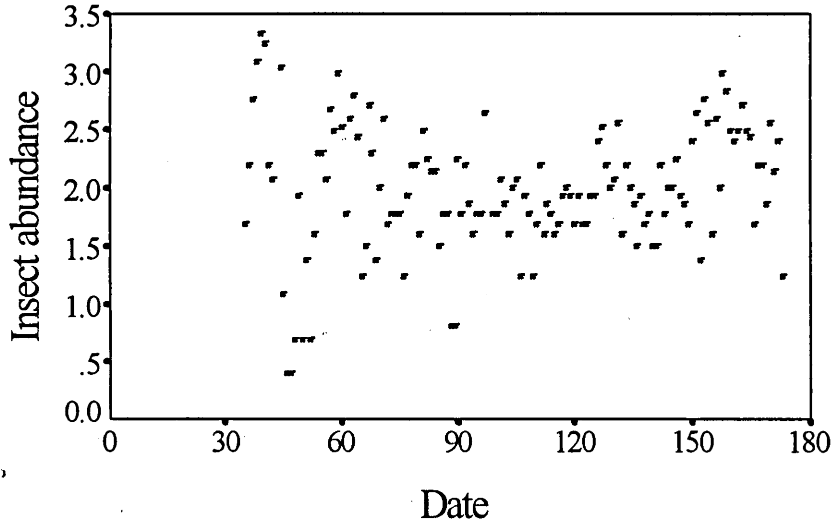


Figure 3.3 **Seasonal variation in daily insect abundance expressed as Vt = ln (v +1), where v is volume of insects caught in the suction trap in cm^3 in (i) 1993 and (ii) 1994. Dates were coded using April 1st =1.**

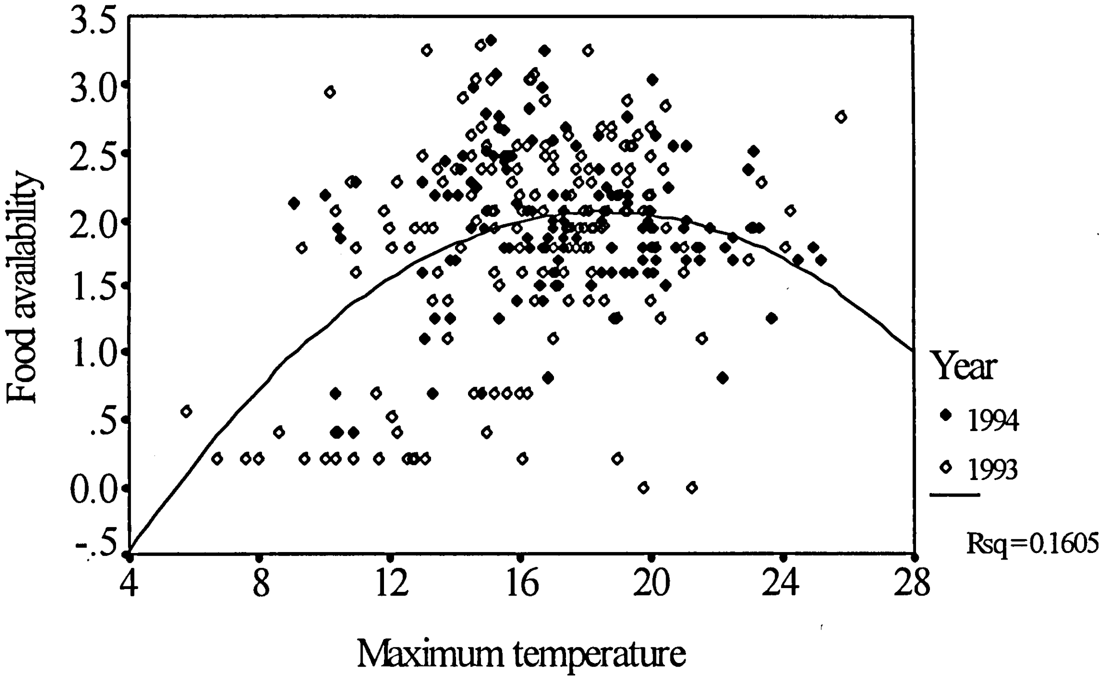


Figure 3.4 **Aerial insect availability (calculated as Vt = ln(v+1) where v is the volume of insects caught by the suction trap in cm^3) in 1993 and 1994, in relation to daily temperature maxima (^oC). In each year, the data were described by a quadratic function. For the combined data, $y = 0.448 - 0.012x - 2.077x^2$. $r^2 = .160$, $F_{(2,313)} = 29.912$, $p < 0.001$.**

3.3.2 Female mass and body condition.

3.3.2.1 Incubation mass in relation to structural size.

Multiple regression techniques and Principal Components Analysis were used to investigate the variation in incubation body mass of female Swallows in relation to their structural size (Section 2.2). 13% of the variation in body mass was explained in a single regression on head-bill length; this was increased to 17% by using a single factor derived from P.C.A.. Thompson (1992) found that live body mass correlated significantly and positively with total body lipid and protein in Swallows and that the standardisation of body mass using structural measures only marginally improved the ability to predict lipid and protein reserves. In the present study, standardisation for body size did not alter the results of the following analyses. Data are, therefore, presented without an adjustment for size.

3.3.2.2 Standardisation for time of capture.

In this study, the majority of females were caught between 16:00 h and 22:00h, mean capture time being 19.6h ± 3.8 (122 captures). There was no correlation between time of capture (expressed in hours after sunrise) and female mass; neither linear, quadratic nor cubic functions could be found to describe the relationship ($p > 0.6$ in all cases) (Figure 3.5).

However, studies of Swallow mass using automated electronic balances (Jones 1985, Ward 1992) have demonstrated the existence of a diurnal cycle of mass change. Between 19:00 and 08:00h, undisturbed incubating Swallows lost an average of 0.15 gh^{-1} ($n = 14$ birds) (Ward 1992). This loss was made good by feeding throughout the hours of daylight. For a single weighing, time of capture would, therefore, be expected to influence observed mass.

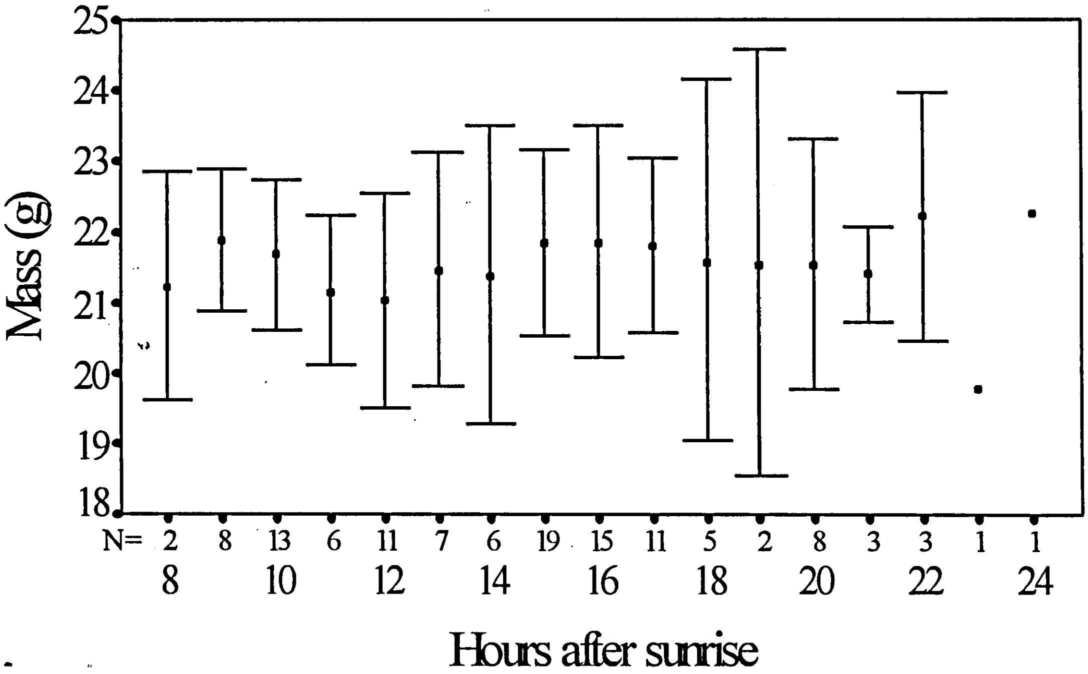


Figure 3.5 **Body mass of incubating female Swallows in relation to time of capture (expressed as hours after sunrise). Data points represent means and error bars represent 1 x s.d., sample sizes (n) are presented below the x-axis.**

Mass data were standardised to the modal capture time (20:00 h) by assuming a loss of 0.15 g h^{-1} from 19:00 to 08:00 h (Ward 1992), and a gain of 0.18gh^{-1} from 08:00 to 19:00h which would recoup this loss. MASSTIME was used in subsequent analyses to represent mass extrapolated to 20:00 h. This calculation adjusted body mass measures by an average of 1.5 % ± 1.3.

3.3.2.3 Incubation mass constancy in Swallows.

Confirming the results of Jones (1985) and Ward (1992), there was no trend of mass depletion through incubation in the Swallow (Figure 3.6). Correlation between female mass and the number of days since clutch completion were not significant (Spearman rank correlation between days since clutch completion and female mass r -.028 , p = 0.756, and female mass adjusted for time of capture (MASSTIME), r = 0.051, p = .578, data from 88 birds at 122 nests).

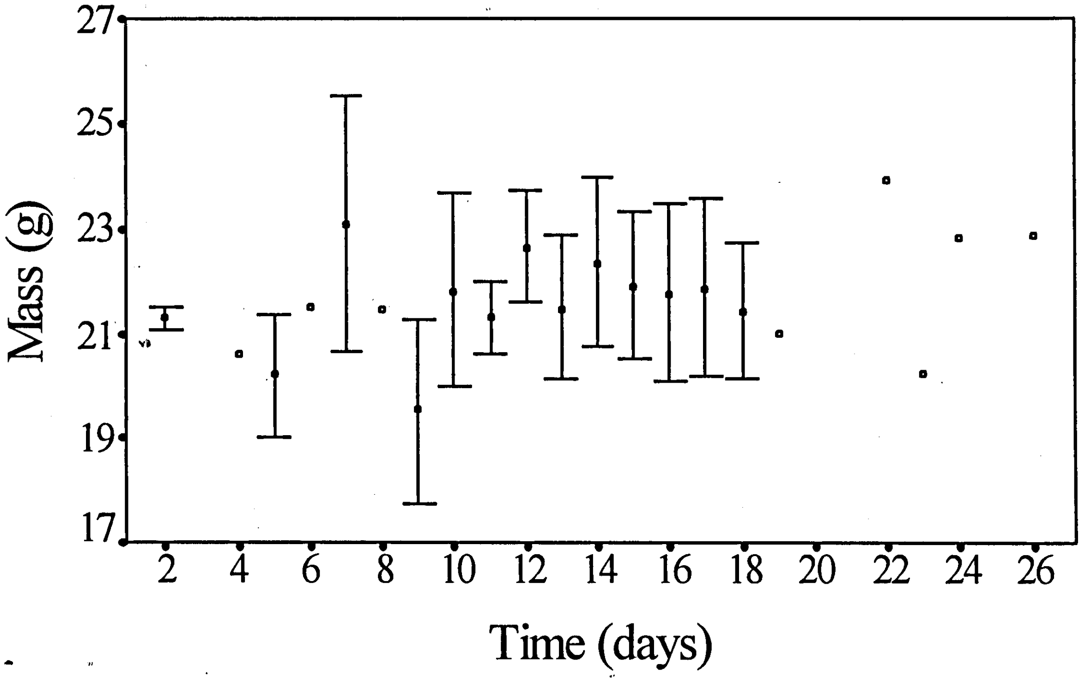


Figure 3.6 **Female mass (adjusted for time of capture) according to the stage of incubation, where date of clutch completion = day 1. Data points represent means ± s.d.**

Linear regression was used to investigate possible factors determining the mass of incubating female Swallows. In a stepwise multiple regression for those birds captured during their first clutches in 1993, female mass was found to be positively correlated with food availability (Vt) on the day of capture and negatively correlated with the date of catch, (Table 3.4). However, in a similar regression for the second clutches of both 1993 and 1994, none of the variables (Vt, date of catch, days since clutch completion, days to hatch, maximum temperature) was entered at the 5% level.

Table 3.4 **Stepwise multiple regression analysis of factors affecting mass of Swallows during incubation of first clutches in 1993 (n = 40 birds). Variables not included at the 5% level of significance were maximum daily temperature (°C), days since clutch completion, days to hatch.**

Independent variable.	**Cum. r^2**	**B**	**t**	**p**	**Beta**
Vt	.285	1.385	4.342	.001	.536
Date of catch (April 1st = day1)	.436	-0.047	-3.139	.0033	-.388
Constant		22.966	17.831	.0000	

3.3.2.4 Effect of clutch size on female mass

Data for female mass in the latter half of incubation were compared across treatment groups. Only those females caught between 9 days prior to and 2 days after hatch date (or predicted hatch date in cases of nest failure) were considered in this analysis. There was no significant difference in female mass between the three treatment groups (Table 3.5). Analysis of variance of either raw mass, or MASSTIME (adjusted for time of capture), gave p values > 0.85 for the complete data set, and for separate analyses performed within first and second broods and years. For females incubating first clutches in 1993, the variability in mass due to date of capture and to food availability could be removed by the multiple regression described in Table 3.4. The variance amongst the residuals differed significantly between treatment groups, (Levene's test for homogeneity of variance, p = 0.006) being greater in the enlarged group. The non-parametric Kruskal-Wallis test was therefore used to test the difference in the residuals from this regression between treatment groups. There was no difference between standardised mass for the treatment groups (p = .982).

There was no significant difference in female mass (or in MASSTIME) at the end of incubation between birds grouped according to experimental or natural clutch size (oneway ANOVA p>0.3 in all cases). However, there was a significant positive correlation between female mass and natural clutch size (Spearman rank correlation $r = .211_{(120)}$, p = 0.021), which was improved by adjusting mass for time of capture ($r = .236_{(120)}$, p = 0.009) (Figure 3.7).

Table 3.5 **Female incubation mass in relation to clutch manipulation. P values were calculated using oneway analysis of variance.**

	No manipulation	Reduced	Control	Enlarged	p
1993:1st clutch	$21.9 \pm 1.9_{(8)}$	$21.7 \pm 1.0_{(12)}$	$21.4 \pm 0.3_{(3)}$	$21.7 \pm 1.5_{(10)}$	0.944
1993:2nd clutch	$21.4 \pm 0.7_{(4)}$	$21.5 \pm 1.2_{(13)}$	$21.4 \pm 1.7_{(6)}$	$21.4 \pm 1.4_{(12)}$	0.995
1994:2nd clutch		$21.6 \pm 2.2_{(17)}$	$21.8 \pm 1.1_{(12)}$	$22.0 \pm 1.5_{(15)}$	0.852
Whole group	$21.7 \pm 1.6_{(12)}$	$21.6 \pm 1.6_{(42)}$	$21.6 \pm 1.2_{(21)}$	$21.7 \pm 1.4_{(37)}$	0.984

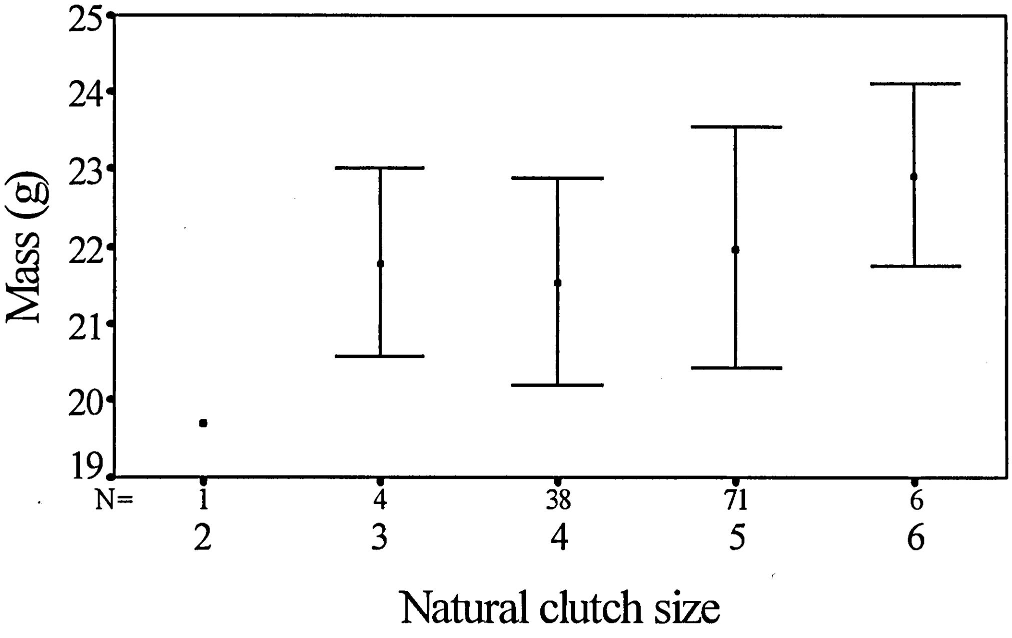


Figure 3.7 **Female incubation mass (standardised for time of capture) according to the number of eggs laid. Data represent means ± s.d., with sample size presented below the graph.**

3.3.2.5 Body condition of incubating Swallows.

The ultra-sonic measurement of pectoralis muscle thickness was used to estimate the extent of the protein reserves (Section 2.2). The following indices were used in subsequent analyses (following Newton 1989):

Eqn 3.1 $$\text{USMEAN} = \frac{(\text{US1} + \text{US2} + \text{US3})}{3}$$

Eqn 3.2 $$\text{USI} = \frac{(\text{US1} + \text{US2} + \text{US3})}{\text{keel}}$$

Eqn 3.3 $$\text{USVOL} = \frac{(\text{US1} + \text{US2} + \text{US3}) \times \text{keel}}{1000}$$

where keel = keel length in mm.

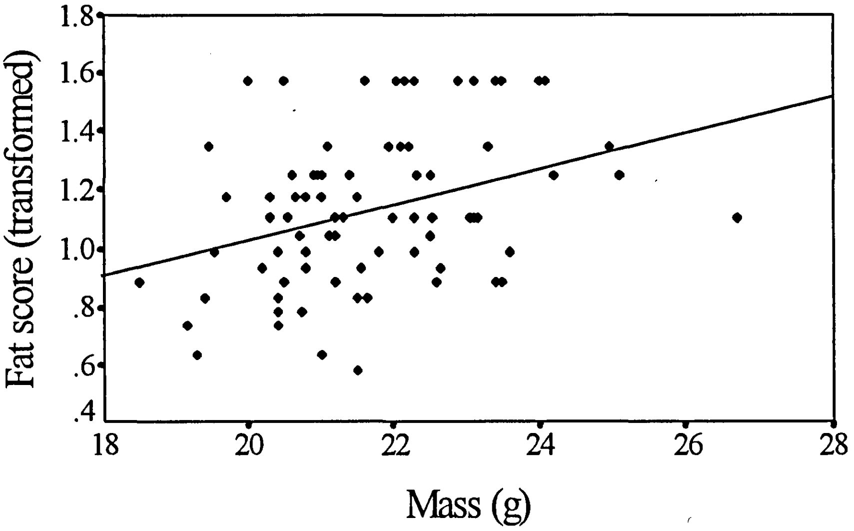
Lipid deposits were estimated from the sum of the scores for anterior and posterior fat deposits (Section 2.2). In order to achieve normality, this figure was expressed as a proportion of the total possible score (10) and transformed using an angular transformation.

Female mass was highly positively correlated with both fat scores and measures of pectoralis thickness, USI being the only exception (Table 3.6, Figure 3.8). Fat scores were positively correlated with measures of muscle thickness, though the relationships did not achieve significance.

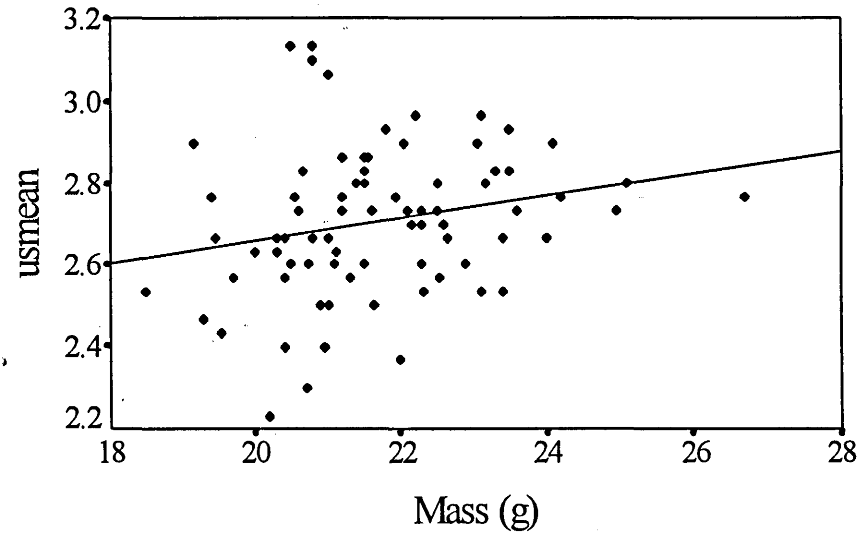
Table 3.6 **Pearson correlation coefficients between female mass, mass standardised for time (masstime), fat score (F.S.), and three measures of size of the pectoralis muscle, described in the text.**

	Mass	Masstime	F.S.	USMEAN	USI
Masstime	.975 (122) ***	.			
F.S.	.324 (121) ***	.350 (121) ***	.		
USMEAN	.223 (119) ***	.240 (119) **	.143 (119) n.s.	.	
USI	-.041 (118) n.s.	-.016 (118) n.s.	.102 (118) n.s.	.781 (118) ***	.
USVOL	.390 (118) ***	.389 (118) ***	.124 (118) n.s.	.804 (118) ***	.258 (118) ***

(i) Fat score.

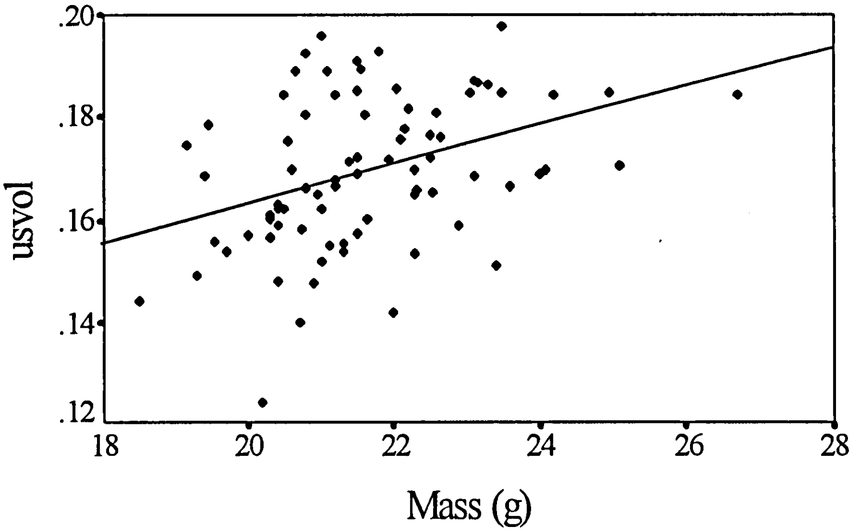


(ii) USMEAN



<u>Figure 3.8</u> (see over).

(iii) USVOL



(iv) USI

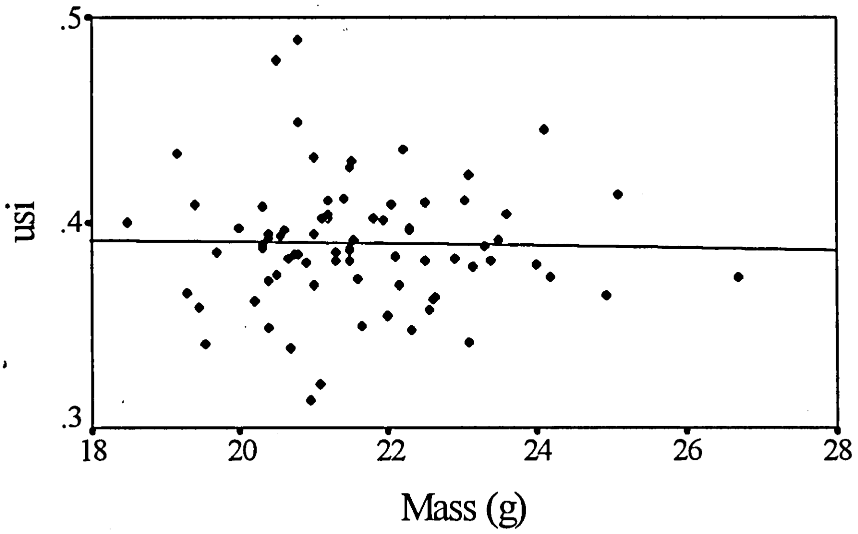


Figure 3.8 (i) Fat scores and three measures derived from the ultrasound reading ((iv) usmean, (iiv) usvol, (iv) usi, defined in the text) with mass of female Swallows during incubation. Fat scores were transformed using an angular transformation.

Significant differences were found in both fat scores and ultrasound measures between broods and years. Oneway ANOVA of all variables was performed to test for differences between three groups: 1st clutches in 1993, 2nd clutches in 1993 and 2nd clutches in 1994. Tukey's honestly significant difference test was used to identify which groups differed. Fat scores were significantly greater for birds with 2nd clutches in 1993 than those in 1994 ($p = .018$). Ultrasound scores were also generally highest in 2nd clutches in 1993. USI was significantly greater for birds with 2nd clutches in 1993 than those with either 1st clutches in 1993 or 2nd clutches in 1994 ($p < .001$). USMEAN was greater for birds with 2nd clutches in 1993 than for those with 1st clutches in 1993 ($p = .013$). However, USVOL was greatest for birds with 1994 2nd clutches and differed significantly from 1993 1st clutches only ($p = .005$).

Lipid deposits increased throughout the season; Pearson correlation coefficient between fat score and date, $r_{(121)} = .202$, $p = .026$ for the whole data set. When analysed separately according to year and 1st or 2nd clutches, this relationship held within 2nd clutches in 1994 only ($r_{(46)} = .510$, $p < .001$). Within both 1st and 2nd clutches in 1993 the relationships were negative, but not significant. In a stepwise multiple regression of transformed fat scores on date for data from all years, food availability (Vt), maximum and minimum temperatures, number of days since clutch completion and number of days to hatch, only Vt was entered as significant (Table 3.7.).

Fat scores are presented according to treatment group in Table 3.9(i). Fat scores did not differ between groups according to either treatment group, manipulated or natural clutch size (analysis of covariance with food availability as covariate, significance of the main effect $p = .519$, $p = .531$ and $p = .866$ respectively).

All three measures of pectoralis thickness were correlated with food availability on the day of capture; USI and USMEAN were also significantly correlated with date and USI with maximum daily temperature. To identify the factors of greatest importance, multiple regression techniques were performed with USMEAN, USI and USVOL as dependent variables and date, maximum and minimum daily temperatures and food availability as independent variables (Table 3.8).

Mean values of USMEAN, USI and USVOL are presented in Table 3.9(ii). The effect of treatment group was assessed for each of these measures, using analysis of covariance for USMEAN, USI and USTHICK, with covariates comprising the variables entered as significant in the above regressions. The data set was restricted to those birds caught at the end of incubation (at least 9 days after clutch completion and no more than 2 days post-hatch). In no case was the effect of treatment group significant ($p>0.2$ in all cases). In similar analyses of covariance, there was no difference between any of the three measures according to either natural ($p >0.1$ in all cases) or manipulated clutch size ($p>0.6$ in all cases).

Table 3.7 **Stepwise multiple regression; factors influencing (transformed) fat scores of incubating Swallows. Date, daily temperature maxima and minima (°C) on the day of capture, number of days since clutch completion and number of days to hatch were not entered as significant at the 5% level. Prey availability was represented by $Vt = \ln(v + 1)$, where v was the volume of insects caught in the suction trap on the day of capture. n = 119 females.**

Independent variable	Cumulative r^2	B	T	p	Beta
Food (Vt)	0.047	0.119	2.403	0.018	0.217
Constant		0.911	9.511	0.000	

Table 3.8 Stepwise multiple regression of three measures of pectoralis thickness on date of female capture (d1 = April 1st), maximum and minimum temperature (°C) and food availability (Vt). Variables not listed were not entered at the 5% level of significance.

(i) USI

Independent variable	Cumulative r^2	B	T	p	Beta
Max. Temp	0.035	-.002	-2.351	.020	-.214
Food (Vt)	.078	.013	2.303	.023	.210
Constant		.413	19.092	0.000	

(ii) USMEAN

Independent variable	Cumulative r^2	B	T	p	Beta
Food (Vt)	.051	.082	2.492	.014	.226
Constant		2.552	40.085	0.000	

(iii) USVOL

Independent variable	Cumulative r^2	B	T	p	Beta
Date	.050	1.31×10^{-4}	2.455	.016	.224
Constant		.155	27.083	0.000	

Table 3.9 Condition indices of incubating Swallows according to clutch manipulation group (i) fat scores and (ii) three measures of US score of pectoralis thickness.

(i) Fat scores.

	No manipulation	Reduced	Control	Enlarged
1993: 1st clutch	7.9±1.3 (8)	7.5±1.4 (12)	8.0±1.0 (3)	7.8±1.6 (10)
1993:2nd clutch	8.1±1.8 (4)	8.7±1.3 (13)	8.8±1.1 (6)	8.4±1.7 (12)
1994:2nd clutch		7.9±1.8 (17)	7.8±2.5 (12)	6.6±2.0 (15)
Mean	8.0±1.4 (12)	8.1±1.6 (42)	8.1±2.0 (21)	7.5±1.9 (37)

(ii) (a) USMEAN (estimate of mean pectoralis thickness)

	No manipulation	Reduced	Control	Enlarged
1993: 1st clutch	2.6±0.2 (8)	2.7±0.2 (12)	2.7±0.1 (3)	2.7±0.1 (10)
1993:2nd clutch	2.7±0.03(3)	2.8±0.2 (13)	2.8±0.1 (6)	2.7±0.2 (12)
1994:2nd clutch		2.7±0.2 (17)	2.7±0.2 (12)	2.7±0.1 (15)
Mean	2.6±0.2 (11)	2.7±0.2 (42)	2.7±0.1 (21)	2.7±0.2 (37)

(b) USI (estimate of pectoralis tissue per unit keel length)

	No manipulation	Reduced	Control	Enlarged
1993: 1st clutch	.39±.03 (8)	.39±.03 (12)	.40±.01 (3)	.38±.03 (10)
1993:2nd clutch	.39±.02 (3)	.42±.03 (13)	.40±.02 (6)	.40±.02 (12)
1994:2nd clutch		.38±.03 (17)	.38±.04 (12)	.38±.02 (14)
Mean	.39±.02 (11)	.40±.03 (42)	.39±.03 (21)	.39±.03 (36)

(c) usvol (estimate of absolute volume of pectoralis tissue)

	No manipulation	Reduced	Control	Enlarged
1993: 1st clutch	.16±.02 (8)	.17±.01 (12)	.17±.01 (3)	.17±.01 (10)
1993:2nd clutch	.17±.00 (3)	.17±.01 (13)	.17±.01 (6)	.17±.02 (12)
1994:2nd clutch		.17±.02 (17)	.17±.01 (12)	.18±.01 (14)
Mean	.16±.02 (11)	.17±.01 (42)	.17±.01 (21)	.17±.01 (36)

3.3.4 The duration of incubation.

The duration of incubation from clutch completion to hatch was calculated to the nearest day for 80 nests, as (date of hatch - date of clutch completion). The mean duration of incubation was 15.25 days ± 1.19, ranging from 12 to 19 days. When the data for both years were combined, there was a significant difference between the duration of incubation in the three manipulated groups, (Kruskal-Wallis test, $p = 0.022$, Table 3.10, Figure 3.9). Enlarged clutches took significantly longer to incubate than reduced clutches, although the difference between either group and the control birds did not attain significance ($p > 0.2$ in both cases, using Dunn's test for non-parametric multiple comparisons (Zar 1984)).

In order to exclude the possibility that a difference in natural clutch size between treatment groups was responsible for the relationship in Table 3.10, the data set was restricted to those birds laying clutches of 5 eggs only. Kruskal-Wallis test detected a significant difference between the groups. Enlarged clutches took significantly longer to incubate than control clutches ($0.02 < p < 0.05$), though the differences between enlarged and reduced, and between reduced and control were not significant.

Table 3.10 **The duration of incubation from clutch completion to hatch for experimental Swallow nests. Kruskal-Wallis test was used to test the differences within each brood and year.**

	Reduced	Control	Enlarged	Kruskal-Wallis test
1993: 1st clutch	$15.5 \pm 0.71_{(2)}$	$15.0 \pm 1.41_{(2)}$	$16.33 \pm 2.08_{(3)}$	$\chi^2_2=.935$, p=.627
2nd clutch	$14.48 \pm 1.13_{(8)}$	$16.0 \pm 0.82_{(4)}$	$15.44 \pm 0.53_{(9)}$	$\chi^2_2=3.412$, p=.182
1994: 2nd clutch	$14.72 \pm 0.67_{(18)}$	$14.77 \pm 1.69_{(13)}$	$15.56 \pm 0.96_{(16)}$	$\chi^2_2=7.475$ p=.024 *
Total	$14.82 \pm 0.82_{(28)}$	$15.05 \pm 1.55_{(19)}$	$15.61 \pm 0.99_{(28)}$	$\chi^2_2=7.600$ p=.022 *

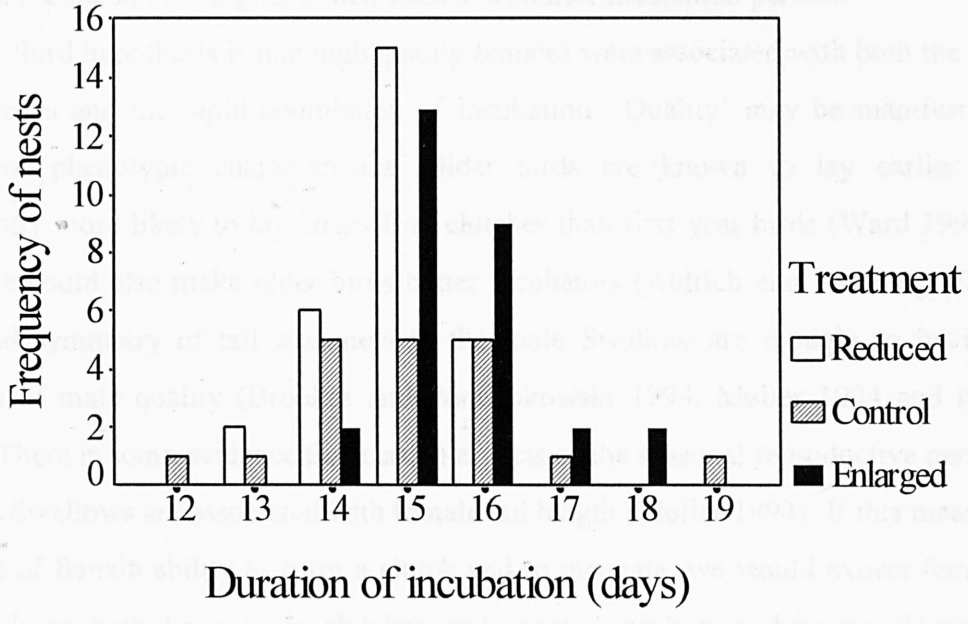


Figure 3.9 **The duration of Swallows incubation from date of clutch completion to hatch according to experimental clutch manipulation.**

The duration of the incubation period was also found to vary with natural clutch size, such that females laying large clutches tended to complete incubation faster (Spearman rank correlation r = -.265, p = .018, n = 80). This correlation may have been an artefact of the experimental technique, since the natural clutch size was slightly lower in the E group (Table 3.3). However, negative correlations persisted between incubation duration and natural clutch size within reduced and control treatment groups, though not in the enlarged group (Spearman rank correlations, Reduced group: r = -.344, p = .073, n = 28; Control: r = -.491, p = .033, n = 19, Enlarged: r = .059, p = .767, n = 28).

The inverse relationship between natural clutch size and incubation period could, perhaps, be explained by mutual correlation with an associated factor such as laying date. Clutch size is known to decline in Swallows throughout the breeding season, both between and within first and second clutches (Ward 1992). However, no significant correlation could be found between the duration of incubation and date of laying or date of clutch completion, analysing either for the whole group or for treatment groups separately (p > 0.2 in all cases). There was no significant difference between the duration of the incubation period of control nests according to 1st or 2nd clutches or year (Kruskal-Wallis test, χ^2_2 = 3.531, p = 0.171).Therefore, there was no evidence to support an alternative hypothesis that better conditions produced both higher clutch sizes and shorter incubation periods.

A third hypothesis is that high quality females were associated with both the laying of large clutches and the rapid completion of incubation. 'Quality' may be manifest via age, territory or phenotypic characteristics. Older birds are known to lay earlier and are subsequently more likely to lay larger first clutches than first year birds (Ward 1992), while experience could also make older birds better incubators (Aldrich and Raveling 1983). The length and symmetry of tail streamers in the male Swallow are thought to be important indicators of male quality (Brookes and Pomiankowski 1994, Møller 1994 and references therein). There is some evidence that certain aspects of the seasonal reproductive performance of female Swallows are associated with female tail length (Møller 1994). If this measure were indicative of female ability to form a clutch and to incubate, we would expect females with longer tails to both have large clutches and shorter incubation duration. There was no evidence to suggest that this was the case: Spearman correlation coefficients between outside tail length and the duration of incubation were not significant for analysis of all birds, or for separate analyses within treatment groups. However, there was some evidence to suggest that the duration of incubation may have been related to structural size: within the control group of birds, duration of incubation was negatively correlated with keel length (r = -.477, p =.045, n = 18). Relationships between structural size and clutch size have been found to be few and conflicting in this population (Ward 1992), suggesting that this was unlikely to be the main link between clutch size and duration of incubation in this study.

In summary, it seems that some aspect of quality is related to both clutch size and ability to complete incubation quickly, but the evidence is insufficient to identify the parameters involved.

3.3.5 Decline in egg density through incubation.

At the 11 nests where eggs were measured daily throughout incubation in 1993, egg mass did not alter significantly during the laying period, before the start of incubation. Small changes in density during this period were attributed to inaccuracies in the weighing equipment. Following clutch completion, eggs lost mass at an average of rate of $0.017gd^{-1}$, a loss of approximately 0.8% of the mass at clutch completion (Figure 3.10). Over 15 days of incubation, this would be equivalent to a 12% decline in initial egg mass.

There was no difference in the rates of decline in egg density measured in 1993 and 1994 (2 tailed t-test, p = .509), so the data were pooled for subsequent analyses.

Linear regressions of egg density on the number of days since clutch completion were computed for each nest. In 93% of cases, these were significant at the 1% level. Table 3.11 shows the rate of decline in egg density compared between the treatment groups for 1993 and 1994. Although the trend in 1993 was consistent with the hypothesis that reduced clutches would develop more quickly, oneway ANOVA showed that there was no significant difference between the slopes measured in each of the three treatment groups, for 1993 or 1994 separately, or for the combined data.

There were no significant correlations between slopes and either manipulated or natural clutch size, or the date of clutch completion. Importantly, the slope of decline of egg density did not correlate well with the duration of incubation, for 54 nests where this was known accurately (Spearman rank correlation, r = 0.004, p = 0.980). This suggest that the rate of decline in egg density cannot be used reliably to indicate the rate of embryonic

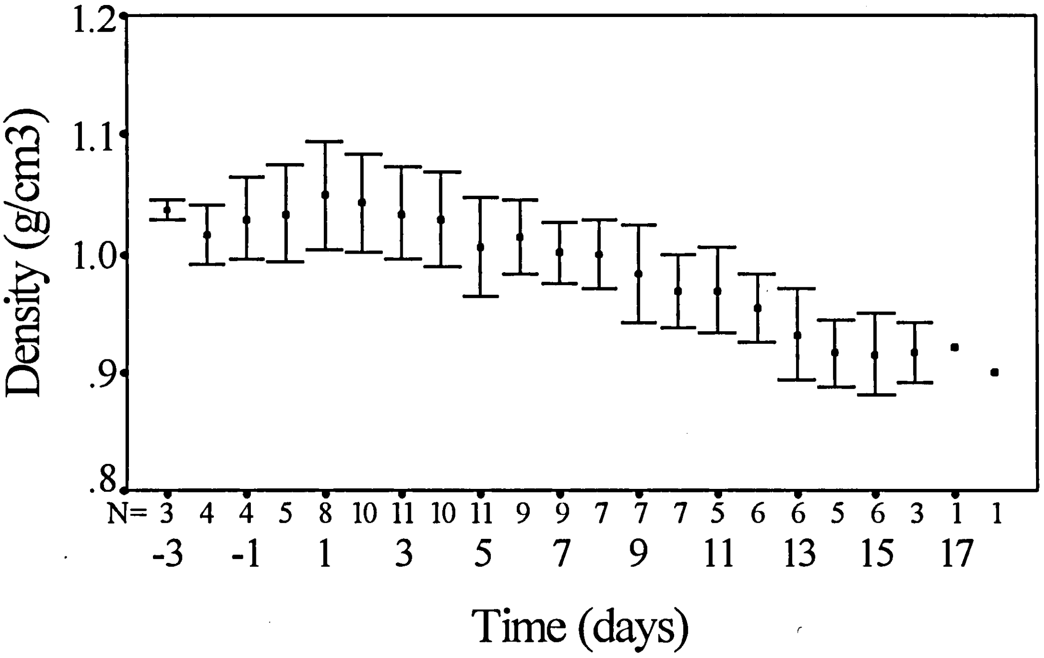


Figure 3.10 Egg density in g / cm^3 (calculated as mass/ (k x l x b^2), where mass (g), k = volume constant, estimated as 0.507 (Hoyt 1979), l = egg length (mm), b = breadth (mm)) of Swallow eggs measured daily in 1993, through laying and incubation. Date of clutch completion = day 1. The data represent mean values ± 1 x s.d., with sample sizes presented below the graph.

Table 3.11 Decline in egg density x 10^{-2} g cm^{-3} (mass/(k x l x b^2) through incubation for nests according to clutch size manipulation. Oneway ANOVA was used to test for a difference between groups.

	Reduced	Control	Enlarged	p value
1993	1.180 ± 0.306 $_{(5)}$	1.166 ± 0.078 $_{(2)}$	1.136 ± 0.223 $_{(4)}$	0.968
1994	1.097 ± 0.308 $_{(18)}$	1.068 ± 0.304 $_{(12)}$	1.079 ± 0.473$_{(19)}$	0.977
Mean	1.115 ± 0.303 $_{(23)}$	1.082 ± 0.283 $_{(14)}$	1.089 ± 0.436 $_{(23)}$	0.953

development in the Swallow. Conditions affecting evaporative losses from the nest, such as temperature, humidity and the exposure of the nest to air movements may be more influential in determining rates of water loss, and hence mass loss, than the rate of development of the embryo.

3.3.6 Hatching success.

Handling and transport of eggs did not alter their hatch rate. The mean hatching success of nests with no clutch manipulation (0.88 ± 0.26 $_{(28)}$) did not differ significantly from that in the control group of birds with manipulated clutches (0.86 ± 0.31 $_{(29)}$); Mann-Whitney U-test, p = 0.535.

Hatching success was generally lower in 1994 than in 1993. In 1993, 88% of all eggs hatched, in 1994, hatching success was 83%; this difference was significant (Mann-Whitney U-test p = 0.047). Exclusion of those cases of whole nest failure gave a hatching success of 94% in 1993 and 90% in 1994; the difference between the two remained significant (p = 0.033).

The proportion of eggs hatching from nests of the three treatment groups is shown in Table 3.12. Part (i) includes all cases of failure to hatch. Part (ii) includes only those nests where at least one egg hatched, excluding cases of predation, nest falls or desertion. The manipulation of clutch size significantly affected the proportion of eggs hatching. Dunn's test for non-parametric multiple contrasts was used to determine amongst which groups the differences were significant. The resulting significance levels were as follows:

(i) including all cases of failure to hatch	R v E: $0.001 < p < 0.002$,
	R v C: $0.05 < p < 0.10$,
	C v E: $p > 0.5$.
(ii) excluding cases of nest failure:	R v E: $p < 0.001$,
	R v C: $0.05 < p < 0.10$,
	C v E: $P > 0.2$.

Hatching success was significantly lower in enlarged than in reduced clutches. While the difference between reduced and control nests approached significance, enlarged clutches did not differ significantly from control nests.

Table 3.12 **The proportion of eggs incubated that hatched successfully, shown according to treatment group. Values of χ^2 and p were calculated using the Kruskal-Wallis test.**

(i) All cases of failure to hatch included.

	Reduced	Control	Enlarged	χ^2	p value.
1993: 1st clutch	.88 ± .28 $_{(13)}$	.82 ± .87 $_{(7)}$	.87 ± .29 $_{(14)}$	0.108	0.947 n.s.
2nd clutch	.86 ± .36 $_{(14)}$	1.00 ± 0 $_{(7)}$	.88 ± .15 $_{(14)}$	4.592	0.100 n.s.
1994: 2nd clutch	1.00 ± 0 $_{(18)}$	.76 ± .33 $_{(15)}$	.72 ± .30 $_{(19)}$	18.430	0.0001***
Mean	.92 ± .25 $_{(45)}$	.83 ± .31 $_{(29)}$	.81 ± .27 $_{(47)}$	13.125	0.001 ***

(ii)Hatching success, excluding those cases where predation, desertion or nest falls led to complete clutch loss.

	Reduced	Control	Enlarged	χ^2	p value.
1993: 1st clutch	.96 ± .10 $_{(12)}$	.96 ± .10$_{(7)}$	.94 ± .15 $_{(13)}$	0.122	0.941 n.s.
2nd clutch	1.00 ± .0 $_{(12)}$	1.00 ± 0 $_{(7)}$	.88 ± .15 $_{(14)}$	9.565	0.008 **
1994: 2nd clutch	1.00 ± 0 $_{(18)}$	.88 ± .13 $_{(13)}$	.81 ± .17 $_{(17)}$	16.659	0.0002***
Mean:	.99 ± .05 $_{(42)}$	.93 ± .12 $_{(26)}$	.87 ± .16 $_{(44)}$	18.378	0.0001***

Table 3.13 **Hatching success of Swallow eggs (proportion of eggs that hatched successfully) in relation to manipulated clutch size.**

(i) Including cases of nest failure.

	2	3	4	5	6	7
1993:						
1st broods.	.	.90±.32 (10)	.83 ±.13$_{(6)}$	.85±.31$_{(22)}$	.83±.35$_{(9)}$	.98 ±.06$_{(6)}$
2nd broods	0$_{(1)}$	.91±.30$_{(11)}$	1.0±0.0$_{(10)}$	.88 ±.28$_{(8)}$	.83±.18$_{(8)}$	.95±.07$_{(6)}$
1994:						
2nd broods.	.	1.00±0$_{(18)}$	.88±.25$_{(4)}$	.72±.34$_{(13)}$	.71±.32$_{(8)}$	.73±.31$_{(9)}$
Mean	0$_{(1)}$	.95±.22$_{(39)}$	.93±.14$_{(20)}$	.81±.32$_{(43)}$	.79±.29$_{(25)}$	.86±.24$_{(21)}$

(ii) Excluding cases of nest failure.

	2	3	4	5	6	7
1993:						
1st broods.		1.0±.00$_{(9)}$	.83 ±.13$_{(6)}$	.93±.16$_{(20)}$	.94±.18$_{(8)}$	.98 ±.06$_{(6)}$
2nd broods	.	1.0±.00$_{(10)}$	1.0±0.0$_{(10)}$	.88 ±.28$_{(8)}$	.83±.18$_{(8)}$	.95±.07$_{(6)}$
1994:						
2nd broods.	.	1.0±0$_{(18)}$	.88±.25$_{(4)}$	.85±.13$_{(11)}$	.81±.15$_{(7)}$	.82±.17$_{(8)}$
Mean	0$_{(1)}$	1.0±.00$_{(37)}$	.93±.14$_{(20)}$	.90±.18$_{(39)}$	.86±.17$_{(23)}$	.91±.13$_{(20)}$

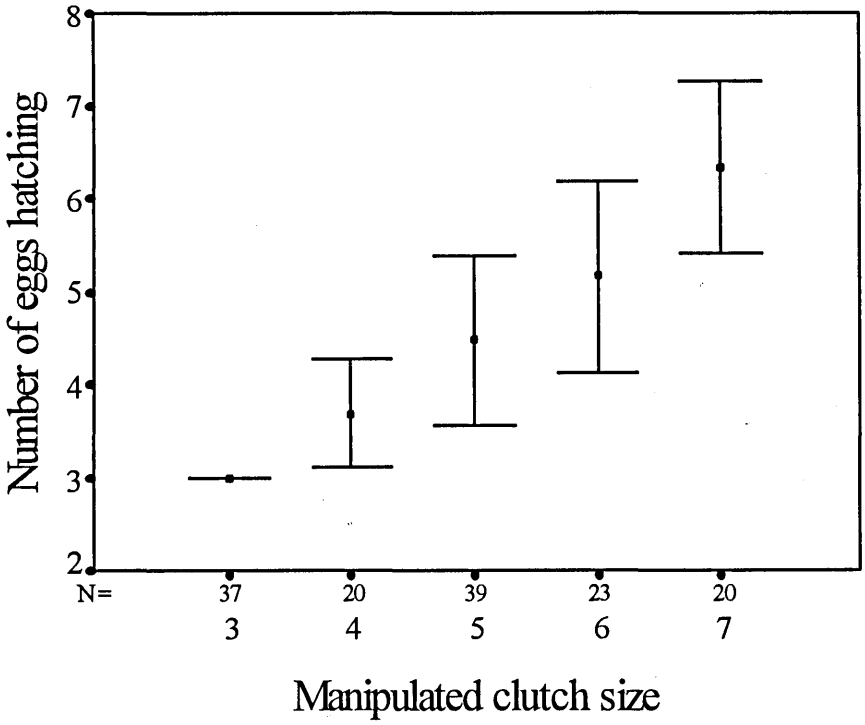


Figure 3.14 The mean (± s.d.) number of young hatching from Swallows' nests of various experimental clutch sizes. Sample sizes are presented at the bottom of the graph.

The proportion of eggs hatching successfully was found to be significantly negatively correlated with the number of eggs in the nest throughout the incubation period, whether cases of nest failure were included (Spearman rank correlation coefficient r = -0.272, n = 149, p = 0.001) or excluded (r = -0.338, n = 139, p < 0.001). Mean values for hatching success at each clutch size are presented in Table 3.13. The significant negative correlation between hatching success and number of eggs incubated resulted from the relationships in second broods in 1993 and 1994, the positive correlation found in first broods in 1993 alone was not significant.

Hatching success did not vary according to the natural clutch size of the female (Spearman correlation coefficient between natural clutch size and hatching success of those nests that did not fail, r = .109, p = .201, n =139). Even controlling for manipulated clutch size, the partial correlation coefficient between hatching success and natural clutch size was not significant (r = .107, p = .138, n = 139).

In spite of the depression in hatching success at increasing clutch sizes, large clutches produced the highest number of hatchlings (Figure 3.14, Spearman correlation coefficient r = .841, p < .001, n = 139).

3.3.7 Patterns of nest attendance.

Female Swallows incubated continuously during the night, but left the nest repeatedly during the day to forage. Values of the duration of incubation sessions, both during intermittent incubation during the active day and during the continuous night-time incubation, and of recess periods were computed for 20 - 24 hours data at each of 16 nests in 1994. The mean values for these parameters for each nest are presented in Appendix 4.

As data from each pair of nests overlapped by a period of at least 20 hours, matched pairs analysis was used to investigate the difference between nest attendance schedules of enlarged versus reduced nests. The mean values derived from the 8 pairs are shown in Table 3.15. There was no significant difference between the parameters measured in the two groups.

Incubation constancy, or the percentage of the active day that a female spent on the nest, was calculated for each bird. Reduced clutches were incubated for 68% ± 3 of the active day, while enlarged clutches were incubated for 71% ± 4. The difference was not significant t = -1.70, p = 0.110).

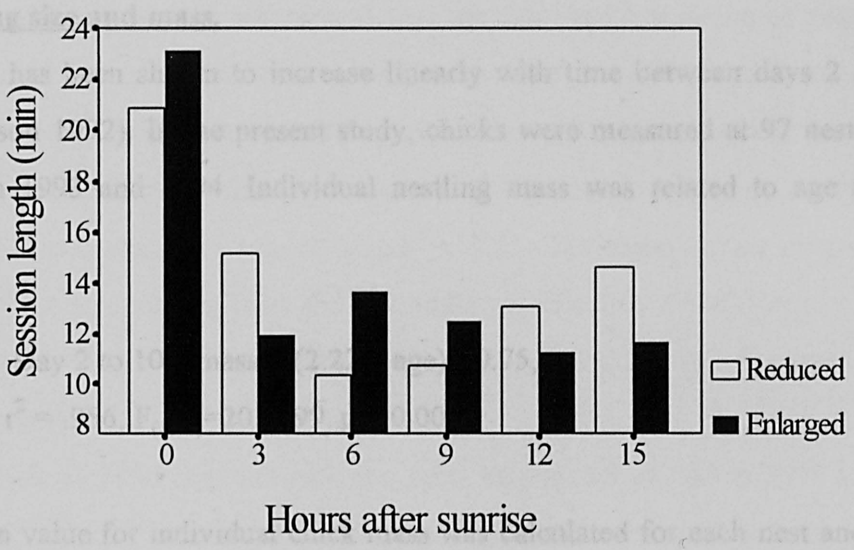
Table 3.15 **Parameters of nest attendance for incubating swallows, grouped according to clutch manipulation. Values represent the average of the daily means (in minutes) recorded at 8 reduced and 8 enlarged nests. Paired t-tests were used to investigate the difference between the two groups; p values are presented in the final column.**

	Enlarged	Reduced	p -value	Mean
Session length (day)	11.52 ± 1.83	11.60 ± 3.11	0.948	11.56 ± 2.47
Session length (night)	581.5 ± 82.3	592.0 ± 50.8	0.766	586.75 ± 66.27
Recess length	5.06 ± 1.78	4.53 ± 1.05	0.509	4.80 ± 1.44

Hourly means were computed at each nest in order to assess whether the two treatment groups varied in the duration of incubation sessions or recess periods throughout the day (Figure 3.11). For clarity, the data were grouped into 3-hourly periods, according to the number of hours after sunrise. At the beginning and end of each day, the majority of birds were incubating continuously (night-time session) so few session or recess periods were recorded. Thus, the long session and recess periods at these times represent the exceptional birds which were engaged in intermittent incubation at this time and are not indicative of generally low incubation constancy at this time.

Repeated measures ANOVA was performed on both session and recess length, with manipulation as the main effect and five repeated measures from each bird, representing the means from each three hourly period between 2 and 16 hours after sunrise. Analysis was restricted to this time period, when over half the birds were engaged in intermittent incubation to avoid inclusion of the few long periods in the early hours. Neither manipulation nor time period had a significant effect on the duration of incubation sessions, nor was there any interaction between the two ($p > 0.17$ in all cases). The duration of recess periods differed significantly between time periods ($p = 0.008$), but the effect of the clutch manipulation was not significant ($p = 0.300$). The relationship between time and recess length did not differ according to manipulation groups (time x manipulation interaction term $p = 0.583$).

(i) Session length.



(ii) Recess length.

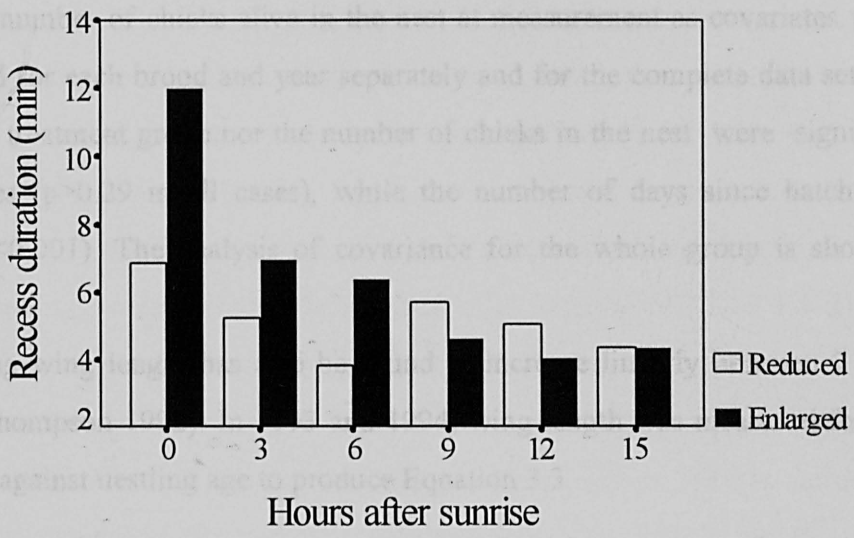


Figure 3.11 **Patterns of nest attendance in the Swallow; (i) session and (ii) recess length according to time of day. Mean values were calculated for three-hourly periods after sunrise, the mid-point each period is shown on the x-axis. Shading of the bars indicates whether clutch size was enlarged or reduced.**

3.3.8 Effects on chick rearing capacity.

3.3.8.1 Nestling size and mass.

Nestling mass has been shown to increase linearly with time between days 2 and 10 after hatch (Thompson 1992). In the present study, chicks were measured at 97 nests within this time period in 1993 and 1994. Individual nestling mass was related to age according to Equation 3.2:

Eqn 3.2 Day 2 to 10; $\text{mass} = (2.22 \times \text{age}) - 0.75$,
$r^2 = .956$, $F_{(1,95)}=2079.99$, $p < 0.0001$

A mean value for individual chick mass was calculated for each nest and an analysis of covariance performed with treatment group as the main effect, the number of days since hatch and the number of chicks alive in the nest at measurement as covariates. The analysis was performed for each brood and year separately and for the complete data set. Neither the main effect of treatment group nor the number of chicks in the nest were significant in any of the analyses ($p>0.29$ in all cases), while the number of days since hatch was always significant ($p<0.001$). The analysis of covariance for the whole group is shown in Table 3.15(i).

Nestling wing length has also be found to increase linearly between 2 and 20 days after hatch (Thompson 1992). In 1993 and 1994, wing length was measured for 109 broods and regressed against nestling age to produce Equation 3.3.

Eqn 3.3 d2-20: $\text{wing} = (5.29 \times \text{age}) - 3.62$
$r^2 = .966$, $F_{(1,107)} = 3035.535$, $p < 0.0001$.

Analysis of covariance was again performed, with treatment group as the main effect, and age and brood size as covariates. In the analyses of the complete data set, and of the data grouped according to year and brood, there was no effect of treatment group on wing length. The number of chicks in the nest did not explain a significant proportion of the variation in wing length. Table 3.15(ii) shows the results of the analysis for the data for 1993 and 1994 combined.

3.3.8.2 Nestling growth rates.

As nestling mass was linear between ages 2 and 10 days (Figure 3.12 (i)) repeat measurements within this time period could be used to give a measure of nestling growth. This measurement was preferred over a single measurement of size or mass, as the latter would be affected by the degree of hatching asynchrony to a greater extent than the former. Nestling mass was measured twice at an interval of 6.3 days ± 1.3, at 41 nests. During this period, chicks gained mass at a rate of 2.3gd^{-1} ± 0.23. The mean values of growth rate for chicks in the three treatment groups did not differ significantly (ANOVA p = .605). Mean daily mass increases were as follows: Reduced: 2.289gd^{-1} ± 0.280 $_{(15)}$, Control : 2.241 gd^{-1} ± 0.210 $_{(12)}$, Enlarged: 2.333 gd^{-1} ± 0.190$_{(14)}$.

There was no difference between the mass increments gained in 1993 for 1st and 2nd broods, or in 1994 for 2nd broods (ANOVA p = .107). Mass increase was lower for nests with a higher brood size, but the relationship was not significant (Spearman rank correlation between mass gain and number of chicks alive in the nest at second measurement, r = -.256, n = 41, p = .107). Mass gain had a tendency to increase through the season; Pearson correlation coefficients with date of hatch were positive but not significant (r = .152, n = 41, p = .342).

Rates of daily increase in wing length were computed for those measurements made within the period of linear growth, between 2 and 20 days after hatch (Figure 3.12(ii)). At 64 nests, where repeated measurements were made at an interval of 8.3 ± 3.4 days, the mean chick wing length increased by an average of 5.16 ± 0.63 mm d^{-1}.

Again, there was no effect of treatment group on the growth of wing length (oneway ANOVA p = .100). Mean values for the three treatment groups were as follows: Reduced: 5.071 ± .705 $_{(22)}$; Control: 5.081 ± .474 $_{(17)}$; Enlarged: 5.259 ± .598 $_{(20)}$ mm d^{-1}.

No differences were detected between growth rates in different broods or years or in broods of different sizes. There was a significant negative correlation between the increase in wing length and the date of hatch, (Spearman rank correlation, r = -.299, n = 64, p = .017), implying that nestling growth rates were slower later in the season. Notably, this trend was in the opposite direction to the observed correlation between increasing mass gain with date of hatch.

Table 3.15 **Analysis of covariance of (i) chick mass between 2 and 10 days after hatch and (ii) wing length between 2 and 20 days after hatch, with treatment group (reduced, control or enlarged) as the main effect. Nestling age in days since hatch and the number of chicks in the nest at the time of measurement were entered as covariates. Data for 1993 and 1994 included.**

(i) Nestling mass.

	Sum of squares	d.f.	Mean square	F	p
Covariates					
chick age	1816.82	1	1816.82	1149.75	.000
brood size	0.54	1	0.54	0.34	.561
Main effects					
Manipulation	2.43	2	1.22	0.77	.467
Explained	1966.25	4	491.56	311.07	.000
Residual	120.09	76	1.58		
Total	2086.35	80	26.08		

(ii) Wing length

	Sum of squares	d.f.	Mean square	F	p
Covariates					
chick age	51036.42	1	51036.42	3240.92	.000
brood size	13.89	1	13.89	0.88	.350
Main effects					
Manipulation	41.05	2	20.53	1.30	.277
Explained	52978.23	4	13244.56	841.06	.000
Residual	1291.30	82	15.75		
Total	54269.53	86	631.04		

3.3.8.3 Peak nestling mass.

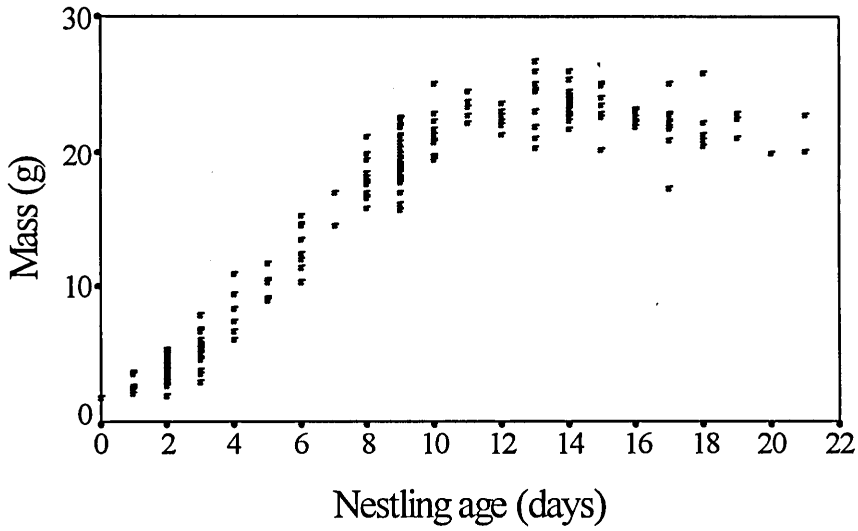
Nestling mass was measured in 50 nests at day 14.10 ± 1.16, at which time the peak in nestling mass occurs (Thompson 1992). There was no effect of treatment group on peak nestling mass. Oneway ANOVA was performed with treatment as main effect, incorporating chick age and brood size at hatching as covariates. The analysis was performed for 1993 and 1994 data combined, and for each brood and year separately. The effect of the clutch manipulation did not attain significance in any analysis ($p > 0.16$ in all cases). Chick age explained a significant proportion of the variation in the 2nd broods of 1993 only ($p = .011$), while brood size was never found to explain a significant proportion of the variation in peak nestling mass. Thompson (1992) found that brood size explained a significant proportion of the variation in nestling mass in some years only.

3.3.9 Inter-brood interval.

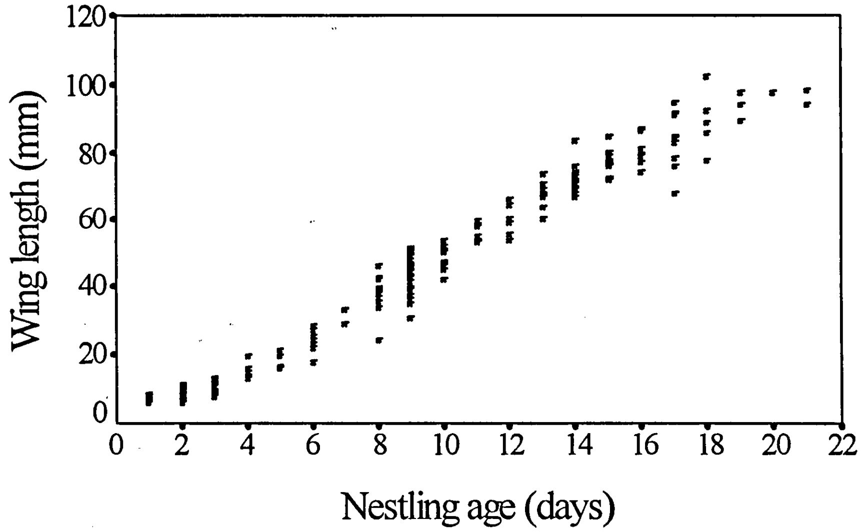
Manipulations of first clutch size in 1993 did not appear to influence the interval between hatching of the first brood and laying the second. The mean interval for all birds for whom second clutches were found was 34.4 days $\pm 4.4_{(41)}$, ranging between 22 and 46 days. Although the longest inter-brood intervals were found in birds with enlarged first clutches, oneway ANOVA showed there to be no significant difference between the four groups ($p = 0.149$) (Table 3.16).

Inter-brood interval was not associated with the number of eggs incubated, the natural clutch size, the number of young at hatch or alive at day 10 after hatch (Spearman rank correlation coefficients all $p > 0.3$ in all cases). There was no significant relationship between the date of hatch of first brood and the inter-brood interval.

Second broods were found for 10/13 nests (77%) whose first clutches were reduced, for 5/6 control (83%) and for 10/13 (77%) enlarged nests. Sample sizes were too few to test these statistics for each group, so the one-tailed hypothesis that there was a lower proportion of second clutches found following clutch enlargement than in all other nests was tested using Fischer's exact test. There was no evidence to reject the null hypothesis that there was no difference in the chance of a second clutch being found for enlarged nests compared to other nests ($p = 0.611$). The mean 2nd clutch size for those birds whose 1st clutches were manipulated and whose 2nd clutches were discovered was 4.7 eggs ± 0.6 for 10 birds with



(i) Nestling mass



(ii) Wing length.

Figure 3.12 (i) Nestling mass (g) and (ii) nestling wing length (mm) with age in days since hatching (day of hatch = day 0).

Table 3.16 **Inter-brood interval (days) in relation to manipulation of the first clutch. Means are presented ± s.d., with the sample size in the subscript. Data for 1993 only.**

	No clutch manipulation.	Reduced clutch	Control	Enlarged clutch
1993	$33.17 \pm 4.31_{(18)}$	$34.89 \pm 2.03_{(9)}$	$32.50 \pm 3.32_{(4)}$	$36.80 \pm 5.59_{(10)}$

reduced 1st clutches; the mean was 4.3 ± 0.4, for 4 birds with control and 4.3 ± 0.6 for 10 birds with enlarged 1st clutches. Non-parametric testing did not reveal any significant difference between groups (Kruskal-Wallis test $p = 0.360$). Larger sample sizes would be needed to investigate this potential tendency for larger 2nd clutch size following incubation of a reduced 1st clutch.

3.4 Discussion.

3.4.1 Evidence of a reproductive cost associated with incubation in the Swallow.

The 'incubation ability' hypothesis suggests that clutch size may be limited by the ability of the incubating parent to maintain eggs from laying through to a successful hatch (Lack 1947(b), Nur 1986). This study aimed to test this hypothesis by examining the intra-seasonal reproductive success of Swallows, whose effort during incubation was manipulated via clutch size adjustment. There was no evidence that female condition at the end of incubation, measured by body mass, or by estimates of the protein and lipid reserves, was affected by the number of eggs incubated, although there was some evidence to suggest that the mass of females incubating enlarged clutches was more variable.

However, the length of the period from clutch completion to hatch was longer for birds whose clutch size was increased than for birds incubating their own or a reduced clutch size. Hatching success was lower for enlarged compared to reduced clutches. These results provide support for the incubation ability hypothesis in Swallows, suggesting that clutch size could be constrained, at least in part, by parental ability to successfully complete incubation.

The existence of reproductive costs which are associated with incubation in the Swallow does not preclude the possibility that reproductive output may also be constrained at other stages in the cycle. Suspensions and interruptions in laying have been reported to be associated with poor weather or low food availability in Swallows (Ward 1992) and other aerial insectivores (Bryant 1975 (a), Lack 1956, O'Connor 1979), suggesting that a continuation of laying during adverse conditions would incur costs to female condition or even to survival (Bryant 1979).

Experimental manipulation of effort during the brood rearing phase in the Swallow was found to affect both current and future reproductive success (Thompson 1992). Within the manipulated broods, Thompson (1992) found nestling mortality to be highest in enlarged broods, whilst nestling mass was inversely related to the experimental brood size and the variation in nestling size was greater in larger broods. Offspring recruitment rates were also lowest from enlarged broods (Thompson 1992). Experimental enlargement of first broods altered the chance of laying, the timing and the success of a second brood. Inter-seasonal effects were also detected, as both males and females rearing reduced broods showed a higher survival than those rearing additional chicks. Studies of time and energy budgets of Swallows have shown that parents may be incapable of gathering sufficient resources for the maintenance and growth of large broods during bad weather (Turner 1983).

Energetic constraints have been postulated to limit reproduction during difficult stages, or 'bottlenecks' (Yom Tov and Hilborn 1981). Physiological machinery or environmental conditions may serve to ultimately constrain reproductive effort in this way at a single stage in the reproductive cycle. However, in evolutionary terms, the most successful individuals are likely to be those that make full use of the resources available at each stage in the cycle. Flexibility in variables such as the period of egg formation, the duration of incubation or the period from hatch to fledging may be exploited, in order to adjust the working level at each stage towards the limit which can be sustained by current resource availability. In this way, reproductive processes may have been adjusted in intensity such that no one stage bears disproportionate responsibility for constraint of the reproductive potential.

The constraining influence of each stage may, nevertheless, vary in importance from year to year, depending on the range of conditions throughout the season. The effects of costs are more likely to be evident in less favourable circumstances (De Steven 1980, Hirshfield 1980, Browne 1982, Feifarek *et al.* 1983). In this study, the extension of the incubation period associated with additional eggs was significant in the 2nd clutches of 1994 only. The depression in hatching success was also evident in 2nd rather than 1st clutches, but in this case, the effect was clear in both 1993 and 1994. Other studies have found evidence that Swallows producing 2nd broods may do so under more taxing conditions. Clutch size, brood size at hatch and the number of young fledged were all lower for second than first clutches (Ward 1992, Thompson 1992).

Maximum daily temperatures became more favourable throughout the season, being both higher and less variable during the production and incubation of 2nd clutches. Food availability also increased to a point towards the end of July, but subsequently declined (Section 4.3.1). A significant proportion of (67% in 1993, 50% in 1994) of the second clutches studied were incubated after this date, when food abundance was declining. The additional stresses of the 2nd clutches probably arise in part from a deterioration in food availability, though the cumulative drain on the resources of the parents, resulting from the stresses experienced during the raising of the first brood may also constitute stress at this time.

Environmental conditions differed between the two years of study. Although maximum temperatures were higher during incubation of the 2nd clutches of 1994 than 1993, they were also more variable. A higher number of rainy days in 1994 probably contributed to the lower insect abundance during 2nd clutch incubation in 1994 than 1993.

Evidence of the reproductive cost in terms of incubation and hatching success was, therefore, largely restricted to periods of poorer weather. During good conditions, it seems that Swallows could successfully incubate additional eggs without evidence of intra-seasonal costs of a magnitude detectable by the study methods employed. Other studies have found similar results, indicating that costs of additional reproductive effort may be effected via an increase in the probability of failure during poor conditions, rather than a ubiquitous depression in success (De Steven 1980, Hirshfield 1980, Browne 1982, Feifarek *et al.* 1983). This implies that it is the safety margin or capacity of the parent to buffer adverse conditions that is reduced by manipulation of reproductive effort within natural limits.

3.4.2 The timing of reproductive costs .

This study found evidence of the effects of manipulated incubation effort during incubation and at the time of hatch. There was no evidence that parental ability subsequent to this stage was affected by the manipulation, as neither parental condition, chick size or growth rates or the number of chicks fledged appeared to vary across the treatment groups. Parents were equally likely to form a second clutch, and there was no firm evidence that the timing or size of the 2nd clutch was altered.

This contrasted with results following manipulation of brood size in Swallows, (Thompson 1992, cited above). Studies of brood manipulation in other species have also detected evidence of both intra-seasonal and inter-seasonal reproductive costs. Studies in which clutch size was manipulated through incubation have generally been restricted to an examination of the effects within the incubation period only (Székely *et al.* 1994, Siikamaki 1995, Moreno and Sanz 1994). In a study of Collared Flycatchers (*Ficedula albicollis*), Moreno *et al.* (1991) compared the effects of the manipulation of the number of offspring at the onset of incubation (clutch manipulation) with manipulations made shortly after hatching (brood manipulation). Their study period encompassed the fledging of chicks. The results were similar to those presented in this study: while the hatching success was depressed for birds with enlarged clutches, no difference was detected between the mass or size of chicks fledged from nests depending on whether the manipulation was made at the clutch or brood stage. However, the study was conducted in one, particularly favourable season. Contrary to studies in more normal years (Gustafsson 1990), there was no evidence of clutch size-dependent variation in nestling size or mass, in either clutch or brood manipulations. The only conclusion to be drawn here is that reproductive costs during incubation or brood rearing did not influence breeding success in good years in this population. Studies encompassing a wider range of conditions may be more likely to uncover reproductive costs.

In this study, conditions during the period of incubation for some 2nd clutches were sufficiently poor for certain reproductive costs to become evident. However, these were apparent only during the incubation period itself (duration of incubation and hatching success), and did not extend beyond hatch. The timing of the payment of reproductive cost may shed light on the decisions faced by the incubating female.

Faced with an experimental increase in clutch size, presumably beyond that which the female 'intended', the female Swallow may find that the energetic demands of incubation increase (Biebach 1981, Haftorn and Reinertsen 1985, Mertens 1977). Balancing of the energy budget will necessitate a shift in behaviour. Her options include: (i) ejection of the eggs from the nest in order to restore energetic balance, or complete nest desertion (ii) reduction in the allocation of time to incubation to allow a greater foraging period in which to satisfy demands, (iii) refusal to elevate the heat input to incubation, with a result that average egg temperature declines and the duration of the incubation period may increase (iv) increase in the depletion of body reserves. The strategies vary in the extent to which parent and offspring take their share of the costs of increased incubation effort.

In option (i), the offspring pay the price early in the equation, and the parent is free to continue reproductive effort at the initial level. Options (ii) and (iii) could be sustained without detriment to female condition, but would increase the likelihood of offspring mortality, via an increase in the risk of egg chilling (Drent 1970), or a greater chance of nest predation, as nests are more frequently unattended or persist for a longer incubation period. Option (iv) differs from the other strategies in that the cost is, initially, borne by the parent, who loses condition in order to sustain adequate care of her offspring. Whether this option translates to a reduction in offspring viability depends on the extent to which the female can use her reserves as a short term buffer, restoring body stores when conditions ameliorate. If adverse weather will subsequently cause parental starvation or necessitate nest desertion to avoid mortality, both parent and offspring will pay the price. Similarly, if parental condition is not recouped before nestling rearing, nestlings may be undernourished and so pay a penalty in terms of reduced viability. However, if reserve depletion can be borne until conditions ameliorate, or until more time is available for self-feeding, neither female survival nor the viability of her offspring will be sacrificed; fitness is not reduced.

In evolutionary terms, the important difference between these strategies is not who pays the price, as the viability of both parent and offspring are important components of genetic fitness. The strategies differ in terms of the degree of certainty attached to the cost. If, as under natural conditions, all eggs were her own, the Swallow would suffer a definite reduction in fitness by ejection of eggs from the nest, whilst, if she is able to buffer the energetic crises with her own reserves, she may be able to complete the season without reproductive losses. Which of these strategies is followed will depend firstly on the availability of each option. If the energy deficit is sufficiently severe, desertion or reduction in nest attendance may be the only option; costs must be paid immediately. Secondly, the advantages of each option depend on the degree of risk attached to them. Rates of predation and egg cooling will determine the viability of option (ii), while the predictability of the food source is important for option (iv). Thirdly, the attraction of certain strategies could be altered if the degree of difficulty at one point in time is a good indicator of conditions at a later stage. If difficulties during incubation usually translate to difficulties feeding the clutch, it would be unwise to sacrifice condition at an early stage, only to have to make further sacrifices later.

The evidence from this study and other studies of the cost of incubation, is that the price is generally paid during the incubation period, rather than being buffered by the condition of the female, to potentially reappear later in the season. Alterations in hatching success or the duration of incubation were more commonly found than changes in female condition (Moreno and Carlson 1989). This study was able to demonstrate no evidence of a reproductive cost acting at a later stage in the season.

What are the reasons behind the timing of payment of the reproductive cost? One possibility is that not all options may be open to the female. If reserve depletion was not possible, because the female was already close to starvation, or because the risk of not being able to recoup losses was too great, then costs must have been paid at the time of incubation. Alternatively, if difficulties during incubation are normally a good indication of the problems to be expected during brood rearing, there may be no point in sacrificing reserves to maintain a clutch, only to pay the penalty later. Experimental differentiation between these alternatives would be problematic.

Whether by strategic planning, or by compulsion, the Swallow pays the reproductive cost of incubation immediately, by lengthening incubation or reducing hatching success. Ejection of eggs was not observed in this study, and nest desertion followed clutch manipulation on only one occasion, when a clutch of 4 was reduced to 2 eggs. Thus the Swallows were opting for an element of risk rather than the certainty of clutch reduction, leaving the possibility that they may be able to hatch all eggs successfully while running the risk of wasting time and energy incubating eggs that ultimately would not hatch. It seems that this strategy paid off in this study. Although the proportion of chicks hatching was lower for enlarged clutches, in both years a higher number of chicks were hatched in total from enlarged nests than control or reduced, and control nests hatched a higher number of eggs than reduced nests.

3.4.3 The shape of the cost curve function.

The shape of the function relating costs to clutch size will be determined by the mechanisms by which the costs are manifest. If energy is limiting, the existence of costs would be related to the energetic demands of incubation. Biophysical modelling has predicted that the energy required to maintain egg temperature will be influenced to a greater extent by the addition of a single egg to a small than to a large clutch (Mertens 1977). Insulation provided by the eggs in the large clutch would be expected to reduce cooling rates (Jones 1985) and hence decrease heat energy requirements per egg.

An alternative response is described by the threshold model (Klomp 1970, Biebach 1984) which predicts that energy expenditure to maintain the heat of eggs remains constant as clutch size increases towards the number of eggs that can be maintained in direct contact with the brood patch simultaneously. Beyond this clutch size, costs will escalate, as eggs have to be repeatedly turned and reheated in order to maintain the temperature of all eggs.

Measurement of the energetic requirements of keeping eggs warm shows a linear increase with clutch size, below the thermoneutral zone (Biebach 1981, 1984, Haftorn and Reinertsen 1985). It has been suggested that this results from a combination of the two processes described above. Field measures of the energetic demands of incubation, incorporating the costs of maintaining egg temperature and other associated activities show a non-linear increase in daily costs with clutch size (Chapter 3, Moreno and Carlson 1989, Moreno *et al.* 1991, Moreno and Sanz 1994).

The reproductive costs associated with the effort of incubation have also tended to be asymmetric; enlarging the clutch more commonly produced a change in reproductive parameters than a comparable clutch reduction. In cases where the duration of incubation was higher for birds with enlarged clutches, no difference was found between control and reduced clutches (Moreno and Carlson 1989, Székely 1994, Siikamaki 1995,). Hatching success has been shown to be reduced by clutch enlargement with Collared Flycatchers, but an associated increase in success with clutch reduction was found in only one of two studies (Siikamaki 1995, Moreno *et al.* 1991).

In this study, the duration of incubation was prolonged by clutch enlargement, but was not shortened by clutch reduction, but hatching success showed a response to both increase and reduction in clutch size. This suggests that, at natural clutch sizes, the length of the incubation period was limited by something other than clutch size in this study and a reduction in clutch size could not further reduce the length of the incubation period. However, birds incubating natural clutches were already incurring some clutch-size dependent penalty in terms of depressed hatch rate, even without clutch enlargement.

The most likely causes of variation in the incubation period and in hatching success between groups are nest attentiveness (Lyon and Montgomerie 1985), or average egg temperatures through incubation (Webb 1987).

Evidence from this study suggests that the mean parameters of nest attendance were not altered by clutch size manipulation. This result is contrary to Jones' (1985, 1987b) study of two incubating Swallows whose clutches were manipulated to 2 and 8 eggs. In his study, the proportion of afternoon time devoted to incubation was higher for the bird incubating an enlarged clutch. Several differences between the two studies could explain the observed differences in response. Firstly, Jones' (1985, 1987b) manipulation of clutch size to 8 eggs extended incubation effort beyond that occurring in the natural population, whereas all birds in this study incubated clutch sizes within the natural range. Secondly, Jones' data relates only to afternoon time. Although there was no significant interaction between time of day and patterns of nest attendance, it is plausible that birds incubating enlarged and reduced clutches differed in their response to diurnal variation in incubation constancy. Restriction of the sampling period may suggest a difference which is not apparent in the complete data set. Thirdly, Jones' study involved clutch size manipulations of restricted duration (3 days), while clutch manipulations were performed during the early stages of incubation in this study and nest attendance monitored during the final week. It is possible that birds responded in the short term to clutch size adjustment by alteration of their incubation schedules, but that in the longer term, the initial schedule was resumed and alternative adjustments in the energetic budget were made.

If the mean parameters of nest attendance were not affected by clutch manipulation, hatching success could still be altered if the range of each variable, particularly of the duration of recess periods were to change. Prolonged nest absences are liable to lead to egg chilling, causing embryo mortality, sub-lethal damage to the embryo or a lengthening of the incubation period (Webb 1985, Mallory and Weatherhead 1993). They also leave the nest vulnerable to predation (Lack 1947, Clark and Wilson 1981). Long recess periods may occur sporadically, associated with low temperatures or food availability. Frequency distributions of the length of recess periods would reveal whether the probability of chilling differs between treatments.

If, as the results of this study indicate, nest attendance did not vary between treatments, the most likely mechanism for prolonging incubation and reducing hatching success is a lower average egg temperature, resulting either from an inability to replace heat losses from a large clutch, or from repeated neglect and re-heating of eggs which cannot be maintained in continuous contact with the brood patch. The first hypothesis would predict a linear response of costs to clutch size, while the latter would be constant over a certain range, and linear only above a threshold value. The results of this study suggest that, while the duration of incubation increases only above a certain threshold, hatching success is linearly related to clutch size throughout the range from 3 to 7 eggs. The scope for further increase in hatching success at clutches of less than 3 eggs is limited, as 100% of eggs from 37 clutches of 3 eggs hatched successfully. It is possible that hatching success also follows a threshold response to clutch size, but that the lower critical point is set at the lowest natural clutch size.

3.4.4 Short-term breeding success as an index of reproductive costs.

When using short terms measures of breeding parameters to estimate the costs of reproduction, caution should be applied for two reasons. Firstly, reproductive success within one brood, or even within one season does not equate to lifetime reproductive success, and it is the latter that is of importance in determining natural selection. Secondly, what is apparent evidence of a cost may be the results of an alternative strategy, which may be of equal fitness value.

The parameters measured in this study were thought to contribute to reproductive success, albeit within certain constraints. The duration of incubation is important, as, in many species, the timing of egg laying is energetically constrained (Martin 1987) whilst early fledging offspring are at an advantage (Perrins 1965, Kikkawa 1980, Arcese and Smith 1985, Cooke *et al.* 1984). The duration of incubation also determines the risk of predation during this vulnerable stage (Lack 1954, Clark and Wilson 1981). Diminished hatching success will result in fewer fledged young, so long as brood reduction or brood-size dependent condition does not reduce this advantage at a later stage. Nestling growth rates are important as larger and heavier individuals form a greater proportion of breeding recruits than do smaller nestlings (Thompson 1992). Inter-brood interval determines when the second clutch is laid; chicks fledged from earlier laid clutches will have longer to prepare for the autumn migration. In exceptional cases, time may be available for production of a third clutch.

The advantages of higher female mass and 'condition' are not so clearly interpreted. Mass change during breeding results from a combination of strategic reserve depletion and enforced condition loss (Moreno 1989). Female Swallows maintain mass throughout incubation then undergo a rapid mass loss after hatch (Jones 1987b). The adaptive value of mass change in reducing flight costs during brood rearing is discussed elsewhere (Chapter 3). Under certain circumstances, the combination of a large clutch and poor environmental conditions has been shown to result in mass loss in an incubating Swallow (Jones 1985, 1987b). However, in the longer term sampled in this study, it seems that incubating Swallows would sacrifice hatching success and incubation duration in preference to loss of mass and condition. A higher degree of variance amongst the masses of females at the end of incubation of an enlarged clutch is, perhaps, evidence that some birds were finding it hard to maintain condition. Maintenance of a certain level of reserves through incubation must be interpreted within the context of the activity patterns of both incubation and the activities that immediately precede (laying) and follow it (rearing). Extreme caution must be applied in the interpretation of mass loss in terms of a reproductive cost (Bryant 1988).

Chapter 4:

Energy expenditure and clutch size in the incubating Dipper.

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4.1 Introduction and aims.

4.1.1 The study of energy expenditure during incubation.

All life-processes depend on the supply of energy. Study of the balance of energy input and output is of considerable value, enabling assessment of an individual's ability to perform various functions, and identification of factors constraining those functions. Studies of energy use by incubating birds can provide a basis for comparisons with other stages in the life-cycle, and for comparison between individuals.

Attempts to measure the energy required for incubation in birds began with the calculation of the heat input necessary to maintain eggs within the temperature range suitable for embryonic development (Kendeigh 1963, Mertens 1977, Walsberg and King 1978). More direct measurements of the energy use during continuous incubation have subsequently been made, by gaseous analysis of oxygen consumption or carbon dioxide production within the nest box (Vleck 1981, Biebach 1981, 1984, Haftorn and Reinertsen 1985). These studies were restricted to measurement of metabolism during periods of continuous 'steady-state' incubation (Biebach 1984), when egg temperature was constant. As such, they excluded the costs of activity off-nest and of re-warming the eggs after periods away from the nest.

The energetic 'cost of living' during the incubation stage, comprising energy required to maintain egg temperature during continuous incubation, to re-heat eggs following recess periods and the energy requirements of all other activities, for example feeding, best represents the energy demands which must be satisfied by the individual and hence is most relevant to the question of an energetic constraint. Measurement of *ad libitum* food intake by incubating birds has been used to estimate this integrated cost (Colemann and Whittall 1988). However, such an approach can not easily be applied to a field situation.

The doubly labelled water technique enables the study of the total daily cost of living of wild birds throughout the daily cycle. The method has been applied to incubating birds (for review, see Weathers and Sullivan 1989,Tatner and Bryant 1993, Section 6.2.2).

Measurements of energy expenditure in the Dipper have been made in this study population for both sexes at five stages in the annual cycle (Bryant and Tatner 1988), enabling evaluation of the cost of incubation in the context of other stages.

4.1.2 Energy expenditure in relation to clutch size.

The 'incubation ability' hypothesis (Nur 1986) proposes that clutch size is constrained by the ability of birds to successfully incubate a given clutch size. It proposes that some costs associated with incubation be dependent on clutch size. The 'energetic limitation' hypothesis (Moreno and Carlson 1989, Moreno *et al.* 1991) proposes that it is the acquisition of energy for incubation that constrains reproduction at this stage. It is important to evaluate whether the energy requirements of incubation vary with clutch size. If the energetic demands of incubation increase with clutch size, some threshold could exist above which the demands become a cost which exceeds any fitness benefits.

Operating at a high level of energy utilisation does not necessarily incur a cost, but fitness could, potentially, be reduced in a number of ways. In order to pay the high energy demands, incubating birds may need to increase foraging time, hence reducing nest attentiveness. This has potential implications for hatching success or the time of hatch (Drent 1975, Zicus *et al.* 1995), while the risk of nest predation is higher while the nest is undefended (Bukacińska *et al.* 1996). The risk of predation to the female may also be increased by the adoption of more risky foraging strategies (Lima and Dill 1990, Skutelsky 1996). Under certain circumstances, energy imbalance could induce a reduction in female reserves, with negative consequences for nestling development (Lifjeld and Slagsvold 1986). Once these types of cost begin to outweigh the advantages gained by laying an extra egg, an upper limit to clutch size can be said to operate.

On theoretical grounds, the energy required to maintain the temperature of a clutch of eggs would be expected to be positively correlated with clutch mass. Empirical evidence seems to support this, as the metabolism of incubating birds, maintaining egg temperature during continuous incubation increased with clutch size (Mertens 1977, Biebach 1981, 1984). In determining whether clutch size could be energetically constrained during incubation, the pertinent question is whether this increase in energy use translates to a significant increase in total daily energy expenditure by incubating birds in the field.

This study aimed to investigate the relationship between daily energy expenditure and clutch size for incubating Dippers. In such a study, it is important to manipulate clutch size in order to avoid the possibility that any difference is due to some correlated factor, perhaps quality of parent or territory, or time of year (Nur 1986). In this study, clutches were manipulated in an 'absolute' manner, adding or removing a single egg for each treatment. Assuming that the clutch laid by the female was related to any endogenous aspects of quality, treatment groups should comprise individuals with a similar level of additional (or reduced) demands, relative to their own 'quality'. Although manipulation by a single egg represents a greater proportional alteration for birds with smaller natural clutch sizes, within the range of clutches studied (3-6 eggs), a single egg was considered to represent the best approximation to a standard alteration of effort achievable, given the indivisible nature of eggs. An alternative method of manipulation would involve the random assignation of a manipulated clutch size to each bird, regardless of natural clutch size (Nur 1984a). The reasons for the choice of a 'relative' manipulation are discussed in Section 6.1.2.

4.2 Methods.

4.2.1 Study species.

The Dippers are unusual amongst passerines in being obligate lotic feeders, which feed by wading or diving in shallow water. Their diet is composed mainly of aquatic invertebrates, with fish and plant material being taken occasionally (Cramp 1988). Birds never move far from flowing water, holding breeding territories which are linear in nature (Johnstone 1994), the length of which is related to altitude, gradient and the presence of rocks, pools, riffles and suitable nest sites (Tyler and Ormerod 1994).

European Dippers (*Cinclus cinclus gularis*) are usually monogamous (Cramp 1988), but occasionally polygynous (Wilson 1996), pairs persisting throughout the breeding season. Nest sites are semi-traditional. Natural sites include cliffs and rocks, but artificial sites are commonly used; nests can be found within crevices or on girders under bridges but are always built by or over running water.

Nest building commences from late February to mid-March. The nest may consist of a large moss dome enclosing a nest cup of grass and wood rush, lined with fallen leaves. In sheltered locations, the dome may be much reduced. Old nests are frequently refurbished. The time taken to complete the entire structure can vary considerably, from 25-32 days in Britain (Shaw 1978). The nest is lined with dead leaves 2-3 days before laying.

Eggs are generally laid in the early morning (though exceptionally eggs are thought to be laid later in the day (Ormerod and Tyler 1994)), at a rate of one per day (Cramp 1988). Interruptions in the laying sequence are recorded occasionally (Shaw 1978), and were recorded at one nest in this study (Section 2.3). Clutch size varies from 1 to 7 eggs (Shaw 1978), though clutches of 1-2 or of 7 eggs are very rare. The modal clutch size was 5 eggs for first clutches in Central Scotland. (Bryant *et al* unpublished).

Incubation generally begins on the day of clutch completion, though the female spends some time in the nest before the clutch is complete (Ormerod and Tyler 1994, Section 2.3) and also roosts in the nest during the laying period. Incubation persists for an average of 16 days until hatch (Cramp 1988), range 12-18 days (Shaw 1978). There is no evidence for male participation in incubation, although males have rarely been observed to cover the eggs in the female's absence (Cramp 1988). Male provisioning of the female during incubation has been reported (Ward 1992, Efteland, cited in Ormerod and Tyler 1994), but is not considered to be significant to the nutrition of the female (Ward 1992). .

4.2.2 Study sites.

Nests were located within the catchments of the River Devon, the Allan Water and the Teith, both on the main river course and on side-tributaries. (Figure 4.1, Plate 1). This area is known to support a dense Dipper population and has been the subject of study for 20 years (Logie *et al.* 1996 in press).

4.2.3 Nesting parameters.

Traditional nest sites and potential new locations were visited on a weekly basis from late February onwards, to record the progress of nest construction. Once nests were complete, checks were made every four days to record egg laying. Nests were always checked after 9 a.m. to minimise disturbance of the female during laying. Before clutch completion, eggs were often found covered lightly with leaves. Females were occasionally found in the nest

Figure 4.1 Field study area for Dippers. Nests were located on the River Devon, the Allan Water and the River Teith and their tributaries. Map reproduced from Ordnance Survey Routemaster Sheet 4, 1989.

Plate 1. **Dipper study site at Glen Devon, Perthshire (NO0101).**

through the laying period (Section 2.3). However, for simplicity, incubation was assumed to commence on the date of clutch completion for all nests. When eggs were discovered after incubation had commenced, the airspace diameter of the eggs was used to estimate laying date (Section 2.3.1). Incubation was assumed to last for 16 days until hatch (Cramp 1988).

4.2.4 Experimental protocol in the context of other studies.

This study was designed to extend the data already available on energy use by incubating Dippers in Central Scotland, gathered initially by Bryant and Tatner (1988b), and extended by Ward (1992). In the original study (Bryant and Tatner 1988b), 8 females were studied (1982 and 1983) using the doubly labelled water technique whilst incubating their natural clutch size. Ward (1992) labelled 5 birds incubating their natural clutch size in 1990 and 5 birds on a either natural or a manipulated clutches in 1991. She introduced the use of the nest temperature monitor to record nest attendance schedules and also recorded individual variations in Time Activity Budgets. As far as possible, this study employed methodology that was consistent with previous studies in order that the resulting data set could be analysed in its entirety.

4.2.5 Manipulation of clutch size.

Clutches were randomly assigned to one of three treatments: an enlarged group ('E'), (addition of one egg), reduced ('R') (removal of one egg), and a control group, whose clutch size was not altered. Ideally, eggs would have been transferred between control nests such that all experimental nests experienced the same amount of disturbance. This was not done, in order to maintain consistency with the previous studies, where such a manipulation did not take place. However, control nests were visited one or two days before the doubly labelled water study period, in order to handle and measure eggs. All birds in the E and R groups resumed normal incubation after clutch manipulation, so it was assumed that the three treatments constituted comparable groups.

Additional eggs for manipulations were supplied from donor nests where the date of clutch completion differed from that of the recipient nest by less than 3 days. Eggs removed from the 'R' group were incubated in recipient nests for the duration of the manipulation. All eggs were marked at the pointed end with fine waterproof marker. Manipulations were carried out two days prior to the experimental protocol. All eggs were returned to their original nests after final capture for the doubly labelled water study.

4.2.6 Nest temperature monitor.

The seven temperature sensitive probes of the nest temperature monitor (Section 2.4) were assembled in and around the nest gradually over a period of a week, using dummy equipment to accustom birds to the presence of the equipment. Probes were positioned as follows:

1. Ambient temperature, in the shade of the bridge or nest, adjacent to the nest .
2. Next to/ on top of eggs
3. Next to/ on top of eggs
4. Under eggs
5. Nest air close to incubating female
6. In nest lining
7. In nest wall

The monitor ran at least throughout the course of the measurement period (48 hours) to give information on time spent on and off the nest by the female.

4.2.7 Doubly labelled water protocol.

During 1993 and 1994, birds were captured for labelling on day 9 of incubation (mean = 9.31d ± 2.09) , where day 1 was the day of clutch completion. Females were caught from the nest using a hand-held net between the hours of 14:30 and 16:00h. During labelling, the nest entrance was obstructed with a bird bag, in order to reduce egg cooling rates and to prevent egg damage by intruders. Egg piercing by other females has been observed in this study area (Bryant *et al.* unpublished).

Females were transported in a cloth bag to a car, where they were dosed with doubly labelled water (Section 2.5.4), measured and weighed (Section 2.2.1). Eggs were also measured at this time if they had not been measured previously. Most birds were already ringed, as a result of a long term study of the individuals in this population (Bryant *et al.* unpub.). Females that were not ringed were given a unique combination of two colour leg bands and one metal ring (BTO), marked with a unique identification number. In order to ease identification of the female with binoculars throughout the following two days, a narrow red stripe was marked onto the white breast feathers using water soluble marker pen.

A period of one hour was allowed to ensure isotopic equilibrium in the body water, after which a blood sample was taken from the leg (Section 2.5.4.). The wound was sealed and the feathers cleaned of any blood. An average of 1.6h after capture, the nest entrance was unblocked, the eggs returned and the female released at the river bank within 20 m. of the nest.

After a period of 48 hours, females were recaptured at the nest in order to obtain a final blood sample. Blood samples were stored until they could be analysed (Section 2.5.5.).

In 1994, an attempt was made to compare the energetic costs of incubation of different clutch sizes <u>within</u> two individuals. For one bird (Sample 304), clutch size was manipulated prior to labelling, as above, but the second capture and blood sampling took place after 24 hours. At this time, the natural clutch size was restored and the bird released to resume incubation. After a further 24 hours, the female was again captured for a third time and a final blood sample taken (to give data for Sample 305). A similar two-day sampling procedure was adopted at another nest (Samples 306 and 307), although in this instance, energy expenditure on the natural clutch size was measured on the first day of the study period, and the clutch manipulated for the second 24 h.

At the first of these two nests, the procedure resulted in the female spending one night away from the nest, before returning to resume incubation at dawn. No other occurrence of this behaviour were recorded for other labelled Dippers. The amount of disturbance was considered to form too high a proportion of the measurement period (12h off nest out of a 24 h study period), so this protocol was not continued in 1995. In order to maintain the statistical criterion of independence of data, one sample from each of these two birds was included in subsequent analyses. Data from the second 24h period (samples 305 and 307) were used as these included more normal behaviour.

4.2.8 Behavioural Observations.

During 1993 and 1994, behavioural observations were made at the nest over the course of the doubly labelled water measurement period. In 1995, additional observations were made of incubating birds without the application of the doubly labelled water technique. This enabled comparison of the behaviour of labelled birds during incubation recess periods with a control group.

Observations were made between dawn and dusk, from a hide 10 -20 m distant from the nest, using binoculars and a telescope. Activities were initially grouped according to Bryant and Tatner (1988b) into four categories: rest, forage, dive and fly, but in 1994 and 1995 these were further subdivided as follows:

Rest: alert / preen / sing
Forage: forage / walk
Dive: swim / dive
Fly: no subdivision.

Activity budgets were recorded using the Time and Event Data Logging Programme (Stirling University Microprocessors) on a Psion Data Organiser. Activities were recorded to a precision of 0.01s, so budgeting was limited by the response time of the observer.

Difficulties were encountered in attempting to monitor the nest to record the female's departure at the beginning of her recess period and behaviour at her chosen foraging site, which was frequently some distance from the nest site. Because of the meandering nature of river courses, it was often not possible to observe both sites simultaneously. Movement of the observer between sites could easily disturb the bird and thus disrupt behavioural observations. This problem was reduced by making preliminary studies in order to establish the location of the female's usual foraging site and subsequently making observations specifically at either this foraging position or from the nest site, in preference to attempting to observe both sites simultaneously. The female's choice of foraging site could not always be reliably predicted, so some excursions were inevitably missed.

Observations based at the nest were also used to gain information on the amount of time the male devoted to nest-guarding, and to record any nest visits or mate-feeding by the male whilst the female was incubating.

4.2.9 Changes in incubation body mass.

Mass was recorded at the beginning and the end of the doubly labelled water period, enabling calculation of the rate of mass change over a 48h period. However, these data were not necessarily representative of normal patterns of mass change during incubation. The stress of handling, labelling and blood sampling could affect mass change, for example by altering feeding behaviour. For this reason, in 1995, a sample of incubating females was caught twice during the course of incubation, without application of the doubly labelled water technique. Stress from capture and handling could also affect these birds, so catches were made at intervals of 3 to 5 days, (mode = 4 days), in order to reduce the proportionate influence of capture on the measurement period. To avoid complications due to any diurnal pattern of mass change, repeat catches were made within 2 hours of the original time of capture.

4.2.10 Environmental data.

Meteorological data were recorded at the Parkhead Meteorological Station, at Stirling University at an altitude of c 40m above sea level (Section 2.7.1.). Study sites were distributed to both the east and west of the station (Figure 4.1), ranging in altitude from c20 - 240m. In order to describe conditions over each doubly labelled water study period in 1993 and 1994, the mean value of maximum and minimum temperature (°C), cloud cover (Oktas), wind speed (knots) and rainfall (mm) were calculated over a three day period, from the day of labelling to the day of re-capture inclusive.

Flow rates have been shown to influence the behaviour of (da Prato 1981, O'Halloran *et al.* 1990) and energy use by (Bryant and Tatner 1988b) Dippers. Information on daily flow rates (m^3s^{-1}) was provided by the Forth River Purification Board, for stations on the River Devon (at Castlehill Reservoir, NO003030) and on the Allan Water (at Kinbuck, NN792053). In order to provide a relative measure of the conditions over the study period which could be compared between sites, a mean value of was computed for a three day period for each doubly labelled water study, using data from the River Devon only. The majority of nest sites were located within the catchment of the R.Devon (58% of nests studied). Over the period 1st March to 10th May, flow rate on the Allan Water was highly correlated with that on the River

Devon (Pearson correlation coefficient, $r > 0.8$, $p < .001$, $n = 70$ for each of the three years 1993, 1994 and 1995).

In 1994, more specific information about the flow rates and river depth at each nest site was gathered by taking measurements during the study period at a point in the river, close to the nest, where the female was known to forage. The width of the river at this point was measured with a tape measure. Four sampling points were then allocated at equal distances across the width of the river. On the smaller side-burns, sampling frequency had to be reduced to a minimum of 2 sampling points.

At these points, depth was measured with a metre rule and a propeller-type flow metre was used to measure flow rate. The propeller was positioned at two-thirds of the river depth from the surface and the number of revolutions of the propeller in 30 seconds was measured three times at each sampling point. The average value was used to calculate the number of revolutions per second (N). Flow velocity (V) in ms^{-1} was calculated for each section according to the equations:

Eqn 4.1.

$$\text{For } N < 0.6: \quad V = 0.229N + 0.020$$

$$\text{For } N \geq 0.6, \quad V = 0.254N + 0.005$$

The total cross-sectional area of the river (A) was estimated assuming that each small section can be approximated by a rectangle, whose width is the interval between sampling points (I), and whose depth is approximated by the depth at each point (d_1 to d_4 in Figure 4.2) (Gordon, McMahon and Finlayson 1992). Flow rate of the whole river in m^3s^{-1} was calculated according to the equation

Eqn. 4.2.

$$Q = \sum_1^4 (A_1 \times V_1)$$

Thus, four parameters were calculated to describe the characteristics of the river at each site: river width (m), average depth (m), speed of flow (ms^{-1}) and flow rate (m^3s^{-1}).

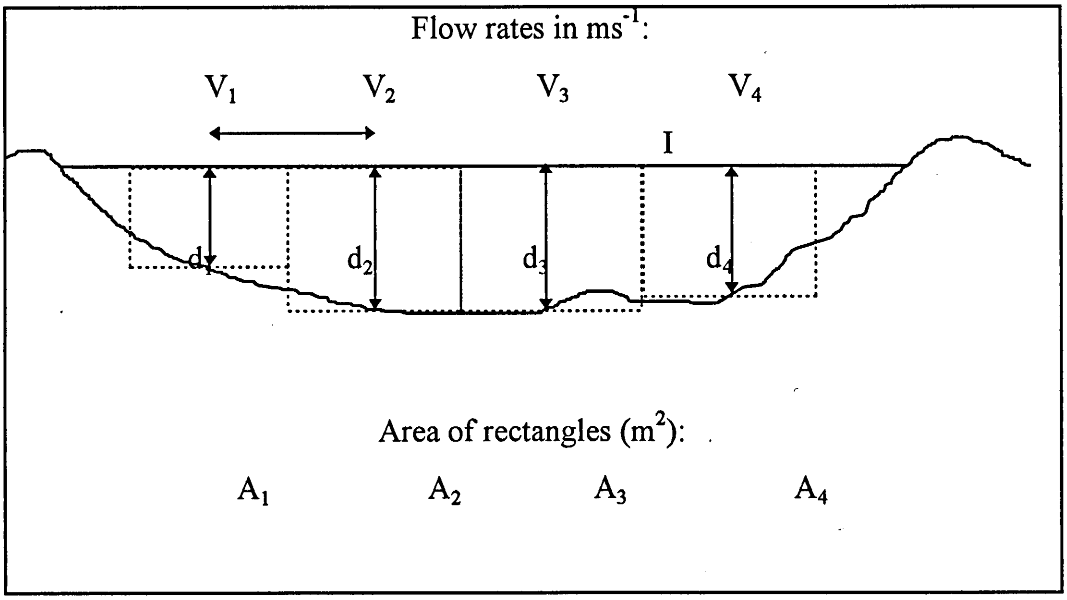


Figure 4.2 **Method of estimation of river flow rate. Depths (d_1 to d_4) were measured at four locations, equidistant at interval I across the width of the river. The cross-sectional area of the river bed was then approximated by the area of the four dashed rectangles ($A_1$to A_4). The products of flow velocity measured at each point (V_1 to V_4 ms^{-1}) and the area of the corresponding rectangle were summed to give an estimate of flow rate in m^3s^{-1}.**

The acidity of rivers is known to affect Dipper behaviour, and hence energy use (O'Halloran *et al.* 1990), and reproductive success (Ormerod et al 1991, Vickery 1992), via the influence on prey availablilty. Data on the pH of the River Devon (at Cambus New Bridge), the Allan Water (at Bridge of Allan) and the Ardoch Burn (at Doune Castle) were provided for the period from 1983 to 1994. Sampling was generally at monthly intervals.

4.3 Results

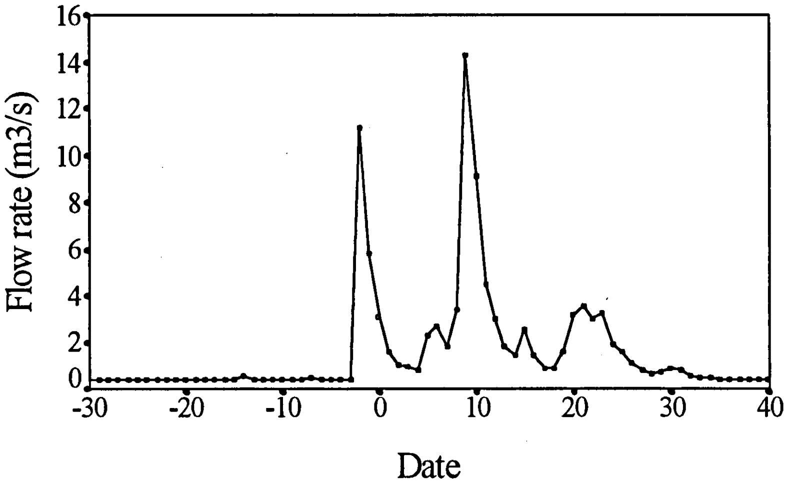
4.3.1 Environmental data.

Flow rates for the River Devon for the period from 1st March to 10th May for the three years of this study (1993 to 1995) are shown in Figure 4.3. Periods of high flow are easily identified in 1993 and 1994. No spates occurred during the study period of 1995.

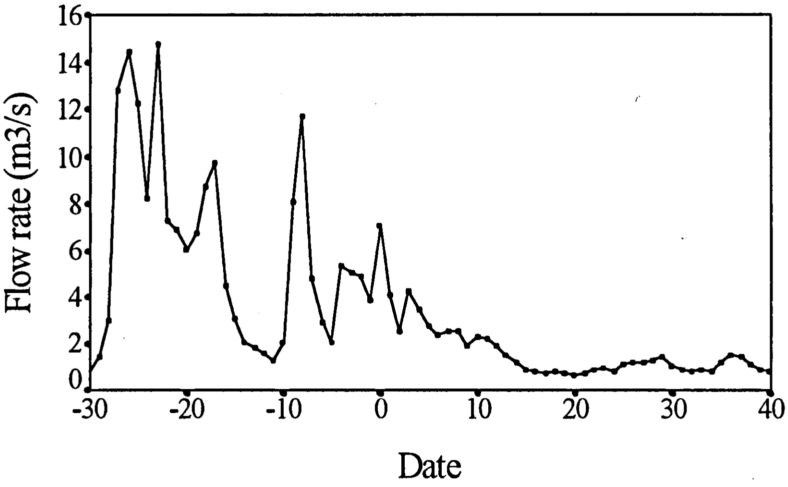
Flow rates were calculated for each sample as the mean flow recorded by F.R.P.B. at Castlehill Dam on the River Devon over the three day measurement period. These data were transformed using a log transformation in order to achieve normality.

During 1994, characteristics of river flow were measured at nine sites close to nests, identified by their use as a foraging site for the female. The width of river was variable, ranging from 1.5 to 14m (mean = 6.6m ± 4.9). The average depth at these sites was 0.23m ± 0.11, and was always under 0.5m. The speed of flow ranged from 0.14 to 0.45 ms^{-1}, and was significantly correlated with the mean F.R.P.B. flow rate (m^3s^{-1}, log transformed)(Pearson correlation coefficient r = .670, n = 9, p = .048). Measured flow rates were not significantly correlated with the F.R.P.B. mean (r = .389, n = 9, p = .300).

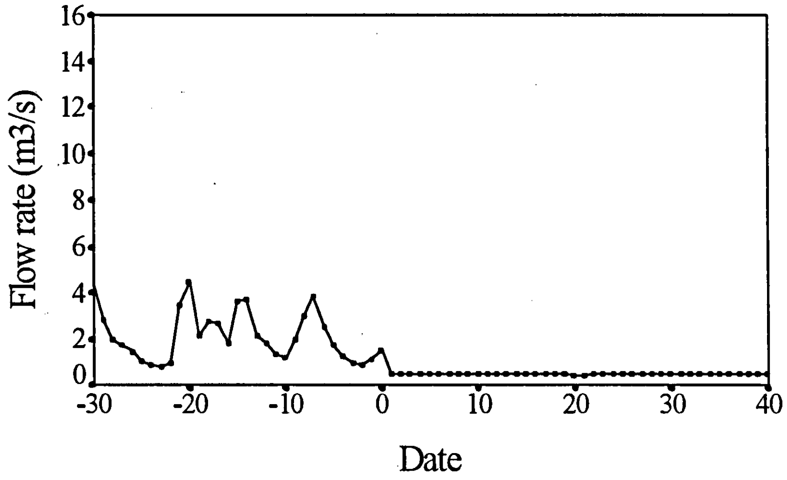
Data on the acidity of the River Devon, the Allan Water and the Ardoch Burn were also provided by the F.R.P.B., for the period from 1983 to 1994. Within this period, pH for all waters averaged over pH6.5, confirming the circumneutral status of the habitat (Ormerod *et al.* 1991).



(i) 1993



(ii)1994



(iii)1995

Figure 4.3 **Flow rate (m^3s^{-1}) of the River Devon over the period from 1st March to 10th May for 1993 to 1995. Data from F.R.P.B.. Dates calculated from 1st April =1.**

4.3.2 Energy use by incubating Dippers.

Energy expenditure was measured on 7 occasions from 5 incubating Dippers in 1993, and on 10 occasions, from 10 individuals in 1994. Appendix 5 lists the ring numbers and dates of study for these birds. Values of average daily metabolic rate, daily energy expenditure and metabolic intensity were calculated according to the assumptions in Section 2.5. The results are presented in Table 4.1, alongside those from the studies of Bryant and Tatner (1988b) and Ward (1992).

The two birds which were caught and sampled on consecutive days in order to examine variation in energy expenditure within individuals (Section 4.2.7), resulted in Samples 304 and 305, and 306 and 307. In order to satisfy the statistical criterion of independence, only one value per birds was entered in these cases. As the first night was spent off nest in Sample 304, it was concluded that data from the second 24h period were less likely to include abnormal behaviour. Samples 304 and 306 were therefore excluded from subsequent analysis and are marked with an asterisk in Table 4.1.

Considering only those birds whose clutch was not manipulated, there was no significant difference between any of the measures of energy expenditure between the three studies (oneway ANOVA $p>0.4$ in each case). The data were, therefore, treated as one sample.

For birds incubating their natural clutch size, average daily metabolic rate was equal to $5.30 \pm 0.74_{(19)}$ $cm^3CO_2g^{-1}h^{-1}$, mean daily energy expenditure was $209.46 \pm 28.68_{(19)}$ $kJ.ind^{-1}d^{-1}$, giving a mean Metabolic Intensity of 3.33 ± 0.46 x B.M.R.($n = 19$), where B.M.R. was calculated according to mass based on the equations of Aschoff and Pohl (1970) (inactive phase, Equation 4.3), or 2.39 x B.M.R., where this was calculated using the species specific data measured for female Dippers from this population (Bryant and Newton 1994, Equation 4.4).

Eqn. 4.3 Basal metabolic rate of passerines, inactive phase: (Aschoff and Pohl 1970)

$$\text{B.M.R.}\ (J\ ind.^{-1}h^{-1}) = 132.832\ W^{.726}$$

where W is mass in g.

Table 4.1 **Body mass, clutch size and energy expenditure, measured by the doubly labelled water technique for incubating Dippers. Data sources are: samples 101-110, Bryant and Tatner (1988b); samples 201 - 208, Ward (1992), samples 301-317, this study. Metabolic Intensity (MI) was derived from daily energy expenditure, expressed as a multiple of basal metabolic rate (calculated using the equations of Aschoff and Pohl 1970, inactive phase MI_{AP}, or using a measured value for female Dippers in this population (Bryant and Newton 1994) (MI_{BN})). Samples labelled with an asterisk were not included in the mean values (for which n = 33) or in subsequent analysis (see text).**

Sample	Mass (g)	Clutch	Clutch manip.	ADMR cm^3CO_2 $g^{-1}h^{-1}$	DEE $kJ\ ind^{-1}d^{-1}$	MI_{AP}	MI_{BN}
101	59.7	5	0	5.93	188.65	3.05	2.25
102	59.1	4	0	4.87	184.48	3.00	2.22
103	63.2	4	0	4.67	189.34	2.93	2.13
104	63.2	5	0	4.96	201.10	3.11	2.26
105	62.3	5	0	4.88	195.04	3.05	2.22
106	60.8	5	0	5.47	213.35	3.40	2.49
107	68.5	5	0	5.18	227.63	3.32	2.36
108	66.5	4	0	5.56	237.02	3.54	2.53
201	62.0	5	0	4.45	177.00	2.78	2.03
202	59.8	4	0	5.58	214.06	3.45	2.54
203	59.9	5	0	4.77	183.14	3.95	2.17
204	57.5	5	0	6.31	232.76	3.86	2.87
205	64.5	4	0	5.25	217.07	3.31	2.39
206	62.6	5	0	5.51	221.28	3.45	2.51
207	58.3	3	-1	7.31	273.16	4.49	3.33
208	60.8	6	+1	8.84	344.52	5.49	4.02
209	60.1	6	+1	8.99	346.32	5.57	4.09
210	61.7	3	-1	4.88	193.00	3.04	2.22
301	66.6	5	0	5.31	224.34	3.35	2.39
302	61.6	3	0	7.55	294.95	4.65	3.40
303	58.7	5	0	4.73	175.95	2.88	2.13
304*	61.8	5	0	5.32	208.70	3.28	2.40
305	60.8	6	+1	8.29	319.75	5.10	3.74
306*	62.5	6	+1	4.29	170.13	2.66	1.93
307	61.9	5	0	4.64	182.27	2.87	2.09
308	63.3	6	0	5.47	219.63	3.40	2.47
309	64.8	4	-1	4.66	191.65	2.92	2.10
310	65.8	3	-1	3.88	162.04	2.44	1.75
311	60.5	6	+1	4.93	189.13	3.02	2.22
312	59.0	6	+1	4.33	162.02	2.64	1.95
313	62.8	5	0	4.43	176.55	2.75	2.00
314	60.5	6	+1	4.14	159.08	2.54	1.87
315	59.0	6	+1	4.24	158.67	2.58	1.91
316	61.2	3	-1	4.05	157.13	2.49	1.82
317	60.3	4	-1	4.39	168.02	2.69	1.98
mean	61.72	4.67		5.41	211.52	3.37	2.44
s.d.	2.60	0.60		1.34	51.25	0.83	0.61

Eqn. 4.4 Basal metabolic rate of female Dippers (Bryant and Newton 1994)

B.M.R. ($J\ g^{-1}h^{-1}$) = 58.68.

Amongst control birds, a positive relationship between daily energy expenditure and mass was found (Bryant and Tatner 1988), though the correlation was not significant ($r = 0.218$, $n = 19$, $p = 0.369$)

4.3.2.1 The effect of clutch size on energy expenditure.

Energy use by incubating Dippers is shown according to treatment group (Table 4.2, Figure 4.4) and 'experimental' clutch size, defined as the number of eggs incubated (Table 4.3, Figure 4.5). The variance of each of the four variables differed both according to treatment group and to experimental clutch size (Levene's test for homogeneity of variance $p<.001$ for ADMR, DEE, MI_{AP} and MI_{BN} for both analyses). Levy's test (cited in Zar 1984) was used to determine which groups differed with respect to variance. Amongst treatment groups, the variance in daily energy expenditure within the enlarged group was found to be significantly greater than that in the control group ($q_{(0.05,\infty,3)} = 5.024$, $p < 0.005$), though the difference between E and R was not significant. Amongst experimental clutch size groups, birds incubating clutch size 6 were significantly more variable than those with clutches of 4 and 5 ($p < 0.01$). The variance amongst birds with clutch size 3 did not differ significantly from those with clutch size 6.

Analysis of variance is generally robust to heterogeneity in variances unless groups have unequal sample sizes (Zar 1984). In this analysis, the degree of heterogeneity amongst variances combined with unequal sample sizes amongst groups was thought to produce a significant deviation from the assumptions of ANOVA. Non-parametric tests were therefore used to investigate the difference between the mean level of energy use according to both treatment groups and experimental clutch size. The results of Kruskal-Wallis test with each variable are presented in Tables 4.2. and 4.3. The rank order of birds by energy use did not differ significantly according to either experimental clutch size ($p > .94$) or to treatment group ($p>.16$).

Table 4.2 Energy use by incubating Dippers according to treatment group. Data from three studies (from 1982 to 1994).

Treatment	Reduced	Control	Enlarged	Kruskal-Wallis
ADMR ($cm^3CO_2g^{-1}h^{-1}$)	4.86± 1.25	5.30 ± 0.74	6.06 ± 2.22	$\chi^2 = 2.34_{(2)}$ p =.309
DEE (kJ ind.$^{-1}$ d^{-1})	190.83± 43.1	209.5 ± 28.7	231.9 ± 87.8	$\chi^2 = 3.44_{(2)}$ p =.179
MI_{AP}	3.01 ± 0.76	3.33 ± 0.46	3.73 ± 1.39	$\chi^2 = 2.34_{(2)}$ p =.309
MI_{BN}	2.20 ± 0.58	2.39 ± 0.33	2.74 ± 1.01	$\chi^2 = 3.59_{(2)}$ p =.166
n	6	19	8	

Table 4.3 Energy use by incubating Dippers according to experimental clutch size. Data from three studies (1982 to 1994).

Clutch (eggs)	3	4	5	6	Kruskal-Wallis
ADMR ($cm^3CO_2g^{-1}h^{-1}$)	5.53 ± 1.77	5.00 ± 0.47	5.12 ± 0.57	6.15 ± 2.17	$\chi^2 = .393_{(3)}$ p =.941
DEE (kJ ind.$^{-1}$ d^{-1})	216.1 ± 64.0	200.2 ± 23.5	199.9 ± 21.4	237.4 ± 85.2	$\chi^2 = .082_{(3)}$ p =.993
MI_{AP}	3.42 ± 1.08	3.12 ± 0.32	3.22 ± 0.39	3.79 ± 1.35	$\chi^2 = .355_{(3)}$ p =.949
MI_{BN}	2.50 ± 0.81	2.27 ± 0.22	2.29 ± 0.24	2.78 ± 0.99	$\chi^2 = .264_{(3)}$ p =.966
n	5	7	13	8	

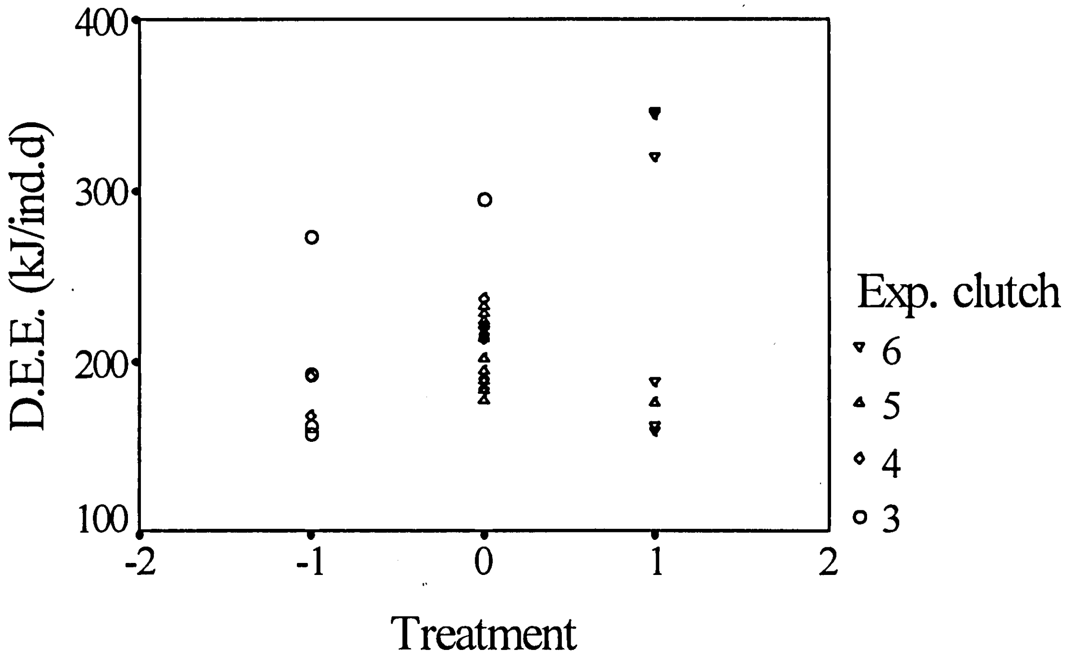


Figure 4.4 Daily energy expenditure (kJ ind^{-1}d^{-1}) of incubating Dippers in relation to treatment, involving the removal (-1) or addition (1) of 1 egg, or no manipulation (0). Markers identify data according to experimental clutch size.

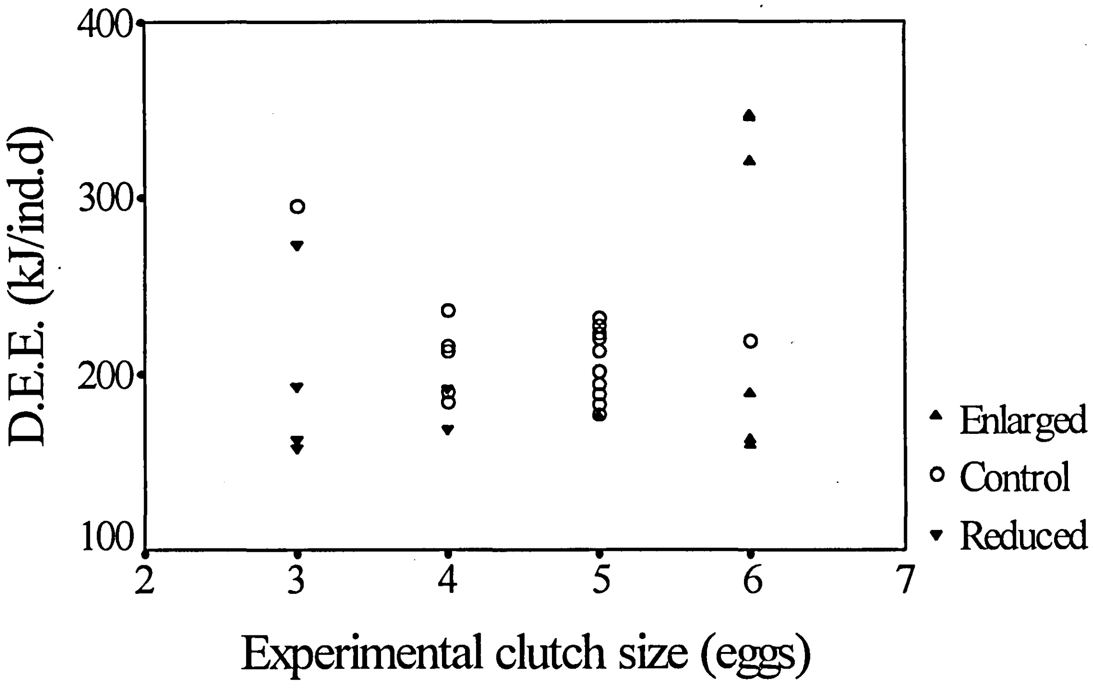


Figure 4.5 Daily energy expenditure (kJ ind^{-1}d^{-1}) of incubating Dippers, shown in relation to the experimental clutch size incubated. Symbols indicate whether the clutch size was increased, reduced or unchanged (control).

4.3.2.2 Flow rates and energy use.

Within the sample of 33 incubating Dippers that were labelled between 1982 and 1994, there was no significant relationship between energy expenditure and mean daily flow rate ($p > 0.9$). However, amongst the 19 females with no clutch manipulation, there was a strong positive correlation between all four measures of energy expenditure and mean flow rate over the period of study, $p < 0.03$ in each case. The relationship between daily energy expenditure and mean flow rate is illustrated in Figure 4.6 and was described by a linear equation. The relationship was not significantly improved by fitting a higher order polynomial. Although costs were high for control birds when flow rates were high, the four birds for which DEE exceeded 250 kJ indiv^{-1} d^{-1} were not sampled under conditions of high flow.

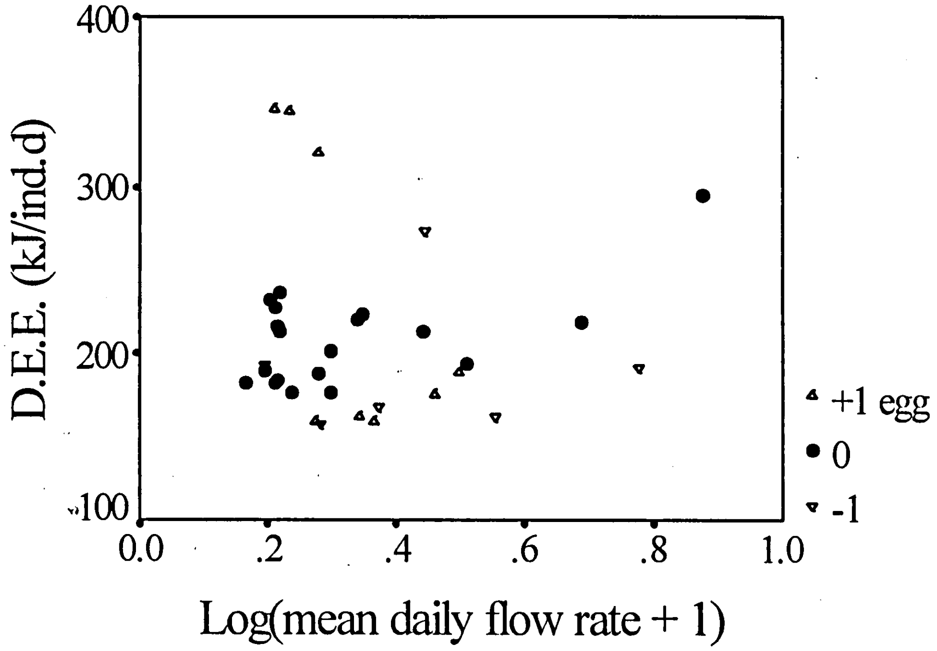


Figure 4.6 **Daily energy expenditure of 33 incubating Dippers with mean flow rate over the study period. Amongst control birds, a linear relationship exists of the form $y = 91.74x + 179.73$, where y = DEE (kJd^{-1}) and $x = \log_{10}$ (mean flow m^{3}s^{-1} + 1), $r^2 = .356$, $F_{(1,17)} = 9.417$, $p = 0.007$.**

At the nine sites where characteristics of flow had been measured, none of the parameters explained any more of the variation in energy expenditure than the use of the mean F.R.P.B. flow rate.(Pearson correlation coefficients between DEE and river width (m), r = .466, p = .206, depth (m), r = -.142, p = .716, velocity (ms^{-1}) r = .534, p = .139, flow (m^3s^{-1}) r = .414, p = .268.) This suggests that site characteristics are perhaps less important in determining day to day variation in energy use by incubating Dippers, than is the relative conditions compared to normal.

4.3.3 Nest attendance.

Nest temperature data for a continuous period of 24 hours, were analysed to determine the patterns of time spent on and off the nest. Females incubated for a continuous period of 631.0 ± 58 minutes duration over night, (n = 25 nights from 13 birds). The remaining ' active day' was divided between, on average, 14 incubation sessions, of 44.6 ± 10.2 minutes duration and 15 recess periods, of 11.5 ± 3.6 minutes (mean of 28 birds). The mean duration of each of these three variables for each bird are presented in Appendix 6.

4.3.3.1 Diurnal variation in nest attendance.

The mean duration of session and recess periods taken in each hour were calculated from all data gathered from 17 samples in 1993 and 1994 (Figures 4.7 and 4.8). Sessions increased to a maximum duration around 13:00h, then became shorter again during the afternoon. Recess periods followed a similar pattern, though the rate of increase was not so steep and the longest recess periods were taken slightly earlier in the day, around 11:00h. The amount of variation explained by these patterns was low for both variables. Incubation constancy (I.C.) was calculated to show the combined effect of these two patterns. I.C. expresses the proportion of each hour spent on the nest. Incubation constancy declined sharply through the early morning, reaching a minimum around the middle of the day before increasing again more gradually before the night incubation session (Figure 4.9).

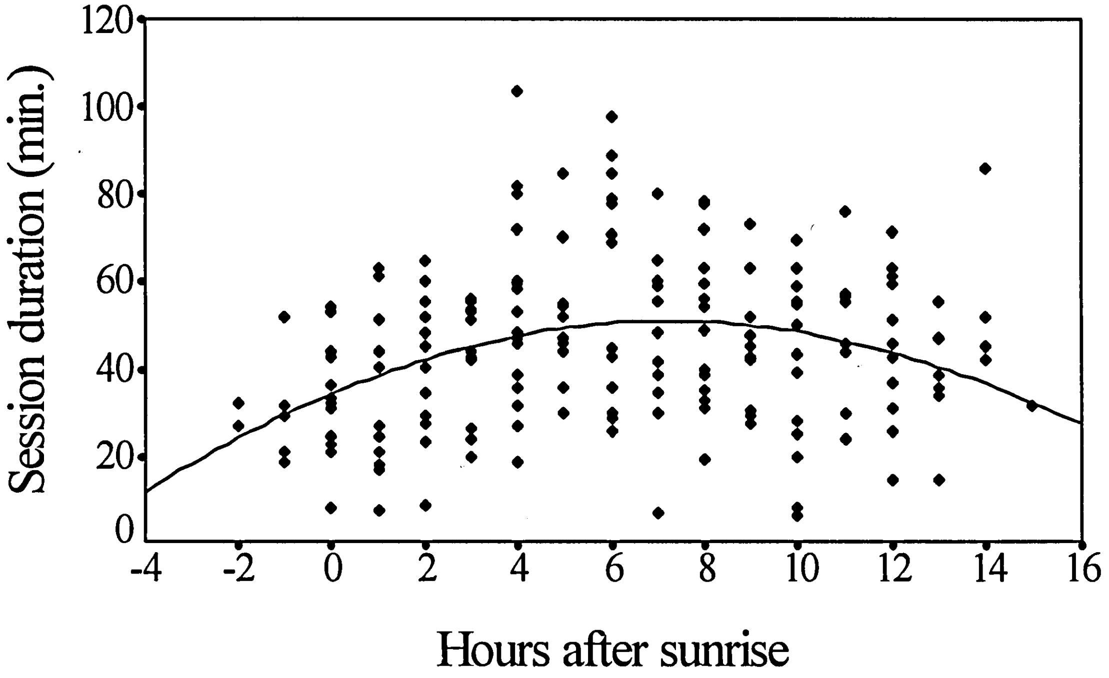


Figure 4.7 **Diurnal pattern of incubation session duration for Dippers. Data points each represent one hourly mean, for 15 birds from 1993 and 1994. The relationship between session duration (min) and time of day (h after sunrise) was described by a negative quadratic: $y = 34.363 + 4.493x - 0.309x^2$, $r^2 = 0.102$, $F_{(2, 181)} = 10.31$, $p < 0.001$.**

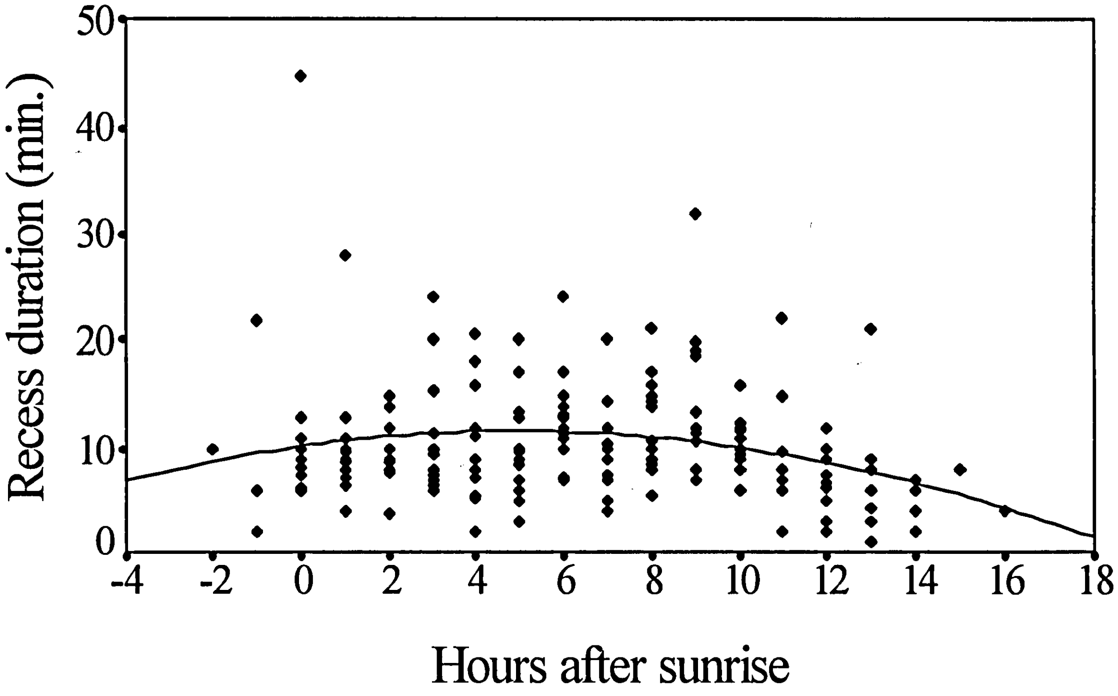


Figure 4.8 Recess duration for incubating Dippers throughout the active day. Each data point represents one hourly mean for each of 15 birds (1993 - 1994). Recess length (min) is related to time (h after sunrise) by a negative quadratic function: $y = 10.250 + 0.575x - 0.059x^2$, $r^2 = 0.063$, $F_{(2,183)} = 6.14$, $p = 0.003$.

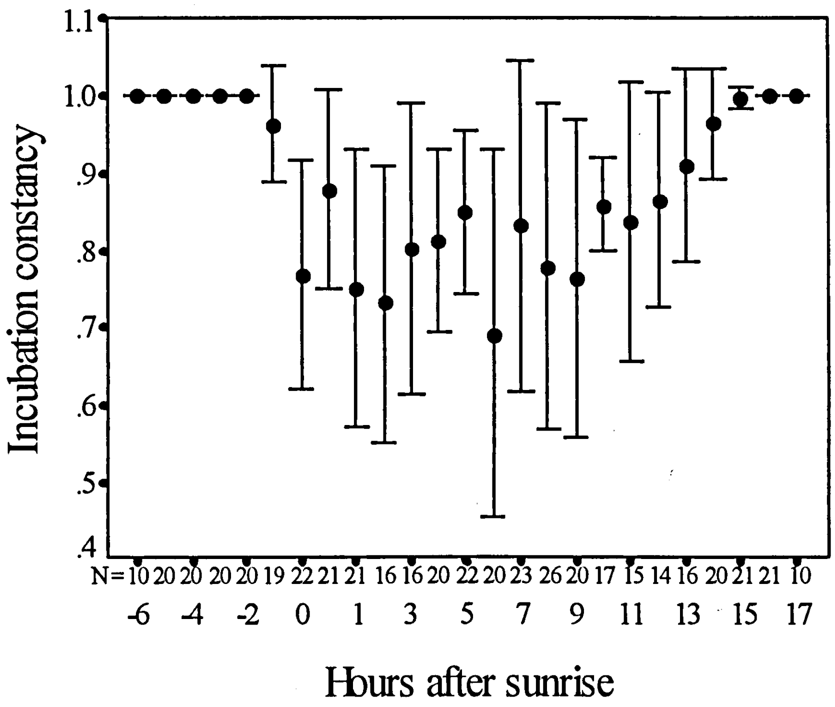


Figure 4.9 **Incubation constancy of Dippers in relation to time of day. Incubation constancy was calculated as a proportion of each hour spent by the female on the nest, as indicated by the nest temperature monitor. Each point represents the mean of a number of hours' data (n values presented below graph) taken from 15 birds. Error bars represent ± 1 x standard deviation.. For the period from one hour before, to 14 hours after sunrise (inclusive), the data was described by a quadratic function; $y = 1.1133 - 0.0698x + 0.0064x^2$, $r^2 = 0.152$, $F_{(2, 325)} = 29.0$, $p < 0.001$, where x is hours after sunrise and y is incubation constancy, transformed using an angular transformation.**

4.3.3.2 Individual variation in nest attendance.

There was no significant correlation between mean session, recess and night session duration amongst females (Pearson correlation coefficients were all positive, r > .2, but non-significant, p >0.2). Neither session nor recess length varied with average mass or mass change over the course of the doubly labelled water period (expressed as final residual mass, standardised for mass at first capture) (p>0.05 in all cases). No significant correlations were found between session or recess duration and any measure of structural size. Session duration increased in a linear manner with female age, while recess duration did not vary (Figure 4.10).

Age or experience could influence a female's ability to incubate efficiently. In Canada Geese, inexperienced females started nesting at a lower body mass than experienced birds and had a lower nest attendance (Aldrich and Raveling 1983). During brood-provisioning, older female Dippers were able to maintain lower levels of daily energy expenditure than younger birds (Bryant and Tatner 1988a).

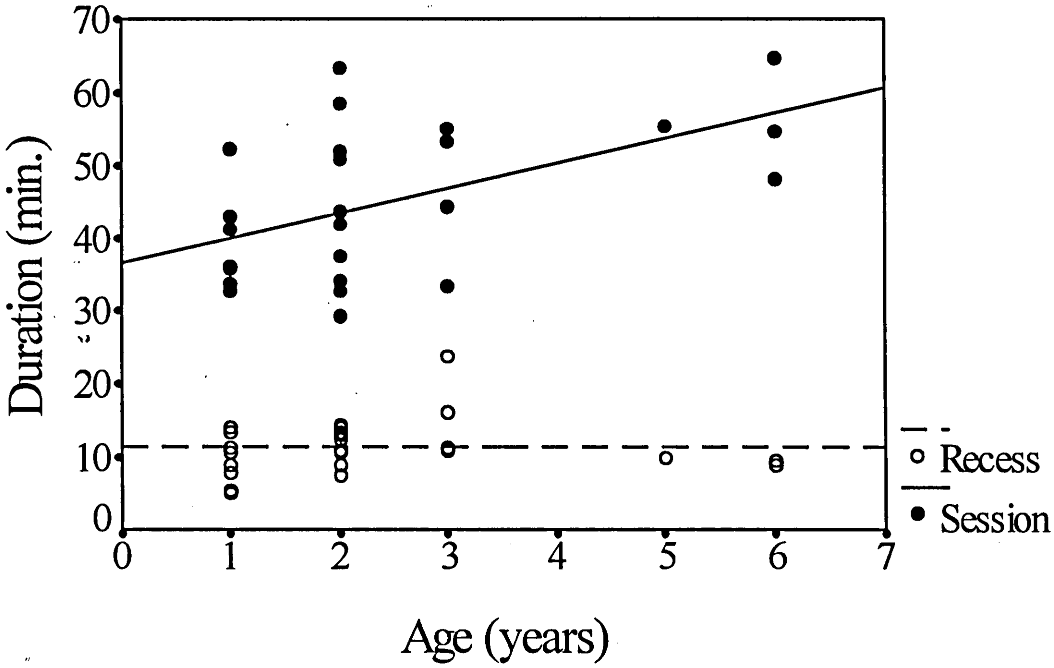


Figure 4.10 **Mean duration of session and recess periods of incubating Dippers against female age in years. The duration of incubation sessions increased with age (Spearman rank correlation r = 0.505, n = 26, p = 0.009).**

4.3.3.3 Nest attendance and energy use.

No significant correlations were found between either the mean session or recess duration and any measure of energy expenditure. However, amongst control females, with no clutch size manipulation, there was a tendency for costs to escalate when incubation sessions were short (Figure 4.11).

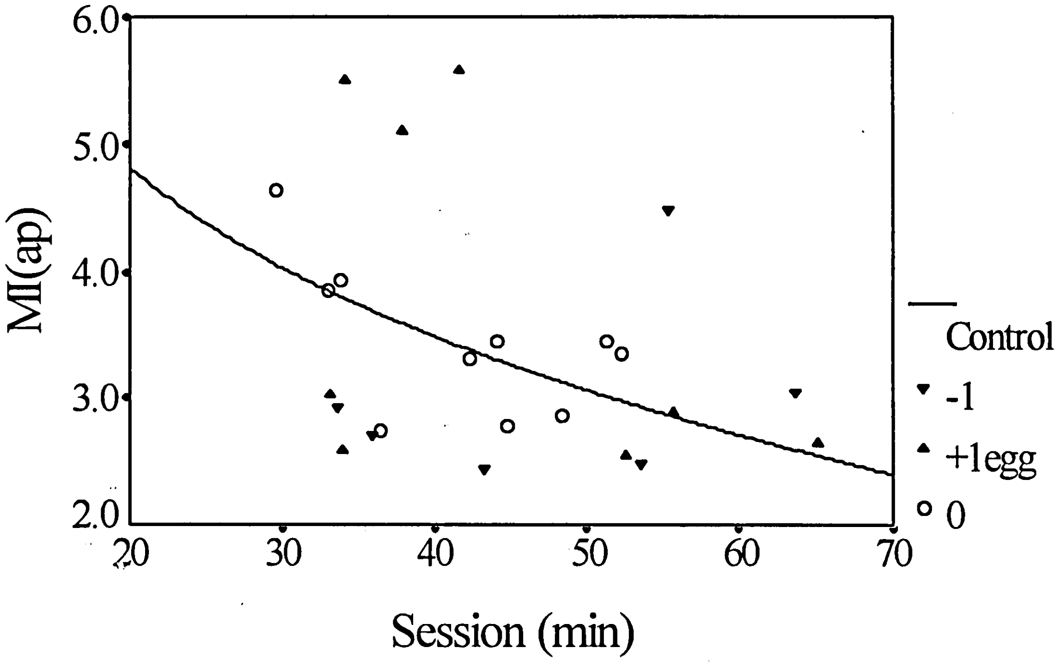


Figure 4.11 **The relationship between metabolic intensity (daily energy expenditure expressed as a multiple of BMR, calculated following Aschoff and Pohl 1970) and daily mean session duration. Amongst females with no clutch manipulation, the relationship was described by a semi-log curve: $y = 10.583 - 4.432x$, where y is MI_{AP} and x is $\log_{10}$ (session in min.); $r^2 = .412$, $F_{(1,8)} = 5.60$, $p = 0.046$.**

4.3.3.4 Relationship between nest attendance and clutch size.

Neither mean session (day or night session), mean recess duration nor Incubation Constancy differed according to the experimental clutch size incubated or between treatment groups (Table 4.4).

Table 4.4 **Patterns of nest attendance for incubating Dippers according to (i) experimental clutch size and to (ii) manipulation group. Incubation Constancy (I.C.) is the proportion of the active day spent incubating.**

(i)

Clutch size	Session (min)	Recess (min)	Night session	I.C.
3	49.1 ± 13.1 (5)	13.5 ± 6.1 (5)	663.6 (1)	.788 ± .055(5)
4	39.0 ± 4.9 (4)	10.9 ± 4.6 (4)	625.3 ± 100.2 (2)	.785 ± .083(4)
5	44.9 ± 7.9 (10)	11.4 ± 2.3 (10)	633.7 ± 95.6 (3)	.794 ± .045(10)
6	41.8 ± 11.4 (8)	10.4 ± 3.0 (8)	620.9 ± 33.8 (5)	.800 ± .061 (8)
Oneway ANOVA p value	.441	.551	.949	.987

(ii)

Manipulation	Session (min)	Recess (min)	Night session (min)	I.C.
-1	47.6 ± 11.9 (6)	13.5 ± 6.2 (6)	625.3 ± 100.8(2)	.782 ± .081(6)
0	41.5 ± 7.2 (13)	11.0 ± 2.2 (13)	610.0 ± 60.2(3)	.789 ± .036(13)
+1	44.3 ± 12.0 (8)	10.5 ± 3.0 (8)	639.9 ± 55.5 (6)	.804 ± .063(8)
Oneway ANOVA p value	.463	.275	.806	.735

4.3.3.5 Nest attendance and flow rate.

There was no evidence to suggest that patterns of nest attendance were affected by flow rates. Pearson correlation coefficients between mean session and recess duration, I.C. and mean flow rates (logarithmically transformed) were all negative, but none attained significance at the 5% level, (p>0.10 in all cases).

4.3.4 Activity during incubation recesses.

Concurrent with the doubly labelled water studies in 1993 and 1994, behavioural observations were made for all labelled birds during recess periods. Activity data were also available for birds labelled by Ward (1992). In 1995, these time-activity data were supplemented by observations of 7 unlabelled incubating Dippers, during recess periods. In all cases, observation periods began as the female left the nest and were continued until the subject moved out of sight and could not be relocated within 10 minutes. The median duration of continuous observation was 1.52 minutes (n = 114).

4.3.4.1 The influence of activity on energy expenditure.

(Labelled birds only).

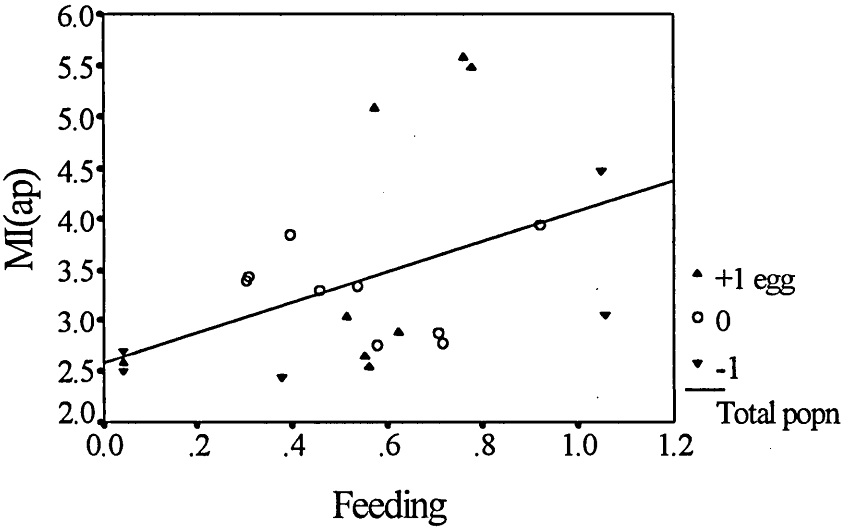
1993 and 1994. For each labelled female for whom over 60s of activity data were available, a single activity budget was constructed, expressing the amount of recess time spent in each activity as a proportion of the total recess time observed (Table 4.5). Very little data were collected for sample number 302 due to poor visibility created by conditions of continuous heavy rainfall and mist during the study period. Data for sample 309 were lost due to equipment failure.

1990 and 1991. Activity budgets are also available for birds labelled in 1990-1991 (Ward 1992). The techniques of observation and recording were comparable between observers (Ward per comm.).

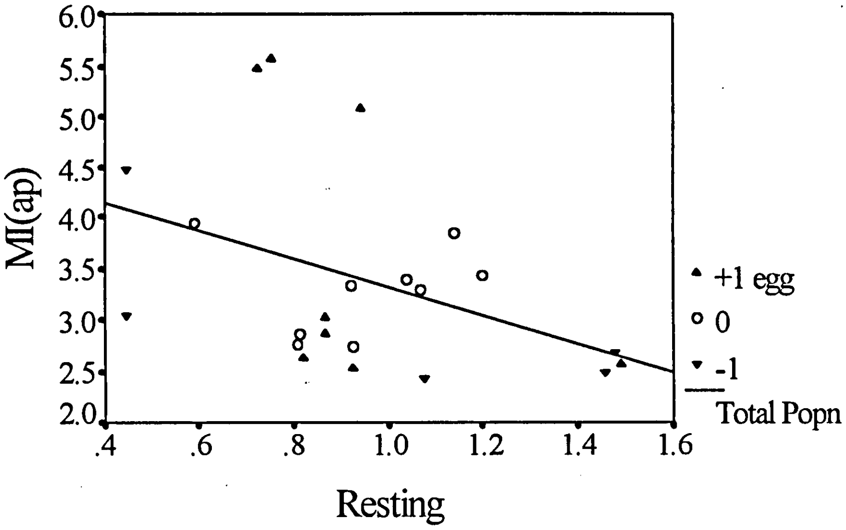
For 22 labelled females, studied between 1991 and 1994, Pearson correlation coefficients were computed between measures of energy expenditure and the proportions of time (transformed using the angular transformation) spent in each activity. The proportion of

Table 4.5 Time-activity budgets for labelled incubating female Dippers, 1993-1994. Activities were grouped into four categories: resting, foraging, diving and flying (Bryant and Tatner 1988b). The data represent the time observed in each activity for each bird, as a percentage of the total time observed for that bird. Samples 304 and 305 derive from the same female, as do 306 and 307 so their activity budgets were grouped. Mean values presented in the final row represent an average of the proportions calculated for each bird ± s.d..

Sample	Rest	Forage	Dive	Fly	Total time (minutes)
301	63.4	14.0	12.1	10.5	32.1
303	58.4	12.5	21.7	7.4	14.1
304/5	65.7	12.8	16.8	4.7	20.4
306/7	52.9	41.8	0.7	4.7	12.7
308	74.2	8.9	0	17.0	6.7
310	77.6	1.2	12.4	8.8	17.6
311	58.0	19.1	5.2	17.6	4.9
312	53.8	18.0	9.6	18.6	10.3
313	63.9	17.6	12.4	6.0	33.7
314	64.0	16.7	11.8	7.5	36.4
315	99.4	0.2	0	0.4	46.1
316	98.8	0.1	0.1	1.0	26.9
317	99.2	0.2	0	0.6	11.8
Mean	71.5 ± 16.6	12.5 ± 11.0	7.9 ± 7.1	8.1 ± 6.1	21.1 ± 12.3



(i)Feeding



(ii) Resting

Figure 4.12 Metabolic Intensity (calculated according to the equations of Aschoff and Pohl 1970) of 24 incubating female Dippers (1990 to 1994) according to the proportion of time spent (i) feeding and (ii) resting. Proportions were transformed using an angular transformation.

time spent flying, foraging and diving were positively correlated with measures of energy expenditure, while the proportion of time resting was negatively correlated. Only one correlation attained significance at the 5% level: the amount of time spent feeding (a combined category, including both foraging and diving) was significantly positively correlated with MI_{AP} ($r = 0.448$, $n = 22$, $p = .037$) (Figure 4.12). A corresponding negative correlation between MI_{AP} and the proportion of recess time spent resting was close to significance ($r = -.412$, $n = 22$, $p = .057$).

4.3.4.2 Influence of activity on mass change.

(Labelled birds only, 1990 to 1994).

Pearson correlation coefficients were calculated between mass change (expressed as mass at final capture, controlled for mass at first capture, ' residual final mass') and the proportion of recess time spent in each activity, for 22 labelled females. Although no correlation attained significance at the 5% level, there was a positive association between the amount of time spent feeding (foraging or diving) and mass change ($r = .381$, $p = .080$). The corresponding correlation between mass change and time spent resting was negative, but not significant ($r = -.330$, $p = .134$) (Figure 4.13).

4.3.4.3 Observations of unlabelled incubating Dippers and their mates

(1995 only)

Behavioural data were supplemented in 1995 by observation of incubating birds outwith a doubly labelled water study. Female behaviour was always recorded in preference to that of her mate, but behaviour of the male was frequently observed while the female was incubating. Male behaviour was generally recorded within the several metres of the nest.

An individual activity budget was constructed using the total data collected for each bird for whom a total of over 60 seconds data was available. Activity budgets for 7 unlabelled females were compared with those collected by the same observer (S.M.B.) for 13 labelled females (1993 and 1994). Labelled birds were more likely to be observed resting than unlabelled birds, and less likely to be observed diving (Table 4.6).

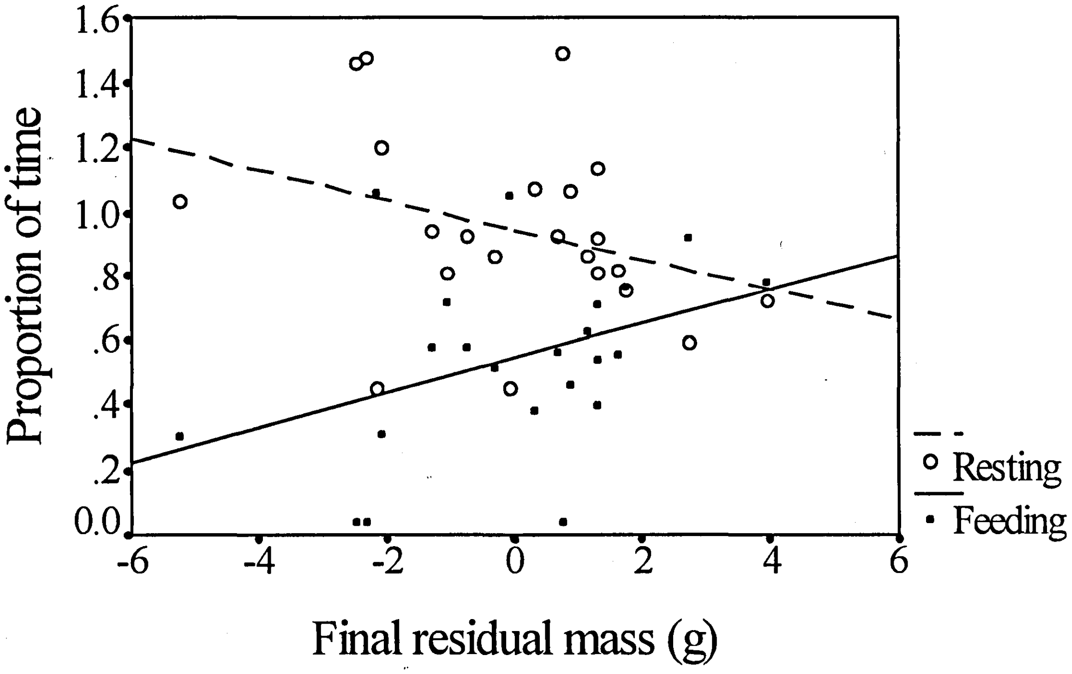


Figure 4.13 The relationship between activity and mass change for incubating female Dippers during the doubly labelled water study period (data from 1990 to 1994). Activities are represented as proportions of the total recess time observed, transformed using an angular transformation.

Although this data could imply abnormal behaviour by labelled birds during incubation, it was thought that a change in observation technique was more likely to underlie the observed differences. In 1993 and 1994, labelled birds were generally observed within site of the nest, whereas in 1995, observations were made both at the nest site and at locations where the female was known to feed. The difference in observed behaviour could result from a higher proportion of feeding behaviour recorded at sites away from the nest for unlabelled birds.

Activity budgets for females observed in 1995, were compared with those of their partners, (Table 4.7). Data for females represent the proportion of recess time spent in each activity, while the data for males represent his behaviour while in close proximity to the nest, whilst the female was incubating. Within these periods, males spent significantly less time diving and flying than females, and significantly more time resting.

4.3.4.4 Effect of flow rate on behaviour.

The effect of flow rate on behaviour was assessed for unlabelled birds (1995). Individual time activity budgets were constructed per bird for each day of observation and compared with the F.R.P.B. measure of flow rate for that day. Pearson correlation coefficients were computed using the angular transformations of the proportion of time in each activity and logarithmically transformed flow rate. Significant correlations were found between flow rate and the proportion of time resting ($r = .3881$, $p = 0.015$, $n = 39$) and diving ($r = -0.4514$, $p = 0.004$, $n = 39$). Correlations with foraging and flying were not significant ($p > 0.1$) (Figure 4.14).

Amongst labelled females (1990 to 1994), flow rate also affected patterns of activity during recess periods. Positive, but non-significant correlations were found between the proportion of time resting and diving with flow rate. The proportion of time spent foraging declined significantly with increasing flow rate ($r = -0.495$, $n = 22$, $p = .019$), while flying increased ($r = 0.486$, $n = 22$, $p = .022$). For the nine nests where site-specific characters of river flow (speed of flow (ms^{-1}), river width (m), average depth (m) and measured flow rate (m^3s^{-1})) were recorded, there were no significant correlations between any of these variables and behaviour.

A similar analysis was performed using data from Dippers in all stages of the annual cycle and in the full range of flow conditions by Bryant and Tatner (1988b) and by

Table 4.6 **Activity budgets during recess periods for incubating female Dippers. Data represent the proportion of recess time (± s.d.) observed in each activity. Labelled birds were observed during doubly labelled water sampling in 1993 and 1994. Unlabelled birds were observed without the doubly labelled water procedure in 1995. Mann-Whitney U tests were used to assess the difference between the budgets.**

	Rest	**Forage**	**Dive**	**Fly**	**n (birds)**
Labelled	.71 ± .18	.13 ± .12	.08 ± .07	.08 ± .06	13
Unlabelled	.53 ± .15	.09 ± .05	.27 ± .15	.12 ± .12	7
Mann-Whitney U test.	z = -2.338 p = .019	z = -0.674 p = .501	z = -2.545 p = .011	z = -0.674 p = .501	

Table 4.7 **Activity budgets for incubating female Dippers during their recess periods (unlabelled birds, 1995 only) and for their partners whilst in the nest environs (1995 only). The proportion of time spent in each activity is presented ± s.d. Mann-Whitney U tests were used to compare the data between sexes.**

	Rest	**Forage**	**Dive**	**Fly**	**n (birds)**
Female	.53 ± .15	.09± .05	.27 ± .15	.12 ± .12	7
Male	.82 ± .12	.10 ± .08	.06 ± .08	.01 ± .01	10
Mann-Whitney U test	z = -2.830 p = .005	z = -0.293 p = .770	z = -2.295 p = .022	z = -2.928 p = .003	

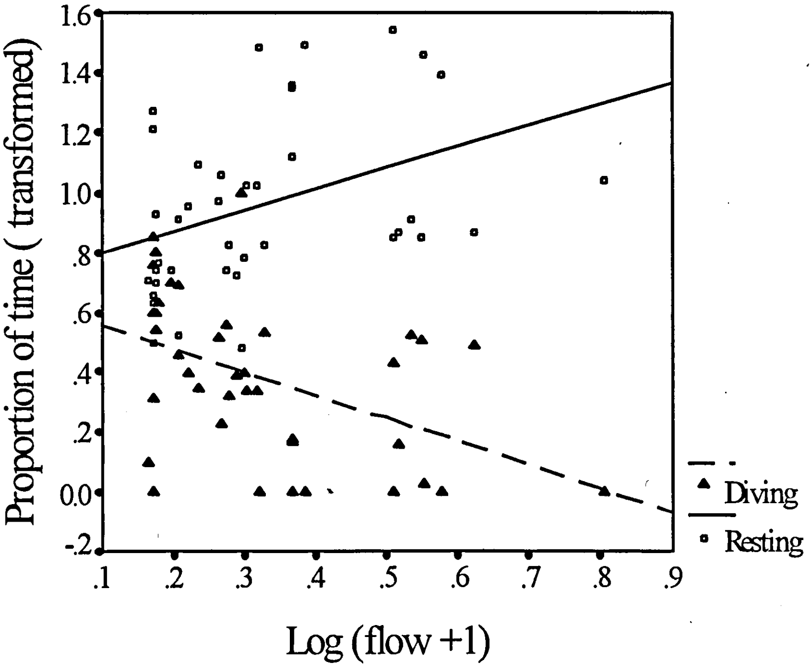


Figure 4.14 **The proportion of the recess time of incubating Dippers spent resting and diving in relation to flow rate (unlabelled birds, 1995 only). Data were transformed using an angular transformation for proportions and a logarithmic transformation for flow rates (m^3s^{-1}). Variables were related by the following regression equations :**

(i) resting: $y = 0.710x + 0.727$, $r^2 = 0.151$, $F_{(1,37)} = 6.562$, $p = 0.015$.

(ii) diving: $y = -0.778x + 0.633$, $r^2 = 0.204$, $F_{(1,37)} = 9.470$, $p = 0.004$

O'Halloran *et al.* (1990). Both studies found evidence of a positive relationship between the proportion of time spent diving and river flow. In the latter study, the amount of time spent diving, foraging and swimming was found to increase with river flow rate up to a threshold level, beyond which turbidity or hydraulic stress apparently necessitated a reduction in feeding behaviour. Da Prato (1980) observed a cessation of male feeding behaviour during spate conditions in February.

4.3.4.5 Diurnal variation in recess behaviour.

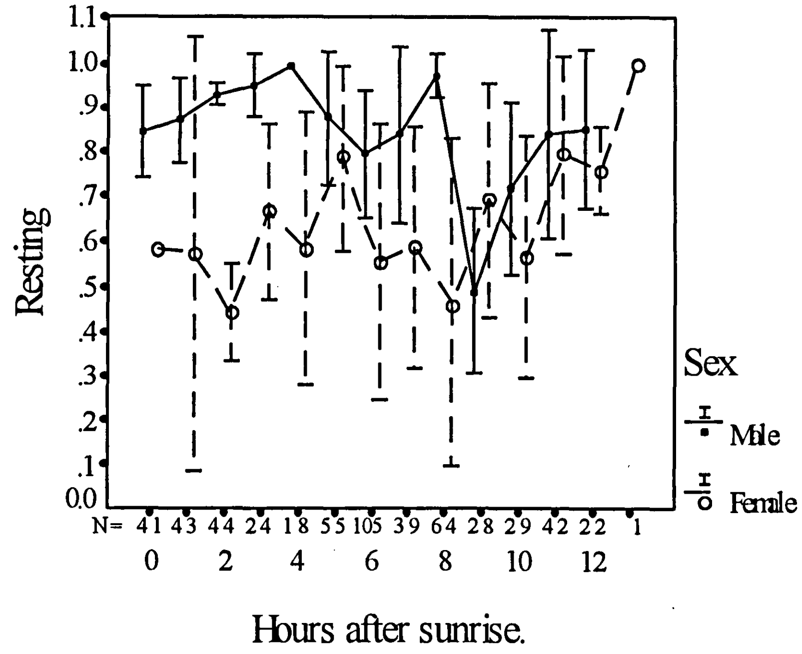
(Labelled and unlabelled birds, 1993 to 1995)

The behaviour of incubating Dippers might vary throughout the day according to the profitability of foraging, hunger motivation or characteristics of the microclimate. In order to assess the diurnal trends in behavioural patterns, a single activity budget was computed from the data available for each hour for each bird, for both incubating females and their partners (Figure 4.15 (i) to (iv)). Data from labelled birds in 1993 and 1994 were combined with data from unlabelled birds observed in 1995. The non-parametric Kruskal-Wallis test was used to test for a difference between activity in each hour. No significant difference could be detected between time periods for any activity group for either sex ($p > 0.05$ in all cases).

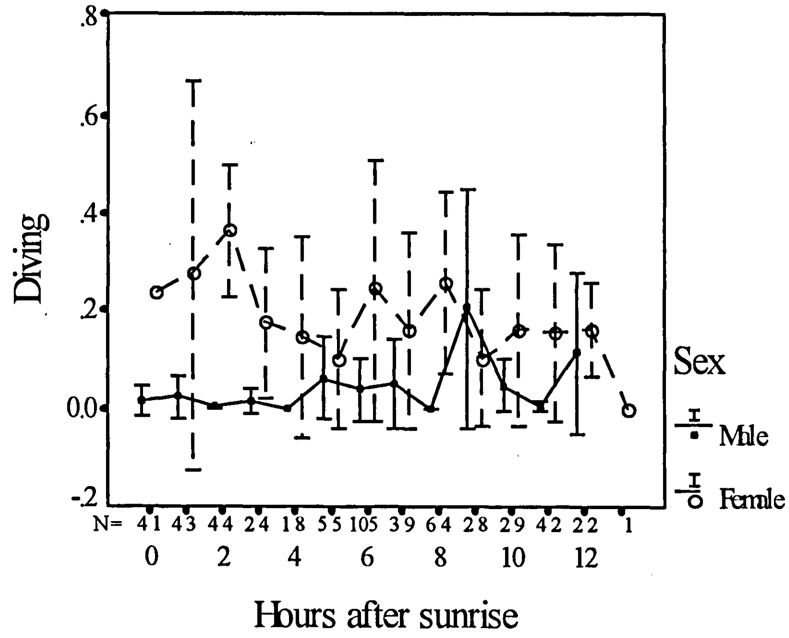
A similar analysis was performed by O'Halloran *et al.* (1990), using data collected from both sexes of Dippers in all months of the year and hence, all stages of the annual cycle. In their analysis, significant differences were established between time intervals, there being a higher proportion of time spent flying and feeding in the first few hours after dawn. In the present study, there was no evidence of a similar allocation of feeding behaviour to a particular time of day, for either females or for males.

In the case of the incubating female, any changes in the costs and benefits of different behaviours, arising from changing hunger motivation of the female or from changes in foraging returns were, perhaps, more likely to affect the time spent off the nest than the behaviour patterns during recess time. That there is some indication of a trend towards the more energetically costly feeding method (diving) in the early hours is perhaps the only evidence of the female's action to replenish reserves following night time incubation.

In the case of the male, the activity budgets recorded in this study represent behaviour within the nest environs only. In some cases, males regularly fed in this location, but several males abandoned nest guarding frèquently to forage elsewhere in the territory.

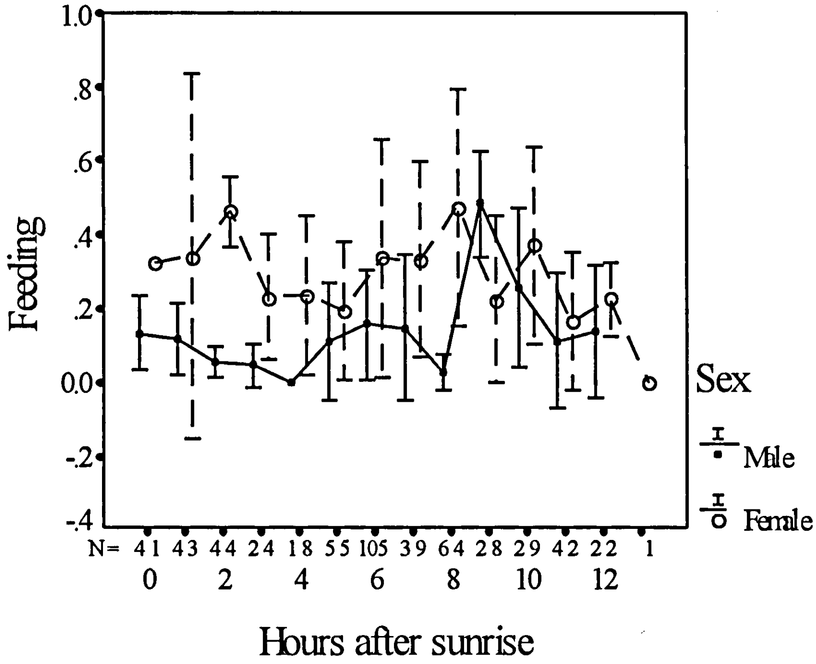


(i)Resting

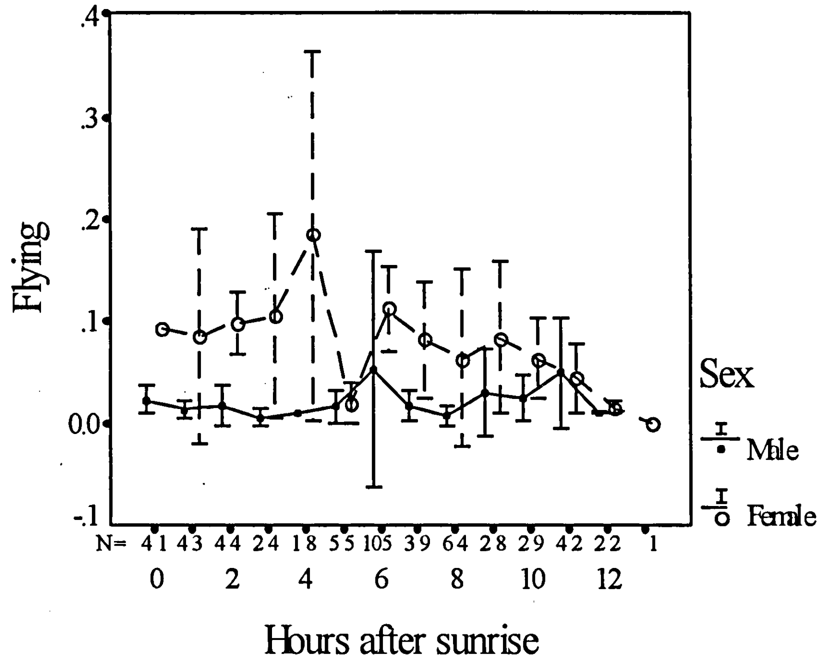


(ii) Diving.

Figure 4.15



(iii) Total time feeding (= foraging + diving)



(iv) Flying.

Figure 4.15 Patterns of behaviour for incubating female Dippers and their mates, according to time (hours after sunrise). Data represent the mean ± s.d. proportion of activity in each of four activity categories. Data were collected from 1993 to 1995.

Thus, for some birds, patterns of feeding behaviour will be reflected more in a change in the proportion of time spent within the nest environs than in a change in activity budget during nest guarding.

4.3.4.6 The significance of female 'resting' behaviour during incubation recesses.

Given the apparent time-constraints acting during incubation, it was surprising to find that 53% of the female's recess time was spent 'resting'. During 1994 and 1995, 'resting' was further subdivided into 'alert' (stationary posture, or characteristic 'dipping'), 'preening' or 'singing'. Of the total time more generally classified as 'resting', females spent 90% alert, 7% preening and 3% singing, while males spent 83% alert, 11% preening and 6% singing.

Periods of inactive behaviour were seldom lengthy; from a sample of 10 observations of female behaviour, the mean duration of periods of 'alert' behaviour was 3.1 seconds ± 5.4 (n = 234 observations). These periods were most frequently observed to follow swimming, diving or preening. Periods of such short duration probably formed an integral part of the more active behaviour, for example, allowing time to breathe or to handle or swallow prey following periods underwater, to ascertain the bird's own position or the location of prey, or to look around for predators.

4.3.4.7 Functional significance of male guarding behaviour.

The partners of incubating females spend a large proportion of their day within a few metres of the nest. During 1995, the proportion of time males spent guarding was analysed by recording whether the male was present or absent within 5 metres of the nest at hourly intervals. This sampling interval was chosen as it exceeded the duration of the female's incubation sessions, so no two observations were taken during the same incubation session. Analysis of the resulting data showed that an observation of a male guarding was equally likely to be followed by a record of male guarding as one of male absent, ($\chi^2 = 6.8 \times 10^{-6}$ on 1.d.f, $p > .999$). Although not strictly independent, these data were therefore assumed to have avoided problems of repeated sampling of the same guarding period.

During 68 hours of observation at 12 nests, males were within 5 metres of the nest for 52% of the 92 records made. Efteland (cited in Tyler and Ormerod 1994) recorded males spending 40% of their time guarding in this manner. Figure 4.16 shows the proportion of nests which were guarded by the male in relation to hours after sunrise. The data were

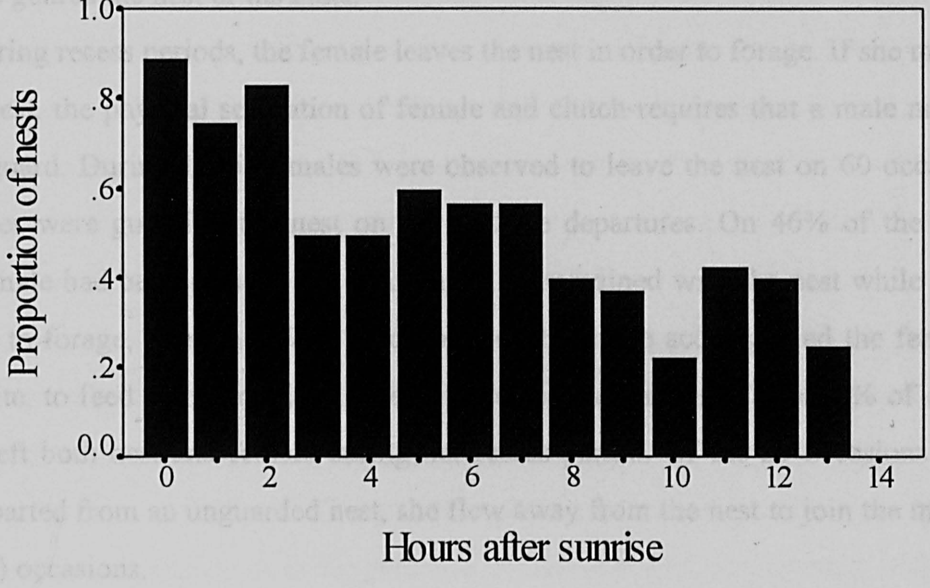


Figure 4.16 **The proportion of Dipper nests with guarding males, (present within 5 metres of nest), in relation to time of day (hours after sunrise). n = 90 observations made hourly at 12 nests from 1993 to 1995.**

Table 4.8 **The frequency distribution of records of guarding males at the nests of incubating Dippers according to the number of hours after sunrise.**

Guarding male	0-3 hours	4-7	8-11	12-15	Total
Present	5	10	22	7	44
Absent	22	11	12	3	48
Total	27	21	34	10	92

grouped into 4 hourly periods (Table 4.8), in order that a Chi-square test could be used to assess the difference in guarding frequency between different time periods. Nest guarding was found to occur at a higher frequency in the early morning ($\chi^2 = 20.97$ on 3 d.f., $p < .001$). The question arises as to the functional significance of the males' guarding behaviour, and whether he guards the nest or his mate.

During recess periods, the female leaves the nest in order to forage. If she moves away from the nest, the physical separation of female and clutch requires that a male must choose which to guard. During 1995, females were observed to leave the nest on 60 occasions at 8 nests. Males were guarding the nest on 39 of these departures. On 46% of the departures when the male had been present, the guarding male remained with the nest while the female flew away to forage, while on 51% of departures, the male accompanied the female to the foraging site, to feed or preen within a few metres of the female. Once, (2% of departures) the male left both nest and female during her recess period. Of the 21 occasions where the female departed from an unguarded nest, she flew away from the nest to join the male to feed on 9 (43%) occasions.

It appears that the advantages to the male of remaining with both the nest and the female are significant. His function at the nest may be to warn of danger. Although no attempts of nest predation were observed in this study, various mammalian (Stoat, Mink, Weasel, Rat) and avian (Crow, Sparrowhawk) species have been recorded to predate Dipper nests in this population (Bryant pers comm.). Wagtails, both Grey and Pied, were frequently observed to forage in the nest environs, and, in 158 hours of observations from all three years of study, were observed to be chased away on three occasions by the male. On a further three occasions, Pied Wagtails were seen to attempt to enter the Dipper nest. All three attempts were deflected by the male; in one instance, he was aided by the female. Intruding Dippers were observed to provoke the guarding male on four occasions, with responses ranging from singing and displaying, to chasing and fighting. At one nest, vigorous singing and displaying by two unidentified birds below the nest was observed one evening. Two of the clutch of 5 eggs were found to be smashed and others pierced on the following day. A case of suspected infanticide has been reported in the Dipper (Wilson 1992). This is the first report of suspected egg piercing. When the nest is unoccupied, it may be susceptible to egg dumping. As eggs are laid early in the morning, the increase in male guarding behaviour during the early hours would coincide with the period of greatest risk.

The advantages of guarding females were not readily apparent. The risk of extra-pair copulations is much reduced following egg-laying. One possible function of the male was to remain alert for predators whilst the female foraged, or to guide her to profitable foraging sites in order to increase her rate of food intake.

During 1994 and 1995, behavioural data were recorded for seven females, both in the presence (defined as being within 10 metres of the female, or within her line of sight, whichever was smallest) and absence of the male. Activity budgets in the presence and absence of the male were compared using a paired t-test on the (angular transformed) data. Though there was a tendency for females to rest less and dive more when their males were present, none of the differences was significant (Table 4.9).

An attempt to quantify the rate of energy gain by observation was made at four sites where catches of medium to large prey (defined by Yoerg (1994) as 'as long as or longer than the bill') could be recorded individually. From observations of 33.0 minutes of time spent feeding, catch rates of 5.74 ± 7.57 and 2.65 ± 2.04 catches per minute were recorded for females when foraging alone or in the presence of their partners, respectively. The difference between the two was not significant (p = 0.285).

Table 4.9 **Activity budgets of incubating female Dippers (1994 and 1995) in the presence (within 10m) or absence of their mates. Data represent the means (with standard deviations) of activity budgets from 8 females. P-values were calculated using a two-tailed paired t-test.**

	Rest	Forage	Dive	Fly
Male present	0.55 ± 0.2	0.09 ± 0.1	0.23 ± 0.2	0.12 ± 0.1
Male absent	0.63 ± 0.2	0.14 ±. 0.2	0.15 ± 0.1	0.08 ± 0.04
p-value	.753	.560	.124	.425

4.3.4.8 Mate feeding during incubation in the Dipper.

During 158 hours of nest observation at 26 nests over the three years, male Dippers were seen to visit the nest on a total of 81 occasions. Food was seen to be presented on only 19 (23%) of these visits, although transfer of small items could not be discounted. At one nest, the male was observed to gather several prey items and to present them to the incubating female on the nest, but to drop down from the nest several seconds later with the prey items still held in the beak. This male then foraged a little more, picking up fresh prey, before returning to the nest, again to be refused. During a five minute period, this pattern was repeated such that the male visited the nest five times in total, after which the female left the nest to forage herself, leaving the male with his collection of prey intact. The same behaviour was observed one week later at the same nest, again with five nest visits without any consumption of prey by the female.

The number of nest visits recorded per hour of nest observation was computed for hourly periods following sunrise. The majority of nest visits took place in the early hours of the day (Figure 4.17). The mean rate of nest visitation was 0.6 ± 0.8 visits per hour. During a 14 hour active day, this would give an average of 8.4 visits per day.

4.3.5 Incubation body mass in the Dipper.

Mass was recorded for all labelled birds (n = 33 between 1982 and 1994) at the first and final capture for the doubly labelled water period, (48h). During 1995, 21 unlabelled females were caught on at least one occasion; 19 of these birds were caught again some days later. Repeat catches were between 3 and 5 days later (mode = 4 days for 16 birds). In all years, repeat catches were synchronised with respect to time of day, so that 79% of repeat catches were within one hour of the time of initial catch, the maximum difference being 1.66 hours.

4.3.5.1 Mass change during incubation for labelled birds.

In the following analysis, mass change was calculated in gd^{-1} for all birds. Sample 109 was excluded on the basis that both the initial mass (72.1g) and mass change ($-5.65gd^{-1}$) were outwith the normal range for other incubating female Dippers (mass range 57.4 - 69.3g, daily

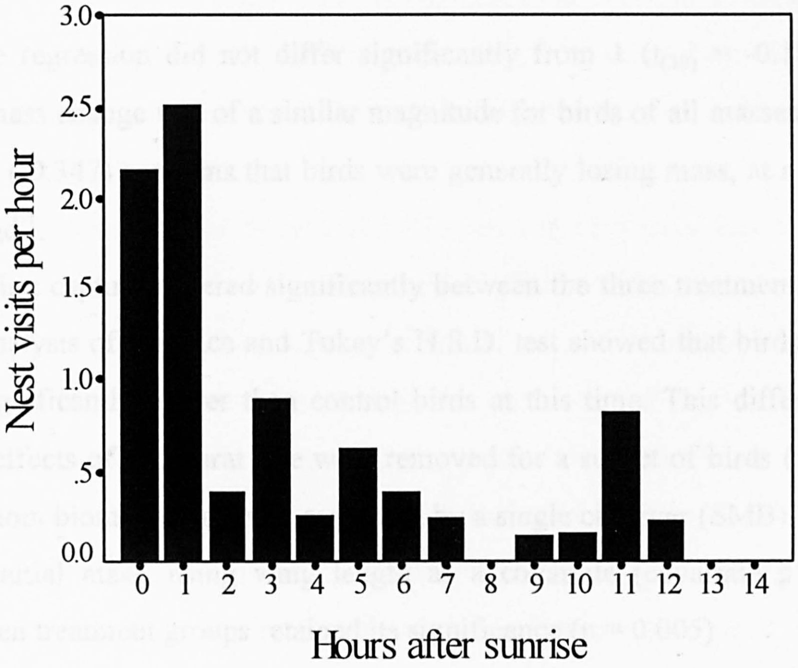


Figure 4.17 **The rate of nest visitation (and presumably mate-feeding), by the mates of incubating Dippers. Data were derived from 158 hours of nest observation at 26 nests.**

mass change range -3.5g - +1.15g). This bird may have been captured initially at the end of the laying period.

The majority of labelled birds lost mass throughout the study period (81%), one bird (3%) maintained the same mass and the remainder (16%) gained mass. Mass change ranged from -3.5 gd^{-1} to +1.15 gd^{-1}, with a mean change of -0.86gd^{-1} ± $0.99_{(32)}$. Figure 4.18 shows mass at first capture against that at second capture 48h later. The relationship was described by a geometric mean regression (Equation 4.5), as the magnitude of error in each measurement was of the same order.

Eqn 4.5 $y = 0.978x - 0.347$

where x was body mass at first capture (g), and y was mass (g) 48h later.

The slope of the regression did not differ significantly from 1 ($t_{(30)} = -0.188$, $p > 0.5$), suggesting that mass change was of a similar magnitude for birds of all masses. The intercept of the regression (-0.347) confirms that birds were generally losing mass, at a rate of 0.347g in 48h, or $0.174gd^{-1}$.

Mass at first capture differed significantly between the three treatment groups (Table 4.10), oneway analysis of variance and Tukey's H.S.D. test showed that birds with enlarged clutches were significantly lighter than control birds at this time. This difference persisted even when the effects of structural size were removed for a subset of birds (1993 and 1994 data only) for whom biometric data was measured by a single observer (SMB). In an analysis of variance of initial mass, using wing length as a covariate (covariate $p = 0.041$), the difference between treatment groups retained its significance ($p = 0.005$).

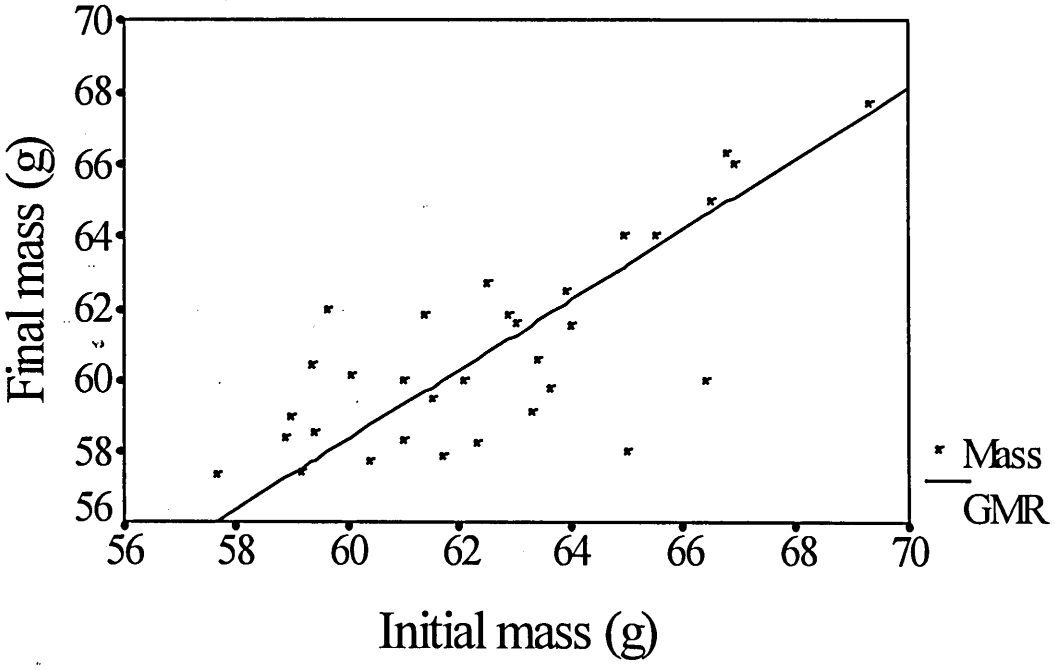


Figure 4.18 **Body mass of incubating Dippers at the beginning and end of the doubly labelled water measurement period (48h). The relationship was described by the geometric mean regression : $y = 0.977x - 0.347$, $n = 32$.**

This difference in body mass between treatment groups could not be explained by date (Pearson correlation coefficient between residual mass and date r = .0483, p = 0.783, n = 35). It was attributed to the fact that clutch size was manipulated between 1 and 2 days prior to initial capture. Birds with enlarged clutches presumably lost more mass than other birds immediately after clutch manipulation and before first capture.

However, this pattern of mass change did not persist throughout the doubly labelled water period. By the time of final capture 48 hours later, there was no significant difference between the mass of birds in the three treatment groups (Table 4.10).

The difference in mass change over the course of the doubly labelled water period between treatment groups was tested by an analysis of variance of mass at final capture. In order to standardise for initial mass, 'residual final mass' was calculated as mass at final capture minus the predicted value of final mass, calculated by inserting initial mass in the geometric mean regression (Equation 4.5). Birds with enlarged clutches showed a tendency to gain mass, while those with reduced clutches lost mass (Table 4.10) but the difference did not achieve significance. There was a significant, positive linear correlation between residual mass and manipulation, expressed as the number of eggs added to the clutch (Figure 4.19)

<u>Table 4.10</u> **Mean body mass of incubating Dippers at the beginning and close of the doubly labelled water measurement period, shown in relation to clutch manipulation Residual final mass represents final mass standardised according to initial mass by Eqn 4.5, and expresses the mass change over the study period. Oneway analyses of variance were performed with all three variables, the resultant p values are presented in the final row.**

Clutch size	Initial mass (g)	Final mass (g)	Residual final mass (g)
-1	63.40 ± 2.58 $_{(6)}$	60.59 ± 3.14 $_{(6)}$	-1.06± 1.38 $_{(6)}$
0	63.31 ± 2.94 $_{(18)}$	61.65 ± 2.97 $_{(19)}$	-0.12 ± 2.16 $_{(18)}$
1	60.13 ± 1.08 $_{(8)}$	59.69 ± 1.14 $_{(8)}$	1.05 ± 1.55 $_{(8)}$
ANOVA p value	.016	.227	.131

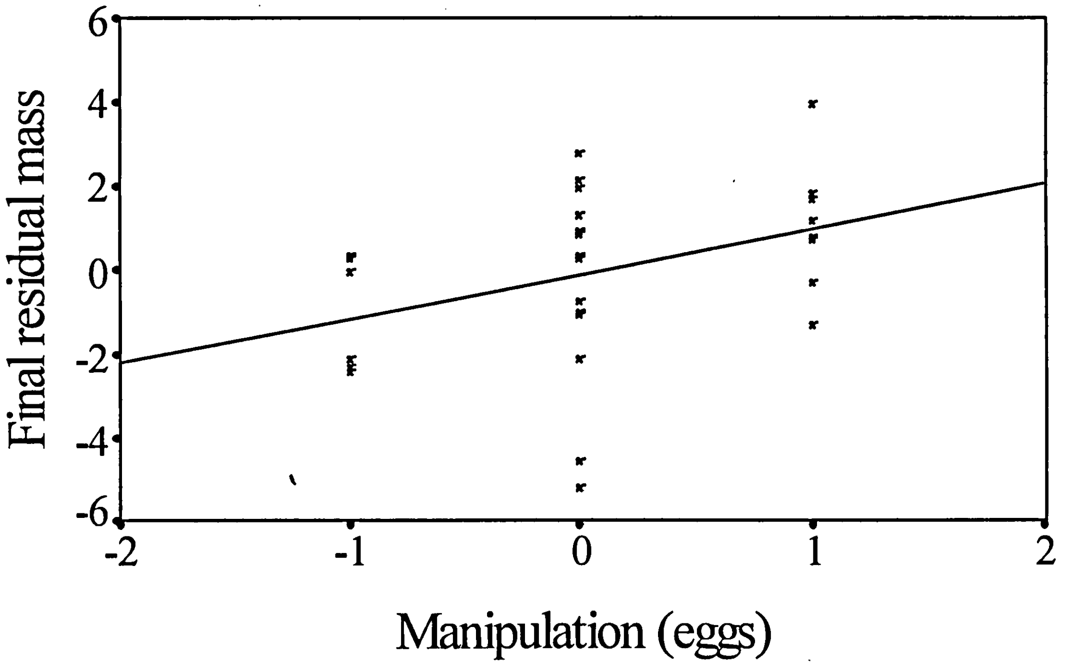


Figure 4.19 **Mass change of incubating Dippers during the doubly labelled water study period (48h) according to the manipulation of clutch size. Mass change is represented by residual final mass, calculated as the difference between mass at final capture and that predicted from mass at first capture using Equation 4.5. The relationship was described by the equation: $y = 1.065x - 0.070$, $r^2 = .130$, $F_{(1,30)} = 4.475$, $p = .043$, $n = 32$; where x was clutch manipulation (eggs) and y was residual mass (g).**

Similar analyses were performed to investigate the effect of experimental clutch size on mass. There was no significant difference between initial or final mass according to experimental clutch size, or in the change in mass between the two captures according to clutch size (Table 4.11). Linear correlation between residual final mass and experimental clutch size did not achieve significance ($p = .168$).

Table 4.11 **Mean body mass of incubating Dippers at the beginning and close of the doubly labelled water measurement period, shown in relation to the number of eggs in the nest. Final mass was standardised according to initial mass using Equation 4.5 (residual final mass) to represent the mass change over the period. Oneway analyses of variance were performed with all three variables, the resultant p values are presented in the final row.**

Clutch size	Initial mass (g)	Final mass (g)	Residual final mass (g)
3	62.80 ± 2.74 $_{(5)}$	60.62 ± 2.90 $_{(5)}$	-.45 ± 1.88 $_{(5)}$
4	64.02 ± 2.53 $_{(7)}$	61.13 ± 3.47 $_{(7)}$	-1.13 ± 2.03 $_{(7)}$
5	62.65 ± 3.28 $_{(12)}$	61.60 ± 3.06 $_{(12)}$	0.66 ± 1.19 $_{(12)}$
6	60.89 ± 1.92 $_{(8)}$	60.08 ± 1.17 $_{(8)}$	0.26 ± 2.70 $_{(8)}$
ANOVA p value	.200	.678	.265

4.3.5.2 Changes in body mass in relation to energy expenditure.

A positive relationship existed between mass change and energy use, such that lower costs were incurred by birds that lost mass. A similar positive association was found between mass change and energy expenditure for Dippers during the nestling rearing stage (Bryant and Tatner 1988b). In this study, all measures of energy expenditure were positively associated with residual final mass (final mass controlled for initial mass by Equation 4.5), though the relationship with MI_{AP} was the only one to attain significance at the 5% level. (Pearson correlation coefficients between final residual mass and ADMR, $r = .301$, $p = .094$; with DEE $r = .312$, $p = .082$, with MI_{AP} $r = .367$, $p = .039$, with MI_{BN} $r = .314$, $p = .080$).The relationship between MI_{AP} and residual mass was best described by a quadratic function (Figure 4.20).

The association between energy expenditure and mass change during incubation is discussed in Section 6.1.4., along with the possibility that such a positive association could arise from spurious assumptions.

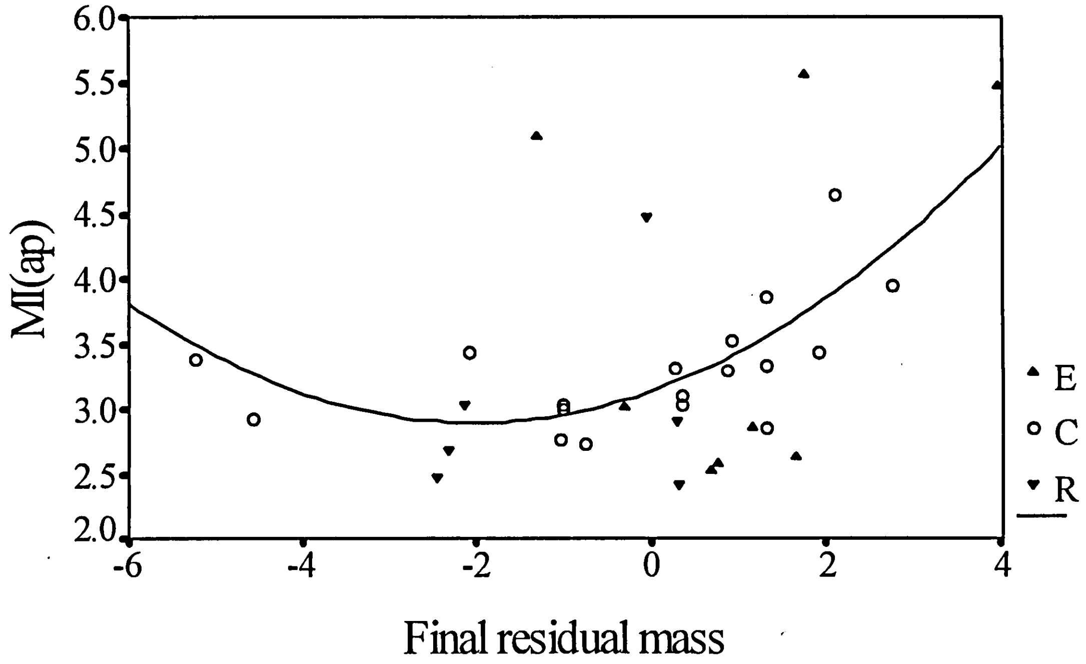


Figure 4.20 **The relationship between Metabolic Intensity (MI_{AP}), calculated according to the equations of Aschoff and Pohl (1970), and residual final mass (g), calculated as the difference between mass at final capture and that predicted from mass at first capture using Eqn. 4.5. A quadratic function provided a significantly (0.05<p<0.02) better description of the data than a linear function $y = .238x + .058x^2 + 3.145$, $r^2 = .285$, $F_{(2,29)} = 5.767$, $p = .008$.**

Pearson correlations and oneway ANOVAs were used to test the relationship between mass change and time activity budgets, patterns of nest attendance, age, date and various environmental data including temperature and mean flow rates over the period of study. The relationship between mass change and behaviour is discussed in Section 4.3.4.2; otherwise, no significant relationships were revealed.

The direction of mass change measured in non-labelled individuals in 1995 was of a similar pattern to labelled birds, with 74% of birds losing mass, 5% staying the same and 21% increasing over the study period. Mean daily mass change was $-0.16gd^{-1} \pm 0.27$ for 19 individuals, ranging from $-0.6gd^{-1}$ to $+0.4gd^{-1}$. Both the mean and variance of mass change differed significantly from that of the labelled sample, (Levene's test for homogeneity of variance, $p = 0.002$, 2-tailed t-test for difference between the means, $p < 0.001$). Both labelled and unlabelled birds were caught and handled, so this result suggests that the additional stress of holding birds, blood sampling or catching at 48 hour intervals rather than 4 days, was associated with a greater and more variable mass loss in the labelled group. The higher level of variance associated with the labelled birds could be due to the shorter period of measurement, during which day to day variation will play a greater part. However, the possibility that 1995 differed because of a year effect cannot be discounted. In a oneway ANOVA of mass change by year, there was a significant difference between years ($p = 0.002$) which was not entirely due to the difference of results from 1995. 1995 differed from 1982 and from 1994, and 1991 differed from 1982 (Tukey's H.S.D. test).

4.3.5.3 Pattern of mass change through incubation.

Because of the difference in mass response over the doubly labelled water study period, mass change through incubation was analysed using data from unlabelled individuals only (1995 only).

The daily rate of mass change was related to the number of days since clutch completion (calculated at the mid-point of the measurement period) by a negative quadratic function (Figure 4.21, Equation 4.6). Mass was lost during the first and last phases of incubation at a greater rate than during the intermediate stages.

Eqn 4.6 The relationship between rate of daily mass change in incubating Dippers and the stage of incubation.

$$y = -1.057 \pm 0.244x - 0.014x^2$$

$$r^2 = .370, F_{(2,16)} = 4.698, p = .025.$$

where x was the number of days after clutch completion, (clutch completion = day1), and y was the rate of daily mass change in gd^{-1}, positive values indicate a gain, negative values a loss of mass.

Mass loss was great in the first days of incubation, which could result from atrophy of the gonads (Breitenbach and Meyer 1959). Patterns of mass loss throughout the middle stages of incubation probably represent some degree of reserve utilisation, while the high loss in the final stages could be of adaptive value in moving birds towards a lighter mass in preparation for the active stage of nestling rearing (Freed 1981, Norberg 1981).

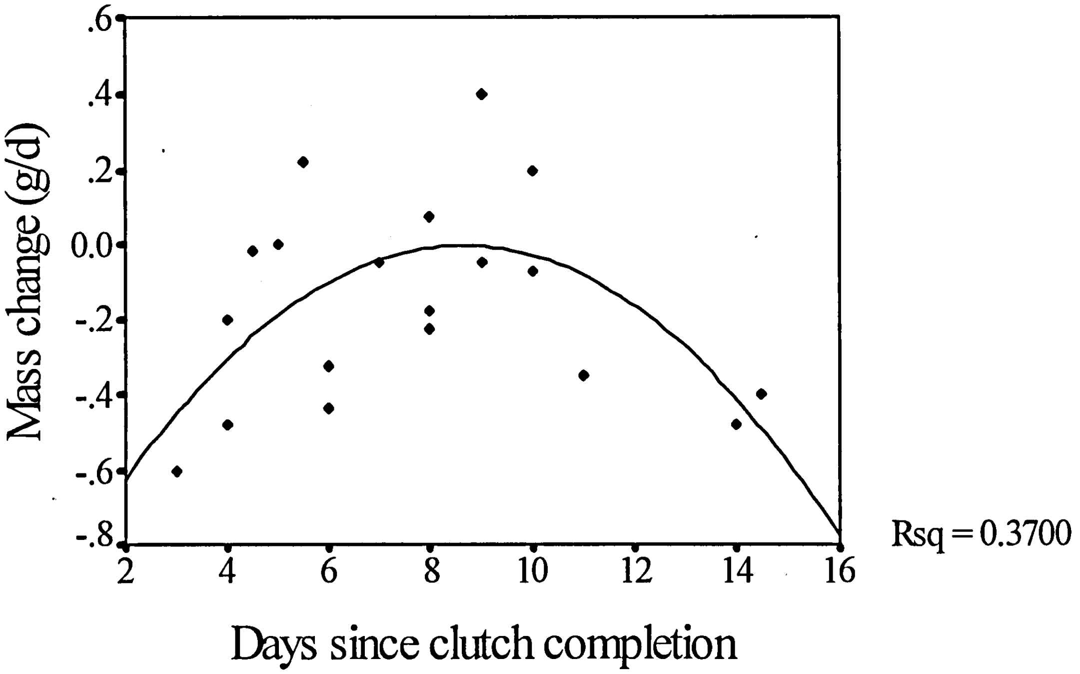


Figure 4.21 **Rate of daily mass change (gd^{-1}) for incubating Dippers (unlabelled birds in 1995 only) in relation to the number of days since clutch completion (where clutch completion = day 1).**

The residuals from Eqn 4.6 were used to look for an effect of clutch size or age on daily mass change. Neither effect was significant (oneway ANOVA $p>0.5$). All birds incubated their own clutch except for two birds. In this case, one egg was removed from the nest of the first bird and added to the nest of the second at the time of first capture, remaining for the duration of the experiment. The date of clutch completion for these birds differed by 1 day, and their nests were located on the same stretch of river. Measures of body mass of both birds were taken within 30 min. of each other. Less mass was lost by the female with the additional egg, (-0.05g d^{-1} for the female with the enlarged clutch, compared with -0.22 gd^{-1} for the female with the reduced clutch). An extension of this experimental 'matched pairs' approach would be instructive.

4.3.5.4 Environmental influence on mass change.

Mean values for maximum and minimum temperature were computed for the duration of each sampling period. Temperatures ranged between -4 to 16 °C. Mass change (standardised for initial mass and stage of incubation) was not significantly correlated with either measure of temperature or with date (Pearson correlations, $p>0.5$ in all cases).

Mean, maximum and minimum flow rates were obtained for the period of measurement between initial and final capture. During 1995, flow rates decreased towards the end of March and remained remarkably constant throughout April (Figure 4.3). No spate conditions were encountered in 1995, flow rates ranging from 0.5 to 1.81 m^3s^{-1}, mean 0.63 ± 0.22 m^3s^{-1}. Within this range, there was no evidence to support a relationship between mass change (standardised for stage of incubation) and flow rate (Spearman rank correlation coefficients, $p = 0.875$, $n = 19$).

In order to extend the data set to incorporate conditions of high flow, daily mass change data for doubly labelled water females (1982 to 1994) were combined with those for the unlabelled birds in 1995. For the combined data, the Spearman rank correlation coefficient between daily rate of mass change (gd^{-1}) and flow rate (ms^{-1}) were negative, but outside significance at the 95% level, $r = -.252$, $n = 51$, $p = .075$.

4.3.6 Sources of individual variation in energy expenditure.

In the preceding sections, river flow rate, the duration of incubation sessions, behaviour during recess periods and mass change have been shown to influence energy expenditure during incubation in the Dipper. Clutch size was also shown to influence the variation in energy expenditure between individuals. In order to assess the effect of these factors in combination, a backwards multiple regression was performed, with daily energy expenditure (kJ $ind.^{-1}d^{-1}$) as the dependent variable and the five variables listed above as dependent variables. Three variables were entered as significant at the 5% level: flow rate, session duration and the proportion of recess time spent diving accounted for 44.8% of the variation in energy expenditure (Table 4.12). The combination of factors still could not account for the highest levels of energy expenditure experienced by some individuals (Figure 4.22).

Table 4.12 Backwards multiple regression of the factors influencing daily energy expenditure (kJ ind.$^{-1}$d^{-1}) in incubating Dippers. 21 birds were entered in the analysis. Factors not entered as significant were clutch size, proportion of recess time spent resting, foraging and flying and mass change (g). Flow rates (ms^{-3}) were transformed using a logarithmic transformation, the proportion of time diving with an angular transformation. Session duration in min.

Variable	B	T	p	Beta weight
Flow rate	-245.877	-2.330	.0324	-.446
Session	-2.934	-2.454	.0252	-.489
Diving	173.329	3.189	.0054	.672
Constant	370.802	4.6245	.0000	

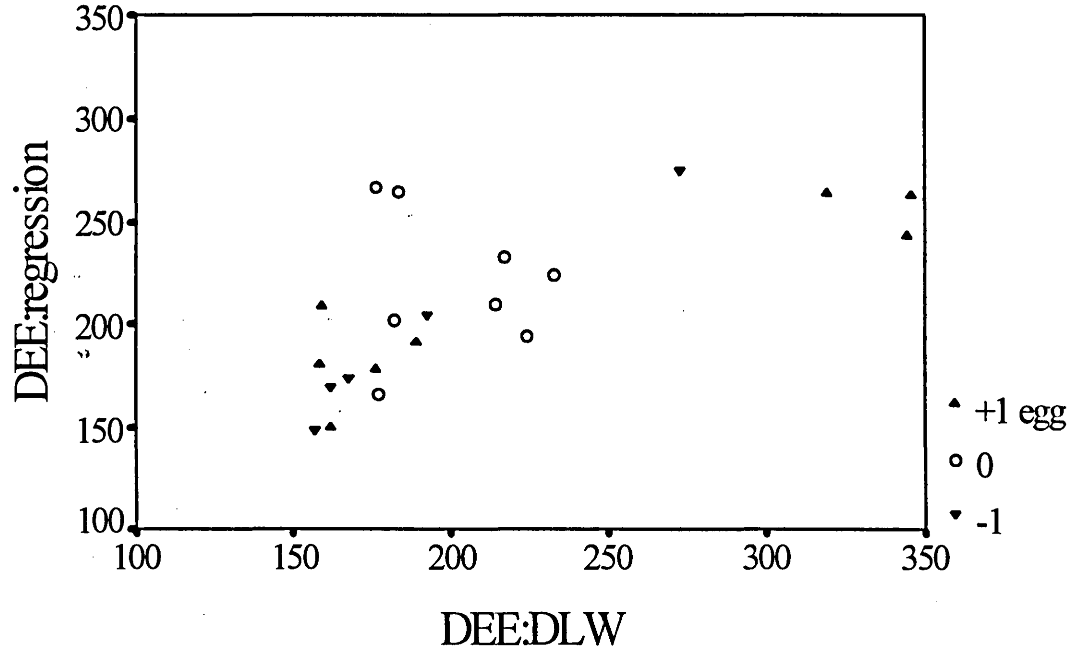


Figure 4.22 Daily energy expenditure, measured by the doubly labelled water technique and the predicted value using the multiple regression analysis in Table 4.12. Markers identify samples according to clutch manipulation. n = 21.

4.3.7 Time Activity Laboratory (T.A.L.) method of estimating energetic demands during incubation in the Dipper.

Data concerning the time activity budgets of free-living individuals, combined with laboratory measurements or estimations of the energetic costs of each component activities, can be used to provide an alternative estimation of the total energy requirements of a particular stage (Mugaas and King 1981). This method can be used for comparison with measures from the doubly labelled water technique, and to investigate the sensitivity of the energy budget to changes in certain parameters.

In order to calculate an estimate of energy expediture by free-living incubating Dippers by this method, cost components were summed according to equation (i), modified from Bryant and Tatner (1988), to give daily energy expenditure in kJ.indiv^{-1}d^{-1}:

Eqn. 4.7

$$DEE = \left\{\left[t_N\left(M_{bN} + TR_N\right)\right] + \left[t_D\left(M_{bD} + TR_D\right)\right] + \left[t_{re} \cdot M_{re} + t_{fo} \cdot M_{fo} + t_{di} \cdot M_{di} + t_{fl} \cdot Mfl\right]\right\}W + I$$

where variables were defined as follows:

t_N and t_D were night and active day lengths in hours; M_{bN} and M_{bD} were basal metabolic rates in kJg^{-1}h^{-1}. In this study, the latter were not differentiated, but were both estimated by night time basal metabolic rate, measured for female Dippers in the Stirling population between September and November, 1989-1991 (Bryant and Newton, 1994), as 58.68 Jg^{-1}h^{-1}. TR_N and TR_D were thermoregulatory components, calculated for night and day, repsectively. t and M denote time (h) and metabolism (kJg^{-1}h^{-1}) respectively, the subscripts representing activity categories: rest (re), forage (fo), dive (di), fly (fl). Thermoregulatory components were assumed to accrue to all activities save flying. Equation 4.7 omits this fact for the sake of clarity. 'W' was body mass in g. 'I' represents the costs accruing to the incubation of the eggs, and was further defined in Equation 4.8.

Eqn. 4.8

$$I = \left\{n \cdot w \cdot h \cdot b \cdot \left(t_e - t_{na}\right) \cdot i \cdot (1 - c \cdot a)\right\} + \left\{p \cdot n \cdot w \cdot h \cdot \left(t_e - t_{ecool}\right)\right\} \times 10^{-3}$$

Equation 4.8 incorporated the cost of keeping eggs warm during incubation sessions, as defined by Kendeigh (1963), and that of reheating eggs following recess time away from the nest (Kendeigh *et al.* 1977). 'I' was calculated in kJ ind^{-1}d^{-1}, 'n' was the number of eggs and 'w' their average mass in g, 'h' was the specific heat capacity of eggs, 'b' was the cooling rate of eggs (^{o}C hour^{-1} ^{o}C^{-1}), 't_e' was mean egg temperature during incubation sessions, while 't_{ecool}' was the egg temperature at the end of a typical recess period; 'i' was the interval of calculation in hours, 'c' was the proportion of this interval that the bird was incubating, 'a' was the proportion of the eggs surface in contact with the brood patch . In order to avoid replication of the cost of re-heating eggs following recess period, the first bracket of the equation was calculated for incubation sessions only, so 'i' was the number of hours spent incubating and 'c' was 1. The number of times eggs must be reheated was equal to the number of recess periods taken during the course of a day (p).

The values used in these calculations are presented in Table 4.13.

4.3.7.1 Sensitivity of the energy budget.

Variables other than those listed in Table 4.13 were assigned the mean value observed in this study, as listed in Table 4.14. The summation of cost components for this model gave a daily energy expenditure of 237.23 kJ ind^{-1}d^{-1}, which is within the range of observed values (157.13 to 346.32 kJ ind^{-1} d^{-1}) but exceeds the mean observed value for all birds (211.52 kJ ind^{-1}d^{-1}) by 25.71 kJ ind^{-1}d^{-1}, or 12%. If the costs of incubation (Kendeigh's costs) were not required in addition to those of basal metabolism and thermoregulation whilst on the nest, but were supplied, at least in part, by the metabolic heat produced as a by-product of these processes (King 1973), this TAL estimate would be likely to overestimate the true energy use during incubation. Kendeigh's costs were removed from the equation for both day and night time incubation, leaving a total DEE of 167.14kJ ind^{-1}d^{-1} for standard metabolism within the nest, re-heating eggs following recess periods and all activity off-nest. The energy use for maintaining egg temperature could be estimated as the difference between the observed mean D.E.E. for incubating Dippers, and the estimated cost of all activities except maintaining egg temperature, or (211.52 - 167.14) = 44.38 kJ ind $^{-1}$d^{-1}. This is equivalent to 63% of the value required for maintaining egg temperature using Kendeigh's (1963) formula.

Table 4.13 **Values derived from literature for use in the modelling of energetic costs of incubating Dippers.**

	Symbol	Value	Source
Basal metabolism(night)	M_{bN}	58.68 $Jg^{-1}h^{-1}$	Bryant & Newton 1994
Basal metabolism (day)	M_{bD}	58.68 $Jg^{-1}h^{-1}$	"
Thermoregulation (night)	TR_N	$45.14-1.646T_{aN}$ $Jg^{-1}h^{-1}$	Bryant *et al.* 1985
Thermoregulation (day)	TR_D	$68.5 - 2.630\ T_{aD}$ $Jg^{-1}h^{-1}$	"
Resting	M_{re}	$31.9+ TR_D$ $Jg^{-1}h^{-1}$	"
Foraging	M_{fo}	$63.8 + TR_D$ $Jg^{-1}h^{-1}$	"
Diving	M_{di}	$262.2 + TR_D$ $Jg^{-1}h^{-1}$	"
Flying	M_{fl}	386.7 $Jg^{-1}h^{-1}$	"
Specific heat capacity of eggs	h	3.3 J g^{-1} °C^{-1}	Kendeigh 1963
Mean egg temperature	t_e	34.4 °C	Ward 1992
Egg cooling rate °C min^{-1}	b	0.906 °C min^{-1}	Ward 1992

Each parameter was altered singly by the addition or subtraction of the standard deviation recorded in Table 4.14 and the effect on calculated daily energy expenditure obtained. In the case of the proportion of recess time spent in each activity, the proportion in each behaviour was varied singly, and the remainder of recess time was distributed amongst the other activity categories according to the ratios observed in the original mean time-activity budget. The results are presented in Table 4.14.

The modelled costs were particularly sensitive to changes in clutch size. As the mean natural clutch size was 4.67 ± 0.60 eggs (n = 33 labelled females), in the model, costs were calculated with 5 eggs as mean, and ranging between 4 and 6 eggs, which is just greater than the standard deviation. Each additional egg increased costs by 7% of the total daily budget.

The model was used to predict daily energy expenditure for all 35 labelled birds, using the measured values of each variable where possible, and substituting the sample mean for any missing variables. The model did not predict individual variation in energy expenditure closely, (Pearson correlation coefficient $r = 0.182$, $p = 0.297$, $n = 35$), although there was no significant difference between the results of the two techniques (paired t-test, $p = 0.201$, $n = 35$).

Figure 4.23 shows the relationship between the TAL estimate and the doubly labelled water measure of costs. Potential errors arise from over-simplifications in the model. The increase in cost concurrent with an increase in clutch size is over-estimated by the T.A.L. method. In the model, clutch cooling rates were represented by a single value, calculated as the mean cooling rate of a fresh Dipper egg with various clutches at a range of ambient temperatures (Ward 1992). In reality, the insulation provided by other eggs in the clutch will reduce clutch cooling rates (and hence reheating costs) in large clutches (Jones 1985). Thermoregulatory costs may be over-estimated in the model, as costs were calculated assuming that birds were fully exposed to the temperature of the nest air. However, an incubating bird, crouching within the nest will receive considerable insulation from the nest cavity. Hummingbirds at 0 - 4.6°C, with one-quarter of their body exposed above the nest walls were thought to avoid 60% of the heat loss from fully exposed birds (Calder 1973).

Table 4.14 **Time Activity Laboratory method of estimating the energy use by incubating Dippers. Sensitivity analysis was calculated by varying the value of each parameter listed by ± 1 s.d.. The predicted costs were compared with those calculated by the original model based on mean values of each parameter (237.23 kJ ind.$^{-1}$d^{-1}).**

Variable	Values (mean ± s.d.)	DEE	% change in costs
Mass	61.72 - 2.60	230.69	-2.8%
	61.72 + 2.60	243.77	+2.8%
Ambt.temp	5.0°C	244.51	+3.1%
	15.0°C	230.00	-3.0%
Session length day (min.)	44.6 - 10.2	240.69	+1.5%
	44.6 + 10.2	234.88	-1.0%
Recess length (min)	11.5 - 3.6	232.85	-1.8%
	11.5 +3.6	241.12	+1.6%
Session length night(min)	631 - 57.8	239.08	+0.8%
	631 + 57.8	235.43	-0.8%
Eggs (5)	4	220.87	-6.9%
	6	253.64	+6.9%
Proportion of recess time:			
Rest	.715 - .172	242.43	+2.2%
	.715 + .172	232.08	-2.2%
Forage	.126 - .115	237.63	+0.2%
	.126 + .115	236.88	-0.1%
Dive	.079 - .074	234.61	-1.1%
	.079 + .074	239.90	+1.1%
Fly	.081 - .063	233.98	-1.4%
	.081 + .063	240.52	+1.4%

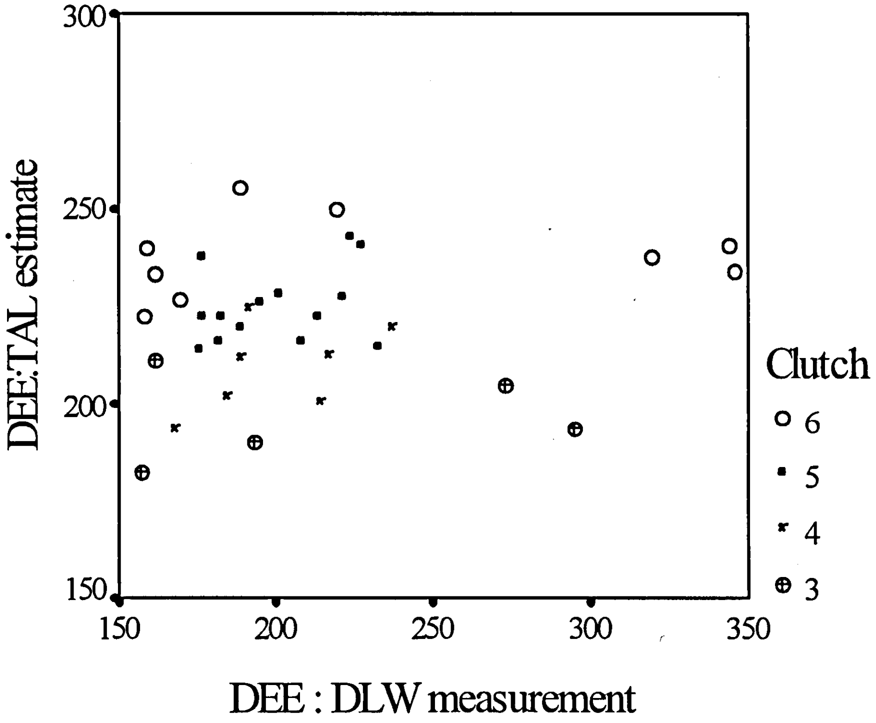


Figure 4.23 **Daily energy expenditure of incubating Dippers (kJ indiv $^{-1}$d^{-1}) calculated using the time-activity lab method against the value measured using the doubly labelled water technique Symbols represent the number of eggs in the clutch incubated.**

The model did not accurately predict the observed variation in energy budgets. There are a number of alternative explanations for this fact. Firstly, the accuracy of the data in the model may be inadequate. Data on nest attendance can be accurately measured using the nest temperature monitor, although session and recess length must be represented in the model by a single mean for each bird, at the expense of losing information concerning the variability in nest attendance. In most cases, data was collected for the entire measurement period. This source of error was not considered to be significant.

Data for time-activity budgets off-nest were likely to be less reliable, as the observed behaviour formed a relatively small proportion of the total time off-nest; the average time observed in 1993-1994 was 21 minutes, whereas an average of 15 recess periods per day, each of length 11.5 minutes would give a total of 345 minutes off-nest over the 48 hour measurement period. The reasons why time activity data of this sort may be subject to bias are discussed in Section 4.2.8 and serve to further detract from the reliability of these data.

A further possibility is that certain factors contribute significantly to the variation in energy expenditure but have not been measured. One possible source of variation that remained unmeasured is the behaviour of the bird on the nest. This includes any egg turning behaviour and consequent resettling, and also any variation in the heat supplied to the eggs. Future studies could assess the extent of this variation by use of temperature sensitive equipment within a dummy egg.

Finally, error inherent in the techniques and sampling of the doubly labelled water methodology would introduce another source of discrepancy. Validation studies have shown the mean absolute discrepancy between doubly labelled water measures of energy expenditure and other measures to be generally less than 10% (Williams 1985, Speakman and Racey 1988b). Thus, individual estimates of daily energy expenditure in this study may be assumed to be within approximately 20kJ indiv^{-1}d^{-1} of that measured by the doubly labelled water technique. Discrimination of low levels of variation between individuals is therefore limited.

4.4. Discussion.

4.4.1.Energetic demands of incubation in the Dipper.

The average metabolic rate of female Dippers incubating an unmanipulated clutch size was 5.30 cm^3 CO$_2$ g^{-1}h^{-1} ± 0.74 (n = 19), which translated to a daily energy expenditure of 209.46 kJ indiv^{-1}d^{-1} ± 28.68. Metabolic intensity, or DEE as a multiple of b.m.r., calculated according to the mass based equations of Aschoff and Pohl (1974) (MI$_{AP}$), averaged 3.33 ± 0.46 for these birds, while MI$_{BN}$, which was based on laboratory measurements of BMR of female Dippers in Central Scotland (Bryant and Newton 1994), averaged 2.39 ± 0.33. The frequency distribution of MI$_{AP}$ for birds with unmanipulated clutches is shown in Figure 4.24. Inclusion of birds whose clutch size was manipulated increased the range of the sample: ADMR; 5.41 cm^3 CO$_2$ g^{-1}h^{-1} ± 1.34, DEE: 211.52 kJ indiv^{-1}d^{-1} ± 51.25, MI$_{AP}$ 3.37 ± 0.83, MI$_{BN}$ 2.44 ± 0.61, n = 33.

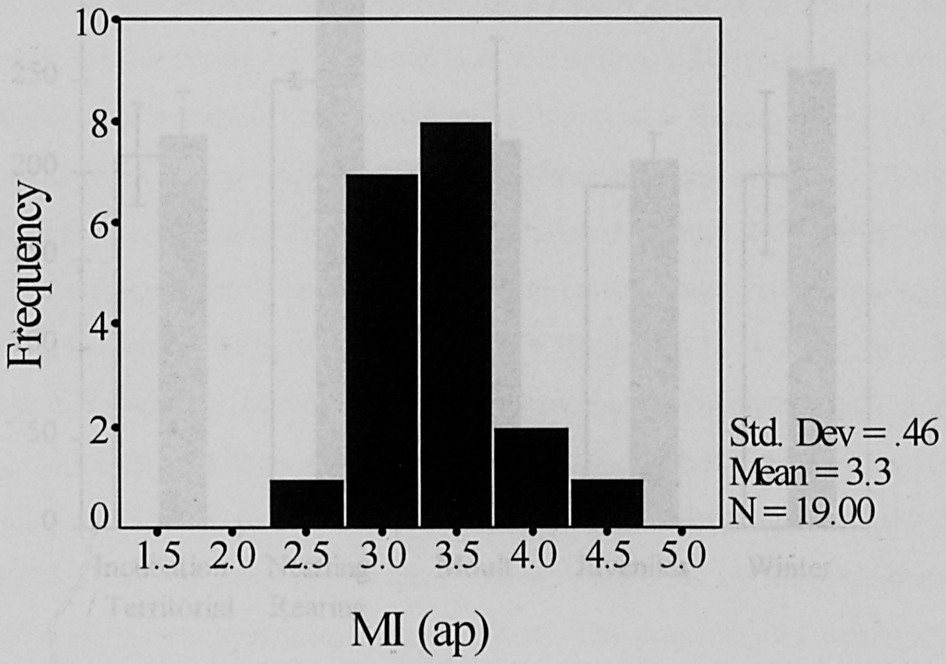


Figure 4.24 **Frequency distribution of the metabolic intensity of Dippers incubating their natural clutch size (expressed as MI_{AP}, a multiple of BMR calculated by the equations of Aschoff and Pohl (1970)).**

Bryant and Tatner (1988) applied the doubly labelled water technique to sample the daily energy expenditure by Dippers at five stages of the life cycle. Data from the present study and from Ward (1992), for birds incubating their natural clutch sizes only, were combined with the data from Bryant and Tatner (1988b) to compare energy use during incubation with other stages in the cycle (Figure 4.25).

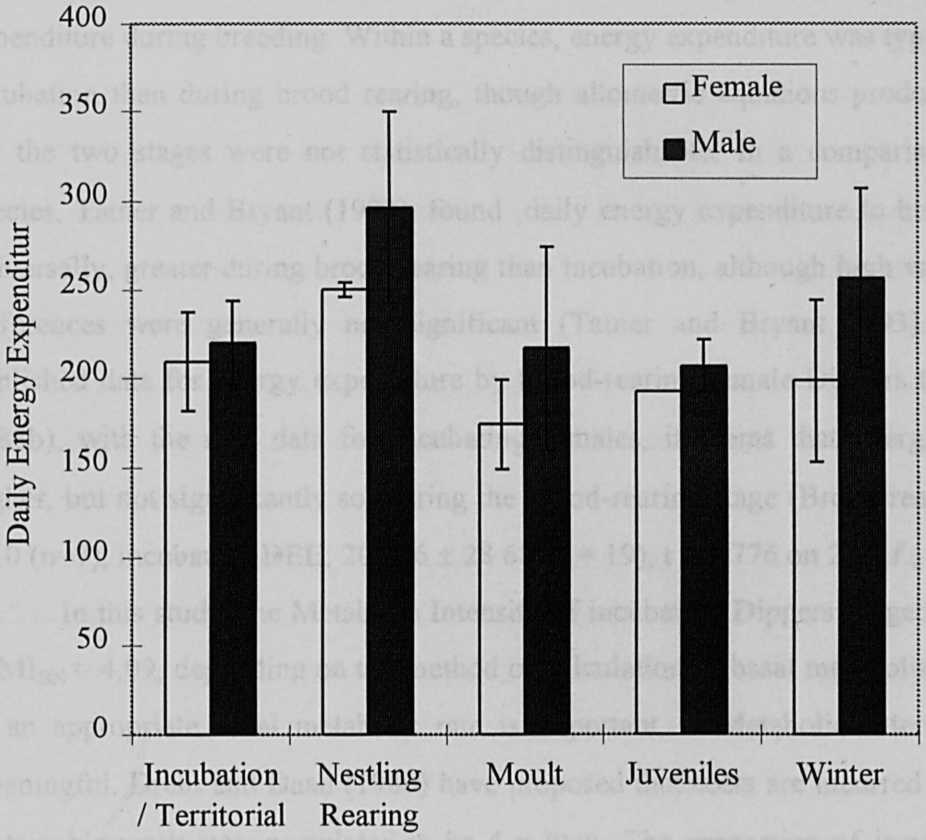


Figure 4.25 **Annual variation in daily energy expenditure (kJ indiv^{-1}d^{-1}) for male and female Dippers throughout the annual cycle. Data for incubating birds represent the results of Bryant and Tatner (1988b), Ward (1992) and the present study; for all other stages data were reproduced from Bryant and Tatner (1988). Error bars represent 1 x s.d.**

Perhaps because of the reduced activity of incubating birds (Skutch 1962), in contrast to the conspicuous activity of parents feeding nestlings, energetic studies of free-living birds have tended to concentrate on the latter stage. An implicit assumption of many studies of energy balance through breeding has been that parents work much harder in rearing the brood than during incubating. However, recent evidence questions this assumption. Weathers and

Sullivan (1989) reviewed published results of doubly labelled water studies of energy expenditure during breeding. Within a species, energy expenditure was typically lower during incubation than during brood rearing, though allometric equations produced within species for the two stages were not statistically distinguishable. In a comparison of data for 17 species, Tatner and Bryant (1993), found daily energy expenditure to be generally, but not universally, greater during brood rearing than incubation, although high variability meant the differences were generally not significant (Tatner and Bryant 1993). Comparing the published data for energy expenditure by brood-rearing female Dippers (Bryant and Tatner 1988b), with the new data for incubating females, it seems that energy expenditure was higher, but not significantly so, during the brood-rearing stage (Brood rearing DEE: 250.1 ± 58.0 ($n=7$), incubating DEE: 209.46 ± 28.68 ($n = 19$), $t = 1.776$ on 24 d.f., $p = 0.091$).

In this study, the Metabolic Intensity of incubating Dippers ranged to $MI_{AP} = 5.57$ or to $MI_{BN} = 4.09$, depending on the method of calculation of basal metabolism. The calculation of an appropriate basal metabolic rate is important, if Metabolic Intensity is biological meaningful. Drent and Daan (1980) have proposed that costs are incurred above a maximum sustainable work rate, postulated to be 4 x BMR. The proportion of incubating Dippers for which costs would be expected to occur will vary according to the estimate of BMR.

A priori, it may be anticipated that MI_{BN} measured in females within the study population should be the more appropriate measure. However, examining the results *a posteriori*, MI_{BN} may be considered less appropriate than MI_{AP}, as the lowest values of MI (1.75 x BMR) were little above the predicted metabolic rates of quiescent birds in the thermoneutral zone (1.2 to 1.8 x BMR (King 1974)). Such a basis for rejection of results is inappropriate. The validity of extrapolating either measure of BMR according to body mass will vary according to body composition. In this respect, BMR, based on measurements from lean birds, may overestimate the value for incubating birds, perhaps with high levels of metabolically inert components, such as lipid and water (Thompson 1992). Both MI_{AP} and MI_{BN} may be susceptible to this error. The best measure of BMR would ideally be made on labelled individuals themselves, ideally concurrent with the doubly labelled water study. Though such techniques are possible (Hashim 1996), they have yet to be applied successfully during incubation.

4.4.2. Individual variation in energy expenditure amongst incubating Dippers.

In this study, correlative evidence has indicated that energy expenditure during incubation in the Dipper was influenced by river flow rate, the duration of incubation sessions, patterns of activity during recess periods and mass change. The variation in energy expenditure between individuals was also found to vary with clutch size. However, none of these factors, either alone or in combination, was sufficient to explain the observed variation in energy expenditure by free-living incubating Dippers. Similarly, time-activity laboratory method of estimating energy expenditure could not explain the highest levels of energy expenditure shown by some individuals.

Amongst the majority of labelled birds, energy expenditure was normally distributed (Komologrov Smirnov test, $p > 0.8$ for all measures), but five birds experienced costs outwith the distribution. Inclusion of these birds destroyed the normality of the distribution ($.031 < p < .286$ for all measures of energy use). Whilst MI_{AP} for the most birds averaged 3.06 ± 0.40 ($n = 28$), MI_{AP} for the five birds in the 'high cost' group was 5.06 ± 0.48 ($n = 5$). Hence, there appears to be a discontinuity between the costs experienced by the majority of birds and this 'high-cost' group, for which MI_{AP} exceeded the proposed sustainable working level (Drent and Daan 1980, Weiner 1992). The inherent level of error in doubly labelled water studies is usually less than 10% (Tatner and Bryant 1989). Discrimination between the daily energy expenditure of individuals which differ by less than the precision of the technique may not be profitable. Within the low cost group, all birds' costs were within 10% of the daily energy expenditure of their nearest cost neighbour. However, the lowest DEE value in the 'high' cost group was 15% greater than the highest value in the 'low' cost group. Within the high cost group, values of DEE were separated by 8% or less. Perhaps the only safe distinction to be made between birds is therefore between these two seemingly discrete groups, defined in Table 4.15.

As correlations failed to explain the energy expenditure for these five birds, the possible reasons why costs should have escalated were examined individually for each bird. The lack of a correlation with any one variable suggests that different factors may be responsible in each case.

Table 4.15 **Description of two arbitrary groups of incubating Dippers, divided according to level of energy expenditure. High cost birds had values of MI_{AP} exceeding 4, while those in the low cost group were all working below 4 x BMR_{AP}.**

Group	Sample numbers	Mean D.E.E.
High cost	207, 208. 209, 302, 305.	315.7 ± 31.7 $_{(5)}$
Low cost	All other birds	192.9 ± 23.4 $_{(28)}$

Exceptional environmental conditions could cause an elevation in energy expenditure. High levels of precipitation during the first week of April in 1993 led to spate conditions on the River Devon, producing flow rates which peaked at 14.3 m^3s^{-1} (Figure 4.3)(compared with a monthly average of $1.92m^3s^{-1}$ for April, based on 10 years data from 1985 to 1994 inclusive). Sample number 302 in the high cost group ($M.I._{A.P.}$ = 4.7 x b.m.r.), nesting at Glensherup, was studied during the peak of these spate conditions. High flow rates are known to be associated with increasing levels of diving, to a threshold level at very high flows where diving presumably becomes inefficient (O'Halloran *et al* 1990). Birds must then either search for more shallow feeding stations, or postpone feeding until conditions ameliorate (da Prato 1981). Although continuous rain and low visibility meant that insufficient time-activity data were collected for a reliable budget to be constructed for this bird, it is likely that the spate conditions produced changes in behaviour which resulted in high costs over the measurement period. High flow rates have been previously related to high levels of energy expenditure throughout the year in the Dipper (Bryant and Tatner 1988b). This bird gained 0.4g over the 48h measurement period, perhaps suggesting an increase in reserve levels for the purpose of insurance in the face of adverse conditions.

The high costs experienced by sample 207, labelled in 1990, could be explained, at least in part, by the high proportion of recess time that this bird spent diving. This female nested over a side-burn, less than 4 metres in width and approximately 20 cm in depth. During this study, she was reported to fly a distance of some 20 metres in order to forage on the main river (Ward pers comm.), where the greater depth necessitated much diving (72% of observed recess time). The combination of flying and diving may have served to increase costs for this female in this sample. The loss of 0.85gd^{-1} by this bird was at the average level for incubating labelled birds (mean = -0.86 gd^{-1} ± 0.99) and indicates, perhaps, that this female was familiar with these conditions and was able to sustain a degree of programmed energy imbalance.

The same bird was labelled at the same site in both 1993 and 1994 (samples 303 and 312). In neither case did costs rise to this level. In both these years, the female was seen to feed to a greater degree in the side burn, within 10 metres of the nest, where the water was much shallower and slower flowing than the main river, leading to more normal patterns of activity and hence lower daily costs (176 and 162 kJ indiv^{-1}d^{-1} for 1993 and 1994 respectively).

Data from sample 305 were gathered as part of the 24h sampling procedure trial (see Methods section 2.7.). The higher level of stress associated with repeat captures within this time scale could be suspected of affecting the results from these birds. The time between release and subsequent return to the nest was 193 minutes for this sample, which falls at the higher end of the range observed for all birds (28 to 210 minutes, mean = 88.6 minutes for 17 birds). However, patterns of nest attendance, activity budgets and mass change for this sample were not exceptional.

Hence, the three remaining birds with unexplained high costs (samples 208, 209 and 305) were all females whose natural clutch of 5 egg was increased by one egg. Such an escalation of costs would be expected if a threshold clutch size exists beyond which costs escalate. If the brood patch is large enough to be in contact with no more than five eggs at one time, costs of maintaining egg temperature would increase in proportion to the additional volume to be incubated at clutch sizes below this threshold. The additional costs per egg could even decrease at larger clutches, as the insulation from other eggs exceeds that from nest air which they replace (Mertens 1977, Biebach 1984). However, when clutch size exceeded this threshold, the maintenance of egg temperature would require repeated rearrangement of the clutch in order to reheat the eggs that were not in contact with the brood patch. Costs of incubation could, therefore, escalate.

Of the 7 birds whose natural clutch of five was manipulated to six eggs, 4 birds were in the low cost group (mean DEE: 167.2 kJ indiv^{-1}d^{-1} ± 14.7) and the remaining 3 were high cost birds (DEE was over twice that for the low cost group, at 336.9 kJ indiv^{-1}d^{-1} ± 14.9).

Thus, it could not be concluded that all birds whose clutch was enlarged to 6 eggs had high costs.

Two alternative hypotheses may explain why some birds on experimental clutches of 6 experienced high costs while some did not. Firstly, the 'external factors' hypothesis postulates that both groups of birds were supplying heat to their clutches of 6 eggs, but differential female or male quality, habitat or environmental conditions meant that some birds were able to maintain low levels of energy expenditure, while for others, problems associated with maintaining energy balance (or programmed energy imbalance) were evidenced by high costs. An alternative hypothesis, the 'cost acceptance' hypothesis, suggests that only the high cost birds were accepting the cost of incubating the extra egg. Low cost birds were failing to adjust to the new clutch size; perhaps failure to turn the eggs with sufficient frequency or a failure to increase levels of heat input to the clutch would result in lower average egg temperature between all eggs. Adjustments in the incubation schedule necessary to accommodate the increase in clutch size may not have been made. The two hypotheses are discussed further in Section 6.2.5.

There was no evidence to suggest that 'external factors', either bird quality or environmental conditions, were producing the difference between the two groups. Neither age, structural size, initial body mass, egg mass nor volume differed between the two groups. Neither flow rates nor maximum or minimum temperatures differed significantly between groups (t-tests, $p > 0.2$ in all cases). All rivers were circumneutral, (pH>6.0, Ormerod *et al.* 1991), although sampling may have been insufficient to detect the existence of acidic episodes.

Under the 'cost acceptance' hypothesis, there should be some evidence of a difference in some aspect of behaviour for the high cost birds, which adjusted to the increased clutch size, while the low cost group continued as though incubating 5 eggs. There was no significant difference in the mean duration of incubation sessions between the two groups ($p = 0.191$), but the variance within the high cost group was significantly lower (Levene's test for homogeneity of variance, $p = 0.036$). Incubation sessions for the high cost group were all towards the lower end of the range exhibited by the low cost group (37.9 minutes ± $3.8_{(3)}$ for high cost group, 47.9 minutes ± $14.0_{(5)}$ for low cost group), suggesting that long sessions were not undertaken by the birds with high costs. Fasting endurance is a function of both reserve storage capacity and rate of energy use (Calder 1974). If the rate of energy use during incubation sessions increased with the acceptance of the costs of the additional egg, high cost birds may not have been able to sustain the longer sessions. However, poor conditions, such as low temperature, could also effect the rate of metabolism during incubation (Haftorn and Reinertson 1985). Lower rates of energy intake during recess periods, influenced by river flow or prey availability, would reduce the size of the nutrient reserve and hence, could also be responsible for an inability to maintain the duration of incubation sessions. Therefore, this result can not distinguish between the two hypotheses. The duration of recess periods did not differ between groups ($p = 0.834$).

Two of the three high cost birds gained mass during the measurement period, the third bird losing mass; in the low cost group, 3 birds lost mass while the fourth retained its mass. The difference between groups in this respect was not significant (Mann-Whitney U test, $z = -0.707$, $p = .480$). The increase in mass could be interpreted as an adjustment over the course of the measurement period towards a higher level of reserve storage, associated with the greater costs of incubation and the greater need for insurance. Again, this result could be interpreted as providing support for either hypothesis.

To distinguish adequately between the two hypotheses would require correlation of the energetic level of working during incubation with measures of incubation ability, such as egg temperature, hatching success and the duration of incubation.

In summary, it appears that increasing clutch size beyond the bird's natural clutch has the potential to increase field metabolic costs dramatically, by some 60%, but that this increase was not always demonstrated. The evolutionary consequences of such an increase in the variability of costs with clutch size are discussed in Section 6.2.5.

4.4.3 Sources of energy for incubating Dippers.

All individuals require an energy input equivalent to their daily energy expenditure if they are to remain in energy balance. Incubating Dippers must therefore assimilate between 157 and 346 kJ of energy per day, with the mean for unmanipulated clutches of 209.5 $kJind^{-1}d^{-1} \pm 28.7_{(19)}$. This requirement is met from a combination of food presented by the mate, utilisation of stored reserves and the remainder from self-feeding.

4.4.3.1 Reserve utilisation.

Mean daily mass change for (unlabelled) incubating Dippers was -0.16gd^{-1} ± 0.27, ranging from +0.4 to - 0.6 gd^{-1}. The maximum contribution to energy requirements would occur if the total mass loss was due to mobilisation of stored lipid. The caloric density of lipids extracted from the adipose tissue of non-migratory House Sparrows in spring was found to be 37.2kJg^{-1} (Johnstone 1970). Thus the energetic contribution from reserve depletion is probably no greater than 5.95 kJd^{-1} (or 3% of the daily budget) for most individuals, with a maximum value of 22.3 kJ d^{-1} (11%), for birds with greater mass loss.

4.4.3.2 Incubation feeding in the Dipper.

Male Dippers were not observed to feed their partners during recess periods, but were observed to visit the nest at an average frequency of 8.4 visits per day (Section 3.3.7.). Although food was observed to be presented on only 23% of these visits, it was not possible to rule out the possibility of feeding on all occasions. A maximum contribution was therefore calculated assuming that prey items were passed to the female on each occasion. In the absence of studies concerning prey items presented by the male during incubation, it was necessary to extrapolate from the studies of Dippers during nestling rearing. Although prey species' availability will differ temporally between incubation and late nestling rearing, in terms of the economies of load carrying, the incubating female can, perhaps, best be compared with nestlings aged over 7 days old. Nestlings younger than this receive a higher proportion of smaller items, perhaps due to their reduced energetic demands and restricted gape size (Ormerod 1985). Trichopterans (82.5%) form the largest part of the nestling diet by mass, followed by Ephemeroptera (14.2%) and Plecoptera (2.0%)(Ormerod 1985). An estimate of 5 prey items per feed was assumed, based on limited observation during incubation. Using the caloric equivalents of these groups (22.5kJg^{-1} dry mass, Cummins and Wuydcheck 1971), and an assimilation efficiency of 71.3% for insectivorous birds (Bryant and Bryant 1988), a mean dry mass per item of 7.78mg (Ormerod 1985), the maximum contribution from mate feeding of 5.2kJ ind^{-1}d^{-1} (or 2.5% of the daily budget) could be achieved if 5 items were successfully presented on 8.4 occasions each day. Given that some visits probably did not include feeding, and on some occasions, food was even refused by the female (Section 3.3.7.), the actual contribution from mate feeding is likely to be, in general, lower than this.

4.4.3.3 Contribution from self-feeding.

If levels of reserve utilisation, mate provisioning and energy expenditure were set at the average levels described above (DEE = 209.5 $kJind^{-1}d^{-1}$, with 5.95kJd^{-1} from endogenous reserves and 5.2 kJd^{-1} from mate feeding), an incubating female would be required to find 198.4 kJ per day (94% of the daily budget) by feeding. The foraging rate necessary to obtain this energy was calculated as follows. Assuming an assimilation efficiency of 71.3%, 278 kJ net energy would be required to provide 198.4 kJ of metabolisable energy. The time available for foraging was equal to the sum of the incubation recesses. An average of 15 recess periods, of mean length 11.5 minutes would give a daily total of 172.5 minutes off-nest. If all this available time were spent feeding, rates of energy gain of 96.70 kJh^{-1} would be necessary. Time-activity data suggest that only 36% of recess time is spent feeding (= foraging + diving, data for unlabelled birds only). The proportion of behaviour in other categories which are essential components of the feeding process, for example looking for prey, flight between feeding patches, is not known. 'Resting' between foraging bouts may be necessary because of physiological factors, such as gut constraints (Kenward and Sibly 1977, Diamond *et al.* 1986, Krebs and Harvey 1986). The true rate of energy intake required must lie between 96.70kJ h^{-1} if all recess time were spent foraging, and 269kJ h^{-1} if only the observed 36% of recess time were devoted to foraging.

Allometric equations have been produced to describe the relationship between foraging rate (kJh^{-1}) and body mass (g) (Bryant and Westerterp 1980). Using this equation, the predicted value for a passerine of body mass equal to that of an incubating female Dipper of 61.73g is 44.35kJh^{-1}. This equation is based on observed foraging rates, which incorporate feeding activity and some element of search time. The most appropriate comparison is, therefore, made with the conservative estimate of required intake rates in the incubating Dipper (96.7 kJ h^{-1}). The required intake rate during incubation is therefore 2.18 x that observed at other stages in the cycle.

Calculations using a similar methodology for all stages of the annual cycle in Dippers (Bryant and Tatner 1988b), but excluding any contribution from mate feeding or reserve utilisation, showed that required rates of energy gain for female Dippers during incubation (263.6 kJ h^{-1}) must exceed those calculated for females at any other stage by at least a factor of 7.

An attempt to quantify the rate of energy gain by observation was made at four sites where catches of medium to large prey (defined by Yoerg (1994) as 'as long as or longer than the bill') could be recorded individually. An average value of 4.2 catches per minute was recorded. Yoerg (1994) reported capture rates of 14.5 medium and 7.3 large items per minute, for adults foraging for nestlings. The tentative conclusion is that Dipper capture rates during nestling rearing exceed those during incubation. This could be due to an increase in effort or an increase in prey availability later in the season. Rates during incubation probably exceed those for brood-rearing adults foraging for themselves, where captures of 1 medium and 0.4 large prey per minute were observed (Yoerg 1994).

Using values of mean prey size and calorific equivalents outlined above, a crude estimation of rates of energy gain can be made. The calculated rate of energy gain, 44.11 kJh^{-1}, probably underestimates the true rate of intake, because captures of small items and crumbs were not recorded. Yoerg (1994) estimates that 78% of the adults' diet is composed of small items and crumbs. If this were the case with incubating birds, the net energy intake rate (100/22 x 44.11 kJh^{-1} = 200.5kJh^{-1}) would be sufficient to meet the remainder of the energy budget during incubation.

4.4.3.4 Food availability during incubation.

Foraging rates will be determined largely by prey availability. The temporal abundance of prey available to Dippers on the River Devon was studied in 1983-84 (Bryant and Gardiner, unpublished). Invertebrate abundance was sampled both on the river bed and from the bank-side vegetation (Figure 4.26). There is evidence that bank-side invertebrates are sometimes taken by Dippers, though plant gleaning and fly catching behaviours are rare. It is thought that some invertebrates become available to Dippers by falling into the water or landing on rocks in the river (Tyler and Ormerod 1994).

The availability of aquatic invertebrates rapidly increased from January to April, reaching a peak in May. The subsequent decline in aquatic invertebrates sampled from the river was off-set in part by the increase in emerging adults available at the bank-side. In this season, 1984, the earliest eggs were laid on the 6th March, the modal date of first egg being 8th April (Logie pers comm.). The earliest birds began to incubate on 10th March, but the modal date for initiation of incubation was 20th April. In this year, egg laying and incubation occurred either in the period of rapidly increasing, or the peak aquatic food availability. The

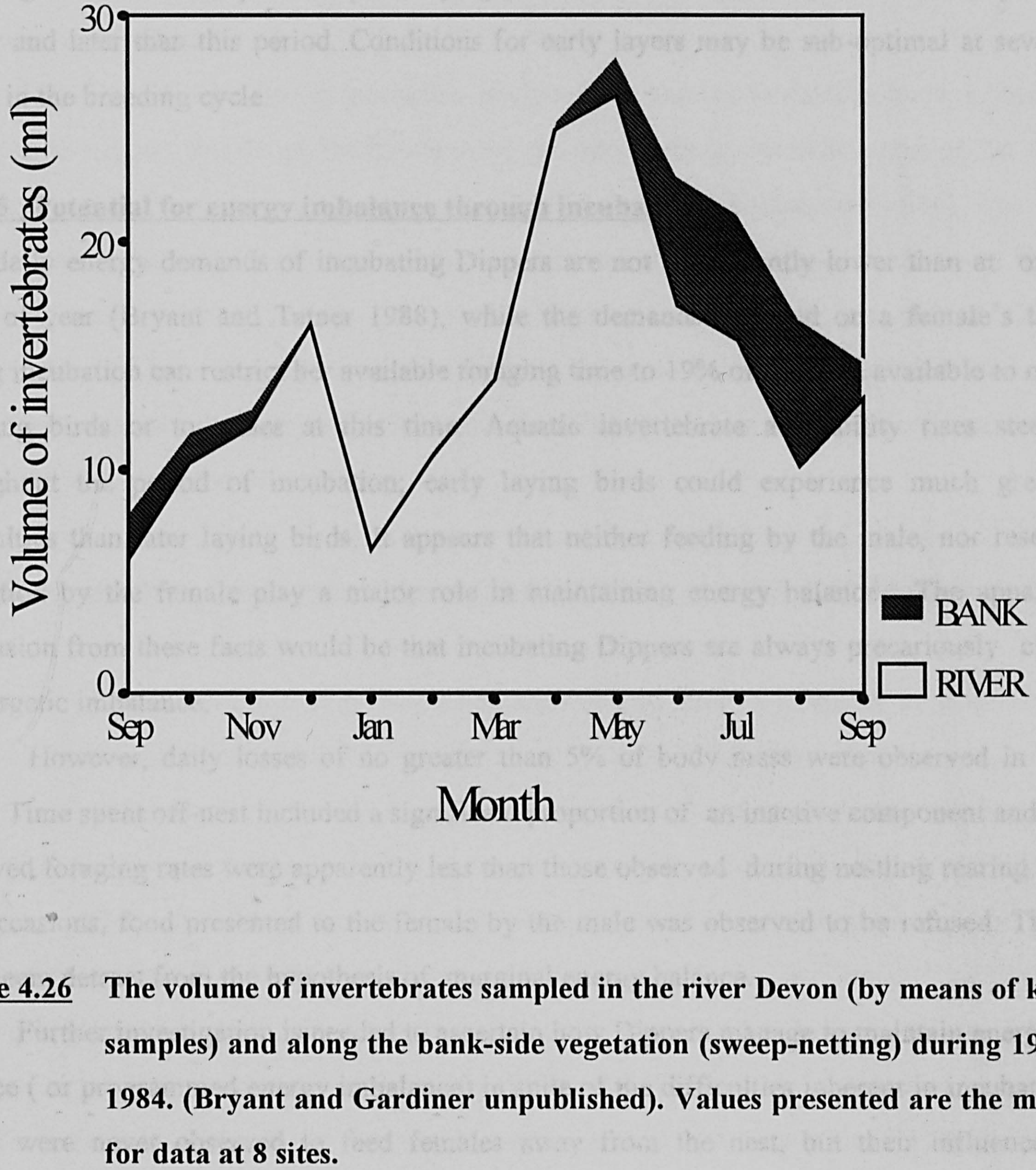


Figure 4.26 **The volume of invertebrates sampled in the river Devon (by means of kick samples) and along the bank-side vegetation (sweep-netting) during 1983-1984. (Bryant and Gardiner unpublished). Values presented are the mean for data at 8 sites.**

predictions of Lack (1954) would lead to the assumption that nestling rearing be coincident with the time of maximal food availability. In this year, at least, for the majority of Dippers this would hold true.

Ormerod and Tyler (1993) have shown that post-fledging survival is greatest for the offspring of birds that lay in the peak laying period and is reduced for those that lay both earlier and later than this period. Conditions for early layers may be sub-optimal at several stages in the breeding cycle.

4.4.3.5 Potential for energy imbalance through incubation.

The daily energy demands of incubating Dippers are not significantly lower than at other times of year (Bryant and Tatner 1988), while the demands imposed on a female's time during incubation can restrict her available foraging time to 19% of the time available to non-breeding birds or to males at this time. Aquatic invertebrate availability rises steeply throughout the period of incubation; early laying birds could experience much greater difficulties than later laying birds. It appears that neither feeding by the male, nor reserve utilisation by the female play a major role in maintaining energy balance. The apparent conclusion from these facts would be that incubating Dippers are always precariously close to energetic imbalance.

However, daily losses of no greater than 5% of body mass were observed in this study. Time spent off-nest included a significant proportion of an inactive component and the observed foraging rates were apparently less than those observed during nestling rearing. On two occasions, food presented to the female by the male was observed to be refused. These facts seem detract from the hypothesis of marginal energy balance.

Further investigation is needed to ascertain how Dippers manage to maintain energetic balance (or programmed energy imbalance) in spite of the difficulties inherent in incubation. Males were never observed to feed females away from the nest, but their influence in guarding the female during feeding may serve to allow greater rates of intake by reducing the time required to be alert for predators. The male could play a role in guiding the female towards good foraging sites, hence reducing search time for prey. The male's function in maintaining a good territory, with suitable foraging sites within a short distance from the nest may be of considerable importance to success during incubation. The partitioning of the territory between the sexes during incubation has been reported in the Spotted Flycatcher

(Davies 1977) and has been proposed to minimise the female's energy expenditure in travel time between nest and feeding station. In the Amazon Kingfisher, Davis and Graham (1991) suggested that partitioning of the breeding territory between the sexes increased the total yield from the territory, as a significant recovery time exists following a dive during which time prey are unavailable. Such territory partitioning could well be operating to advantage during incubation in the Dipper.

The Dipper's diet during incubation has not been studied in detail, although there is evidence to suggest that large Trichopteran larvae may form a significant part of the diet, declining in importance through nestling rearing (Efteland and Kyllingstad 1984). The size and energetic content of prey items available during incubation could have a significant effect on the potential rate of energy intake. Investigation of these factors should enable understanding of how Dippers manage to achieve energetic balance during incubation, and hence their vulnerability to biotic or environmental changes at this time (e.g. removal of the mate, acidification of the watercourse).

4.4.4 Costs and benefits of reserve storage and utilisation in incubating Dippers.

The mass of laying and incubating Dippers exceeds that of females in any other stage in the life cycle (Galbraith and Broadley 1980, Bryant and Tatner 1988). This high mass is due, in part to hypertrophy of the reproductive organs, but evidence suggests that birds also increase their lipid reserves and the mass of other visceral organs before laying in order to provide the machinery and the fuel to support laying (Breitenbach and Meyer 1959). In common with many other species (Ricklefs 1974, Moreno 1989b), female mass declines by the time of nestling rearing. Mass loss during breeding has been interpreted as being indicative of the energetic drain associated with reproduction (Ricklefs 1974), but the timing and pattern of mass loss does not appear to be completely consistent with this view (Freed 1981, Gaston and Jones 1989, Jones 1994, Sanz and Moreno 1995). Mass loss during incubation has been shown to persist despite provision of supplementary food (Sherry *et al.* 1980, Hillstrom 1995).

Mass loss during breeding could have adaptive value (Freed 1981). Lowering body mass during nestling rearing could make considerable energetic savings by lowering the power requirements for flight (Freed 1981, Norberg 1981, Croll *et al.* 1991). Animals are known to exhibit programmed anorexia (or a reduction in appetite) under certain circumstances (Mrosovsky and Sherry 1980), by altering the balance between mass loss and motivation to feed. The programmed anorexia hypothesis (Gaston and Jones 1989) suggests that mass loss during breeding occurs through the regulated depletion of reserves.

The regulation of body mass through incubation has been interpreted as the results of the transition between high mass during egg laying and lower mass at nestling rearing (Moreno 1989b). Moreno (1989b) suggested that species may be grouped into two categories according to the pattern of female body mass during incubation: IMC = 'incubatory mass constancy' species maintain a constant body mass through incubation, then lose mass rapidly at hatch, while IML = 'incubatory mass loss' species undergo a progressive depletion of reserves throughout the incubation period. IML is usually associated with larger birds, commonly those species with uniparental incubation and high levels of nest attentiveness, and is generally demonstrated by precocial species. IMC is more commonly found in smaller, altricial species. Partners may aid by feeding the incubating parent or by taking their share in incubation, or levels of uniparental nest attentiveness are reduced.

The Dipper loses an average of 0.25% of its initial body mass daily over 16 days of incubation, or 4% of body mass throughout the incubation period. IMC species can lose up to 30% of their body mass through incubation (Moreno 1989b). The weights of female Dippers at the end of incubation (mean = 60.6g, minimum = 59.1g) are greater than those observed during nestling rearing (57.2g (Bryant and Tatner 1988)), suggesting that females also lose mass at the time of hatch. Dippers therefore, share features in common with both I.M.C. and I.M.L. species, exhibiting a limited programmed depletion of mass throughout incubation and undergoing rapid mass loss at the time of hatch.

The variation in an individual's body mass, or the level of reserves it carries, reflects the balance of both the costs and benefits associated with the reserve (Witter and Cuthill 1993). Costs and benefits will vary for each stage in the life cycle, so it is likely that the resulting optimal mass will do the same. For incubating birds, both the current balance of costs and benefits and the role as a transition between high mass at egg laying and the low mass at nestling rearing will be important.

The main benefit of fat storage is the possession of an energy store. For incubating birds, this store increases fasting endurance time (Section 5.4.6.4, Calder 1974, William 1992) and thus enables longer periods of uninterrupted incubation. Longer session lengths enable a higher overall degree of nest attentiveness and are associated with lower daily costs, as re-heating costs are reduced (Chapter 5).

Subcutaneous fat deposits may also have benefits in providing insulation and subsequently reducing thermoregulatory demands (Blem 1990). Although incubating Dippers receive a degree of insulation from the nest, nest air temperatures remain below the thermoneutral zone, so additional benefits may be gained from the insulative properties of fat. The reduction in activity during incubation means that the contribution of metabolic heat to thermoregulation is limited at this time. The advantage of insulation to an incubating bird may outweigh any disadvantage manifest in the restricted dissipation of heat during flight. Time budgets show that 87% of each 24 hour period is spent trying to maintain heat balance on the nest and only 1.5% is spent in flight.

A third advantage of fat storage is as insurance against poor conditions. The amount of fat stored should reflect the level of risk of encountering situations where sufficient food can not be obtained to balance the energy budget (M^{c}Namara and Houston 1990). Incubating birds, with restricted time in which to forage, increase their risk of failure to satisfy demands. Birds are known to increase mass when time constraints limit feeding (Kacelnik 1979, Bednekoff 1992). The Dipper's habitat incorporates an additional element of unpredictability, on top of the risk of low temperatures to which most birds are susceptible. High river flows can decrease the returns from foraging and diving to such an extent that feeding may be temporarily suspended under spate conditions (da Prato 1981). The risk of a flood is high during March and April and declines rapidly thereafter (Figure 4.3). The possession of a fat store could, therefore, enable a bird to continue incubation through conditions that would otherwise lead to nest abandonment. This type of response will be subject to strong selection.

The disadvantages of fat storage are mainly related to an increase in metabolic costs associated with a greater mass. The cost of flight increases as an accelerating function of mass, and foraging may become less efficient as mass increases in some species (Witter and Cuthill 1993). The relationship between the cost of diving in the Dipper and body mass has yet to be investigated. During nestling rearing, females can spend up to 5% of each 24 h period flying (Bryant and Tatner 1988), and a further 55% feeding. During incubation, when flight constitutes only 1.5% and feeding 4.6% of each 24h period, the increased energetic costs of these activities is of reduced significance. Any impairment in flight performance and hence ability to escape predation (Witter *et al.* 1994) may be of reduced importance to a bird spending 87% of its time within the confines of the nest. However, an impairment in take-off velocity (Witter *et al.* 1994) could be significant to incubating birds attempting to escape predators at the nest.

In summary, the balance of costs and benefits of mass during incubation in Dippers seems to be in favour of maintenance of fat stores at this time. The decline in reserves observed throughout the course of incubation may result from the adoption of some degree of reserve utilisation, enabling the adoption of an incubation schedule that may be sustained only by programmed energy imbalance. Alternatively, the gradual loss of mass may reflect the decrease in the need for insurance as incubation progresses. Throughout the season, conditions are progressively ameliorating: temperatures are increasing steadily and daylength increases rapidly at this time of year. Food availability also escalates towards its peak (Figure 4.26), while the risk of flood conditions decreases (Figure 4.3). This would lead to a predicted decline in mass throughout the season, irrespective of the stage of breeding. The need for insurance may also be related to the progress of incubation. The risk of encountering conditions severe enough to interrupt incubation will be proportional to the amount of time when birds are vulnerable, or the length of incubation remaining. As the number of days to hatch decreases, so the risk of interruption is lessened and the need for insurance declines. The combination of these factors could alter the balance of costs and benefits of fat storage in favour of a lower optimal mass towards the end of incubation.

Sherry *et al.* (1980) have demonstrated how programmed loss of mass during incubation is regulated in Burmese Red Junglefowl (*Gallus gallus spadiceus*) in the face of unexpected conditions. When birds were deprived of food for 6 days in the early stages of incubation, they lost mass at a greater rate than control birds, fed *ad libitum* throughout. On resumption of feeding, the experimental birds were observed to increase their food intake until their weight exceeded that of the controls, from which point they resumed their programmed loss of mass at a similar rate to controls. This response is consistent with the 'insurance hypothesis', their perception of the increased risk in obtaining food being associated with their increase in insurance stores. When the same manipulation was carried out at a later stage of incubation, intake rates again increased on resumption of feeding, but mass levels were then maintained, for the final few days of incubation, at a level corresponding to that shown at hatching in controls (see Section 6.1.3.2). The difference in

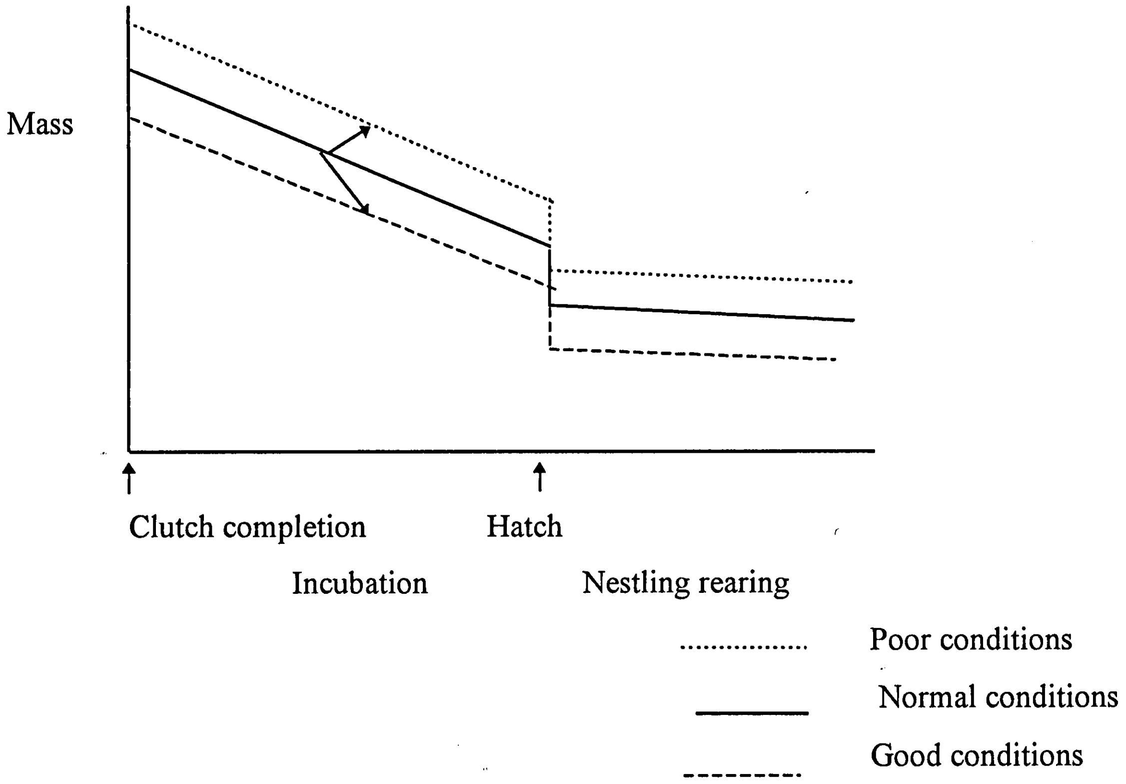


Figure 4.27 Schematic representation of body mass of female Dippers during incubation. Under normal conditions, mass declines slightly through incubation to reflect the systematic depletion of fat deposits, the reduced insurance necessary as the season progresses and the length of time to hatch decreases. In a poor year, reserves are maintained at a higher level throughout, while a lower fat store is necessary in a good year. The arrows indicate the change in reserves over a two day period as birds adjust to poor conditions or increased clutch size (arrow up), or to amelioration of conditions or a reduction in clutch size (arrow down).

response of the two treatments is again consistent with the greater value attached to insurance reserves earlier in incubation.

When incubating Dippers are faced with a deterioration in conditions, be this a decline in temperature, increase in flow rates or acceptance of the additional costs associated with incubation of an extra egg, they may respond by striving to guard against desertion by increasing feeding behaviour (and hence energy expenditure) until reserves may be stored. In this study, Dippers which gained mass showed a tendency towards spending more time feeding (Figure 4.13) and an associated increase in daily energy expenditure (Figure 4.20, see also Bryant and Tatner 1991).

There was some evidence that an increase in clutch size led to an immediate reduction in mass, possibly resulting from an inability to maintain energy balance, followed by an adjustment period during which birds with enlarged clutches gained more mass than control birds. Incubating Goldeneye demonstrated the ability to adjust the rate of reserve depletion on a daily basis: daily mass change tended to be opposite to the change on the preceding day, i.e. birds that lost mass one day compensated by maintaining or even gaining mass during the following day (Mallory and Weatherhead 1993).

Thus, the pattern of body mass change through incubation may result from a combination of programmed anorexia, occasional increased mass loss due to reproductive stress and strategies to increase an insurance reserve in the face of adverse conditions, where this is possible. Figure 4.27 illustrates this hypothesis.

4.4.5.Could energetic costs impose an upper limit on clutch size?

In accordance with Lack's (1947, 1948) hypothesis, the most common brood size found on circumneutral streams in the Dipper has been shown to be the most productive, in terms of numbers of surviving young (Ormerod and Tyler 1993). What factors are responsible for the proximate constraint of clutch and brood size to this optimal level?

Because of their potential to constrain reproductive output, the costs associated with increasing clutch and brood size have been the subject of many studies. The majority of studies dealing with clutch size have investigated changes in nesting parameters, such as duration of incubation, hatching success or number of young fledged in relation to manipulation of the clutch (Székély 1994, Sìikamaki 1995). Studies of the energetic changes associated with clutch size were, formerly, restricted to measuring the steady state costs of periods of continuous incubation, i.e. the energy required to maintain the temperature of the clutch (Biebach 1981, 1984, Haftorn and Reinertsen 1985). Until the advent of the doubly labelled water technique, measurements of the daily metabolic costs associated with incubation and all its associated behaviours had to be made indirectly through studies of food intake (ElWailly 1966, Colemann and Whitall 1988), thus normally excluding free-living birds.

The form of the relationship between the energetic costs of incubation and clutch size has been studied by means of biophysical modelling (Mertens 1977) and by open-flow gas analysis (Biebach 1981, Biebach 1984, Haftorn and Reinertsen 1984). These studies showed that the energetic cost of incubating the clutch increases with clutch size.

The energy required to maintain the temperature of a certain mass increases in proportion to that mass. Therefore, if costs were solely related to maintaining the temperature of a certain clutch mass, the energy required would increase in a linear fashion with clutch size. However, heat loss from a single egg to the other eggs in a large clutch is less than that to nest air in a small clutch, so the incremental cost of incubating an extra egg at large clutch sizes should be lower (Mertens 1977). On the other hand, an incubating bird may not be able to cover a large clutch with the brood patch, so an extra egg at large clutch sizes could increase the incremental costs by necessitating repeated rearrangement of the clutch and re-heating of cooled eggs. Biebach (1984) found a linear increase in costs of 3-5% with each additional egg in incubating Starlings and suggested that this relationship resulted from the combination of the two processes.

Because of the difficulties of measuring fluctuating levels of metabolism, the nest box has only been used as a chamber calorimeter to estimate the costs of the steady-state metabolism that occurs within the nest. Studies of field metabolic rate give an integration of the costs of incubation and associated behaviours as they occur in the wild. When assessing the potential for an energetic constraint during incubation, it is the bird's ability to satisfy the summation of all cost components that is important, so doubly labelled water studies provide a unique insight into the energetic state of the birds.

Accepting that the energetic cost of keeping eggs warm does increase in a linear fashion, does it follow that an increase in field metabolic rate during incubation will be associated with an increased clutch size? If costs of maintaining egg temperature increases by only 4% and these costs comprise approximately 30% of total daily budget (from T.A.L. modelling), such an increase could go undetected in the inherent variability of values of daily energy expenditure, if costs of associated behaviours do not increase concurrently. Alternatively, an increase in the cost of maintaining egg temperature, combined with increased clutch re-heating costs could be magnified by the need to raise feeding costs in order to satisfy demands.

Daily energy expenditure during incubation has been measured in relation to clutch size in three passerine species, the Dipper (Ward 1992, this study), the Pied Flycatcher (Moreno and Carlson 1989, Moreno and Sanz 1994) and the Collared Flycatcher (Moreno *et al* 1991). In each case, the effect of an increase in clutch size has been to increase the variation in field metabolic rate (see Section 6.2.5), as costs escalated for some birds with enlarged clutches, but did not for others. If reproductive costs are associated with high levels of energy expenditure (Drent and Daan 1980), birds with large clutches will be subject to a greater probability of incurring a cost than those with a more conservative clutch size. Such a mechanism could restrain the evolution of large clutches.

The reasons why some birds incur high costs at large clutches while others do not should provide a focus for future research. In the Collared Flycatcher, Moreno *et al.* (1991) found some evidence that birds laying larger clutch sizes would not accept the additional energetic demands of an increased clutch size, providing some support for the 'threshold hypothesis', that costs escalate because of the additional turning and re-heating necessary when clutch size exceeds brood patch capacity. However, the variation amongst energy use in Dippers incubating clutches of 5 + 1 eggs provides evidence against the threshold hypothesis, unless the threshold is variable amongst individuals. Study of the variation in energy use during incubation, and in incubation ability between individuals is necessary to establish the degree to which energy expenditure represents a reproductive cost during incubation.

Chapter 5:

Energetic constraints on incubation in the Great Tit.

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Energetic constraints on incubation in the Great Tit.

5.1 Introduction.

In species such as the Great Tit, incubation is undertaken by one parent alone, so the incubating parent must balance the requirements of maintaining egg temperature with the need to meet her own nutritional demands. Although the energy demands of incubation have been the subject of some controversy, recent evidence suggests that incubation is not a period of reduced energy demands (Weathers and Sullivan 1989, Tatner and Bryant 1993), as was originally proposed (Calder 1973, Smith *et al.* 1974, Walsberg and King 1978a, b). High levels of nest attendance (Skutch 1962) require that rates of energy intake during periods off the nest are several times higher than those necessary to achieve energy balance at other times of the year (Walsberg and King 1978a, Williams 1992). Breeding is often timed such that parents exploit any peak in food availability in order to supply the demands of chicks in the nest (Lack 1954, Nager and van Noordwijk 1995). Incubation must precede this, and so, in first broods at least, incubation may be undertaken when food availability is sub-optimal .

Selection pressure may favour those individuals which are able to minimise the duration of incubation. Whilst incubating, both parent and eggs may be vulnerable to predation (Clark and Wilson 1981). There are also advantages in producing fledglings early in the season (Perrins 1965). Strategies are therefore likely to develop whereby females invest heavily in incubation, such that the development of the embryos proceeds at the maximum rate which is physiologically possible. For gynelateral incubators, therefore, incubation may well pose a serious challenge in maintaining positive energy balance (Yom Tov and Hilborn 1981).

Small birds may be especially prone to energetic stress, as they are unable to store large metabolic reserves. If birds are constrained in their application to incubation by the availability of energy, incubation may constitute an 'energetic bottleneck' in the reproductive process (Yom Tov and Hilborn 1981).

Several hypotheses have been proposed to explain the evolution of avian clutch size (Chapter 1). The 'incubation ability' hypothesis (Lack 1954) suggests that reproductive output is constrained by the number of eggs which a bird can successfully incubate. Attempts to evaluate the hypothesis have concentrated on the success of incubation in relation to clutch size, either in natural clutches (reviewed by Klomp 1970) or in those in which clutch size was experimentally manipulated (Moreno 1989a, Smith 1989, Moreno *et al.* 1991, Székély *et al.* 1994, Siikamaki 1995). Chapters 3 and 4 describe experiments in which reproductive effort was manipulated, via alteration of clutch size, in Swallows and in Dippers, and the effect on incubation ability assessed in terms of energy use, female condition and short term reproductive success.

Indirect manipulation of reproductive effort can provide information on the factors which constrain the reproductive process. For example, by manipulation of energy availability, by the provision of supplementary food during the pre-laying period, the time of laying has been advanced (Kjällander 1974, Yom Tov 1974, Nilsson 1994,), and/or clutch size has been increased (Högstedt 1980, Dijkstra *et al.* 1982, Sanz and Moreno 1995, Soler and Soler 1996), indicating the proximate influence of food availability in decisions concerning laying (Martin 1987). In this study, the availability of energy to incubating Great Tits was experimentally increased by an alternative method, in order to ascertain whether incubating birds were energetically constrained during incubation.

5.2 Aims.

The aim of this study was to examine the hypothesis that female Great Tits are constrained in their application to incubation by the energy available to fuel this activity, the 'energetic limitation hypothesis'. To this end, the experimental technique was designed to manipulate the supply of a potentially limiting resource (energy) and to monitor any response which could reflect incubation ability.

Manipulation of the energy budget can be achieved either by increasing the inputs to the system, or by reducing demands which compete for energy. The provision of supplementary food is one method of increasing the energy availability through incubation (Nilsson and Smith 1988, Moreno 1989a, Smith *et al.* 1989, Hillström 1995, Merkle and Barclay 1996). However, current food availability may be an important variable on which birds base their predictions of the availability and temporal distribution of future food resources, (Nilsson 1994, Sanz and Moreno 1995). It may be difficult to separate the behavioural responses arising from an adjustment in the prediction of future conditions, from the proximate effects of energy supplementation.

Manipulation of thermoregulatory demands provides an alternative method of directly influencing the energetic budget of the bird. This can be achieved, in hole nesting species, by modification of nest air temperature (Nager and van Noordwijk 1992, Yom Tov and Wright 1993). As the machinery of heat production is common to both the control of body temperature and the provision of warmth to eggs, pathways are likely to exist whereby energy savings from thermoregulation could be diverted towards incubation (Hammond *et al.* 1994).

It is conceivable that current temperature may also be used by birds to anticipate future conditions (Nager and van Noordwijk 1995). In this experiment, therefore, the nest microclimate was manipulated during the hours of darkness only. Variables such as day-time temperature, day-length and current prey availability were not manipulated. It was thought that birds were unlikely to alter reproductive decisions on the basis of nocturnal nest temperature alone, in the presence of conflicting information from these other variables.

In this study, incubating birds were treated to a thermoregulatory saving by warming the nest box overnight and their behavioural and energetic response was then compared to that of a control group of birds, incubating under comparable conditions. Under the energetic-limitation hypothesis, birds would respond to the removal of an energetic constraint by increasing their investment in incubation. Alternatively, if birds are not energetically constrained, the rate of incubation perhaps being determined by the time period between achievable laying dates and optimal hatch dates, or the nest attendance schedule being determined by an inherent, inflexible programme, incubation should proceed in the same manner as for control birds and the energy budget should be reduced by the amount of the thermoregulatory saving.

There are two possible ways in which a female Great Tit could, potentially, increase the rate of incubation. Optimal embryonic development occurs when eggs are maintained between approximately 35 and 37°C (Drent 1972a). Time to hatch can be reduced if eggs are maintained within this range for as long as possible (Haftorn 1983). Therefore, females may increase the control of egg temperature whilst on the nest, either by varying the contact between eggs and brood patch (White and Kinney 1974), or by varying the heat flow and blood flow through the brood patch (Haftorn and Reinertsen 1982). However, during continuous nocturnal incubation in the Great Tit, mean egg temperatures of 36 °C are generally achieved (Haftorn 1983). Thus, it seems that, during continuous incubation, birds are generally able to maintain egg temperature within the optimal range for development.

A second approach would be the modification of the nest attendance schedule. During the active day, female Great Tits must leave the nest repeatedly in order to forage. Egg temperature may drop below development temperature whenever the female leaves the nest (Haftorn 1983). A reduction in the amount of time spent off-nest could increase the average daytime egg temperature, increasing the overall rate of embryonic development and so shortening the time to hatch.

The latter response was monitored by recording incubation schedules, or time spent on and off the nest each day. The prediction of the 'energetic-limitation' hypotheses was that, having spent a night in a warmed nest, birds would devote a greater amount of time to incubation than control birds during the following day.

The response to the treatment was also examined by measuring total energy use by birds in heated and control nests, by the application of the doubly labelled water technique. Under the energetic limitation hypothesis, it was predicted that the energy saved from thermoregulation would be re-invested to incubation, so no difference between the total daily energy expenditure of birds in the control and heated groups would be apparent. This prediction was tested by the use of the doubly labelled water technique to measure total daily energy expenditure for incubating birds in the each of the two treatment groups.

5.3 Methods.

5.3.1 Study species and sites.

The Great Tit (*Parus major*) is a hole nesting passerine, which readily accepts artificial nest boxes, and thus is amenable to manipulation of the nest microclimate. A detailed account of the breeding biology of this study population is given by Hashim (1996). Breeding territories are established by early spring (Cramp 1988). In this population, nest building generally commences in mid- to late-March (Hashim 1996). Clutch size may vary between 3 and 18 eggs (Cramp 1988), but ranges from 3 to 10 eggs in this study population, with a modal clutch of 6 or 7 eggs (Hashim 1996). In common with most other passerines, eggs are generally laid daily (Gibb 1950), though occasional interruptions in the laying sequence of between 1 and 3 days occur (Dhondt *et al.* 1983), often associated with periods of inclement weather (Yom Tov and Wright 1993). The male begins to provision his partner with food around the time that the first egg is laid and continues until the eggs hatch (Royama 1966).

Incubation often begins gradually, from 3 days after the laying of the first egg, though it may be suspended until several days after clutch completion (Haftorn 1981a,b). The period from clutch completion to hatch averaged 13 days in this population (n = 75 nests over 3 years of study (Hashim 1996)). The hatch is generally asynchronous, varying according to the amount of incubation undertaken before clutch completion; the average hatching period in the South of England was recorded as 3.0 days (Cramp 1988).

Incubation is undertaken by the female alone, and though she is, generally, fed by the male throughout incubation, energy requirements must be supplemented by self-feeding off the nest. The female alone broods the newly hatched young, but both parents feed the nestlings, who remain in the nest for 16-22 days from hatch to fledging (Cramp 1988).

The study was conducted in Central Scotland, with Great Tits nesting in approximately 100 boxes situated in 160 ha around the campus of Stirling University (NS9086) (Figure 5.1). The site comprises open areas of parkland, buildings and a loch, and two areas of mixed woodland, consisiting mainly of mature Sycamore (*Acer pseudoplatanus*) with some Oak (*Quercus petraea*), Ash (*Fraxinus excelsior*) and Spruce (*Picea norvegicus*). Rhodendron (*Rhodedendron ponticum*) forms dense cover in some areas. The larger woodland block, Hermitage Wood, is situated on a steep south-facing slope (Plate 2), while the Abbey Craig woodland in the south of the study site, covers the north-easterly slopes of a rocky outcrop. The nest box population was established in 1989 (Johnson 1990) and damaged or stolen boxes were replaced prior to each breeding season.

Nest boxes were visited at intervals of five days from mid-April onwards, and the progress of nest construction recorded. Eggs are generally laid before or around sunrise (Kluijver 1950), so nest visits were performed after 9.00 a.m. in order to minimize disturbance to laying birds. After the commencement of laying, nests were visited every other morning, to establish the completed clutch size and the date of clutch completion. During the laying period, eggs were frequently found covered with nest material, such as fur or hair. The first stages of incubation were indicated by the finding of warm, uncovered eggs. However, using the above method of checks, it was not

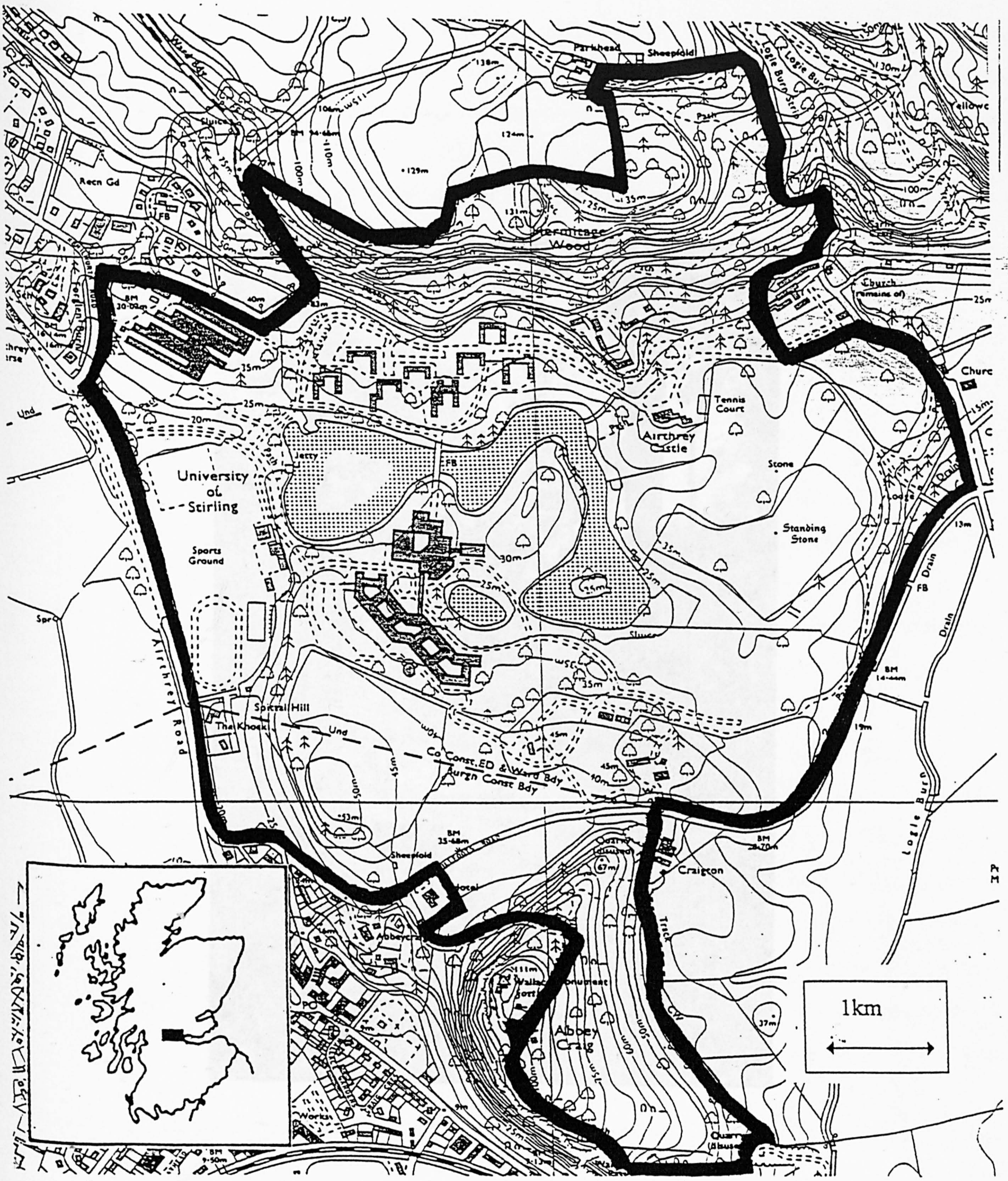

Figure 5.1 Great Tit study site, comprising the campus of Stirling University (Central Scotland), with the woodlands of Hermitage Wood to the north and the Abbey Craig to the south. Map reproduced from Ordnance Survey Landranger Series: Sheet 57, 1991. Scale 1:50,000.

Plate 2. **Great Tit study site: Hermitage Wood in the Spring.**

possible to accurately determine the date of commencement of full incubation, as eggs could be found uncovered and warm when incubation behaviour was only partially developed. For simplicity therefore, the start of full incubation was assumed to coincide with the date of clutch completion.

Nests were assigned alternately to either a 'heated' or a 'control' group, according to date of discovery. Assembly of equipment was commenced gradually around the nest during the pre-laying period and early incubation. 'Heated' nests were equipped with the heating assembly outlined below, while control nests were fitted with dummy equipment, to replicate the level of disturbance.

5.3.2 Nest box heating apparatus.

The heater assembly is shown in Figure 5.2, and was modified from equipment used by Yom Tov and Wright (1993) in their study of laying interruptions in the Blue Tit. It comprised a small candle (a night-light) positioned at the bottom of an aluminium food can. This was surrounded by a wind-shield formed by a second, larger can. The entire unit was suspended below the nest. The inner can was positioned open side down and fitted with a plastic lid, which could be removed to insert a lighted night-light. Both the plastic lid and the upper perimeter of this inner can were perforated to allow adequate air flow for the candle. This inner unit was fixed flush to the bottom of the nest box by threading garden wire through the upper holes and wrapping it tautly around pins positioned at the sides of the box. The windshield was fitted over the inner can, open side up and was held in place by small springs looped over the side pins. Experimentation with the design showed this unit to be the best compromise between adequate air-flow for the candle and sufficient shelter from the wind. Nails placed alongside the box in the supporting tree were used to prevent lateral movement of the box in wind, which could result in the wick being extinguished by molten wax.

Heated nests were supplied with lighted candles between 1 and 2 hours before dusk. Control nests were disturbed in a similar manner at this time. The aim of the experimental protocol was to provide sufficient heat to the nest of the ‘heated’ treatment group throughout the night in order to provide a significant thermoregulatory saving during the course of the 48h doubly labelled water protocol. Heating was not applied throughout the entire incubation period, as this was thought to increase the predictability of the warmed night. If birds began to anticipate the continuation of

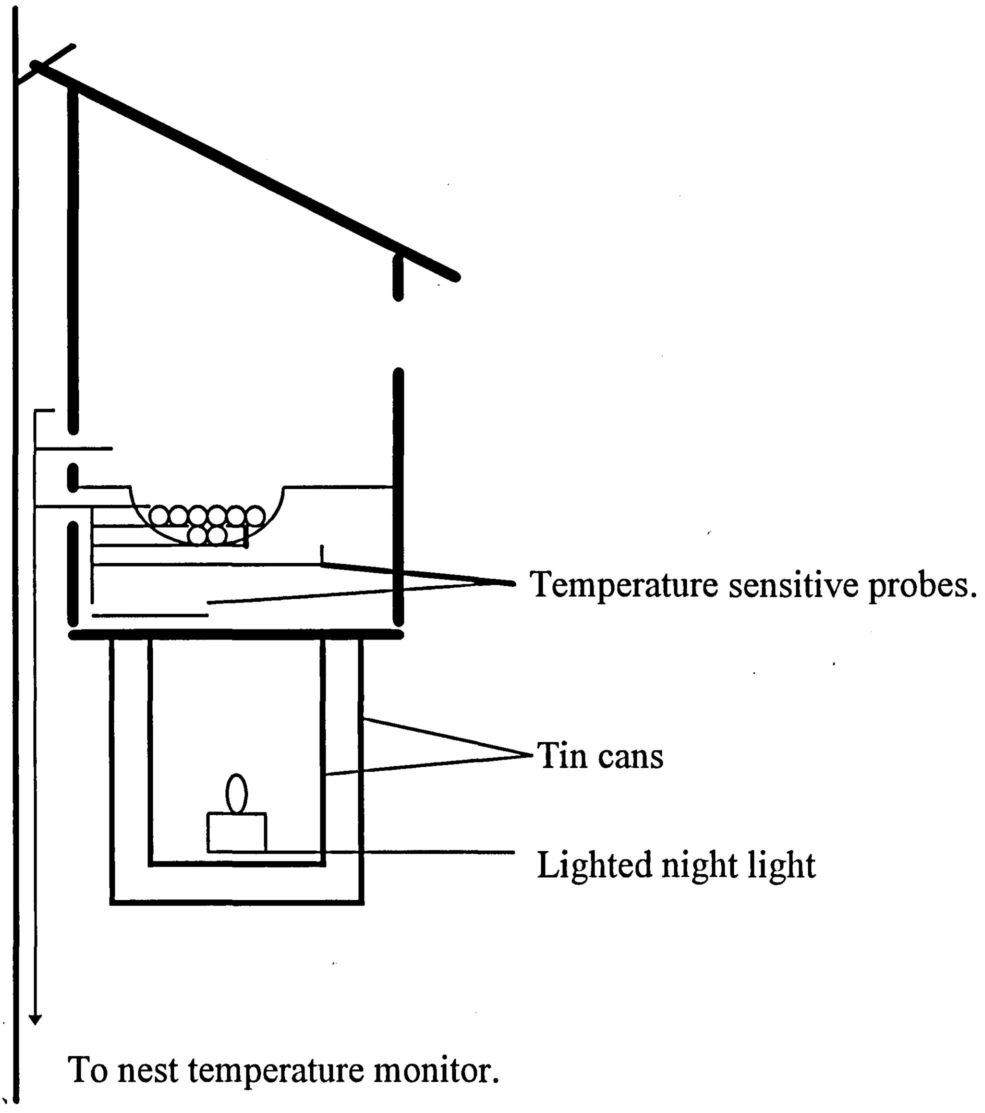

Figure 5.2 Nest box heating apparatus, modified from Yom Tov and Wright (1993). The lighted night-light was supported in a can below the nest box. Holes in this can allowed adequate air flow for the candle. The outer can formed a wind-shield.

mild nocturnal conditions, they could modify incubation behaviour according to alterations in their prediction of future conditions. Such responses would be difficult to distinguish from those resulting from an energetic saving *per se*.

However, in order to reduce the number of novel and potentially stressful influences on females during the doubly labelled water study period, nests in the heated group were supplied with a lighted candle for one night preceding the doubly labelled water study. Control birds were also disturbed in a similar manner at this time. Treatments were also applied on the two following nights within the doubly labelled water study period, but were discontinued after final capture.

5.3.3 Nest temperature monitor.

Temperature sensitive probes were used to monitor ambient temperature adjacent to the box and nest air temperature, and hence to establish patterns of incubation, as described in Section 2.4. Dummy equipment was used to acclimatize the birds to the presence of the probes in the nest, the real probes being inserted immediately before the doubly labelled water study. Six probes were inserted through two small holes drilled in the back wall of the nest box, and positioned as shown in Figure 5.2., to record temperature at the following positions:

1. Nest air adjacent to, but not in contact with, female.

2, 3 and 4. Air between eggs.

5. Nest material.

6. Nest floor

The seventh probe was positioned outside the nest, in the shade of the box to record ambient temperature. The logging equipment was left at the base of the supporting tree or an adjacent one and was concealed by vegetation.

5.3.4 Doubly labelled water protocol.

The study was conducted on day 6.9 ± 2.8 days (n = 31), where day 1 was the day of clutch completion. In 1994, attempts were made to synchronize the study to days 8-10 of incubation, in order to limit variation in patterns of incubation or energy requirements, due to the progression of incubation and increasing metabolism of embryos (Mertens 1977). However, in 1995, this was sacrificed in order to increase the sample size.

Females were caught from the nest between 14:00 and 16:00h, using the methods outlined in Section 2.2. In 1995, experiments were paired, such that one 'control' and one 'heated' female were captured and labelled on each day. Females were transported a maximum distance of 2.5 miles to the lab. In most cases, eggs were removed from the nest along with the female and transported to the lab, to be measured and maintained at approximately 35°C. However, following desertions at four consecutive nests in 1995, it was considered that eggs should not be handled, and were instead covered with a thick layer of cotton wool to prevent heat loss and left in the nest. In all cases, the nest entrance was blocked with a dark cloth to further reduce the cooling rate of eggs and to prevent intruders.

Birds were dosed with doubly labelled water as outlined in Section 2.5. During the period of isotopic equilibration, measures of structural size were recorded (Section 2.2.1.) for both female and for eggs. Visible deposits of subcutaneous fat were scored in 1995 only (Section 2.2.2.). After an initial blood sample had been taken, eggs were returned to the nest (or cotton wool was removed), the nest entrance was unstopped and the female released within 10 metres of the nest. The mean period from first capture to release was 1.6h ± 0.2 (n = 28).

After a period of 48 hours, the female was recaptured from the nest and transported to the lab in order to take a final blood sample. The mean time between second capture and release was 0.65h ± 0.2 (n = 22).

At intervals throughout the doubly labelled water study period, observations were made from a hide positioned 10-20 metres from the nest, in order to validate the nest attendance schedules recorded by the nest temperature monitor and to observe the number of feeds supplied by the male to the female while on the nest (1995 only). Otherwise, the nest site was visited only to light the candles and to download data from the nest temperature monitor, the latter involving one visit every 20 hours.

Following the final release of the female, equipment was removed gradually from the nest over a period of days.

5.3.5 Mate feeding observations.

Observations were made at 2 nests in 1994, and at 12 nests in 1995, in order to investigate the extent to which male Great Tits provisioned their partners during incubation. Observations were made between 09:30 and 15:00, a period when levels of mate feeding are reportedly high (Royama 1966).

Males delivered food to their partners both when they were incubating and during their recess periods. Due to the nature of the habitat however, dense foliage prevented observation of almost all behaviour away from the nest box. Attempts were made to record feeding off-nest, in the immediate vicinity of the box, where possible, but bias due to site characteristics such as foliage density could not be eliminated. The probability of a feed taking place unobserved differed markedly according to whether the female was on or off the nest. Differences in the nutritional value to the female between feeds delivered on or off nest (Nilsson and Smith 1985) could make direct comparison of the two difficult. Analysis was therefore restricted to periods when the female was known to be incubating. Feeds recorded outside the box were only included at one exceptional nest, where the male repeatedly called to the incubating female from near the nest entrance and fed her immediately outside the nest box as she came out. She then returned directly to the box and presumably resumed incubation within a matter of seconds. At this nest, the male was never seen to enter the box. This behaviour was considered to be comparable to feeding within the nest box at other sites, both in terms of behaviour of the female and ease of observation, so feeds of this nature were included in the analysis.

All nest visits by the male were recorded as feeds, although in some cases, prey items were not visible and the duration of the visit was under 2 seconds. Royama (1966) notes that males sometimes feed the female at the nest entrance, so it was considered that feeding could have occurred on even the shortest nest visits.

Nests were observed for periods ranging from 20 - 60 minutes (mean 30.62 ± 18.6 min.) in order to observe a period of continuous incubation of 10 minutes' duration. The number of visits observed in this 10 minute period was recorded.

5.4 Results

5.4.1 Nesting parameters.

Egg laying and incubation commenced slightly earlier in 1995 than in 1994 (Figure 5.3). The day of laying of the first egg (DOFE) was significantly earlier in 1995 than in 1994. Dates were expressed numerically, where day 1 = April 1st . mean DOFE for 1994 was 34.63 ± 3.77 $_{(16)}$, for 1995 was 29.63 ± 5.98 $_{(27)}$, independent samples t-test, p = .005. The day of clutch completion (DOCC) was also earlier in 1995: mean DOCC in 1994 was 41.13 ± 4.40 $_{(16)}$, for 1995 was 36.82 ± 6.60 $_{(27)}$, independent samples t-test p = .025.

Clutch size ranged from 5 to 10 eggs in this study. Mean clutch size was slightly larger in 1995 than 1994 (mean clutch size for 1994 was 7.00 ± 0.32 (n = 16), for 1995 was 7.67 ± 0.23 (n = 27)) (Figure 5.4) The modal clutch size was 7 in each year.

In the Great Tit, clutch size is often maximal at the beginning of the season (Perrins 1965). A large portion of the subsequent decline is due to the smaller size of repeat clutches and second broods, where these are undertaken. A slight decrease has also been detected throughout first broods in some years (Kluijver 1951, Lack 1958). In this population, females were single brooded. A significant decline in clutch size according to the date of laying the first egg (DOFE) was apparent when data from both years were combined (Figure 5.3.). Analysis of covariance of clutch size by year, with day of laying as covariate, showed that the larger clutch size in 1995 was a result of the earlier laying in this year; covariate p = .034, main effect of year, p = .370.

The mean clutch size of birds labelled in each year was representative of the wider sample for that year: in 1994, mean clutch size in the labelled birds was 7.22 ± 1.30 (n = 9), for 1995, mean clutch size was slightly, but not significantly higher at 7.68 ± 1.25 (n = 19) for labelled birds. Clutch size did not differ between treatment groups (control: 7.69 eggs ± 1.03 (n = 13), heated: 7.40 eggs ± 1.45 (n = 15), independent samples t-test t_{26} = .60, p = .551; Levene's test for homogeneity of variance, F = 1.262, p = .271).

Interruptions in the daily laying sequence were recorded at three nests, lasting between 1 and 3 days. The length of incubation from clutch completion to hatch date (inclusive) was determined for a sample of nests: the mean duration was 14.93d ± 0.41 $_{(14)}$. The incubation period recorded in nests that experienced the heating treatment for 3 nights (14.5d ± 0.50 $_{(4)}$) did not differ significantly from that in control nests (15.1d ± 0.55 $_{(10)}$, Mann-Whitney U-test, p = .84).

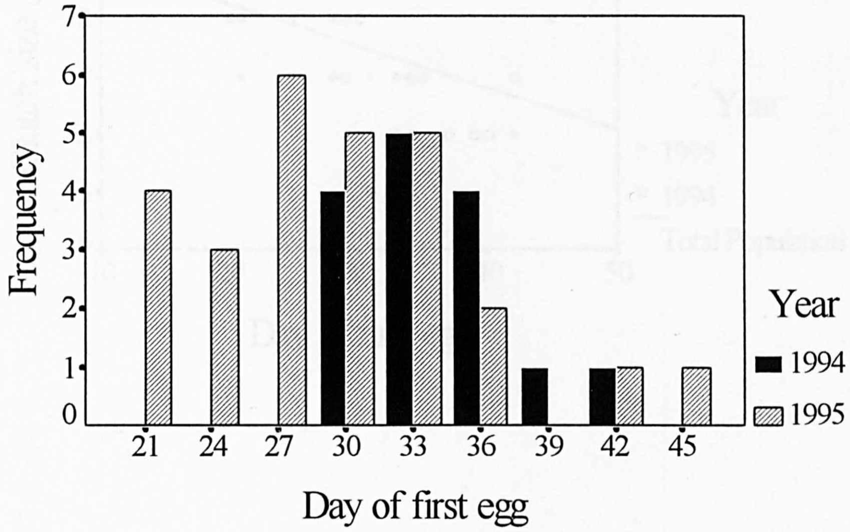

Figure 5.3 **Frequency distribution of laying in Great Tits in 1994 and 1995. The day of laying the first egg of the clutch was expressed numerically, where April 1st = day 1.**

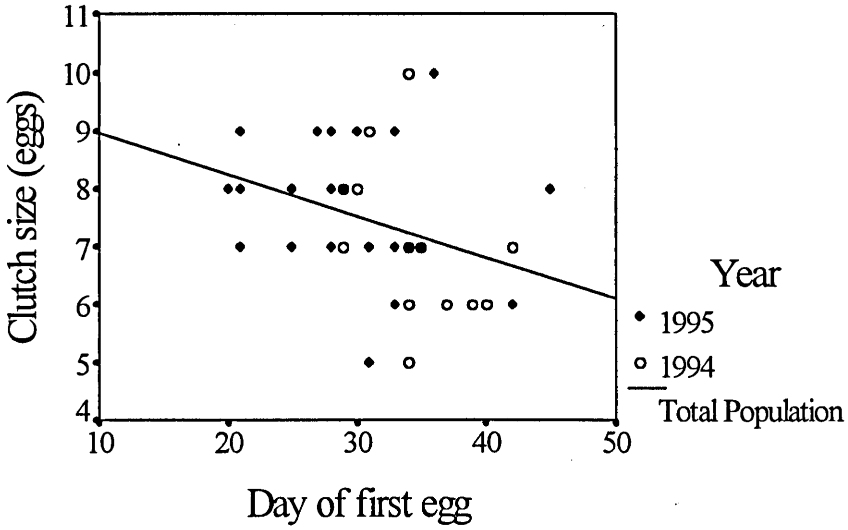

Figure 5.3 **Seasonal decline in Great Tit clutch size according to the date of laying of the first egg. For the data for both years combined: y = 9.65 - 0.07x, where y = clutch size (eggs) and x is the day of laying the first egg (where day 1- April 1st), r^2 = .110, $F_{(1,41)}$ = 4.832, p = .034.**

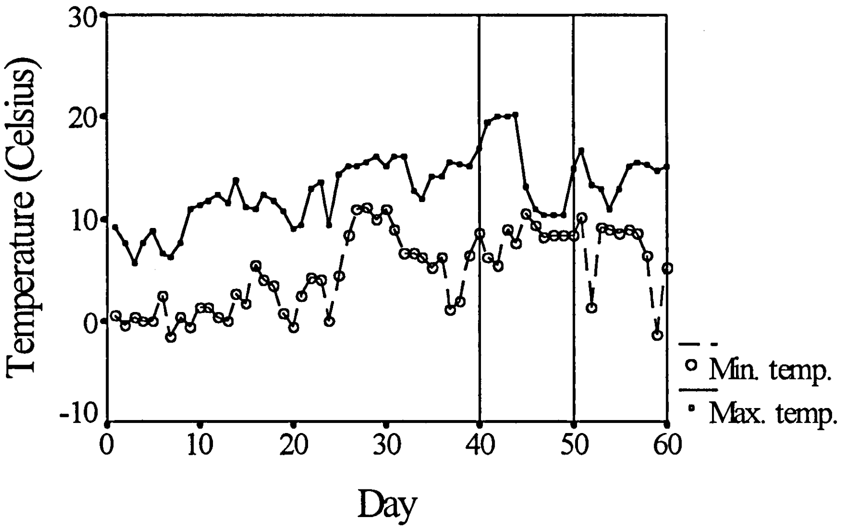

Figure 5.5 (a) 1994.

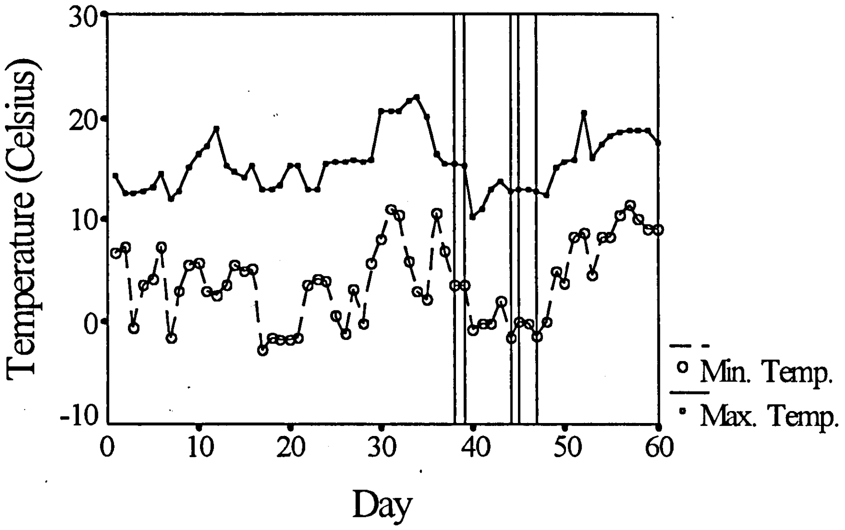

Figure 5.5(b) 1995.

Figure 5.5 **Maximum and minimum daily temperature (°C) for the period from 1st April to 30th May in (a) 1994 and (b) 1995. Day calculated as day 1 = April 1st. Vertical lines represent laying interruptions and cases of nest desertion.**

5.4.2 Environmental Conditions.

Figures 5.5. (a) and (b) show the daily maximum and minimum temperatures recorded at Stirling University Weather Station, for the period from 1st April to 30th May, for the two study years. During the egg laying and incubation period, minimum temperature was variable in both years.

Minimum temperatures were significantly lower in 1995 than in any of the years from 1991 to 1994 inclusive (oneway ANOVA p = .004, with Tukey's Honestly Significant Difference Test).

Low temperatures seemed to adversely affect the birds' response to the doubly labelled water treatment. Though most birds returned to the nest to resume incubation within 2 hours of release, on 5 occasions, females roosted away from the box on the first night following release, returning to the nest at dawn the following day. On 6 further occasions, labelled birds did not resume incubation, but deserted the nest completely. The mean minimum temperature throughout the doubly labelled water period was found to be significantly lower for those birds that deserted the nest (1.2 °C $\pm 2.5_{(6)}$) than for those who resumed incubation within 2 hours (5.5 °C $\pm 3.9_{(17)}$) or those that returned to the nest at dawn (8.1°C $\pm 2.1_{(5)}$) , (oneway ANOVA, p = .007 with Tukey's h.s.d. test).

Minimum temperatures generally occur during the hours of darkness. Cold nights could create problems of energy imbalance for laying and incubating birds (Yom Tov and Wright 1993). The times of laying interruptions and nest desertions following the first capture of the doubly labelled water procedure during incubation are represented in Figure 5.5 by vertical lines. In 1994, a laying interruption was recorded on day 40, and a desertion on day 50; in 1995, laying interruptions were observed on day 44 and 47, and desertions on day 38 (2 nests), day 39 (2 nests) and day 45.

5.4.3 Temperature profile in nest box overnight: effects of heating treatment.

Data from the output of the nest temperature monitor revealed that night-lights remained alight for approximately 6 hours and elevated nest temperature above the corresponding values for control nests for an average of 10 hours. In order to calculate the magnitude of the temperature elevation, mean nest air temperature was calculated on an hourly basis for a

Table 5.1. **Temperature elevation resulting from heating of Great Tit nest boxes. Hourly nest air temperatures are presented as the mean elevation above ambient temperature (°C), ± standard deviation with the number of hours data presented as subscript.**

Time (h)	Control	Heated	Ambient
18:00	$2.99 \pm 0.66_{(8)}$	$2.75 \pm 0.59_{(21)}$	$10.05 \pm 0.78_{(29)}$
19:00	$2.74 \pm 0.57_{(12)}$	$3.46 \pm 0.55_{(21)}$	$9.61 \pm 0.66_{(33)}$
20:00	$2.76 \pm 0.51_{(14)}$	$6.32 \pm 0.84_{(21)}$	$8.99 \pm 0.63_{(35)}$
21:00	$2.81 \pm 0.54_{(14)}$	$7.19 \pm 0.97_{(21)}$	$8.12 \pm 0.64_{(35)}$
22:00	$2.70 \pm 0.52_{(14)}$	$7.36 \pm 0.91_{(21)}$	$7.69 \pm 0.65_{(36)}$
23:00	$2.67 \pm 0.52_{(14)}$	$8.02 \pm 1.24_{(22)}$	$7.28 \pm 0.64_{(36)}$
24:00	$2.62 \pm 0.52_{(14)}$	$8.16 \pm 1.41_{(22)}$	$6.95 \pm 0.64_{(36)}$
01:00	$2.60 \pm 0.52_{(14)}$	$7.74 \pm 1.24_{(22)}$	$6.63 \pm 0.65_{(36)}$
02:00	$2.65 \pm 0.53_{(14)}$	$7.39 \pm 1.22_{(22)}$	$6.34 \pm 0.66_{(36)}$
03:00	$2.61 \pm 0.56_{(14)}$	$7.19 \pm 1.18_{(22)}$	$6.30 \pm 0.69_{(36)}$
04:00	$2.61 \pm 0.56_{(14)}$	$6.95 \pm 1.21_{(22)}$	$6.13 \pm 0.72_{(36)}$
05:00	$2.32 \pm 0.57_{(15)}$	$4.75 \pm 0.90_{(22)}$	$6.62 \pm 0.62_{(37)}$
06:00	$2.59 \pm 0.61_{(14)}$	$2.55 \pm 0.58_{(22)}$	$7.36 \pm 0.51_{(34)}$
07:00	$2.68 \pm 0.57_{(14)}$	$2.75 \pm 0.51_{(22)}$	$8.27 \pm 0.47_{(34)}$
08:00	$3.08 \pm 0.56_{(14)}$	$2.37 \pm 0.53_{(21)}$	$9.49 \pm 0.47_{(35)}$
09:00	$2.81 \pm 0.52_{(14)}$	$2.09 \pm 0.64_{(19)}$	$10.20 \pm 0.46_{(33)}$
10:00	$2.92 \pm 0.52_{(12)}$	$1.91 \pm 0.41_{(15)}$	$10.81 \pm 0.44_{(27)}$
11:00	$3.02 \pm 0.51_{(12)}$	$2.13 \pm 0.40_{(12)}$	$11.55 \pm 0.52_{(24)}$
12:00	$2.92 \pm 0.53_{(12)}$	$2.08 \pm 0.30_{(9)}$	$12.41 \pm 0.57_{(21)}$

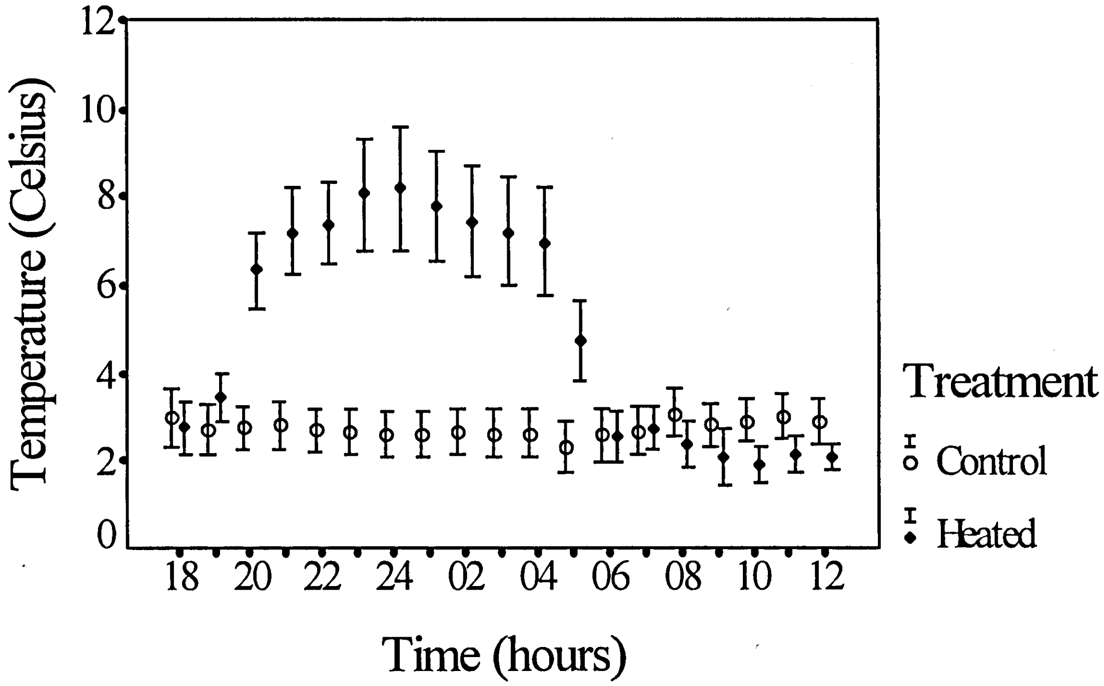

Figure 5.6 **Elevation of nest air temperature above ambient for control and heated Great Tit nests throughout the night (bars represent ± standard error).**

sample of nights from each nest, using data from the probe positioned close to the female. Mean hourly ambient temperature was calculated in a similar manner, using the data from the probe positioned just outside the box. Table 5.1. shows the hourly elevation of nest air temperature above ambient for control and for heated nests, alongside the mean ambient temperature throughout the night. These data are represented graphically in Figure 5.6.

Using data from 8 control and 8 heated nests for which a complete record of hourly temperature means were available from 18:00 to 12:00 h inclusive, repeated measures analysis of variance was used to investigate the effect of the heating treatment on the elevation of nest temperature above ambient '(n-a)'. The value of (n-a) varied significantly with time (within subjects 'time' effect F = 2.23, p = .003). The shape of this response was significantly different for heated than for control nests (interaction of 'time' x 'treatment' F = 2.88, p< .001). Figure 5.6 shows how the temperature of heated nests was approximately 6°C warmer than that in control nests for a period of 9 hours.

Nest air temperature in heated nests was more variable than that in control nests. This variation probably arose from differences between the burning characteristics of the night-lights.

Heat loss from the heating apparatus and the box could differ according to environmental parameters such as ambient temperature, air movements or relative humidity. The metabolism and hence heat loss to the box by the incubating female could also vary with temperature (Mertens 1987). The effect of the heating apparatus on nest air temperature could, therefore, have varied according to environmental conditions. However, there was no correlation between the elevation of nest air temperature above ambient recorded at midnight and the mean ambient temperature recorded at this time, Pearson correlation coefficient $r = -.308$, $p = .164$, $n = 22$, so there was no evidence to suggest that the treatment affected birds differently according to ambient temperature.

5.4.4 Allocation of time: nest attendance schedules.

In order to determine the allocation of time and hence energy within the daily budget, nest attendance was monitored for 11 control and 11 'heated' birds. Patterns of time spent on and off the nest were determined from the output of the nest temperature monitor. In order to avoid bias towards any one portion of the day, a continuous record of as close to 24 hours as possible within the doubly labelled water period was selected for each bird. For each bird, the mean duration of session and of recess periods during the active day, and the duration of the night time incubation session were determined. Nest attendance was also expressed by incubation constancy, I.C., calculated as the percentage of the active day spent on the nest. Analyses of I.C. were performed after angular transformation of the data to ensure normality (Komologrov-Smirnov test for normality, $z = .742$, $p = .640$, $n = 22$).

Nest attendance parameters for each of the 22 birds sampled are shown in Appendix 7. There was no difference in the duration of session or recess periods or of night sessions between 1994 and 1995 (Table 5.3) so in subsequent analyses, the data from both years were considered together.

Five birds spent the first night away from the nest following the first capture. For each of these birds, the length of session and of recess periods during the active day following the night spent off the nest were compared with values calculated from the active day following a night spent incubating (Table 5.2). When data for all five birds were analysed together, paired t-tests showed there was no significant difference between the duration of either session or recess periods. The mean session duration following a night spent on the nest was 37.5 min ± 8.8 $_{(5)}$, and that following a night off, 30.9 min ± 8.1$_{(5)}$, paired t-test, p = .27; mean recess duration following a night on was 8.5 min ± 1.7$_{(5)}$, following a night off, 7.5 min ± 1.0 $_{(5)}$, p = .08. In four of the five cases, nests were in the heated group. When the four heated birds were analysed separately, session length was found to be significantly shorter following the night spent away from the nest (night on: 41.0 ± 6.1$_{(4)}$, night off: 39.5 ± 8.4 $_{(4)}$, p = .009). Recess duration did not differ significantly (on : 8.3 ± 1.8$_{(4)}$, off: 7.4 ± 1.1 $_{(4)}$, p = .20). Data sampled from days following a night spent away from the nest were therefore considered unrepresentative and not included in subsequent analyses.

Table 5.2 **The duration of session and recess periods (min.) for five incubating Great Tits during the active day following a night spent away from the nest (off) compared to that subsequent to a night spent incubating (on).**

Sample	Treatment	Session		Recess	
		on	off	on	off
4	H	45.7 ± 28.3$_{(14)}$	35.1 ± 15.2 $_{(16)}$	6.1 ± 3.0 $_{(14)}$	6.4 ± 2.9 $_{(17)}$
7	H	47.9 ± 34.8$_{(8)}$	37.6 ± 22.3 $_{(18)}$	7.4 ± 3.6 $_{(9)}$	6.2 ± 4.2 $_{(19)}$
104	H	32.9 ± 28.4$_{(18)}$	15.9 ± 7.3 $_{(33)}$	10.0 ± 7.3 $_{(18)}$	8.8 ± 6.9 $_{(31)}$
116	H	37.5 ± 17.2$_{(17)}$	29.3 ± 10.5 $_{(17)}$	10.0 ± 5.9 $_{(19)}$	8.2 ± 8.4 $_{(18)}$
117	C	23.6 ± 17.3$_{(16)}$	36.7 ± 24.5 $_{(15)}$	9.3 ± 11.6$_{(17)}$	7.8 ± 2.8 $_{(16)}$

Table 5.3 **Nest attendance for incubating Great Tits in 1994 and 1995. The duration of session and recess periods is given in minutes. Incubation constancy (I.C.) was calculated as the proportion of the active day spent incubating. Independent samples t-tests were used to assess the difference between years. I.C. was transformed using an angular transformation prior to testing.**

	Session	**Recess**	**Night session**	**I.C.**
1994	$38.2 \pm 11.6_{(7)}$	$7.8 \pm 2.5_{(7)}$	$647.7 \pm 101.6_{(7)}$	$.81 \pm .05_{(7)}$
1995	$32.6 \pm 9.0_{(15)}$	$8.1 \pm 1.7_{(15)}$	$605.7 \pm 57.3_{(15)}$	$.78 \pm .05_{(15)}$
t-test	p = .253	p = .706	p = .253	p = .147
Mean	$34.4 \pm 10.2_{(22)}$	$8.0 \pm 1.19_{(22)}$	$619 \pm 76.9_{(22)}$	$.79 \pm .05_{(22)}$

5.4.4.1 Effect of heating treatment on nest attendance schedule.

The difference in nest attendance between control and heated birds was assessed by independent samples t-tests on the duration of session, recess and night session periods and on incubation constancy. A single mean value for each variable per bird was used in analysis. Although the treatments were generally paired with respect to date, deficiencies in the data due to periodical failures in the records of the nest temperature monitor, and occasional aberrant behaviour following nights spent away from the nest (Section 5.4.4) meant that the 24 hour periods considered did not coincide well enough for a paired sample t-test to be used.

Incubation sessions were significantly longer for birds following a night spent in a heated nest than for those in control nests, the mean difference being 9.5 min.(Table 5.4). The length of recess periods did not differ significantly between the two groups. Night sessions were also longer for birds in a heated box, though the difference did not attain significance at the 5% level. However, as one 24 hour period only was included for each bird in this analysis, the data included only one night-time session per bird. In order to reduce the level of variability due to sampling error, the night session data set was extended to include all the available data on night session duration, varying from 1 to 5 nights for each of the 22 birds, recorded during or after the doubly labelled water measurement period. A single mean value was calculated per bird and an independent samples t-test performed on these means. Using this extended data set, the difference between night sessions spent in heated and control boxes attained significance at the 5% level (Table 5.4).

The difference in session duration associated with the heating treatment resulted in a significantly greater proportion of the active day being spent on the nest by heated birds; I.C. for heated birds was, on average, 6% higher than for control birds.

<u>Table 5.4.</u> **Patterns of nest attendance for incubating Great Tits in 1994 and 1995 according to treatment group (C = control and H = heated nests). Mean session, recess, night session[1] length (min.) and I.C. were calculated from means derived from 24 h data (Appendix 7). Night session [2] was derived from an extended data set, comprising between 1 and 5 nights' data per bird. I.C. (incubation constancy) was calculated as the proportion of the active day spent incubating.**

	Session (min.)	Recess (min.)	Night session[1] (min.)	Night session [2] (min.)	I.C.
C	29.6 ± 8.9 $_{(11)}$	8.1 ± 1.5 $_{(11)}$	589.4 ± 56.5$_{(11)}$	590.2 ± 41.2 $_{(11)}$	0.76 ± 0.04$_{(11)}$
H	39.2 ± 9.3 $_{(11)}$	7.8 ± 2.4 $_{(11)}$	648.7 ± 82.9$_{(11)}$	647.0 ± 68.3 $_{(11)}$	0.82 ± 0.04$_{(11)}$
t-test	p = .029	p = .80	p = .076	p = .036	p = .010

The difference in night session length resulted from birds leaving the nest later in the morning following a heated night (Figure 5.7 (a) and (b)). The start of the night session did not vary with date (Pearson correlation coefficient r = .048, p = .746, n = 49) so the number of minutes before sunset when birds began the night session was compared directly for the two treatment groups. The difference was not significant: heated birds began the night session 113.9 min ± 52.5 $_{(25)}$ and control birds 100.9 min ± 54.9 $_{(24)}$ before sunset, (t-test, p = .401).

The timing of the end of the night session did vary with date. For the range of dates shown in Figure 5.7b, the relationship was described by a quadratic function (Equation 5.1).

Eqn 5.1. $y = 11.057 - 0.246x + .003x^2$

$r^2 = .143$, $F_{(2,46)} = 3.842$, $p = .029$.

where x was the time (h) when the birds first left the nest in the morning, and y was the number of days from April 1st.

The time when birds ended their night session (and hence began their active day) was standardised for date by calculating the residuals from Equation 5.1. These residuals differed significantly according to treatment group: heated birds: residual time = 0.202 min ± .664 $_{(25)}$, control birds, -.211 min ± .435 $_{(24)}$, independent samples t-test, t = -2.56 on 47 d.f., p = .014, suggesting that heated birds remained on the nest for longer after sunrise than did controls.

In summary, control birds spent an average of 590 minutes (9.83hours) incubating throughout the night, leaving an 'active daylength' of 850 minutes (14.17 hours). This was partitioned between periods of incubation, averaging 29.6 minutes, and periods away from the nest, of 8 minutes duration. The active day was, therefore, divided between, on average, 22.3 sessions, interspersed with 23.3 recesses. According to these calculations, the control birds spent 20.85 hours on the nest (86.9% of each 24 hour period), leaving 3.15 hours off-nest in which to satisfy foraging requirements.

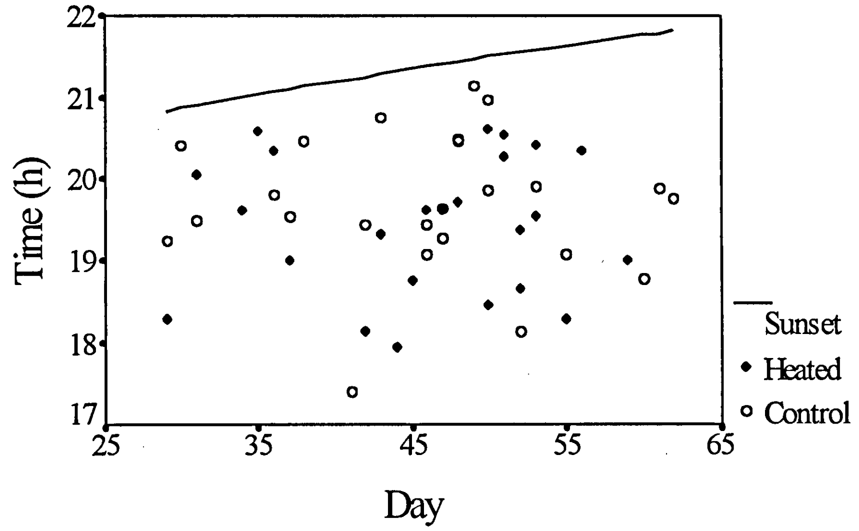

Figure 5.7(a).

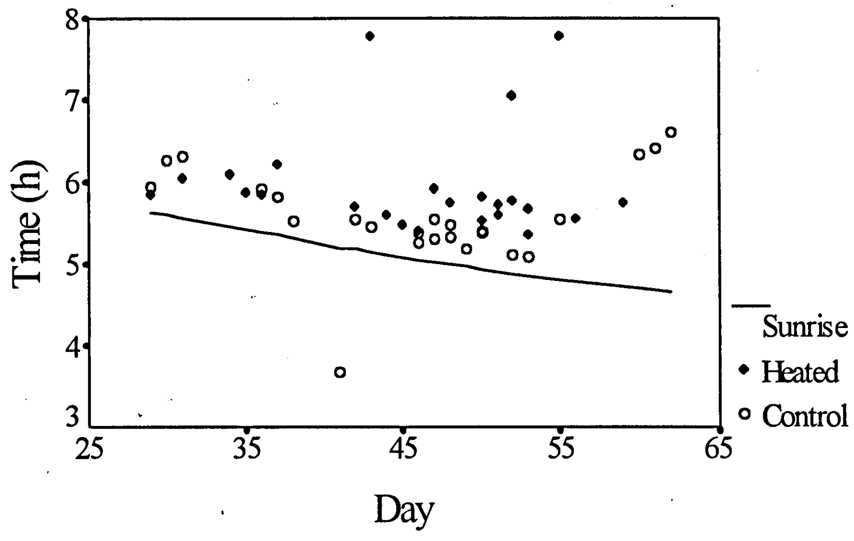

Figure 5.7(b).

Figure 5.7 **(a) Start and (b) end of the night session of incubating Great Tits in relation to sunset and sunrise. Markers represent treatment groups.**

Heated birds had a longer mean night session, of 647 minutes (10.8 hours), leaving a shorter active day: 793 minutes (13.2 hours). This was divided into 16.7 sessions, of average length 39.2 minutes, and 17.7 recess periods, averaging 8 minutes each. Thus, throughout a warmed night and the following day, incubating Great Tits spent a total of 1302 minutes incubating (21.7 hours, or 90.3% of the 24 hour period) and spent the remaining 2.3 hours off-nest foraging. In short, the heating treatment induced birds to increase their total time allocation to incubation by an extra 51 minutes per day.

5.4.4.2 Seasonal variation in the length of the active day.

In Central Scotland, reproduction in the Great Tit takes place during a period of rapidly increasing daylength. Early layers were subject to daylengths approximately two hours shorter than those laying at the end of the season. Incubating Tits could be expected to utilise the extra hours of daylight available as the season progresses, by returning to the nest later in the evening and leaving earlier in the morning, in order to make use of the extended foraging opportunity.

The active daylength was expressed as a proportion of the total hours of daylight (Figure 5.8). For heated birds, the proportion of daylight spent 'active' did not vary with date, (Pearson correlation coefficient between the angular transformed proportion of daylight hours active and days since April 1st, $r = -.309$, $n = 25$, $p = .133$). Heated birds spent 83% ± 7 $_{(25)}$ of the daylight hours active. However, for control birds, the proportion of the day spent active declined significantly as the season progressed. The relationship was best described by a quadratic function, Equation 5.2.

Eqn. 5.2. $y = .289 + .043x - .0005x^2$

$r^2 = .411, F = 7.316_{(2,21)}, p = .004.$

where y was the proportion of daylight hours spent active, transformed using an angular transformation, and x was days after April 1st.

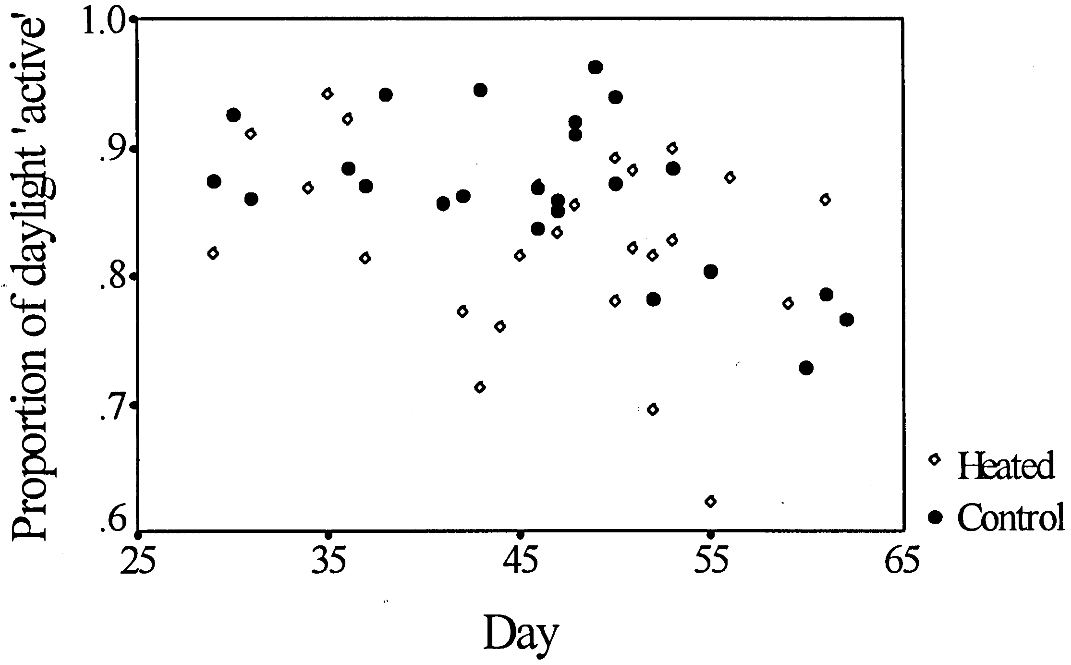

Figure 5.8 **The proportion of the hours of daylight spent in intermittent incubation (the 'active day') by incubating Great Tits, in relation to the number of days after April 1st.**

During the early hours of daylight, foraging is unlikely to be profitable, due to low temperatures, low insect mobility and low light intensity (Kacelnik 1979a,b, Avery and Krebs 1984). This may explain why the active day length never equals daylength. Due to the high cost of re-warming eggs after recess periods, the continuous night session is energetically more efficient than intermittent incubation (Drent 1970), so it is advantageous for birds to maximise the length of the night session. The active daylength could reflect the ability of the incubating bird to sustain a lengthy period of continuous incubation at night. As temperatures and food availability rose throughout the season, reducing thermoregulatory demands and increasing foraging efficiency, the extension of the night session could be possible. This would explain the observed reduction in the proportion of daylight spent active later in the season. It is interesting to note that the timing of peak caterpillar abundance recorded in deciduous woodland (recorded as c22nd May (represented by day 52 on the graph) for Dutch oak woodlands, van Balen 1973) coincides with the point of inflexion on the graph, suggesting that birds may have responded to an increase in foraging efficiency by extending the length of the night session.

5.4.5.Energy use by incubating Great Tits.

Twenty-eight incubating females were captured and labelled, 9 in 1994 and 19 in 1995. Of these, 6 birds deserted the nest before second capture, 4 of which were controls, 2 were heated birds. Five additional birds were captured in order to obtain blood samples for calculation of the background isotopic composition of the population. Details of the ring numbers and dates of sampling for Great Tits caught for the purposes of this study are presented in Appendix 8.

Energy expenditure was calculated following the methodology and assumptions outlined in Section 2.5. Values of Average Daily Metabolic Rate, (ADMR) in $cm^3\ CO_2\ g^{-1}h^{-1}$, Daily Energy Expenditure (DEE) in kJ $indiv^{-1}d^{-1}$, and Metabolic Intensity (MI) (which expresses DEE as a multiple of BMR) for each bird are shown in Table 5.5. Metabolic Intensity is sensitive to the use of different estimates of BMR, (Bryant and Tatner 1985), and is ideally based on measurements of BMR taken from the same study population. Hashim (1996) measured the basal metabolic rate of female Great Tits from the Stirling study population between 1991 and 1993. Basal metabolic rates were, therefore, predicted using both Hashim's (1996) species-specific equation (Eqn 5.3) and using Aschoff and Pohl's (1970) inter-specific equation (Equation 5.4), for passerines during the inactive phase. Metabolic intensity was expressed as a multiple of both Aschoff and Pohl's prediction of BMR ($MI_{A.P}$) and Hashim's calculation ($MI_{.H}$).

Eqn. 5.3 Basal metabolic rate of female Great Tits in the summer (Hashim 1996):
$y = 18.463x^{1.46}$
where y is BMR in Jh^{-1} and x is body mass in g.

Eqn 5.4 Basal metabolic rate of passerines, inactive phase (Aschoff and Pohl 1970)
$y = 132.832x^{0.726}$
where y is BMR in Jh^{-1} and x is body mass in g.

Although most birds resumed normal incubation within two hours of the labelling procedure, in five cases, females stayed away from the nest for the first night of the doubly labelled water study period, returning to resume incubation at dawn. These cases are marked with an asterisk in Table 5.5. Although the energy expenditure of these birds did not differ significantly from those that returned to the nest before nightfall (t-test, $p > 0.36$ for all measures of energy expenditure), differences in the pattern of nest attendance suggested that roosting away from the nest significantly altered the behaviour of incubating Great Tits during the following day (see Section 5.4.4). The energy expenditure of these birds was therefore not included in subsequent analyses

5.4.5.1 Effect of heating treatment on energy use.

The mean energy expenditure of the remaining sample of 17 birds (with data from the 5 birds who spent one night off-nest removed) is presented in Table 5.6, according to treatment group. There was no significant difference in energy expenditure between the two groups as measured by any of the four parameters. However, ADMR, DEE, MI_{AP} and MI_H were all significantly more variable in the control than in the heated group of birds.

Table 5.5 **Energy expenditure of incubating Great Tits, as measured by the doubly labelled water procedure in 1994 and 1995. Treatment group 'C' were control birds, 'H' were heated. MI$_{A.P.}$ was calculated according to the equation of Aschoff and Pohl (1970), passerine inactive phase. MI $_{H}$ was calculated using the equation of Hashim (1996) for female Great Tits in summer. Birds marked with an asterisk spent the first night of the study period off the nest.**

Sample	Mass (g)	Group	A.D.M.R (cm^{3}CO$_2$g^{-1}h^{-1})	DEE (kJ/indiv.d)	MI$_{AP}$	MI$_H$
*1	21.06	C	6.96	92.95	3.19	2.45
2	19.74	C	7.15	89.53	3.22	2.59
3	21.43	C	5.19	70.53	2.39	1.81
4	22.01	H	7.63	106.54	3.54	2.63
5	24.78	H	6.00	94.42	2.88	1.96
6	21.85	H	6.08	84.28	2.82	2.11
*7	20.88	H	5.84	77.38	2.67	2.07
8	20.76	H	6.53	85.96	2.98	2.32
105	21.75	C	6.51	89.86	3.01	2.26
107	21.59	C	5.65	77.73	2.61	1.98
109	23.21	C	6.73	99.15	3.17	2.27
111	21.49	C	12.26	167.18	5.65	4.28
113	21.83	C	8.76	121.28	4.05	3.04
115	20.53	C	13.39	174.38	6.10	4.77
*117	21.52	C	14.04	191.67	6.47	4.90
*104	22.34	H	7.70	109.18	3.59	2.64
106	21.29	H	6.02	81.33	2.77	2.11
108	21.68	H	6.39	88.39	2.96	2.23
110	21.67	H	7.68	104.65	3.54	2.65
112	21.18	H	7.69	103.36	3.53	2.70
114	21.42	H	8.28	112.45	3.81	2.89
*116	21.08	H	8.83	118.08	4.05	3.11
Mean (n=22)	21.49 ± 1.01		7.79 ± 2.43	106.38 ± 32.18	3.59 ± 1.11	2.72 ± 0.87

Table 5.6 **Energy expenditure for incubating Great Tits according to treatment group. Means are presented ± s.d.. Levene's test for homogeneity of variance (Var. test) was conducted with each variable, and an independent samples t-test conducted, based on an appropriate estimate of the population variance.**

	n	Mass (g)	ADMR cm^3 CO_2/gh	DEE kJ/ind.d	MI_{AP}	M_H
Control	8	21.44 ± 1.01	8.20 ± 3.06	111.2 ± 39.77	3.78 ± 1.39	2.88 ± 1.09
Heated	9	21.85 ± 1.16	6.92 ± 0.89	95.71 ± 11.31	3.21 ± 0.40	2.40 ± 0.33
Mean	17	21.66 ± 1.08	7.53 ± 2.22	103.0 ± 28.63	3.48 ± 1.01	2.62 ± 0.80
Var. test		p = .945	p = .007	p = .004	p = .006	p = .010
t-test		p = .459	p = .285	p = .318	p = .293	p = .270

5.4.5.2 Environmental influence on energy expenditure.

Spearman rank correlation coefficients were calculated between measures of weather data, taken from Stirling University Meterological Station, and of energy expenditure of incubating Great Tits, as measured by the doubly labelled water procedure in 1994 and 1995 (Table 5.7).

Energy expenditure was significantly related to mean minimum temperature over the doubly labelled water study period. Figure 5.9 shows the relationship between MI_H and mean minimum temperature, for the two treatment groups. Considering the whole data set together, the relationship was best described by a quadratic function, (Equation 5.5), which explained 61% of the variation in Metabolic Intensity. However, when considered separately, the Metabolic Intensity of heated birds was best described by a linear function (Equation 5.6); the relationship was not significantly improved by fitting a higher order polynomial (p>.2). The data for control birds were best described by a second quadratic function, (Equation 5.7), which increased the explained variation to over 90%.

Relationship between $MI_{(H)}$ and minimum temperature for incubating Great Tits.

Equation 5.5 All data (1994 and 1995):

$y = 3.356 - 0.521x + 0.043x^2$, $r^2 = 0.610$, $F_{(2,14} = 10.935_)$, $p = .001$, $n = 17$.

Equation 5.6 Control group:

$y = 4.064 - 0.874x + 0.074x^2$ $r^2 = 0.955$, $F_{(2,5)} = 52.660$, $p < .001$, $n = 9$

Equation 5.7 Heated group:

$y = 2.626 - 0.077x$ $r^2 = 0.613$, $F_{(1,6)} = 11.074$, $p = .013$ $n = 8$

where y is MI_H and x is the mean minimum temperature (°C) over the doubly labelled water measurement period.

Minimum temperatures are most likely to occur at night, so the mean minimum temperature is probably a good indicator of the conditions which birds faced during incubation at night. Energy expenditure escalated at low temperatures for incubating Great Tits under normal conditions, but the increase in energy use by heated birds at low temperatures was restricted compared to the control group. The extra warmth in the heated nests enabled birds to avoid the high levels of energy use required by birds under natural conditions.

Table 5.7 **Spearman's rank correlation coefficients between measures of weather and of energy expenditure by incubating Great Tits, measured by the doubly labelled water technique in 1994 and 1995 (n = 17 birds). ADMR in $cm^3CO_2g^{-1}h^{-1}$, DEE in kJ $indiv^{-1}$ d^{-1}, MI_{AP} and MI_H are unitless. Average values of maximum and minimum daily temperature (°C), rainfall (mm) and cloud cover (oktas) were calculated for the 48h period of study.**

DEE	.920 p < .001						
MI_{AP}	980 p<.001	.963 p < .001					
MI_H	993 p= .000	.887 p < .001	.998 p < .001				
Maximum Temperature	-.297 p = .247	-.239 p = .355	-.316 p = .217	-.260 p = .313			
Minimum Temperature	-.769 p = ..000	-.650 p = .005	-.728 p = .001	-.786 p < .001	.520 p = .032		
Rainfall	.031 p = .907	.055 p = .835	.045 p = .865	.010 p = .969	-.173 p = .506	-.069 p = .793	
Cloud	-.147 p = .573	.-116 p = .657	-.099 p = .706	-.177 p = .498	-.562 p = .019	.156 p = .550	.269 p=.296
	ADMR	**DEE**	**MI_{AP}**	**MI_H**	**Max Temp**	**Min Temp.**	**Rain**

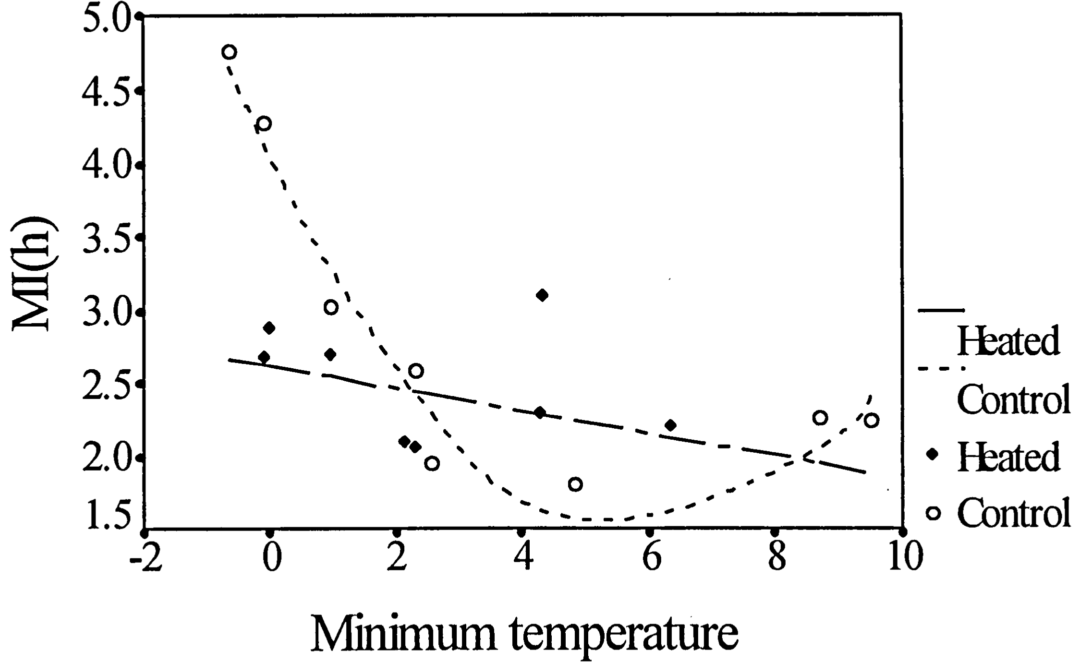

Figure 5.9 **Metabolic intensity (calculated according to the BMR equations of Hashim (1996)) of incubating Great Tits in relation to mean minimum temperature over the study period. A quadratic functions best describes the data for control birds (Equation 5.6) and a linear function describes that for heated birds (Equation 5.7).**

5.4.6 Body mass of incubating Great Tits.

The structural size of incubating female Great Tits measured in 1994 and 1995 is described in Section 2.2.1. 24% of the variation in average mass could be explained by a linear regression on tail length (Equation 5.8).

Eqn 5.8 Linear regression of mass of incubating Great Tits on tail length:

$y = 0.185x + 10.388 \quad r^2 = .243, F = 7.710_{(1,24)} \; p = .011.$

where y is mass (g) and x is tail length (mm).

In the following analyses, the body condition of incubating Great Tits was estimated by both raw mass data, and by 'residual mass', standardised for structural size by the application of Equation 5.8.

Mass was recorded post-defecation where this occurred during handling. In three cases where mass was measured immediately before and after defecation, measurements differed by 0.52g ± $0.21_{(3)}$. As all captures were made between 14:00 and 17:00h, mass was not adjusted for time of capture.

Mean mass at first capture was 21.58 ± 1.07g for 31 captures. There was no significant difference between mass at first capture in 1994 (21.72g ± $1.50_{(9)}$) and that in 1995 (21.52g ± 0.87), (t-test, p =.636). Likewise, residual mass (standardised for structural size) did not differ between years (p = .736). There was no difference in the mass at first capture (p = .185) or in residual mass (p = .141) between treatment groups.

Birds which deserted the nest during the doubly labelled water procedure were lighter at first capture than those that did not; this result attained significance at the 5% level when mass was standardised for structural size (Table 5.8)

Table 5.8 **Mean mass of incubating Great Tits at first capture in relation to whether they subsequently deserted the nest.**

	Mass at first capture (g)	**Residual mass**
Deserted during d.l.w.	20.91 ± $0.82_{(6)}$	-0.65 ± 1.13 $_{(6)}$
Did not desert.	21.73 ± $1.07_{(25)}$	0.18 ± $0.77_{(25)}$
t-test.	p = .090	p = .037

5.4.6.1 Mass change through incubation.

A second mass reading was made at the end of the doubly labelled water period of 48h. This final mass was plotted against initial mass at first capture (Figure 5.10). Because both axes contained a similar amount of error, a geometric mean regression was used to describe the relationship (Equation 5.9). The slope of this regression did not differ significantly from 1 ($t_{(2, 0.05, 20)} = 0.02$, $p > 0.5$), suggesting that there was no difference in mass change between heavy and light birds. The intercept of the regression (-0.175g) suggests that birds were, on average, losing mass over the study period.

Eqn. 5.9 Geometric mean regression equation describing mass of incubating Great Tits at the beginning and end of a 48h period.

$$y = -0.175 + 1.003x$$

where y was mass at final capture (g) and x was mass at first capture (g).

No evidence of a linear trend in mass with stage of incubation could be demonstrated. Neither mass, nor residual mass at first capture was significantly correlated with the number of days since clutch completion (for 31 first captures, Pearson correlation coefficient between days since clutch completion with mass $r = .071$, $p = .704$, with residual mass $r = .047$, $p = .800$).

5.4.6.2 Effect of treatment on mass change.

Mass change was expressed by 'final residual mass', or the residual mass (g) at final capture, after controlling for mass at first capture using Equation 5.9. Only birds which resumed normal incubation before first nightfall were considered. An independent samples t-test showed a trend for heated birds to lose more mass (standardised mass change = $-0.52 \pm 0.43_{(9)}$) than control birds ($0.05 \pm 0.76_{(8)}$), although the difference was not significant at the 5% level ($t_{(15)} = 1.93$ $p = .064$). Mass change also tended to be more variable in the control group (Levene's test for homogeneity of variance $p = .064$).

The five birds which spent the night away from the nest did not differ from other birds in mass at first capture (t-test, $p = .546$), in mass at first capture controlled for structural size ($p = .428$) or in mass change, represented by final residual mass ($p = .164$).

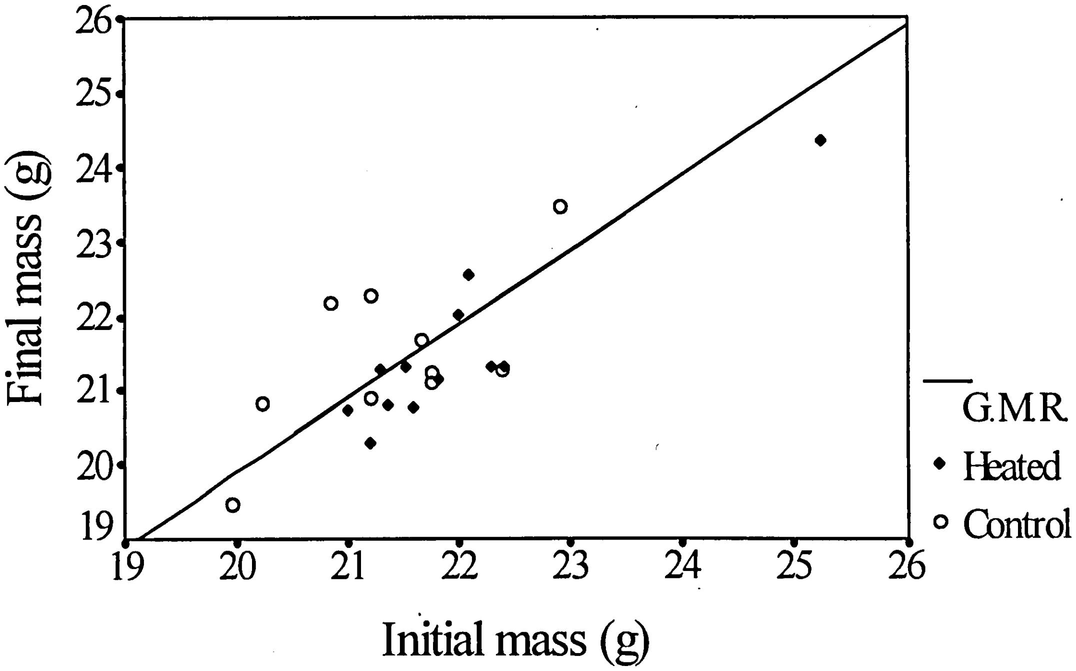

Figure 5.10 **Mass (g) of incubating Great Tits at first capture and at final capture 2 days later. The relationship was described by a geometric mean regression as: $y = -0.175 + 1.003x$. Markers label the birds according to treatment group.**

5.4.6.3 Lipid reserves during incubation.

Subcutaneous fat deposits were scored for females in 1995 only, using a 10 point system for deposits in the inter-clavicular pit and at the posterior edge of the sternum (Section 2.2.2). Scores at the beginning and end of the measurement period were obtained for 9 birds, 5 control and 4 heated (Figure 5.11). The mean score at the start of the period was 6.7 ± 2.4; mean score at the close was 7.3 ± 2.1. Only 1 (heated) bird (11%) scored lower at final than at first capture. Three birds, (33%: 2 heated and 1 control) showed no change, while the remaining 5 birds (56%) were scored for higher fat deposits at final capture. The mean change was a daily increase of 0.7 units ± 1.4 over the 48h period. Final fat scores were highly correlated with initial fat scores; Spearman rank correlation coefficient = 0.776, p = .014, n = 9. Heated birds showed a trend towards depletion of fat scores (mean change = - 0.3 ± $1.3_{(4)}$ units), where control birds tended to accumulate fat (mean = + 1.4 ± $1.1_{(5)}$ units), however this difference did not attain significance in a non-parametric test: Mann-Whitney U-test, z = -1.775, p=0.076.

5.4.6.4 Relationship between mass and nest attendance.

Between species, larger birds are able to maintain longer incubation sessions by virtue of their greater nutrient reserve and hence longer fasting endurance (Williams 1992). Within incubating Great Tits, birds that were heavier at first capture were able to sustain longer incubation sessions than lighter birds (Figure 5.12). The relationship was best described by a log-log function. Recess length was also greatest for heavy birds, although the relationship attained significance at the 5% level when considering mass at final capture only (Figure 5.13) . In neither case were the regressions improved by controlling for structural size. Night session length was not significantly correlated with any measure of body mass (p >.12 for all measures)..

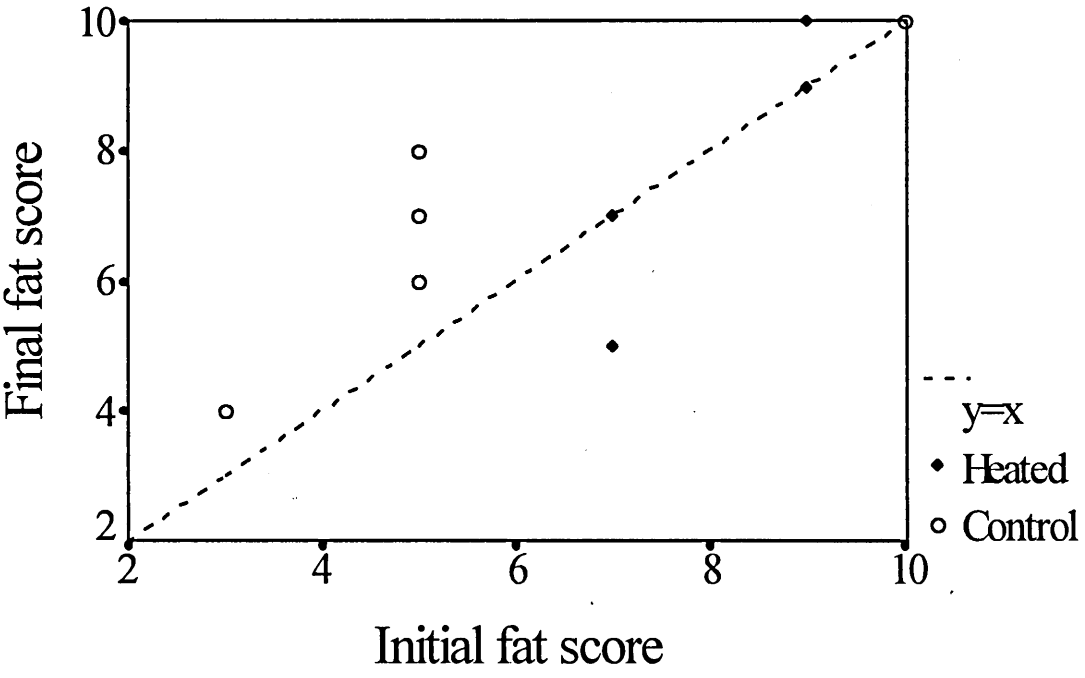

Figure 5.11 Fat scores at the beginning and end of the 48h DLW study period for incubating Great Tits according to treatment group.

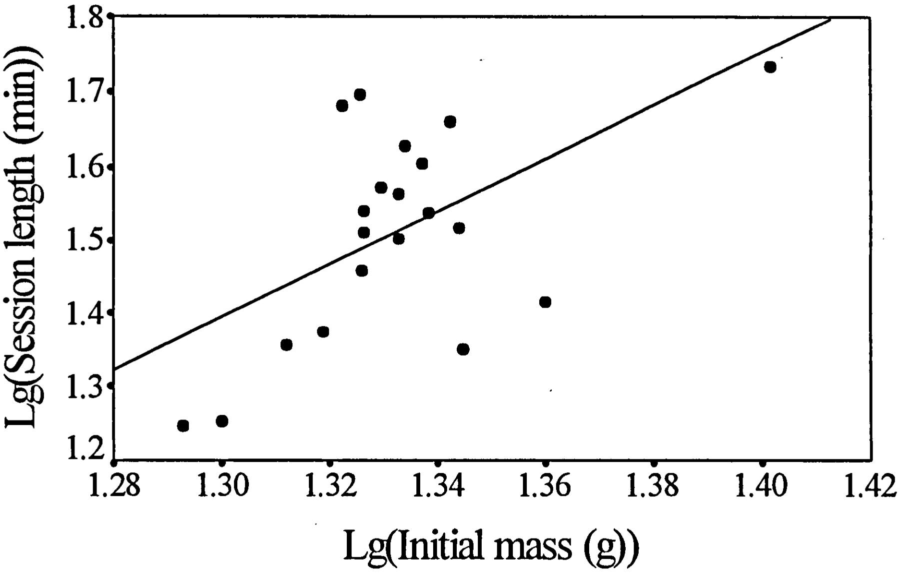

Figure 5.12 Session length (daily mean in min.) in relation to body mass (expressed as mass at first capture (g)) of incubating Great Tits. The relationship was described by the equation: $y = -3.205 + 3.538x$, $r^2 = .317$, $F = 8.365_{(1,18)}$, $p = .010$, where y was $\log_{10}$ (session length (min)) and x was $\log_{10}$(initial mass (g)).

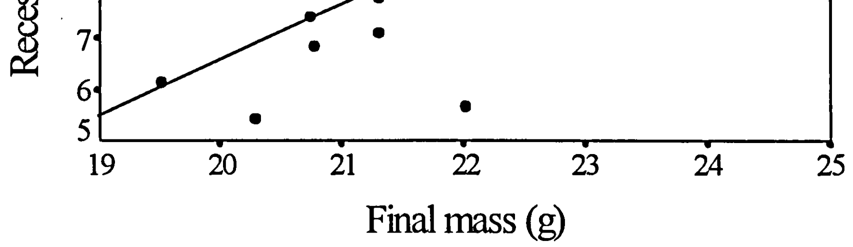

Figure 5.13 Recess length (daily mean in min.) as a function of body mass, measured at the final capture of the doubly labelled water measurement period for incubating Great Tits. The relationship was described by the equation: $y = 1.094x - 15.303$, $r^2 = .505$, $F_{(1,13)} = 13.261$, $p = .003$, where y was recess length (min) and x was mass at final capture (g).

5.4.6.5 Body mass and energy expenditure.

There was no significant correlation between body mass and daily energy expenditure (Spearman correlation coefficient between $\log_{10}$ body mass and $\log_{10}$ D.E.E. r = -0.17, p = 0.44, n = 22). However, daily energy expenditure was greatest for those birds which gained mass, although the relationship was just outside significance at the 5% level (Pearson correlation coefficient between D.E.E. and mass change in gd^{-1}, r = .410, n = 22, p = .058, Figure 5.14). A similar positive relationship between D.E.E. and mass change was found for the House Martin, the Swallow and the Dipper during brood rearing, although for brood-rearing Great Tits, no significant relationship was established (Bryant 1988). Birds which utilised stored reserves could have reduced energy expenditure by reducing foraging activity. The alternative possibility, that the result may have arisen from errors in the estimation of the Respiratory Quotient, is discussed in Section 6.1.4.

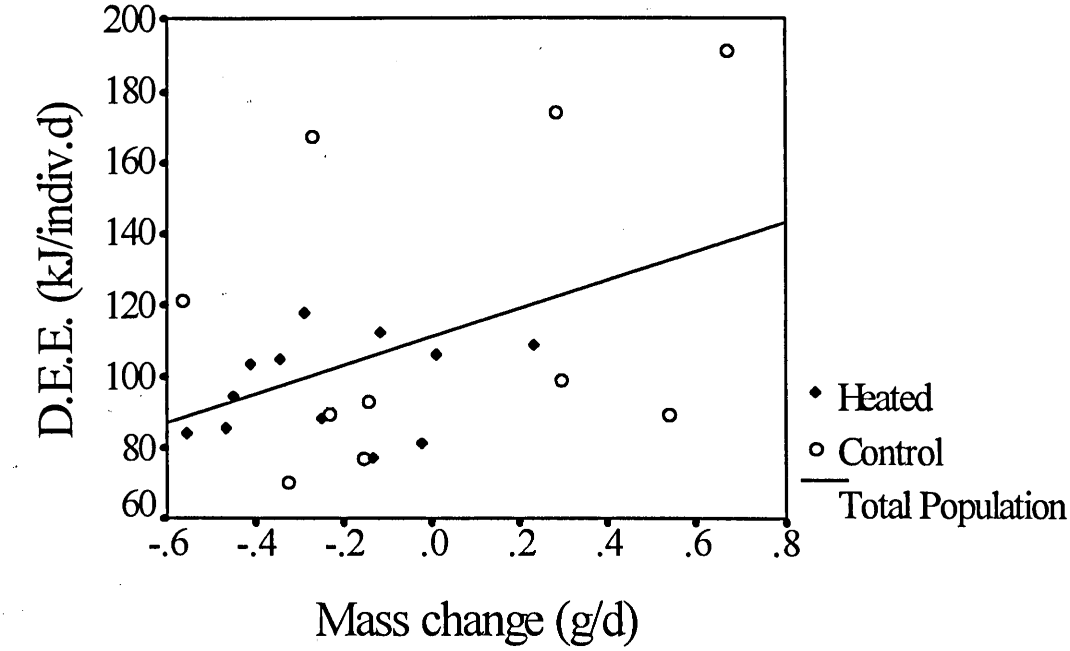

Figure 5.14 **Daily energy expenditure (kJ $ind.^{-1}d^{-1}$) of incubating Great Tits as a function of daily mass change. y = 37.812x + 111.128, r^2 = .168, $F_{(1,20)}$ = 4.034 p = .058, n = 22.**

5.4.7 Model of the energy budget of an incubating Great Tit.

In order to provide a framework for the interpretation of the results, energetic costs were modelled using the environmental and behavioural parameters recorded in the study. Total daily energetic costs were calculated on an hourly basis according to the constituent demands of various activities.

5.4.7.1 Assumptions and calculations.

The energy requirements of birds in the two treatment groups were modelled under the following assumptions:

Nest Temperatures.

Nest air and ambient temperatures were calculated from the hourly means derived from the records of the nest temperature monitor. The mean elevation of nest air temperature over ambient (n-a) was calculated on an hourly basis for both control and heated nests (Table 5.1, Figure 5.6). Between the hours of 20:00-04:00, temperature was higher in heated nests, so the hourly means recorded in heated nests in this period were used to represent nocturnal temperatures for heated birds. Elsewhere, for control birds at night and for both groups during the day, a single mean value of 2.85°C was used to represent the elevation of nest air above ambient.

Costs of incubation.

The energy use by incubating Blue Tits has been studied by measurement of the rate of oxygen consumption during periods of continuous incubation (Haftorn and Reinertsen 1985). The relationship between oxygen consumption and air temperature was described (1983 data for a female incubating 13 eggs) by Equation 5.10. Extrapolation from the results of a single bird clearly risks the results being unrepresentative, either of Blue or of Great Tits.

Eqn. 5.10 Metabolism of incubating Blue Tits (from Haftorn and Reinertsen 1985):

$$y = 15.35 - 0.62x,$$

where y is oxygen consumption in ml $g^{-1}h^{-1}$, and x is temperature in °C.

This equation was adjusted to represent the metabolism of an incubating Great Tit as follows.

The energy equivalent of 1 ml O_2, based on a R.Q. of 0.75, is 19.8J (Brody 1945). The equation was adjusted for mass, as an incubating Great Tit is heavier (21.49g this study) than an incubating Blue Tit (12.04g, Prys-Jones in Tatner and Bryant 1993). Field metabolism for individuals is thought to scale with body mass with a slope of 0.75 (on a $\log_{10}$-$\log_{10}$ plot) amongst passerines (Nagy 1987), which is equivalent to a slope of -0.25 for mass specific metabolism with mass (on a $\log_{10}$- $\log_{10}$ plot). Thus, the elevation of Equation 5.10 was adjusted for the larger size of the Great Tit by multiplying the intercept by a factor of ($21.49^{-0.25}/12.04^{-0.25}$). The slope of the Equation 5.10 describes the thermoregulatory component of the cost of incubation. The thermal conductance of a bird has been shown to scale with body mass to the power of -0.536 (Herreid and Kessel 1967). It was assumed that the thermoregulatory component of incubation costs scaled with body mass in a similar manner. The slope of the equation relating metabolism by incubating Great Tits with temperature was therefore calculated by multiplication of the slope of Equation 5.10 by a factor of ($21.49^{-0.536}/12.04^{-0.536}$). The resulting equation for metabolism during time on the nest in the Great Tit was Equation 5.11.

Eqn. 5.11 Metabolism during steady-state incubation in the Great Tit.

$$y = 262.949 - 8.999x,$$

where y is metabolism in $Jg^{-1}h^{-1}$ and x is temperature in °C.

This equation accounted for incubation in the 'steady-state' only, i.e. when egg temperature remained constant and so was applied for the night time incubation session and periods of continuous incubation during the day. It did not include the costs of re-warming eggs following time away from the nest. The energy required to raise a body of mass m (g) and specific heat capacity c ($Jg^{-1}\ {}^{\circ}C^{-1}$) through a temperature increase of $\theta\ {}^{\circ}C$, can be calculated by Equation 5.12

Eqn. 5.12 Energy required (E in J) to raise the temperature of mass m (g) with s.h.c. c ($Jg^{-1}\ {}^{\circ}C^{-1}$), through $\theta\ {}^{\circ}C$

$$E = m.c.\theta\ {}^{\circ}C,$$

Thus, for a clutch of average size (8 eggs), each of mean mass 1.72g (this study), with specific heat capacity of 3.3J g^{-1} $°C^{-1}$ (Kendeigh 1963), the energy required depends on the temperature to which eggs have cooled. Egg temperature during continuous incubation averaged 35 °C in the Great Tit (Haftorn 1983). The temperature to which eggs would cool from an initial temperature of 35°C, during a recess period of average length (8 minutes), (termed t_{cool}) at various times of the day was calculated according to the cooling coefficient of Great Tit eggs measured in this study as 1.985 °C $°C^{-1}$ h^{-1} (Section 2.6.). t_{cool} was found to vary in a linear fashion according to nest air temperature:

Eqn. 5.13 Great Tit egg temperature at the end of an 8 minute recess period:

$$t_{cool} = 0.236\, t_{na} + 26.734$$

where t_{cool} is the temperature to which eggs cool after an 8 minute recess period, at a nest air temperautre of t_{na} °C.

The required elevation in egg temperature on the female's return to the nest, $\Delta\,°C$, was then calculated as $(35 - t_{cool})°C$.

Re-heating costs were applicable after every recess period, so were added to the costs of maintaining egg temperature throughout the active day, at a frequency determined by the duration of session and recess periods.

Energy use during recess periods depends on the activities undertaken during time off the nest. Observations of activity during recess periods were not made during this study, because of the difficulties in following birds away from the nest. Time-activity budgets were therefore extrapolated from a study of radio-tagged individuals in the Stirling study site during October and November (Whitaker 1994). Although temperature and food availability differs between mid-winter and spring, it was thought that the time constraints imposed by short daylengths in the winter would make these data a suitable comparison for time-constrained incubating females. Birds foraged for 85% of time, spent 2% of time in flight, and spent the remaining 13% perching, singing, being aggressive, preening or in miscellaneous activities (Whitaker 1994).

Energy costs were assigned to these activities as follows. Flight costs were estimated from doubly labelled water studies of the cost of short flights in the Willow Tit (Carlson and Moreno 1992) as 11.7 x BMR. Basal metabolism has been measured for female Great Tits during the summer in the Stirling study population by Hashim (1996) and was described by the equation:

Eqn. 5.14 BMR for female Great Tits in the summer (Hashim 1996):

$y = 1.46x - 2.29$,

where y is $\log_{10}$ (BMR) in Watts and x is $\log_{10}$(body mass) in g.

The costs of foraging and other activities were extrapolated from respirometry measures of non-flight activity in the Robin (Tatner and Bryant 1986). Foraging costs were estimated by the cost of hopping in the Robin and the results adjusted for the larger body mass of the Great Tit by the scaling procedure described above. The intercept (and hence elevation) of Tatner and Bryant's (1986) equation was adjusted by multiplication by a factor of $21.49^{-0.25}/18.6^{-0.25} = 0.965$ to account for the reduction in mass-specific metabolism with increasing size (Nagy 1987). The slope of the equation was scaled to account for the reduction in mass-specific thermal conductance with increasing body mass (Herreid and Kessel 1967), under the assumption that the mass-specific costs of thermoregulation during periods of activity are related to body mass by the same exponent as is thermal conductance. The slope of the robin equation was therefore multiplied by a factor of $21.49^{-0.536}/18.6^{-0.536} = 0.926$. Equation 5.15 expresses the cost of foraging in the Great Tit, after conversion to an energy equivalent of 26.44 cm^{-3} CO_2 $g^{-1}h^{-1}$, based on an R.Q. of 0.75 (Brody 1945).

The cost of other activities was estimated by the cost of alert behaviour in the Robin (Taner and Bryant 1986), which, after conversion to an energy equivalent based on an R.Q. of 0.75 and adjustment for the smaller mass of the Robin (18.6g) by the conversion factors for slope and intercept presented above produced Equation 5.16.

Eqn. 5.15 Foraging metabolism in the Great Tit (based on Tatner and Bryant 1986):

$y = 219.426 - 3.075x$

y is metabolic rate in $Jg^{-1}h^{-1}$ and x is ambient temperature, °C.

Eqn. 5.16 'Alert' metabolism in the Great Tit (based on Tatner and Bryant 1986):

$y = 107.875 - 1.795x$

y is metabolic rate in $Jg^{-1}h^{-1}$ and x is ambient temperature, °C.

Activity costs were assumed to increase linearly with time spent off-nest. In reality, the relationship was more likely to be described by a decelerating function, increasing in a near linear fashion only for the period of time where foraging is profitable and can be maintained at a steady rate. Beyond some threshold, factors such as gut constraints or patch depletion would limit the profitability of foraging. A behavioural switch towards lower cost activities may follow, resulting in a decline in the slope of the cost-time curve. Observational data were not available to confirm this supposition, so it was assumed that recess length did not generally exceed this threshold level, and therefore that the relationship between costs and time off-nest approximated to linearity.

All costs, except that of flight, incorporated a thermoregulatory component. Only flight was thought to generate sufficient heat to satisfy thermoregulatory requirements without additional energy use (Bryant and Tatner 1988b).

The lower critical point of the thermoneutral zone is generally greater for incubating than for non-incubating birds (Biebach 1981).While 10°C was thought to be the lower critical temperature for the thermoneutral zone of an incubating Starling (Biebach 1981), and 15°C for the Blue Tit (Haftorn and Reinertsen 1985), for the Zebra Finch the value was as high as 28°C (Vleck 1981). Mertens (1977) observed that an incubating Great Tit fluffed out her feathers and put her head under her wing at a nest temperature of 27.5°C. Neither ambient nor nest air temperature exceeded 16°C in the model, so it was considered that all birds remained below the thermoneutral zone throughout incubation.

For those activities undertaken in the nest, costs were based on nest air temperature, while for activity during recess periods, ambient temperature data were applied.

The various cost components were applied to the appropriate portions of the day, based on the average nest attendance schedule of incubating Great Tits from control and heated groups. The parameters used in the model are summarised in Table 5.9.

5.4.7.2 Effects of nest box heating on the modelled energy budget.

The daily energy expenditure by incubating birds in the two treatment groups calculated according to the model were below (within 26%) the values determined by the doubly labelled water studies (Table 5.10). This indicates that an unidentified component of the Great Tit's energy budget had been omitted or, more likely, one or more of the component costs was underestimated.

The energy saved resulting from the heating treatment was calculated by considering costs deriving from the period between 20:00 and 04:00 hours, when the bird was incubating through the night and the heating apparatus was effective. The total cost calculated for control birds over this period was 38.53 kJ, and for heated birds was 28.03 kJ. Thus an energy saving of 10.50 kJ can be attributed directly to the heating treatment.

Even though heated birds had a longer night incubation session than control birds, the heating treatment enabled them to reduce their total energy expenditure through the night session by 8.5 kJ (Table 5.10). The energy devoted to incubation during the active day was approximately equal for the two groups, but heated birds were able to make further energy savings of 3.8kJ, as re-heating costs and the cost of activity were both reduced due to the shorter active day and fewer recess periods.

The effect of nest attendance on energy expenditure was further investigated by varying the duration of daytime incubation sessions for control birds in the model, other parameters being held constant. Daily energy expenditure showed a decelerating decline with increasing session length (Figure 5.15). Thus birds which are able to maintain long incubation sessions were also able to maintain a low daily energy expenditure. However, energy expenditure would escalate for those birds whose incubation sessions were short. The qualitative nature of these conclusions is independent of the apparent inaccuracy in the absolute values of the budget.

5.4.8 Incubation feeding in the Great Tit.

The number of nest visits observed in a 10 minute period during an incubation session varied from 0 to 3 (Figure 5.16). The mean visit rate (and presumed feeding rate) was 0.76 visits in 10 minutes. Incubating Great Tits spent, on average 660 minutes incubating during the course of the active day (mean for control birds, Section 5.4.4.1). Thus, at the observed feeding rate, an incubating female could expect to receive 50 feeds (on average) per day during the time she was on the nest.

Although heated birds received fewer feeds than control birds (means in 10 minutes, control = 0.83, heated = 0.68), the difference was not significant (Mann Whitney U-test, $z = -0.264$, $p = 0.792$, $n = 37$ observation periods from 14 birds).

Table 5.9 **Parameters used to model the energy budget of incubating Great Tits according to treatment group. Sources are described in the text. T_a represents the temperature to which the bird is exposed (°C).**

	Control	Heated
Body mass (g)	21.49	21.49
Clutch size	8	8
Egg mass (g)	1.72	1.72
Nest attendance:		
Night session length (min)	590	647
Session length (min)	29.6	39.2
Recess length (min)	8.0	8.0
% recess time spent :		
Foraging	85	85
Flying	2	2
Other	13	13
Cost ($Jg^{-1}h^{-1}$)		
Incubation	$262.949 - 8.999T_a$	$262.949 - 8.999T_a$
Foraging	$219.426 - 3.075T_a$	$219.426 - 3.075T_a$
Flying	11.7 x BMR	11.7 x BMR
Other	$107.875 - 1.795T_a$	$107.875 - 1.795T_a$
Basal metabolic rate ($Jg^{-1}h^{-1}$)	$18.468\ mass^{.46}$	$18.468\ mass^{.46}$

Table 5.10 **Modelled energy budget of incubating Great Tits according to treatment group. Costs are expressed in kJ ind. $^{-1}$d^{-1}.**

	Control	Heated	Difference (C- H)
'Steady-state' incubation (night)	34.27	25.76	+8.51
'Steady-state' incubation (day)	31.38	31.61	-0.23
Re-warming eggs	4.92	3.75	+1.17
Recess period activity	12.20	9.58	+2.62
Total D.E.E. (estimate)	**82.77**	**70.70**	**+12.07**
D.E.E. measured by doubly labelled water.	**111.20**	**95.71**	**+15.49**

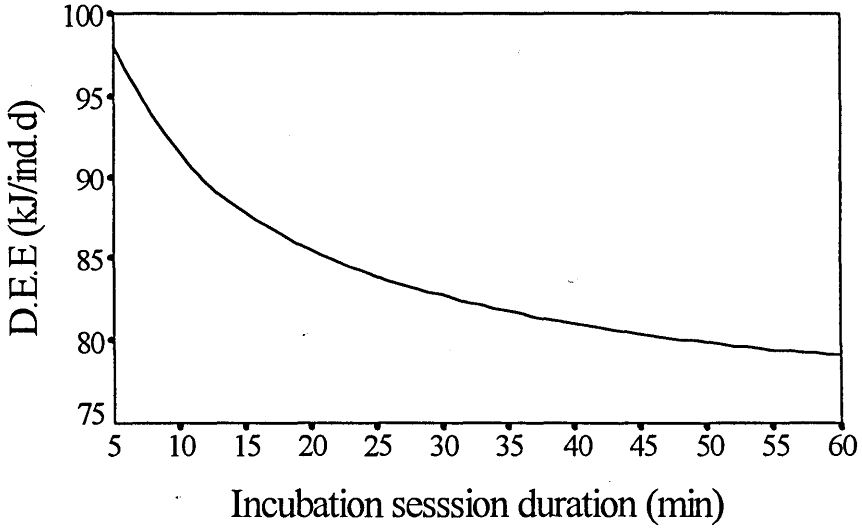

Figure 5.15 **Modelled daily energy expenditure (kJ ind. $^{-1}$d^{-1}) of incubating Great Tits in relation to the duration of incubation sessions (min).**

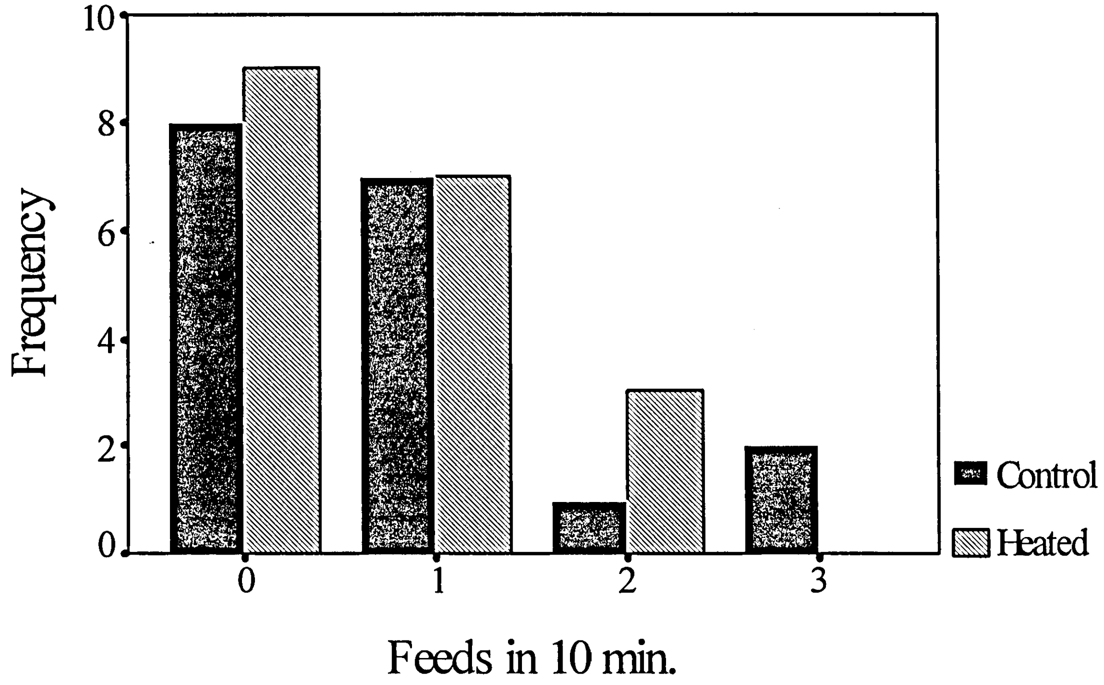

Figure 5.16 Frequency distribution of the number of nest visits by male Great Tits during 10 minutes of continuous incubation by the female. Bars show the results according to treatment group.

5.5 Discussion.

5.5.1 Field metabolism of incubating Great Tits.

This study is the first to measure energy expenditure for incubating Great Tits by means of the doubly labelled water technique. In the past, the energy demands of incubation in the Great Tit have been estimated by biophysical modelling of the heat resistance of the incubating bird, egg and nest (Mertens 1977). Mertens' model predicted a metabolic rate of approximately 0.6W at an ambient temperature of 8°C, or 1.75 x BMR, based on the predictions of Aschoff and Pohl (1970) for a 21.5g passerine. A new technique, employing the use of heat flux discs to measure the heat output of an incubating bird (Mertens 1980), measured levels of metabolism in incubating Great Tits up to 1W, or 2.95 x BMR $_{(AP)}$,during continuous nocturnal incubation, at temperatures below 8°C. The present study was the first to measure the costs of behaviour both on and off the nest during incubation, by applying the doubly labelled water technique to measure field metabolic rate during free-living incubation in the Great Tit. It suggests an average daily rate of working that exceeds previous estimates in this species.

The results of this study were consistent with the idea that incubation represents a three-fold increase above basal levels of metabolism for hole-nesting passerines (Westerterp and Bryant 1984, Westerterp and Drent 1985, Moreno and Carlson 1989, Moreno *et al.* 1991, Williams 1991, Moreno and Sanz 1994). In this study, mean Metabolic Intensity was measured at 3.47 x BMR$_{AP}$ (Aschoff and Pohl 1970) or 2.63 x BMR $_{H}$ (Hashim 1996).

The daily energy expenditure of incubating female Great Tits was greater than that measured by the doubly labelled water technique for females feeding nestlings in the Netherlands (Tinbergen and Dietz 1994), although the difference was not significant (for control birds in this study, mean D.E.E. = 111.2 kJ ind^{-1}d^{-1} ± 39.77 $_{(8)}$, for females feeding nestlings (Tinbergen and Dietz 1994), D.E.E. = 95.1 kJ ind^{-1}d^{-1} ± 14.8$_{(32)}$, $t_{(38)}$ = 1.126, p >0.2). This result is consistent with recent evidence obtained by the doubly labelled water technique that energy expenditure during incubation is generally comparable to that during nestling rearing (Weathers and Sulllivan 1989, Tatner and Bryant 1993).

The sensitivity of Metabolic Intensity to measures of BMR is exemplified by the difference between MI$_{AP}$ and MI$_{H}$. Although the two measures were highly correlated

(Pearson correlation coefficient, $r = .996$, $n = 22$, $p < .001$), MI_{AP} was significantly higher than MI_H, (paired samples t-test, $t_{(21)} = 15.73$, $p < .001$), the mean difference being 0.872 ± .260 units. Both measures were of equal value for comparative analysis within the data set, but comparisons with MI taken from other studies should be made with caution.

Of particular comparative interest is the concept of a maximum sustainable work rate (Drent and Daan 1980). It has been suggested that energy expenditure above a certain work rate cannot be maintained without incurring some somatic cost. Drent and Daan (1980) proposed that costs occur above some threshold level, and postulated a Metabolic Intensity of 4 x BMR as this threshold. For 14% of incubating Great Tits in this study, MI_{AP} exceeded this proposed maximum working level, whereas all values of MI_H were within the limit. If costs are incurred in a threshold manner, and the threshold is indeed related to basal metabolic rate, it is important to determine which measure of BMR is more accurate. Incubating birds may contain a high proportion of metabolically inert tissues (water, lipid, Mertens 1987). Estimates of basal metabolism derived from results relating basal metabolism to the body mass of non-breeding birds, but based on incubation mass would give an inflated estimate of true basal metabolism, and hence an underestimate of M.I.

5.5.2 Factors affecting body mass in incubating Great Tits.

Great Tits are known to undergo progressive mass loss throughout the incubation period (Van Balen 1973, Mertens 1987). In this study, there was no evidence that either mass, or residual mass (corrected for structural size) of birds at first capture declined with the stage of incubation. However, 68.2% of birds lost mass over the course of the doubly labelled water measurement period.

The magnitude of daily mass loss has been estimated by means of repeated captures by van Balen (1973) in Oosterhout, as 0.11g d^{-1} ± 0.05, during the early incubation period (until 9.4 days after last egg laid), rising to 0.18 g d^{-1} ± 0.06 during the transitional period from incubation to nestling rearing, and falling to 0.06 g d^{-1} ± 0.07 during the latter stages of nestling rearing. For birds near Arnhem, Mertens (1987) estimated mass loss of incubating female Great Tits to total 1.913g, 0.002g and 1.449g over the course of incubation in 1980, 1981 and 1982 respectively. He notes that 1981 was an exceptional year in terms of high temperatures during the incubation period. If we assume 1980 and 1982 to be more representative samples, and more comparable in terms of temperature with Stirling, and assume an average period of 14 days for the duration of incubation (Gibbs 1950), the average mass loss calculated (0.121 g d^{-1}) is of similar magnitude to that measured by Van Balen (1973). Mass change for incubating females in this study (mean change was -0.125g d^{-1}) was similar to these values, suggesting that the doubly labelled water technique did not affect behaviour in such a way as to influence mass.

The maximum calorific contribution of this mass loss can be estimated under the assumption that all mass loss was due to lipid metabolism. Avian adipose tissue yields 37.2 kJ g^{-1} (Johnstone 1970), so a mass loss of 0.125 gd^{-1} could provide, at most, 4.65 kJ, or 4.2% of the average daily energy expenditure, based on the doubly labelled water results for the control group.

Mass loss through incubation has been interpreted as evidence that birds are unable to balance their energy budget at this time (Ricklefs 1974). An alternative explanation is that females deliberately reduce their mass through incubation in order to reduce flight costs during the nestling rearing period (Freed 1981, Norberg 1981), at the same time enabling incubation schedules which can only be achieved via some degree of programmed energy imbalance (Sherry *et al.* 1980, Moreno 1989b). It is possible that these two hypotheses are not mutually exclusive (see Section 6.1.3.2 for discussion).

The timing of mass loss during incubation in the Great Tit favours the 'mass - adjustment' hypothesis, as mass loss is most rapid before the peak demands of nestling rearing (van Balen 1974). However, the extent of reserve depletion through incubation increased at low temperatures (Mertens 1987). During cold periods, an inability to satisfy the energy budget may result in loss of mass to an extent that would not occur under better conditions. The advantages in maintaining a high incubation mass were evidenced by the positive association between both session and recess length and incubation mass. Birds in better condition were able to sustain a schedule of lengthy incubation sessions, which is energetically more efficient than repeated periods of foraging and subsequent re-heating of eggs. In other species, high female body mass during incubation has been shown to be an important determinant of nest attendance (Aldrich and Raveling 1983, Jones 1987b), and of the probability of nesting success (Gloutney and Clark 1991, Mallory and Weatherhead 1993,Chaurand and Weimerskirch 1994, Jones 1994, Sanz 1995, Wiggins *et al.* 1996), and to be associated with short incubation length (Lifjeld and Slagsvold 1986, Siikamaki 1994, Sanz

1995), higher hatching success and the production of more and/or larger fledglings (Lifjeld and Slagsvold 1986, Sanz 1995).

Experimental manipulation of nocturnal temperature could provide an opportunity to evaluate the extent to which body mass in incubating Great Tits is determined by programmed reserve utilisation or an inability to satisfy the energy budget. In this study, the difference in mass change between the two treatment groups did not achieve significance (p= .06), though heated birds tended to lose more mass than control birds. Several factors could influence the different strategies in the two groups.

The primary difference between the control and heated birds could arise from the thermoregulatory saving overnight. During cold periods in mid-winter, birds respond to a decline in temperature by increasing their daily weight gain in order to support a higher rate of nightly weight loss (Haftorn 1989). Even if a similar response occurred during incubation, the heating treatment cannot be compared simply to milder natural conditions. Under natural conditions, nocturnal temperatures can be predicted from those of the preceding day (Kessel 1976), and, similarly, daytime temperatures may be predicted from those of the preceding night. In this experiment, day time and night time temperatures were uncoupled over a period of time that was too short for nocturnal temperature to become predictable.

Wansink and Tinbergen (1994) studied the response of Great Tits to nocturnal temperatures which were uncoupled from those experienced during the day. In their experiment, night temperature was maintained at either 16°C (warm) or -8 to -5°C (cold) for periods of seven nights. Mass loss overnight was found be greater on cold nights (warm nights: -1.08g; cold nights: -1.60g). Consistent with the response observed in mid-winter (Haftorn 1989), daily net food consumption and metabolisable energy intake both increased with decreasing night time temperatures. If incubating birds in the present study responded in a similar manner, a greater amplitude of diurnal mass cycle for the control birds compared to the heated would result. In the present study, the two measurements of mass were made in late afternoon, 48 hours apart in this study. If control birds were accumulating more mass during this time, in order to attain a higher peak mass at the end of the active day than heated birds, the observed tendency for heated birds to lose more mass by the end of the measurement period could be explained.

In the present study, birds were exposed to the heating treatment for 3 nights only, so the predictability of night temperature was less than for Wansink and Tinbergen's (1994) birds. The risk of being unable to balance the energy budget is greater when conditions are unpredictable, so birds may be expected to guard against this risk by carrying greater fat reserves as insurance (McNamara and Houston 1990). The response of Great Tits to unpredictable temperatures has been studied by Bednekoff *et al.* (1994). The fat reserves of control birds, maintained at 8.5 ° C, were compared with birds exposed to a pseudo-random sequence of warm (15.5 ° C) and cold (1.5 ° C) periods, the temperature changing each evening after 'lights off'. Conditions differed from the present study in that temperatures were manipulated throughout complete 24 hour periods, rather than at night only. Bednekoff *et al.* (1994) found that evening weights were indeed higher under the regime of unpredictable temperatures than under the constant temperatures, but the result was mostly due to an increased weight gain on cold days, rather than an overall maintenance of higher nutrient reserves. It was concluded that birds were not able to respond to the artificial, unpredictable manner of the environment.

If incubating females were similarly regulating their mass according to short term conditions only, both heated and control birds would anticipate and prepare for the same night time conditions, based on the conditions they both experienced during the preceding day. Any difference in reserve depletion overnight could be evidenced by a gradual depletion of mass in control birds compared to heated birds, but equally, could be obscured by differential incubation and foraging schedules by the two groups during the following day. By the time mass was measured in late afternoon, both heated and control birds could again be prepared for the same type of night, and hence carry the same reserves.

These factors serve to confuse the response of incubation mass in this study. A better test of the effect of energy imbalance on the incubation mass of Great Tits would remove the element of unpredictability in the experiment, by the application of the heating treatment throughout the incubation period. It would also be necessary to monitor the amplitude of the diurnal mass change throughout the course of the day, by the use of automated nest balances. Two snapshots, taken 48 hours apart cannot provide information regarding the mass gains and losses experienced through the day and night for the two groups.

5.5.3 Extent of male provisioning during incubation in Great Tits.

In Great Tits, as in many other species, the male provides his partner with food during the course of reproduction. The male begins provisioning around the time that the first egg is laid and continues throughout the course of incubation (Royama 1966). Feeding occurs both while the female is on and off the nest; the latter accounting for the major part of provisioning (Royama 1966).

In his study of Great Tits in Japan, Royama (1966) estimated that feeding occurs on average, once for every minute that the female spends away from the nest, giving a mean value of 160 feeds off-nest per day. Feeding on the nest was more variable, ranging from 33 times in 9 days, to 164 times in 5 days (Royama 1966). In this study, nest visit rates were calculated to give a mean of 50 visits per day. This value could exceed that measured by Royama (1966) because of differences between the Scottish and Japanese populations, because sampling in this study coincided with the highest feeding rates of the day, or because not all nest visits involved feeding.

Royama (1966) observed that food items which were brought to the nest differed from those presented away from the nest, estimating that one 2-3 cm caterpillar (of approximately 30 mg dry weight) was offered at each nest visit, whereas smaller eucosomid larvae (average 15 mg) were presented at each feed away from the nest.

Assuming the calorific value of invertebrate material to be 22 kJ/g dry weight (Cummins and Wuydcheck 1971), and assuming an assimilation efficiency of 71.3% for insectivorous species (Bryant and Bryant 1988), the energetic value of food provided by the male would range from 39.4 kJ per day at the lower rate quoted to 53.1 kJ per day at the higher rate. The daily energy expenditure of incubating females was 111 kJ per individual per day under normal (control) conditions, so males may be responsible for the provision of somewhere between 35 and 48 % of the female's daily requirements.

The functional significance of courtship feeding has been variously proposed as a predictor of mate quality (Nisbet 1977), to strengthen the pair bond (Lack 1940, Klujver 1950) and to aid the nutrition of the female during periods of high energy use (Royama 1966). In the Great Tit, the extension of mate feeding beyond fertilisation and the significance of the male's contribution to the female's nutritional requirements provides support for the third 'nutritional' hypothesis. In other gynelateral incubators, incubation feeding has been shown to be important to the condition of the female (Lifjeld and Slagsvold 1986), to nest attendance (Lyon and Montgomerie 1985) and to the length of the incubation period (Lyon and Montgomerie 1985, Nilsson and Smith 1988).

5.5.3.1 Female 'manipulation' of male provisioning rate.

Mate feeding can have important advantages to the female. Females can increase their energy intake rate when foraging off-nest by two and a half fold by receiving food from the male rather than solely feeding themselves (Krebs 1969). By receiving food on the nest, they avoid the need to leave eggs unprotected and pay re-heating costs, while satisfying their energy budget. Royama (1966) introduced the notion, developed by Smith (1980), that rates of provisioning by the male could be under the influence of manipulation by the female. Certainly, a potential mechanism for manipulation exists, as the female's begging calls elicit provisioning behaviour in the male, in much the same way as nestlings or fledglings encourage their parents' feeding response. There are likely to be advantages for the male who adjusts provisioning effort to the requirements of his mate, in order to maximise reproductive benefits to the pair (Lyon and Montgomerie 1986). Lifjeld and Slagsvold (1986) attempted to investigate the extent to which female Pied Flycatchers manipulated their partners by handicapping certain females, by removing certain primary and tail feathers, in an attempt to lower their body condition. They predicted that handicapped birds should induce their partners to feed them more frequently, but no such effect could be demonstrated. However, in the same species, Smith *et al.* (1989) were able to demonstrate that males decreased their rate of incubation feeding according to female nutritional state, when females were experimentally provided with additional food.

Heating of nest boxes could provide an excellent opportunity to study the roles played by males and females in decisions relating to courtship feeding. The effects of a heated night, whatever form these take, will accrue to the female alone. Any resultant change in male behaviour must be mediated through transfer of information regarding the female's requirements to the male. Under the 'demand' hypothesis, control birds would demand and receive more feeds from their partners than would heated birds. The results of this study, although consistent in direction with this hypothesis, are not sufficient to provide conclusive support.

The restriction of observation in this study to periods when the female was on the nest neglects feeding off-nest, which is thought to be nutritionally more important (Royama 1966). Not only is feeding on the nest more limited and more variable than that away from the nest during recess periods (Royama 1966), but the former is generally initiated by the male, whereas the latter is much more susceptible to female manipulation by variation in begging behaviour. The use of radio-tagging to aid in observations of both females and males during recess periods could enable study of this important variable.

5.5.4 Energy saving associated with the heating treatment.

In this experiment, the nests of incubating Great Tits were warmed throughout the night in order to produce an energy saving in the demands of thermoregulation for 'heated' birds. Records of the nest air temperature throughout the night (Figure 5.6), show that the treatment had the desired effect on nest box temperature. Several assumptions should be discussed before the heating treatment can be assumed to represent a thermoregulatory saving.

If the heating effect was to produce the same energy saving for birds at all temperatures, it is necessary that two conditions hold. Firstly, the increase in temperature should not differ according to ambient temperature, and secondly, the metabolic rate of the female should be linearly related to temperature within the range of nest air temperatures in both control and heated boxes.

Evidence suggested (Section 5.4.3.) that the heating treatment produced a simple increase in nest air temperature and did not vary according to ambient temperature. Amongst incubating temperate zone passerines, a linear relationship between metabolism and temperature has been shown to be common (Mertens 1977, Vleck 1981, Biebach 1984, Haftorn and Reinertsen 1985). In this study, all nest temperatures were assumed to fall below the lower critical temperature of the thermoneutral zone. For non-incubating female Great Tits in summer, the lower critical temperature is 29°C (Hissa and Palokangas 1969), and incubation generally has the effect of raising the lower critical temperature (Biebach 1984). Mertens (1977) estimated that female Great Tits, incubating a clutch of 7 or more eggs, must increase their heat production at temperatures below 25°C. In this study, the maximum hourly mean nest air temperature recorded for control birds was 26.11 °C, with 99.6 % of temperatures below 25 °C. For heated boxes, the maximum recorded temperature was 32.03 °C, with 98.7 % of hourly means below 25 °C . Thus, experimental conditions did, in general, lie below the lower critical temperature, though occasionally, a combination of mild conditions and effective heating treatment produced temperatures within the thermoneutral zone. For the most part, the effect of the heating treatment could be assumed to produce a standard increase in nest air temperature, resulting in a standard reduction in the costs of thermoregulation for all birds.

Recent evidence has shown that, under certain circumstances, the rate of nocturnal metabolism may depend more on the level of reserves present at evening than on overnight temperature (Reinertsen and Haftorn 1986, Bednekoff *et al.* 1994). Given that a certain degree of flexibility in the relationship between metabolism and temperature exists, the assumption that birds have no choice but to accept the energy saving produced by the heating treatment needs to be examined. Great Tits have been shown to demonstrate nocturnal hypothermia, but only in situations where access to food has been restricted such that evening weights are depressed below their normal level (Reinertsen and Haftorn 1986). During spring in Britain, it is unlikely that either temperatures or food scarcity are severe enough to induce this behaviour. The only evidence for nocturnal hypothermia amongst incubating birds comes from Orange Breasted Sunbirds (Williams 1993). Their exceptionally high rate of working (Metabolic Intensity = 6.5) could make them highly susceptible to inclement conditions and hypothermia may be an essential means of avoiding energy imbalance. However, amongst the Hummingbirds, which generally use torpor at night as an energy conserving mechanism outwith the breeding season, homeothermy has been found to be maintained during incubation in both Anna's (Howell and Dawson 1954) and the Calliope Hummingbird (Calder 1971).

It seems safe to conclude that the experimental protocol has achieved the aim of reducing thermoregulatory costs by a significant proportion of the daily budget.

5.5.5 Alternative effects of heating the box.

Heating the box may have affected birds in ways other than the simple energetic saving and its effect on female reserves at dawn. It has been suggested that birds use various environmental cues, for example increasing day-length, bud burst (Van Balen 1974), prey abundance (Perrins 1965) and temperature (Kluyver 1952, van Balen 1973, Nager and van Noordwijk 1995) to anticipate future conditions and modify reproductive strategies accordingly. It is possible that night-time temperature may be used as a predictor of future conditions, either of environmental conditions during the following day, or of resource availability later in the season and hence the optimal time to rear chicks. The possibility that the behavioural response to the heating treatment was due to an alteration in strategy, based on modified predictions of future conditions must be considered, before interpreting the results as the effects of the thermoregulatory saving *per se*.

Following a heated night, the first decision that an incubating female must make is when to leave the nest for the first recess of the day. This decision is probably under the control of a variety of motivational stimuli. Ambient temperature and direct sunlight affect invertebrate activity and ease of capture and, hence, Tit foraging efficiency (Kacelnik 1979a,b, Avery and Krebs 1984), so altering the benefit function of time spent off the nest. Ambient temperature also, independently, affects the rate of egg cooling, so modifying the costs associated with a recess period. Mate behaviour, such as singing near the nest, could encourage female departure (Kendeigh 1952). Female reserve level at the end of the night, perceived at a proximate level by hunger motivation, is likely to play a large part in the decision.

In this study, two of these stimuli may have differed between the two treatment groups: perception of ambient temperature and female reserve level. Before leaving the nest, the female's perception of ambient temperature must be based exclusively on her perception of conditions within the nest box. Heated birds may have estimated that conditions outside the box were warmer than, in reality, they were. Higher ambient temperature would serve to increase foraging efficiency and to decrease egg cooling rates, so the balance would be in favour of time off-nest, and the predicted shift would be towards earlier nest departure. Under natural conditions, Haftorn (1981a) found evidence for birds to leave the nest earlier on warmer days.

Conversely, if the decision was influenced primarily by female reserve depletion throughout the night, we would expect heated females, with a reduced nocturnal metabolic rate, to reach a threshold level of depletion some time after control birds, and so to postpone the first foraging trip of the active day.

In this study, heated females were observed to leave the nest later than their control counterparts, so the evidence supports the 'reserve depletion' hypothesis.

Under natural conditions, night time temperature is correlated with conditions the following day (Kessel 1976) and may provide a reliable cue to those conditions. Using environmental data for the period from 1st April to 31st May, for 1991-1995 inclusive from Parkhead weather station, it was possible to demonstrate a positive correlation between minimum nightly temperature and maximum temperature the following day, controlling for the effect of date, (Pearson's partial correlation coefficient $r = 0.20$, $p = 0.001$, $n = 300$), although the predictive quality of this association was low ($r^2 = .04$). Based on this relationship, if maximum temperature were to be predicted from the minimum temperatures experienced overnight by a control and a heated bird, the latter 6°C warmer than the former, predictions of maximum temperature the following day would differ by 1.2°C.

Could the observed change in incubation schedule result from an adjustment in behaviour of heated birds in line with their expectations of an additional 1.2°C during the day? Haftorn (1981a) found some evidence that incubation sessions decreased in length with increasing ambient temperature, while recess periods increased in length. If heated birds were adjusting their behaviour to their expectations of daytime temperature, they would demonstrate a reduced nest attendance, contrary to the observed effect.

The application of the heating treatment to the hours of darkness only was intended to allow both groups of birds access to the same environmental variables during the daytime. Responses which are based on night time conditions, but not flexible to modification in the face of unexpected daytime conditions, seem highly unlikely to be successful in a population that is adapted to the unpredictable nature of the British weather.

Finally, birds may use night temperatures to predict the timing of the caterpillar peak, and hence the optimal time for eggs to hatch. Caterpillar development is dependent on temperature (Perrins 1979), so temperature early in the season may give a good indication of the timing and size of the peak in food availability. If heated birds anticipated that the optimal time of hatch was sooner than that detected by control birds, they could increase their nest attendance, in order to shorten the time to hatch. Such a scenario would provide results consistent with those observed in this study.

However, Nager and van Noordwijk (1995) failed to find any significant correlation between spring temperature and caterpillar peak date. Once again, the access of the two treatment groups to the same environmental variables, temperature, daylength and prey abundance, would seem to count against this possibility. Decisions based on one variable, such as night time temperature, in spite of conflicting information from other sources, would be likely to be unreliable and not established in the population.

5.5.6 Diversion of energy to incubation.

It seems safe to assume that the heating treatment did induce an energy saving overnight. So, following a heated night, incubating birds would find themselves with greater reserves than control counterparts. Birds could use this saving to prevent mass loss, or to reduce the pressure on foraging throughout the subsequent day, either by a reduction in foraging rate, or in the time devoted to foraging. Given the time constraints imposed on incubating birds, foraging rates may be high (Walsberg and King 1978b) and potentially energetically costly, inefficient or highly risk-prone (Lima and Dill 1990). Heated birds were found to increase their nest attendance, and consequently to reduce their total foraging time in preference to decreasing the foraging rate or preventing mass loss, indicating the high priority attached to nest attendance.

Several birds with low body mass deserted the nest at first capture (Table 5.8). If these cases represented those individuals who were in poor condition or of low quality, then the remaining individuals, for whom data on energy use and nest attendance were available, would not form a representative sample of the total population. If, in reality, the population included a higher proportion of low quality individuals than did the present sample, the energetic constraint of incubation could be more significant than indicated by this study.

5.5.7 The advantages of high nest attendance

In this study, birds responded to a reduction in the competing demands for energy by increasing nest attendance. Future studies should extend the heating treatment throughout incubation, in order to determine whether the altered incubation schedule could enable birds to reduce the length of the incubation period.

A reduction in the total time spent away from the nest would not only reduce the risk of eggs chilling (Webb 1987) and of nest predation (Clark and Wilson 1981), but would also increase the average egg temperature during the active day. A negative correlation has been found between average egg temperature and the duration of incubation in the Great Tit (Haftorn 1983), suggesting that birds may be able to influence the rate of embryonic development by altering their incubation schedules and so manipulate time to hatch. Zicus *et al.* (1995) found total incubation length to be positively related to the mean daily recess period in the Common Goldeneye. The total time spent on the eggs was nearly identical between birds, regardless of the total duration of the period from laying to hatch, suggesting that incubation in this species required a minimum of nest attendance and that time to hatch was prolonged by increasing the time spent away from the nest.

An additional advantage of the incubation schedule adopted by heated birds is that the extended night session and lengthy daytime incubation sessions constitute an energetically efficient way of maintaining egg temperature (Section 5.4.7). Total energy budgets were lower for heated birds during periods of cold weather than for control birds. Thus heated birds would avoid some costs arising from particularly high levels of energy use (Drent and Daan 1980).

5.5.8 Translation between the currencies of energy and time.

The thermoregulatory saving attributed to the heating treatment has been estimated as 10.50kJ per individual (Section 5.7). Thus, heated birds find themselves with an 'extra' 10.50kJ in reserves over their control counterparts at dawn. This saving enables the devotion of an additional 51 minutes to incubation during the following day.

The time equivalence of 10.50 kJ of metabolisable energy can be expressed in two ways. Firstly, the saving can be expressed as the length of time for which incubation could be sustained by 10.50kJ. From Equation 5.11, which describes metabolism during steady-state incubation in the Great Tit, incubation by a 21.49g bird, requires 3.72kJh^{-1}, so the given saving could, theoretically, sustain 2.82 h of continuous incubation. A second approach is to calculate the time which a control bird would need to spend foraging in order to gather 10.50kJ of metabolisable energy. Foraging rates were calculated using the allometric equation of Bryant and Westerterp (1980), Equation 5.17.

Eqn. 5.17 Allometric equation for foraging rate in passerines (Bryant and Westerterp 1980)

$$F_r = 0.92\, W^{.94},$$

F_r is foraging rate in kJh^{-1} and W is body mass in g.

Net metabolisable energy gain was calculated by subtracting the energy costs of foraging (described in Equation 5.9), and assuming an assimilation efficiency of 0.713 for insectivorous birds (Bryant and Bryant 1988). In this way, foraging was calculated to yield

9.89 kJ h^{-1} net metabolisable energy, hence it would take a control bird 1.06h of foraging in order for its nutrient status to be equivalent to that of the heated birds.

The observed difference in nest attendance between heated and control birds (mean = 51 minutes) corresponds most closely with the estimate of time taken to forage for extra reserves. That the increase in nest attendance was slightly less than the estimated foraging time suggests, perhaps, that heated birds did not respond to the energy saving by re-investing maximally in incubation, but that part of the saving was used elsewhere in the budget. The foraging rate during recess time may have been reduced to a less risk-prone level.

It is interesting to note that the higher nest attendance of heated birds was manifest via a lengthening of both day-time and night-time sessions, whilst no change in recess length was apparent. Perhaps recess length is more a product of the balance between egg-cooling rates and foraging efficiency than a response to internal reserve level.

5.5.9 Energetic limitation during reproduction in the Great Tit.

Though traditionally viewed as a low cost activity (Walsberg and King 1978b), the possibility of energetic limitation during incubation was recognised by Yom Tov and Hilborn (1981). Modelling the energy reserves of the female Great Tit through the breeding cycle, these authors found that incubation constituted the first and most severe of two 'energy bottlenecks' or low points in energy reserves, in the reproductive process. The present study provides evidence that female Great Tits were, indeed, limited in the amount of time they could devote to incubation by the energy available to them.

However, other stages in the reproductive cycle are also subject to proximate constraints and it is important to see incubation in the context of these stages.

The timing of egg laying in the Great Tit may also be subject to an energetic constraint, as evidenced by the response to both temperature and food availability. It is well established that spring temperature is significantly correlated with the date of laying in both Great and Blue Tit populations (Kluyver 1952, Perrins 1965, Van Balen 1973, Nager and van Noordwijk 1995). Even small differences in nest box temperature can have a significant effect on lay date (O'Connor 1978, Dhondt and Eykerman 1979). Experimental manipulation of nest box temperature, using methodology similar to the present study during the pre-laying and laying periods, provided additional evidence in support of energetic-limitation during the egg-laying stage. In the Great Tit, increasing nocturnal temperature, in one study, advanced the onset of laying, while birds in cooled boxes commenced laying later than controls in one of two years (Netherlands Ins. Ecol.1993-1994). However, in a second study, laying date was not advanced in heated boxes compared to cooled birds (Nager and van Noordwijk 1992), but females in cooled boxes produced smaller eggs than did females in heated boxes. Nocturnal temperature increase during the laying period in the Blue Tit was found to reduce the frequency of laying interruptions (Yom Tov and Wright 1993).

Food availability also has important consequences for the timing of laying (Martin 1987). Laying has been advanced in the Great and Blue Tits by the experimental provision of supplementary food (Kjällander 1974, Nilsson and Svensson 1993, Nilsson 1994). An inadequate supply of either energy or of specific nutrients, such as calcium (Graveland 1996, Perrins 1996) during laying can delay the onset of egg production.

The consequences of this delay are evidenced in the decline in reproductive success that is apparent throughout the season. The negative correlation between post-fledging survival and date of hatch can be quite dramatic in the Great Tit (Perrins and M^{c}Cleery 1989). Correlations of this sort in natural populations could result, at least in part, from differences in the quality of birds laying at different times in the season (reviewed by Barba *et al.* 1995). However, female Great Tits that were delayed from egg-laying by the presence of Sparrow nests occupying the available nest site (a factor presumably unrelated to the quality of the Great Tit pair) were found to produce smaller clutches which took longer to hatch, fledged fewer and lighter young and were less likely to lay a replacement clutch than birds which were not delayed (Barba *et al.* 1995), suggesting a real cost to a delay in egg-laying.

Recent evidence has demonstrated that a reproductive cost of breeding too early also exists. Nager and van Noordwijk (1995) have shown that the most successful breeders are the birds which synchronise the period of brood rearing with the period of maximal food availability, rather early breeders *per se.* In two studies, Blue Tits (Nilsson 1994) and Pied Flycatchers (Sanz and Moreno 1995) were 'tricked' into laying earlier (Nilsson 1994) or laying more eggs (Sanz and Moreno 1995) by the experimental provision of food, which was removed by incubation. In both cases, manipulated birds suffered from reproductive costs, which probably arose from an inability to incubate successfully. Experimental Blue Tits (Nilsson 1994) postponed the onset of incubation relative to clutch completion, and were subsequently liable to a higher rate of total clutch loss than control birds. A reduction in nest attendance, leading to egg chilling or nest predation could explain the latter result. In the case of the Pied Flycatcher (Sanz and Moreno 1995), hatching success was reduced for experimental birds, again indicating problems in incubating adequately.

The emerging picture is one where reproduction involves the interplay of constraints at various stages in the reproductive cycle. It seems that both egg-laying and incubation are energetically constrained such that birds generally cannot lay earlier than they do, while the time of hatch must be matched to the peak in food availability as closely as possible. The duration of incubation is, therefore, very important in matching the achievable laying date with the optimal hatch date as closely as possible. The factors governing these constraints, food availability and temperature will vary from year to year, while the timing of optimal hatch date may not be well predicted by the conditions during laying (Nager and van Noordwijk 1995). The ability to regulate the rate of incubation, varied by nest attendance, could provide an important degree of flexibility, or phenotypic plasticity in the time period between the lay and hatch dates.

Evidence for such a flexibility is very difficult to distinguish from evidence of an energetic constraint during incubation. The results of Nilsson (1994) cited above, show that birds delayed incubation in response to a removal of supplementary food. In the corollary of this situation, Blue Tits were experimentally provisioned with food only after clutch initiation (Nilsson and Svensson 1993). In this case, females responded by advancing the onset of incubation in relation to clutch completion. Both results could provide support for the 'flexibility' hypothesis: birds responded to a change in food supply by anticipation of an alteration in optimal hatch date and hence altered the onset of incubation. However, the same results could also be explained by the 'energetic limitation' of birds through incubation : females were unable to achieve normal incubation under current conditions of temperature and food availability. The results of the present study are open to the same differences in interpretation.

However, reductions in hatching success (Sanz and Moreno 1995) and increases in total clutch loss (Nilsson 1994) could not result solely from an alteration in the progress of incubation. These results suggest that the response is due to an inability of birds to incubate effectively under current conditions, and confirms the 'energetic limitation' hypothesis.

Chapter 6:

General Discussion.

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6.1 Measuring the costs of reproduction.

6.1.1 Methodological approach.

In order to examine the forces that determine strategies of reproductive investment, it is important to identify both the costs and the benefits of a certain reproductive programme. Considerable effort has been invested to the identification of reproductive costs in a variety of species (reviewed by Bell and Koufopanou 1985, Lessells 1991, Stearns 1992, Roff 1992 and, for birds, by Lindén and Möller 1989). Reznick (1985) classified studies of the cost of reproduction according to the methods employed, defining four groups:

(i) **phenotypic correlations** based on non-manipulated populations,
(ii) **experimental manipulation** of reproductive effort and examination of a phenotypic response, compared to that of a control group,
(iii) **genetic correlations** between reproduction and some component of fitness, obtained by sib. analysis
(iv) genetic correlations under conditions of **artificial selection** for a particular reproductive trait.

There has been some controversy over the value of each method. As Reznick (1992) argued, natural selection will only act on characters exhibiting genetic variation, so only the last two methods are able to provide conclusive proof of a cost of evolutionary significance. Indeed, the proportion of studies finding evidence of costs is higher for the last two methods than those based on phenotypic characters (Roff 1992). However, Partridge (1992) has defended phenotypic studies and Lessells (1989) describes how the scale on which phenotypic experiments can be performed mean that useful data can be obtained.

Studying correlations between phenotypes in certain natural populations, a link has been demonstrated between reproductive effort and mortality (Bryant 1979, Ekman and Askenmo 1986). However, such studies are unable to distinguish causation from correlation. The results may be confused by the existence of confounding variables. Several studies have failed to find a negative correlation between reproduction and survival, some have even demonstrated a positive correlation between the two (Högstedt 1980, 1981).

In the present study, the second approach was employed, involving phenotypic manipulations and monitoring the subsequent response. Reproductive effort was manipulated in the Dipper and the Swallow via alteration of the clutch size (Chapters 3 and 4). In both these experiments, individuals were randomly assigned to one of three treatment groups (Enlarged, Control or Reduced) and the clutch duly manipulated by the addition, exchange or removal of eggs. It has been argued that such manipulations are 'artificial' (Klomp 1970), but clutch size in this study was manipulated within the natural range only. Ideally, comparisons should be made between groups of manipulated birds only (Nur 1984a). For Swallows, eggs in the control group were transferred between nests, so that nests in all treatment groups were subject to the same degree of disturbance. Comparisons between the 'E', 'C' and 'R' groups were, therefore, considered to be valid. The Dipper work extended previous studies, which differed somewhat in their aims. In order to maintain comparable methodology as closely as possible between studies, Dipper eggs were not transferred between nests in the control group. However, all Dippers continued normal incubation following clutch manipulation, suggesting that the transfer of eggs was accepted by the birds.

6.1.2. How should clutch size be manipulated?

The majority of clutch or brood size manipulations were carried out by addition or removal of a certain number of eggs or nestlings, as outlined above (Hegner and Wingfield 1987, Pettifor *et al.* 1988, Dijkstra *et al.* 1990, Lerkelund *et al.* 1993, Wheelwright *et al.* 1991). However, some controversy surrounds the validity of this approach, which assigns a manipulated clutch size that is some function of the original (or natural) clutch size (a 'relative' manipulation). An alternative approach, the random allocation of a manipulated clutch size to each bird, irrespective of the original clutch size (an 'absolute' approach), was advocated by Nur (1984a) as being the best way to separate the effects of individual quality and clutch size, and has been applied by several experimentors (Lessells 1986, Reid 1987). Unfortunately, determination of which type of manipulation is most valid relies on information that is generally not available to an investigator.

If natural clutch size is determined by aspects of the quality of an individual or habitat which persist from egg-laying to incubation (Individual Optimisation Hypothesis), the appropriate manipulation would alter the clutch size by a discrete amount (e.g. one or two eggs). In this way, all birds in each treatment group would experience the same additional costs relative to their own 'quality', or incubation ability and circumstances. In this situation, allocation of a random clutch would produce heterogeneous groups of birds, incubating the same number of eggs, but consisting of some individuals whose effort had been increased beyond that which they 'intended' and others whose effort was reduced.

However, if natural clutch size does not reflect the quality of the bird or habitat during incubation, a 'relative' manipulation, involving addition or removal of one egg would be inappropriate, as it would inflict a range of costs on the individuals within a treatment group, dependent on the clutch size which they 'happen' to have inherited from the laying stage. In this case, a random allocation of clutch size, independent of natural clutch would be more valid.

There is no way to determine, *a prioiri*, which of these scenarios best describes the actual situation. Correlations between clutch size and variables such as female age, breeding density and date of laying (Ward 1992, van Balen 1973) would suggest that some factors are involved in the determination of clutch size which could also influence incubation ability. However, short term factors, such as immediate food availability during laying, also seem to play a part in the determination of clutch size (in the Swallow, Ward 1992). If conditions during laying were to change by the time birds were incubating, a 'relative' manipulation would result in treatment groups of mixed effect, with manipulated clutch size determined by stochastic events that occurred pre-incubation. A similar situation could arise if the costs of incubation are dependent to a greater extent on the number of eggs (or clutch mass) to be heated, than on the incubation ability of the parent. In this case, an 'absolute' manipulation, involving the random allocation of clutch size and comparison of the results amongst groups of birds with the same manipulated clutch size would be more appropriate.

In the present study, it was decided that in the three species concerned, at least some part of the costs during incubation would be determined by factors that similarly affect natural clutch size, and manipulations were, therefore, made relative to the natural clutch size for Dippers and Swallows. In an experiment with the Great Tit, clutch size was not manipulated, but the availability of energy to the incubating bird was increased by a reduction in the demands of thermoregulation. In this case, reproductive effort was considered to be constant amongst birds, maintained at the level appropriate to the natural clutch.

6.1.3 Short term correlates of lifetime fitness.

Ideally, having manipulated the reproductive trait in question, the consequences should be assessed in terms of the lifetime fitness of the individual, or more correctly, the genotype. Inclusive fitness (Hamilton 1964) is determined by the reproductive output of the individual at the current and all future breeding attempts, as well as the survival and fecundity of all offspring arising from each brood. In practice, measurement of inclusive fitness in a natural population is almost impossible. Components of fitness which can be readily measured are therefore normally chosen, to give a short-term measure which is likely to correlate with fitness in the longer term. Three types of variable were employed in this study to provide such an index of reproductive cost;

(i) measures of reproductive success at the current breeding attempt,
(ii) measures of mass or 'body condition',
(iii) rates of energy expenditure.

6.1.3.1 Measures of current reproductive success.

A suite of variables was chosen on the grounds that changes in each could influence reproductive success at the current attempt.

Patterns of nest attendance were monitored in all three species. These can influence the duration of the incubation period and hatching success (Lyon and Montgomerie 1985), the effects presumably being mediated via the influence of attendance on egg temperature (Haftorn 1983). As average egg temperature falls below the optimal range, the rate of development may slow and the risk of embryonic abnormality or mortality through chilling increases (Webb 1983). An unattended nest is also more likely to fall to predators (Bukacińska *et al.* 1996).

The influence of nest attendance on egg temperature. For each species, the range of egg temperature at which optimal development takes place is rather narrow; typically peak survivorship occurs at continuous exposure to temperatures between 36°C and 40°C (Webb 1987). Above this range, the risk of death through hyperthermia rapidly becomes important, while limited exposure to temperatures below the optimum is less likely to be lethal. A lower critical temperature, known as the physiological zero temperature (Drent 1975) is usually defined as between 25-27 °C (White and Kinney 1974). Below this temperature, embryonic development ceases, but may be resumed after moderate periods of exposure if normal incubation temperatures are restored. If egg temperature is maintained for long periods in the range above the physiological zero but below the temperatures of optimal development, there is a high risk of sub-lethal effects, such as abnormalities in organ growth in the developing embryo.

Egg temperature during normal incubation generally averages somewhat less than the optimum. For gynelateral incubators such as the Dipper, Swallow and Great Tit, this results in part, from the periodic cooling of eggs throughout the day as the bird leaves the nest to forage.

The influence of recess duration on egg temperature was investigated for the Dipper, the Swallow and the Great Tit by modelling egg cooling rates within a normal recess period. Egg temperature during continuous incubation averages 36.0°C for the Great Tit (Haftorn 1983), 34.4°C for the Dipper (Ward 1992) and 35.7°C for the Swallow (Turner 1985). These values were assumed to represent egg temperature at the start of a recess period. Average cooling rates were calculated from the literature for Swallows (Jones 1985) and Great Tits, (this study), based on the average temperature of the nest air amongst the eggs throughout the active day, as recorded by the probes of the nest temperature monitor. These cooling rates, presented in Table 6.1, represent a conservative estimate of actual cooling rate, as air temperatures will quickly fall below the daily mean as the female leaves the nest, by a combination of air disturbance and convection. Cooling rates for Dipper eggs represent a mean value measured in the field across the natural range of temperatures (Ward 1992).

Eggs cool most rapidly following the initial departure of the female, as the temperature gradient between eggs and nest is then greatest. Accurate data on the optimum temperature range for development in these species was not available, but results from other species (Webb 1983) suggests that egg temperature drops below the optimal range within 1 or 2 minutes following departure. For each species, the time taken for egg temperature to drop below 25°C (assumed physiological zero) was calculated ('tPZ'). The results are shown in Table 6.1.

Figure 6.1 illustrates this time period (tPZ) in relation to the frequency distribution of recess periods for the three species. For the Great Tit and the Swallow, recess periods were rarely long enough for eggs to cool to physiological zero; 98.2% and 99.5% respectively of the observed recesses were within the calculated tPZ. However, in the case of the Dipper, only 71.1% of recesses were within tPZ: in 29% of Dipper recesses, egg temperature would have dropped below physiological zero before the female returned to the nest.

This difference between species could result from a difference in measurement of egg cooling rate. For the Great Tit and Swallow, this was extrapolated from laboratory studies, to give a cooling rate based on the average temperature of air between the eggs, while measurement for the Dipper took place in the field, and was an average value for the range of temperatures recorded (Ward 1992). However, even if less conservative cooling rates were calculated for the Great Tit and Swallow, assuming eggs were exposed to air temperatures equivalent to the mean values of nest air temperature, measured above the incubating female, rather than that between the eggs, 98.2% of Great Tit and 99.5% of Swallow recess periods still remained within the modified estimate of tPZ. It seems that Dipper eggs are more commonly exposed to temperatures below physiological zero than those of Swallows and Great Tits. Factors such as the level of humidity or air turbulence in a Dipper's nest close to running water may be responsible for the observed rapid cooling rate.

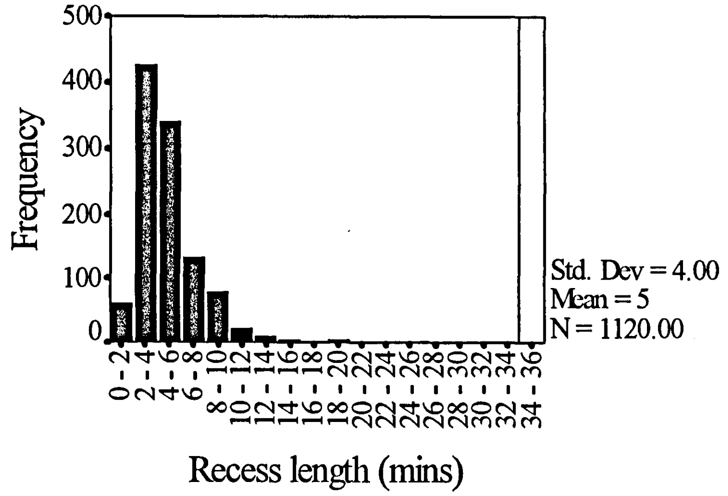
Factors that serve to increase the length of recess periods in Swallows and Great Tits are likely to affect the risk of embryonic abnormality, by prolonging the time that egg temperature remains between 25 and 35 °C. In the Dipper, longer recess periods may be more likely to influence the duration of incubation, by extending the amount of time that embryonic development is suspended at temperatures below physiological zero. In each case, reduced nest attendance can have a detrimental effect on hatching success.

Table 6.1 **Initial egg temperature, average cooling rates and the calculated time for eggs to cool to physiological zero (tPZ) for Swallows, Dippers and Great Tits. The percentage of observed recess periods falling within this time period are presented in the final column.**

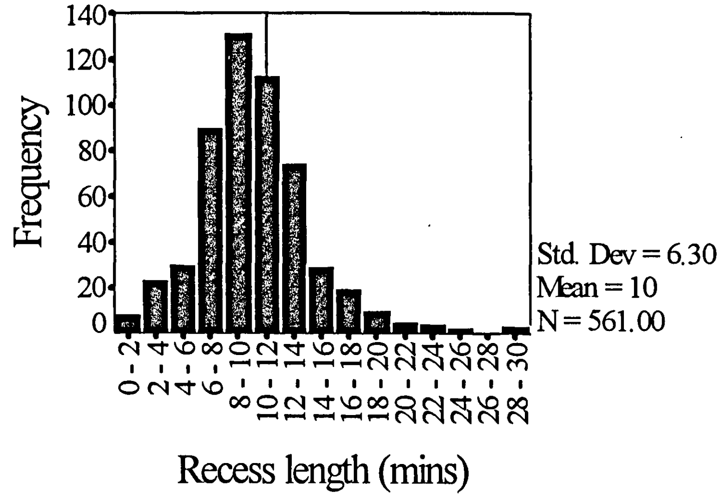
Species	Egg temperature during incubation (°C)	Average temperature of air amongst eggs. (°C)	Cooling rate (°C min^{-1})	tPZ	% of recess periods within tPZ.
Swallow	35.7	20	0.397	27	99.5
Dipper	34.4	22	0.906	11	71.1
Great Tit	36	18	0.766	20	98.2

Figure 6.1. **(overleaf) . Frequency distribution of recess length (min) for (i) Swallows, (ii) Dippers and (ii) Great Tits in relation to the time taken for eggs to cool to physiological zero at average nest temperatures (tPZ), shown by the solid line. Data represent 1120 observations from 17 Swallows, 561 observations from 26 Dippers and 393 observations from 22 Great Tits. The illustrated distributions represent at least 99% of each data set, recess periods of over 30 minutes duration being excluded for clarity.**

(i) Swallow



(ii) Dipper



(iii) Great Tit.

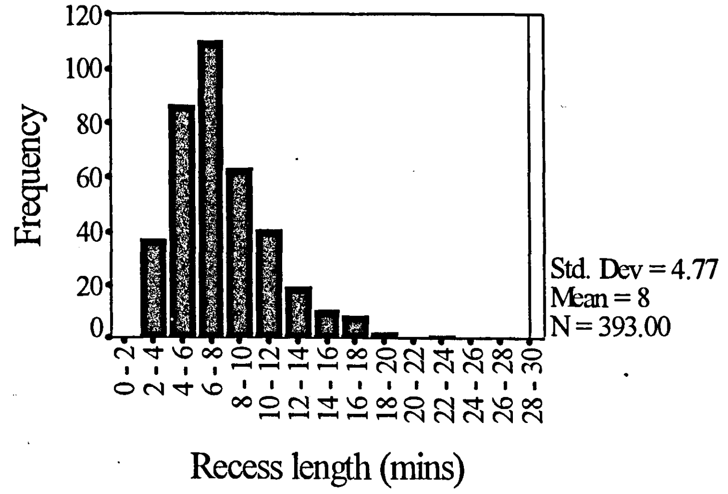


Figure 6.1.

The duration of the incubation period. This was measured in relation to clutch size in the Swallow. There may be selective advantages in minimising the time between clutch completion and hatching of the young, as the fledgings from early hatching broods are more successful in some species, (Perrins 1979, Kikkawa 1980. Arcese and Smith 1985). The incubation period can be associated with a high risk of predation for both the female and her eggs (Clark and Wilson 1981), hence minimising the duration of incubation can reduce this risk of mortality (Bukaciñski *et al.* 1996).

The decline in egg density. This results from the loss of water throughout incubation and has been used in some studies as an index of the rate of development of embryos and hence the duration of incubation (Székély *et al.* 1994). The mass lost over the course of incubation, reported for a range of species with various incubation periods, has been shown to vary little between 14 and 18% of fresh egg mass (Rahn and Ar 1974, Drent 1975, Rahn *et al.* 1976, Morgan *et al.* 1978). This relationship can be represented as follows:

Eqn 6.1. $M_{H2O} \times I = K$

where M_{H2O} is the daily rate of water loss, expressed as a proportion of fresh egg mass, I is the incubation length in days and K is a constant, between 14 and 18%. Although the relationship seems to hold between species (Rahn *et al.* 1976), it has yet to be validated within a species.

The rate of water loss from developing eggs, to which the loss of egg mass during incubation can be almost entirely attributed (Grant *et al.* 1982), depends on a species-specific water vapour conductance, based on the pore geometry of the shell, the water vapour pressure in the egg (determined by egg temperature) and that in the nest (regulated by nest ventilation) (Rahn *et al.* 1976). The importance of nest-attendance schedules, and the influence of nest position on air movements and nest air humidity in accounting for variation in K within a species has yet to be quantified.

In this study, the duration of incubation was not well represented by the rate of decline in egg density in the Swallow (Spearman rank correlation coefficient, r = 0.004, Section 4.3.4). This suggests that, in this species, factors other than the duration of incubation may influence the rate of mass loss in such a fashion that egg density is not a valid indicator of the rate of embryonic development.

Hatching success. This can provide a measure of the incubation ability of a bird. In order for this to translate to an increase in fitness, parents should be able to successfully feed the number of chicks hatched. Increased effort during incubation could reduce the feeding ability of parents, so it is important to investigate the longer term implications of reproductive effort at this particular stage. Most studies which investigated the evidence of reproductive costs associated with increased effort during incubation were not concerned with reproductive success following hatch. (Moreno and Carlson 1989, Smith 1989, Székély 1994, Moreno and Sanz 1994, Siikamaki 1995). Those that were (Moreno *et al.* 1991, this study) have found no evidence that incubation effort affects brood-rearing capacity. However, the condition of the female at hatch, which may be affected by the effort during incubation (Moreno and Carlson 1989), can influence the condition of the young at fledging (Lifjeld and Slagsvold 1986), so it would seem profitable to continue investigation of effects beyond the incubation period in future studies.

Subsequent breeding performance. Ideally, the future survival and success of both parents and offspring following manipulation of reproductive effort would be investigated in subsequent breeding seasons. The effects of increased reproductive effort during the brood rearing stage have been identified in the subsequent survival and performance of both parents (Nur 1984a, Reid 1987, Lessells 1989, Wernham 1992) and offspring (Pettifor *et al.* 1988, Smith *et al.* 1989) at subsequent breeding attempts. This study was the first to evaluate the influence of incubation effort with a first clutch on the production of a second clutch: there was no evidence to suggest an intra-seasonal cost associated with incubation effort in the Swallow. However, more studies are needed to investigate whether the mechanisms underlying the long-term effects of brood-rearing effort could result in similar costs being associated with incubation effort.

6.1.3.2 Body condition as a measure of reproductive cost.

The 'body condition' of an individual is a measure of its nutrient reserves. Although difficult to measure (Piersma and Davidson 1991), body condition is commonly used to represent energy balance. Body condition may be crudely estimated by body mass. However, body mass is a function of many other variables as well as energy reserves: structural size, gut contents, body water and growth or senescence of tissues may all be influential. A size-adjusted measure of body mass attempts to control for structural size. Further refinement of the estimate can be made by measuring specific body components, particularly the size of the protein and lipid reserves. Lipids constitute the major energy reserve in birds (Blem 1990), as their breakdown is both efficient and productive in terms of energy yield (37.7-39.7 kJ g^{-1} for avian adipose tissue (Johnstone 1970)). Although protein is not routinely used as an energy store as its breakdown is inefficient (Blem 1990), the protein reserve may be depleted under extreme conditions to yield 18 kJ g^{-1} . Various studies have reported the importance of the protein reserve in the control of aspects of reproduction (Jones and Ward 1979, Ankney and MacInnes 1978, Houston *et al.* 1993, Houston *et al.* 1995 and references therein) as well as in the timing of moult and migration (Blem 1990).

While the analysis of carcass composition can yield detailed information on the size of body components, (Jones and Ward 1979), its destructive nature precludes study of the subsequent behaviour or physiology of individuals. In live birds, the extent of lipid reserves have been estimated with variable accuracy by scoring the visible deposits of subcutaneous fat (Krementz and Pendleton 1990, Scott *et al.* 1995). Protein reserves are commonly estimated in live birds from the size of the pectoralis muscle. A variety of techniques is available to determine the size of this muscle. The ultrasonic flaw detector has produced accurate and repeatable measures of muscle thickness (Baldassare *et al.* 1980, Sears 1988, Newton 1993). Measures of the muscle profile can be made using solder-wire (Bolton *et al.* 1991) or alginate gel (Selman and Houston, in press). A measure of total body lean mass can also be obtained by measurements of the total body electrical conductivity (TOBEC) (Walsberg 1988).

In this study, body condition was estimated by raw mass data, or size-adjusted mass, where this removed a significant proportion of the variation. In the Swallow, fat reserves were also estimated by scoring subcutaneous fat depots and an ultrasonic flaw detector was employed to measure pectoralis muscle thickness. Mass, fat score and measures of pectoralis thickness (USMEAN) and volume (USVOL) were all inter-correlated (see Section 4.3.1.6). For a sample of 35 incubating females which were captured more than once, the change in mass was associated with a corresponding change, in both direction and in magnitude, in fat score ($r = .5146$, $p < .001$) and in the size of the pectoralis muscle (mass change with USMEAN $r = .5057$, $p=.002$, with USI $r = .5895$ $p < .001$).

In many species of bird, female mass increases prior to laying and subsequently declines throughout the breeding season. This loss of mass is due, in part, to the regression of the reproductive organs, but the remainder has commonly been interpreted as evidence of reproductive stress (Nice 1938, Ricklefs 1974) (the 'reproductive stress hypothesis'). Indeed, high levels of reproductive effort have been linked with higher levels of mass loss in certain species, (Hussell 1972, Askenmo 1977, Bryant 1979, De Steven 1980, Nur 1984a, Finke *et al.* 1987, Hegner and Wingfield 1987, Tombre and Erikstad 1996).

However, certain lines of evidence suggest that reproductive stress cannot be solely responsible for the patterns of mass loss exhibited during breeding. Firstly, the most rapid loss of mass is commonly observed shortly after eggs hatch, before the peak in energetic demands which is associated with nestling rearing, (Freed 1981, Jones 1987, Jones 1994). Secondly, experimental studies have shown mass loss to persist in spite of supplementary feeding (Sherry *et al.* 1980, Hillstrom 1995, Merkle and Barclay 1996), casting doubt on the 'reproductive stress' hypothesis. An alternative explanation, the 'mass adjustment hypothesis', postulates that the reduction of mass, apparently triggered by hatching (Gaston and Perin 1993) has adaptive value, as it can reduce the high, mass-dependent flight costs experienced during the active stage of nestling rearing (Freed 1981, Norberg 1981, Croll *et al.* 1991).

Moreno (1989b) has categorised species according to the pattern of mass change demonstrated throughout the breeding season. In some, generally small species, mass remains relatively stable throughout the incubation period, declining sharply post-hatch (defined as incubatory mass constant or I.M.C.). Other species exhibit a gradual decline in mass from egg laying to the nestling rearing period (incubatory mass loss or I.M.L.). In the latter group, patterns of behaviour, namely high nest attendance, that can only be achieved via controlled energy imbalance, are made possible by the programmed use of reserves.

If some component of mass loss during breeding is adaptive, is it valid to interpret a decline in body mass or condition as evidence of a reproductive cost? Evidence suggests that maintaining a certain mass during incubation can have important consequences for reproductive success. Nest attendance declined dramatically below a certain condition threshold for both Swallows (Jones 1987) and Canada Geese (Aldrich and Raveling 1983). Nest desertions were more common amongst individuals with low body mass in Common Goldeneyes (Mallory and Weatherhead 1993), Blue Petrels (Chaurand and Weimerskirch

1994), Kestrels (Meijer *et al.* 1989), Pied Flycatchers (Sanz 1995) and Collared Flycatchers (Wiggins *et al.* 1996). Successful nesters were heavier than unsuccessful individuals in Mallards and Northern Shovelers (Gloutney and Clark 1991) and in Least Auklets (Jones 1994). In the Pied Flycatcher, females that were heavier during incubation had shorter incubation periods (Lifjeld and Slagsvold 1986, Siikamaki 1994, Sanz 1995) and a higher hatching success, and produced more and larger (Sanz 1995), or heavier (Lifjeld and Slagsvold 1986) fledglings. In the Wood Duck, females that were heavy during incubation had a greater chance of surviving to breed the next year in one of three years of study (Hepp *et al.* 1990).

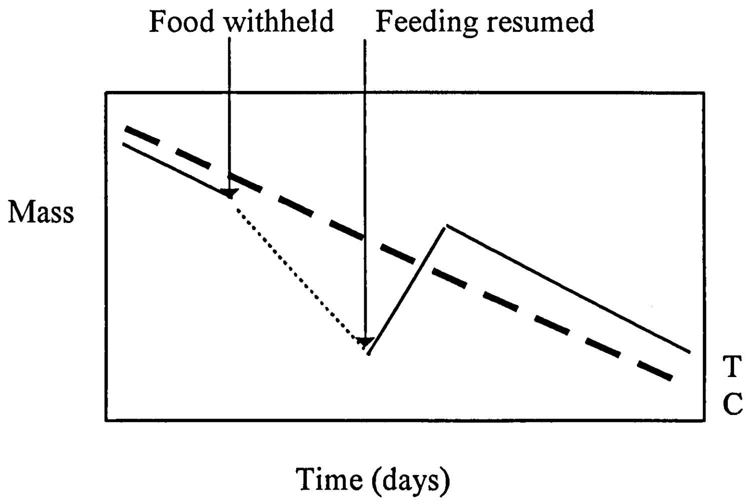
Thus, maintaining mass at least above a certain level seems to be advantageous during incubation. Hillström (1995) suggested that the 'reproductive stress' hypothesis and the 'mass adjustment' hypothesis may not be mutually exclusive. A limited decline in body mass may be of adaptive value, where it allows adjustment from the high levels of reserve storage during egg laying to the lean, flight-adapted individual for nestling rearing. In IML species, mass loss may also sustain an incubation schedule that would be impossible without some degree of energy imbalance. However, if mass falls below some critical threshold, a somatic cost arises that can reduce fecundity or survival.

Thus, in a combined hypothesis, birds aim to regulate their degree of mass loss in order to achieve a final optimum mass for nestling rearing, but can be disrupted from this pattern by environmental or reproductive stress. In the Canada Goose, the variation in mass at the onset of incubation is greater than that at the end, heavier females losing more mass (Aldrich and Raveling 1978), providing evidence that, in this IML species, birds aim towards a final optimal mass. The Pied Flycatcher maintains a constant mass throughout incubation, then loses mass rapidly during brooding to achieve a lower constant mass through brood-rearing (IMC) (Sanz and Moreno 1995). Manipulation of the length of the brooding period in this species did not affect the total mass loss between incubation and nestling rearing. Instead, the rate of mass loss was adjusted, such that birds with enlarged brooding periods lost mass less rapidly over a longer period of time. In the same species, Hillström (1995) found no reduction in the rate of female mass loss when food was experimentally added to territories. Merkle and Barclay (1996) demonstrated a similar result for breeding Mountain Bluebirds. These manipulative studies suggest that mass loss is programmed to attain an optimal mass for nestling rearing, whether this be fixed or variable in relation to environmental conditions.

Certain studies suggest that conditions of stress can lead to 'undesirable' mass loss in incubating birds. Environmental conditions, such as low temperature, can perturb birds from their mass schedules. Mertens (1987) showed that the energy content of incubating Great Tits declined by 2.3kJ for every °C decrease in average temperature over an 11 day period preceding sacrifice. The mass of incubating Swallows was lower on days of low insect availability (Jones 1987, this study), suggesting that birds experience difficulties in achieving their optimal mass under such conditions. Direct evidence of the response of an incubating bird to a perturbation from its schedule of mass loss was found by Sherry *et al.* (1980), working under laboratory conditions with the Burmese Red Junglefowl. In this IML species, the programmed schedule of mass loss was disturbed by an enforced fast, in two treatments, either early or late in the incubation period. The results of the study are presented diagramatically in Figure 6.2 (a) and (b). In both cases, fasting increased the rate of mass loss compared to a control group. On resumption of feeding following the fast, both early and late fasters gained mass for a period, whilst control birds were losing mass, in order to return to the programmed mass loss exhibited by control birds.

If birds show this kind of response to environmental stress, it may be anticipated that a change in reproductive effort, for instance a change in clutch size, could produce a similar effect on mass. Such a response has recently been presented for lactating Red Squirrels (Humphries and Boutin 1996). Lactating mammals commonly accumulate energy reserves during the early stages of reproduction in order to supplement foraging during periods of energetic stress during late reproduction. Augmentation of the litter size in lactating Red Squirrels produced a greater increase in mass during the early stages of lactation, attributed to an increase in fat stores. However, 'augmented' females subsequently lost more mass during the latter stages of lactation, so the net mass loss did not differ from the control group. These results suggest that Squirrels use the demands of early lactation to predict the demands later in the season, and store an appropriate level of reserves to accommodate those demands. Despite the energetic demands of lactation, it seems that Squirrels are able to rapidly adjust their energy budgets in response to manipulation of reproductive effort, to increase energy balance from a slight positive, to a highly positive balance. In this study, manipulated clutch size was found to have no significant effect on the mass, fat score or size of the pectoralis of Swallows at the end of incubation. Similar results were found in the Bengalese Finch (Colemann and Whittall 1980), the Kentish Plover (Székély 1994) and the Collared

(a) Starvation during early incubation.



(b) Starvation during late incubation.

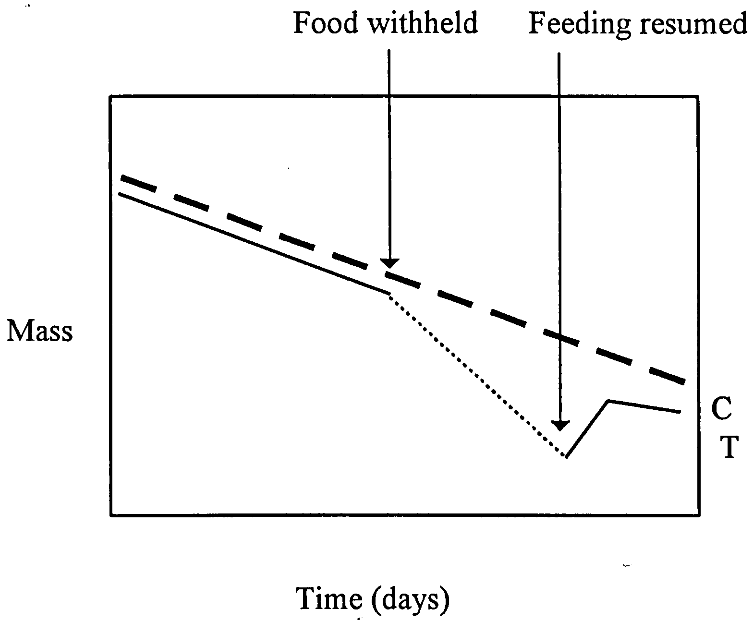


Figure 6.2 Diagrammatic representation of the results of Sherry *et al.* (1987), on the mass of incubating Burmese Red Junglefowl. The treatment group (T) were exposed to periods of starvation, represented by the pecked line in either (a) early or (b) late incubation. The mass of a control group of incubating birds (C) is represented by a heavy dashed line in each figure. Time in days since the start of incubation is shown on the x-axis.

Flycatcher (Moreno *et al.* 1991). In the Pied Flycatcher, two studies found no evidence of a response of mass to clutch size, (Siikamaki 1994, Moreno and Sanz 1994), while in a third (Moreno and Carlson 1989), birds with enlarged clutches lost significantly more mass than those with reduced clutches.

The general inability to detect difference in condition between females with manipulated clutches at the end of incubation could be interpreted as indicating the lack of an effect of clutch size on female condition. However, the results of Sherry *et al.* (1980), illustrated in Figure 6.2, and those for Red Squirrels (Humphries and Boutin 1996) suggest a temporally complex pattern of mass changes, which would only be detected by repeated measurements throughout the incubation period. Most studies measure mass at the end of incubation, or, at best, changes in condition between the end of laying and hatch and, as such, would not detect such changes. Use of automated precision balances under the nest throughout the incubation period enables the detection of losses of condition that are subsequently recouped (Jones 1985, 1987b).

In Dippers, an increase in clutch size seemed to produce an immediate increase in the rate of mass loss occurring between clutch manipulation and first capture 1-2 days later, resulting in a significantly lower mass for birds with enlarged clutches at first capture. This was followed by a tendency for ‘enlarged’ birds to gain mass during the subsequent 2 days of the doubly labelled water measurement period, compared to controls, although the difference did not attain significance. During this time, birds who gained mass spent a higher proportion of their recess time foraging and had higher daily energy expenditure. These results are consistent with those of the Junglefowl. Following an initial decline in body condition resulting from disruption of the energy budget, birds increased their foraging effort, increasing daily costs as necessary, in order to restore mass to the ‘programmed’ level.

Maintenance of an adequate lipid store during incubation is likely to be advantageous in providing insurance against unsuccessful foraging during the little time spent off-nest (Witter and Cuthill 1993). It can also enable longer incubation sessions by increasing fasting endurance (Williams 1992), and is probably less disadvantageous in terms of increased flight costs than at other stages in the annual cycle as the incubating bird is sedentary for a high proportion of the day (Witter *et al.* 1994).

While the results of single mass measurements can provide an insight into the average response of a large number of birds, the complexity in the temporal response pattern of incubation mass to stress requires continual mass recording in order to give more valuable information. Use of an automated nest balance under incubating Swallows on enlarged and reduced clutches showed how birds at both nests were generally able to maintain a similar mass, but in a period of poor weather, the female on the enlarged clutch lost mass while the female with a reduced clutch gained mass (Jones 1987). Such a response could remain undetected by a single capture event.

In summary, losses in mass or condition through breeding must be interpreted as evidence of stress with caution (Bryant 1991). Continuous recordings may give insight into short term responses which may be missed by individual measurements (Jones 1985, 1987b). Manipulative experiments, involving comparisons with a control group are essential in order to separate the influence of reproductive or environmentally induced stress and adaptive mechanisms.

6.1.3.3 Energy expenditure as an index of reproductive costs.

The energy required for a particular activity is sometimes referred to as the energetic 'cost' of that activity. This can be misleading, as high levels of energy use do not always impose a cost. In some situations, for example brood rearing, high levels of energy expenditure are associated with high rates of brood provisioning, which can represent a benefit to fitness in terms of offspring quality, rather than a cost (Bryant 1992). Under what circumstances can high levels of energy expenditure be costly?

High levels of energy expenditure demand a suitably large energy supply, if energy balance is to be maintained. Thus an increase in energy expenditure may be associated with an increase in the time allocated to foraging, perhaps at the expense of other fitness-enhancing activities, such as nest attendance. More risk-prone foraging behaviours may be adopted in order to balance the budget (M^{c}Namara and Houston 1987, Lima 1988, Lima and Dill 1990, Skutelsky 1996), entailing an increased risk of predation. An inability to balance the energy budget from exogenous sources could increase reliance on endogenous reserves, incurring penalties in body condition that may subsequently reduce fecundity or survival.

Drent and Daan (1980) proposed that energy expenditure increases towards some threshold limit, beyond which a cost is incurred. Hence, energy expenditure is constrained within a 'maximum sustained working limit' (M.S.W.L.). The M.S.W.L. is normally expressed as a multiple of the basal metabolic rate of an individual, as the same metabolic machinery is thought to determine both basal and maximal working rates, (but see Ricklefs 1996 for a discussion of the validity of this assumption).

The maximum level of energy expenditure at which an individual can work is related to the amount of time for which the work rate must be sustained. Thus, while a human athlete can endure bursts of activity, fuelled by anaerobic respiration, at the level of 100 times resting metabolism, this level can be sustained for no more than a few seconds (Peterson *et al.* 1990). Respiring aerobically, wolves can maintain working levels of 32 x resting metabolic rate (RMR) for periods of minutes or even hours (Peterson *et al.* 1990). The aerial forays of Robins require energy expenditure at a rate of 23 x BMR, but periods of such activity are short and interspersed with periods of less costly behaviour (Tatner and Bryant 1986). Peterson *et al.* (1990) suggest that sustained working levels should be defined over time periods that are long enough for metabolism to be fuelled by food intake rather than the transient depletion of energy reserves. Based on this definition, values of sustained metabolic rate for a range of 37 species under various conditions were all under 7 x RMR, and were mostly in the range from 1.5 to 5 x RMR (Peterson *et al.* 1990). Bryant and Tatner (1991) found the field metabolism of free-living birds to range from 1 to 7 x BMR.

Maximum limits to sustained metabolic rate have been estimated by measuring the metabolic rate of animals which are working at levels assumed to be close to their limit. Drent and Daan (1980), studying the energy expenditure of birds feeding nestlings, suggested a level of 4 x BMR as a maximum sustainable working level. Kirkwood (1983) reviewed studies of the metabolisable energy intake of 12 avian and 9 mammalian species under various conditions of stress, and found the resulting intake rate to correlate closely with body mass according to the equation $ME_{max} = 1713 /W^{0.72}$, where ME is metabolisable energy intake in $kJ\ d^{-1}$ and W is body mass in kg.

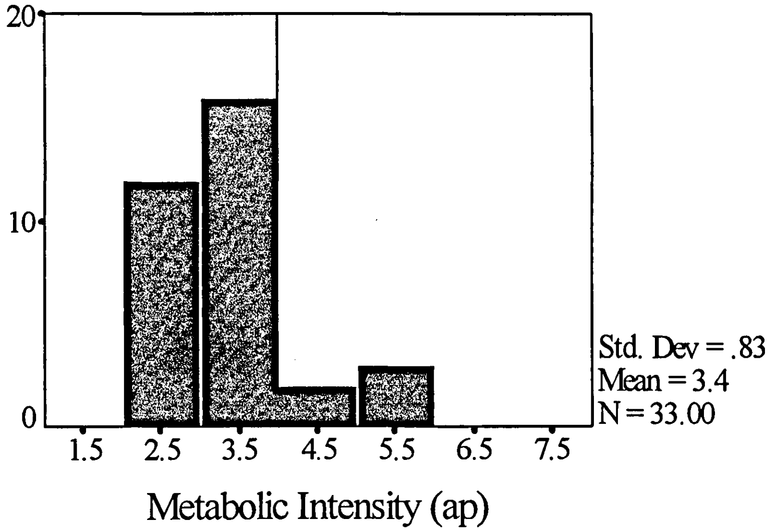
However, Bryant and Tatner (1991) reviewed the energy expenditure of 28 species of small bird, measured at all stages of the annual cycle using the doubly labelled water technique and showed that the ratio of field metabolism to basal metabolism (FMR/BMR) or metabolic intensity (MI) exceed the predictions of Drent and Daan (1980) in up to 48% of species, and up to 30% of individuals, and exceeded the predictions of Kirkwood (1983) for 50% of species and 28% of individuals. The frequency distribution of metabolic intensity for all incubating Dippers and Great Tits labelled in this study is shown in Figure 6.3(a) and (b). Metabolism exceeded 4 x BMR (based on the allometric predictions of Aschoff and Pohl

1970) for 15% of incubating Dippers and for 23% of incubating Great Tits. While this evidence must lead to the rejection of the proposals of Drent and Daan (1980) and of Kirkwood (1983) as to the level of a maximum sustainable working limit, it does not rule out the possibility that such as threshold does exist, but suggests that it may vary in magnitude between species, or even between individuals, or that it may not be described as a simple multiple of basal metabolic rate.

Upper limits to energy expenditure could be maintained in a population if higher energy expenditures were associated with a reduction in lifetime reproductive success. In the House Martin, birds with higher levels of energy expenditure measured during the breeding season had a lower chance of returning to breed the next year than birds with more conservative budgets (Bryant 1992). Reyer (1984) presents evidence of a negative correlation between survival and energy expenditure during breeding in the Pied Kingfisher. Lifetime reproductive success was greatest for birds with intermediate levels of energy expenditure, declining both above and below these levels.

Energy expenditure could also be limited by physiological constraints, for instance, foraging bouts of the Hummingbird are constrained by the time required for the crop to empty (Diamond *et al.* 1986). Periods of inactivity are necessitated in this species by digestive constraints (Krebs and Harvey 1986). Such constraints may be of especial significance for uniparental incubators. The foraging rates of incubating Dippers, Swallows and Great Tits required in order to satisfy the energy budget are discussed in Section 6.3.2. Incubating birds must forage between 1.5 and 3 x the rates observed at other times of the year. Given this fact, it is surprising to find that, for 65% of the time spent off-nest, incubating Dippers are 'inactive'. A large proportion of this time is probably spent looking for predators or for suitable foraging sites, or preening, but gut constraints may also play a significant part. Kenward and Sibly (1977) found that the Woodpigeons, feeding on brassicas, spent around 50% of their time 'resting'. The duration of these rest periods was not reduced when feeding time was experimentally restricted such that birds could no longer maintain their mass. The limiting digestion rate prevented birds from attaining energy balance when feeding time was constrained.

(a) Dippers



(b) Great Tits.

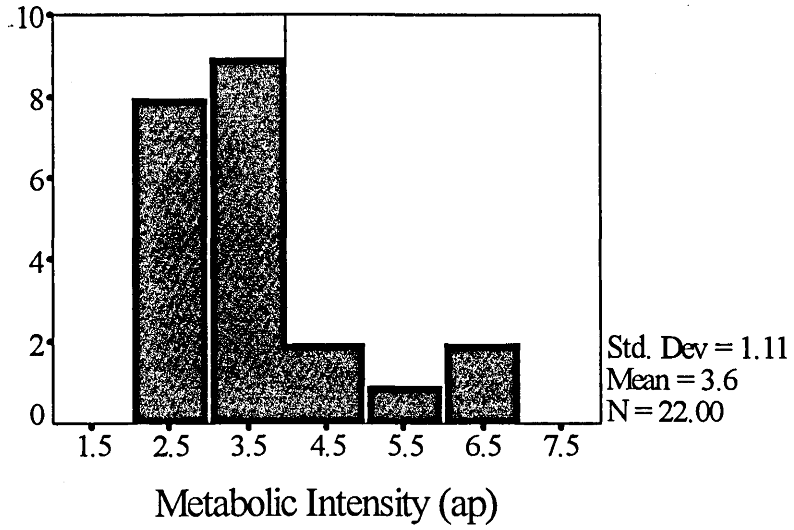


Figure 6.3 **Frequency distribution of metabolic intensity (DEE/BMR calculated according to the equations of Aschoff and Pohl 1970) of incubating (a) Dippers and (b) Great Tits. x-axis labelled by the mid-point of the range. Vertical lines represent a proposed maximum sustainable working limit of 4 x BMR (Drent and Daan 1980).**

The Dipper's digestive system is likely to be adapted to the mode of foraging that operates throughout the majority of the year, where feeding bouts are interspersed between other behaviours throughout the active day. During incubation, the pattern of feeding is atypical, in that resources to last for over an hour must be gathered in approximately 12 minutes. Such intense feeding activity could plausibly require periods of rest to allow for digestive constraints, or to compensate for the intense burst of energetically expensive diving and flying.

In this study, energy expenditure was compared between individuals of the same species at the same stage in the reproductive cycle. Although many of the factors that serve to complicate the comparison of energy use are eliminated in such a sample, the question remains as to the validity of the use of energy expenditure as a correlate of reproductive costs. The daily energy expenditure of incubating Great Tits treated to a nocturnal reduction in the demands of thermoregulation, did not differ significantly from a control group of birds. In this case, observations of nest attendance were required to demonstrate the difference in reproductive investment between the two groups. This example highlights the need for time activity information to supplement data on total energy use in order to construct an energy allocation schedule, before levels of energy use can be interpreted in terms of reproductive costs (Bryant 1988).

6.1.4. The relationship between mass and energy expenditure in relation to costs.

Bryant (1988) suggested that high levels of energy expenditure may be more reliably indicative with a reproductive cost where they are associated with loss in mass or condition. However, where patterns of reserve utilisation are complex (see Section 6.1.3.), a combination of high energy expenditure with mass gain, increased time devoted to foraging and a concurrent reduction of time spent on the nest may indicate a reduction in the benefits gained from the current nesting attempt for incubating birds.

In both Dippers and Great Tits, birds with high daily energy expenditure tended to gain in mass, while those that lost mass had low rates of energy use. Bryant (1988) studied the association between daily mass change (gd^{-1}) and daily energy expenditure for birds during the brood-provisioning phase. The relationship between the two variables differed according to species. For Dippers, a positive correlation between mass change and daily energy expenditure was found, consistent with the results for incubating birds, (though $p>0.05$ for both incubation and brood provisioning). No significant association was found for Great Tits. The results are summarised in Table 6.2.

These results could indicate the kind of cost indicated above, or could arise from differing levels of reserve utilisation, those birds relying more heavily on endogenous reserves losing more mass, whilst those birds that spend time and energy foraging were more likely to gain mass. However, there are two mechanisms by which a spurious association could arise, which should be discussed.

The doubly labelled water technique assumes that the size of the body water pool remains constant throughout the measurement period. In this study, the body water pool was estimated as a constant proportion of the average body mass over the measurement period. However, if the size of the body water pool changes in parallel with the changes in body mass observed above, this assumption would be invalid. The observed correlation between DEE and mass change could be an artefact arising from systematic errors in the calculation of DEE dependant on the size and direction of mass change.

Table 6.2 **Pearson correlation coefficients between changes in body mass (g d $^{-1}$) and daily energy expenditure. Data for the brood-provisioning period are from Bryant (1988), those for the incubation period are the results of this study.**

	Incubation	**Brood provisioning**
Dipper	r = .31	r = .41
	p = .08	p = .06
	n = 32	n = 16
Great Tit	r = .42	
	p = .05	N.S.
	n = 22	n = 17

The effects of such a change on the estimation of energy expenditure was investigated using the equations for carbon dioxide production in the case of a linear change in body water volume described by Nur (1980) (Equation (2)). Under the assumption that the body water pool comprises a constant proportion of body mass, the estimated value of DEE was calculated for certain hypothetical values of isotope enrichment. The resulting estimation of daily energy expenditure was related to the mass change by a negative quadratic curve, almost symmetrical about the origin.

If DEE were calculated under the assumption that mass did not change, while, in reality, mass (and hence body water pool) increased linearly, the calculated value of daily energy expenditure would overestimate the true value. The magnitude of the error would depend on the fraction of body mass that constitutes the body water pool, the mass of the individual and the isotopic enrichment. As a result of the symmetry of the curve, the error would be of the same magnitude and direction were the bird to lose mass in reality. Inappropriate use of the 'constant mass' equation would, therefore, produce over-estimates for cases of both mass increase and mass loss.

Thus, the observed correlations between DEE and mass change cannot be explained by invalidity of the assumptions of the doubly labelled water technique.

Inaccuracies in the correlation between mass change and field metabolism could also result from errors in the estimation of the respiratory quotient, based on diet. For both Dippers and Great Tits in this study, an R.Q. value of 0.75 was adopted to represent mixed protein and lipid metabolism (Brody 1945) for all individuals. However, birds which lost mass were relying, in part, on the metabolism of body fat, so an R.Q. value closer to that of lipid metabolism (0.71 for mixed fat (Brody 1945)) would be more appropriate.

The doubly labelled water technique measures the rate of carbon dioxide production, which is related to an energy equivalent according an estimated R.Q. value. As the energetic equivalent per unit CO_2 production is greater for lower values of R.Q., an over-estimation of R.Q. for those birds which metabolised body fat, would lead to an underestimation of energy expenditure. Weathers and Sullivan (1989) discuss this effect, and describe how the doubly labelled water technique will also overestimate energy expenditure for birds which are gaining mass, in which fat synthesis is significant.

If these problems affected the data for incubating Dippers and Great Tits, energy expenditure would be underestimated for birds who lost mass and overestimated for those that gain mass. Thus the observed relationship could arise through inaccuracies of this type. Weathers and Sullivan (1989) estimated that the problem was insignificant for birds whose mass changed by under 5% per day. As all incubating Dippers and Great Tits were within this range, the effect was unlikely to be problematic in this study.

6.2 The 'cost of living' during incubation.

6.2.1 Measuring the energy demands of incubation.

A certain amount of controversy has surrounded the question of whether incubation is energetically costly, arising in part from disagreement concerning whether the rates of metabolism during incubation should be compared with those of a bird resting within or outwith the nest (Walsberg 1983). However, the energetic cost of incubation can only be determined by comparing the energy use during incubation with the rate of energy acquisition. Comparisons of the energy requirements for different activities are of limited use if temporal or behavioural differences mean that the availability of energy is not constant. An accurate assessment of the energetic constraints during incubation requires information on rates of both energy use and energy gain at this time. A variety of methods have been employed to measure the level of energy use during incubation (reviewed by Grant 1984). In order to account for the difference in energy use between species of various body mass, metabolism during incubation is conventionally expressed as a multiple of basal metabolic rate (but see Gales and Grant 1990).

The earliest attempts to measure the energy requirements of incubation were based on biophysical modelling of the energy required to maintain egg temperature. Kendeigh (1963) developed a formula, described elsewhere (Chapter 4), to calculate the energy input necessary to balance the heat lost from a clutch during incubation. Using this method, the energy requirements of incubation for five species of bird were calculated to range from 15 to 120% of BMR (Drent 1972, Ricklefs 1974). Kendeigh proposed (Kendeigh 1973) that this energy must be produced in addition to the normal requirements of the incubating bird, but King (1973) suggested that at least part of this energy could be supplied as a by-product of metabolism.

An alternative method of calculation was pioneered by West (1960), who considered the clutch mass as though it were an equivalent mass of body tissue. Calculations using this clutch mass method are based on the assumption that the insulative properties of the eggs resemble those of the plumage. Estimates of the cost of incubation for the same five species using this method ranges from 1-153% of BMR (Ricklefs 1974).

The heat budgets of incubating birds of various species have been modelled, by incorporating measures of the heat resistance of bird, clutch and nest. In this way, Walsberg and King (1978a, b) calculated that the insulative properties of the nest were sufficient to compensate for the heat loss to the clutch. For White-crowned Sparrows, Red-winged Blackbirds and Willow Flycatchers, their calculations predicted that energy use during steady state incubation would be 15-18% lower than that of a bird perched outwith the nest. Calder (1973) modelled the thermal environment of the nest of the Broad-tailed Hummingbird, and calculated that a nesting bird could reduce her metabolic rate to one-third of the costs measured at rest at an equivalent ambient temperature, by virtue of the insulative properties of the nest. Similarly, Smith *et al.* (1974) produced biophysical models to estimate the level of nocturnal energy expenditure by incubating Anna's Hummingbirds, and found the calculated metabolism to be somewhat lower than values previously reported for resting birds, due to the maintenance of a high nest temperature during incubation.

Mertens (1977) modelled the heat budget of the incubating Great Tit. His calculations indicated that resting metabolism would be insufficient for the incubation of a clutch of 7 or more eggs, whenever temperatures fell below 25 °C. Metabolic rates of 1.5 x BMR would be required for incubation at the temperatures commonly experienced in the Netherlands during incubation. He estimated that energy demands would decline in the latter stages of incubation when the metabolism of the embryos could make a significant contribution to heat production.

These indirect methods of measurement were restricted to periods of uninterrupted incubation and were limited in accuracy by the knowledge of the thermal system and the precision with which variables such as heat resistance could be measured. Direct measures are potentially more accurate and a number of methods have been applied to incubating birds.

Mertens (1980) developed a novel approach to the question, by using the quantity of heat lost from the nest as a measure of the metabolic rate of an incubating bird. A series of 35 heat-flux discs was positioned in the roof, walls and floor of a Great Tit nest box, and heat loss through the various sections of the box was measured nightly, from the period of nest building until the nestlings fledged. The heat loss, at an ambient temperature of 8 °C was found to be roughly three times greater for incubation than for a resting bird, this increase being much greater than that predicted by his own models (Mertens 1977).

Several authors have attempted to measure the metabolic rate of incubating birds by direct measures of gas exchange. This technique involves the use of the nest as a metabolic chamber. Air can be drawn through the box over the incubating bird and the gaseous composition analysed in order to determine rates of oxygen consumption and/or carbon dioxide production, from which metabolic rate may be calculated. Two early studies, calculating metabolism during incubation in this way, reported figures below those predicted for basal metabolism for Starlings (Ricklefs 1974) and Great Tits (Mertens, cited in Drent 1972). However, the authors suspected inaccuracies in the technique, resulting from air loss through the nest box entrance (Mertens 1977), which would cause rates of metabolism to be underestimated.

Since these early studies, the technique has been applied successfully to several species. The results of this technique have generally provided evidence that, at temperatures below a thermoneutral zone, incubation requires greater energy use than resting metabolism. Haftorn and Reinertsen (1985) were able to compare the metabolic rate of a female Great Tit whilst incubating with that of the same female resting in a non-incubating posture in a nest box within the course of a single night, and found that incubation required significantly more energy at all temperatures below 15° C. Biebach (1981, 1984) obtained a similar result with Starlings, incubation requiring the production of additional energy below 20°C. In addition, both studies found evidence that metabolism increased with clutch size. Vleck (1981) found the energy requirements of incubation to represent an increase of about 20% of the metabolic rate of non-incubating birds, at temperatures below 28° C.

However, other studies have produced conflicting results. Gessaman and Findell (1979) studied incubation in the American Kestrel and, using the same technique, measured the metabolic rates of two females and one male. In agreement with the three studies cited above, the data for one female showed an elevation in energy use during incubation compared to resting metabolism, at temperatures below 15 ° C. However, incubation seemed to pose no additional demands for the other female or for the male. Similarly, studies of two Procellariiform species, the Laysan Albatross and Bonin Petrel measured levels of metabolism during incubation that were lower than those measured for resting, non-incubating birds (Grant and Whittow 1983).However, in the latter study, measurements were only made within the thermoneutral zone. The metabolic rate of the Wandering Albatross was found to be higher during incubation than at rest within the thermoneutral zone (Brown and Adams 1984), but in this study, different techniques were used to measure resting and incubation metabolism, so the difference cannot be unambiguously attributed to incubation (Grant 1984).

The studies outlined above measured the energetic demands of activities within the confines of the nest. Because of the difficulties associated with measuring fluctuating levels of metabolism resulting from intermittent incubation during the day, measurements were generally restricted to periods of constant incubation during the night. The only study to address daytime incubation (Biebach 1981, 1984) was restricted to periods of 'steady state' behaviour, i.e. when egg temperature remained constant. Thus, the measured demands neglect the energy required for re-heating eggs following periods away from the nest, which Vleck (1981) suggested was energetically demanding. Energy use for other constituent behaviours conducted off-nest was also excluded.

Other methods have been used to measure the inclusive demands of all behaviours covered by incubation, in the wider sense of the word, including maintenance of egg temperature during incubation sessions, restoration of egg temperature following periodic absence from the nest and associated off-nest activities. Food intake has been used as a correlate of energy demands, and was found to be greater for incubating Zebra Finches than for resting birds (El Wailly 1969), and to increase with clutch size for Bengalese Finches (Colemann and Whittall 1985). This method relies on the assumption that food intake tracks energy utilisation and makes no allowance for differential strategies of tissue synthesis or reserve utilisation.

In species where birds undertake prolonged fasts during incubation, mass loss can be used to estimate energy consumption, so long as information concerning the composition of the material lost and its energetic value is available. Grant (1984) has reviewed data cited by Croxall (1982) and presents estimates of the metabolic rate of 14 species of Procellariiformes during incubation, derived from rates of mass loss. Calculations were based on an assumption that 55.5% of mass loss can be attributed to fat, 9.2% to protein and the rest to water. Calorific equivalents were assigned to each body component. The resulting ratio of 'incubation metabolic rate' to BMR ranged from 0.85 to 2.31, with 50% of the values within 25% of unity (Grant 1984).

Although the experiments outlined above provide some conflicting evidence, the picture that emerges, at least for small birds, is that a thermoneutral zone exists, over which temperature range the energy demands of incubation can be satisfied without the generation of additional heat above resting metabolism. Incubation has the effect of raising the lower critical temperature (L.C.T.) of this zone, compared to that of resting metabolism, so that incubating birds will have to increase their heat production at temperatures at which non-incubating birds remain within their thermoneutral zone. At temperatures below the L.C.T., incubating birds must increase their metabolism, in order to generate sufficient heat to maintain egg temperature, to a level which is dependent on both ambient temperature and on clutch size (Haftorn and Reinertsen 1985, Biebach 1981, 1984).

6.2.2 The doubly labelled water technique to measure energy expenditure for free-living birds during incubation.

The studies cited above failed to determine the total energy budget of incubating birds in the field. Techniques were, therefore, required which allowed the determination of the metabolic rate of incubating birds in their natural environment, with limited disturbance to the normal behavioural routine. The doubly labelled water technique fulfilled these aims and has been successfully applied to several species of bird during incubation. Tatner and Bryant (1993) provide a review of studies of 17 species of birds, ranging in body mass from 12 - 85g, for which the doubly labelled water technique has been used to evaluate metabolism during incubation. Their comparison amongst species shows that a large part of the variance in daily energy expenditure can be explained by body mass (r^2 = 82.6%), and that much of the residual variation can be attributed to temperature and levels of activity. Table 6.3 shows the average values of daily energy expenditure in kJ indiv^{-1} d^{-1} for the 17 species reviewed by Tatner and Bryant (1993) plus 6 additional species. The data from this study for Dippers have been combined with the results of Bryant and Tatner (1988) and Ward (1992). Data on Great Tits from this study provides the first evidence of the field metabolism of incubating females of this species.

In each case, basal metabolic rates were extracted from the best estimate provided in the literature where these were available, or were calculated according to the equations of Aschoff and Pohl (1970) (inactive phase). Values of metabolic intensity (MI) were then calculated by expressing daily energy expenditure as a multiple of BMR.

The findings of these studies are that field metabolism during incubation is equivalent to an average of three times the basal metabolism of the species. In order to set these values in context, rates of metabolism may be compared between species (Tatner & Bryant 1993), between stages (Bryant and Tatner 1988, Masman *et al.* 1988) or, arguably most profitably, they may be compared with rates of energy gain and the ability to satisfy demands.

Across species, M.I. varies between 1.0 and 6.6 x BMR (Table 6.3). The low values for some seabirds are an artefact of the protracted incubation shifts undertaken by these large birds. Doubly labelled water measurements often relate to time on the nest only and do not include the costs of foraging and other activity during periods away from the nest (Tatner & Bryant 1993). For those species where measurements were also made during periods at sea, the two values may be combined under the assumption that birds spent approximately the same time incubating as foraging, by computing an average of the two values. This produced results more directly comparable with the remaining studies. Average values calculated in this way were in the range of 2 - 3.2 x BMR for the Wilson's Storm Petrel, Leach's Storm Petrel, the Sooty Tern, the Little Penguin and the Laysan Albatross (for references see Table 6.3). Exceptionally high values of M.I. were obtained for the Ringed Plover and the Orange Breasted Sunbird. Tatner and Bryant (1993) suggested that characteristics of the open ground-based nest of the Ringed Plover and possibly also behaviour away from the nest may contribute to this high value. Rates of energy use by the Orange Breasted Sunbird are high even in comparison with other small nectarivorous birds (Williams 1993); their habit of nesting during the austral winter, when temperatures are low and rainfall is common may contribute to this value. Energy expenditure during incubation could differ markedly between species where both sexes share incubation and those where the female incubates unaided; the costs of re-warming eggs and the demands of off-nest activities are likely to be more significant in the latter, while the thermoregulatory demands of maintaining egg temperature will be greater for the former, as they cover eggs for longer. However, Tatner and Bryant (1993) compared species of both types and found no significant difference in the (mass-adjusted) costs of the two groups.

Table 6.3 Daily energy expenditure (kJ indiv^{-1} d^{-1}) during incubation for 17 species of bird (from Tatner and Bryant 1993). Metabolic intensity expresses mean daily energy expenditure as a multiple of basal metabolic rates, where these were (a) taken from the literature or (b) calculated using the equations of Aschoff and Pohl (1970) inactive phase:

passerines BMR (kJ/d) = $24 \times .1329 \times W^{.726}$;

non-passerines : BMR (kJ/d) = $24 \times .0805 \times W^{.734}$, where W is mass in g.

Species	Sex	Mass (g)	Dee (kJ/d)	n	M.I.	Source.
Orange Breasted Sunbird	F	9.5	66.2	10	6.5[a]	Williams (1993)
Blue Tit	F	12.04	61.13	4	3.2[b]	Prys-Jones in Tatner & Bryant (1993)
Pied Flycatcher	F	14.97	68.01	6	3.3[a]	Moreno & Carlson (1989)
Pied Flycatcher	F	14.31	60.70	9	3.0[a]	Moreno & Sanz (1994)
Sand Martin	F	14.30	81.7	3	3.6[a]	Westerterp & Bryant (1984)
Pacific Swallow	F	14.68	53.16	8	2.4[b]	Tatner & Bryant (1993)
Collared Flycatcher	F	15.93	78.6	12	3.3[a]	Moreno *et al* (1991)
House Martin	M/F	18.70	80.69	11	2.9[a]	Westerterp & Bryant (1984)
Yellow-eyed Junco	M/F	19.93	68.99	14	1.9[a]	Weathers & Sullivan (1989)
Savannah Sparrow	F	20.4	79.3	3	2.6[a]	Williams (1987)
Barn Swallow	F	20.5	120.59	1	4.1[a]	Westerterp & Bryant (1984)
Barn Swallow	F	20.8	105.4	6	3.7[a]	Ward (1992)
Robin	F	20.6	75.09	3	2.6[b]	Tatner & Bryant (1993)

Table 6.3 (cont.) **Daily energy expenditure (kJ indiv^{-1} d^{-1}) during incubation for 17 species of bird (from Tatner and Bryant 1993).**

Species	Sex	Mass (g)	Dee (kJ/d)	n	M.I.	Source.
Great Tit	F	21.5	111.20	8	3.8[b]	This study
Tree Swallow	F	22.6	118.91		4.0[a]	Williams (1988)
Dipper	F	63	209.4	20	3.3[b]	Bryant & Tatner (1988) plus this study.
Starling	F	81.4	183.16	6	2.4[a]	Westerterp & Drent (1985)
Starling	F	85	228.16	4	2.9[a]	Ricklefs & Williams (1984)
European Kestrel	F	275.2	301	2	3.0[a]	Masman et al (1988)
Blue-throated Bee-eater	M/F	35.4	90.37	9	2.1[b]	Tatner & Bryant (1993)
Wilson's Storm Petrel	M/F	42.3	81	3	2.2[a]	Obst et al (1987)
Leach's Storm Petrel	M/F	45	51.5	12	1.2[a]	Ricklefs et al (1986)
Common Sandpiper	M/F	54.65	135.47	3	3.7[b]	Tatner & Bryant (1993)
Ringed Plover	M/F	74.8	301.5	4	6.6[b]	Tatner & Bryant (1993)
Sooty Tern	M/F	188	141.2	9	1.6[a]	Flint & Nagy (1984)
Little Penguin	M/F	1107	731.7		1.9[a]	Gales & Green (1990)
Laysan Albatross	M/F	3073	689.1	8	1.0[b]	Pettit et al (1988)

6.2.3 Energy expenditure through the annual cycle.

Comparisons of the energetic demands of various stages in the life-cycle of a species may reveal potential points of crisis, particularly if data on energy intake are also available. Two studies have combined doubly labelled water measurements with time activity budgets for each sex at each stage in the annual cycle (Bryant and Tatner 1988, Masman *et al.* 1988). For Kestrels, Masman *et al.* (1988) showed that, while males experienced an extended annual peak in energy use during reproduction, females tended to have reduced costs during the early stages of the breeding cycle, with demands peaking during late nestling rearing. In the Dipper (Bryant and Tatner 1988), the highest demands for both males and females occurred during the nestling rearing stage. The additional data for incubating Dippers obtained during the present study did not alter this finding. Both studies suggested that incubation is energetically inexpensive, compared to other stages. Studies based on time budgets alone have demonstrated a similar result (Ettinger and King 1980, Mugaas and King 1981, Wijnandts 1984).

Of particular comparative interest is the relationship between energy expenditure during incubation in relation to that during the brood provisioning period, when birds are traditionally thought to be working maximally. Weathers and Sullivan (1989) and Tatner and Bryant (1993) made allometric comparisons of the rate of energy use by birds during the incubation and brood rearing stage. Using data for 34 species, within a mass range of 14 - 8417 g, Weathers and Sullivan (1989) were able to demonstrate no significant difference between the equations relating energy expenditure to mass during the incubation / brooding stage compared to the nestling rearing stage. Using data from 17 species, mass range (12 - 85 g), Tatner and Bryant (1993) similarly found no significant difference between the slope of the relationship between energy expenditure and mass during the two stages, although the intercept, and hence elevation of the regression was significantly lower during the brood-rearing than the incubation period. An alternative statistical approach, comparing the difference in DEE between the incubation and brood rearing stages within a species, showed that energy expenditure was generally greater during brood-rearing, though the difference was not significant in the majority of cases (Tatner and Bryant 1993). It is apparent that the widely held assumption that energy use is maximal during brood rearing is not well-founded.

6.2.4 The effect of clutch size on energy use during incubation.

In order to evaluate the possibility that energy expenditure during incubation plays a role in the limitation of clutch size, it is necessary to establish whether energy use varies with clutch size. As long as the surface area:volume ratio does not change, an increase in clutch mass should require a proportionate increase in the energy required to heat the clutch. The energy (E, kJ) required to elevate a mass (m) of a substance, with specific heat capacity c (kJ $^{o}C^{-1}g^{-1}$) by θ (^{o}C) can be expressed as $E = m \times c \times \theta$. Mertens (1977) described two factors which may affect the shape of the relationship between the energy required for incubation and clutch size. Firstly, it may be expected that mass-specific costs of maintaining clutch temperature will decrease with increasing clutch size, as the insulative effect of adjacent warm eggs reduces heat loss to air or to nest materials. Secondly, as the number of eggs that can remain in contact with the brood patch at any one time is limited by the size of the brood patch, clutches larger than a threshold size will require periodic re-heating of eggs which have cooled, thus increasing energy expenditure above the linear relationship with increasing clutch size.

Experimental studies measuring the energy required to maintain egg temperature during continuous nocturnal incubation have provided evidence that energy expenditure does increase with clutch size in a linear fashion (Biebach 1981, 1984, Haftorn and Reinertsen 1985). Energy expenditure measured by gas exchange increased by approximately 5% with each additional egg in the Starling (Biebach 1984), and by 6-7% for incubating Blue Tits (Haftorn and Reinertsen 1985). In both studies, the lower critical temperature of the thermoneutral zone was increased with increasing clutch size, such that birds with large clutches had to generate additional heat for incubation at temperatures when those with smaller clutches were still within the thermoneutral zone. This result was predicted by the models of heat-resistance generated for the Great Tit by Mertens (1977).

Evidence from these studies, although limited, suggests that the cost of maintaining egg temperature does increase linearly with increasing clutch size. Of more relevance to the question of clutch size limitation, is whether this increase is of sufficient magnitude to be of any significance in the total energy budget.

In order to investigate this question, the energetic demands of maintaining egg temperature and of re-heating eggs following recess periods were calculated using the equations of Kendeigh (1963) for an incubating Great Tit, according to clutch size. Assumptions were as outlined in Section 5.4.7 and the model was based on the average incubation schedule of a Great Tit from the control group, with 590 minutes of continuous incubation during the night, and an active day comprising 22 sessions of length 30 minutes, and 23 recess periods, of length 8 minutes. Although egg cooling rates were measured for the Great Tit in this study (Chapter 2), no data was gathered on the effect of clutch size on cooling rate. In order to adjust for the effect of clutch size, the rates measured for Swallow eggs, by Jones (1985), in clutch sizes ranging from 1 to 8 eggs at an ambient temperature of 15 °C, were therefore used in this calculation. His results were best described by a quadratic function :

Eqn 6.2. cooling rate ($^{\circ}$C min^{-1}) $= -.0424n + .0016\, n^2 + .5844$

where n is the clutch size (eggs).

An additional re-heating cost of 144J per hour of incubation was incurred for each egg above clutch size 7, to account for re-heating of eggs not in continuous contact with the brood patch (Mertens 1977).

The energy required for incubation of the eggs calculated in this way ranged from 2.2 to 19.0 kJ indiv $^{-1}$ d^{-1} additional to the costs of resting metabolism, as clutch size increased from 5 to 10 eggs, the range observed in this study. Assuming a total daily energy budget of 111 kJ indiv $^{-1}$ d^{-1}, based on the doubly labelled water results for the control group of birds incubating a range of natural clutch sizes (Section 5.2), the percentage of total daily energy expenditure attributed to maintaining egg temperature and re-heating would range from 2 to 17%. Addition of a single egg between clutch size 5 and 7 would, therefore, increase energy expenditure by 1% of total daily energy expenditure, while the additional demands of re-heating at larger clutch sizes would increase costs by 3% of the total budget. Assuming that the doubly labelled water technique is accurate to within 10% (Bryant and Tatner 1989), changes of this magnitude may be undetected in the total energy budget.

A similar analysis was performed for incubating Dippers, although, in this case, no information was available concerning the relationship of egg cooling rate to clutch size. Because of the difference in egg size between the Swallow and the Dipper, and because the measured cooling rate for Dipper eggs (0.906 °C min^{-1} (Ward 1992)) differed significantly from results for Swallow eggs, Jones' (1985) data were not considered a suitable approximation, so the observed cooling rate was employed for all clutch sizes. No data were available to determine how many eggs could be in simultaneous contact with the brood patch in this species, so additional costs above a certain threshold were not applied.

The energy required for incubation in the Dipper, calculated using Kendeigh's equation for maintaining egg temperature plus the additional cost of re-heating eggs after recess periods ranged from 24.4 to 48.7 kJ indiv^{-1} d^{-1}, representing 12 to 23% of the average daily budget of 209 kJ indiv. $^{-1}$d^{-1} (as measured using the doubly labelled water technique, Section 4.4). Each additional egg raised energy demands by 4% of total daily energy expenditure. Again, changes of this magnitude would not be reliably detected by the doubly labelled water technique.

If manipulation of clutch size were to influence the demands of maintaining egg temperature and re-heating costs only, detection of a significant difference between the daily energy expenditure of birds incubating various clutch sizes would be unlikely, given the influence of additional factors, such as the level of activity off nest and ambient temperature in contributing to the variation in energy expenditure (Tatner and Bryant 1993).

However, changes in the energy requirements for incubation may necessitate other behavioural changes, which could magnify the change in total daily energy expenditure. In order to increase energy intake, foraging time may be extended, and nest attendance reduced. The length of time spent off-nest is determined by the energy balance of incubating birds (Moreno 1989b, Moreno and Carlson 1989, Smith *et al.* 1989). Section 5.5. describes how energy expenditure may be expected to escalate if the length of incubation sessions is reduced. Thus, a reduction in nest attendance could contribute to an escalation of energy expenditure.

Energy intake could also be increased if activity during recess periods was altered towards more energetically costly modes of foraging. For example, diving in the Dipper requires approximately 4 times as much energy as foraging in more shallow water (Tatner and Bryant 1988). More profitable foraging sites could be exploited by increasing the travel time to the patch, increasing the amount of energetically expensive flying. Section 3.3 shows how the total daily energy expenditure of incubating Dippers is sensitive to changes in the proportion of time off-nest spent in costly activity.

Thus, increases in energy expenditure on the nest could increase the necessary energy expenditure off-nest to provide the fuel for incubation. Such a positive feedback mechanism could lead to an escalation in daily energy expenditure for birds with large clutch sizes, especially if clutch size is experimentally increased outside that which matched the quality of individual birds.

6.2.5 Studies of field metabolism in relation to clutch size during incubation.

To date, few studies have examined the effect of changes in clutch size on the total daily energy budget during incubation. Colemann and Whittall's (1988) study of incubating Bengalese Finches compared the food intake of birds incubating clutches manipulated to either 2 or 8 eggs. Although food intake was an average of 6% higher for birds incubating the larger clutch size, the experiment gave no information on the shape of the relationship between clutch size and energetic demands.

The doubly labelled water technique has been used to measure field metabolism of three species of passerine incubating manipulated clutch sizes; the Dipper (Ward 1992 and this study), the Pied Flycatcher (Moreno and Carlson 1989, Moreno and Sanz, 1994) and the Collared Flycatcher (Moreno *et al.* 1991). The results of each study are summarised in Table 6.3.

In each study, the results indicate that daily energy expenditure increased in a non-linear manner with an increase in clutch size. As yet, the data are insufficient to determine whether the response is associated with the number of eggs incubated *per se* or the deviation of the clutch size from the original, although the results for Pied Flycatchers suggest that the response to clutch manipulation varied according to the original clutch size (Moreno *et al.* 1991). In the latter study, birds laying 5 eggs, whose clutch size was reduced by 1 egg showed a reduction in daily energy expenditure, and an increase of 1 egg was associated with a corresponding increase. However, those birds that laid an initial clutch of 6 eggs did not appear to increase energy expenditure if clutch size was experimentally increased to 7, suggesting, that they were unwilling, or unable, to accommodate such an increase in expenditure. No such distinction could be demonstrated for Dippers.

Table 6.4 shows the mean value of daily energy expenditure for birds according to clutch manipulation. Mean daily energy expenditure was generally (though not always significantly) higher for enlarged clutches. Perhaps of equal significance is the fact that the coefficients of variation were also higher for enlarged clutches in every study but the first, where sample sizes were too small for coefficients of variation to be meaningful.

Birds incubating large clutches showed a greater degree of variation in their level of working: energy expenditure was high for some individuals in the group, but not for others. Two hypotheses were proposed to account for this variation in the Dipper (Section 5.5). The first hypothesis ('external factors') proposes that the variation in energy expenditure within the enlarged clutch group was due to variation in external factors. Some individuals adjusted their heat input in order to incubate the enlarged clutch, without making significant changes in the incubation schedule or foraging behaviour, and so exhibited a daily energy expenditure that was comparable with birds incubating smaller clutches. Others found it necessary to adjust the pattern of nest attendance or foraging behaviour in order to satisfy the demands of warming extra eggs, and a rise in overall daily energy expenditure was apparent. Factors such as food availability, temperature, quality of mate, or the size of nutrient reserves available may determine which of these behaviour patterns applied to which birds. In other words, the effect of an increase in clutch size would be to make birds more susceptible to external influences on energy supply.

An alternative explanation (referred to as the 'cost acceptance' hypothesis) is that some birds did not accept an increase in clutch size and made no adjustment to heat input or to incubation behaviour to allow for the change, while others, which did accept the additional demands, necessarily increased their total energy expenditure. Such a response would be associated with an increase in hatching failure and/or a lengthening of the incubation period for birds who maintained low energy expenditure when presented with enlarged clutches, as incubation would not be effective for all eggs. To distinguish adequately between the two alternative hypotheses would require a comparison of incubation parameters, such as hatching success, duration of incubation and egg temperature of birds with enlarged clutches in relation to their energy expenditure during the incubation period.

The two hypotheses are summarised in Figure 6.3. In the first graph, representing the external factors hypothesis, the energy required for maintenance of egg temperature is represented by a single line for both high and low cost birds. This energy use increases in a linear manner with clutch size. However, the energy demands of all other activities, for example foraging, re-heating the eggs after recess periods, differ between the two groups.

Table 6.4 **Summary of the results of doubly labelled water studies investigating the effect of clutch size manipulation on daily energy expenditure. Values represent the mean daily energy expenditure in kJ indiv^{-1}d^{-1}. The coefficient of variation and the sample size are also presented. References: [1] Moreno and Carlson (1989), [2] Moreno and Sanz (1994), [3] Moreno *et al.* (1991) [4]this study, including data from Bryant and Tatner (1988) and Ward (1992). In studies [1] and [3], manipulations involved the addition or removal of 2 eggs, elsewhere, manipulations involved a single egg.**

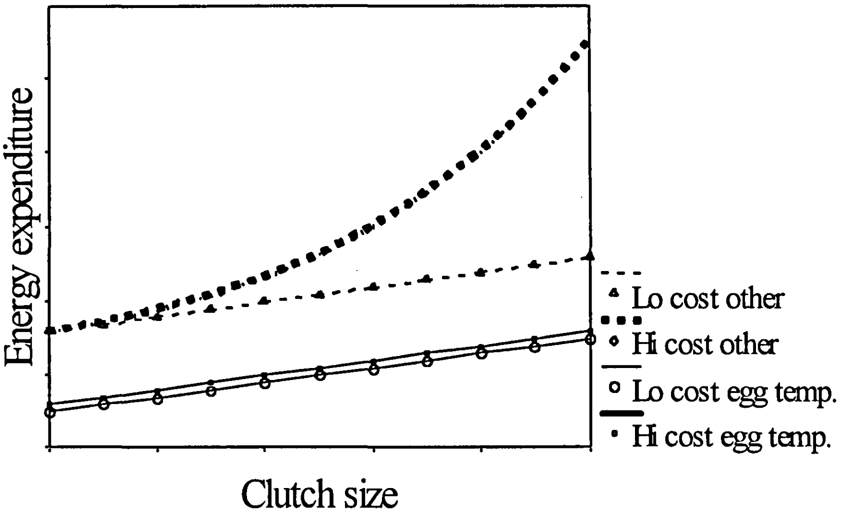
Species	Reduced	Control	Enlarged
Pied Flycatcher[1]	70.37 c.v.= 4% (n = 2)	56.61 c.v. = 28% (n = 2)	77.06 c.v. = 10% (n = 2)
Pied Flycatcher[2]	54.87 c.v.= 10% (n = 7)	64.35 c.v. = 8 % (n = 3)	64.42 c.v. = 12% (n = 8)
Collared Flycatcher[3]	75.22 c.v.= 4% (n = 4)	72.13 c.v. = 4% (n = 3)	85.10 c.v. = 14% (n = 5)
Dipper[4]	190.83 c.v.= 23% (n = 6)	209.46 c.v. = 14% (n = 19)	231.93 c.v. = 38% (n = 8)

Difficulties due to 'external factors' mean that these 'other' costs escalate for high cost birds, while for low cost birds, the increase remains proportional to the increase in clutch size.

The second graph, the 'cost acceptance' hypothesis, shows the cost of maintaining egg temperature and of other activities increases for high cost birds, as they accept the demands of heating an enlarged clutch. For low cost birds, the costs of both maintaining egg temperature and other activities are independent of clutch size, as the birds fail to adjust to the extra egg.

Two predictions of the hypotheses are presented below the graphs. Under the external factors hypothesis, both groups of birds will maintain an average egg temperature suitable for embryonic development. The cost acceptance hypothesis may be distinguished as those birds with lower levels of energy use would exhibit lower average levels of egg temperature. Under

(i) H_1: External factors.



(ii) H_2: Cost acceptance.

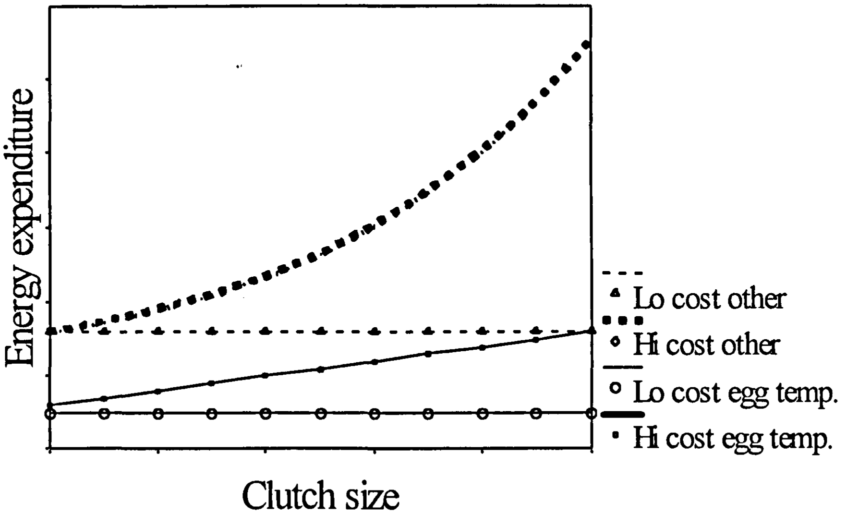


Figure 6.3 **Schematic representation of energy use by incubating birds according to two hypotheses (see text). The energy demands for maintaining egg temperature are represented by "Hi' and 'Lo' cost egg temp' for high and low cost birds respectively. Energy use for foraging and other off-nest activity, along with costs due to changes in the incubation schedule are shown by "Hi' and 'Lo' cost other'. See text for further explanation.**

Predictions.

		Maintain egg temperature?	**Risk high energy expenditure dependent costs?**
H_1: External factors	Lo cost	Yes	No
	Hi cost	Yes	Yes
H_2: Cost acceptance	Lo cost	No	No
	Hi cost	Yes	Yes

both hypotheses, 'high cost' birds are at risk of incurring any costs that depend on levels of energy use (Drent and Daan 1980).

Whichever hypothesis is correct, the result was that birds incubating enlarged clutches were more likely to exhibit high levels of daily energy expenditure, and were therefore more likely to incur any energy expenditure-dependent cost, such as an inability to satisfy demands or an increase in risk-prone behaviour (see Section 6.1.3.3.). If costs are incurred above some threshold level of working (Drent and Daan 1980), birds with large clutches also have a higher probability of incurring these physiological costs than those with smaller clutches.

Metabolic intensity, which expresses energy expenditure in terms of basal metabolic rate, can be used to provide results which may be compared amongst species. Figure 6.4 shows the metabolic intensity of the three species of incubating birds according to clutch manipulation, derived from the doubly labelled water studies referenced in Table 6.3. Drent and Daan (1980) proposed that costs be incurred when metabolic intensity exceeds 4 x BMR. This value is shown in Figure 6.4 as a solid line on each graph. The probability of exceeding a metabolic intensity of 4 x BMR for all studies combined was 1/18 or $p = .056$ for reduced clutches, 1/27 or $p = 0.037$ for control clutches and 4/18 or $p = 0.222$ for enlarged clutches. Because sample sizes were small, Fischer's exact test was used to calculate the one-tailed probability that birds with enlarged clutches were more likely to exhibit values of M.I. exceeding 4 x BMR. The difference did not attain significance at the 5% level, $p = 0.076$. More data would be necessary to confirm the trend that the probability of exceeding such a value, or indeed any such threshold value, is least for reduced clutches and greatest for enlarged clutches.

In summary, the evidence suggests that an experimental increase in clutch size can lead to an increase in the mean and the variance in energy expenditure by incubating birds, via the potential escalation of energy consuming activities initiated by the additional cost of egg-warming. Natural selection can act on the variance as well as the mean of certain parameter (Gillespie 1977, Slatkin 1974, De Steven 1980), favouring those strategies that result in lower variability for a given mean. If costs are incurred in a probabilistic manner, dependent on the level of daily energy expenditure, birds with larger clutch sizes have a higher probability of incurring reproductive costs. It seems, therefore, that an energetic constraint on clutch size is plausible during incubation.

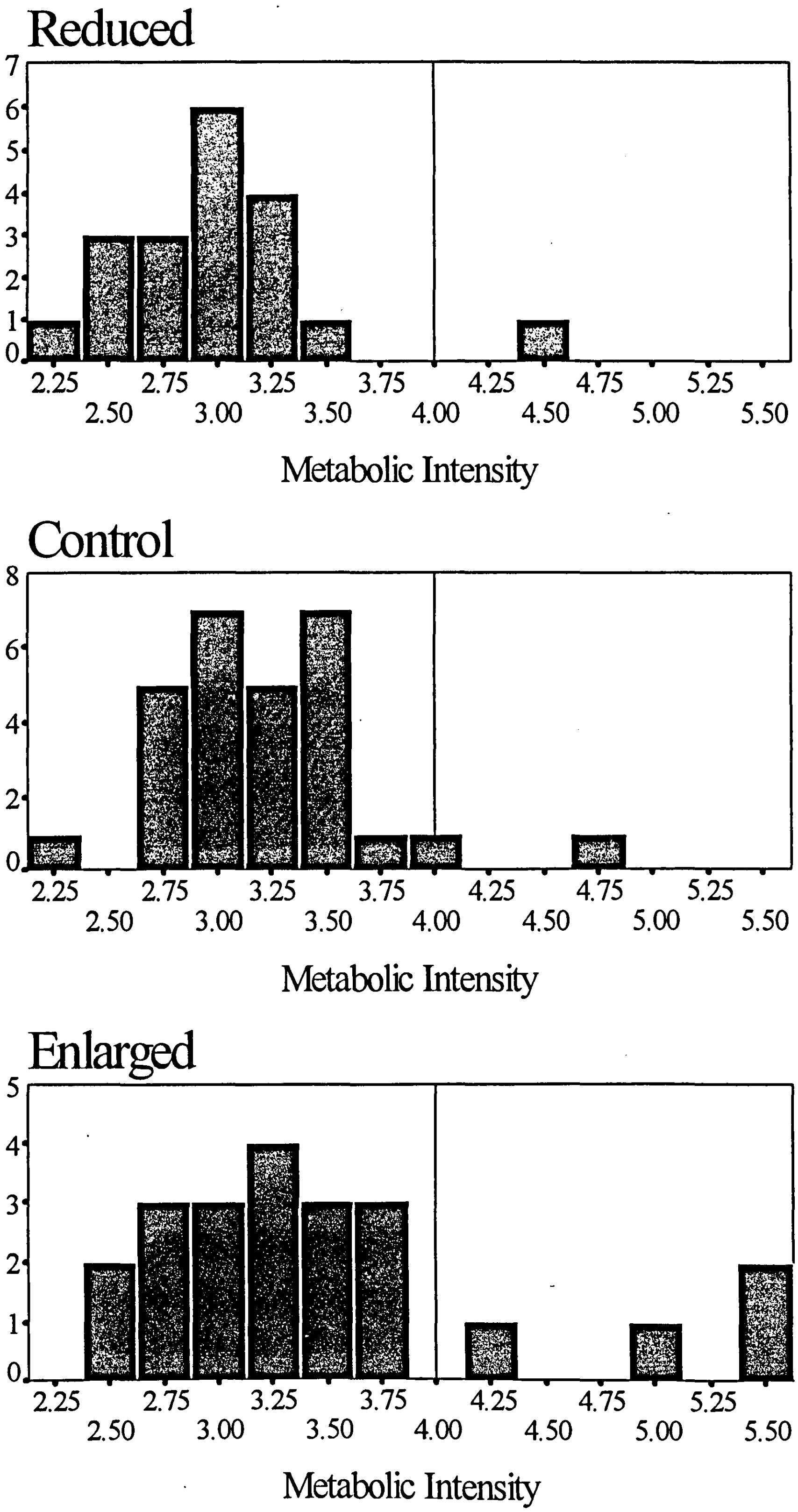


Figure 6.4. **Frequency distribution of metabolic intensity (Daily energy expenditure expressed as a multiple of BMR), for incubating Dippers, Pied and Collared Flycatchers according to clutch size manipulation. Data derived from references in Table 6.3.**

6.3 Factors determining clutch size in birds: the incubation ability hypothesis.

Numerous hypotheses have been proposed to explain the selective forces which determine clutch size in birds. Factors constraining the escalation of clutch size have been proposed to exist at almost every stage in the breeding cycle. Lack's (1954) proposal that the number of eggs laid by an altricial bird corresponds to the maximum number of young which can be adequately fed has received much attention, and the majority of cross-fostering studies designed to evaluate the cost of reproduction have concentrated on this stage alone (e.g. Askenmo 1979, Gustafsson and Sutherland 1988,). More recently, both energetic, and specific nutritional constraints have been proposed to limit the timing of laying, the number and/or the quality of eggs laid (Jones and Ward 1976, Bolton *et al.* 1992, Nager and van Noordwijk 1992, Nilsson and Svensson 1993, Yom Tov and Wright 1993, Monaghan *et al.* 1995, Perrins 1996, Graveland 1996). Nest predation (Slagsvold 1982) or nest size (Karlsson and Nilsson 1976) have also been postulated as factors constraining clutch size. Episodes of energetic stress have been postulated for parents caring for recently fledged young (Morehouse and Brewer 1968, Smith 1978, Moreno 1984, Hegner and Wingfield 1987, Lindén 1988, Verhulst *et al.* 1996) and for young that are recently independent (Weathers and Sullivan 1989).

In his consideration of the factors controlling clutch size, Lack (1954) discussed the 'incubation ability' hypothesis, that clutch size is limited by the number of eggs which a bird can successfully cover. He dismissed the idea, on the grounds that birds are commonly able to incubate clutch sizes which are larger than the norm. He did not consider that birds incubating larger clutches could experience costs such as reduced hatching success or decline in parental condition, or that clutch size may be adjusted to an individual's own quality and resources. Klomp (1970) extended his argument, by comparing the hatching success of various natural clutch sizes. Consistent with the incubation ability hypothesis, hatching success has been shown to be significantly negatively correlated with clutch size in some species (Pied Flycatcher, Potti and Merino 1996), whilst in other studies, no correlation has been found (Blackbird, Snow 1958; Swift, Lack and Lack 1951). In some cases, higher hatching success has been associated with larger clutch sizes (Herring Gull, Paynter 1949, Lesser Black Backed Gull, Harris 1964). Such phenotypic correlations may be subject to confounding influences and cannot easily be interpreted as evidence of a reproductive cost.

Manipulative studies are able to break the link between the number of eggs laid and reproductive effort during incubation. Females incubating enlarged clutches have been shown to have a lower hatching success (Moreno *et al.* 1991, Siikamaki 1995) compared to control or reduced clutches, an increase in the duration of incubation (Moreno and Carlson 1989, Smith 1989, Székely *et al.* 1994, Siikamaki 1995) and/or a greater rate of mass loss during incubation (Moreno and Carlson 1989). In this study, the hatching success and duration of incubation in Swallows were found to be affected by manipulation of clutch size.

Indirect manipulations during incubation can also reveal evidence that birds are energetically constrained. In two recent studies, birds were induced to lay earlier (Nilsson 1994) or to lay more eggs (Sanz and Moreno 1995) by the provision of supplementary food before (Nilsson 1994) and during (Sanz and Moreno 1995) the laying period, but removed thereafter. In both studies, females managed to complete laying successfully, but when feeding was withheld, incubation was postponed relative to clutch completion in Blue Tits (Nilsson 1994) while in Pied Flycatchers, eggs were less likely to hatch compared to a control group of non-provisioned birds. Either birds were energetically constrained in their ability to incubate or their behaviour was modified in response to alterations in their anticipation of future conditions.

Reproductive effort during incubation was increased, independently of clutch size, by extending the duration of the incubation period in Barnacle Geese (Tombre and Erikstad 1996). Prolonged incubation was found to be costly in terms of loss to predators and female condition.

Recognition that the time-constraints imposed on an incubating bird can severely restrict foraging opportunities led to the suspicion that energy could be limiting through incubation (Yom Tov and Hilborn 1981). The importance of food supply in determining incubation ability was suggested by the positive correlation between the rate of food provisioning by males and nest attendance in the Pied Flycatcher (von Haartman 1958). Rates of mate provisioning were negatively correlated with the duration of incubation in the Marsh Tit (Nilsson and Smith 1988). Removal of a feeding partner led to a reduction in nest attendance (von Haartman 1958, Lyon and Montgomerie 1985), a decline in hatching success and a lengthening of the incubation period (Lyon and Montgomerie 1985). Experimental food supplementation during incubation has confirmed the constraining influence of food availability on incubation. Supplementary feeding during incubation has been associated with an increase in nest attendance (Smith *et al.* 1989, Moreno 1989), a reduction in the duration of the incubation period (Nilsson and Smith 1988, Moreno 1989) and an increase in hatching success (Nilsson and Smith 1988).

In this study, indirect manipulation of the energy budget was achieved via a reduction in the thermoregulatory demands of incubating Great Tits. Nest attendance was similarly increased as energy was more readily available.

6.3.1 Satisfying the energy budget.

Incubating birds derive their energy from foraging, during periods away from the nest, from any contribution provided by their mate and from the mobilisation of stored reserves. The importance of each of these components varies between species, for example, reliance on external food supply is more important for species with smaller body mass (Moreno 1989b) as fasting endurance increases with body size (Calder 1974). 'Incubation feeding' by a mate can have significant nutritional benefits to an incubating bird. Even in those species where the incubating bird leaves the nest intermittently to forage herself, the contribution to the nutrition of the female is thought to be significant, in addition to any pair-bonding function or an indication of male quality (Royama 1966, Nisbey 1973, Lyon and Montgomerie 1985, Lifjeld and Slagsvold 1986, Nilsson and Smith 1988, Smith *et al.* 1989).

The time constraints acting on an incubating bird can restrict available foraging time, resulting in an 'energy bottleneck' during incubation (Yom Tov and Hilborn 1981). Table 6.5. displays the average daily energy expenditure (as calculated by the doubly labelled water technique) for incubating Swallows, Dippers and Great Tits. The total time available for foraging, calculated from the results of the nest temperature monitor, as the average total time spent off-nest each day, probably overestimates the true time spent feeding. Some time must be allocated to travel to and between feeding patches and to behaviours other than feeding. The rates of metabolisable energy gain (kJ h^{-1}) necessary to achieve energy balance (FR_A) were calculated most simply as D.E.E./ time off-nest.

The rate of required rate of energy intake for energy balance was modified for Dippers and Great Tits, to incorporate the energy derived from reserve depletion and from mate feeding (FR_B). The Swallow does not lose mass consistently over the course of incubation (Jones 1985). No study has recorded mate provisioning during incubation in the

Table 6.5 **Foraging rates necessary to maintain energy balance in incubating Swallows, Dippers and Great Tits. F.R.$_A$ was calculated as D.E.E./ time off-nest, FR$_B$ was calculated accounting for the contribution of reserve depletion and mate feeding. Both foraging rates were expressed as metabolisable energy gain in kJ per hour. Comparison was made with FR$_{calc}$ predicted from the equation of Bryant and Westerterp (1980), where FR = 0.92 mass (g) $^{0.94}$. This figure was converted to metabolisable energy gain assuming an assimilation efficiency of 71.3% for insectivorous birds (Bryant and Bryant 1988).**

	Mass (g)	D.E.E. (kJ.d^{-1})	Time off-nest (h)	F.R.$_A$ (kJ. h^{-1})	Contribution from reserves (kJ.d^{-1})	Contribution from mate (kJ.d^{-1})	FR$_B$ (kJ. h^{-1})	Fr$_{calc}$ (kJ. h^{-1})	FR$_A$ / FR$_{calc}$	FR$_B$/FR$_{calc}$
Swallow	20.8[a]	105.4[a]	4.14[c]	25.5	.	.	25.5	11.4	2.24	2.24
Dipper	61.7[b]	209.4[b]	2.88[c]	72.8	6.0[c]	5.6[c]	68.8	31.6	2.30	2.18
Great Tit	21.8[c]	111.0[c]	3.10[c]	35.8	4.1[d]	53.1[e]	17.35	11.9	3.01	1.46

Sources of data: [a] Ward (1992); [b] this study, including data from Bryant and Tatner (1988)and Ward (1992); [c] this study; [d] Van Balen (1973); [e] Royama (1966).

Swallow; feeding was not observed in the present study and was considered to be absent or negligable.

The calculated rates of energy gain were compared with average rates calculated from allometric equations (Bryant and Westerterp 1980)(FR_{calc}). These rates were derived from data on energy gain in birds either feeding young or self-feeding at various times of the year. The predicted foraging rate probably represents an average rather than a maximal rate of energy gain.

In reality, foraging rates probably exceed FR_A and FR_B, as the amount of time spent feeding is less than the total time spent off-nest. However, some of the time spent in non-feeding activity must comprise integral parts of 'feeding' activity: travel to and between foraging sites, searching for prey, remaining alert to the presence of predators. These elements will also be incorporated to some degree in FR_{calc}. Although there is some suggestion that incubating Dippers may spend a high proportion of recess time resting (see Section 6.1.3.3.), the result has not been confirmed for other species. The calculation of an average rate of energy gain per unit time spent off-nest provides a conservative estimate with which to make comparisons.

In order to satisfy the energetic demands of incubation by foraging alone, rates of energy intake of 2.2 x FR_{calc} (Swallow), 2.3 x FR_{calc} (Dipper) and 3.0 x FR_{calc}(Great Tit) would be required. Accounting for the contribution from reserve utilisation and from provision by the mate, the required rates of energy intake fell to 1.5 x FR_{calc} for the Great Tit, and 2.2 x FR_{calc} for the Dipper.

Provisioning by the male Great Tit was calculated to account for 48% of the total energy requirements in this species. However, this calculation was based on the highest level of provisioning observed by Royama (1966), and could overestimate the general importance of this energy source.

In summary, in order to satisfy the energy budget, incubating Dippers, Swallows and Great Tits must increase their rate of metabolisable energy gain to between 1.5 and 3 times the rates observed at other times of the year when circumstances elicit a high rate of food gains. These results confirm the suggestion (Walsberg and King 1978) that rates of energy gain during incubation must substantially exceed those in non-breeding birds if energy balance is to be maintained. Rates of energy gain based on estimates of the daily energy expenditure of incubating Magpies, Black-throated Blue Warblers and Phainopeplas showed approximately a 4-8 fold increase in foraging rate during incubation compared to non-breeding birds (Walsberg and King 1978). Observations in the Black-throated Blue Warbler (Black 1975, cited in Walsberg and King 1978) and in other parulids (Morse 1968) have confirmed that the foraging rate (indicated by the number of foraging manoeuvres per unit time), peaked during incubation, exceeding rates during nestling rearing. The risk of predation or of accident can increase at high foraging rates (Lima and Dill 1990, Skutelsky 1996). With energy intake rates at their highest level of the annual cycle, at a time when food supplies are often sub-optimal, it seems likely that rates of energy acquisition may constrain reproductive investment during incubation.

6.3.2 Energy saving mechanisms during incubation.

If energy acquisition during incubation does constitute a 'bottleneck' which limits the reproductive process, selection should favour the evolution of energy saving mechanisms. The construction of a well-insulated nest, positioned to exploit solar radiation could reduce the thermoregulatory demands throughout the breeding period, enabling a greater devotion of energy to the reproductive process (O'Connor 1978, Dhondt 1979). Although nest construction may be energetically demanding (Withers 1977), the cost can be easily outweighed by the benefits gained from insulation and protection from predation. Repeated use of nests also reduces the cost per brood. Importantly, the energetic demands of nest building are paid prior to any energy bottlenecks arising during reproduction. The nest can therefore be seen as a 'pre-payment', which not only spreads the total cost of reproduction over a longer period, reducing the height of the peak in demands, but also reduces the total cost by means of thermoregulatory savings.

For species where the accumulation of endogenous nutrient reserves is possible, the programmed use of stored reserves during incubation may be another method of 'spreading the energetic load' . An increase in foraging effort before the onset of egg-laying not only enables the bird to carry a suitable reserve store to buffer against unpredictable conditions during laying and incubation; the programmed mobilisation of reserves between egg-laying and hatch can also reduce an incubating bird's need to participate in energetically expensive foraging. By increasing fasting endurance, the bird is also able to increase session lengths, which in turn reduces egg re-heating costs, thus enabling the maintenance of a lower overall energy budget (Chapter 5).

The partner of an incubating bird can reduce its energetic demands considerably by incubation feeding. Within individuals, the degree of provisioning by a mate can determine the extent to which a bird can remain on the nest (von Haartamn 1958, Lyon and Montgomerie 1985). Between species, levels of nest attendance are also correlated with the degree of provision by the male, although it is not clear which of the two variables drives the relationship, or whether the association is a product of phylogenetic history.

Partners can also help to satisfy the energy budget of the female in other ways. In territorial species, the defence of good quality feeding stations in close proximity to the nest can reduce the incubating parent's travel and search time, and increase overall foraging efficiency (Davies 1977). Male 'guarding' behaviour during a female's recess periods may be motivated by a desire to prevent extra-pair copulations, but could also function to relieve the female of her need to be alert for intruders or predators, leaving her better able to forage maximally during the short time off-nest. Evidence suggests that female Dippers are more willing to dive for prey when males are present, and spend more time 'resting' when their partners are absent (Section 3.3.6).

6.3.3. Perspectives.

The focus of investigations into reproductive constraints in birds is shifting, from the original hypothesis that the ability to feed nestlings limits the reproductive output of altricial birds, to an acceptance that other stages in the breeding cycle may also be costly. This should not be unexpected. If reproductive output were ultimately constrained by only one period of the reproductive process and other stages were performed with relative ease, selection would act on those individuals that use their 'spare capacity' during the slack times for productive work. For example, if birds were ultimately constrained by the number of young which they could feed, and incubation were relatively cheap, strategies which manage to increase the rate of incubation to the maximum which physiological constraints (such as embryonic development) allow, would be favoured, as the advantages of early hatching would be theirs. Reproduction would then be a sequence of processes, each one proceeding at maximum rate. Identification of which stage posed the original constraint to clutch size would not be easily apparent. It may even be true that reproductive constraint may vary between species, individuals or years, according to the life-history parameters of the species concerned, the variation in the ability of individuals or fluctuations in environmental conditions.

Future studies should extend this area of investigation into the factors that determine energy use by incubating birds and to investigate the way in which these energy demands are met. Identification of the factors affecting mass accumulation and depletion during incubation, and the bird's response (both short and long term) to these factors is important. Continuous monitoring of mass, using automated balances is necessary to detect changes in response which are of limited duration, and to identify responses which may only occur under certain conditions. Manipulation of stresses and comparison with a control group of birds is essential if the adaptive component of mass loss is to be separated from that attributed to reproductive stress.

The study of energy use during incubation is of greatest use if time-activity budgets are obtained simultaneously and the energy budget can be apportioned appropriately. Studies of the foraging behaviour of incubating birds in comparison to behaviour at other stages, of food availability and diet during incubation, of the role of mates during foraging periods are needed to establish how an incubating bird can achieve an increase in energy intake rate of up to 3 x the average for other times of the year and what level of risks it accepts in so-doing. Physiological studies of digestion under conditions of restricted feeding time (c.f. Kenward and Sibly 1977) would establish the possibility that gut constraints determine energy intake rates during incubation.

Chapter 7:

Summary.

Chapter 7:

Summary.

1. Field studies were conducted with incubating Swallows, Dippers and Great Tits in Central Scotland between 1993 and 1995, in order to investigate the possibility that an energetic constraint limits reproduction during incubation.
2. All three species exhibit gynlateral intermittent incubation. Incubation during the night is continuous, but during the active day, time and energy must be allocated between the conflicting demands of reproduction and self-maintenance. Incubating Great Tits and Dippers are provisioned to a limited extent by their mates. There was no evidence of mate provisioning during incubation in the Swallow.
3. The doubly labelled water technique was employed to measure energy use by free-living incubating Dippers and Great Tits. The assumptions of the doubly labelled water technique were discussed with special reference to incubating birds. An experimental study suggested that gas accumulation in the nest box of an incubating Great Tit would not be sufficient to invalidate the results of doubly labelled water studies on incubating birds.
4. The 'airspace diameter' technique for the estimation of egg age was tested. The degree of error between observers was found to significantly affect the accuracy of predictions for Swallow and Dipper eggs. However, construction of an equation specific to the observer for Swallow eggs, enabled predictions that were sufficiently accurate to be of use in the field (90% of errors within 2 days).
5. Patterns of nest attendance were determined for all three species by deployment of temperature sensitive probes and automated logging equipment around the nest. In all three species, the active day was partitioned between periods of incubation and recess periods away from the nest, while a single period of continuous incubation occurred at night. The mean duration of day sessions, recess periods and night sessions for each species were: Swallows: 11.6 : 4.8 : 587 min.; Dippers: 44.6 : 11.5 : 631 min.; Great Tit: 34.4 : 8.0 : 619 min..
6. The cooling rate of Great Tit eggs was measured in the laboratory. The mean cooling rate of 1.987 $^{\circ}C\ ^{\circ}C^{-1}\ h^{-1}$ was calculated for eggs in a clutch of 8, in a fully lined nest.

7. Incubating Swallows were studied with their first and second clutches during 1993 and with second clutches only in 1994. Reproductive effort was manipulated by the alteration of clutch size within experimental trios of clutches. Two eggs were removed from reduced clutches to control nests, from which a further two eggs were transferred to enlarged nests. Clutch size was manipulated on day 2.9 ± 1.9 of incubation (where day 1 was the date of clutch completion) and the manipulation was reversed on day 14.2 ± 2.0. Thus, reproductive effort was manipulated through incubation alone.
8. The duration of the incubation period from clutch completion to hatch was significantly greater in those clutches which were enlarged (15.61d) than those which were reduced (14.82 d). The effect was most pronounced within the second clutches of 1994, when a measure of the aerial insect availability, obtained by suction trap catch volume, was lower than in the corresponding period in 1993.
9. Hatching success was depressed by clutch manipulation, differing significantly between enlarged (81%) and reduced (92%) nests. Despite this, enlarged nests still produced the greatest number of hatched young. Again, the effects were more pronounced within 2nd clutches in 1994, when overall hatching success was significantly lower than in 1993.
10. During incubation, Swallow eggs lost mass at a rate of $0.017 g d^{-1}$, equivalent to a 12% decline in mass at clutch completion over the course of the incubation period. The rate of decline in egg density was not well correlated with the duration of the incubation period, suggesting that, in this species, the decline in egg density was not a valid indication of the rate of embryonic development. It is suggested that factors such as relative humidity and air movements may affect the rate of water loss independently of embryonic development.
11. Clutch manipulation did not affect the body condition of incubating Swallows at the end of incubation. There was no difference between mean mass, mass standardised for structural size, the size of the pectoralis muscle (measured using an ultrasonic flaw detector) or visible lipid deposits according to treatment group. However, for birds with 1st clutches in 1993, the variation in mass was greatest for individuals with enlarged clutches. This result was interpreted as evidence that birds with enlarged clutches were more likely to incur short term problems of energy imbalance than other birds (Jones 1987b). The mass of incubating Swallows, lipid deposits and protein reserves were all found to increase with aerial insect availability.

12. Contrary to the results of Jones (1987b), clutch size manipulation did not influence patterns of nest attendance. The results from the present study were thought to be more valid as they were based on a wider sample of times, birds and conditions, while clutch size was restricted within the natural range. It was concluded that the effects of clutch size manipulation on hatching success and the duration of incubation were more likely to be manifest via a decline in average egg temperature than via an alteration in the patterns of nest attendance.
13. No effects of effort during incubation could be detected post-hatch. Clutch size manipulation did not affect nestling size or mass, nestling growth rate or peak nestling mass. For birds whose first clutches were manipulated, neither the probability of forming a second clutch, nor the timing or size of a second clutch was related to clutch size during incubation.
14. Faced with an increase in the demands of incubation, Swallows suffered egg losses and an extension in the incubation period rather than incur penalties to female condition. It was suggested that postponing the costs of increased effort during incubation was either not possible, due to imminent energy imbalance, or was not profitable, as high costs during incubation may indicate high costs at later stages.

15. A shorter term study was conducted to investigate the effect of clutch size on energy use during free-living incubation in the Dipper. Clutch size was manipulated by one egg over a 48 hour period, during which time rates of field metabolism were measured using the doubly labelled water technique. Patterns of nest attendance and behaviour off-nest were also recorded to enable partitioning of the energy budget.
16. Data were combined with the results of females labelled in 1982 -1983 (Bryant and Tatner 1988b) and 1990 to 1991 (Ward 1992) to give a sample size of 33 birds. For the 19 birds incubating their natural clutch size, energy use was described as follows: ADMR 5.3 ± 0.74 $cm^3CO_2\ g^{-1}h^{-1}$, DEE 209.46 ± 28.68 kJ $ind.^{-1}\ d^{-1}$, MI_{AP} (DEE expressed as a multiple of BMR calculated by the equations of Aschoff and Pohl (1970)) 3.33 ± 0.46, MI_{BN} (using Bryant and Newton's (1994) equation for BMR for female Dippers in this study population) 2.39 ± 0.33.
17. While neither enlargements nor reductions in clutch size significantly affected mean energy use by incubating Dippers, the variation in costs amongst the enlarged group

significantly exceeded that amongst control birds. This result was consistent with findings for other incubating passerines (Carlson and Moreno 1989, Moreno *et al.* 1991, Moreno and Sanz 1994) and suggests that reproductive costs could act at high clutch size in a probabilistic manner.

18. Energy expenditure during incubation in the Dipper was influenced by river flow rate, the duration of incubation sessions and activity during periods off-nest. However, the combination of these factors in multiple regression or by Time-Activity Laboratory modelling was not sufficient to explain the observed variation amongst all birds.
19. It was suggested that birds with enlarged clutches could be divided into two distinct groups, those with high levels of energy use (>4 x BMR) and the remaining birds. Two hypotheses were proposed to account for the difference between groups: the 'external factors' hypothesis suggested that those birds with high levels of energy use encountered problems in obtaining the energy necessary to incubate an enlarged clutch, due to either low female quality or environmental conditions and were obliged to increase total energy use in order to avoid energy imbalance; the 'cost acceptance' hypothesis proposed that only those individuals with enlarged clutches and high levels of energy use were accepting the cost of the additional egg. Methods of distinguishing between the hypotheses were suggested.
20. During recess periods, incubating female Dippers spent 53% (unlabelled birds) of their time off-nest 'resting'. The duration of these inactive periods was generally short (mean = 3.1s) and it was proposed that they may arise from the need for vigilance during feeding bouts. The proportion of female time spent resting was reduced (though not significantly) when partners were within 10m, suggesting that males may contribute to rates of energy gain by the female by assuming responsibility for vigilance for the pair during feeding. An alternative explanation, that periods of inactive behaviour were evidence of the existence of a physiological constraint limiting feeding rate (Kenward and Sibly 1977) was also examined. Experimental investigation of the latter was suggested.
21. Male Dippers were found to be within 5 m of the nest on 52% of observations, the highest frequencies being observed early in the morning. Nest guarding may function to prevent intruders, both predators and conspecifics. Males were observed to enter the nest and presumably to feed the female at a rate of 0.6 times per hour, or 50 times a day, with

the highest frequencies again occurring shortly after sunrise. At this rate, male feeding could contribute 5.2kJ $ind^{-1}d^{-1}$, or 2.5% of the average daily energy budget.

22. Female incubation mass in the Dipper was studied in both labelled and unlabelled birds. Body mass declined at a rate of $-0.16gd^{-1}$ (unlabelled birds), the most rapid rates of mass loss occurring in the first and last stages of incubation. If all mass loss were due to lipid metabolism, reserve depletion could account for 3% of the daily energy expenditure of female Dippers. There was some evidence that birds responded to a clutch enlargement by an initial increase in the rate of mass loss in the first day after clutch manipulation, suggested to represent an element of reproductive stress. The mass change over the subsequent 2 days was influenced by clutch manipulation in the opposite direction, birds with enlarged clutches tending to gain mass while those with reduced clutches showed the most rapid mass losses. This response was interpreted as a paradoxical increase in mass in response to the increased costs of reproduction, determined by the greater need for reserves as 'insurance' with larger clutches (Humphries and Boutin 1996). The costs and benefits of mass storage and utilisation during incubation in the Dipper were discussed.
23. The Time Activity Laboratory method was used to provide an alternative estimate of the costs of incubation in the Dipper. Comparison of the results between T.A.L. and D.L.W. estimates suggested 27% of the energy needed to maintain egg temperature (Kendeigh 1963) was met by heat produced as a by-product of metabolism (King 1973).

24. A third study, conducted with incubating Great Tits, aimed to manipulate the energy budget and hence availability of energy to incubation by a reduction in the thermoregulatory demands of an incubating bird. The nest boxes of a treatment group of birds were heated overnight, resulting in the elevation of nest air temperature of approximately 4°C over the corresponding temperature at a control group of nests, for a period of 9 hours. The thermoregulatory saving was calculated to reduced energy requirements by 10kJ per night.
25. The effects of this heating treatment were subsequently monitored, employing nest temperature monitors to record patterns of nest attendance and the doubly labelled water technique to measure the total energy use by birds in each group.
26. Following a heated night, birds extended the night incubation session by leaving the nest later in the morning (mean night session duration for controls was $590.2 \pm 41.2_{(11)}$ min.,

for heated birds was 647.0 ± $68.3_{(11)}$ min). Throughout the following day, incubation sessions were also longer for birds whose nests were heated (control: 29.6 ± $8.9_{(11)}$ min., heated: 39.2 ± $9.3_{(11)}$ min.). In total heated birds were able to devote an additional 51 minutes per day to incubation.

27. Energy expenditure for 22 incubating Great Tits averaged: ADMR 7.79 ± 2.43 cm^3 $CO_2g^{-1}h^{-1}$, DEE 106.38 ± 32.18 kJ $ind.^{-1}d.^{-1}$, MI_{AP} 3.59 ± 1.11, MI_H (based on the basal metabolism of female Great Tits in the study population in the summer (Hashim 1996)) 2.72 ± 0.87. The mean energy use did not differ according to treatment group, but the variation amongst individuals was significantly reduced by the heating treatment.
28. This result was explained by the differential relationship between energy use and nightly minimum temperature according to treatment group. Costs escalated for control birds when the nightly minimum temperature fell below 2°C. The relationship was described by a quadratic function. Costs for heated birds increased to a limited extent only with decreasing nightly temperature, the relationship being described by a linear equation.
29. The rate of mass loss observed ($-0.125gd^{-1}$) was of a similar magnitude to that found in other studies of incubating Great Tits (van Balen 1973, Mertens 1987) and was sufficient to account for 4.65 kJ or 4.2% of the daily budget, if all mass loss could be attributed to lipid metabolism. Mass change over the study period was more variable within the control group. Patterns of mass change in the heated group were attributed to the combined effects of warmer temperatures and the alteration of the predictability of conditions. A positive relationship between mass and both session and recess length was demonstrated.
30. Modelling of the components of energy use during incubation for Great Tits showed how costs may escalate with short incubation sessions. It was suggested that the heating treatment enabled longer incubation sessions by the increased fasting endurance of heated birds, by means of their greater reserves. This increase enabled the restraint in levels of energy use at low temperatures compared to levels observed amongst control birds.
31. Energetic limitation during incubation was concluded to form one component of the constraint of reproduction in the Great Tit. It was postulated that flexibility in the duration of incubation sessions, determined at a proximate level by energy availability, could be an important element of plasticity in the timing of achievable lay dates and optimal hatch dates, under conditions of limited predictability (Nager and van Noordwjik 1995).

32. The doubly labelled water technique was advocated as providing the best measure of field metabolism during incubation. The results for Dippers and Great Tits confirm the suppositions that incubating passerines work at approximately 3 x BMR (Moreno and Carkson 1989, Moreno *et al.* 1991, Moreno and Sanz 1994) and that metabolism during incubation does not differ significantly from that during brood-rearing (Weathers and Sulllivan 1989, Tatner and Bryant 1993). Studies with both Dippers and Great Tits emphasised the importance of an appropriate choice of BMR measurement. Concern was aroused over the extrapolation of BMR measures based on lean birds to individuals with substantially differing body composition.
33. Body condition of incubating birds was suggested to be influenced by four factors, differing in importance amongst species; a 'mass adjustment' component, as the high level of reserves necessary during laying are depleted through incubation to create the lean flight-adapted body form necessary for nestling rearing (Freed 1981, Norberg 1981); an element of programmed reserve utilisation, enabling otherwise unsustainable incubation constancy (Sherry *et al.* 1980); an component resulting from reproductive stress, evidenced particularly in the short term, before birds adjust to changes in the energy budget; an insurance reserve, maintained as a buffer against poor conditions, suggested by a longer term response to an increase in reproductive costs, or a decline in conditions, increasing the value of insurance reserves (M^{c}Namara and Houston 1990).
34. Rates of energy intake necessary to balance the energy budget during incubation were calculated to be between 1.5 and 2.2 times those measured at other demanding stages in the annual cycle (Bryant and Westerterp 1980), even when the contribution from mate feeding and reserve utilisation were taken into account. It was suggested that information on foraging strategies, prey selection and diet during incubation is lacking and may contribute to the question of how incubating birds satisfy energetic demands.

35. In summary, the species studied showed evidence of being energetically constrained during incubation. The energetic demands of incubation increased with clutch size for some birds in such a manner that they were not able to incubate the whole clutch adequately. In these passerine species, the evidence, therefore, supports the existence of an energetic constraint during incubation.

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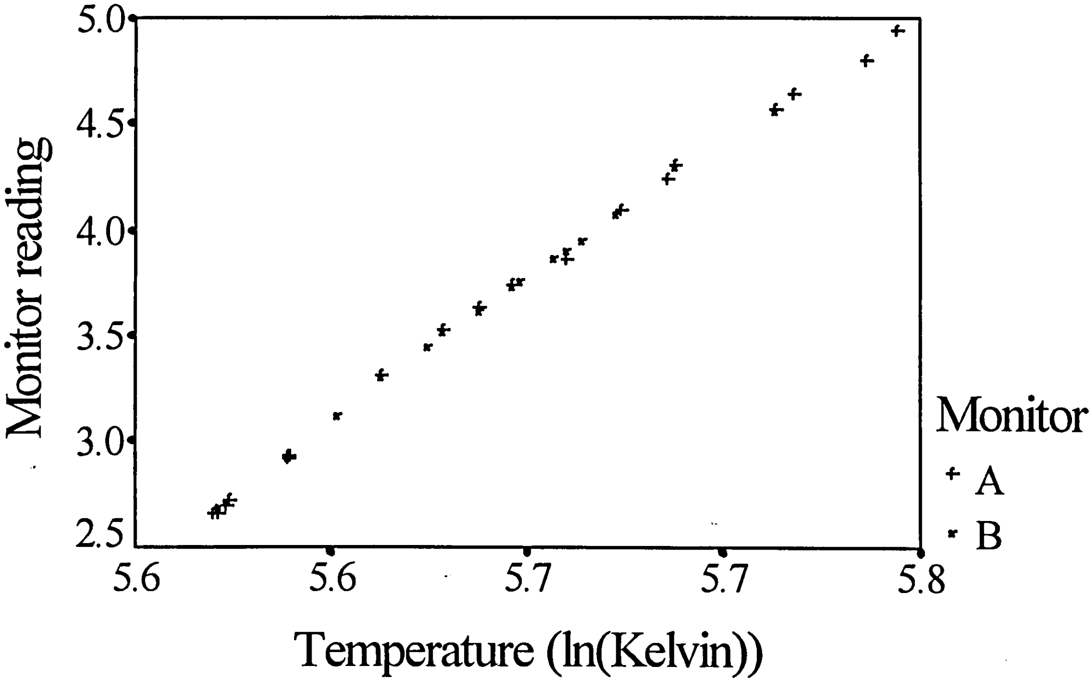
Appendix 1.

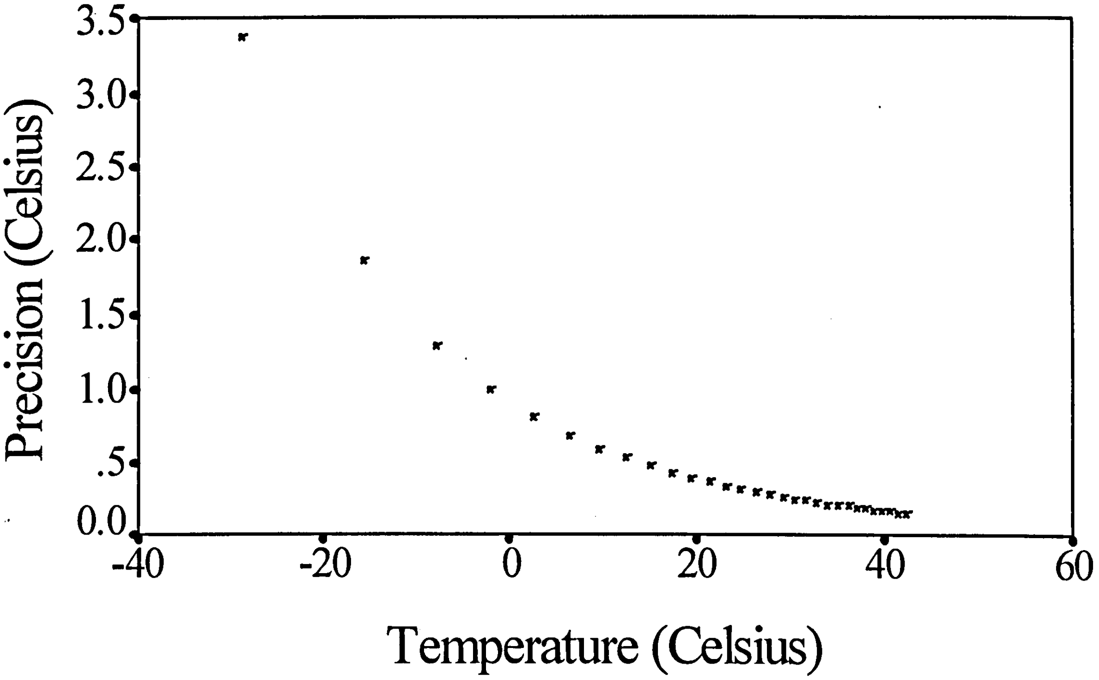
1993 calibration of the two nest temperature monitors. The monitor reading was plotted against temperature in Kelvin on a $\log_e$- $\log_e$ scale. Temperature was measured in Centigrade using a digital thermometer accurate to 0.01°C, before conversion to Kelvin. Analysis of covariance showed that there was no significant difference between the two regressions in this year ($p = .169$), so temperatures were calculated from the monitor output using a single equation: $r^2 = .997$, $p < .001$,

$$\ln(\text{monitor reading}) = (13.271 \times \ln(\text{Kelvin})) - 71.356.$$



Appendix 1. (cont.)

Precision of the nest temperature monitor. The graph shows the temperature increment represented by 1 unit on the monitor output at each temperature on the x-axis, shown in Celsius for clarity. The relationship was best described by a $\log_e$-$\log_e$ regression: ln (precision) = -12.041 ln(temperature) + 67.450 , where precision and temperature are both in Kelvin.



Appendix 2.

1. Calibrations of mass spectrometers for analysis of doubly labelled water results.

Calibration lines were derived for each mass spectrometer for each period of analysis, using international standards within the range of natural abundance (e.g.SLAP, SMOW) and a range of gravimetrically prepared enriched standards (Tatner and Bryant 1989). Equations for deuterium and oxygen-18 are presented for each year alongside the number of standard samples which contributed to each regression. The mass spectrometers used for each analysis are indicated (Sira 2, 9 or 10). In 1994, a change of reference gas (A to B) part way through analysis made it necessary to re-calibrate the mass spectrometer with a second set of samples.

N.B.

1.All regressions were significant at the 0.1% level.

2. Low enrichment values were converted to ppm using the regressions listed and the equations presented below.

3. δ^{18}Ocom is a value derived from δ^{18}Oraw, incorporating an adjustment for the low enrichment calibration of the mass spectrometers computed by geologists at S.U.R.R.C..

September - October 1993 (Analysis by S.Bryan).

Deuterium: Sira 9:	Low enrichment	[1,2]δDsmow = (δDraw - 77.7406)/0.9802 (n = 4)
	High enrichment	ppm = (δDraw +1134.2739)/6.9156 (n = 12)
Oxygen-18: Sira 2.	Low enrichment	ppm = (δ^{18}Osmow+924.4289)/0.4570 (n = 4)
	High enrichment	[3]ppm = (δ^{18}Ocom + 994.4615)/0.4873 (n = 5)

October - November 1994 (S.Bryan).

Deuterium: Sira 9.	Low enrichment.	δDsmow = (δDraw - 82.9645)/1.0434
		(n = 6)
	High enrichment.	ppm = (δDraw + 1208.33)/7.0152
		(n = 9)
Oxygen-18:Sira 2/10.	Low enrichment.	δ^{18}Osmow = (δ^{18}Ocom -2.2952)/0.9832
		(n = 21)
	High enrichment:	ppm = (δ^{18}Oraw + 942.6329)/0.4567
	(reference gas A).	(n = 10)
	High enrichment:	ppm = (δ^{18}Oraw + 925.3507)/0.4676
	(reference gas B).	(n = 4)

June 1995. (J.Weir.)

Deuterium: Sira 9.	Low enrichment.	δDsmow = (δDraw - 59.0757)/0.88854
		(n = 8)
	High enrichment.	ppm = (δDraw + 944.8538) /6.2754
		(n = 20).
Oxygen-18: Sira 10.	Low enrichment.	δ^{18}Osmow = (δ^{18}Ocom -14.2229)/1.0236
		(n=5).
	High enrichment.	ppm = (δ^{18}Oraw + 896.0505)/0.4530
		(n = 8)

Standard equations relating δDsmow and δ^{18}Osmow to ppm.

Deuterium:

$$\text{(i) } \partial D_{smow} = \left[\frac{D/H_{sample}}{D/H_{smow}} - 1 \right] \times 10^{3}$$

$$\text{(ii) } D/H_{sample} = \left[\frac{\partial D_{smow}}{1000} + 1 \right] \left[\frac{155.76}{10^{6} - 155.76} \right]$$

$$\text{(iii) } ppm_{sample} D = \frac{10^{6}\, D/H_{sample}}{1 + D/H_{sample}}$$

Oxygen-18:

$$\text{(i) } \partial\, {}^{48}O_{smow} = \left[\frac{{}^{18}O/{}^{16}O_{sample}}{{}^{18}O/{}^{16}O_{smow}} - 1 \right] \times 10^{3}$$

$$\text{(ii) } {}^{18}O/{}^{16}O_{sample} = \left[\frac{\partial\, {}^{18}O_{smow}}{1000} + 1 \right] \left[\frac{2005.2}{10^{6} - 2005.2} \right]$$

$$\text{(iii) } ppm_{sample}\, {}^{18}O = \frac{10^{6}\, {}^{18}O/{}^{16}O_{sample}}{1 + {}^{18}O/{}^{16}O_{sample}}$$

Background Samples.

Background concentration of deuterium and oxygen-18 in the blood of Dippers and Great Tits; mean values are presented ± standard deviation, the number of birds sampled is presented in parenthesis.

Species.	Year	Deuterium (ppm)	^{18}O (ppm)
Dipper	1993	$148.12 \pm 1.16_{(2)}$	$2021.06 \pm 4.39_{(2)}$
Dipper	1994	$145.61 \pm 0.67_{(3)}$	$1993.58 \pm 1.45_{(3)}$
Great Tit	1994	$149.49 \pm 2.28_{(2)}$	$2025.89 \pm 25.27_{(2)}$
Great Tit.	1995	$152.65 \pm 2.90_{(3)}$	$1991.02 \pm 3.64_{(3)}$

Replicate accuracy.

Ppm values for both H/D and ^{18}O were within 3% for Dipper samples and within 5% for Great Tit samples (with one exception which differed by 7%).

Appendix 3.

Alphabetical list of animal species mentioned in the text: common and scientific names.

Amazon Kingfisher	*Chloroceryle amazoma*
American Kestrel	*Falco sparverius*
Anna's Hummingbird	*Calypte anna*
Arctic Skua	*Stercorarius parasiticus*
Barn Swallow	*Hirundo rustica*
Barn Swallow (N. American subspecies)	*Hirundo rustica erythrogaster*
Barnacle Goose	*Branta leucopsis* Bechstein
Bengalese Finch	*Lonchura striata var. domestica*
Black Throated Blue Warbler	*Dendroica caerulescens caerulescens*
Blackbird	*Turdus merula*
Blue Petrel	*Halobaena caerulea*
Blue-throated Bee-eater	*Merops viridas*
Blue Tit	*Parus caeruleus*
Bonin Petrel	*Pterodroma hypoleuca*
Broad-tailed Hummingbird	*Selasphorus patycercus*
Burmese Red Jungle Fowl	*Gallus gallus spadiceus*
Calliope Hummingbird	*Stellula calliope*
Canada Goose	*Branta canadensis moffitti*
Cliff Swallow	*Petrochelidon pyrrhonota*
Coal Tit	*Parus ater*
Collared Flycatcher	*Ficedula albicollis*
Common Goldeneye	*Bucephala clangula*
Common Sandpiper	*Actitis hypoleucos*
Crow	*Corvus corone*
Dipper	*Cinclus cinclus*
Great Skua	*Catharacta skua*
Great Tit	*Parus major*

Grey Wagtail	*Motacilla cinerea*
Herring Gull	*Larus argentatus*
House Martin	*Delichon urbica*
House Sparrow	*Passer domesticus*
House Wren	*Troglodytes aedon*
Kentish Plover	*Charadrius alexandrinus*
Kestrel (European)	*Falco tinnunculus*
Laysan Albatross	*Diomedea immutabilis*
Leach's Storm Petrel	*Oceanodroma leuchorhoa*
Least Auklet	*Aethia pusilla*
Lesser Black Backed Gull	*Larus fuscus*
Little Penguin	*Eudyptula minor*
Magpie	*Pica pica*
Mallard	*Anas platyrhynchos*
Marsh Tit	*Parus palustris*
Mink	*Mustela vison*
Mountain Bluebird	*Sialia currucoides*
Mouse	*Mus musculus*
Northern Flicker	*Colaptes auratus*
Northern Shoveler	*Anas clypeata*
Orange Breasted Sunbird	*Nectarinia violacea*
Pacific Swallow	*Hirundo tahitica*
Phainopeplea	*Phainopepla nitens*
Pied Flycatcher	*Ficedula hypoleuca*
Pied Wagtail	*Motacilla alba*
Rat	*Rattus norvegicus*
Red-winged Blackbirds	*Agelaius phoenicus*
Red Squirrel	*Tamiasciurus hudsonius* Erxleben
Ringed Plover	*Charadrius hiaticula*
Robin	*Erithacus rubecula*
Sand Martin	*Riparia riparia*
Savannah Sparrow	*Passerculus sandwichensis*

Sooty Tern	*Sterna fuscata*
Sparrowhawk	*Accipiter nisus*
Spotted Flycatcher	*Muscicapa striata*
Starling	*Sturnus vulgaris*
Stoat	*Mustela erminea*
Swallow	*Hirundo rustica*
Swift	*Apus apus*
Tree Swallow	*Tachycineta bicolor*
Wandering Albatross	*Diomedea exulans*
Weasel	*Mustela nivalis*
Wheatear	*Oenanthe oenanthe*
White crowned sparrow	*Zonotrichia leucophrys*
Willow Flycatcher	*Empidonax traillii*
Willow Tit	*Parus montanus*
Wilson's Storm Petrel	*Oceanites oceanus*
Wolf	*Canis lupus*
Wood Duck	*Aix sponsa*
Wood Pigeon	*Columba palumbus*
Yellow-eyed Junco	*Junco phaeontus*
Zebra Finch	*Poephila guttata*

Appendix 4.

Nest attendance of incubating swallows in relation to clutch manipulation. The mean duration of incubation sessions and recess periods are presented ± s.d., with sample size in the subscript. Means were derived from 20-24 hours data for each bird recorded for second broods in 1994. Dates were calculated from April 1st = 1.

Date	Enlarged				Reduced			
	Nest	Session (day)	Session (night)	Recess	Nest	Session (day)	Session (night)	Recess
72-73	3	$9.6 \pm 5.4_{(54)}$	$519_{(1)}$	$3.5 \pm 2.1_{(56)}$	1	$9.6 \pm 4.6_{(53)}$	$612_{(1)}$	$4.0 \pm 2.5_{(54)}$
75-76	5	$11.7 \pm 7.0_{(31)}$	$699_{(1)}$	$6.1 \pm 3.3_{(32)}$	4	$12.7 \pm 9.1_{(44)}$	$556_{(1)}$	$3.9 \pm 1.6_{(44)}$
81-82	6	$12.5 \pm 5.7_{(35)}$	$653_{(1)}$	$6.8 \pm 5.4_{(35)}$	4	$15.2 \pm 9.5_{(39)}$	$546_{(1)}$	$3.4 \pm 1.4_{(40)}$
91-92	7	$10.4 \pm 6.1_{(55)}$	$567_{(1)}$	$3.1 \pm 1.6_{(55)}$	8	$7.7 \pm 5.8_{(60)}$	$598_{(1)}$	$3.9 \pm 2.5_{(60)}$
105-106	14	$13.0 \pm 11.7_{(47)}$	$472_{(1)}$	$6.7 \pm 9.3_{(48)}$	9	$10.4 \pm 6.1_{(54)}$	$507_{(1)}$	$4.5 \pm 3.1_{(54)}$
109-110	17	$14.8 \pm 9.6_{(40)}$	$627_{(1)}$	$7.1 \pm 7.4_{(40)}$	15	$12.6 \pm 16.0_{(38)}$	$626_{(1)}$	$5.7 \pm 5.1_{(40)}$
113-114	20	$9.5 \pm 5.6_{(49)}$	$490_{(1)}$	$3.1 \pm 1.4_{(50)}$	21	$16.3 \pm 11.2_{(29)}$	$638_{(1)}$	$6.5 \pm 4.6_{(28)}$
120-121	48	$10.8 \pm 4.9_{(44)}$	$625_{(1)}$	$4.1 \pm 2.2_{(45)}$	45	$8.3 \pm 5.4_{(54)}$	$653_{(1)}$	$4.2 \pm 2.4_{(56)}$
Mean		$11.5 \pm 1.8_{(8)}$	$581.5 \pm 82.3_{(8)}$	$5.1 \pm 1.8_{(8)}$		$11.6 \pm 3.1_{(8)}$	$592.0 \pm 50.8_{(8)}$	$4.53 \pm 1.05_{(8)}$

Appendix 5.

Sample and ring numbers, age and year of study for all incubating female Dippers captured for doubly labelled water analysis. Study 1= Bryant and Tatner (1988b), 2 = Ward (1992), 3 = this study.

Sample	BTO Ring number	Study.	Date of first capture.	Age (yrs)	Site
101	XK29292	1	14.4.82	≥2	Dungeon E.
102	XK29293	1	26.4.82	1	Glendey Culvert
103	XJ21005	1	31.5.82	2	Auchlinsky Lower
104	XK29292	1	30.3.83	≥3	Dungeon East
105	XS44821	1	5.4.83	1	Auchlinsky Lower
106	XJ21005	1	18.4.83	3	Auchlinsky Lower
107	XS44827	1	20.4.83	1	Glendey Culvert.
201	RB93651	2	24.4.90	3	Blackford
202	XR68715	2	30.4.90	2	Greenhill.
203	XR68572	2	3.5.90	1	Wharry
204	XR68830	2	11.5.90	≥2	Gannel
205	XR68808	2	15.5.90	≥2	Merryhills
206	XR68590	2	26.3.91	2	Doune Castle
207	XR68810	2	16.4.91	≥3	Cromlix House
208	XR68814	2	21.4.91	2	Auld Dalbreck
209	RS03029	2	24.4.91	1	Ardoch Hide
210	XR68821	2	30.4.91	2	Alva 5th Bridge
301	RS03083	3	1.4.93	2	Fossaway
302	XR68737	3	7.4.93	2	Glensherup
303	XR68810	3	18.4.93	≥5	Cromlix House
304	RS03079	3	29.4.93	2	Lendrick Burn
305	RS03079	3	30.4.93	2	Lendrick Burn
306	XP05214	3	9.5.93	6	Cromlix Girder Bridge
307	XP05214	3	10.5.93	6	Cromlix Girder Bridge.
308	RS03079	3	24.3.94	3	Blue Pipe Bridge
309	RS03095	3	30.3.94	3	Quoigs
310	RS03198	3	5.4.94	1	Hunthall
311	SX74004	3	9.4.94	1	Dollar Sign
312	XR68810	3	13.4.94	≥6	Cromlix House
313	RS03403	3	18.4.94	1	Spinney-burn
314	RS03413	3	21.4.94	1	Vicar's Bridge
315	RS03207	3	27.4.94	1	Glensherup
316	SX30621	3	30.4.94	≥3	Corbs
317	RS30697	3	6.5.94	1	Cromlix Graveyard.

Appendix 6.

Nest attendance parameters for incubating Dippers. Data were derived from a period of 24 hours, studied using the nest temperature monitor (data for samples 301 and 310 were collected by nest observation alone; the period of observation was therefore less than 24h). Data for session and recess duration were available for 1990, 1991, (Ward 1992, pers. comm.), 1993 and 1994 (this study). Data for the night-time session were available for 1993 and 1994 only. For each value, standard deviations are shown in brackets and the number of observations contributing to the mean shown as a subscript.

Sample	Clutch size	Session	Recess	Night session	Hours of study
201	5	$44.7\pm19.1_{(20)}$	$11.1\pm2.0_{(22)}$	.	.
202	4	$44.0\pm27.8_{(34)}$	$11.2\pm4.8_{(39)}$	.	.
203	5	$33.8\pm20.0_{(27)}$	$9.2\pm2.4_{(31)}$	.	.
204	5	$33.0\pm15.7_{(12)}$	$13.3\pm8.2_{(14)}$	.	.
205	4	$42.3\pm14.9_{(36)}$	$10.9\pm4.9_{(40)}$	.	.
206	5	$51.3\pm25.6_{(6)}$	$14.2\pm5.4_{(10)}$	.	.
207	3	$55.4\pm15.8_{(17)}$	$11.5\pm3.2_{(21)}$	.	.
208	6	$34.2\pm6.6_{(33)}$	$9.1\pm3.0_{(36)}$	.	.
209	6	$41.7\pm23.1_{(28)}$	$8.2\pm3.0_{(30)}$	.	.
210	3	$63.7\pm33.3_{(7)}$	$13.5\pm2.8_{(8)}$	.	.
211	5	$44.4\pm25.6_{(14)}$	$9.1\pm3.5_{(16)}$	.	.
212	6	$35.6\pm10.83_{(12)}$	$9.7\pm2.6_{(14)}$	.	.
301	5	$52.2\pm19.3_{(6)}$	$14.5 \pm 3.5_{(6)}$	.	8
302	3	$29.6\pm10.1_{(18)}$	$7.8\pm4.6_{(20)}$	$663.6\pm22.1_{(2)}$	24
303	5	$55.7\pm28.1_{(11)}$	$10.2\pm3.2_{(10)}$	$734.8\pm39.9_{(3)}$	24
304	5	$58.8\pm28.6_{(9)}$	$12.5\pm5.4_{(10)}$	$592.0_{(1)}$	24
305	6	$37.9\pm10.8_{(7)}$	$14.9\pm7.3_{(7)}$	$622.5\pm53.0_{(2)}$	22
306	6	$54.9\pm17.7_{(9)}$	$9.3\pm1.3_{(9)}$	$694.0_{(1)}$	24
307	5	$48.3\pm25.8_{(12)}$	$9.3\pm4.4_{(15)}$	$544.8\pm41.7_{(3)}$	24
309	4	$33.7\pm28.0_{(9)}$	$16.4\pm7.6_{(5)}$	$696.5\pm152.0_{(2)}$	24
310	3	$43.3\pm21.4_{(8)}$	$10.7\pm4.7_{(7)}$	.	7
311	6	$33.1\pm17.1_{(16)}$	$14.2\pm18.8_{(17)}$	$622.3\pm12.7_{(3)}$	24
312	6	$65.1\pm17.2_{(16)}$	$9.7\pm3.5_{(9)}$	$667.0_{(1)}$	24
313	5	$36.5\pm19.4_{(12)}$	$13.6\pm10.8_{(13)}$	$621.5\pm50.2_{(2)}$	24
314	6	$52.6\pm10.2_{(12)}$	$11.7\pm7.1_{(13)}$	$621.0\pm22.6_{(2)}$	24
315	6	$34.0\pm15.3_{(20)}$	$5.8\pm3.2_{(19)}$	$571.5\pm19.1_{(2)}$	24
316	3	$53.6\pm24.0_{(7)}$	$23.8\pm13.3_{(6)}$	.	24
317	4	$36.0\pm28.9_{(19)}$	$5.2\pm3.5_{(20)}$	$554.0_{(1)}$	24
Mean	**4.8**	**$44.6\pm10.2_{(28)}$**	**$11.5\pm3.6_{(28)}$**	**$631.0\pm57.8_{(13)}$**	**.**

Appendix 7.

Nest attendance parameters for incubating Great Tits according to treatment group. **Mean session, recess and night session duration (min) were calculated over a 16-24h period in 1994 and 1995. Incubation constancy (I.C.) represents the proportion of the active day for which the female was on the nest.**

1994.

Sample	Treatment	Session	Recess	Night session	I.C.	n
1	C	$28.8 \pm 12.8_{(23)}$	$9.1 \pm 5.2_{(24)}$	485	.75	23
2	C	$17.9 \pm 9.7_{(32)}$	$6.2 \pm 3.2_{(33)}$	658	.74	24
3	C	$40.3 \pm 4.3_{(17)}$	$8.1 \pm 2.9_{(18)}$	619	.83	24
4	H	$45.7 \pm 7.6_{(14)}$	$5.7 \pm 3.2_{(15)}$	665	.88	24
5	H	$54.3 \pm 11.9_{(6)}$	$12.4 \pm 4.5_{(7)}$	553	.79	16
7	H	$47.9 \pm 34.8_{(8)}$	$7.4 \pm 3.6_{(9)}$	810	.85	23
8	H	$32.6 \pm 10.8_{(16)}$	$4.1 \pm 5.2_{(24)}$	744	.84	24

Appendix 7 (cont.)

1995.

Sample	Treatment	Session	Recess	Night session	I.C.	n
101	C	$22.7 \pm 15.6_{(28)}$	$6.9 \pm 6.5_{(28)}$	589	.77	24
105	C	$34.7 \pm 15.2_{(6)}$	$10.0 \pm 4.8_{(7)}$	649	.75	21
109	C	$37.9 \pm 11.0_{(12)}$	$11.1 \pm 3.2_{(14)}$	617	.75	24
117	C	$23.6 \pm 17.3_{(16)}$	$9.3 \pm 11.6_{(17)}$	531	.71	24
121	C	$17.8 \pm 10.3_{(21)}$	$6.9 \pm 3.3_{(22)}$	545	.71	24
122	C	$22.5 \pm 11.0_{(27)}$	$7.2 \pm 3.0_{(28)}$	523	.75	24
123	C	$36.1 \pm 14.7_{(12)}$	$7.1 \pm 2.2_{(14)}$	616	.81	22
124	C	$43.8 \pm 19.0_{(12)}$	$7.2 \pm 2.3_{(14)}$	651	.84	24
103	H	$36.7 \pm 16.0_{(17)}$	$5.9 \pm 3.4_{(18)}$	559	.85	24
104	H	$32.9 \pm 28.4_{(18)}$	$10.0 \pm 7.3_{(18)}$	601	.77	24
108	H	$24.1 \pm 15.5_{(21)}$	$7.2 \pm 2.7_{(22)}$	571	.76	22
112	H	$42.6 \pm 20.9_{(11)}$	$6.9 \pm 2.9_{(13)}$	748	.84	20
114	H	$26.7 \pm 15.5_{(22)}$	$6.0 \pm 2.3_{(24)}$	588	.80	24
116	H	$37.5 \pm 17.2_{(17)}$	$10.0 \pm 5.9_{(19)}$	625	.7	23
120	H	$49.7 \pm 28.0_{(12)}$	$9.8 \pm 3.1_{(12)}$	672	.81	24
Mean: 1994 and 1995		$34.4 \pm 10.2_{(22)}$	$8.0 \pm 1.9_{(22)}$	$619 \pm 76.9_{(22)}$	.79	

Appendix 8.

Sample and ring numbers and dates of capture of incubating Great Tits studied using the doubly labelled water technique. The treatment groups were control birds (C), and those who nests were heated overnight (H). Birds labelled 'B' were sampled in order to determine the background concentration of D and ^{18}O.

Sample number	Ring number.	Date of first capture.	Date of recapture.	Treatment.
3	J390128	16.05.94	18.05.94	C
6	F781396	18.05.94	20.05.94	H
1	J390129	19.05.94	21.05.94	C
4	F781178	19.05.94	21.05.94	H
9	J390130	20.05.94	-	C
8	VK51653	22.05.94	24.05.94	H
2	H227930	24.05.94	26.05.94	C
7	H227578	24.05.94	26.05.94	H
5	J390131	26.05.94	28.05.94	H
10	J390132	28.05.94	-	B
11	J390133	29.05.94	-	B
104	H227576	30.04.95	02.05.95	H
105	J390128	30.04.95	02.05.95	C
106	J815156	04.05.95	06.05.95	H
107	J815157	04.05.95	06.05.94	C
108	H227907	05.05.95	07.05.94	H
109	J815078	06.05.95	08.05.95	C
118	J815158	08.05.95	-	H
119	VK51703	08.05.95	-	C
120	J815159	09.05.95	-	H
121	J815089	09.05.95	-	C
110	J815087	11.05.95	13.05.95	H
111	J815160	11.05.95	13.05.95	C
112	VK51603	12.05.95	14.05.95	H
113	J815161	12.05.95	14.05.95	C
114	J815165	15.05.95	17.05.95	H
122	J815164	15.05.95	-	C
115	J815167	17.05.95	19.05.95	C
101	H227930	18.05.95	-	B
102	F781178	19.05.95	-	B
116	VK51653	19.05.95	21.05.95	H
117	H227950	19.05.95	21.05.95	C
103	J815159	23.05.95	-	B