Energetics of laying and incubation in birds: studies of Swallows *Hirundo rustica*, Dippers *Cinclus cinclus* and Japanese quail *Coturnix coturnix*

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Doctor of Philosophy

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ABSTRACT

The doubly labelled water technique was validated for captive-bred, laying Japanese quail (*Coturnix coturnix*), and used to measure the energy expenditure of free-living laying swallows (*Hirundo rustica*). Swallows had a slightly higher energy expenditure during laying than during incubation or nestling-rearing. Energy expenditure and the net cost of incubation increased sharply for female dippers (*Cinclus cinclus*) when clutches were enlarged to 6 eggs.

Lipophilic dyes were used to determine the rate of ovarian follicular growth and the volume of yolk deposited in a 24 h period by captive quail and free-living swallows. Rates of yolk deposition were related to daily energy expenditure in quail, but not in swallows.

Balances placed under swallow nests recorded an increase in female mass from 5 d before the first egg was laid. Female mass peaked on the evening before the first egg and declined as eggs were laid. Mass changes during laying were equal to the mass of the oviduct and developing ova. However, body composition also changed, as a lipid reserve was built up in the final 4 d before the first egg was laid, whilst body water content declined. This substantially increased the peak energy requirement for biosynthesis in a laying swallow. The lipid reserve was catabolized during the remainder of the laying period. The lipid reserve was likely to serve as an insurance against a drop in food intake during laying. Shortage of food on the day before the first egg was laid led to a reduction in clutch size for some swallows. There was no evidence for use of a protein reserve by laying swallows.

A model was developed from which it was predicted that egg production by swallows, and probably all other insectivorous birds, would be constrained by energy rather than crude lipid or protein requirements.

It was concluded that laying patterns and clutch sizes were sometimes constrained by food availability during egg-laying, and that an upper limit to clutch size could be set by the capacity of an incubating bird to cover the eggs.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Professor David M. Bryant at the University of Stirling, and Dr. Murdo G. MacLeod at the AFRC Institute of Animal Physiology and Genetics Research, Roslin, for their advice and constructive criticism throughout this study.

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The Microcomputer and Media Technology group at Stirling University - Gordon Brewster, John Broom and Jeff Wotherspoon - designed and built the nest temperature monitor and wrote the Psion Organiser "Time and Event logger"

program. James Weir calibrated the mass spectrometer for the chamber calorimetry work with swallows. Lewis Taylor provided an excellent photographic service, Sandy Gardiner collected the insect suction trap catches and Carole Campbell kept an eye on the soxhlet apparatus. Simon Booth, Rob Ibister, Rob Marshall and Marilyn Scott helped me with computing problems. The staff of the animal house were very helpful in setting up the incubator and turning the swallow eggs.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Some life history theory assumes a trade-off between parental investment in the current breeding attempt and future reproductive output (Williams 1966, Charnov and Krebs 1974). Investigation of such theories requires that variation in parental investment is examined in relation to residual reproductive value (Pianka and Parker 1975). Yet quantification of parental investment is difficult, since different types of reproductive costs cannot be measured in a single currency. Measurement of daily energy expenditure provides a possible short term correlate of longer term reproductive costs (Bryant 1988a), although this cannot measure costs related to predation rate, for example. It can, however, synthesise the effort invested in parental care and exploiting food. A major aim of this study was to establish if egg-laying is a costly activity for free-living birds by comparison with incubation and nestling-rearing. If so, it would suggest that more effort should be diverted to identifying fitness trade-offs for egg production rather than offspring number, which has been the focus of most recent work (reviewed by Partridge 1989).

Egg synthesis has often been proposed to be potentially demanding for female birds in terms of energy or nutrient requirements (Perrins 1970, King 1973, Ricklefs 1974, Jones and Ward 1976, Ankney and MacInnes 1978, Murphy 1978, Fodgen and Fogden 1979, Drent and Daan 1980, Houston *et al* 1983, Robbins 1983, Walsberg 1983a,b, Järvinen and Väisänen 1984, Martin 1987, Blem 1990), although other authors have questioned this (Scott and Ankney 1980, Krementz and Ankney 1986, Breitwisch 1989). It was not possible, prior to the present study, to assess whether or not laying was particularly energetically demanding as there was no satisfactory measurement of the energy expenditure of a free-living laying bird. Time budget studies had suggested that laying females might have the highest energy expenditure of any stage in the annual cycle (reviewed by Walsberg 1983a), however estimation of energy expenditure from time budgets can be very inaccurate

(Weathers and Nagy 1980) particularly when some elements of the budget, such as the energy requirement for egg production, are unknown. Two previous studies have used the doubly-labelled water (DLW) technique (Lifson and McClintock 1966, Tatner and Bryant 1989) to make a direct measurement of energy expenditure of a laying bird (Bryant and Westerterp 1980, Meijer *et al* 1989), however neither study produced the required information as the birds in the first study suspended egg formation and those in the second were part of a captive group. In this study, the DLW technique was validated for a (captive) laying bird by comparison with chamber calorimetry, and applied to measurement of the energy expenditure of a free-living species.

The second major aim of this study was to investigate the source of energy and nutrients for egg formation. If egg formation was especially energetically or nutritionally demanding, it might be expected that reserves would be required to supplement daily food intake during the laying period (Jones and Ward 1976, Fogden and Fogden 1979, Houston *et al* 1983, Ankney and Alisauskas 1991, Drobney 1991). If eggs were formed from daily food intake alone, this would support the idea that egg formation was not especially demanding (Ankney and Scott 1980, Ricklefs and Hussell 1984, Arnold and Rohwer 1991). Correlations between potential clutch size and reserve levels have led to suggestions that clutch size might be determined by the level of the nutrient reserve built up prior to laying (Jones and Ward 1976, Houston *et al* 1983, and reviewed by Ankney *et al* 1991, Arnold and Rohwer 1991, Drobney 1991). Alternatively, such a correlation could be interpreted as birds laying down reserves appropriate to the clutch size to be laid (Arnold and Rohwer 1991).

If clutch size or egg quality were under energetic constraint during laying and a substantial part of the nutrient requirement of laying birds came from daily food intake, correlations between food supply and reproductive output would be expected. Alternatively, clutch size might be determined by the number of offspring which the parents could feed (Lack 1954, 1968), rather than by ability to lay or incubate eggs. Lack's hypothesis has been modified by subsequent workers, as the most productive

clutches were often larger or laid earlier than average (Perrins 1965, Perrins and Moss 1975, De Steven 1980, Nur 1984, Gustaffson and Sutherland 1988), and supplementary feeding generally advanced laying date and increased clutch size (reviewed by Martin 1987). These studies suggested a widespread relationship between food supply and reproductive output, although, again, energetic constraint during laying was not necessarily implied, as clutch size could be adjusted in anticipation of the level of demand which could be supplied during nestling-rearing.

The third major aim of this study was to extend knowledge of the energetics of incubating birds, both for comparison with laying and because of dispute over the living costs of uniparental incubators. There has been much controversy as to whether warming the clutch during incubation entails an additional energy expenditure for a bird. Much of this was related to differences in the non-incubating standard with which the incubating bird was compared. Activity budget models and nest box calorimetry showed that incubating birds had lower energy requirements than birds perched in the open at the same ambient temperature, but greater requirements than non-incubating birds within the nest microclimate (El-Wailly 1966, Biebach 1981, Walsberg and King 1978a,b, Vleck 1981, Walsberg 1983a). More recently, measurement of metabolic rate using the doubly labelled water (DLW) technique has allowed comparison of the living costs of free-living incubating birds with those at other stages of the annual cycle. These studies showed that incubating birds had lower metabolic rates than those rearing nestlings (Bryant and Westerterp 1980, Ricklefs and Williams 1984, Bryant and Tatner 1988, Williams 1988), and at other times of the year (Bryant and Tatner 1988). This did not necessarily mean that incubating birds could easily achieve energy balance, as time available for foraging would be greatly reduced (Yom-Tov and Hilborn 1981).

In this study, an energetics approach was adopted to assess the questions of whether reproductive output was constrained during laying or incubation.

1.2 Definition of terms

In order to clarify terms which have sometimes been used with different meanings, and to avoid repetition in the text, the following terms and abbreviations as used in this thesis, are defined below:

Stages in the reproductive cycle:

Pre-laying: before the start of egg formation.

Laying: between the start of formation of the first egg and laying of the final egg. This period included:

Rapid follicular growth (RFG): period between initiation of yolk deposition on ovarian follicles and ovulation of the first egg,

Albumen formation: period between ovulation of the first egg and laying of the final egg. Birds with a clutch size of greater than 1 egg would deposit both yolk and albumen during this period.

Characteristics of birds:

Nutrient reserves: that part of the bird which could potentially supply nutritional demands during periods of energy imbalance.

Body size or **structural size**: the nutrient reserve-independent size of a bird (following Piersma and Davidson 1991).

Body condition: a measure of nutrient reserve mass (following Piersma and Davidson 1991).

Parental investment: extent to which care of offspring reduces the future reproductive output of the parent (following Clutton-Brock 1991).

Energetics:

Gross energy (GE): total heat of combustion of a material as determined by a bomb calorimeter.

Metabolisable energy (ME) or **assimilable energy**: the portion of GE utilised by the animal, i.e., GE excluding energy excreted as faeces, urine or (for ruminants) methane.

Net energy: the portion of ME used for work or tissue synthesis, excluding the heat

increment incidental to nutrient digestion and metabolism.

Production efficiency: defined by Equation 1.1.

1.1

$$\text{Production efficency} = \frac{\text{energy content of production}}{\text{energy content of production + cost of biosynthesis}} \times 100$$

Average daily metabolic rate (ADMR): mean mass specific rate of use of energy during a 24-hour cycle ($cm^3CO_2g^{-1}h^{-1}$).

Basal metabolic rate (BMR): mass specific rate of use of energy of a resting bird, which was not digesting food or synthesising tissue, in the thermoneutral zone. Determined from Aschoff and Pohl (1970), inactive phase, as $0.1326 \times \text{mass(g)}^{0.726}$ kJd^{-1} for passerine birds ($cm^3CO_2g^{-1}h^{-1}$).

Daily energy expenditure (DEE): measure of the total amount of energy used each day by an animal (kJd^{-1}).

Metabolic intensity (M): ADMR/BMR. Used to compare the relative rate of use of energy expenditure of birds of different mass (no units).

1.3 Statistics

Statistical procedures follow Zar (1974) and were performed with the assistance of SPSSX and Minitab. Normality of data was assessed using graphical methods and by correlation of the data with the normal scores: data sets with correlations of greater than 0.95 were taken to be normally distributed. Small data sets and those which were not normally distributed (and normality was not achieved by transformation) were analyzed using non-parametric statistics. The 0.05 level of probability using two-tailed tests were accepted as the level of statistical significance throughout.

CHAPTER 2

STUDY SPECIES AND STUDY SITES

2.1 Introduction

This chapter introduces the three study species. Methods of capture, ageing and measurement of structural size and body condition of individual birds, and assessment of food abundance for swallows, are described here in order to avoid repetition, as each technique will be used in several other chapters.

Fieldwork was carried out with swallows and dippers in Central Scotland. (Latin names of all species are given in Appendix 1). Both these passerines are widespread, are easy to observe, and are tolerant of capture and visits to the nest. The dipper breeding season is earlier than that of the swallow, so work with these two species extended the period when field work with laying and incubating birds was possible. Swallows are aerial foraging passerines which are summer migrants to the study area (Cramp 1988). The breeding biology of this population of swallows has been described by Turner (1980), Jones (1985) and Thompson (1992).

The dipper is a resident passerine which lives and feeds in watercourses. Pairs defend a territory of 0.5-2 km in length during the breeding season (Cramp 1988). The population dynamics of dippers in this area have been described by S.F. Newton (1989).

Laboratory work involved captive-bred Japanese quail. These are gallinaceous birds which are bred commercially to produce eggs. Japanese quail were used to develop and validate the techniques subsequently applied to free-living swallows.

2.2 Methods

2.2.1 Study species and study sites

2.2.1.1 Swallow

Swallows arrived in the study area in late April and eggs were laid between early May and August. The cup-shaped nests, constructed from mud and lined with

feathers, were typically situated on rafters in farm buildings. Each pair defended only a small area around the nest (Turner and Rose 1989). Farms in the study area were evenly spaced amidst mixed arable and grazing land within 10 km of Stirling, Central Region, Scotland (Fig. 2.1). Farms had 1-12, usually 2 or 3, pairs of swallows (Thompson 1992).

Swallows were typically double brooded in the study area, although exceptionally 3 broods were reared (Thompson 1992). New pairs appeared throughout the first half of the breeding season. These birds, and pairs which started to breed late in the season, had only one clutch. Replacement clutches were laid if nesting attempts failed.

Farms were visited to locate swallow nests between the beginning of May and the end of July. At the start of the season, each farm was visited twice weekly to observe which buildings were entered by swallows, and to search for new nests or old nests with fresh lining material or wet mud on the rim. Fresh swallow droppings on the floor could be used to indicate a roost site. This was often associated with a pair nesting nearby. Some nests were located by M. Thompson and A.V. Newton during concurrent work in the same area.

Nests were visited daily from completion of the nest rim until the day after final egg of the clutch was laid (no new egg added to the clutch for two days and eggs found warm). Each new egg was numbered at the blunt end with waterproof ink. Nests were visited weekly during the incubation and nestling-rearing periods, to identify cases of failed breeding. Nests were visited in the same way as before the laying of the first clutch from a week after fledging of the first brood or failure of a breeding attempt, to determine the laying date of second or repeat clutches. All visits to nests to determine dates of laying were made after 0800 hours, to ensure that the day's egg would have been laid before the nest was visited.

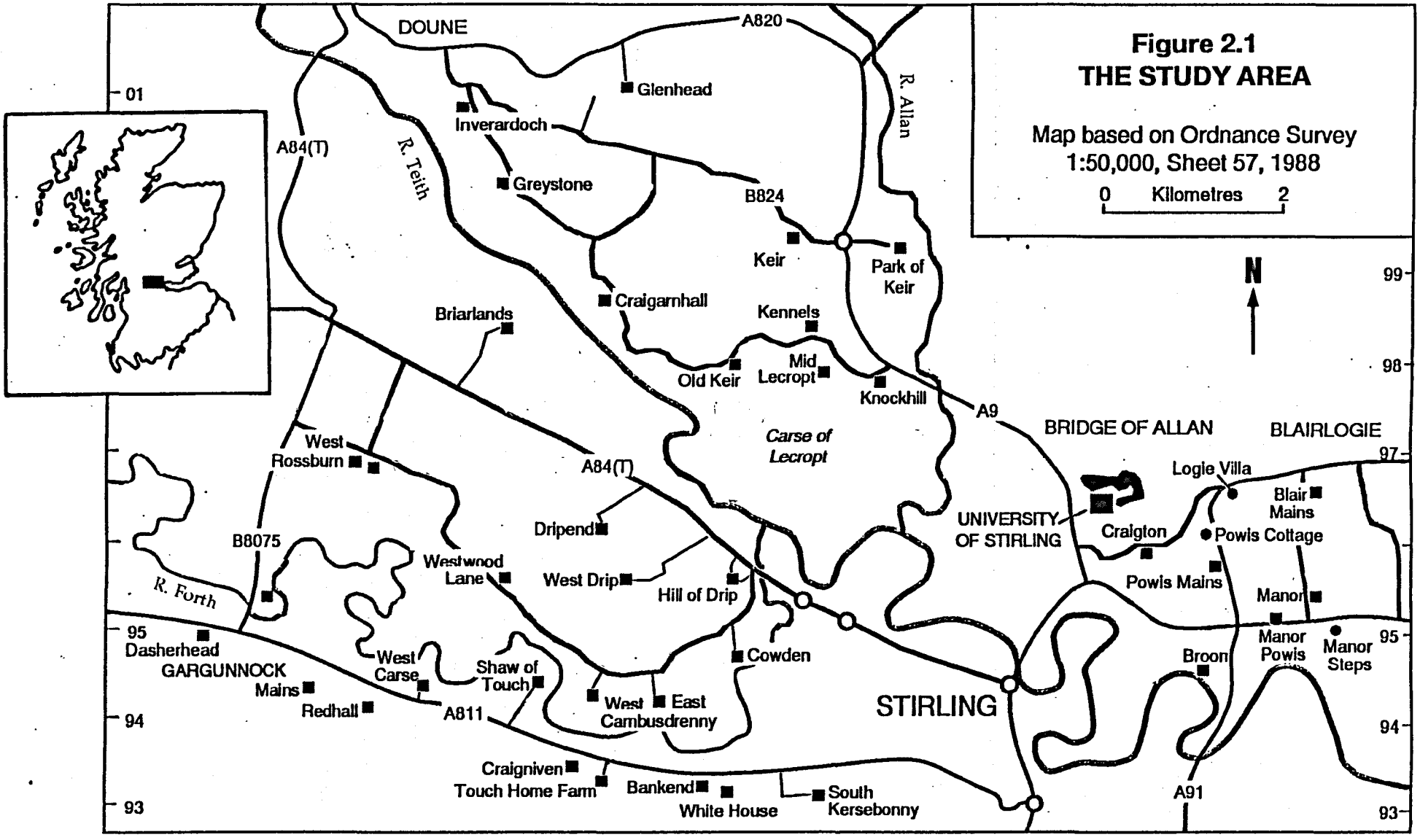


Fig. 2.1 Map of the swallow study area, showing farms at which swallows were studied, roads and rivers.

2.2.1.2 Dipper

Dipper nests were built under bridges, in culverts or at natural sites which overhung water. The spherical nests were constructed of moss and other vegetation with an entrance below the nest cup which was lined with grass and leaves. Clutches were laid between mid-March and mid-May. Dippers were usually single brooded, although pairs which started to breed earliest in the season laid a second clutch (S. F. Newton 1989).

Breeding territories were categorised as upland or lowland using the criteria of S.F. Newton (1989). Lowland sites were on or close to the rivers Devon, Ardoch, or Allan, in Central Region, Scotland (Fig. 2.2). Upland sites were on the smaller tributaries of these rivers in the Ochil Hills. Birds laid 2-3 weeks later in the upland territories than on the main rivers (S.F. Newton 1989).

Searches for dipper nests began in early March, when pairs begin to build or refurbish nests on lowland sections of the river. Nests were located by checking sites known to have been used in previous years and by systematic searches of river banks to find a nest for each pair of birds. Some nests were located by D.M. Bryant, I.G. Johnstone, A.V. Newton and S.F. Newton during concurrent work in the same area.

A sample of lowland sites was visited each week once nest building or refurbishment had begun, and every other day between completion of the nest lining and when the first egg was laid. Daily visits were made from the day the first egg was laid until the day after incubation began (no new egg added to the clutch for 2 days and eggs found warm). Each new egg was numbered at the blunt end with waterproof ink. Upland territories and additional lowland nests were visited during the incubation period (mid-May). Searches were made for second or repeat clutches from about a week after fledging or failure of the first breeding attempt, until mid-May.

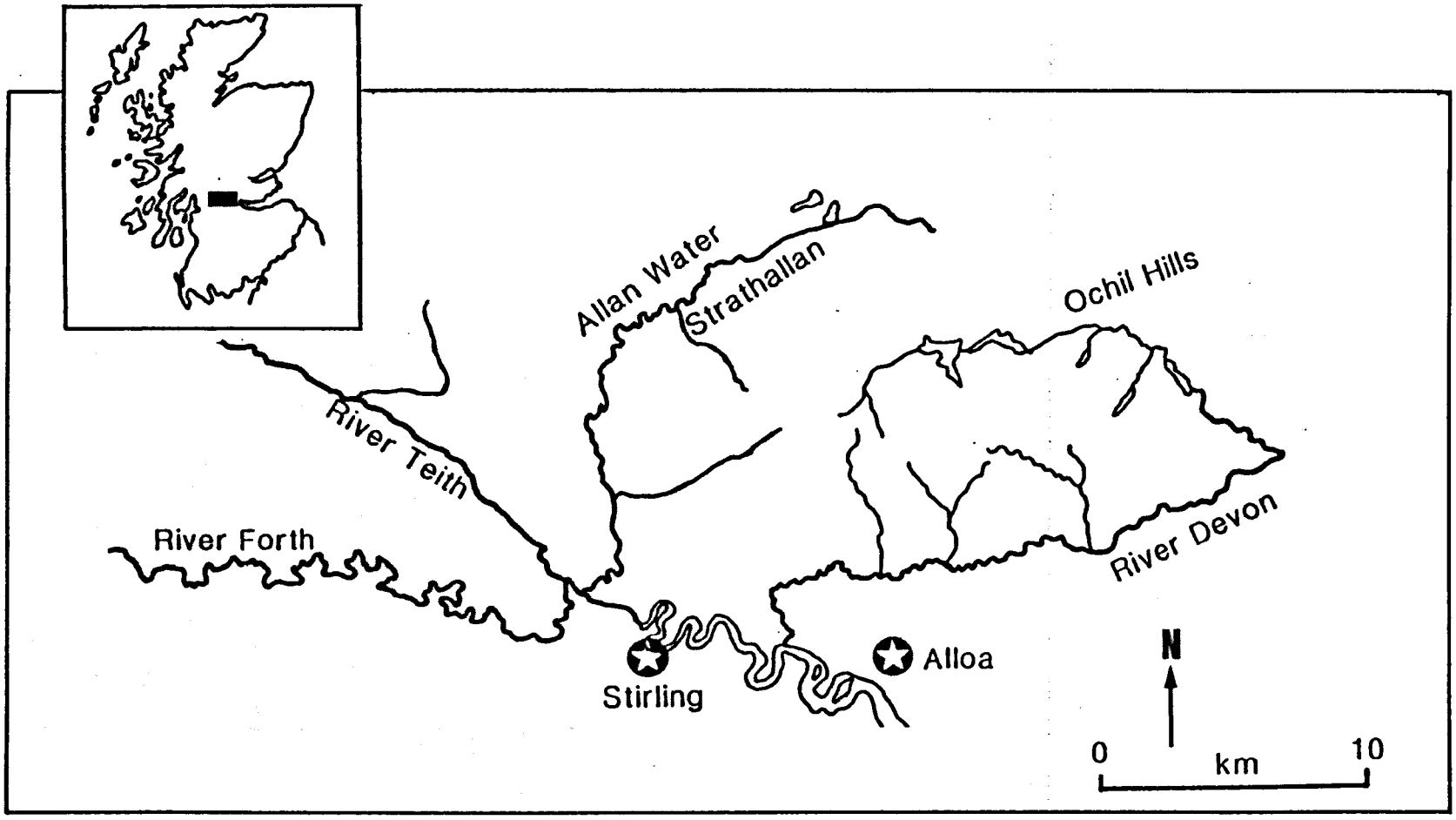


Fig. 2.2 Map of the dipper study area showing the main rivers and their tributaries.

2.2.1.3 Japanese quail

Captive female Japanese quail laid year round and produced infertile eggs if kept separately from males. Mature body size was attained at 6-7 weeks and females started to lay at this age. The birds in this study were reared from stock at the AFRC Institute of Animal Physiology and Genetics Research, Roslin, Midlothian. Although the birds had been in captivity for many generations, they had not been bred selectively. Quail were hatched at weekly intervals, so that stock of the same age, from the same group of parents, were available at the start of each experiment.

2.2.2 Capture techniques and examination of individual birds

2.2.2.1 Swallow

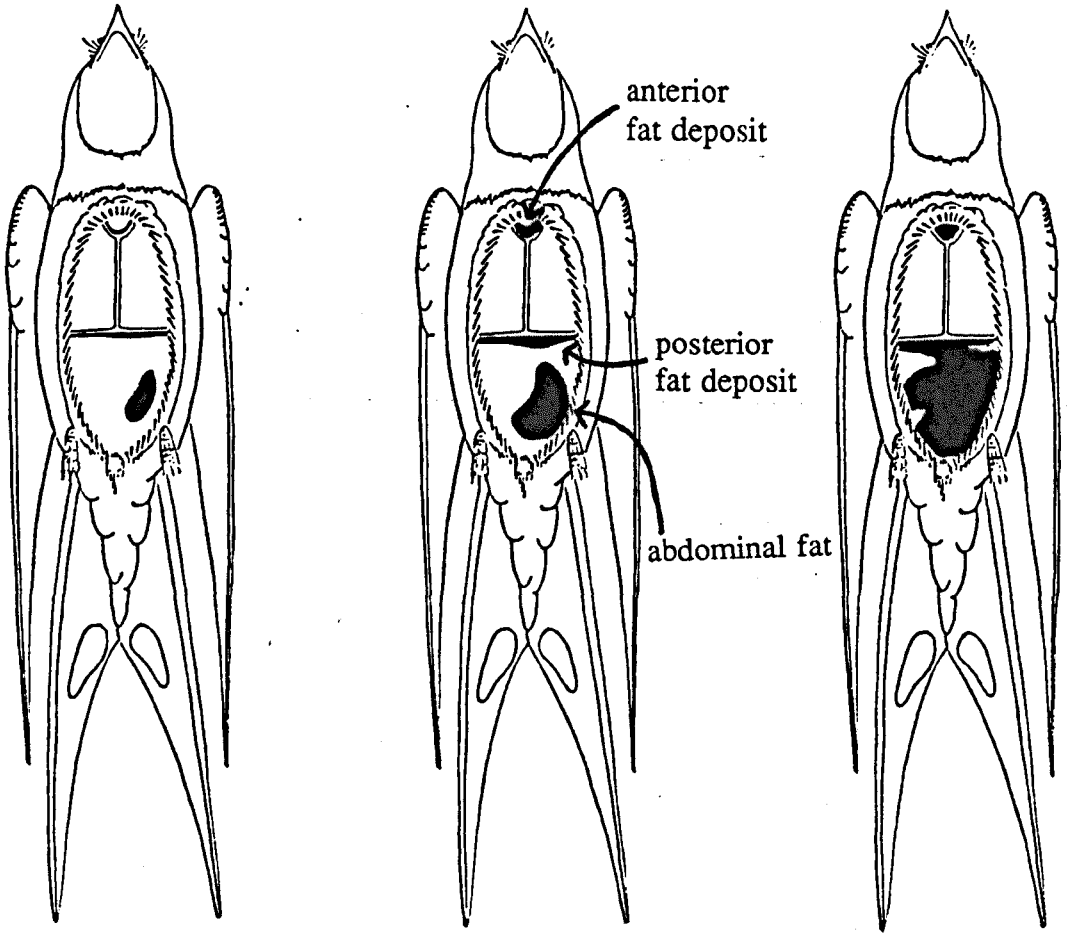
Female swallows were caught at least once each season, to determine the identity of the female at each nesting attempt. Additional captures were made to measure body condition. Most swallows were caught at night, at or close to their nests, by placing a small hand-held mist net on a wire frame over the bird. Additional mist nets were deployed across entrances to the building, in case birds evaded the hand net. Each bird was weighed, and measurements of structural size and body condition made shortly after capture. Birds were held in cloth bags until dawn, when they were released close to the farm at which they nested. Some swallows were caught using mist nets suspended within farm buildings during the day and a few incubating birds were caught on their nests using the hand-held net in daylight. These birds were released within 10 min of capture.

No instances of clutch desertion or failure to hatch followed capture of the parents, except for one incubating female caught twice within a few hours which deserted her clutch. Pairs caught in the pre-breeding period, early in the season, sometimes moved to a different farm. These birds might have been prospecting at the farm at which they were originally caught, but it was thought more likely that capture before these birds were committed to a nest site caused them to move.

The following measurements were made for each female swallow:
mass (to nearest 0.05 g, using 50 g Pesola spring balance),
wing length (maximum chord, using a 15 cm stopped rule, to nearest mm),
length of outermost, second and innermost tail feathers (from base of tail feather in undertail covets, to nearest mm),
and using a dial reading vernier calliper, to nearest 0.1 mm,
tarsus length (tarso-metatarsus bone),
head+bill length (back of skull to tip of bill),
keel length (anterior notch to posterior edge of sternum).

Visible subcutaneous fat deposits were scored on a 5 point scale in the tracheal/claviculo-coracoid deposit (anterior fat score) and in the deposit at the posterior edge of the sternum (posterior fat score) (after Jones 1985). The two values were added to give fat score 1 (range 0-10). For some birds, the percentage cover of abdominal lipid was estimated. This was arcsine transformed to provide fat score 2. The fat scoring system is shown diagrammatically in Fig. 2.3, with examples of birds and their fat scores in Plates 2.1a and 2.1b.

Pectoralis muscle thickness was measured using a portable Krautkramer ultrasonic flaw detector (model USK7) with an Alpha2 Aerotech 10MHz transducer probe. This emitted an ultrasonic sound, and the time taken for reflected signals to return to the probe was displayed as a series of peaks on a screen. The first peak represented the time taken for the signal to travelled once through the muscle and back. Signals were reflected when they met a change in the medium through which they travelled: in this application the muscle/bone interface at the keel. The time taken to receive a reflection was read in arbitrary "ultrasound units" along the x-axis of the display screen. The calibration of the machine was checked each day by measurement of the thickness of a piece of perspex. The principles of the ultrasound technique have been described by Sears (1988) and S.F. Newton (1989).



Anterior	1	3	5
Posterior	1	3	5
Abdominal	10%	50%	90%
Fat score 1	2	6	10
Fat score 2	5.7	30.0	64.2

Fig. 2.3 Diagrammatic representation of visual fat scoring of female swallows. Fat scores were made on a 0-5 point scale at the anterior and posterior edge of the keel and as percentage cover of abdominal fat.

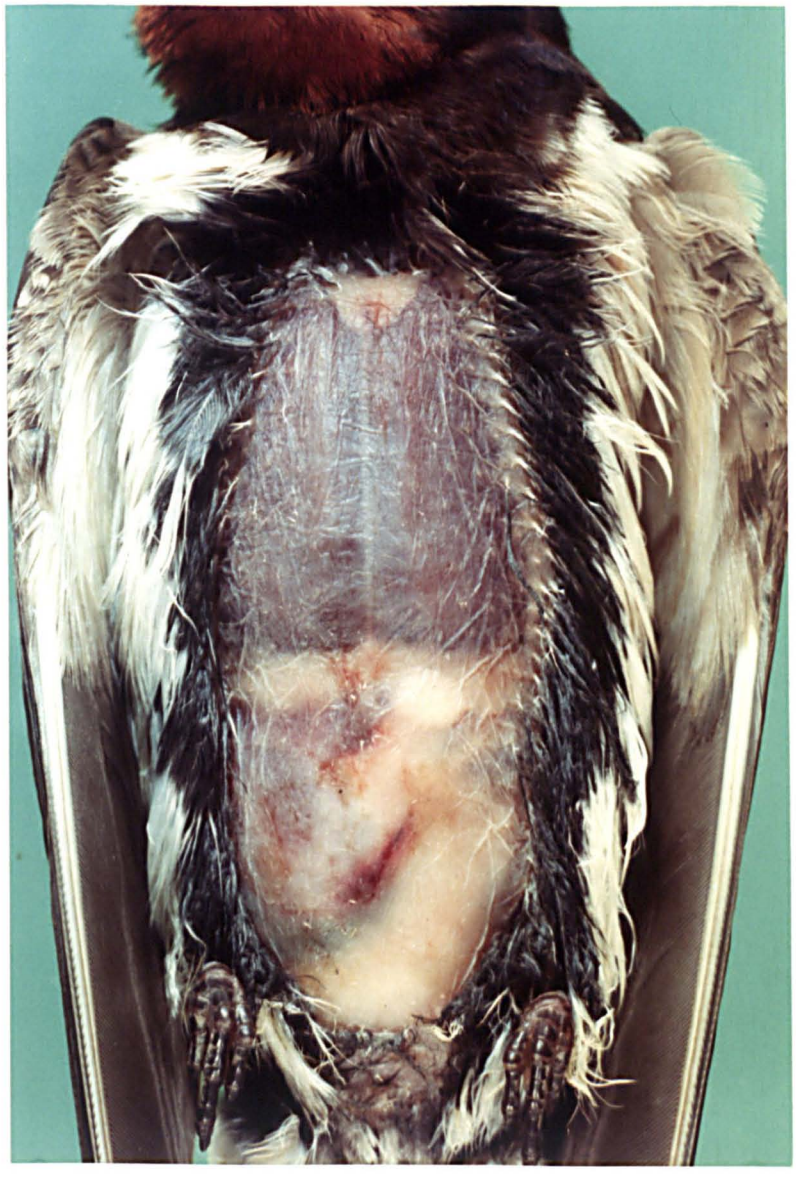


Plate 2.1a A laying female swallow (bird 2, Appendix 7) sacrificed for carcass analysis on the night after she laid her first egg of her second clutch in 1991. The breast feathers have been dampened and moved aside to show the cream coloured anterior, posterior and abdominal fat deposits which were scored as 4,4,70%. Mass before death=24.30 g. Compare with the starved bird in Plate 2.1b.

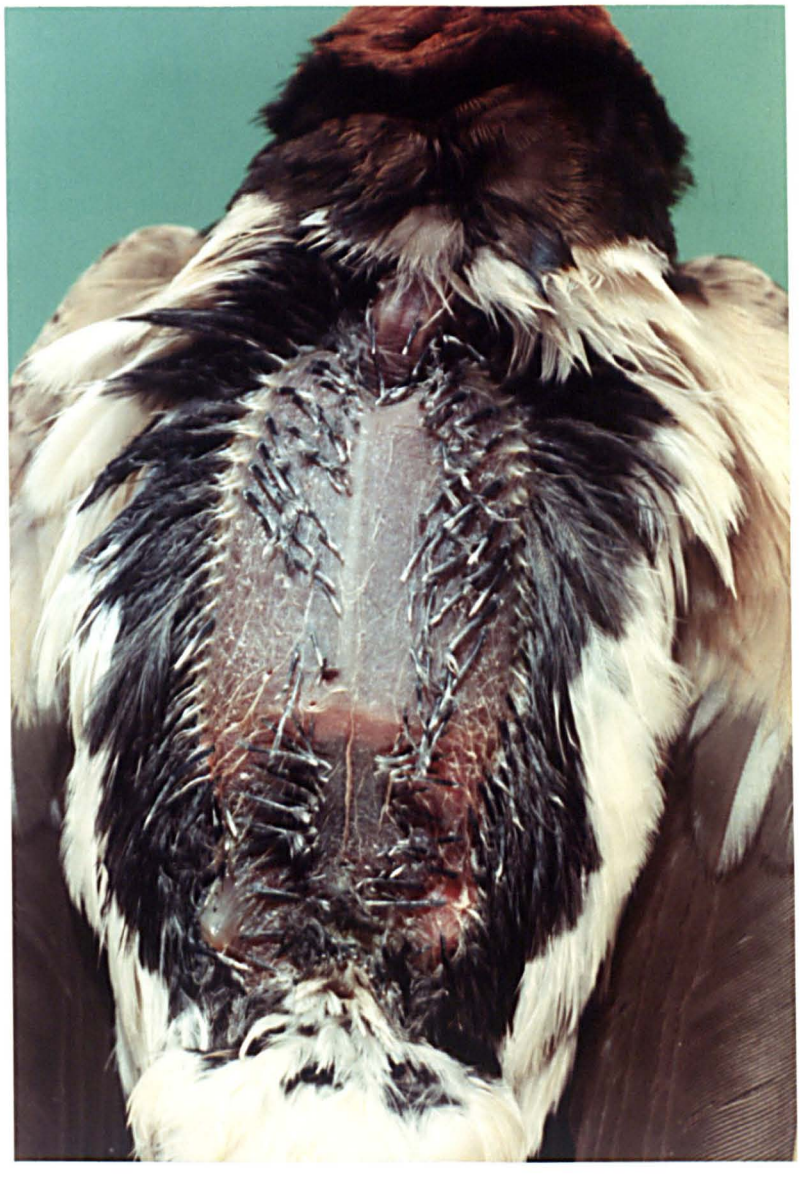


Plate 2.1b A nestling-rearing female swallow (bird 3, Appendix 7) which had become entangled in fishing line used as nest lining. She was killed when found close to death from starvation. The anterior, posterior and abdominal fat deposits were scored as 0,0,0%. This bird had a lower fat score and pectoralis thickness than any healthy bird caught during the study. Note regrowth of feathers on the brood patch (typical after the end of incubation duties for the season), the prominence of the keel, the lack of cream coloured fat reserves and the contracted abdomen when compared with the laying bird in Plate 2.1a. Mass 1 day after death=16.00 g.

Breast feathers were dampened with alcohol and brushed to one side whilst the probe was placed normal to the muscle surface, adjacent to the keel (Fig. 2.4, Plate 2.2). The interface between the probe and the skin of the bird was also wetted with alcohol as the signal does not transmit through air. The pectoral muscle thickness of swallows was measured at 2 positions on each side of the keel in 1989 and 1990. In 1991 measurements were made on the right pectoralis muscle only in order to reduce handling time, as it was shown that measurements from either pectoralis muscle were correlated (Section 2.3.1.6, S.F. Newton 1989).

Ultrasound measurements of pectoral muscle thickness were used to assess the size of the pectoral muscle using three indices: ultrasound thickness (USTHICK), ultrasound volume (USVOL), and ultrasound index (USI) (Equations 2.1, 2.2 and 2.3).

$$USTHICK = us1 + us2 \qquad 2.1$$

$$USVOL = \frac{(us1 + us2) \times keel\ length}{1000} \qquad 2.2$$

$$USI = \frac{(us1 + us2)}{keel\ length} \qquad 2.3$$

where us1 and us2 were the two measurements of muscle thickness made on the (bird's) right hand pectoral muscle (arbitrary ultrasound units).

Swallows were sexed by the presence of a brood patch on the female, or by tail length and plumage coloration for birds caught before the development of a brood patch. Males had brighter plumage and longer tails (Thompson 1992). Male swallows were not aged or measured as part of this study, because only the female swallow incubated the eggs (Cramp 1988).

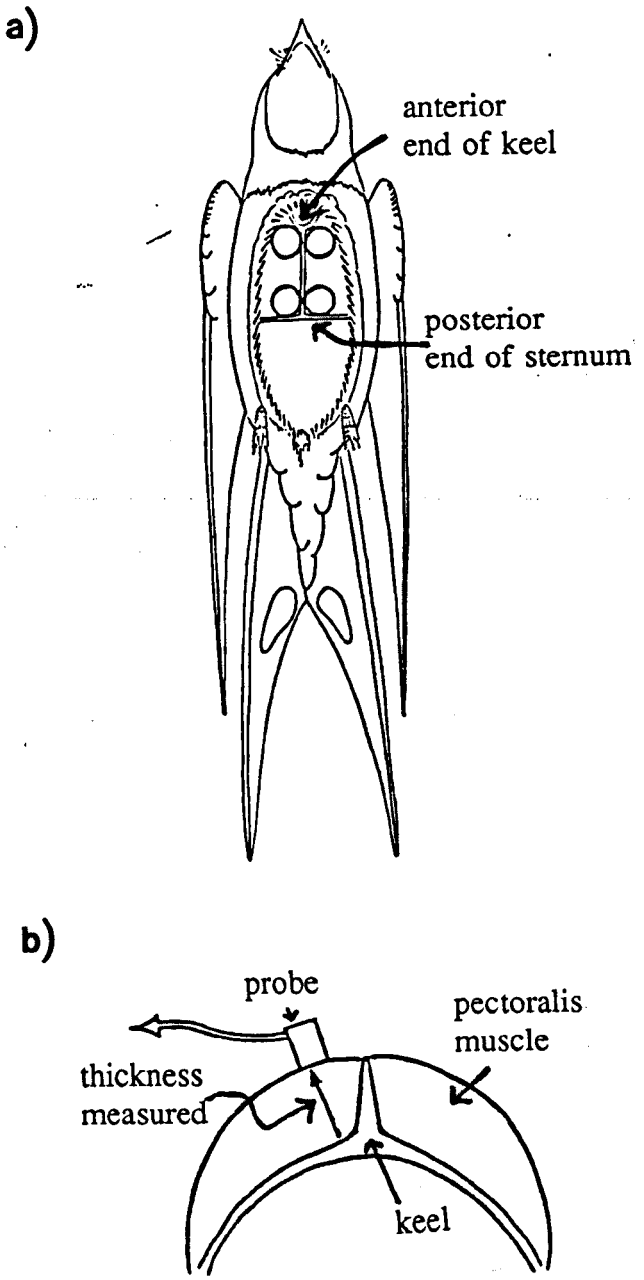


Fig. 2.4 Diagrammatic representation of a) locations of ultrasound probe for measurement of pectoralis thickness on a swallow, b) muscle thickness measured by ultrasound.

Plate 2.2 Use of the ultrasound probe to measure the thickness of the pectoral muscle left upper position of a female swallow on the night after she laid her first egg.

2.2.2.2 Dipper

Female dippers were caught in daylight during the incubation period by placing a fisherman's landing net over the entrance to the nest. Each female was caught once each year to obtain mass and size measurements and determine age from ringing history or plumage. First year birds had white-tipped wing coverts (Svensson 1984). Birds were released close to their nests within 10 min of capture. No clutch desertions followed this procedure.

The following measurements were made for each bird:
mass (to nearest 0.1 g, using 100 g Pesola spring balance),
wing length (maximum chord, to nearest mm with a 15 cm stopped rule),
and using a dial reading vernier calliper, to nearest 0.1 mm,
tarsus length (tarso-metatarsus bone),
head+bill length (back of skull to tip of bill),
bill length (from tip of bill to the end of feathering on the top surface of the upper mandible),
bill depth (measured vertically at anterior of nares),
keel length (anterior notch to posterior end of sternum).

Dippers can be sexed readily during incubation by the presence of a brood patch on the female. No male dippers were caught during this study as only the female incubated the eggs (Cramp 1988).

2.2.2.3 Japanese quail

The following measurements were taken from male quail at the start and end of each experimental period, to determine whether the birds grew structurally or the size of pectoralis muscle or fat reserves changed during the experiment:
mass (to nearest 0.1 g, using a 2000 g Ohaus balance),
wing length (maximum chord, to nearest mm, using a stopped 30 cm rule)

and using a dial reading vernier calliper, to nearest 0.1 mm,
tarsus length (tarso-metatarsus bone),
keel length (anterior notch to posterior end of sternum),
head+bill length (back of skull to tip of bill).

Sub-cutaneous fat was scored as pinched thickness (mm) in the subalar fat deposit (under the wing) where Japanese quail are lightly feathered. Pectoral muscle thickness was measured at three sites on either side of the keel using ultrasound (Section 2.2.2.1). Female quail were not measured, as changes in size and composition were not involved in the experimental program.

2.2.3 Ageing techniques

2.2.3.1 Swallow

Swallows which had not been ringed as pulli were aged using the assumption that birds which were caught in the study area for the first time were 1 year old, if all adults of that sex had been ringed at the farm in the previous breeding season. Other birds were classified as at least 1 year old. Less than 5% of adult birds moved between farms within the study area (Thompson 1992), so this was a reasonable assumption. Most swallows in the study area were ringed during previous or concurrent studies on the population (Thompson 1992, D.M. Bryant and A.V. Newton), so all breeding swallows were ringed at most of the farms in the area. Swallows were placed in the following age categories:

1 - 1 year old, ringed as a pullus in previous year,

1a - assumed 1 year old, unringed adult on farm where all females had been ringed previous year,

1b - at least 1 year old, first year found breeding, but of unknown age as not all female swallows were ringed at the farm in the previous year,

2 - 2 year old, ringed as a pullus 2 years ago,

2a - assumed 2 year old, in category 1a in previous year,

2b - at least 2 years old, in category 1b in previous year,

3 - 3 year old, ringed as a pullus 3 years ago,

3a - assumed 3 year old, in category 1a 2 years ago,

3b - at least 3 years old, in category 1b 2 years ago,

4 - 4 year old, ringed as a pullus 4 years ago,

4a - assumed 4 year old, in category 1a 3 years ago,

4b - at least 4 years old, in category 1b 3 years ago.

2.2.3.2 Dipper

Most of the dippers in the study area were ringed during previous or concurrent studies (S.F. Newton 1989, D.M. Bryant, I.G. Johnstone, A.V. Newton), so most birds could be aged from their plumage or ringing history. Dipper age categories were:

1 - 1 year old, ringed as pullus or juvenile previous season, or unringed with white tipped wing covets,

1a - at least 1 year old, unringed, but wing covets too worn to see whether they had white tips,

2 - 2 years old, in category 1 in previous year,

2a - at least 2 years old, in category 1a in previous year,

2b - at least 2 years old, unringed with no white tips to wing covets,

3 - 3 years old, in category 1 2 years ago,

3a - at least 3 years old, in category 1a 2 years ago,

3b - at least 3 years old, in category 2b previous year,

4 - 4 years old, in category 1 3 years ago,

4a - at least 4 years old, in category 1a 3 years ago,

4b - at least 4 years old, in category 2b 2 years ago,

5 - 5 years old, in category 1 4 years ago,

5a - at least 5 years old, in category 1a 4 years ago,

5b - at least 5 years old, in category 2b 3 years ago.

2.2.4 Food supply of aerial feeding birds

The food supply of aerial feeding birds was measured using a 12.2 m high suction trap (Taylor and Palmer 1972). Although swallows often fed closer to the ground than this, especially in poor weather conditions, values of insect abundance from hand net sampling at 0.2-2 m above the ground were significantly correlated with suction trap volume (Turner 1982, Jones 1985). This allowed use of the suction trap catch as a measure of the food supply available to swallows, although it obviously could not reflect local variation in food availability in the vicinity of each nest. Taylor (1973) demonstrated that suction trap catch measured insect abundance over a wide area so data would be representative of the study area in general.

The suction trap was sited on the University of Stirling campus, 0.5-10 km from the swallow study sites. The trap was run continuously and samples were removed at 1000 hours daily and stored in 10:1 methanol:glycerol solution. Large, nocturnal insects were removed from the catch, and the settled volume (Vcm^3) estimated in a measuring cylinder. Volumes were transformed ($\log_e (V+1)$) as hirundine foraging rate is thought more likely to have a logarithmic than a linear relationship with insect abundance (Bryant 1978b).

2.2.5 Weather data

A daily record of maximum and minimum temperature and rainfall was made at the Parkhead recording station on the University campus, adjacent to the suction trap. Mean temperature was calculated as the mean of the maximum and minimum temperatures on each day.

2.3 Results

2.3.1 Structural size of female swallows

2.3.1.1 Comparison of measurements made by two observers

Measurements of structural size of 19 female swallows were made by M. Thompson (MT) and myself (SW) in 1989, without prior knowledge of the other person's measurements. There were no significant differences between our measurements of tail length, but my wing and head+bill lengths were significantly shorter and keel lengths significantly longer than those of MT (Table 2.1). The mean difference between our measurements was used to adjust wing, head+bill and keel lengths measured by MT to be consistent with SW measurements using Equations 2.4 to 2.6.

$$wing_{sw} = wing_{mt} - 0.789 \ (mm) \qquad 2.4$$

$$head{+}bill_{sw} = head{+}bill_{mt} - 0.153 \ (mm) \qquad 2.5$$

$$keel_{sw} = keel_{mt} + 0.084 \ (mm) \qquad 2.6$$

where $_{sw}$ indicates a size measured by myself and $_{mt}$ indicates one made by M. Thompson.

There were no significant differences between measurements made by SW and the adjusted measures made by MT (Wilcoxon matched pairs signed ranks test, both $p>0.6$). Subsequent analyses involving the structural size of female swallows include measurements made by SW, and adjusted measurements made by MT.

Table 2.1 Comparison of measurements of structural size (mm) of female swallows made by two observers (Wilcoxon matched pairs signed ranks tests, n = 19 birds).

Size measurement	Z	Significance
Wing length	2.73	<0.007
Head+bill length	2.82	<0.005
Keel length	2.56	<0.011
Outer tail[a]	0.76	>0.44
Second tail[b]	1.14	>0.25
Inner tail[c]	0.18	>0.85

[a] length of outermost tail feather

[b] length of second tail feather

[c] length of shortest tail feather

2.3.1.2 Variation in structural size of the population between years

There were no significant differences in the mean structural size (wing or keel lengths) of female swallows, between 1987 and 1991 (Table 2.2). Each swallow was included in this analysis in each year in which she was caught. Subsequent analyses involving structural size included birds measured from any of the three years of this study (1989-1991).

There was no significant change in the structural size of female swallows between 1987 and 1991, although the breeding population in the study area increased in 1988 (Thompson 1992) and decreased in 1991 (pers. obs.). The breeding population in 1991 was reported as 50-75% down on the 1990 level, nationally (Mead 1991). Sand martins showed a decrease in population mean keel length during a crash in numbers in 1984 and similar results were found for house martins (Jones 1987d). The factors which caused the reduction in the swallow breeding population in 1991 did not exert a comparable selection pressure upon body size.

2.3.1.3 Description of the structural size of female swallows

The mean structural size of female swallows caught during this study are shown in Table 2.3. Birds which bred in more than one year were only included once in this analysis (the year which was included was determined at random). Wing and tail feather lengths were highly correlated with one another (Table 2.4). The three skeletal measures were not correlated with each other. Wing length was significantly correlated with tarsus length and head+bill length, but not with keel length. Head+bill and keel lengths were significantly correlated with tail feather lengths.

A principal components analysis was performed using the structural size measurements of female swallows, to provide a composite measure of size (Table 2.5). The first principal component (PC1) was interpreted as a measure of wing and tail feather length and the second component (PC2) was a measure of skeletal size.

Table 2.2 Measurements of a) wing length and b) keel length of female swallows 1987-91. 95% confidence intervals for both measures show overlap between all years and both observers.

a) Wing length (mm)

Year	Mean	Standard error	n	95% confidence interval Max.	Min.
1987[a]	125.4	0.26	98	125.92	124.88
1988[a]	125.9	0.20	98	126.30	125.50
1989[a]	125.7	0.22	149	126.13	125.27
1989[b]	125.1	0.35	68	125.80	124.40
1990[b]	125.0	0.33	61	125.66	124.34
1991[b]	124.9	0.72	15	126.43	123.36

b) Keel length (mm)

Year	Mean	Standard error	n	95% confidence interval Max.	Min.
1987[a]	21.2	0.07	98	21.34	21.06
1988[a]	21.1	0.05	98	21.20	21.00
1989[a]	21.1	0.05	149	21.20	21.00
1989[b]	21.1	0.08	68	21.26	20.94
1990[b]	21.2	0.09	61	21.38	21.02
1991[b]	21.5	0.20	15	21.93	21.07

[a] measurements made by MT, adjusted to be compatible with SW measurements.
[b] measurements made by SW.

Table 2.3 Measurements of structural size (mm) of female swallows caught between 1989 and 1991. Individuals caught in more than one year were included once in this analysis (year selected randomly). Sample sizes varied between measurements, as a few birds had damaged tail feathers, a reliable keel measurement was not obtained for two birds, and tarsus lengths were not included for birds measured by MT.

Size measurement	Mean	sd	n
Wing length	124.86	2.64	125
Head+bill length	29.82	0.57	125
Keel length	21.18	0.73	123
Tarsus length	11.61	0.41	90
Outer tail[a]	90.50	6.11	121
Second tail[b]	62.14	2.72	124
Inner tail[c]	46.02	2.46	124

[a] length of outermost tail feather

[b] length of second tail feather

[c] length of shortest tail feather

Table 2.4 Pearson correlation co-efficients between measurements of the structural size (mm) of female swallows. Sample sizes are given in brackets. *** = p<0.001, ** = p<0.01, * p<0.05, ns = not significant. [a] length of outermost tail feather, [b] length of second tail feather, [c] length of shortest tail feather

Size measurement	Wing	Head+bill	Keel	Tarsus	Outer tail[a]	Second tail[b]
Head+bill length	0.33 (125) ***					
Keel length	0.15 (123) ns	0.17 (123) ns				
Tarsus length	0.28 (90) **	0.07 (90) ns	-0.02 (88) ns			
Outer tail[a]	0.57 (121) ***	0.27 (121) **	0.14 (119) ns	0.02 (86) ns		
Second tail[b]	0.51 (124) ***	0.24 (124) **	0.14 (122) ns	-0.02 (89) ns	0.62 (121) ***	
Inner tail[c]	0.39 (124) ***	0.13 (124) ns	0.26 (122) **	-0.03 (89) ns	0.32 (121) ***	0.39 (124) ***

Table 2.5 Factor loading scores from principal components analysis of measures of the structural size of female swallows (mm). % variance is percentage of total variance in body size explained by each component. n = 90.

Size measurement	Principal component PC1	PC2
Wing length	0.312	0.207
Head+bill length	0.153	0.135
Keel length	0.129	-0.483
Tarsus length	0.010	0.663
Outer tail[a]	0.312	0.141
Second tail[b]	0.320	0.021
Inner tail[c]	0.240	-0.335
% variance	36.2	16.5

[a] length of outermost tail feather

[b] length of second tail feather

[c] length of shortest tail feather

2.3.1.4 Relationship between structural size and age of female swallows

Eighteen female swallows bred in more than one year, and 2 of these bred in all three years of the study. There were no significant changes in any of the measures of structural size between years (Wilcoxon matched pairs signed ranks tests, all $p>0.1$).

For swallows of known age, wing length, PC1, outer and second tail feather lengths were positively correlated with age (Table 2.6). Measures of skeletal size were not significantly correlated with age (Table 2.6).

2.3.1.5 Relationship between structural size and mass of incubating swallows

The mass of incubating swallows caught during the night was adjusted to midnight mass using an overnight rate of mass loss of 0.15 gh^{-1} (sd=0.04, n=9 nights) of an undisturbed incubating female swallow weighed using the nest balance system (Chapter 8). There was no significant trend in mass for females caught during the day, so these masses were not adjusted for time.

There was no significant difference in mass between female swallows weighed whilst incubating their first and second clutches (t-test, $t=0.95$, $p=0.3$, $n=99$). The mass of female swallows was not significantly correlated with day of incubation (Pearson correlation, $r=0.14$, $p=0.14$, $n=62$). All birds caught during incubation were therefore included in an analysis of the relationship between the mass and structural size of female swallows.

In a multiple regression analysis, head+bill and keel length accounted for 14.6% of mass variation of incubating female swallows (Table 2.7). This was more than the 12.1% accounted for by PC1 if mass was instead regressed upon this composite measure of size. Size-adjusted incubation mass was therefore calculated from keel and head+bill lengths. Residual mass was calculated from mass minus incubation mass predicted from the regression equation in Table 2.7. Residual mass was not correlated with day of incubation ($r=0.12$, $p=0.18$, $n=62$).

Table 2.6 Spearman correlations between age and measures of the structural size (mm) of female swallows. ** = $p<0.01$, ns = not significant

Size measurement	r_s	p	n
Wing length	0.30	**	72
Head+bill length	-0.04	ns	72
Keel length	0.18	ns	70
Tarsus length	0.00	ns	56
Outer tail[a]	0.33	**	69
Second tail[b]	0.27	**	71
Inner tail[c]	-0.06	ns	71
PC1[d]	0.37	**	51
PC2[e]	0.15	ns	51

[a] length of outermost tail feather

[b] length of second tail feather

[c] length of shortest tail feather

[d] first component of a principal components analysis of swallow body size (Table 2.5)

[e] second component of a principal components analysis of swallow body size (Table 2.5)

Table 2.7 Stepwise multiple regression analysis of the relationship between the mass and structural size of incubating female swallows. n = 68 birds. Variables are tabulated in the order of inclusion in the regression model. Variables not included at the 5% level of significance were tarsus, wing and tail feather lengths, day of incubation, and age.

Independent variable	Cumulative r^2	Regression equation	t	p	Beta weight
Head+bill	0.09	$0.615x_1$	2.44	<0.02	0.277
Keel	0.15	$0.744x_2$ -12.359	2.34	<0.03	0.266

2.3.1.6 Relationship between measurements of thickness made on the left and right pectoralis muscles of swallows

Thicknesses of the left and right pectoralis muscles were highly correlated (Pearson correlation r=0.85, p=0.001 for anterior position, r=0.78, p=0.001 for posterior position, n=74). The mean of the left and right muscle thicknesses was used to calculate us1 and us2 for birds for which measurements were made on both sides of the keel.

2.3.2 Structural size of female dippers

The size measurements of female dippers caught during this study are shown in Table 2.8. Birds caught in more than one year were included once in this analysis (year of inclusion selected at random). This sample of birds was too small to warrant detailed analysis of the relationships between structural size measurements and age. These analyses have been performed with a larger sample of birds (S.F. Newton 1989).

2.3.3 Structural size of male quail

The structural size of male quail used in the experiment in Chapter 5 are shown in Table 2.9. There were positive correlations only between wing and head+bill lengths (Pearson correlation, r=0.51, p=0.007, n=28), and between keel and wing lengths (r=0.38, p=0.05, n=28).

2.3.4 Relationships between weather and food supply of aerial feeding birds

Maximum, minimum and mean temperature were significantly positively correlated with each other in each of the three years (Pearson correlations, all r>0.54, p<0.001, 1st April to 31st August in 1989 and 1990, n=153 d, 1st April to 30th

Table 2.8 Structural size measurements (mm) of female dippers 1989-91, n=34.

Size measurement	Mean	sd
Wing length	89.1	2.21
Tarsus length	28.2	0.84
Head+bill length	45.5	0.85
Bill length	16.2	0.87
Bill depth	4.6	0.37
Keel length	26.8	1.14

Table 2.9 Structural size measurements (mm) of 10-week-old male quail.

Size measurement	Mean	sd	n
Wing length[a]	103.8	2.7	28
Tarsus length	25.0	1.2	45
Head+bill length	36.0	1.0	45
Keel length	36.1	1.6	45

[a] wing length could not be measured for all birds due to abrasion of the tips of the feathers

June 1991, n=90 d). In 1989 and 1991, minimum temperature was significantly positively correlated with rainfall (r=0.17, p=0.04 in 1989, r=0.25, p=0.02 in 1991 and r=-0.03, p=0.7 in 1990). Insect availability, measured by the suction trap catch ($\log_e(V+1)$), was significantly positively correlated with maximum, minimum and mean temperature in each of the three years (all $r>0.37$, $p<0.001$). Daily variation in maximum and minimum temperature and insect suction trap catch are shown for each year in Appendix 2.

As the relationships between suction trap catch, temperature and rainfall were similar in all years, data from the three years was combined to show the correlations between maximum, minimum and mean temperature, suction trap catch, date and rainfall (Table 2.10). Partial correlations between suction trap catch and date, controlling for mean temperature (r=0.23, p=0.001, n=391), and between suction trap catch and temperature controlling for date (r=0.25, p=0.001, n=391) showed that suction trap catch was more highly correlated with mean temperature than with date.

The three measures of temperature and suction trap catch would measure similar environmental characteristics, whilst rainfall would have an independent effect. Temperature could have a direct effect upon swallow energy expenditure by increasing the metabolic requirement for thermoregulation as temperature fell, and an indirect effect via decreased food availability at low temperatures. Heavy rainfall would also be predicted to reduce food availability and foraging success (Turner 1982, Hails and Turner 1985).

2.4 Discussion

2.4.1 Choice of an appropriate measure of structural size for swallows

Choice of an appropriate measure of structural size was necessary in order to adjust mass for the effects of variation in structural size prior to consideration of the effects of other factors upon mass. Freeman and Jackson (1990) recommended use of principal components analysis to describe structural size, but more of the variation in the mass of incubating female swallows was accounted for by head+bill and keel

Table 2.10 Pearson correlation co-efficients between daily maximum, minimum and mean temperature (°C), rainfall (mm), food supply (insect trap catch, $\log_e$ (V+1)) and date (days after 1st April). Data pooled for the years 1989-1991, n = 394 days. 1st April to 31st August in 1989 and 1990, 1st April to 30th June in 1991.) *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$, ns = not significant.

	Suction trap catch	Maximum temperature	Minimum temperature	Mean temperature	Rainfall
Maximum temperature	0.47 ***				
Minimum temperature	0.47 ***	0.62 ***			
Mean temperature	0.52 ***	0.92 ***			
Rainfall	0.03 ns	-0.04 ns	0.11 *	0.03 ns	
Date	0.51 ***	0.65 ***	0.66 ***	0.73 ***	0.17 **

length than by PC1 (Section 2.3.1.5). Use of skeletal measures of structural size was also preferable to use of a size correction based on principal components analysis for swallows because PC1 increased with age whilst the skeletal measures did not (Table 2.6). Incubation mass was therefore predicted from head+bill and keel lengths using the regression equation in Table 2.7.

Freeman and Jackson (1990) recommended tarsus length as the best single external metric of body size. Tarsus length was a good measure of variation in structural size in the dipper (Bryant and Tatner 1988). However, tarsus length was not included as a significant variable in the regression model of swallow mass (Table 2.7). Possibly the aerial habit of swallows reduced the importance of the relationship between mass and the legs which must support the bird.

2.4.2 Accuracy of fat scoring as measure of body lipid content in swallows

Krementz and Pendleton (1990) found an r^2 of 0.443-0.697, n=20, for a regression of body lipid content upon fat score in five passerine species. Fat score was a much better predictor of body lipid content in the swallow (r^2=0.749, n=11 female swallows, laying birds excluded, Thompson 1992). Fat score was also a reliable indicator of body lipid content in the house martin (r=0.87, n=23, Bryant and Westerterp 1983a) and the sand martin (r=0.79, n=32, Jones 1987c). Fat scoring was particularly accurate with breeding females as lipid deposits were easily visible due to defeathering of the brood patch (Plate 2.1). Visual fat scoring was concluded to be particularly accurate in assessment of the body lipid content of hirundines.

CHAPTER 3

EGG COMPOSITION AND RATE OF EGG FORMATION

3.1 Introduction

Until recently, the form and timescale of egg formation by birds was poorly known. Dissection of laying females demonstrated that although many species laid one egg per day, the formation of each egg required several days; hence a bird deposited yolk in several rapidly developing ovarian follicles each day. Albumen and shell were deposited around each ovulated follicle over 24 h (Johnson 1986). Comparison of the size of the hierarchy of ovarian follicles, and assumption that the difference in size between follicles was the same as the daily growth increment of a single follicle, allowed production of models of egg formation (King 1973, Ricklefs 1974, Walsberg 1983a). In these models, it was assumed that ovarian follicular growth followed a sine curve, and that all eggs in a clutch followed the same pattern of growth and required the same number of days to form. The energy content of egg formed on any day could be calculated by summation of the energy contents of yolk deposited in each rapidly developing follicle.

The use of lipophilic dyes to mark a discrete layer in rapidly developing follicles shortly after the dye was fed to the laying female (Gilbert 1971), and of staining techniques to show daily increments in follicular growth (Grau 1976), can now allow refinement of models of the pattern of egg formation. These techniques allow determination of the pattern of egg formation in free-living birds without harming the female. The volume of egg formed by individual females each day during production of a clutch can be determined by examination of the eggs after laying.

In this chapter, the use of lipophilic dye to stain the outside of each rapidly developing follicle is described for captive Japanese quail and free-living swallows. The technique has been used previously with Japanese quail (Bacon and Koontz 1971), but this is the first application with a small free-living passerine. The technique was developed in Japanese quail, so that dyed layers in follicles delimited

the volume of yolk deposited during simultaneous measurement of energy expenditure of laying females by chamber calorimetry and the doubly labelled water technique (Chapter 6). In swallows, the dye was used both to determine the mean pattern of follicular growth in this species for the first time (this chapter), and to determine the volume of egg deposited during measurement of energy expenditure by the doubly labelled water technique (Chapter 7). The composition of Japanese quail and swallow egg yolk and albumen was determined to allow calculation of the energy content of egg formed each day. Variation in the rate of growth of swallow follicles was analyzed in relation to clutch size, position in the clutch and environmental factors during follicular growth. Swallow egg composition was analyzed in relation to environmental factors before and during egg formation.

3.2 Methods

3.2.1 Analysis of egg composition

Japanese quail eggs analyzed for lipid, protein and ash content were infertile eggs laid during chamber calorimetry of captive-bred birds (Chapter 6). Sixty-five swallow eggs from 25 nests were collected for analysis of composition under licence from the NCC in 1990 and 1991. These were the same eggs as used in Section 3.2.2 for determination of the rate of egg formation and in Chapter 7 for calculation of energy output of female swallows for which energy expenditure was measured using the doubly-labelled water technique. Eggs from which albumen samples were removed for isotopic analysis (Chapter 7), were not included in this analysis.

Eggs were weighed immediately after collection, 1-4 h after laying (fresh mass). Egg volume was determined from the increase in volume of a measuring cylinder partly filled with water. The eggs were hard boiled (2-3 min for quail eggs, 1-2 min for swallow eggs) taking care to increase water temperature slowly so that the eggs did not crack. Eggs were allowed to dry and were then weighed (boiled mass). Egg yolk, albumen and shell were separated, weighed (wet mass) and frozen for storage. Shell thickness was measured using a dial reading vernier calliper

(accurate to 0.1 mm). Each component was freeze-dried and weighed (dry mass). The lipid component was extracted from quail egg yolk and each component of swallow eggs using 5:1 ether:chloroform solvent in a Soxhlet apparatus, before components were again freeze-dried and weighed (lean dry mass). The nitrogen content of quail egg yolk and albumen was determined by the Kjeldal process and protein content calculated by multiplication of the nitrogen content by 6.25 (Brouwer 1965). For swallow eggs, the lean dry component of albumen and yolk was assumed to be protein and that of the shell to be of mineral content. The energy content of protein was taken as 23.7 kJg^{-1} and that of lipid as 39.2 kJg^{-1} (Znaniecka 1967)

3.2.2 Use of lipophilic dyes to determine rate of egg formation

Commercial producers can vary the colour of egg yolk to suit consumer expectation by varying the colour of feed provided to domestic fowl. Xanthophylls and caratenoids from plant material normally present in the diet are responsible for the normal yellow colour of the yolk (Romanoff and Romanoff 1949). These pigments colour the yolk because they are fat soluble. A single feed of a lipophilic dye of a different colour to a laying bird can be used to alter the colour of a layer in the yolk in follicles undergoing the period of rapid follicular growth (Gilbert 1972). These dyes bind to the lipoproteins synthesised by the liver and are deposited on the outside of each rapidly developing follicle as a discrete layer.

The inside diameter of the dye band was taken to mark the diameter of the follicle at the time of the dye feed and yolks were assumed to be effectively spherical. The volume of yolk deposited in a follicle in a given period could be calculated from Equation 3.1.

$$V = \frac{4}{3}\pi \left[\left(\frac{x}{2}\right)^3 - \left(\frac{y}{2}\right)^3 \right] \qquad 3.1$$

where V = volume of yolk deposited (mm^3),
x = internal diameter of band from first dye feed (mm),
y = internal diameter of band from second dye feed (mm).

3.2.2.1 Japanese quail

If a mature female Japanese quail was dissected, the ovary would contain a hierarchy of follicles undergoing the rapid growth phase, with a further egg undergoing albumen or shell deposition in the oviduct (Plate 3.1). If, instead, the bird was fed a capsule of lipophilic dye, the eggs which were subsequently laid could be sectioned to show the diameter of the coloured rings which marked the diameter of each of the follicles undergoing the rapid growth phase at the time of the dye feed (Plate 3.2).

Lipophilic dye was fed to quail in gelatin capsules (0.02 $gfeed^{-1}$ for Sudan B and 0.05 $gfeed^{-1}$ for Scarlet R). The dye began to stain the outside of some follicles within an hour of the dye feed (pers. obs.), although 4 h was required before all rapidly developing quail or domestic fowl follicles had dyed outer layers (Bacon and Koontz 1971, Gilbert 1972). Eggs were usually laid shortly after 0700 hours when the lights came on each day. The dye capsules were fed between 0900 and 1000 hours. Lipophilic dye was fed daily to mature female quail, so that each egg contained a series of coloured layers, deposited at 24-hour intervals.

3.2.2.2 Swallows

Laying swallows were fed lipophilic dyes mixed in raw mince (in a mince to dye ratio of approximately 10:1). Females were caught on their roosts at dusk and 3 to 4 billfulls of the dye mixture were placed in the back of the throat using blunt tweezers. If necessary, each billfull was accompanied by a drop of water to assist swallowing the rather dry mince mixture. If the dye mixture was fed at dawn rather than at dusk, it passed through the digestive system very quickly. One bird fed dye at 0400 hours produced dyed droppings within 15 min. Females fed dye at dawn laid eggs with much fainter dyed layers than those of birds fed dye in the evening.

Plate 3.1 Ovarian follicles (top) and oviducal egg (lower right) dissected from a Japanese quail. A fully formed egg (lower left) laid the previous day, is shown for comparison. The hierarchy of yellow ovarian follicles undergoing the rapid growth phase, a large number of small pale follicles which had not begun the rapid growth phase, and two "collapsed" post ovulatory follicles can be seen.

Plate 3.2 Hard boiled, sectioned Japanese quail eggs laid on consecutive days by a female fed the lipophilic dye, Scarlet R, 2 d before the egg in the top left hand corner was laid. The eggs were laid daily in the sequence, top row left to right followed by bottom row, left to right. The dye marked the outer layer of each ovarian follicle at the time at which the dye was fed. The egg in the bottom right hand corner, laid 8 d after the dye feed, contained no dyed ring, as the rapid growth phase of this follicle had not begun at the time of the dye feed.

3.2.2.3 Dippers

It was hoped that female dippers might self feed on the lipophilic dyes, if mealworms or maggots containing the dye were placed on stones near the nest or close to where the birds were observed feeding. This would have allowed a different colour of dye to be fed each day during egg formation without the need to catch the bird. Although both members of three pairs of dippers found the food provided, only male dippers ate it, possibly because the natural food supply was so abundant that females had no need to try a new food source. No female dippers were fed lipophilic dye. Investigation of the rate of egg formation of dippers would be an area for future work.

3.3 Results

3.3.1 Egg composition

3.3.1.1 Japanese quail

The composition of quail eggs is presented in Table 3.1. Egg composition was similar to that determined by Ricklefs (1977).

3.3.1.2 Swallow

The composition of swallow eggs is presented in Table 3.2. Composition was similar to that found for other altricial birds (Sotherland and Rahn 1987). Differences in sample sizes were due to extraction of albumen from some of the eggs for analysis of stable isotope ratios (to measure the energy expenditure of the laying swallow using the doubly labelled water technique, Chapter 7) and to omitting to weigh some of the yolk and albumen samples immediately after boiling. These samples were frozen and weighed later, but had by then lost some of the original water content.

Table 3.1 Quail egg composition. Energy contents were calculated using values of 39.2 kJg^{-1} for lipid and 23.7 kJg^{-1} for protein (Znaniecka 1967).

Variable	Mean	sd	n
Fresh mass (g)	8.31	0.66	69
Length (mm)	29.30	1.65	91
Breadth (mm)	22.96	0.87	91
Volume (cm^3)	7.79	0.65	30
Internal volume (cm^3)	7.18	0.79	91
Shell mass (g)[1]	1.50	-	15
% egg[1]	15.2	2.3	15
Yolk mass (g)	2.53	0.22	11
% egg	26.36	1.82	11
Diameter (mm)	15.81	0.62	87
Volume (cm^3)	2.08	0.24	87
Density (gcm^{3-1})	1.07	0.11	10
Water (%)	48.29	1.32	11
Lipid (%)	31.12	0.90	11
Protein (%)	16.31	0.78	11
Energy content (kJ)	40.68	3.25	11
Energy content (kJg^{-1})	16.06	0.35	11
Energy content ($kJcm^{3-1}$)	17.18	1.72	11
Albumen mass (g)	5.81	0.30	11
% egg	60.45	1.82	11
Volume (cm^3)	5.13	0.67	87
Density (gcm^{3-1})	1.13	0.06	11
Water (%)	77.47	0.75	11
Lipid (%)[1]	0.03	0.10	15
Protein (%)	10.22	0.65	11
Energy content (kJ)	14.07	1.07	11
Energy content (kJg^{-1})	2.42	0.15	11
Energy content ($kJcm^{3-1}$)	2.74	0.21	11
Yolk lipid/protein	1.91	0.12	11
Yolk/albumen (wet)	0.437	0.04	11
Yolk/albumen (dry)	1.003	0.10	11
Yolk/albumen (kJ)	2.91	0.38	11
Total lipid (g)	0.79	0.07	11
% egg	8.2	0.70	11
Energy content (kJ)	40.68	3.25	11
Total protein (g)	1.01	0.04	11
% egg	10.5	0.40	11
Energy content	23.83	0.94	11
Total energy content (kJ)	54.74	3.07	11
Energy content fresh egg (kJg^{-1})	5.70	0.25	11

[1] data from Ricklefs (1974), - data not available.

Table 3.2 Swallow egg composition. Energy contents were calculated using values of 39.2 kJg^{-1} for lipid and 23.7 kJg^{-1} for protein (Znaniecka 1967).

Variable	Mean	sd	n
Fresh mass (mg)	1955.7	196.65	46
Length (mm)	19.70	0.93	59
Breadth (mm)	13.73	0.49	59
Volume (mm^3)	1912.7	176.43	59
Internal volume (mm^3)	1838.7	171.84	59
Shell mass (mg)	46.7	0.44	45
% egg	5.1	0.45	45
Yolk mass (mg)	433.3	45.85	21
% egg	23.3	2.03	21
Diameter (mm)	9.10	0.42	58
Volume (mm^3)	396.6	52.78	58
Density ($mgmm^{3-1}$)	1.007	0.10	20
Water (%)	55.3	2.41	22
Lipid (mg)	126.58	16.1	21
(%)	29.2	1.97	22
(J)	4962	632	21
Protein (mg)	67.50	6.6	21
(%)	15.5	0.87	22
(J)	1600	259	21
Energy content (J)	6562	774	21
(Jmg^{-1})	15.140	0.877	21
(Jmm^{3-1})	15.189	1.65	21
Albumen mass (mg)	1193.6	129.75	21
% egg	65.4	3.42	19
Volume (mm^3)	1432.0	184.25	55
Density ($mgmm^{3-1}$)	0.935	0.07	20
Water (%)	88.6	1.05	19
Lipid (mg)	13.86	2.49	21
(%)	1.14	0.18	19
(J)	543	97.6	21
Protein (mg)	124.90	20.4	21
(%)	10.26	1.00	19
(J)	2960	484.8	21
Energy content (J)	3504	546	21
Energy content (Jmg^{-1})	2.880	0.26	19
Energy content (Jmm^{3-1})	2.738	0.29	20
Yolk lipid/protein	1.87	0.14	21
Yolk/albumen (wet)	0.357	0.04	19
Yolk/albumen (dry)	1.429	0.26	21
Yolk/albumen (J)	1.947	0.35	21
Total lipid (mg)	140.4	16.95	21
% egg	7.55	0.84	20
Energy content (J)	5505	664	21
Total protein (mg)	192.4	21.77	21
% egg	10.3	0.69	20
Energy content	4560	514	21
Total energy content (J)	10065	949	21
Energy content fresh egg (Jmg^{-1})	5.410	0.33	20

3.3.2 Relationships between laying date, egg size, yolk and albumen content of swallow eggs

The volume, wet mass, dry mass, lean dry mass and energy content were significantly correlated for the yolk and albumen components of swallow eggs (Tables 3.3 and 3.4). In this sample of eggs, fresh mass, length and breadth were negatively correlated with laying date (Pearson correlation, all $r<-0.35$, all $p<0.006$, $n=60$), although there was no significant relationship between egg size and laying date in the larger sample of eggs measured (Chapter 4).

Yolk volume was positively, and albumen volume negatively correlated with laying date (Pearson correlations, $r=0.39$, $p=0.003$, $n=59$ for yolk volume with laying date and $r=-0.63$, $p=0.001$, $n=56$). Yolk volume was negatively correlated with albumen volume ($r=-0.32$, $p=0.02$, $n=56$). Wet, dry and lean mass of yolk and albumen were not significantly correlated (all $r<0.11$, $p>0.6$, $n=64$). There were no significant correlations between wet or dry mass of yolk or albumen and laying date (all $r>-0.16$, $p>0.19$). Albumen volume was calculated by difference between the internal shell volume and yolk volume, therefore any error in measurement of yolk volume would lead to an error of opposite sign in the calculation of albumen volume. Mass, rather than volume, was a better measure of the size of the yolk and albumen as this was determined independently for each component.

3.3.3 Relationship between component and fresh mass of swallow eggs

Regressions of the logarithm of component mass on the logarithm of fresh egg mass were calculated to determine the allometric relationships among egg components. If the slope of this regression was not significantly different from 1.0, then that component did not differ significantly as a proportion of fresh egg mass over the range of egg size analyzed.

As egg size increased, the proportion of wet albumen increased but that of yolk decreased (Table 3.5). Albumen mass was highly correlated with egg mass, but

Table 3.3 Pearson correlation co-efficients between volume, wet mass, dry mass, lean dry mass and energy content of swallow egg albumen. Sample sizes are given in brackets. *** = $p<0.001$

	Wet mass	Dry mass	Lean dry mass	Energy content (kJ)
Volume (mm^3)	0.73 (20) ***	0.52 (55) ***	0.46 (55) ***	0.56 (55) ***
Wet mass (g)		0.79 (21) ***	0.80 (21) ***	0.78 (21) ***
Dry mass (g)			0.99 (62) ***	0.99 (62) ***
Lean dry mass (g)				0.96 (62) ***

Table 3.4 Pearson correlation co-efficients between diameter, volume, wet mass, dry mass, lean dry mass and energy content of swallow egg yolks. Sample sizes are given in brackets. *** = $p<0.001$, * = $p<0.05$.

	Volume	Wet mass	Dry mass	Lean dry mass	Energy content (kJ)
Diameter (mm)	1.00 (58) ***	0.49 (21) *	0.31 (58) *	0.27 (58) *	0.31 (58) *
Volume (mm^3)		0.47 (21) *	0.30 (58) *	0.26 (58) *	0.30 (58) *
Wet mass (g)			0.89 (22) ***	0.86 (22) ***	0.88 (22) ***
Dry mass (g)				0.93 (62) ***	0.99 (62) ***
Lean dry mass (g)					0.91 (62) ***

Table 3.5 Allometric (log-log) regressions of egg component mass on swallow fresh egg mass. a and b are the intercept and slope in the equation $\log_e Y = a + b \log_e X$, s_b the standard error of b, 95%CI the 95% confidence interval of b, and r^2 the coefficient of determination. Signif. indicates whether b was not significantly different from 1 (ns), significantly greater (gt), or significantly less (lt) than 1. n = 47.

	a	b	s_b	95%CI Lower	95%CI Upper	r^2	Signif.
Albumen							
Wet	-2.785	1.31	0.159	1.036	1.584	0.79	gt
Dry	-3.915	1.174	0.179	0.874	1.474	0.49	ns
Lean dry	-3.847	1.151	0.190	0.832	1.469	0.45	ns
Lipid	-1.136	0.167	0.509	-0.713	1.047	0.01	ns
Water	4.832	-0.051	0.036	-0.113	0.011	0.09	lt
Yolk							
Wet	2.230	0.511	0.261	0.060	0.962	0.05	lt
Dry	-2.178	0.991	0.164	0.716	1.266	0.45	ns
Lean dry	-3.509	1.027	0.129	0.811	1.243	0.59	ns
Lipid	-2.654	0.094	0.119	-0.111	0.299	0.03	lt
Water	4.736	-0.095	0.066	-0.209	0.019	0.10	lt
Whole egg							
Dry	-2.08	1.049	0.087	0.903	1.195	0.76	ns
Lean dry	-2.975	1.093	0.106	0.915	1.271	0.70	ns
Lipid	-1.901	0.924	0.152	0.669	1.179	0.45	ns
Water	1.292	0.793	0.054	0.702	0.884	0.82	lt

yolk mass was not. Dry mass and lean dry mass of yolk, albumen and whole egg remained in proportion with egg mass, and were highly correlated with egg mass. The lipid content of albumen and whole egg changed in proportion with egg mass, whilst larger eggs had less yolk lipid. The water content of yolk, albumen and whole egg deceased as egg mass increased. In summary, although larger eggs contained absolutely more of each component, they had a significantly greater proportion of albumen and a lower proportion of yolk, lipid and water.

3.3.4 Relationships between swallow egg composition and environmental factors before and during formation

Fresh egg mass and yolk dry mass, lean dry mass and lipid mass were positively correlated with food abundance and negatively correlated with rainfall during the period of rapid follicular growth (Table 3.6). Temperature before or during the formation of each yolk was not correlated with yolk dry, lean dry or lipid mass, but the rainfall in the 3 d prior to the start of the period of rapid follicular growth was negatively correlated with yolk dry mass. There were no significant correlations between the water content of swallow egg yolk and environmental factors before or during the period of rapid follicular growth.

Fresh egg mass and albumen water content were negatively correlated with rainfall on the day the albumen was formed (Table 3.7). Food availability and temperature on the day the albumen was formed were not correlated with fresh egg mass or albumen composition.

3.3.5 Pattern of egg formation

3.3.5.1 Japanese quail

Mature captive-bred Japanese quail laid daily and almost continuously. Lipophilic dye was fed to 65 birds and the diameter of dyed layers from 426 follicles used to determine the duration of the period of rapid follicular growth. The

Table 3.6 Pearson correlation co-efficients between fresh egg mass, yolk dry mass, lean dry mass, lipid content and water content (g) of swallow eggs, and environmental factors a) during the 3 days before the start of rapid follicular growth, b) during the period of rapid follicular growth (days -7 to -2, where an egg is laid in the morning of day 0) (Section 3.3.5.2), c) during the period of rapid follicular growth when most yolk was deposited (days -5 to -2). Food is $\log_e$ (insect suction trap catch volume +1). Sample sizes are given in brackets. *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$, ns = not significant. n = 63.

a) Before egg formation (day -10 to -8)

	Fresh egg mass	Dry mass	Lean dry mass	Lipid content	Water content
Food	0.29 ns	0.20 ns	0.16 ns	0.20 ns	0.17 ns
Max. temp.(°C)	0.00 ns	0.01 ns	0.03 ns	0.00 ns	0.30 ns
Min. temp.(°C)	-0.23 ns	0.05 ns	0.06 ns	0.03 ns	0.14 ns
Mean temp.(°C)	-0.11 ns	0.04 ns	0.03 ns	0.05 ns	0.34 ns
Rainfall (mm)	-0.38 **	-0.35 **	-0.34 **	-0.33 **	-0.30 ns

b) During yolk formation (day -7 to -2)

	Fresh egg mass	Dry mass	Lean dry mass	Lipid content	Water content
Food	0.23 ns	0.34 **	0.35 **	0.31 *	0.13 ns
Max. temp.(°C)	0.08 ns	-0.02 ns	0.10 ns	-0.08 ns	0.18 ns
Min. temp.(°C)	-0.04 ns	0.07 ns	0.24 ns	-0.01 ns	0.19 ns
Mean temp.(°C)	-0.06 ns	0.02 ns	0.18 ns	-0.05 ns	0.19 ns
Rainfall (mm)	-0.37 *	-0.29 **	-0.31 *	-0.27 *	-0.21 ns

Table 3.6 continued

c) During yolk formation (day -5 to -2)

	Fresh egg mass	Dry mass	Lean dry mass	Lipid content	Water content
Food	0.34	0.36	0.41	0.32	0.21
	*	**	**	*	ns
Max. temp.(°C)	-0.14	-0.16	-0.02	-0.21	0.22
	ns	ns	ns	ns	ns
Min. temp.(°C)	0.00	0.09	0.23	0.02	0.12
	ns	ns	ns	ns	ns
Mean temp.(°C)	-0.09	-0.05	0.10	-0.12	0.19
	ns	ns	ns	ns	ns
Rainfall (mm)	-0.32	-0.25	-0.28	-0.22	-0.23
	*	*	ns	ns	ns

Table 3.7 Pearson correlation co-efficients between fresh egg mass, albumen dry mass, lean dry mass, lipid content and water content (g) of swallow eggs and environmental factors on the day when the albumen was formed (day-1). Food is $\log_e$ (insect suction trap catch volume +1), * = $p<0.05$, ns = not significant. n = 63.

	Fresh egg mass	Dry mass	Lean dry mass	Lipid content	Water content
Food	-0.07	0.01	0.04	-0.16	0.37
	ns	ns	ns	ns	ns
Max. temp.(°C)	-0.17	-0.03	-0.01	-0.08	0.08
	ns	ns	ns	ns	ns
Min. temp.(°C)	-0.05	-0.16	-0.19	0.08	-0.05
	ns	ns	ns	ns	ns
Mean temp.(°C)	-0.14	-0.11	-0.12	-0.01	0.03
	ns	ns	ns	ns	ns
Rainfall (mm)	-0.32	-0.07	-0.08	-0.09	-0.44
	*	ns	ns	ns	*

mean pattern of yolk and albumen formation for one egg is shown in Fig. 3.1. Eggs required 7 d for yolk formation and a further day for deposition of the albumen and shell. As quail laid daily, the equivalent of an entire egg (54.74 kJ) would require to be formed each day, although yolk deposition would be spread between 7 ovarian follicles and albumen would be deposited around a further, ovulated, follicle.

3.3.5.2 Swallows

Swallows normally laid one egg each morning and produced a clutch of 4-6 eggs. Twenty-two swallows were fed dye during the laying and pre-laying periods, and 50 egg yolks which contained layers were subsequently analyzed. The diameter and volume of follicles at the time of the dye feed are shown in Fig. 3.2. The cumulative volume of yolk deposited with time between day -7 and day -1 was best fitted by a second order polynomial (r^2=0.85) of the form:

$$Y = a + bX + cX^2$$

where a = 75.56,

b = -91.85,

c = 21.78,

X = days after the start of rapid follicular growth,

Y = volume of the ovarian follicle (mm^3).

The relationship between the co-efficients b and c fitted the same line as those from polynomial equations describing follicular growth in seabird eggs (Astheimer and Grau 1990). (The co-efficients given above need to be divided by the density of yolk (1007, Table 3.2) to convert from mm^3 to g, the units used in the graph of Astheimer and Grau 1990).

The pattern of yolk deposition by swallows was similar to that of quail, except that only 6 d of rapid follicular growth were required (Fig. 3.3). The albumen and shell were deposited on the seventh day after the start of the period of rapid follicular growth, and the egg was laid on the morning on the eighth day.

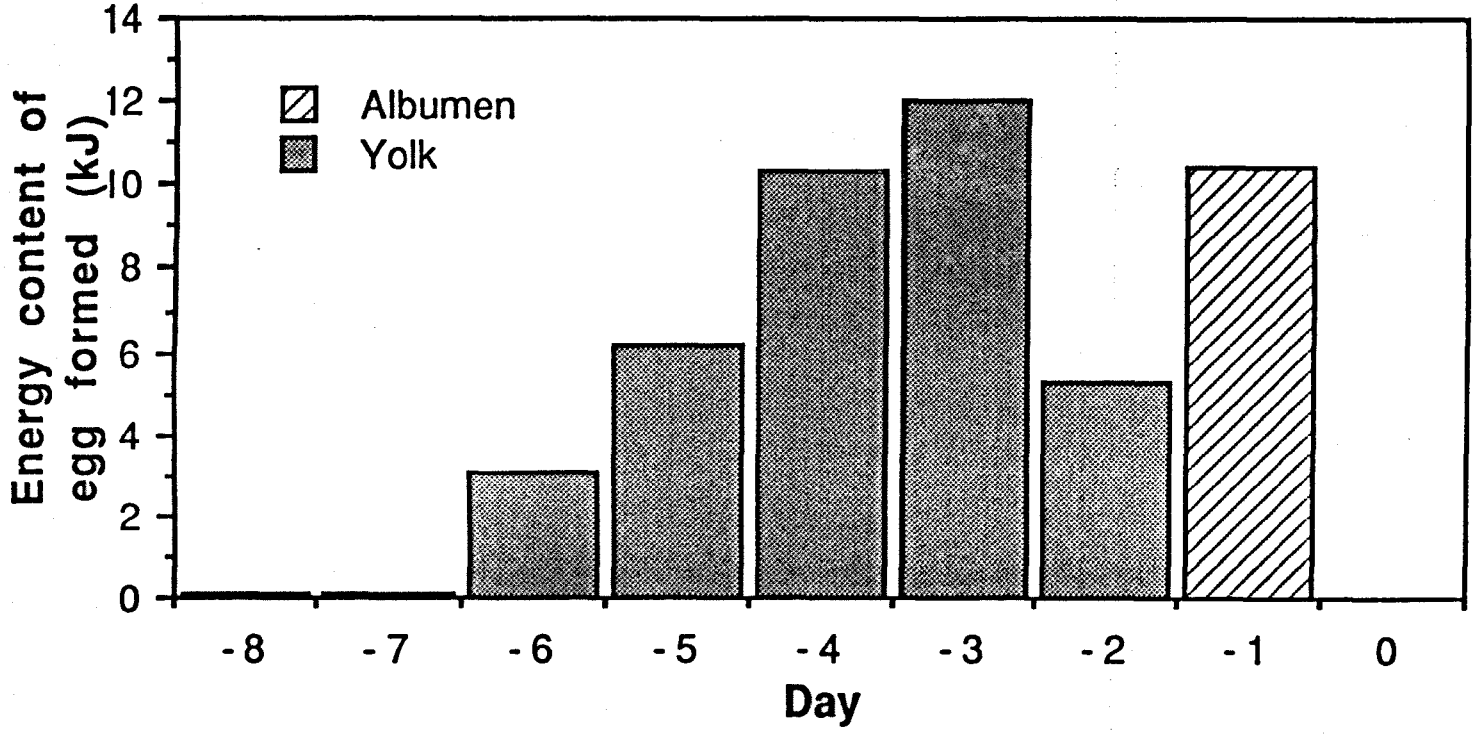


Fig. 3.1 Mean pattern of Japanese quail egg deposition, determined from layers of lipophilic dye in egg yolks. The dye was used to mark a coloured layer on the outside of each rapidly developing ovarian follicle at the time of the dye feed. The energy content of the volume of yolk and albumen formed in each 24 hour period was determined from multiplication by the energy density (Table 3.1). n=426 follicles.

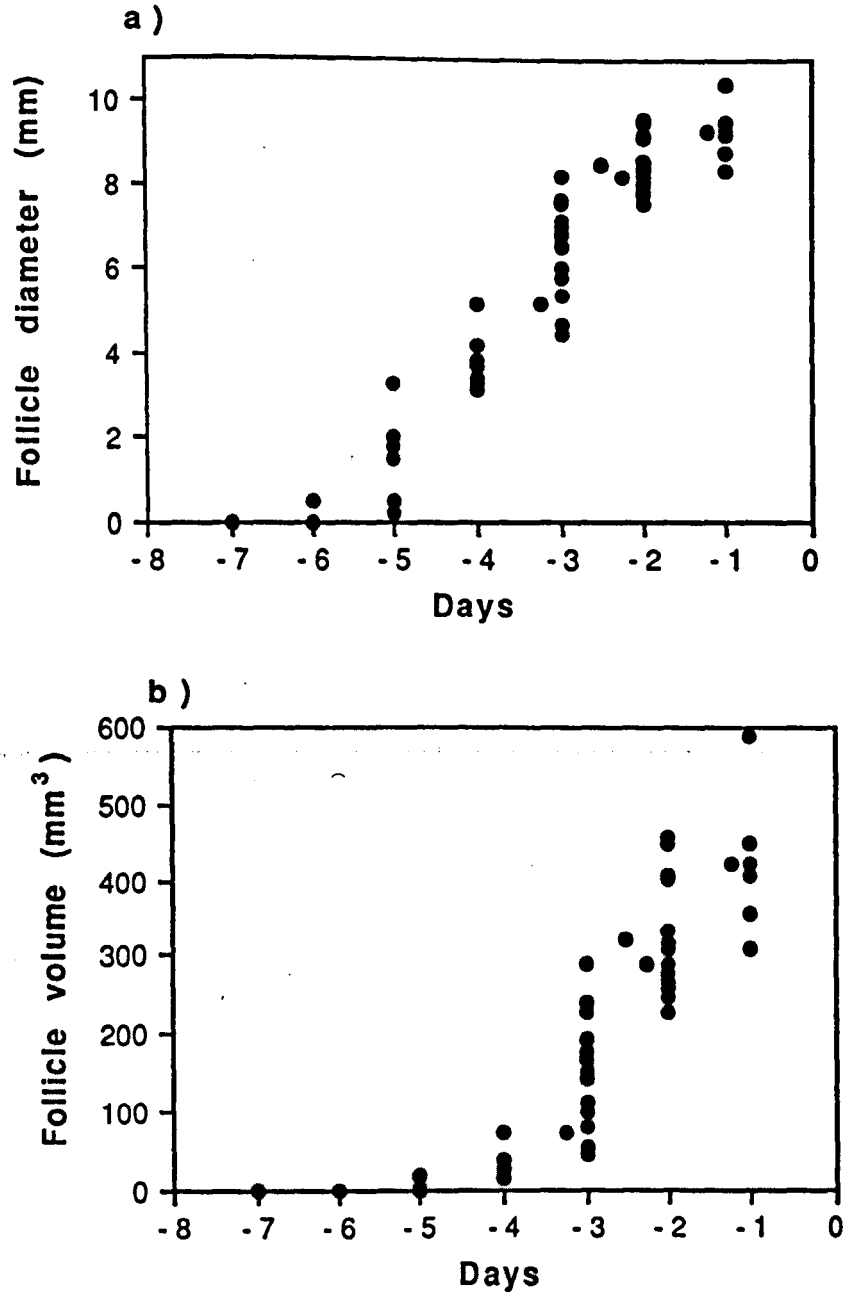


Fig. 3.2 a) Diameter and b) volume of swallow ovarian follicles during the rapid growth phase, determined by feeding lipophilic dye to the female during yolk deposition. This dyed a layer on the outside of each rapidly developing ovarian follicle. The first egg was ovulated in the morning on day -1 and laid on day 0. n=50 eggs.

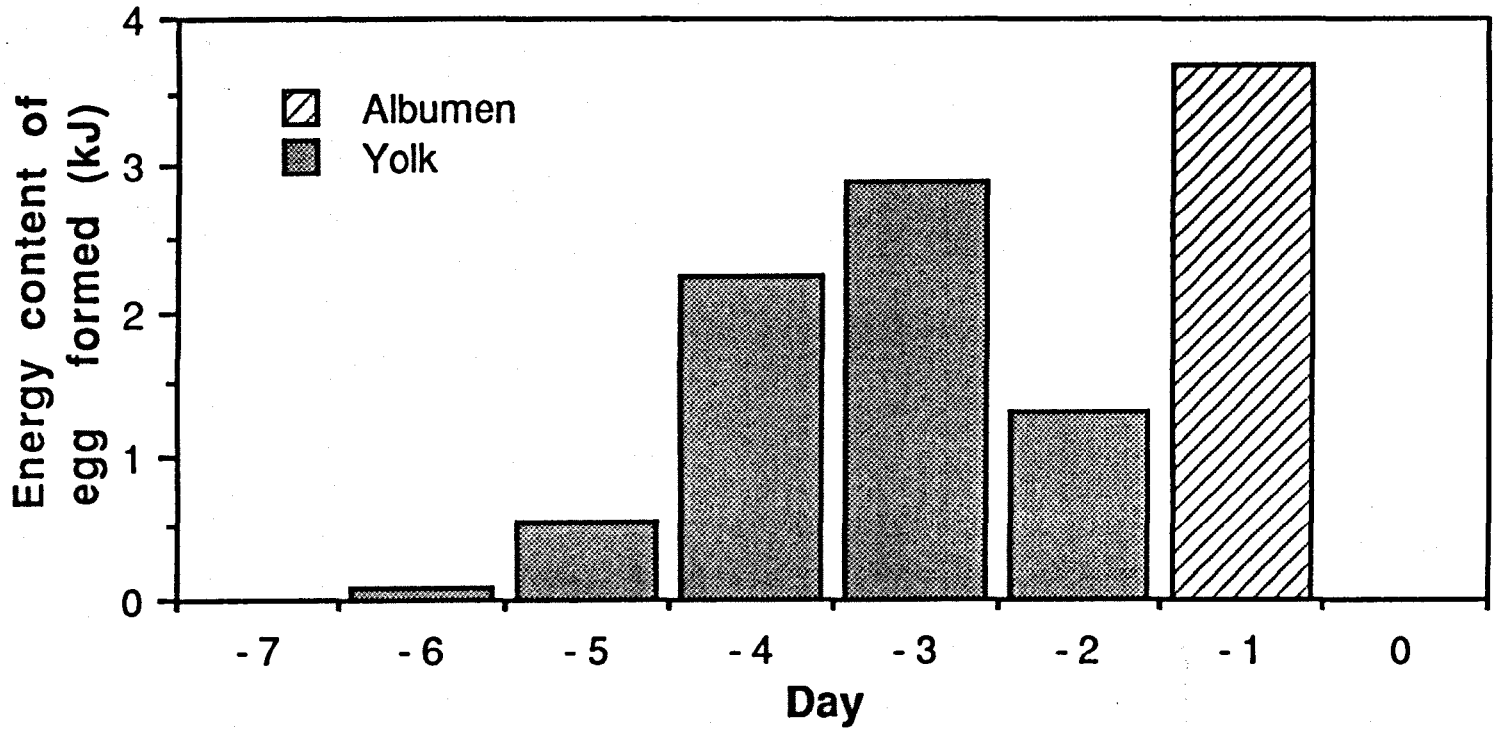


Fig. 3.3 Mean pattern of yolk deposition on a swallow egg, determined from layers of lipophilic dye in egg yolks. The dye was used to mark a coloured layer on the outside of each rapidly developing ovarian follicle at the time of the dye feed. The energy content of the volume of yolk and albumen formed in each 24 hour period was determined from multiplication by the energy density of yolk and albumen (Table 3.2). Some yolk was formed on day -7, but this was too small an amount to be shown on a graph of this scale. n=50 eggs.

Unlike the continuously laying quail, clutch size influenced the amount of egg formed by swallows each day. Assuming that each yolk followed the mean pattern of follicular growth (Fig. 3.3, but see Section 3.3.6 for investigation of this assumption), the total energy content of egg formed each day could be calculated for clutches of 3 to 6 eggs (Table 3.8, Fig. 3.4). The greatest mass of yolk, and the greatest energy content of yolk and albumen combined were required on day -1, the day before the first egg was laid, irrespective of clutch size.

An increase in clutch size from 3 to 4, 5 or 6 eggs required an increase in the energy content egg formed on day -1 by 27.1, 33.6 and 34.6% respectively (Table 3.8). These figures might suggest a large increase in maximum daily energy content of egg formed as clutch size increased, however, only a few birds laid clutches as small as 3 eggs, so a more relevant comparison would be between the peak daily energy content of egg formed on day -1 for clutches of 4, 5 and 6 eggs. A clutch of 5 eggs required a peak of 5.1% more energy than one of 4 eggs, a clutch of 6 required 5.8% more than a clutch of 4 and 0.7% more than a clutch of 5 eggs. Clutch size therefore had a more important effect upon the length of the period of egg formation than upon the peak energy content of egg formed. Both the total period of egg formation and the period when more than 50% of the maximum daily energy content of egg was formed (i.e., more than 9.62/2=4.81 kJ) lasted an extra day for each increase in clutch size of 1 egg (Table 3.8).

The mean mass of lipid and protein deposited on each follicle each day, and the total daily lipid and protein content of egg deposition, were calculated from Tables 3.2 and 3.8 (Table 3.9).

3.3.6 Variation between swallow eggs in the pattern of yolk deposition

Feeding lipophilic dyes to swallows during the period of rapid follicular growth provided the potential to examine variation in the rate of yolk deposition between different eggs in a clutch, between different clutch sizes and between eggs formed during different environmental conditions.

Table 3.8 Mean energy content (kJ) of swallow yolk and albumen deposited during each day from the start of the period of rapid follicular growth to the end of albumen formation. The first egg was laid in the morning on day 0.

a) Mean energy content of yolk and albumen formed for each egg each day.

Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Egg 1	0.00	0.07	0.46	1.94	2.52	1.13	3.50					
Egg 2		0.00	0.07	0.46	1.94	2.52	1.13	3.50				
Egg 3			0.00	0.07	0.46	1.94	2.52	1.13	3.50			
Egg 4				0.00	0.07	0.46	1.94	2.52	1.13	3.50		
Egg 5					0.00	0.07	0.46	1.94	2.52	1.13	3.50	
Egg 6						0.00	0.07	0.46	1.94	2.52	1.13	3.50

b) Mean total energy content of egg formed each day for clutches of different sizes. Figures in bold show days when more than 50% of the maximum daily energy content of egg material was formed.

Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Clutch = 3	0.00	0.07	0.53	2.47	**4.92**	**5.59**	**7.15**	4.63	3.50			
Clutch = 4	0.00	0.07	0.53	2.47	**4.99**	**6.05**	**9.09**	**7.15**	4.63	3.50		
Clutch = 5	0.00	0.07	0.53	2.47	**4.99**	**6.12**	**9.55**	**9.09**	**7.15**	4.63	3.50	
Clutch = 6	0.00	0.07	0.53	2.47	**4.99**	**6.12**	**9.62**	**9.55**	**9.09**	**7.15**	4.63	3.50

c) Mean energy content of entire clutches of different sizes.

Clutch = 3	28.83
Clutch = 4	38.48
Clutch = 5	48.10
Clutch = 6	57.72

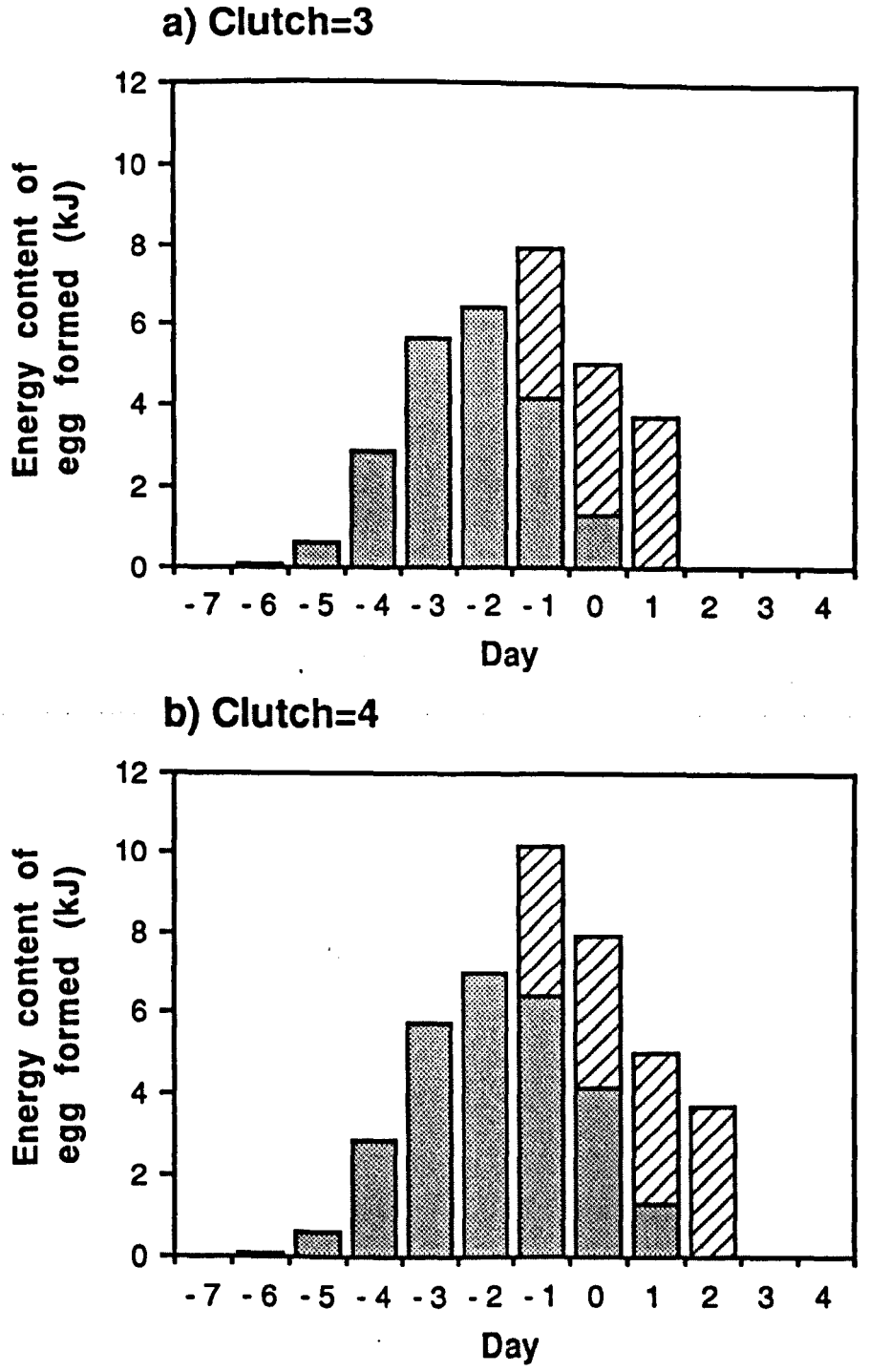
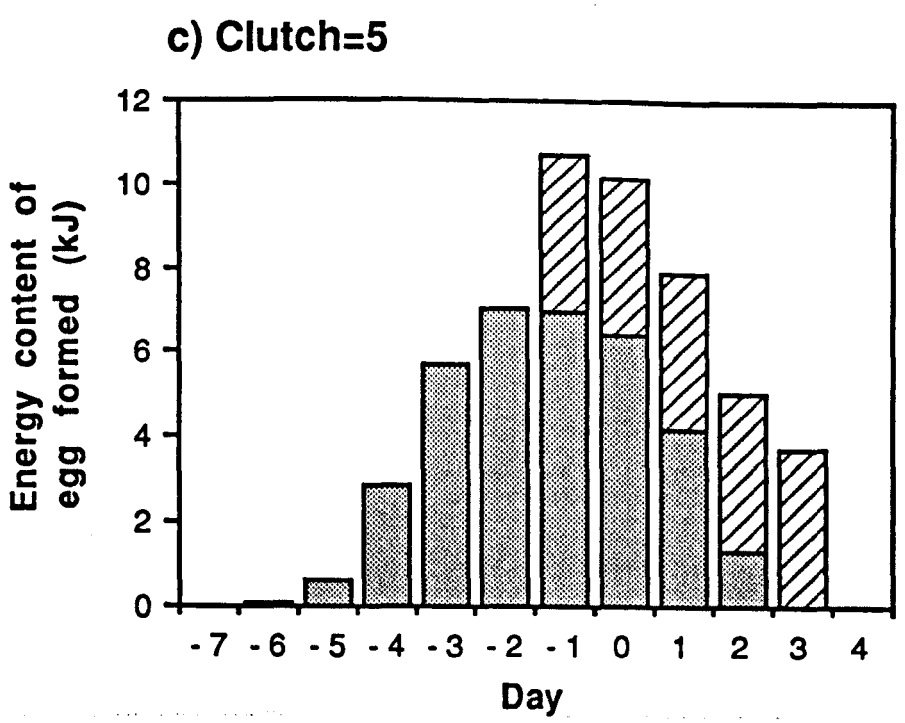


Fig. 3.4 Total daily energy content of egg deposited by swallows during the formation of clutches of different sizes (a-d). Each egg was assumed to be of mean mass (1.97g) and to be deposited according to the same pattern (Fig. 3.3). The energy content of yolk formed each day is shown by the stippled area and the energy content of albumen by the hatched area. Some yolk was formed on day -7, but this was too small an amount to be shown on graphs of this scale.



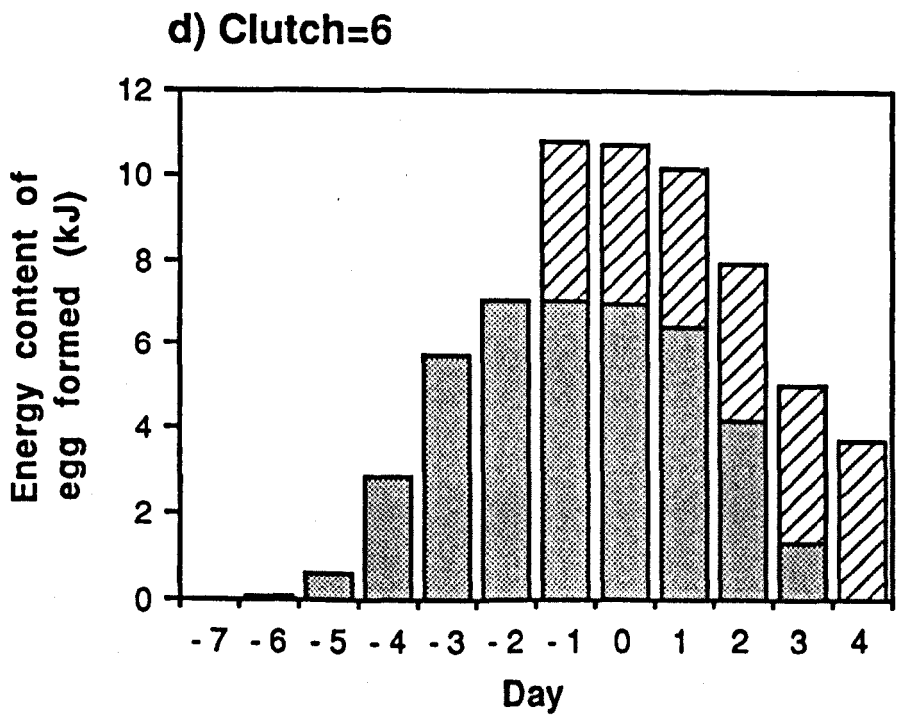


Fig. 3.4 continued

Table 3.9 Mean content (mg) of a) lipid and b) protein of swallow egg yolk and albumen deposited on each egg each day, and for entire clutches of different sizes. The first egg was laid in the morning on day 0. Figures in bold show days when more than 50% of the maximum daily lipid or protein was deposited.

a) Lipid

Mass of lipid deposited on each egg each day.

Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Egg 1	0.01	1.35	8.76	36.58	47.44	21.37	13.86					
Egg 2		0.01	1.35	8.76	36.58	47.44	21.37	13.86				
Egg 3			0.01	1.35	8.76	36.58	47.44	21.37	13.86			
Egg 4				0.01	1.35	8.76	36.58	47.44	21.37	13.86		
Egg 5					0.01	1.35	8.76	36.58	47.44	21.37	13.86	
Egg 6						0.01	1.35	8.76	36.58	47.44	21.37	13.86

Total lipid content of egg yolk and albumen deposited each day during formation of clutches of different sizes.

Clutch = 3	0.01	1.36	10.12	46.69	**92.78**	**105.39**	**82.67**	35.23	13.86			
Clutch = 4	0.01	1.36	10.12	46.70	**94.13**	**114.15**	**119.25**	**82.67**	35.23	13.86		
Clutch = 5	0.01	1.36	10.12	46.70	**94.14**	**115.50**	**128.01**	**119.25**	**82.67**	35.23	13.86	
Clutch = 6	0.01	1.36	10.12	46.70	**94.14**	**115.51**	**129.36**	**128.01**	**119.25**	**82.67**	35.23	13.86

Table 3.9 continued

b) Protein

Mass of protein deposited on each egg each day.

Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Egg 1	0.00	0.72	4.65	19.42	25.18	11.34	124.90					
Egg 2		0.00	0.72	4.65	19.42	25.18	11.34	124.90				
Egg 3			0.00	0.72	4.65	19.42	25.18	11.34	124.90			
Egg 4				0.00	0.72	4.65	19.42	25.18	11.34	124.90		
Egg 5					0.00	0.72	4.65	19.42	25.18	11.34	124.90	
Egg 6						0.00	0.72	4.65	19.42	25.18	11.34	124.90

Total protein content of egg yolk and albumen deposited each day during formation of clutches of different sizes.

Clutch = 3	0	0.72	5.37	24.79	49.25	55.94	**161.42**	**136.24**	**124.90**			
Clutch = 4	0	0.72	5.37	24.79	49.97	60.59	**180.84**	**161.42**	**136.24**	**124.90**		
Clutch = 5	0	0.72	5.37	24.79	49.97	61.31	**185.49**	**180.84**	**161.42**	**136.24**	**124.90**	
Clutch = 6	0	0.72	5.37	24.79	49.97	61.31	**186.21**	**185.49**	**180.84**	**161.42**	**136.24**	**124.90**

Most of the birds which were fed lipophilic dye laid clutches of 4 or 5 eggs, so comparisons of growth rates of eggs from different sizes clutches and from different positions in the laying order were performed with eggs from these clutches. For each of the eggs in positions 1 to 4 in the clutch, there were no significant differences between the volume, or proportion of the total volume, of yolk already formed or the volume subsequently deposited from clutches of 4 and 5 eggs on the same number of days before the egg was laid (Mann-Whitney U-tests, all $Z<1.409$, $p>0.16$). There were no significant differences with laying order in the volume or proportion of the total volume already formed or the volume or proportion of volume of yolk subsequently deposited on ovarian follicles (Kruskal Wallis one-way analysis of variance, all $p>0.16$). Sample sizes in these analyses were small (1-5 eggs in each category) so although there was no evidence for differences in the rate of yolk deposition between follicles from clutches of different sizes or at different positions in the laying order, analysis of the rate of formation of a larger sample of eggs, would be an area for future work.

Since yolk mass was correlated with environmental factors during the period of rapid follicular growth (Table 3.6), this was also investigated for the ovarian follicles at the time of dye feeds. As the diameter of only a small number of follicles was marked by dye on the same day of rapid growth period, the difference between actual and mean follicle volume on each day and between actual and mean volume of yolk still to be formed before ovulation were calculated from Equations 3.2 and 3.3.

$$\Delta \text{ volume of yolk formed} = \text{vol}_{(\text{day } n)} - \text{mean vol}_{(\text{day } n)} \qquad 3.2$$

$$\Delta \text{ volume of yolk to be formed} = \text{yolk vol} - \text{vol}_{(\text{day } n)} - \text{mean yolk vol} - \text{mean yolk vol}_{(\text{day } n)} \qquad 3.3$$

where Δ = difference in,

$vol_{(day\ n)}$ = volume of the follicle when the dye was fed on day n (mm^3),

mean $vol_{(day\ n)}$ = mean follicle volume on day n (mm^3),

yolk vol = final volume of the yolk in which the dye ring was deposited on day n (mm^3),

mean yolk volume = mean volume of all swallow egg yolks (mm^3).

This provided two measures from each yolk as to whether it was formed faster or more slowly than the mean "expected" rate of growth.

Follicles were larger than expected when the maximum or mean temperature was greater during the period over which they were deposited (Table 3.10). Final yolk volume was greater when the minimum temperature was greater. There were no significant correlations between the size of ovarian follicles and food availability or rainfall. Temperature therefore had an important influence on the rate of yolk deposition.

3.4 Discussion

3.4.1 Variation in swallow egg composition

Albumen content was the constituent most strongly related to fresh egg mass (Table 3.5). Egg mass was strongly correlated with the mass of water in the egg, moderately correlated with the protein content but not significantly correlated with yolk or lipid content. Larger eggs contained proportionately more water and less yolk and energy, although the larger eggs still had an absolutely greater content of all components. These results were similar to those found for the starling (Ricklefs 1984), ring-billed gull (Meathrel and Ryder 1987), and puffin (Birkhead and Nettleship 1984).

Yolk mass, lipid and protein contents were influenced more than albumen mass or composition by environmental factors during the period of yolk or albumen formation (Tables 3.6 and 3.7). Fresh egg mass was positively correlated with food supply during the period when the majority of yolk was formed and negatively correlated with rainfall during the periods of yolk and albumen formation. These

Table 3.10 Pearson correlation co-efficients between the "difference in volume of yolk formed" (Equation 3.1), and "difference in volume of yolk to be formed" (Equation 3.2), by swallows and mean insect suction food and temperature on the days of yolk deposition. Spearman correlation co-efficients were presented for the relationships with rainfall. Column A refers to correlations with environmental factors during the period when the majority of the yolk was deposited on follicles (i.e., excluding the first 2 days of rapid follicular growth). Column B refers to correlations with environmental factors during the entire period of rapid follicular growth. Food = $\log_e(V+1)$ where V = insect suction trap volume. * = $p<0.05$, ** = $p<0.01$, ns = not significant.

	Difference in volume of yolk formed		Difference in volume of yolk to be formed
	A	B	
n	36	51	51
Food	0.04 ns	0.01 ns	0.00 ns
Maximum temperature (°C)	0.31 ns	0.37 **	-0.01 ns
Minimum temperature (°C)	0.30 ns	0.06 ns	0.33 *
Mean temperature (°C)	0.35 *	0.26 ns	0.17 ns
Rainfall (mm)	0.16 ns	0.17 ns	-0.51 ns

results were consistent with the hypothesis that egg size was reduced in the case of a deficiency in the total energy available for egg synthesis, and not due to a lack of protein. A protein deficiency in the diet of the domestic fowl showed the opposite trend: egg size decreased with a reduction in protein supply, but the yolk and shell mass changed less than that of the albumen (Fisher 1969).

There have been few studies of the affect of food supply on the composition of the eggs of free-living birds. Egg and clutch size, rather than composition, have received more attention (Chapter 4). Variation in food supply altered the proportion of yolk in common tern eggs (Nisbet 1978). In the common guillemot, the albumen content of eggs increased and yolk content decreased in a year of more favourable environmental conditions, although egg size did not differ significantly between years (Hatchwell and Pellatt 1990). Supplementary feeding influenced only the water content of magpie eggs (Hochachka 1988).

In summary, swallow egg size and composition were related to environmental conditions during the period of rapid follicular growth. The size and composition of the yolk were influenced more by environmental factors than those of the albumen. Larger eggs had a lower water content and a proportionately greater albumen content than smaller eggs. These results were similar to those found for other species and supported the hypothesis that the total energy available for egg formation, rather than a specific requirement for albumen synthesis, limited egg formation when environmental conditions were poor.

3.4.2 Rate of egg formation

3.4.2.1 Japanese quail

Japanese quail eggs weighed approximately 9.5 g (pers. obs.). Rapid follicular growth required 7 d, although only a small amount of yolk was deposited on the first 2 d. This was within the period of rapid follicular growth of 6.10 ± 1.12 d determined by Bacon and Koontz (1971) using the same technique, but longer than implied by the normally 5 (range 4-7) pairs of light/dark rings stained by Grau

(1976) where each pair of rings indicated 24 hours' growth. A period of 5.5 d was predicted by Walsberg's (1983a) equation, which calculated the length of the period of rapid follicular growth from egg size. The sources used to construct this equation probably did not include the first 2 d of rapid follicular growth, as very little yolk was deposited. If 2 d were added to the duration of rapid follicular growth estimated from Walsberg's equation, the result would be in good agreement with that calculated in this study.

Discrepancies between estimates by different authors of the period of rapid follicular growth almost certainly occur because the start of this period is difficult to define. Slow ovarian growth occurs over several weeks or months prior to laying of the eggs in some species (Johnson 1986). A female kestrel dissected one month before laying normally commenced already had follicles 16.8% of final yolk diameter (Meijer *et al* 1989). Similar results were found for house sparrows (Pinowska 1976 in Schifferli 1980), pied flycatcher (Ojanen 1983) and lesser black-backed gulls (Houston *et al* 1983).

3.4.2.2 Swallow

Swallows laid eggs with a mean fresh mass of 1.97 g (Chapter 4). The period of rapid follicular growth lasted 6 d, although on the first two days very little yolk was formed. This was longer than the 3.6 d of rapid follicular growth predicted from the Walsberg's (1983) equation. Rapid follicular growth was also estimated to require only 4 d in the bank swallow (Petersen 1955). The first 2 d of rapid follicular growth, although included in the present study, would probably have been excluded from Petersen's and Walsberg's calculations as the increase in follicle diameter was so small (Section 3.4.2.1).

The form of the growth curve of quail and swallow follicles was approximately sigmoid, although when the daily energy content of yolk deposited in each follicle was plotted through the period of rapid follicular growth, the peak was skewed to the right (Figs. 3.1 and 3.2) rather than forming a sine function as

assumed in King's (1973) model of the total energy requirement for egg formation. This fitted a model in which the yolk deposition was constrained by the ability of follicle cells to deposit yolk material, rather than one in which yolk production was limited by the rate of production of yolk precursors (in which case a linear increase in yolk mass would have been predicted) (Astheimer and Grau 1990).

The maximum daily energy content of egg formation, when the equivalent of an entire egg would have been formed in a day, was not reached by swallows, as egg formation required 7 d, and the largest clutch laid without a laying interruption was of 6 eggs. The energy content of yolk deposited on the first 2 d of rapid follicular growth was so small however that if the energy requirement for deposition of this part of the yolk was not included, the near maximum energy requirement for egg formation would need to be sustained for one day by a swallow laying a clutch of 5 eggs, and for 2 d by a bird laying a clutch of 6 eggs.

The peak daily energy content of egg material produced by swallows did not vary much between clutches of 4, 5 or 6 eggs, although a considerable reduction was obtained by birds which laid clutches of 3 eggs (Section 3.3.5.2). A small difference in the peak daily energy requirement for egg production for clutches of different sizes was also predicted by Ricklefs (1974) and Bryant (1975).

Calculation of the energy content of daily yolk deposition on ovarian follicles during the period of rapid follicular growth and of the albumen during passage through the oviduct was only part of the information required to determine the daily energy requirement for egg formation. The energy requirement for egg production by the laying female will be greater than the energy content of the egg material formed, as the efficiency of biosynthesis must be taken into account. The effect of a range of plausible efficiency factors on the net energy requirement for egg production and oviducal recrudescence, which occurred at the same time as rapid follicular growth, will be discussed in Chapter 7.

The technique described by Grau (1976) in which yolks were fixed and stained to show dark and light rings which corresponded to yolk deposition during the day and night was not tried during this study, although this has been shown to be

successful in determination of the duration of yolk formation in seabirds and shorebirds (Roudybush *et al* 1979). A combination of dye feeds and yolk staining has been used successfully to determine the duration of the rapid phase of yolk deposition in the Adélie penguin (Astheimer and Grau 1985). Dye feeds to captive kestrels have been used to show a pattern of increase in the diameter and volume of kestrel eggs very similar to that of swallows (Meijer *et al* 1989). Lipophilic dye feeds have been used to show the same pattern of follicular growth in domestic fowl (Romanoff 1931 in Ricklefs 1974), California quail (Anthony 1970 and Lewin 1963 in Ricklefs 1974) and the starling (Ricklefs 1974).

The duration of the period of rapid follicular growth may vary between eggs at different positions in the laying order, or according to environmental conditions during yolk formation (Grau 1976). Common guillemots formed their yolks more rapidly in a year when environmental conditions were more favourable than in a less favourable year (Hatchwell and Pellatt 1990). In the silver gull, Meathrel (1991) found variation between 7 and 14 d in the length of the period of rapid follicular growth and the rate of yolk deposition varied independently between eggs in the same clutch. Although second eggs required the same number of days to form as first eggs, the amount of yolk deposited each day was smaller as second eggs were smaller than first eggs. This differed from the result of Astheimer and Grau (1990), who found that the rate of yolk enlargement was uniform for each species of seabird in their study. Environmental factors have therefore ben demonstrated to influence the rate of egg formation in a variety of other species, as well as swallows. It was concluded that egg formation in general may be constrained by food availability.

CHAPTER 4

VARIATION IN EGG AND CLUTCH SIZE AND LAYING DATE

4.1 Introduction

Since Lack's (1966, 1968) proposal that clutch size was adjusted to produce the largest number of chicks which the parents could raise, a number of studies have shown that the most common clutch size was in fact smaller than the most productive one (e.g., Perrins 1965, Perrins and Moss 1975, De Steven 1980, Nur 1984, Gustaffsson and Sutherland 1988). This apparent paradox might be accounted for in three ways. Birds might optimise clutch size so that each pair laid the number of eggs which was most productive in their circumstances (Pettifor *et al* 1988). Alternatively, there might be a trade-off between present and future reproductive output, so that greater reproductive output in one breeding attempt would be counterbalanced by a reduction in the survival or future reproductive success of parents or offspring (Williams 1966, Charnov and Krebs 1974). A number of studies have provided evidence that a future "cost" to present reproduction does exist (reviewed by Nur 1990). These aspects of the determination of clutch size are beyond the scope of this study, which will examine the evidence for a third hypothesis; that proximate constraints act at the time of egg formation to determine egg and clutch size and laying date. According to this hypothesis, birds which laid smaller clutches or laid later in the season would do so because of a constraint which acted during the period of egg formation, rather than due to anticipation of difficulty later in the breeding season or a cost of reproduction which would not be manifest for some time.

It has frequently been proposed that egg production might be limited by energetic or nutrient constraints. Perrins (1970) suggested that birds laid as early in the year as possible, and that this was determined by the time at the food supply increased sufficiently for females to have an excess energy intake from which to form eggs. The hypothesis that egg formation is limited by food supply has been supported by experiments in which food supply was increased prior to or during laying where either egg or clutch size increased or laying date was advanced (reviewed by Martin

1987). Studies of populations over several years, or in several areas with differing food abundance, have also found a link between food supply and reproductive output (Hussell and Quinney 1987, Anderson 1977, Gibbs and Grant 1987, Rotenberry and Wiens 1991).

In this chapter, evidence for energetic constraint of swallow and dipper egg or clutch size will be assessed by examination of variation in egg and clutch size and laying date in relation to female age, structural size, territory, and environmental factors (temperature, food supply and rainfall) prior to and during egg formation. Relationships between female body condition prior to laying and reproductive output will be considered in Chapter 8.

4.2 Methods

4.2.1 Determination of laying date

A sample of freshly lined swallow and dipper nests was visited daily to determine the date upon which the first egg was laid. Daily visits were continued until the day after no new eggs were found and incubation had begun (eggs found warm). Cases of interrupted (up to 4 d gap between laying of consecutive eggs) or suspended (more than 10 d gap) laying were recorded. The date upon which the first egg of a clutch was laid was used as the laying date of that clutch in subsequent analyses. Nests which were first found when they contained more than one freshly laid, unincubated egg were assigned to a laying date on the assumption that one egg had been laid each day and that there had been no egg dumping. These were reasonable assumptions, as there were only occasional cases of laying anomalies (13 of 84 swallow clutches, 15.5%, and none of the 11 dipper nests visited daily). No more than one new egg appeared in any of these nests on each day, so egg dumping was assumed to be rare, if it occurred at all, in these populations. Swallows which nested in much larger colonies (Møller 1987a,b), other colonial hirundines (Brown and Brown 1988) and other species of birds (reviewed by Birkhead and Møller 1992) do lay eggs in the nests of conspecifics.

Laying date was calculated for clutches found during the incubation period from the hatching date of the clutch, using the assumptions that one egg had been laid per day, that incubation started on the day the final egg was laid and that incubation lasted 16 d. Incubation lasted 16.0 d (sd=1.2, range 13-19 d) in 93 swallow clutches for which the dates of the start of incubation and hatching were known. The mean length of incubation was also 16 d in the dipper (Cramp 1988).

The hatching date of clutches found during incubation was estimated from the mean diameter of the airspace at the blunt end of the eggs in order that the nest could be visited when the clutch was due to hatch. The airspace could be seen clearly in fresh eggs or those in the early stages of incubation, and by candling eggs in the second half of the incubation period. Swallow and lowland dipper nests were visited on the day the chicks were predicted to hatch, and thereafter on alternate days if the chicks had not hatched, so that exact hatching dates could be determined. Upland dipper nests were visited once during incubation and once during the nestling-rearing period. For these nests, laying date was calculated as described above, except that hatching date was determined from the mean of the dates predicted from the airspace diameter during incubation, and the relationships between age and nestling mass and wing length (Feltham 1987).

4.2.2 Order of laying of eggs within a clutch

All eggs were numbered using a waterproof ink on the first day they were found. This allowed determination of laying order in the clutches visited daily during the pre-laying and laying periods. The diameter of the airspace of these eggs was measured each day. As airspace diameter increased even before the last egg was laid, the laying order of eggs in unincubated clutches could be determined even after more than one egg had been laid. Airspace diameters of the clutch became more similar after the start of incubation so the eggs could not then be placed reliably in order of laying (although airspace diameter could be used to predict hatching date, Section 4.2.1).

4.2.3 Measurement of egg size

The maximum length (L) and breadth (B) of swallow and dipper eggs were measured with vernier callipers to the nearest 0.1 mm. Fresh (unincubated) egg mass was recorded to the nearest 0.01 g, using a 5 g Pesola balance for swallow and dipper eggs and an Ohaus 2000 g balance accurate to 0.1 g for quail eggs. The relationship between fresh egg mass and LB^2 was calculated for unincubated eggs (Equation 4.1). This allowed calculation of fresh mass from egg dimensions for eggs first found during incubation.

$$mass = constant \times LB^2 \qquad 4.1$$

Fresh egg mass was used as the measure of egg size in subsequent analyses. Clutch mass was the sum of the fresh masses of all eggs in each clutch. Mean egg mass and relative difference in egg weight (RDEW) were calculated for each clutch. RDEW was a measure of variation in egg mass within a clutch (Equation 4.2).

$$RDEW = \frac{(heaviest\ egg\ mass - lightest\ egg\ mass)}{mean\ egg\ mass} \qquad 4.2$$

4.2.4 Hatching date and hatching success

A majority of swallow and dipper clutches hatched synchronously, but if hatching was spread over more than one day, the day when the majority of the eggs hatched was taken as the hatching date. Swallow nests and lowland dipper nests were visited on the day the eggs were expected to hatch, 16 d after the start of incubation. Any un-pipped eggs were candled to determine whether they were infertile, had failed during development of the embryo or contained chicks which were about to hatch. Eggs containing fully developed embryos were checked during

the next few days to determine whether they hatched. Unhatched eggs were classified as infertile (yolk still visible upon candling), failed during development (partly grown embryo) or failed at hatching (apparently fully developed embryo which did not hatch). In 1990 and 1991 unhatched dipper eggs were collected for analysis of possible pesticide and heavy metal contamination, in collaboration with Dr. S.J. Ormerod (Ormerod and Tyler 1992).

4.2.5 Artificial incubation of swallow eggs

In order to determine whether there was a relationship between egg size and chick size, it was necessary to weigh and measure freshly hatched chicks from known eggs. It was difficult to do this if chicks hatched in their nests, as clutches tended to hatch synchronously so that hatchlings could not be matched with their eggs. Parents might also provide the first feed before the hatchling was weighed.

Some unfed (no dark coloured insect bolus visible through skin) hatchling swallows which could be matched with their eggs were measured in the field, but a larger sample of eggs was hatched in an incubator. No account was taken of the degree of moisture on the feathers, as a chick which was hatched in the incubator and weighed immediately had lost less mass than could be detected using the Pesola balance when its feathers had dried (accurate to 0.01 g).

Swallow eggs were moved to the incubator on day 14 or 15 of incubation under licence from the NCC. Female swallows did not desert if at least 3 eggs were left in their nest (Thompson 1992). These could be their own eggs or infertile eggs moved from other nests. The incubator was kept at 35-40°C and contained a tray of water to provide a moist atmosphere. The eggs were placed in a cardboard (chicken) egg tray and turned through 45°, 4-6 times a day. The incubator was checked for the emergence of new hatchlings every 2 to 8 h. The eggs hatched after 1-3 d in the incubator. The hatchlings were returned to their nests. Eggs and hatchlings were transported in plastic cups loosely filled with tissue paper. The feathers of the chicks hatched in the incubator were kept marked with non-toxic felt tipped pen until

fledging, to determine whether hatching in the incubator affected survival.

4.2.6 Swallow hatchling morphometrics

The following measurements were made for each swallow hatchling:
mass (to nearest 0.01 g, 5 g Pesola balance),
mass of shell (as above),
wing length (maximum chord, to nearest 0.1 mm, vernier callipers),
tarsus length (as for adult swallows, Chapter 2),
area of yolk visible on the belly (categorised by eye as rectangles or circles, which were measured using vernier callipers, and their areas summed).
The following information was also recorded:
incubator or naturally hatched,
clutch size,
age and structural size of female parent (Chapter 2),
laying date of the egg,
time and date of hatching of the egg,
time since the incubator was last visited,
time since the egg was moved to the incubator,
duration of incubation (d).

4.3 Results

4.3.1 Swallow

4.3.1.1 Use of airspace diameter to determine order of laying of unincubated clutches

The airspace began to form at the rounded end of each egg immediately after laying, due to the contraction of the contents of the egg as it cooled. After this the diameter increased due to loss of water (Fig. 4.1a). Airspace diameter would have correctly predicted the order of laying in 21 of 27 complete swallow clutches where

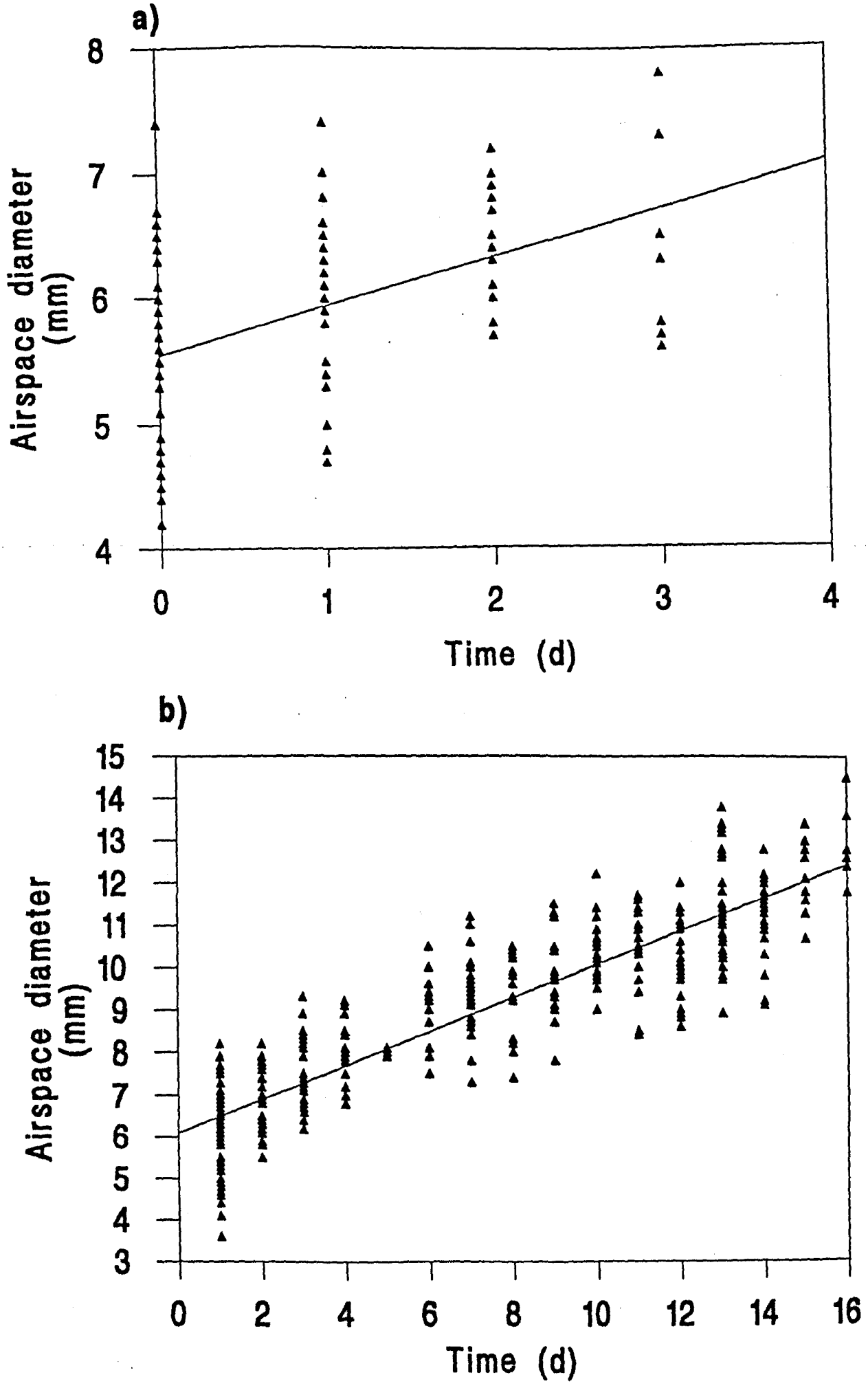


Fig. 4.1 Increase in airspace diameter at the blunt end of swallow eggs a) between laying and the start of incubation ($y=0.387x + 5.55$, $r^2=0.24$, $p=0.0001$, $n=98$), b) during incubation ($y=0.296x + 6.09$, $s_b=0.99$, $r^2=0.76$, $p=0.0001$, $n=383$).

this was measured before the start of incubation, and order of laying was known. In the six clutches in which airspace diameter did not reflect laying order the last egg to be laid would have been identified correctly. Nest visits on alternate days would therefore allow eggs to be placed in the correct laying order, and laying order could often be predicted correctly for entire unincubated clutches.

4.3.1.2 Use of airspace diameter to calculate hatching date

The diameter of the airspace of swallow eggs which had been incubated for known numbers of days was regressed upon day of incubation (Fig. 4.1b). The regression equation allowed prediction of the number of days for which clutches first found during incubation had been incubated ±2 d (95% confidence interval of b from regression equation, Fig. 4.1b).

The mean rate of mass loss of swallow eggs during incubation was $0.02gd^{-1}$ (sd=0.004, range 0.016 to 0.026, n=18 eggs from different nests). Day of incubation could have been calculated from mean daily mass loss, and the difference between mass on the day an egg was found and fresh mass calculated from egg dimensions. The airspace diameter method of prediction of day of incubation was preferred, as this did not require calculation of fresh egg mass which could itself introduce additional error into the calculation of the day of incubation.

4.3.1.3 Egg size

Eggs re-weighed each day after laying did not lose mass before the start of incubation. Mass must have decreased due to water loss (demonstrated by the increase in diameter of the airspace), but this appeared to be less than could be detected using a spring balance. All eggs weighed before the start of incubation were therefore used to calculate the value of the constant in Equation 4.1.

The constant differed significantly between years (Table 4.1). Fresh egg mass calculated from Equation 4.1 using a separate constant for each year provided a

Table 4.1 Constant relating swallow fresh egg mass to egg length and breadth, in the relationship mass = constant $\times LB^2$, a) in each year 1989-91, b) t-tests showing significant differences in the constant between years. Tabulated values are t, followed by the significance level. *** = $p<0.001$, ** = $p<0.01$

a)

Year	Mean	sd	n
1989	0.522	0.01	192
1990	0.537	0.01	208
1991	0.531	0.01	37
All years	0.531	0.02	440

b)

	1990	1991
1989	13.4 ***	4.6 ***
1990		2.9 **

slightly better match with actual fresh mass than if data from the three years was pooled to calculate the constant (Pearson correlations, r=0.92 using the separate constants for each year, r=0.91 using the mean constant for all years, both $p<0.0001$, n=440).

Possible reasons for the differences in the constant between years were that eggs indeed differed in shape between years, that I was particularly careful not to crush eggs in 1989, resulting in slightly larger dimensions than in subsequent years when I was accustomed to handling eggs, and that the sample of eggs measured in 1991 was small. Mean egg mass (mean of mean egg mass for each clutch) was 1.97 g (sd=0.14, n=247 clutches). Mean egg length was 19.7 mm (sd=0.81) and breadth 13.7 mm (sd=0.40). Mean egg mass was the same as that determined for swallows nesting in the same area by Turner (1980).

4.3.1.4 Egg size and hatching success

There was no significant difference in mass between eggs which hatched and those which were infertile or failed during development of the embryo (t-test, t=0.41, p=0.6, n=766 eggs known to hatch (mean mass=1.97 g, sd=0.15) and 99 which did not (mean mass=1.96 g, sd=0.12)). Eggs which failed to hatch due to human interference, predation of the nest or female, collapse of the nest or desertion during a spell of particularly bad weather were excluded from this analysis (n=139, including 69 eggs collected during the study under licence from the NCC).

4.3.1.5 Factors with a potential effect on egg and clutch size or laying date

1. Year

There were no significant differences between years in mean egg mass (Table 4.2) or clutch size (Table 4.3) of either first or second clutches. Similar analyses showed no significant differences between years in RDEW and laying date of both first and second clutches (all $t<1.71$, $p>0.09$). Data for egg and clutch sizes and laying dates were therefore pooled for the three years in subsequent analyses.

Table 4.2 Effect of year on swallow **egg mass**, a) first clutches 1989-91, b) second clutches 1989-90. Each clutch was treated as an independent unit, so the mean egg mass for each clutch was entered in the analysis. ns = not significant. n = number of clutches.

a) First clutch egg mass

Year	**Mean**	**sd**	**n**
1989	1.96	0.14	49
1990	1.96	0.12	62
1991	2.00	0.12	20

t-tests

	1990	**1991**
1989	0.06 ns	1.15 ns
1990		1.32 ns

b) Second clutch egg mass

Year	**Mean**	**sd**	**n**
1989	1.96	0.14	56
1990	1.96	0.14	35

t-test

	1990
1989	0.11 ns

Table 4.3 Effect of year on swallow **clutch size** for a) first clutches 1989-91, b) second clutches 1989-90. T-tests show no significant effect of year upon first or second clutch size. ns = not significant. n = number of clutches.

a) First clutch size

Year	Mean	sd	n
1989	4.84	0.77	49
1990	4.81	0.91	63
1991	5.10	0.55	20

t-tests

	1990	**1991**
1989	0.17 ns	1.38 ns
1990		1.34 ns

b) Second clutch size

Year	Mean	sd	n
1989	4.39	0.77	63
1990	4.33	0.68	43

t-test

	1990
1989	0.49 ns

2. Clutch number

There was no significant difference in mean egg mass of all first and second clutches (t-test t=0.33, p=0.7, n=131 first clutches of mean egg mass 1.97 g, sd=0.13, and n=93 second clutches of mean mass 1.96 g, sd=0.14) or between the first and second clutches of double brooded females (paired t-test, t=1.96, p=0.06, n=60, mean first clutch egg mass=1.98 g, sd=0.13, mean second clutch egg mass=1.95 g, sd=0.15).

First clutch size was significantly greater than second clutch size for all females (t-test, t=4.24, p=0.001, mean first clutch size=4.86, sd=0.82, n=132, mean second clutch size=4.43, sd=0.73, n=94) and for double brooded females (paired t-test, t=4.66, p=0.001, n=71, mean first clutch size=4.90, sd=0.93, mean second clutch size=4.36, sd=0.76).

It was concluded that there was no difference in egg size between first and second clutches, but that first clutches were significantly larger.

3. Relationships between egg and clutch size, RDEW and laying date

There was no significant correlation between mean egg mass and clutch size (Pearson correlation, r=-0.06, p=0.15, n=247 clutches). Mean egg mass was weakly but significantly negatively correlated with RDEW (r=-0.18, p=0.004, n=247). RDEW was positively correlated with clutch size (r=0.16, p=0.02, n=247).

Clutch size declined through the season if first and second clutches were considered separately or if all clutches were included in a single analysis (Fig. 4.2). Clutch mass was correlated with laying date (Pearson correlation, r=-0.36, p=0.001 for first clutches, r=-0.27, p=0.007 for second clutches; r=-0.44, p=0.001, n=238 for all clutches). There were no significant relationships between laying date and mean egg size of first, second or all clutches (all $r<0.1$, $p>0.5$).

4. Laying order

There were no significant differences between the mass of eggs laid first or last in a clutch and mean egg mass for that clutch (paired t-tests, both $t<1.0$, $p>0.3$,

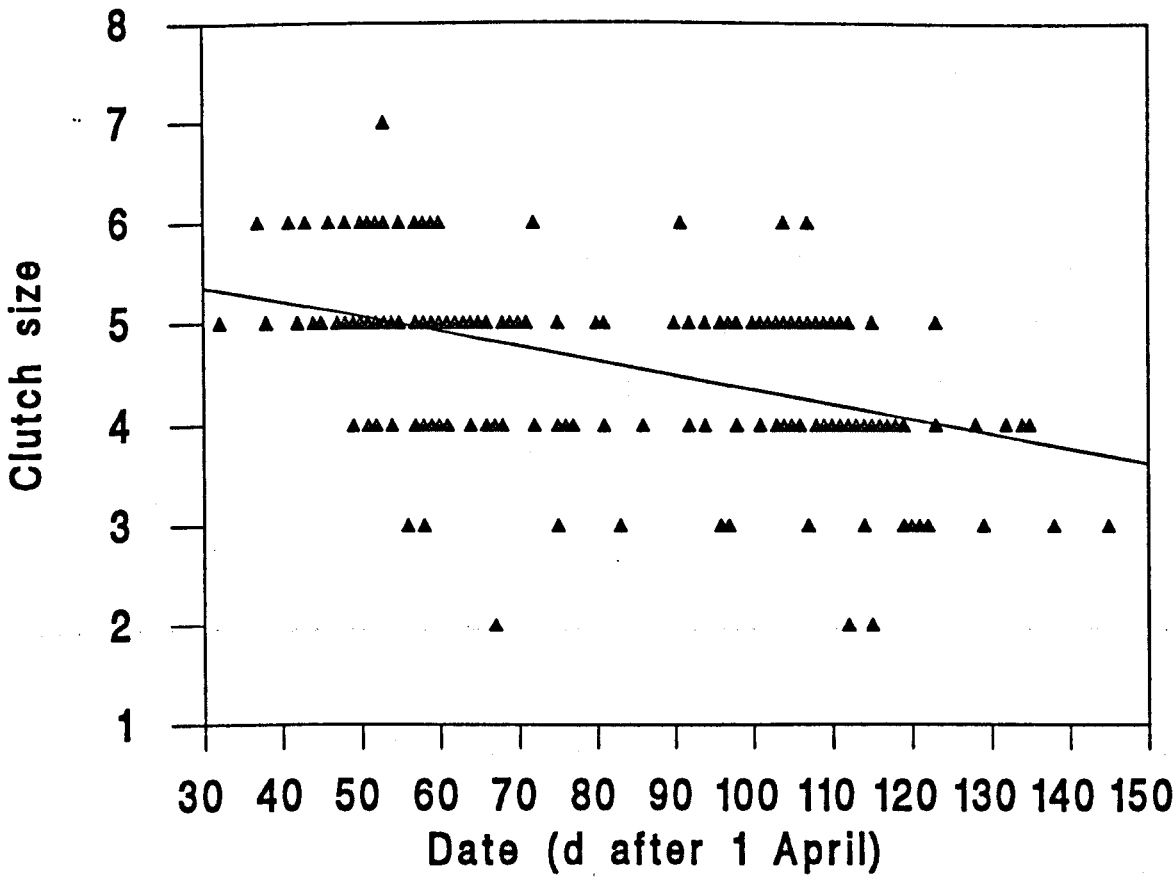


Fig. 4.2 Decline in swallow clutch size during the breeding season ($y=-0.015x + 5.750$, $r^2=0.20$, $p=0.0001$, $n=240$). First, second and replacement clutches included.

n=93). There was no significant difference in egg size with order of laying for clutches of 4, 5 or 6 eggs (analyses of variance, all $p>0.05$).

5. Farm

There were no significant differences in egg or clutch size or laying date either between individual farms or between 3 sections of the study area (the carse to the W of Stirling (10-15 m a.s.l.), higher ground to the NW of Bridge of Allan (60-80 m a.s.l.) and farms to the E of Stirling (10-15 a.s.l.) (analyses of variance, all $p>0.05$).

6. Female

Egg size was measured in first and second clutches laid by individual females in the same year, and in consecutive years. Mean egg size was highly correlated between first and second clutches laid by a female in the same year (Pearson correlation, $r=0.71$, $p=0.001$, $n=60$), as was mean egg size (mean all eggs laid by a female in each year) in consecutive years (Fig. 4.3a). The size of second clutch eggs was more highly correlated between years than that of first clutch eggs (Fig. 4.3b,c). This suggested that although there was a strong genetic component in the determination of egg size, other factors were also involved in the determination of egg size which were more important during the laying of first than second clutches. It was possible that a site factor might be involved, as individual females laid at the same farm in successive years (pers. obs., Thompson 1992), but this seemed unlikely as there was no significant effect of site on egg size in general, and because of the difference in correlation between the size of first and second clutch eggs.

An analysis of variance showed that within female variation in egg size was significantly less than between female variation (each egg laid by each female treated as the sampling unit) (Table 4.4). Repeatability of egg size was 0.54, calculated after Lessells and Boag (1987), from Table 4.4.

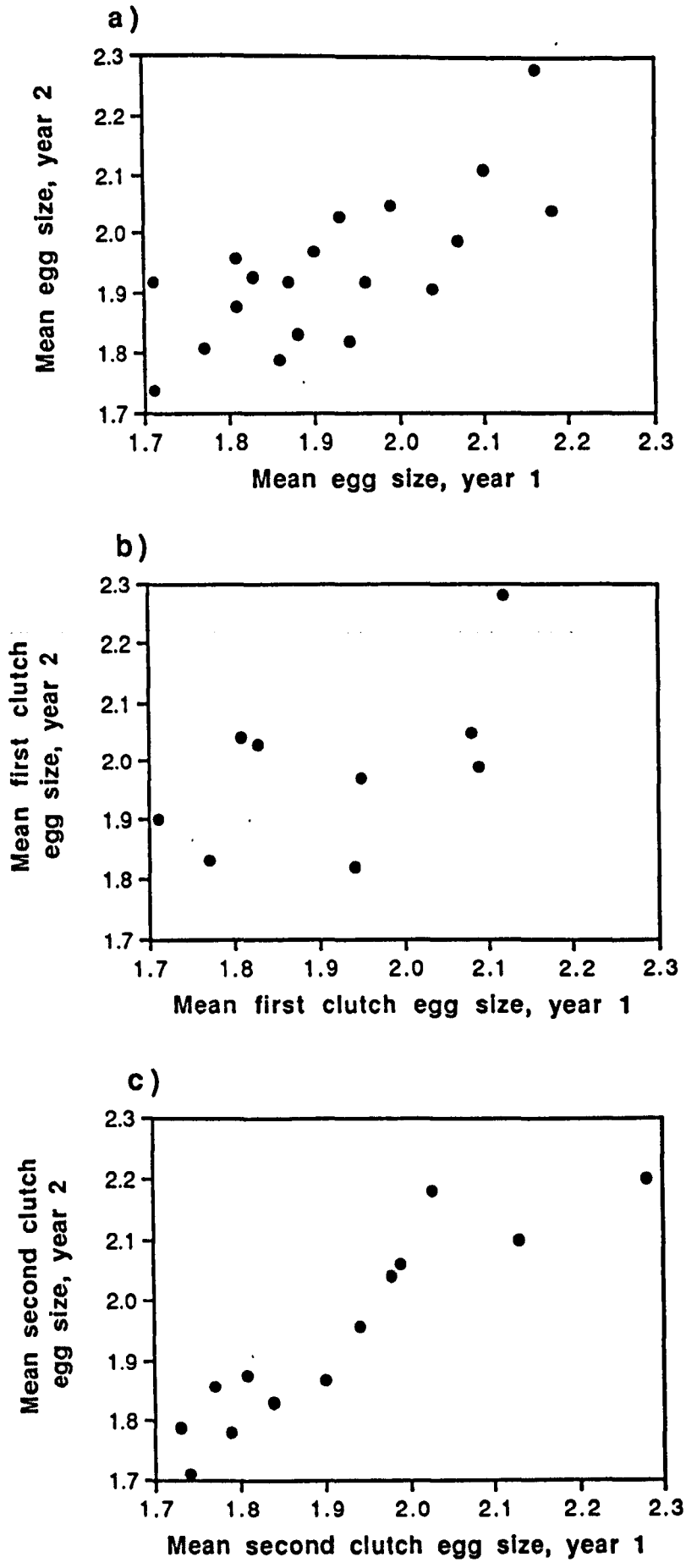


Fig. 4.3 Correlation between years of the mean egg size of female swallows which laid in the study area in consecutive years. a) Mean size of all eggs laid in each of the years (Pearson correlation=0.70, p=0.002, n=19), b) mean first clutch egg size in consecutive years (r=0.50, p=0.2, n=9), c) mean second clutch egg size in consecutive years (r=0.92, p=0.001, n=13).

Table 4.4 Analysis of variance of swallow egg mass within and between individual females. All eggs laid by each female over all years of the study were included in the analysis.

Source	D.F.	Sum of Squares	Mean squares	F ratio	F prob.
Between clutches	125	15.3107	0.1225	10.0522	<0.0001
Within clutches	851	10.3694	0.0122		
Total	976	25.6801			

7. Female age

There was no consistent change in egg or clutch size between consecutive years by the 18 female swallows which bred in more than one year (Sign tests, both $p>0.4$). There was, however, a significant correlation between female swallow age and the size of first and second clutches (Pearson correlations, $r=0.30$, $p=0.01$, $n=73$ for first clutches, $r=0.29$, $p=0.05$ $n=50$ for second clutches). This was a result of older females laying earlier than first year birds (Table 4.5), since a partial correlation between age and clutch size, controlling for laying date, revealed no significant effect of age upon clutch size (partial correlations, $r=0.2$, $p=0.1$ for first and second clutches). There were no significant correlations between egg size or RDEW and age in either first or second clutches (Pearson correlations and partial correlations controlling for date, all $r<0.1$, $p>0.5$)

There was no significant difference in egg size between first year and older females but first year birds laid their first clutches later and had smaller second clutches than older birds (Table 4.5). 53.8% of first year birds had a second clutch, whilst significantly more (74.0%) of older birds did so (Chi-square test, $\chi^2=6.42$, $p=0.02$, $n=57$ first year and 75 older birds).

8. Female body size

There were no significant correlations between measures of female body size (wing length, keel length, head+bill length, tarsus length, outermost, second or innermost tail feather lengths, PC1 or PC2) and clutch size or mean egg mass, however first clutch mass was positively correlated with keel length (Table 4.6). Controlling for laying date, a partial correlation between mean egg size, first and second clutch size and clutch mass revealed a positive correlation between first clutch size and tarsus length and a negative correlation between inner tail length and first clutch size or mass (Table 4.7).

There were no significant correlations between female swallow body size and egg size, and those between clutch size and body size were weak and gave conflicting conclusions as to whether body size was positively or negatively

Table 4.5 Egg and clutch size, and laying date, of first year and older female swallows and a comparison between the two groups using t-tests. ** = $p<0.01$, * = $p<0.05$, ns = not significant.

	First year			Older				
	Mean	**sd**	**n**	**Mean**	**sd**	**n**	**t**	**p**
Mean egg size (all clutches)	1.96	0.14	63	1.97	0.12	101	0.57	ns
First clutch size	4.75	0.68	57	4.95	0.51	75	1.35	ns
Second clutch size	4.06	0.74	34	4.53	0.69	74	3.13	**
Laying date of first clutch[1]	61.3	11.1	56	55.9	8.7	73	2.92	**
Laying date of second clutch[1]	110.6	12.6	34	105.4	10.3	68	2.09	*

[1] Laying date coded with day 1 = 1st April of each year.

Table 4.6 Pearson correlation co-efficients between measures of female swallow body size and mean egg size (all clutches), first and second clutch size and first and second clutch mass. Details of body size are given in Chapter 2. Sample sizes are given in brackets.

	Mean egg size	First clutch size	First clutch mass	Second clutch size	Second clutch mass
Wing length (mm)	0.06	0.14	0.11	0.09	0.07
	(141)	(112)	(112)	(99)	(98)
	ns	ns	ns	ns	ns
Keel length (mm)	0.08	0.16	0.20	0.10	0.03
	(139)	(110)	(110)	(98)	(97)
	ns	ns	*	ns	ns
Head+bill length (mm)	-0.02	-0.05	-0.06	0.06	-0.02
	(141)	(112)	(112)	(99)	(98)
	ns	ns	ns	ns	ns
Tarsus length (mm)	0.03	0.10	0.08	0.13	0.11
	(102)	(89)	(89)	(67)	(67)
	ns	ns	ns	ns	ns
Outer tail (mm)[a]	0.04	0.11	0.05	0.11	0.06
	(135)	(108)	(108)	(96)	(95)
	ns	ns	ns	ns	ns
Second tail (mm)[b]	0.13	-0.07	-0.04	0.07	0.05
	(139)	(110)	(110)	(99)	(98)
	ns	ns	ns	ns	ns
Inner tail (mm)[c]	0.13	-0.15	-0.14	0.19	0.18
	(139)	(110)	(110)	(99)	(98)
	ns	ns	ns	ns	ns
PC1	0.10	-0.04	-0.09	0.08	0.06
	(94)	(83)	(83)	(63)	(63)
	ns	ns	ns	ns	ns
PC2	-0.07	0.07	0.02	-0.01	-0.03
	(94)	(83)	(83)	(63)	(63)
	ns	ns	ns	ns	ns

[a] length of outermost tail feather
[b] length of second tail feather
[c] length of shortest tail feather

Table 4.7 Partial correlation co-efficients between measures of female swallow body size and mean egg size (all clutches), first and second clutch size and clutch mass, controlling for laying date.

	Mean egg size	First clutch size	First clutch mass	Second clutch size	Second clutch mass
Wing length (mm)	-0.03	-0.07	-0.11	-0.01	-0.01
	(76)	(79)	(79)	(55)	(55)
	ns	ns	ns	ns	ns
Keel length (mm)	0.08	0.12	0.13	0.12	0.03
	(76)	(79)	(79)	(55)	(55)
	ns	ns	ns	ns	ns
Head+bill length (mm)	-0.05	-0.12	-0.14	-0.02	-0.10
	(76)	(79)	(79)	(55)	(55)
	ns	ns	ns	ns	ns
Tarsus length (mm)	0.06	0.19	0.14	0.19	0.06
	(76)	(79)	(79)	(55)	(55)
	ns	*	ns	ns	ns
Outer tail (mm)[a]	-0.12	0.05	-0.08	-0.08	-0.11
	(76)	(79)	(79)	(55)	(55)
	ns	ns	ns	ns	ns
Second tail (mm)[b]	-0.13	-0.13	-0.13	-0.14	-0.11
	(76)	(79)	(79)	(55)	(55)
	ns	ns	ns	ns	ns
Inner tail (mm)[c]	-0.03	-0.24	-0.22	0.15	0.18
	(76)	(79)	(79)	(55)	(55)
	ns	*	*	ns	ns
PC1	0.04	-0.11	-0.17	-0.02	-0.03
	(72)	(79)	(79)	(55)	(55)
	ns	ns	ns	ns	ns
PC2	-0.10	0.13	0.06	0.03	-0.01
	(72)	(79)	(79)	(55)	(55)
	ns	ns	ns	ns	ns

[a] length of outermost tail feather
[b] length of second tail feather
[c] length of shortest tail feather

correlated with first clutch size. There were slightly fewer significant correlations between measures of body size and egg or clutch size than would be expected by chance (0.05×95=4.5 significant correlations expected, compared with 4 significant correlations observed). It was concluded that there was no important effect of female body size upon the number or size of eggs laid.

9. Food supply and weather

There were no clear relationships between egg size and female attributes, year, farm, laying order and laying date. Of these factors only laying date was related to clutch size. The observed variability in egg and clutch size might be explained by environmental factors before or during egg formation. In the investigation for effects of environmental factors upon egg or clutch size, relationships with food availability (insect suction trap catch ($\log_e(V+1)$), rainfall and maximum, minimum and mean temperature were examined. A significant relationship between egg or clutch size and any of these variables before or during egg formation would imply that energy or nutrient availability acted as a proximate constraint upon egg formation, either directly through a reduction in food availability or indirectly, due to increased thermoregulatory energy requirement of the female at lower temperatures or increased activity costs when food was less abundant.

The temperature was significantly lower (t-tests, all $t>3.39$, $p<0.001$ for maximum, minimum and mean temperature) and suction trap catch significantly more variable during the laying period of first clutches when compared with that of second clutches (analysis of variance, $F=2.09$, $p=0.001$). RDEW was slightly but not significantly greater for first clutch than second clutches (paired t-test, $t=1.81$, $p=0.08$, mean RDEW=0.096, sd=0.047 for first clutches, 0.082, sd=0.05 for second clutches, n=53 double-brooded females which laid without laying anomalies). These results were consistent with the hypothesis that variation in daily food intake had some effect on variation in egg size.

Correlations between egg or clutch size and environmental factors were examined during periods which were likely to be critical during egg formation. The

critical periods described below are also shown in relation to each other and oviducal, yolk and albumen formation in Table 4.8.

a) immediately before the start of egg formation: the 3 d just before the start of the rapid follicular growth (days -10 to -8, where the first egg is laid in the morning on day 0). The duration of this period was selected arbitrarily. Environmental factors during this period might be related to egg or clutch size if egg formation depended upon an energy or nutrient reserve obtained immediately before the start of egg formation.

b) period of rapid follicular growth and enlargement of the oviduct: the 6 d prior to ovulation of the first egg (days -7 to -2). Environmental factors would be important during this period if mean egg or clutch size was constrained by availability of energy or nutrients for yolk formation, or if the demands of oviducal recrudescence limited resource availability for egg formation.

c) the more demanding part of the period of rapid follicular growth and enlargement of the oviduct: the 4 d prior to the ovulation of the first egg (days -5 to -2). As b), but omitting the first two days as only a small part of yolk deposition and oviducal growth occurred on these days.

d) period of lipid reserve deposition (days -3 to -1, derived in Chapter 8). Egg and clutch size might be affected by environmental factors during this period if the size of the lipid reserve built up on these days was influenced by resource availability and the size of the lipid reserve on day -1 determined egg or clutch size.

e) the day upon which the rapid growth phase of the ovarian follicle destined to become the fifth egg of the clutch would be initiated (day -3). Environmental factors on this day could determine whether a clutch of 4 or of 5 eggs was laid, if swallows began to form only those follicles which they went on to lay and clutch size was determined by resource availability on this day.

Table 4.8 Periods during which environmental factors might be important in the determination of egg or clutch size. See text for explanation of the periods a) to j). The first and second days of the yolk formation (Y1 and Y2) are shown in brackets, as little energy input is required on the first two days of rapid follicular growth. W indicates that an egg albumen was formed on that day. O1 to O6 indicate the days during which the oviduct enlarged (Appendix 3).

Day	**-10**	**-9**	**-8**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**
Oviduct				O1	O2	O3	O4	O5	O6							
Egg 1				(Y1)	(Y2)	Y3	Y4	Y5	Y6	W						
Egg 2					(Y1)	(Y2)	Y3	Y4	Y5	Y6	W					
Egg 3						(Y1)	(Y2)	Y3	Y4	Y5	Y6	W				
Egg 4							(Y1)	(Y2)	Y3	Y4	Y5	Y6	W			
Egg 5								(Y1)	(Y2)	Y3	Y4	Y5	Y6	W		
Egg 6									(Y1)	(Y2)	Y3	Y4	Y5	Y6	W	
Period																
a)	x	x	x													
b)				x	x	x	x	x	x							
c)						x	x	x	x							
d)								x	x	x						
e)								x								
f)									x							
g)										x						
h)										x	x	x	{x}	[x]		
i)										x	x	x	x	{x}	[x]	
j)								x	x	x	x	{x}	[x]			

x indicates a day which was part of the period for clutches of 4, 5 or 6 eggs.
{x} indicates a day which was part of the period for clutches of 5 or 6 eggs.
[x] indicates a day which was part of the period only for clutches of 6 eggs.

f) the day upon which the rapid growth phase of the ovarian follicle destined to become the sixth egg of the clutch is initiated (day -2). As d), but environmental factors on this day might determine whether a clutch of 5 or of 6 eggs was laid.

g) the day before the first egg was laid (day -1): the day of maximum energy and material requirement for egg formation (Tables 3.8 and 3.9). Environmental factors on this day would be important in determination of the size of the first egg, if egg size was related to resource availability on the day upon which the albumen was deposited. Clutch size or mean egg size could be determined on this day if energy or nutrient limitation caused follicles which would have formed the yolks of later eggs in the clutch suffered atresia, reducing clutch size, or their rate of formation could be slowed, reducing mean egg size.

h) mixed yolk and albumen formation: the days upon which both albumen and yolk were formed (days -1 to 1, for clutches of 4, -1 to 2 for clutches of 5 and -1 to 3 for clutches of 6 eggs). Environmental factors during this period would be related to clutch size or mean egg size if the demands of simultaneous yolk and albumen formation limited reproductive output, and eggs were formed from daily food intake.

i) albumen formation: the days upon which albumen was deposited (days -1 to 2, for clutches of 4, -1 to 3 for clutches of 5 and -1 to 4 for clutches of 6 eggs). Environmental factors during this period could be related to egg or clutch size if albumen formation was sensitive to daily food intake. This was possible as albumen must be deposited during one day, whilst yolk formation was spread over several. Environmental factors during albumen formation could be related to mean egg size, or the size of individual eggs could be related to conditions on the day upon which the albumen was formed. A period of poor weather could precede an interruption in laying if yolk and albumen deposition was suspended until conditions improved so that ovulation of the largest follicle was delayed. A period of poor weather could precede termination of laying if follicles were resorbed rather than ovulated.

j) period of more than 50% of the maximum energy content of egg deposition: days -3 to 0 for clutches of 4, days -3 to 1 for clutches of 5, days -3 to 2 for clutches of 6. Environmental factors were most likely to be related to egg or clutch size during this period if the availability of energy for egg production limited reproductive output.

Correlation between egg or clutch size and environmental factors or rainfall upon a certain day or days could have a variety of interpretations, due to the overlap between many of the periods described above (Table 4.8). In the following description of the results of these analyses relationships between suction trap catch, rainfall and maximum, minimum and mean temperature and egg or clutch size were analyzed for first (n=129) and second (n=100) clutches separately. Clutch sizes were smaller although insect suction trap catch and temperature were greater during the period when second clutches were laid, so effects of energy or nutrient limitation to egg production were most likely to be found for first clutches. Pearson correlations were performed between environmental factors and egg size. As clutch size declined during the breeding season (Fig. 4.2) partial correlations, controlling for date, were performed between clutch size and environmental factors. Results of correlations not specifically mentioned in the text were non-significant ($p>0.05$). The results are summarised in Table 4.9 and 4.10.

a) There were no significant correlations between environmental factors during the pre-egg formation period and mean egg size of first or second clutches (all $r<\pm0.16$, $p>0.08$ n=131 first clutches and 91 second clutches). Partial correlations, controlling for date, showed no significant correlations between clutch size and environmental factors (all $r<\pm0.12$, $p>0.16$, n=132 first and 106 second clutches). There was no evidence that environmental factors during the pre-laying period influenced egg or clutch size.

b) There were no significant correlations between environmental factors during the period of rapid follicular growth and the mean size of first or second clutch eggs (all

Table 4.9 Pearson correlation co-effecients between **mean egg size** of a) first and b) second clutches and environmental factors (food = insect suction trap catch ($\log_e$ (V+1)), maximum, minimum and mean temperature (°C) and rainfall (mm)) during the periods before and during egg formation. The duration of each period a) to j) is shown in Table 4.8 and explained in the text. - indicates that correlation between egg size and environmental factors during this period was not appropriate. *** = p<0.001, ** = p<0.01, * = p<0.05, ns = not significant.

a) First clutch

	Food	Max. temp.	Min. temp.	Mean temp.	Rain
a)	ns	ns	ns	ns	ns
b)	ns	ns	ns	ns	ns
c)	ns	ns	ns	ns	ns
d)	0.18*	ns	ns	ns	ns
e)	-	-	-	-	-
f)	-	-	-	-	-
g)	ns	ns	ns	ns	ns
h)	ns	0.25**	ns	0.23**	ns
i)	ns	0.25**	ns	0.24**	ns
j)	ns	0.26**	ns	0.24**	ns

b) Second clutch

	Food	Max. temp.	Min. temp.	Mean temp.	Rain
a)	ns	ns	ns	ns	ns
b)	ns	ns	ns	ns	ns
c)	ns	ns	ns	ns	ns
d)	ns	ns	ns	ns	ns
e)	-	-	-	-	-
f)	-	-	-	-	-
g)	ns	ns	ns	ns	ns
h)	ns	0.23*	ns	0.23*	ns
i)	ns	ns	ns	0.22*	ns
j)	ns	ns	ns	ns	ns

Table 4.10 Partial correlation co-effecients, controlling for laying date, between **clutch size** and environmental factors (food = insect suction trap catch ($\log_e$ (V+1)), maximum, minimum and mean temperature (°C), and rainfall (mm)) for a) first and b) second clutches. The duration of each period a) to j) is shown in Table 4.8 and explained in the text. During periods e), f) and g), t-tests between environmental factors during laying of clutches of 4, 5 and 6 eggs. corr = results of partial correlations. t-test = results of t-tests. For correlations, *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$, ns = not significant. For t-tests 0 indicates no significant difference in the environmental factor for clutches of different sizes, +ve indicates that the factor was significantly greater ($p<0.05$) for larger clutches, and -ve that the factor was significantly smaller for larger clutches. Results shown in brackets show trends in the opposite direction to those expected if larger eggs were formed during more favourable conditions.

a) First clutch

		Food	Max. temp.	Min. temp.	Mean temp.	Rain
a)	corr	ns	ns	ns	ns	ns
b)	corr	ns	ns	ns	ns	ns
c)	corr	ns	ns	ns	ns	ns
d)	corr	ns	ns	0.24**	0.19*	ns
e)	t-test	0	0	0	0	(-ve)
f)	t-test	+ve	+ve	0	0	0
g)	corr	ns	ns	0.31***	0.19*	ns
g)	t-test	+ve	ns	+ve	ns	ns
h)	corr	ns	ns	ns	ns	ns
i)	corr	ns	ns	ns	ns	ns
j)	corr	ns	ns	0.18*	ns	ns

b) Second clutch

		Food	Max. temp.	Min. temp.	Mean temp.	Rain
a)	corr	ns	ns	ns	ns	ns
b)	corr	(-0.22*)	ns	(-0.23*)	ns	ns
c)	corr	(-0.23*)	ns	(-0.22*)	ns	ns
d)	corr	ns	ns	ns	ns	ns
e)	t-test	0	0	0	0	0
f)	t-test	0	0	0	0	0
g)	corr	ns	ns	(-0.22*)	ns	ns
g)	t-test	0	0	0	0	0
h)	corr	ns	ns	ns	ns	-0.24*
i)	corr	ns	ns	ns	ns	-0.25*
j)	corr	ns	ns	ns	ns	-0.26*

$r<0.14$, $p>0.11$). Surprisingly, second clutch size was negatively correlated with suction trap catch and minimum temperature, suggesting that larger clutches were laid in poorer conditions (both $r=-0.23$, $p<0.04$).

c) There were no significant correlations between mean first or second clutch egg size and environmental factors during the main period of rapid follicular growth (all $r<\pm 0.15$, $p>0.08$). Second clutch size was again negatively correlated with minimum temperature and suction trap catch ($r=-0.22$, $p=0.04$).

d) Mean egg size was significantly correlated with suction trap catch during the period of lipid reserve deposition ($r=0.17$, $p=0.05$), but first clutches were significantly larger when the mean and minimum temperature were higher during this period (both $r>0.19$, $p<0.03$).

e) There was significantly greater rainfall on the day upon which the rapid growth phase of the follicle for the fifth egg began for first clutches 5 than of 4 eggs (t-tests, $t=3.05$, $p=0.003$, $n=25$ clutches of 4 and 78 clutches of 5 eggs). This result was contrary to the expectation that larger clutches would be initiated when the weather was more favourable as heavy rain depressed foraging rates (Turner 1982, Hails and Turner 1985). There were no significant differences in the other environmental factors on this day prior to laying clutches of 4 and 5 eggs (all $t<1.11$, $p>0.25$).

f) The maximum temperature was higher on the day upon which the rapid follicular growth would begin for the of the sixth egg before clutches of 6 eggs were laid than prior to laying of clutches of 5 eggs (t-test, all $t=2.07$, $p=0.04$, $n=78$ clutches of 5 eggs and 20 clutches of 6). The suction trap catch was significantly greater on this day prior to the laying of a clutch of 6 than one of 5 eggs, but only if a 1-tailed significance test was applied ($t=1.6$, $p=0.05$, 1-tailed test). These results suggested that clutches of 6 eggs were initiated when conditions were particularly favourable.

g) The suction trap catch was significantly greater on the day before the first egg was laid of first clutches of 5 than of 4 eggs (t-test, $t=2.78$, $p=0.007$, $n=25$ clutches of 4 and 78 clutches of 5 eggs). There were no differences in temperature or rainfall on this day prior to the laying of clutches of 4 and 5 eggs (all $t<0.28$, $p>0.6$). Minimum temperature was significantly greater on the day before laying of the first of a clutch of 6 than of 5 eggs ($t=2.42$, $p=0.02$, $n=78$ clutches of 5 and 20 clutches of 4 eggs). Both minimum temperature and suction trap catch were significantly lower on the day before a clutch of 4 than of 6 eggs was laid ($t=2.23$, $p=0.03$ for minimum temperature and $t=3.03$, $p=0.005$ for suction trap catch).

Controlling for laying date, minimum and mean temperature on the day before the first egg was laid were correlated with first clutch size ($r=0.31$, $p=0.001$ and $r=0.19$, $p=0.03$, respectively). A significant negative correlation between minimum temperature on the day before the first egg was laid and clutch size was found for second clutches ($r=-0.22$, $p=0.03$). Mean first and second clutch egg size showed no significant correlation with environmental factors on the day before the first egg was laid (all $r<0.16$, $p>0.07$).

These results showed that food supply and temperature on the day before the first egg was laid were related to the size of the clutch subsequently laid. This day was likely to be the most critical in egg formation, since the maximum energy content of egg formation must be deposited on this day. As clutch size was related to environmental factors on the day before the first egg was laid, rather than on the days when the rapid growth phase of ovarian follicles began, birds probably began the rapid growth phase of more follicles than they would necessarily ovulate and adjusted clutch size to the resources which they were able to devote to egg formation on the day of maximum energy requirement. It would therefore be predicted that laying female swallows would have atretic follicles in their ovaries, which could be used as a source of energy and nutrients for the continued formation of the rest of the clutch if conditions deteriorated. To test this hypothesis would require the sacrifice of laying female swallows following a day of poor weather on the day before their first egg was laid to determine whether their ovaries contained atretic follicles.

h) Second clutch size was correlated with rainfall during the period of mixed yolk and albumen formation ($r=-0.24$, $p=0.03$). There were no other significant correlations between environmental factors during this period and first or second clutch size.

This was an important period in the determination of egg size, as mean egg size of first and second clutches was correlated with maximum and mean temperature (all $r>0.22$, $p<0.04$). This suggested that egg size was influenced by environmental factors during the period of mixed yolk and albumen formation.

i) Mean first and second clutch egg sizes were correlated with mean temperature during the period of albumen formation (both $r>0.20$, $p<0.05$). Mean first egg size was correlated with maximum temperature ($r=0.25$, $p=0.008$). First clutch size was not correlated with environmental factors during this period (all $r<0.14$, $p>0.1$), but second clutch size was negatively correlated with rainfall ($r=-0.25$, $p=0.02$).

This period had considerable overlap with period h). The significant positive correlation between egg size and temperature again suggested a direct effect upon egg size of food supply and temperature.

j) Environmental factors during the period of more than 50% of the maximum daily energy deposition on eggs were significantly correlated with egg and clutch size of first clutches and with clutch size of second clutches. Mean first clutch egg size was correlated with mean and maximum temperature (mean temperature, $r=0.24$, $p=0.008$, maximum temperature, $r=0.26$, $p=0.004$). First clutch size was weakly but significantly correlated with minimum temperature during this period ($r=0.18$, $p=0.05$). Second clutch size was correlated with rainfall ($r=-0.26$, $p=0.02$).

The correlation between environmental factors and mean egg size of first clutches was higher during this period than during either periods h) or i), which suggested that this was the period when environmental factors had the greatest influence upon egg size. Clutch size too was correlated with environmental factors,

suggesting that the period of maximum energy demand could influence reproductive output both via the number and the size of the eggs formed. This provided strong evidence for the hypothesis that the majority of resources for egg formation were obtained during the days when the majority of energy was required for egg formation. Variation in environmental conditions at this time could therefore exert a direct effect on reproductive output.

As anticipated, correlations between egg and clutch size and environmental factors were found mainly for first rather than second clutches. This confirmed the conclusion drawn from the higher degree of correlation between second than first clutches laid by the same female in consecutive years.

First clutches of five and six egg were initiated when the suction trap catch and maximum temperature were higher than prior to laying of smaller clutches. These clutches were laid when temperature and food availability were lower than later in the season, when smaller clutches were laid. This, and the unexpected negative correlations between environmental factors during second clutch formation and clutch size, suggested that environmental factors do not have as important an influence on second as first clutch formation.

Minimum and mean temperature on the day before the first egg was laid were important in the determination of clutch size as was the minimum temperature during the period when more than 50% of the maximum energy requirement for egg production. The greater the minimum temperature, the earlier in the morning and later in the evening a large number of insects were likely to be airborne and therefore available to swallows. Larger clutches were therefore more likely to be formed when insects were abundant for a longer period during the day.

Partial correlation co-efficients (controlling for laying date) between first clutch size and mean temperature on each day before and during egg formation increased as the day when the first egg was laid approached, and decreased sharply thereafter (Fig. 4.4). There was a significant correlation between the energy content of yolk deposited each day (calculated from Table 3.8) and the partial

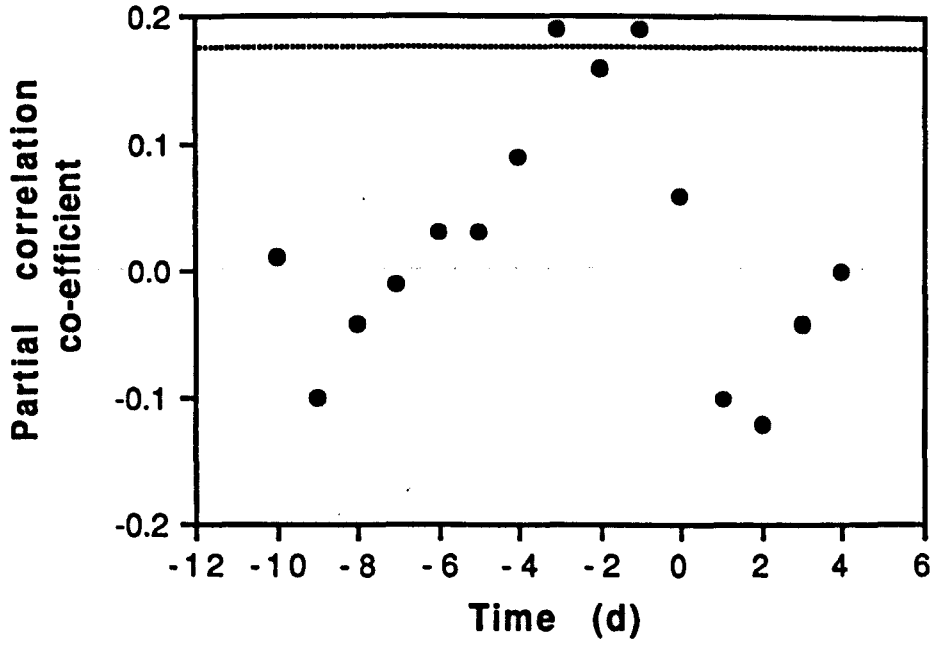


Fig. 4.4 Partial correlation co-efficient (controlling for date) between mean temperature and clutch size on each day during the egg formation period. The first egg was laid on day 0. The dotted line represents the level at which the partial correlations became significant ($p<0.05$).

correlation co-efficient (controlling for laying date) between clutch size and mean temperature on that day (Fig. 4.5). This demonstrated that the importance of environmental factors on a particular day was proportional to the energy content of egg formed on that day. The number of correlations between environmental factors and egg size increased as the energy requirement for egg formation increased. Environmental factors were most important in the determination of mean first clutch egg size during the period when more than 50% of the maximum energy requirement was required for egg formation. This period overlapped substantially with the period of albumen deposition, and temperature during this period was only slightly less highly correlated with mean egg size. Temperature might affect mean egg size by influencing albumen formation only or by limiting energy in excess of maintenance available for reproduction. Overall, 10.0% of the correlations and t-tests summarised in Table 4.9 and 4.10 produced significant results which were consistent with a constraint on egg production by environmental factors during the period of egg formation, double the number which would be expected by chance. Only 2.7% of correlations, almost half the number expected by chance, showed significant correlations of the opposite sign to that expected. This supported the hypothesis that egg production could be constrained by food intake during egg formation.

Much of the variation in size between clutches was attributable to the effect of the female (Table 4.4), so this might mask variation in egg size due to environmental factors. To reduce the effect of female on egg mass, the mean egg mass for each clutch was subtracted from mass of each egg to provide the "deviation in egg mass" from that expected for that female. Positive correlations were therefore expected between deviation in egg mass and suction trap catch or temperature on the day upon which albumen was formed, and negative correlations between deviation in egg mass and rainfall. There were some significant correlations between deviation in egg mass and environmental factors (Table 4.11), but as there were both positive and negative correlations between deviation in egg mass and temperature, suction trap catch and rainfall, this did not support the hypothesis that intra-clutch egg size variation was accounted for by environmental conditions on the day of albumen formation.

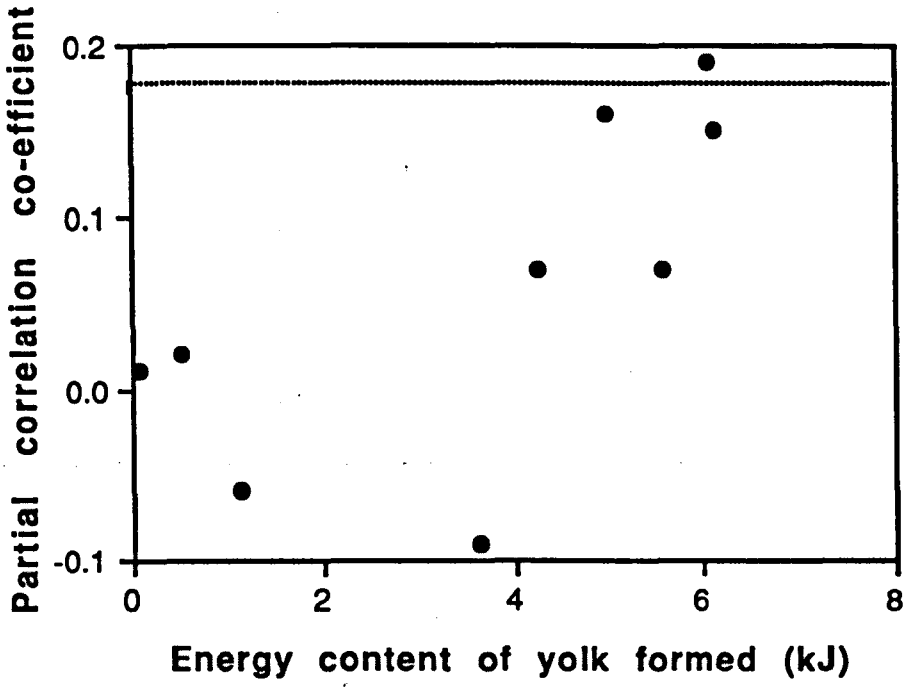


Fig. 4.5 Relationship between the partial correlation co-efficient (controlling for date) between mean temperature and clutch size on each day during the yolk formation period and the energy content of yolk formed each day (for a clutch of 5 eggs, Table 3.8). There was a significant correlation between the partial correlation co-efficient and the energy content of yolk formed ($r=0.66$, $p=0.05$). The dotted line represents the level at which the partial correlations became significant ($p<0.05$).

Table 4.11 Significant Pearson correlation co-effecients between "deviation in egg mass" of each egg of a swallow clutch (egg mass-mean egg mass for that clutch) and insect suction trap catch ($\log_e(V+1)$), maximum, minimum and mean temperature, and rainfall on the day upon which the albumen was formed. Variables named indicate a significant correlation between that variable and the deviation in egg mass. (Rain = rainfall, min. t. = minimum temperature, mean t. = mean temperature, food = suction trap catch.) Variables shown in brackets indicate significant correlations which were in the opposite direction to those expected (egg size positively correlated with food availability and temperature and negatively correlated with rainfall). 0 indicates no significant correlations. - indicates no data in this category.

Egg	First clutch	Second clutch
1	(Rain)	0
2	0	0
3	(Rain)	0
4	0	Min. t. Mean. t.
5	0	(Food)
6	0	-

4.3.2 Dipper

4.3.2.1 Use of airspace diameter to determine order of laying of a clutch

Airspace diameter increased each day before the start of incubation (Table 4.12) and could be used to determine the laying order of unincubated clutches. Airspace diameter was measured in 16 clutches before the start of incubation for which the laying sequence was known. In 10 of these airspace diameter would have predicted laying sequence correctly, and the last egg to be laid would always have been predicted correctly.

4.3.2.2 Use of airspace diameter to calculate hatching date

The diameter of the airspace of dipper eggs which had been incubated for a known number of days was regressed upon day of incubation (Fig. 4.6). This provided an equation from which to predict the number of days for which a clutch had been incubated. Hatching date could be predicted from airspace diameter for clutches which were found during incubation with an accuracy of ±2 d (95% CI from regression equation, Fig. 4.6). The mean daily rate of mass loss of dipper eggs during incubation was 0.054 g (sd=0.037, n=17) but, as explained in Section 4.3.1.2, airspace diameter, rather than mass loss, was preferred as a measure from which to calculate hatching date.

4.3.2.3 Egg size

Fresh (unincubated) dipper eggs were weighed in each of the years 1989-91. Most (78%) of these eggs were weighed in 1990, so data were pooled for the three years to calculate the constant in Equation 4.1. Further work would be required to determine whether the constant varied between years. The mean constant was 0.526 (sd=0.018, n=87).

Table 4.12 Airspace diameter of unincubated dipper eggs on each day after the eggs were laid.

Days after laying	Mean	sd	n
0	7.20	1.98	18
1	8.87	0.46	11
2	9.02	0.73	9
3	9.71	0.45	7
4	10.4	0	1

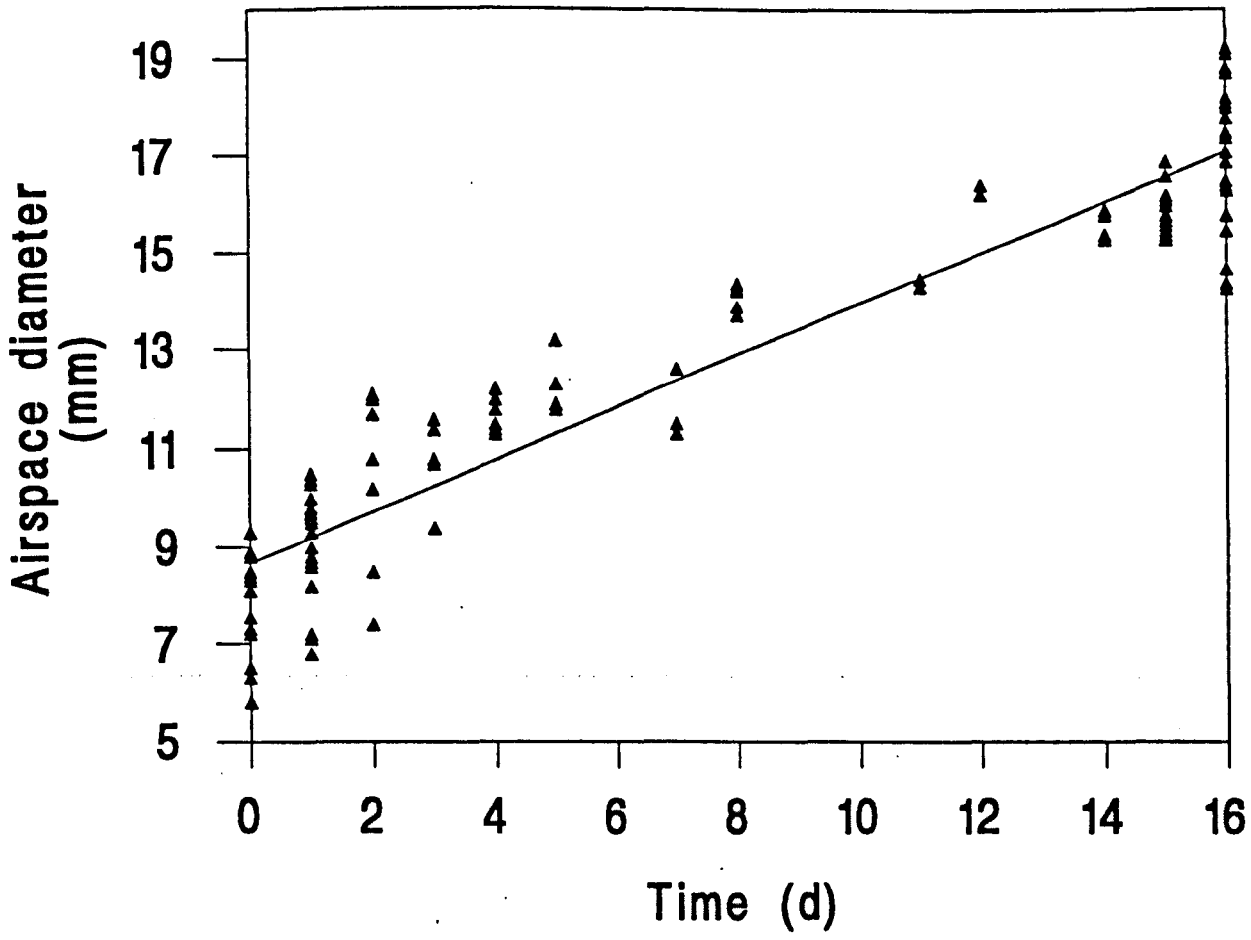


Fig. 4.6 Increase in airspace diameter at the blunt end of dipper eggs during incubation ($y=0.527x + 8.678$, $r^2=0.90$, $s_b=1.17$, $p=0.0001$).

4.3.2.4 Egg size and hatching success

There was no significant difference in egg size between eggs which hatched successfully and those which failed to hatch (t-test, $t=1.61$, $p=0.1$, n=238 eggs which hatched and 24 which were infertile or failed during embryonic development).

4.3.2.5 Factors with a potential effect on egg and clutch size and laying date

1. Year

There were no significant differences in mean egg or clutch size or laying date between the three years (t-tests, all $t<1.45$, $p>0.15$, n=83 clutches). Data collected in each the three years were therefore pooled in subsequent analyses. Mean egg mass (mean of clutch mean egg masses) was 4.87 g, sd=0.35, n=83 clutches.

2. Relationships between egg and clutch size and laying date

There was no correlation between mean egg size and clutch size (Pearson correlation, $r=0.01$, $p=0.4$, n=73), or between laying date and either egg or clutch size (both $r<\pm0.01$, $p>0.9$, n=73).

3. Laying order

There was no significant effect of laying order upon egg mass (analysis of variance, $p>0.05$).

4. Territory

Birds laid significantly later in upland than lowland territories (t-test, $t=5.4$, $p=0.001$, n=52 lowland and 28 upland territories). The mean laying date (first clutches) was 4th April for birds on lowland territories, and 15 d later for upland birds. Only birds on lowland territories which laid before 25th March had second clutches. There were no significant differences in mean egg size or clutch size between upland and lowland territories (both $t<1.00$, $p>0.3$).

5. Female

Fifteen female dippers bred in the study area in more than one year. Mean egg size in the second year was highly correlated with egg size in the first year (Fig. 4.7). There was no correlation of clutch size or laying date between years (Pearson correlations, both $r<0.4$, $p>0.2$, $n=13$).

Most of the variation in dipper egg size occurred between rather than within dipper clutches (Table 4.13). Mean RDEW was 0.09 g (sd=0.07, range=0.00 to 0.51 g, n=74 clutches). Repeatability of egg size of individual females was 0.68 (calculated after Lessells and Boag 1987, from Table 4.13).

6. Female age

There was a significant positive correlation between age and clutch size if birds for which age was not known exactly were placed in the youngest year group to which they could belong (Pearson correlation, $r=0.25$, $p=0.04$, $n=71$). If only birds which were of exactly known age were included in the analysis the correlation was not significant ($r=0.21$, $p=0.14$, $n=48$). There were no significant correlations between age and mean egg size or laying date for either sample of dippers (all $r<\pm0.20$, $p>0.09$). A t-test between the clutch sizes of first year and older birds showed a slightly, but not significantly larger clutch size for older birds ($t=1.7$, $p>0.09$, mean=4.51, n=35 for first year and mean=4.75, n=36 for older birds).

It was concluded that dipper clutch size increased with age, but that differences in clutch size between age categories were small.

7. Female body size

There were no significant relationships between mass or measures of female dipper body size (wing, keel, head+bill and tarsus lengths or bill depth, PC1, PC2 or PC3), and mean egg or clutch size (Pearson correlations, all $r<\pm0.24$, $p>0.15$, $n=38$).

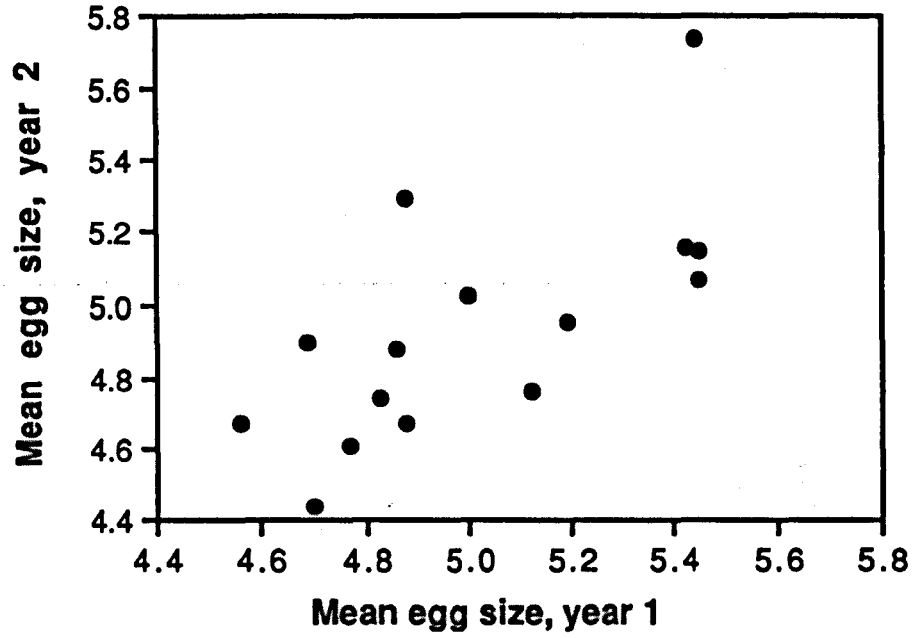


Fig. 4.7 Mean egg size of female dippers which laid in the study area in consecutive years (Pearson correlation=0.75, p=0.001, n=15).

Table 4.13 Analysis of variance of dipper egg mass within and between individual females.

Source	D.F.	Sum of squares	Mean squares	F ratio	F prob.
Between clutches	53	39.44	0.74	13.55	0.0001
Within clutches	271	14.88	0.05		
Total	324	54.32			

4.3.3 Relationship between swallow egg size and hatchling size

There was no significant difference in hatchling mass between swallows from 9 eggs which hatched in their nests and 58 hatched in the incubator (t-test, t=0.19, p=0.8). There were no significant relationships between hatchling mass and hatching date, length of time since the incubator was last checked or duration of incubation (Pearson correlations, all $r<\pm 0.18$, $p>0.4$). Artificial incubation of swallow eggs during the final few days of incubation was therefore thought not to affect hatchling mass, and the nest and incubator hatched chicks were combined in subsequent analyses.

There were significant relationships between hatchling mass and pipped egg mass (hatchling mass=0.80 × pipped egg mass + 0.15, $r^2=0.81$, p=0.0001, n=50), and between hatchling mass and fresh egg mass (Fig. 4.8). Heavier hatchlings were structurally larger (wing length) and had a greater supply of yolk (greater area of yolk on the stomach) (Figs. 4.9 and 4.10). In a regression analysis of factors which might affect hatchling mass, none of the factors listed in Section 4.2.6 was included at the 5% level of significance after pipped egg mass, or alternatively fresh egg mass, was included as an independent variable.

4.3.4 Significance of laying anomalies in swallows

No instances of interrupted laying were recorded for dippers, presumably because their food supply was sufficiently stable and predictable that interruptions were not necessary. In the event of a large increase in water level during egg-laying, food would be less accessible and dippers might experience laying interruptions, however there were no floods during dipper laying periods in the present study.

Swallows often experienced large variations in food supply between and within days. These events were not predictable, although they were less frequent in the middle of the breeding season (Appendix 2).

Thirteen cases of laying anomalies were recorded during this study: 7 of 49

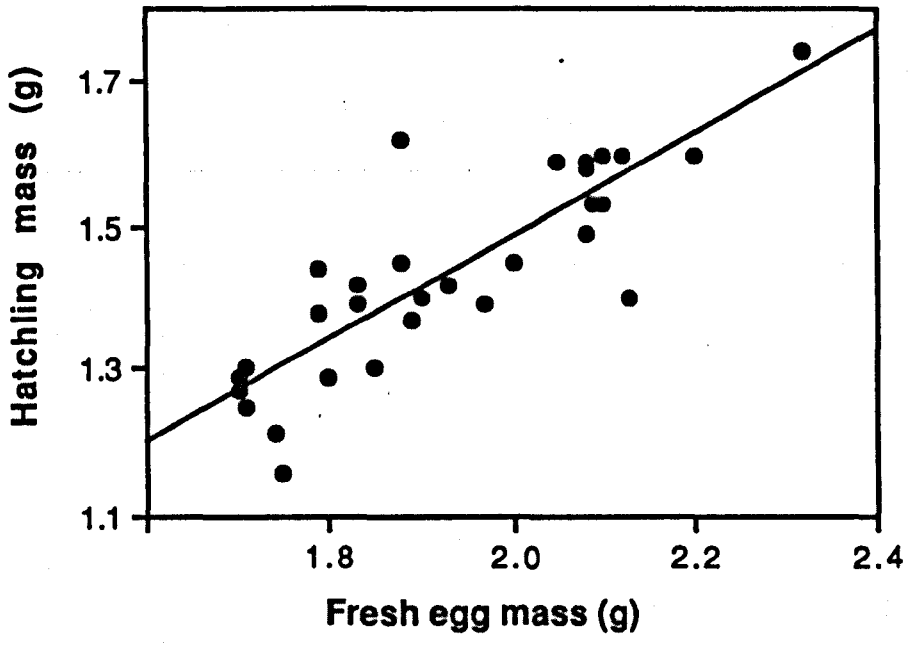


Fig. 4.8 Relationship between swallow fresh egg mass and hatchling mass ($y=0.76x - 0.03$, $r^2=0.78$, $p=0.0001$, $n=52$).

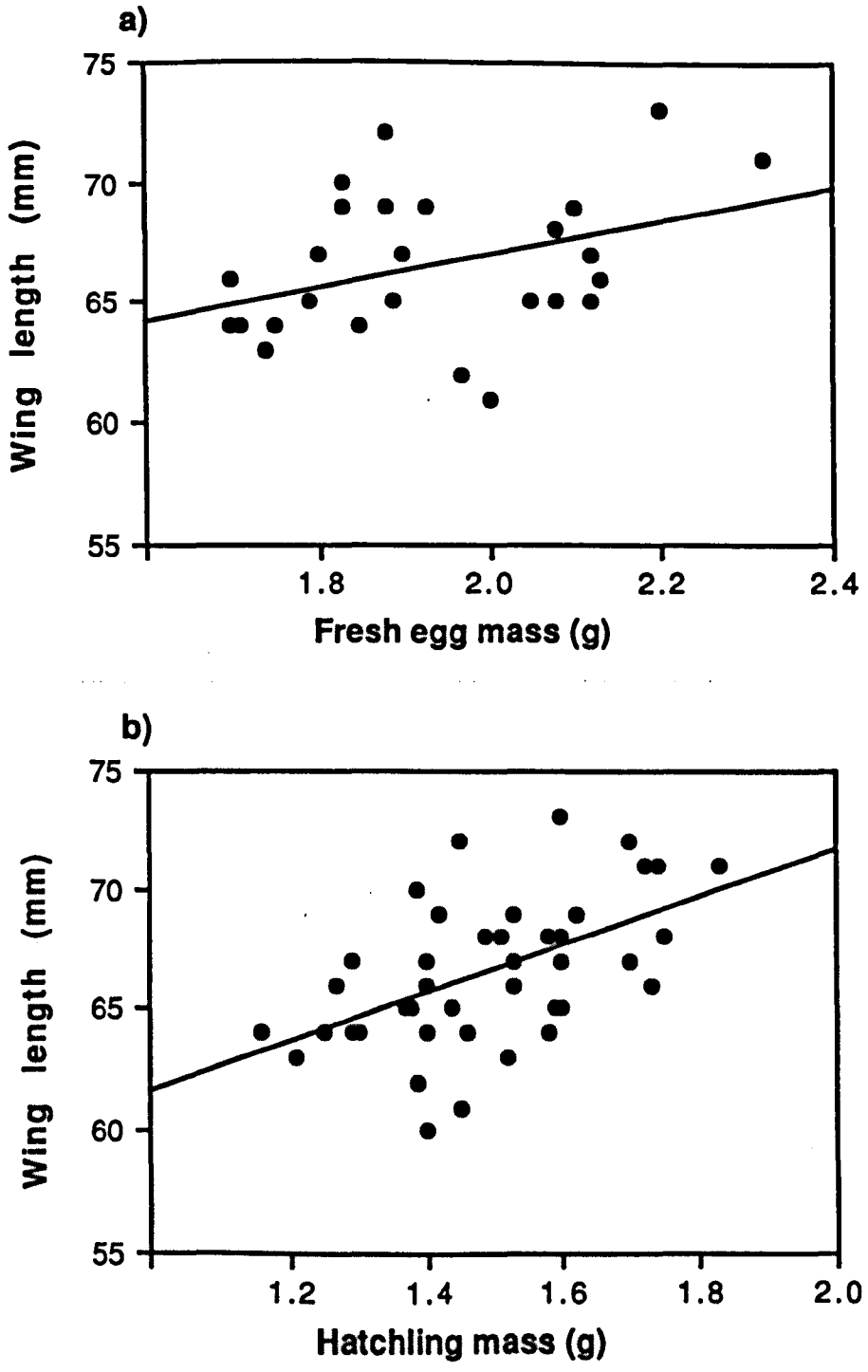


Fig. 4.9 Relationships between a) swallow fresh egg mass and hatchling wing length ($y=6.89x + 52.68$, $r^2=0.14$, $p=0.008$, $n=49$, b) swallow hatchling mass and wing length ($y=9.87x + 51.67$, $r^2=0.25$, $p=0.0001$, $n=52$).

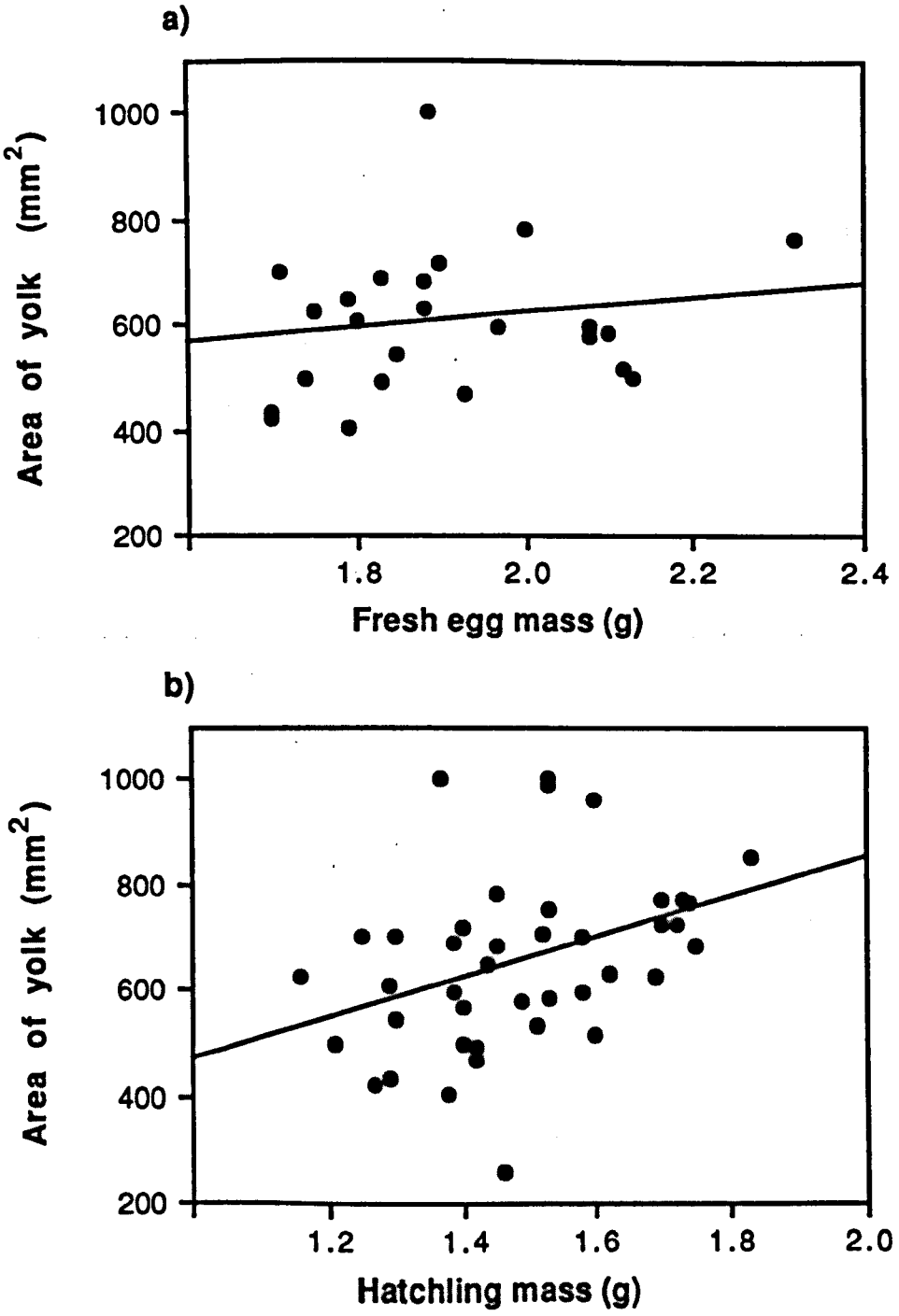


Fig. 4.10 Relationships between a) swallow fresh egg mass and the area of yolk visible on the stomach of the hatchling ($y=354.2x - 43.1$, $r^2=0.16$, $p=0.008$, $n=45$), b) swallow hatchling mass and the area of yolk visible on the stomach of the hatchling ($y=281.1x + 245.7$, $r^2=0.07$, $p=0.06$, $n=47$).

first clutches which were visited daily during laying (14.2%), and 6 of 33 second clutches (18.2%) (Table 4.14). These figures could be overestimates for two reasons. Cases where no egg was laid in the nest for only one day when this was not preceded by poor feeding conditions could be caused by disturbance from farm activities (which I did not know about) which prevented the bird from laying. This was known to occur once in 1990, when a female was unable to reach her nest because a tool shed door had been closed overnight. The door was reopened the next day, but not until mid-morning by which time the swallow had laid her egg on the floor below a perch in an adjacent building. Swallows were normally tolerant of farm activity so this was an unusual event.

Interruptions in laying might also have been due to my activities. Five of the laying interruptions were of birds which had their nests placed on balances (Chapter 8), however two of these involved the Blair Mains female (Table 4.14) which showed atypical behaviour in other respects, abandoning several partly incubated clutches and one partly grown brood. She only reared one brood in two years, despite starting five clutches. If the Blair Mains bird was excluded, the proportion of balance birds with laying interruptions (13.0%) was the same as in the rest of the population (13.1%), so it seemed that use of nest balances did not alter the probability of an interruption in laying.

Three of the laying anomalies recorded were associated with a period of bad weather in 1990 (Tables 4.15 and 4.16). There was unfavourable weather on days 66, 67 and 68 (Table 4.16) including strong wind and thunder storms on day 68, the day that the Bankend bird laid her first egg and the Manor Steps bird her second (Table 14.6). The only three birds which were known to be laying at this time all failed to lay an egg on day 69 or day 70, although one of them (the Bankend bird) resumed laying on day 71. Presumably the weather improved sufficiently on day 70 for her to be able to resume egg production. The Bankend bird went on to lay eggs on days 71, 72 and 73 and incubated her clutch successfully. The Manor Steps and Blair Mains birds abandoned their breeding attempts. Two incubating females also deserted their clutches during this period.

Table 4.14 Laying anomalies recorded in swallow clutches 1989-91. Numbers in brackets refer to the duration of the laying interruption (in days). X indicates that the breeding attempt was abandoned at that point. The Blair Mains site was occupied by the same female in 1989 and 1990. The ML Skylight site was used by different females in 1989 and 1991.

Year	Clutch number	Site	Nest balance present	Laying dates (d after 1st April)						
1989	1	Menstrie	N	56	57	(1)	59	X		
	2	Kennels	N	97	(14)	112	113	114	115	116
	2	Logie Villa	N	98	99	100	(1)	102		
	2	Blair Mains	Y	101	102	(1)	104	105		
	2	ML Skylight	Y	108	109	110	111	(1)	113	
	2	Old Keir	N	109	110	111	(6)	118	119	120
1990	1	Powis Cottage	N	47	48	49	50	(1)	52	53
	1	ML Passage	N	61	62	63	64	(1)	66	
	1	Manor Steps	N	67	68	X				
	1	Bankend	Y	68	(2)	71	72	73		
	1	Blair Mains	Y	68	X					
	2	West Carse	N	96	97	98	99	(1)	101	
1991	1	ML Skylight	Y	62	63	(1)	65	66	67	

Table 4.15 Suction trap catch ($\log_e(V+1)$), temperature (°C) and rainfall (mm) during a spell of bad weather in 1990. The weather was considered favourable for swallows before and including day 65 and after and including day 71. X indicates the day upon which the birds in Table 4.16 stopped laying.

					X		
Day	65	66	67	68	69	70	71
Suction trap catch	3.0	2.2	0.9	2.6	1.9	2.4	2.4
Rainfall	1.4	22.9	2.8	4.7	0.0	0.0	0.0

Table 4.16 Clutch size, mean egg mass and RDEW of the five swallows in the study area laying during a period of bad weather in 1990. The Manor Steps and Blair Mains birds abandoned the breeding attempt and the Bankend bird interrupted laying for two days. The Inverardoch A and Inverardoch Tool Shed clutches were found freshly laid on day 72 and must have been laid during the period of bad weather. It was not known whether these birds interrupted laying. - indicates that no egg was laid on this day, ? that laying dates were not known.

Bird	**Clutch size**	**Mean egg mass**	**RDEW**	**Laying dates**					
Manor Steps	2	2.26	0.12	67	68				
Blair Mains	1	1.89	0.00	68					
Bankend	4	2.21	0.09	68	-	-	71	72	73
Inverardoch A	5	1.83	0.10			?			
Inverardoch Tool Shed	5	2.04	0.21			?			

The Bankend female had either larger eggs or lower RDEW than 2 other swallows (Inverardoch A and Inverardoch tool shed) which must have been laying during the period of bad weather but for which it was not known whether laying interruptions occurred (Table 4.16). This suggested that the Inverardoch tool shed bird might have continued to lay during the period of bad weather, and suffered a high RDEW as a result. The disadvantages of a high RDEW are discussed in Section 4.4.4. The Inverardoch A bird laid small eggs, but did not have a high RDEW, so she probably did interrupt laying, so that egg size was conserved at the (slight) expense of starting incubation later in the season.

This demonstrated that prolonged periods of poor feeding conditions can prevent that continuation of egg formation and incubation by female swallows. Swallows were also able to interrupt laying, which could be resumed after a few days if conditions improved. This meant that they did not suffer reduced reproductive success as a result of brief spells of poor weather. It also confirmed the view that swallows formed a substantial part of egg material from daily food intake, as they were unable to continue daily egg production when intake fell.

Eggs from clutches with laying interruptions did not have lower hatching success than other clutches (Chi-squared test, χ^2=0.08, p=0.7, 2 df). One egg hatched to produce a chick which fledged having survived a laying suspension of 14 d (Kennels nest, Table 4.14). Unincubated swallow eggs did not appear to show a decrease in viability with time, as had been found for American coot eggs (Arnold *et al* 1987). Females which interrupted laying therefore incurred no great cost, and gained some important benefits.

Swallows sometimes interrupted laying without an obvious reduction in food supply, particularly before the last egg of a clutch was laid (Table 4.14). This could be due to a long laying interval so that the final egg missed the "open period" for laying on the day after the penultimate egg was laid (Meijer 1992) rather than energetic stress.

4.4 Discussion

4.4.1 Usefulness of airspace diameter to determine laying order or day of incubation of eggs

Airspace diameter could be used to determine the laying order in clutches of un-incubated dipper and swallow eggs, as airspace diameter increased each day after an egg was laid. This characteristic of eggs was used to save time during this study, as nests could be visited on alternate days, rather than every day, without losing information on the order in which the eggs were laid. Knowledge of laying order was important if patterns of intra-clutch egg size variation were to be related to environmental factors during egg formation, and to determine whether egg size varied systematically with laying sequence.

Another technique used to determine the order of laying of clutches, was the number of nest parasite droppings on the shells of house martin eggs (D. M. Bryant, pers. comm.). This technique would only be useful with white eggs. There was no noticeable increase in marking of the shell of the brown-spotted swallow eggs during incubation, although dipper eggs did become marked by the vegetation used to line the nest.

Airspace diameter could also be used as a measure of the time for which eggs had been incubated. In this case it was necessary to candle eggs to see the airspace through the opaque shell of incubated eggs. This allowed prediction of hatching date to within ±2 d, so that the nest could be visited at the right time to observe the exact hatching date. This was an alternative to methods which predicted hatching date from the reduction in egg density during incubation. The airspace diameter method was simpler than submersion of eggs in water to see how they floated (Lundberg and Väisänen 1979), and would not involve any risk of harming the embryo (by cooling from the water), and would be more suitable for small eggs than an estimation of laying date from a reduction in density of eggs weighed in air (Furness and Furness 1981).

Techniques from which to predict hatching date would be useful in studies

where the objective was to return to ring chicks shortly after hatching, or commence a study during the nestling period, without the need to make repeated visits to the nest during laying and incubation. The advantages of the airspace technique were that it would not harm the embryo and it allowed calculation of hatching date independently of any error in weighing eggs in windy environments or for small eggs when the daily mass loss during incubation might be close to the limit of measurement precision. Prediction of hatching date from one nest visit during incubation would be most valuable for species where the nest was either inaccessible, or the parents were particularly prone to desertion if frequent visits were made during the laying or incubation periods.

4.4.2 Comparison of egg size variation and frequency of laying anomalies between species with constant and variable food supplies

If food supply during the egg formation period was an important constraint upon egg production, species with a constant food supply might show less variation in egg and clutch size than those with a food supply which varied unpredictably. In this study, dippers were used as an example of a species which was assumed to have an abundant, predictable food supply. Swallows, however, fed on aerial insects which varied in abundance unpredictably according to weather conditions.

Food supply was more variable, and temperature lower during the period when swallows laid first than second clutches (Section 4.3.1.5). Swallows which laid early in the season therefore had a greater probability of encountering unfavourable weather conditions which would influence egg or clutch size. The difference in the stability of food supply between swallows and dippers, and between the laying periods of first and second swallow clutches, should be reflected in different patterns of egg and clutch size variation, if the level of daily food intake is important for egg formation. Alternatively, if eggs were mainly formed from reserves accumulated by pre-laying females, variability in food supply during laying should have little relationship to patterns of egg and clutch size variation.

Egg size was highly repeatable for individual swallows and dippers but there was a much closer match between the size of second clutch than of first clutch swallow eggs laid in consecutive years (Fig. 4.3). High levels of repeatability and heritability of egg size between individuals and generations have also been found in great tits (Ojanen *et al* 1979, Perrins and Jones 1974, Van Noordwijk *et al* 1981), waders (Nol *et al* 1984, Grant 1991), red grouse (Moss and Watson 1982) and Darwin's finches (Grant 1982). Egg size would therefore appear to have a strong genetically determined component in birds in general. The reduced correlation between swallow first clutch egg size between years suggested that egg size was also influenced by environmental factors during the egg formation period, as eggs of a more variable size were laid during a period of more variable food supply (Section 4.3.1.5).

Swallow clutches showed greater within clutch variability in egg size than dipper clutches (RDEW=0.097, sd=0.056, n=247 for swallows, RDEW=0.083, sd=0.044 n=82 for dipper clutches). The greater RDEW of swallows when compared with dippers, and of first than second swallow clutches were also compatible with the hypothesis that food supply had a proximate influence on egg size.

No laying anomalies and no clutch desertions (other than due to disturbance or predation) were recorded for dippers, whilst swallows had interruptions or suspensions in laying, and would desert clutches during laying or incubation when feeding conditions were poor. Dipper egg and clutch size variation appeared unlikely to be due to variation in food supply, at least during this study, as there were no floods during the laying period. Swallows also often had an abundant supply of food, but poor weather could reduce this unpredictably. When this happened, possible options were for egg production to be suspended until conditions improved, for an unusually small clutch to be laid or for follicles within the ovary to be resorbed. Investigation of which option was used under different circumstances would be an area for future study.

Suspensions and interruptions in laying, associated with poor weather or food supply, have been reported widely for other birds. Amongst aerial foragers, house

martins (Bryant 1975a), sand martins (M.A.S. Alves, pers. comm.) and swifts (Lack 1956, O'Connor 1979) had laying interruptions following poor weather. Unseasonal snowfall was followed by laying interruptions for pied flycatchers and magpies (von Haartman 1990, Birkhead 1991), laying anomalies in American coot were reduced by supplementary feeding (Arnold 1991), and cold weather delayed the initiation of laying in the great tit (Perrins and McCleery 1989). Detection of laying interruptions requires daily visits to large numbers of nests during the laying period, something which was difficult or impractical in many studies, so occurrence of laying anomalies is probably under recorded. This was taken into account by (Dhondt *et al* 1983) in their study of the frequency of occurrence of laying anomalies in blue and great tits. In these species, the frequency of laying interruptions was not correlated with clutch size, laying date or breeding density, but the blue tit had more anomalies after a cold winter, and the great tit more when the rate of the seasonal decline in clutch size was greater. Both these variables were indirect measures of food availability to the laying female. Laying anomalies were less frequent in the swallow (14.0%) than in the house martin (35%, Bryant 1979), but more frequent than in the tits studied by Dhondt *et al* (1983) (6.7%). This was consistent with the prediction that aerial foraging birds would have a less predictable food supply than other passerines, which would make them more prone to laying interruptions. The probability that there would be an inadequate food supply for a swallow during laying of first and second clutches will be discussed in Chapter 10.

Laying anomalies would be disadvantageous as they prolonged the period required for breeding, reduced the probability that the pair would lay a second clutch and made the fledging date of the chicks later in the season. Yet, otherwise birds which interrupted laying would incur little loss of reproductive output (Section 4.3.4). The alternatives would be for swallows to stop laying when food supply dropped and suffer a reduction in reproductive output due to reduced clutch size, or to continue laying and either produce an egg which was small in comparison with the rest of the clutch which might have a low chance of survival, produce an inadequately provisioned egg which would not be viable, or reduce the energy

reserve of the female so much that her survival was endangered. This last possibility would be seem to be very unlikely, but one female house martin which laid a normal clutch during poor weather when no other birds were laying was found dead in her nest shortly after the clutch was completed (Bryant 1979).

Interruptions or suspensions in laying presumably occurred instead of laying eggs which were either too small or too poorly provisioned to develop successfully. There was no significant relationship between egg size and hatchability for swallows or dippers, which suggested that birds did indeed avoid laying eggs which were too small to be viable. A relationship has been found between egg size and hatchability for a few species (Murton *et al* 1974), but not for a larger number of others (Parsons 1970, De Steven 1978, O'Connor 1979, Moss *et al* 1981, Bancroft 1984).

4.4.3 Causes of variation in laying date, egg and clutch size

4.4.3.1 Interaction between egg and clutch size and laying date

A negative correlation between egg and clutch size would be predicted if birds have a limit to the resources which they can devote to reproduction and so must produce either many small or a few large eggs, if other factors such as individual quality and food supply were equal (Lack 1967). A negative correlation between egg and clutch size has been found in comparisons between species (for waterfowl, Blackburn 1991 and for tits Ekman and Johansson-Allende 1990), but there is little evidence that this trade-off occurs within species (Sections 4.3.1.5 and 4.3.2.5, Rohwer 1988, Moss *et al* 1981, Greig-Smith *et al* 1988, Ekman and Johansson-Allende 1990, Blackburn 1991).

One explanation for the lack of the correlation between egg and clutch size predicted by life history theory would be that stored reserves were of little importance in egg formation. If eggs were formed directly from daily food intake and food intake was sufficient to provide for the day of maximum energy and material requirement for egg production (the day before the first egg was laid) then laying could be indeterminate as an increase in clutch size would not increase the

maximum daily energy requirement for egg production. Other factors, such as a limit on the number of young which could be fed, would prevent indeterminate laying. Indeterminate laying has been found in some birds species and was more common in precocial than altricial species (Kennedy 1991), despite the assumed higher cost of egg production by precocial birds (King 1973, Ricklefs 1974).

Female swallows could have increased their clutch size by decreasing egg size without increasing total clutch mass. The lightest viable swallow egg laid during this study weighed 1.50 g, only 65% of the mass of the heaviest egg. If female swallows had laid 1.50 g eggs, rather than those of mean mass (1.97 g), clutches of 4, 5 or 6 eggs could have been increased by one egg whilst actually decreasing total clutch mass. The difference in mass between the smallest and mean egg dipper eggs was not sufficiently great to have allowed this species to lay an extra egg without an increase in clutch mass. Why did swallows lay eggs which were so large, when compared with the smallest viable egg, when they could have increased their clutch size by decreasing the size of each egg? Possibly egg size was genetically determined, so that swallows could not "decide" to lay more smaller eggs, or there may be disadvantages to the production of small hatchlings (Section 4.4.4). It was most likely that resources for egg formation in a bird such as the swallow did not have a finite limit, as eggs were formed from daily food intake rather than reserves. This would mean that there was no advantage to a reduction in egg size, as this would not have provided resources for the production of additional eggs.

Swallow clutch size was influenced strongly by laying date: the later clutches were initiated, the smaller they were (Fig. 4.2).

4.4.3.2 Age and breeding experience

Birds breeding for the first time frequently show a lower reproductive output than older birds. The lower reproductive output of first year birds could be due to a number of factors, including later arrival of migrants at the breeding grounds, inexperience, and competition with older birds for territories, food or nest sites.

An increase in reproductive performance with age is typical in long lived species such as seabirds which do not start to breed until they are several years old (Wooller *et al* 1990). The reproductive output of passerine birds also increased with age. First year blue and great tits laid later and had smaller clutches than older birds, but females became "old" at 4 and 5 years respectively, when they laid later, had smaller broods and their chicks had lower post-fledging survival (Dhondt 1989). Female age was related to egg size in magpies, younger females laid later in the season and produced smaller eggs (Birkhead 1991). Older song sparrows raised more young to independence than yearlings, and in this species it was demonstrated that the increase in reproductive output of older birds was due to the differential survival of more successful breeders (Nol and Smith 1987).

Relationships between age, laying date, egg and clutch size have been found for other aerial foragers. Both clutch and egg size were smaller for yearling female tree swallows than for older birds, and yearlings laid later in the season (De Steven 1978). Egg mass and clutch size both increased between the first and second year of breeding (Wiggins 1990). Older house martins laid more eggs and reared more young, as they bred earlier in the season and this was associated with laying larger clutches and a greater probability of laying a second clutch (Bryant 1979). Swallow breeding performance was consistent with these trends. Clutch size of female swallows increased with age, but this was only because first year birds laid later in the season. Later laying of their first clutch also meant that a lower proportion of first year birds had a second clutch.

In summary, clutch size increased and birds laid earlier in the season as they became older or more experienced. Egg size, however, increased with age in only one study (Wiggins 1990). In most species, clutch size and laying date were again more flexible than egg size, which was apparently determined genetically.

4.4.3.3 Structural size

As older females laid earlier and swallow wing and tail length increased with

age, it might have been expected that clutch size or laying date would be positively correlated with wing or tail length as a consequence of the relationships with age. This was not the case. There were no correlations between the structural size of swallows or dippers and their laying date egg or clutch size. There was a weak positive correlation between swallow first clutch mass and keel length (Table 4.7). This disappeared in a partial correlation controlling for laying date (Table 4.8), although a weak positive correlation was then found between tarsus length and first clutch size. Inner tail length was, however, negatively correlated with first clutch mass and size (Table 4.8).

Most studies of other species confirmed the lack of a relationship between structural size and egg or clutch size (De Steven 1980, Murphy 1986, Leblanc 1989, Wiggins 1990, Grant 1991). A few studies have shown a positive correlation between structural size of the female and egg size: great tit (*), American oystercatchers (Nol *et al* 1984), spruce grouse (Naylor and Bendell 1989) and a suggestion that this might be the case for Darwin's finches (Grant 1982).

Smaller females, which need an absolutely smaller food intake, might be able to obtain a sufficient surplus of food to allow egg formation earlier in the year than larger individuals (Dunn, in Perrins 1970). Smaller eastern kingbirds did indeed lay earlier in the season than larger females (Murphy 1986), but larger redshank laid earlier than small ones (Summers and Underhill 1991). It was concluded that there was no strong evidence for any relationship between structural size of female birds and their egg size, clutch size of laying date.

4.4.3.4 Intra-clutch egg size variation

The final egg in the clutch of a number of species of birds was consistently larger or smaller than the mean for the clutch (Slagsvold *et al* 1984). A larger than average final egg was seen as a means to enhance the survival of a chick which must compete with siblings which hatched earlier. Alternatively, a small final egg would increase the hierarchy caused by hatching asynchrony, and facilitate brood

reduction. Neither swallow nor dipper clutches showed a consistent pattern of egg size variation. A similar lack of relationship between egg size and order of laying was found for the house martin (Bryant 1975a), although egg size did increase slightly toward the end of the clutch in the tree swallow (Wiggins 1990).

4.4.3.5 Food supply during egg formation

Food supply and temperature have been shown to have an important influence upon laying date, egg and clutch size, in swallows (Section 4.3.1.5) and other aerial foragers. The clutch size of tree swallows differed between sites and years of high and low insect abundance (Hussell and Quinney 1987). Swifts which laid during favourable weather had larger clutches (O'Connor 1979), and house martin clutch size was positively correlated with the abundance of aphids in May (Bryant 1975a). Examples of relationships between food supply and reproductive output from other foraging guilds include tree sparrows which increased their clutch size in a year during which food was superabundant due to an emergence of cicadas (Anderson 1977), Brewer's sparrows which had larger clutches during wetter, more biologically productive years (Rotenberry and Wiens 1991) and great tits which laid earlier at warmer sites (Nager 1990, O'Connor 1978b). Darwin's finches prolonged their breeding season and produced more than twice the drought-level number of clutches in response to an increased food supply during El Nino (Gibbs and Grant 1987). Domestic fowl responded to variation in food supply, by varying the number rather than the size of the eggs laid (Romanoff and Romanoff 1949).

Variation in food abundance due to natural fluctuation in small mammal populations, or to experimental manipulation of food supply, demonstrated that the clutch size and laying date of birds of prey are dependent upon food supply (Dijkstra *et al* 1982, Korpimaki and Hakkarainen 1991). Earlier laying, an increased clutch size and a shortened interbrood interval have been observed as a result of supplementary feeding (reviewed by Martin 1987). Effects upon egg or clutch size or laying date were not demonstrated in all studies involving supplementary feeding, or

in all years, possibly because natural food was so abundant that additional food could not be utilised in some circumstances (Högstedt 1980, Djikstra *et al* 1982).

These results suggested that there was an effect of food supply upon clutch size, and the timing and frequency of breeding attempts of birds, but could not specify the particular period during which food supply was important. This study is the first to look in detail at the importance of food supply in relation to demands at specific times in the laying cycle, rather than at the level of a general relationship between food supply and reproductive output. This was important in order to distinguish between the hypotheses that birds built up reserves from which they subsequently formed eggs (in which case conditions prior to laying would be important), and that eggs were formed directly from daily food intake (in which case environmental factors during egg formation would be related to reproductive output). The analysis in Section 4.3.1.5 demonstrated that environmental factors during, and not before, egg formation, were important in the determination of egg size, and that the importance of environmental factors increased with the nutrient demand for egg formation.

This demonstrated the particular importance of daily food intake on certain days of the laying cycle for the first time. This confirmed the hypothesis that food intake was important for egg formation. The relationship between days when daily food intake was most important and the nutrient demands of egg formation, will be discussed further in Chapter 10.

4.4.4 Consequences of variation in egg and clutch size and laying date

Larger swallow eggs hatched into chicks which were heavier, structurally larger and probably had a greater reserve of yolk (Section 4.3.3). Larger eggs have been shown to hatch into larger, heavier chicks which have better growth and survival than chicks from small eggs in a sufficiently large number of species, that this appears to be a general relationship for birds (Table 4.17). These relationships were apparent despite the lack of correlation between egg size and yolk mass found

Table 4.17 Summary of studies showing relationships between egg size and hatchling characteristics. ✓ indicates a significant correlation between egg mass or size and the hatchling characteristic. × indicates no significant relationship. - indicates that the relationship between egg size and this hatchling characteristic was not examined in that study. Order of list according to Sibley and Monroe (1990).

		Hatchling				
Species	**Mass**	**Structural size**	**Energy reserve**	**Growth**	**Survival**	**Reference**
Red grouse *Lagopus lagopus scoticus*	✓	-	-	-	✓	Moss *et al* (1981)
Snow goose *Chen caerulescens*	✓	-	✓	-	✓	Ankney (1980)
Wood duck *Aix sponsa*	✓	-	×	-	-	Hepp *et al* (1987)
Mallard *Anas platyrhynchos*	✓	-	-	-	-	Eldridge & Krapu (1988)
Swift *Apus apus*	✓	✓	×	-	✓	O'Connor (1979)
Coot *Fulica atra*	✓	×	-	-	-	Horsfall (1984)
American coot *Fulica americana*	✓	✓	×	-	-	Hill (1988)
Whimbrel *Numenius phaeopus*	✓	✓	-	-	✓	Grant (1991)
Lapwing *Vanellus vanellus*	✓	✓	✓	-	✓	Galbraith 1988
Great skua *Catharacta skua*	✓	✓	-	×	✓	Furness (1983)

Table 4.17 continued

Species	Hatchling Mass	Structural size	Energy reserve	Growth	Survival	Reference
Herring gull *Larus argentatus*	✓	×	✓	-	✓	Parsons (1970)
Lesser black-backed gull *Larus fuscus*	✓	✓	×	-	✓	Bolton (1991)
Black-headed gull *Larus ridibundus*	✓	-	-	-	✓	Lundberg & Väisänen (1979)
Roseate tern *Sterna dougallii*	-	-	-	✓	✓	Nisbet (1978)
Common tern *Sterna hirundo*	-	-	-	✓	✓	Nisbet (1978)
Common guillemot *Uria aalge*	✓	×	✓	-	-	Birkhead & Nettleship (1984)
Thick-billed murre *Uria lomvia*	✓	×	✓	✓	-	Birkhead & Nettleship (1982)
Brünnich's guillemot *Uria lomvia*	✓	×	✓	-	-	Birkhead & Nettleship (1984)
Razorbill *Alca torda*	✓	×	✓	-	-	Birkhead & Nettleship (1984)
Puffin *Fratercula arctica*	✓	×	✓	-	-	Birkhead & Nettleship (1984)
Shag *Phalacrocorax aristotelis*	✓	✓	-	-	✓	Amundsen & Stokland (1990)

Table 4.17 continued

Species	Hatchling Mass	Structural size	Energy reserve	Growth	Survival	Reference
Fork-tailed storm-petrel *Oceanodroma furcata*	-	-	-	-	✓	Boersma *et al* 1980
Magpie *Pica pica*	✓	✓	×	-	-	Birkhead (1991)
Hooded crow *Corvus corvus cornix*	✓	✓	✓	-	-	Rofstad & Sandvik (1987)
Great tit *Parus major*	✓	-	-	✓	×	Schifferli (1973)
Tree swallow *Tachycineta bicolor*	✓	-	-	-	×	De Steven (1978)
Swallow *Hirundo rustica*	✓	✓	✓	-	-	This study

in some species, which led to suggestions that egg size might not be an indicator of egg quality (Ricklefs 1977, 1984, Hochachka 1986). Correlations between egg size and chick growth or survival could be spurious, due an effect of parental or territory quality which might effect both egg size and chick growth or survival. This point was investigated by Bolton (1991), in a chick transfer experiment with lesser black-backed gulls. Large eggs produced larger chicks with a higher rate of survival even if reared by parents which had laid small eggs, although the survival of hatchlings was greatest when chicks from large eggs were reared by parents which had laid large eggs. Egg size and parental quality therefore had independent effects upon the survival of hatchlings.

Egg size was, therefore, a measure of the reproductive value of the chick, as structural size, level of reserves and growth rate were all expected to be positively correlated with survival. In most of the studies summarised in Table 4.17, chicks from large eggs had better survival than those from smaller eggs, although egg size was often important for survival only during the first part of the nestling period. Mass at fledging was positively correlated with survival (Perrins 1965, 1970, von Haartman 1971, Loman 1977, Murphy 1978, Garnett 1981), although initial differences in size were only one factor which might lead to differences in fledgling mass (Johnston 1990).

The consequence of within clutch egg size variation would be to produce hatchlings of different sizes. This would interact with degree of hatching asynchrony to either increase or minimise the extent of the brood hierarchy. If the growth of hatchlings was followed through the nestling period the influence of hatchling size upon growth, survival and position in the brood hierarchy was important mainly during the first few days after hatching (Parsons 1970, Schifferli 1973, Nisbet 1978). Growth rates of nestlings were so rapid that the small initial differences in size were of relatively little importance. In species were brood reduction occurs regularly, initial differences in hatchling size would be larger and of greater importance, but in these species the effects of hatching asynchrony would be greater than those of egg size. Laying small eggs, especially if accompanied by a large range in intra-clutch

egg size, was therefore seen as disadvantageous, and likely to result from food shortage during the egg formation period. A similar result was found by Bryant (1978a).

Swallows could have increased their clutch size by one egg without increasing the total mass of egg formed if they had decreased egg size to that of the smallest viable egg found in this study. As mean swallow egg size was 31% greater than the smallest viable egg there must be either no advantage or some disadvantage of a reduction in egg size. Aerial feeding birds in particular might benefit from production of heavier hatchlings because variability in the food supply meant that chicks needed an energy reserve in order to be able to survive periods when little food is brought into the nest (resource storage strategy of O'Connor 1978a, Bryant and Gardiner 1979). Birds would also benefit from a reduction in the period for which the chicks required brooding or a reduction in the length of the nestling period when the chicks are liable to predation. Although these factors might not confer a great advantage to an egg size larger than the minimum required, there was probably no advantage to a reduction in egg size if the majority of energy and nutrients for egg production came from daily food intake rather than from body reserves. In this case the size of each egg would have little influence on the resources available for the rest of the clutch.

The consequences of laying date for seasonal reproductive output were clear: swallows which laid later in the season had smaller clutches, and those which laid their first clutches latest did not have time to lay a second clutch. Laying suspensions would be disadvantageous, as this would reduce the probability of laying a second brood. Interruption in laying for 1-2 d led to an unimportant delay, and would be more advantageous than an attempt to continue laying when conditions were poor, which might result in laying small eggs which had a lower reproductive value, or even lead to the death of the female (Bryant 1979).

The influence of clutch size and timing of laying upon the probability of recruitment of the chicks into the breeding population and the consequences for the lifetime reproductive output of the parents and offspring, were beyond the scope of

this study. The advantages of large rather than small clutches, early rather than late laying, and laying two rather than one clutch, must therefore be inferred from other studies.

Studies of two other aerial foragers, house martins and tree swallows, provide information on some of the consequences of variation in clutch size and laying date. Both these species exhibited a seasonal decline in clutch size which was not associated with a decline in food supply. Seasonal decline in clutch size has also been found in many other passerines (reviewed by Daan *et al* 1989). The supply of food for aerial foragers did become less predictable toward the end of the summer, but saving time by laying smaller clutches was not likely to be an important factor in the determination of swallow clutch size. For birds such as tits, survival of chicks declined for those fledged later in the season and the caterpillar food supply displayed a predictable seasonal drop (Perrins 1965, Kluyver 1971). Yet by delaying the start of incubation by a day to allow formation of an extra egg great tits would almost always have raised more young (Perrins and McCleery 1989).

Laying the first brood early in the season allowed a female swallow time to rear a second clutch, but exposed her to greater probability of poor weather conditions during laying and incubation of the first clutch and the nestling period of the second brood. Females which laid later in the season had only one brood, but were less likely to experience poor weather at any stage in their breeding cycle. Older birds were more likely to adopt the former strategy. Presumably their greater experience allowed them to breed under conditions which were less favourable than those required by yearlings. The two breeding strategies and their division between the age classes were also found for house martins (Bryant 1988b). Double-brooded females in this species suffered greater overwinter mortality than single brooded birds (Bryant 1979). The greater mortality of double-brooded females would be offset by the production of a larger number of young in the year in question. In swallows, double-brooded birds did not have a greater overwinter mortality than individuals which had a single clutch, but the offspring of single-brooded birds were more likely to be double-brooded than those of double-brooded parents (Thompson

1992). This result highlighted the importance of long term studies in the determination of reproductive success, for consideration only of the number of offspring would lead to the conclusion that double-brooded parents were more successful in passing on their genes. If the contribution to the second generation were also taken into account, the fitness of single and double-brooded parents would be more similar.

Studies of many altricial birds have shown that pairs can raise more offspring than their natural clutch size (Chapter 1). Reductions in swallow clutch size associated with lower temperature and poor feeding conditions at the time of laying was therefore considered to be disadvantageous, as the birds were apparently capable of laying, incubating and raising more young, but were constrained by lack of energy and material resources during egg production. At least part of the adjustment of clutch size was therefore a proximate response to food supply. The consequences of a reduction in clutch size under these circumstances were likely to be a reduction in lifetime reproductive output of the parents.

In summary, a reduction in clutch size due to poor weather during the egg formation period was seen as disadvantageous, as this would decrease annual reproductive output, probably without increasing future survival or fecundity of parents or offspring. Small egg size, especially if accompanied by a large intra-clutch egg size variation was a disadvantage, and likely to be caused by a limit to the energy or nutrient supply during egg formation. This analysis also isolated the time at which food intake for egg production was most critical. This was on the days when most of the egg was deposited, which supported the hypothesis that swallows formed eggs primarily from daily food intake, rather than from reserves. This point will be considered further in Chapter 10.

CHAPTER 5

COMPARISON OF THREE TECHNIQUES OF ENERGY EXPENDITURE MEASUREMENT FOR MALE JAPANESE QUAIL

5.1 Introduction

To establish the compatibility of different ways of measuring energy expenditure, it was measured simultaneously in captive Japanese quail by chamber calorimetry, the doubly labelled water (DLW) technique, and from food input/excreta output + comparative slaughter. Only the most recently developed of these methods, the DLW technique, can be used to measure the energy expenditure of free-living birds. The three methods of energy expenditure measurement were compared under laboratory conditions to determine whether the results were in agreement, and in particular, whether the DLW technique gave similar results to the two traditional methods.

The DLW technique has been validated for more than 30 species of bird, with a mean deviation of 7% when compared with chamber calorimetry (reviewed by Tatner and Bryant 1989, Speakman 1990). The present experimental programme aimed to compare the results of the DLW technique with the input-output technique and a different chamber calorimetry system for a new species, the Japanese quail. This was followed by validation of the DLW technique for quail during laying, a stage in the breeding cycle not previously validated, nor indeed investigated in detail using the DLW technique (Chapter 6).

5.2 Methods

The energy expenditure of 14 10-week-old male Japanese quail was measured simultaneously by open circuit chamber calorimetry (Section 5.2.1), the DLW technique (Section 5.2.2), and the food intake/excreta output + comparative slaughter technique (Section 5.2.3) at a range of temperatures between 14 and 36°C.

Quail were hatched at weekly intervals to provide a cohort of the same age

from the same group of parents at the start of each experiment. Birds were reared on a lighting regime of 23:1 light to dark. When the birds were 9 weeks old, ten males were taken at random from each cohort, and kept together for a week at 25°C in the experimental lighting regime of 14:10 light to dark. Lights came on at 0700 hours. When the birds were ten weeks old, 5 control birds were slaughtered and 5 experimental birds placed singly in the calorimeter chambers at 25°C with food and water provided *ad lib.* The experimental birds were allowed two days to become accustomed to the chambers. Temperatures of the chambers were then adjusted to the experimental temperatures (high, medium and low, within the given temperature range, arranged according to a latin square design, with an additional column in which the temperatures were at random). Daily energy expenditure was measured for the next 4 days by the three techniques. The birds were slaughtered at the end of this period. One of the 15 experimental birds died for unknown reasons. This bird was excluded from analyses. Only 5 birds could be accommodated singly in the calorimeter chambers at one time, so the birds used in this experiment were from 3 cohorts.

5.2.1 Indirect chamber calorimetry

5.2.1.1 Principle of measurement of energy expenditure by indirect calorimetry

Open-circuit indirect chamber calorimetry involves calculation of metabolic rate of the subject from the rate of oxygen consumption, and/or the rate of carbon dioxide production. This is done by comparison of the concentration of oxygen and carbon dioxide in air which has passed though a chamber containing the subject with ambient air.

The technique is based on the relationship between the amount of heat released and the volumes of oxygen and carbon dioxide consumed and produced during combustion of foodstuffs. The same volumes of gases are exchanged and amounts of energy released when the same substrate is oxidised by an animal. Measurement of the volumes of oxygen consumption and carbon dioxide production

therefore allow calculation of the amount of energy released by the respiring animal.

The formula given by Romijin and Lockhorst (1961), converted to SI units, was used to calculate metabolic rate from oxygen consumption and carbon dioxide production (Equation 5.1).

$$DEE = 16.18\ O_2 + 5.02\ CO_2 \qquad 5.1$$

where DEE=daily energy expenditure ($kJbird^{-1}d^{-1}$),
O_2 and CO_2 = the volumes of oxygen consumption and carbon dioxide production (ld^{-1}, at stp).

The shortened form of the equation was used, which does not take protein catabolism into account, as it has been shown that this simplification introduces negligible error (Romijin and Lockhorst 1961).

5.2.1.2 The Roslin multi-calorimeter

The open-circuit multi-calorimeter system at Roslin (AFRC Institute of Animal Physiology and Genetics Research, Roslin, Midlothian) has been described in detail by Lundy *et al* (1978) and MacLeod *et al* (1985). The computer software controlling the calorimeter has been updated more recently.

Ambient air and gases from up to ten chambers can be sampled in turn. Only the five smaller chambers (0.16 m^3) were used in work with Japanese quail. Each chamber was continuously ventilated by air at a rate of 1 $lmin^{-1}$. The main features of the calorimeter chambers are shown in Fig. 5.1. A wire mesh partition was placed to keep the birds in the front half of the calorimeter chambers so that they remained in the area scanned by the Doppler-radar activity meter. The chambers were designed to house domestic fowl, so the floor area was larger than necessary for a quail. The gases leaving each chamber were sampled for 10 min in each hour. This sampling regime has been shown to provide good agreement with continuous sampling for domestic fowl (Lundy *et al* 1978), and allowed the energy expenditure

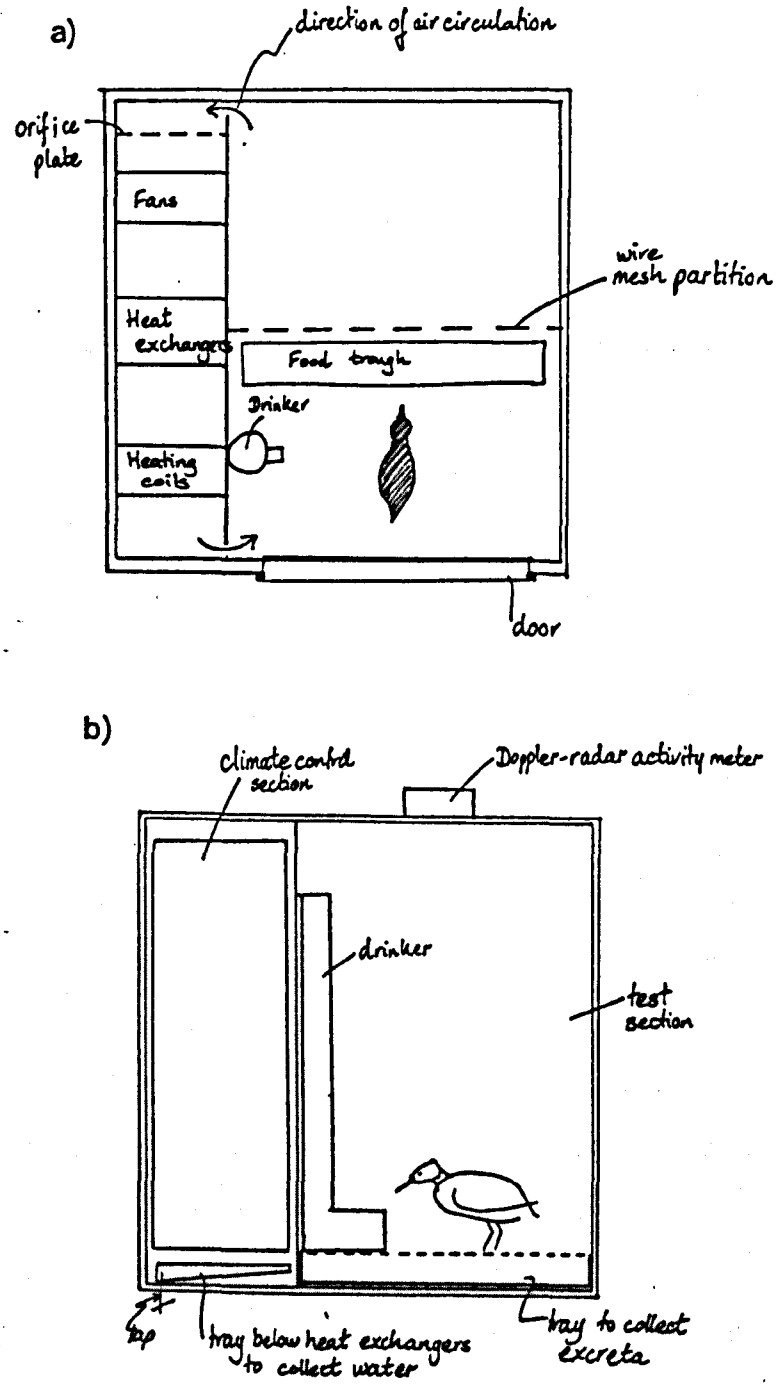


Fig. 5.1 Diagram showing a) in plan view, b) vertical cross section of a chambers in the Roslin multi-calorimeter.

of birds in 5 chambers to be measured over the same period. The oxygen and carbon dioxide analyzers were calibrated at the start of each experiment.

The birds were removed from the chambers in turn between 0900 and 1000 hours each day to allow weighing, collection of excreta and recording of food and water consumption. Blood samples required for measurement of energy expenditure by the DLW technique (Section 5.2.2) were also taken during this period.

The calorimeter was used to measure energy expenditure for less than entire 24-hour periods during the 4 days of simultaneous energy expenditure measurement. No calorimeter measurements could be made whilst the birds were removed from the chambers and a further hour was allowed after the chambers were resealed to allow equilibration of gases before calorimetry measurements were recommenced. Additionally, maintenance of the calorimeter system during the experiment reduced the time for which data could be collected. Mean hourly energy expenditure was calculated during the photoperiod and multiplied by 14 to give photoperiod energy expenditure. The calorimeter always operated during the 10 h scotoperiod.

5.2.2 Doubly labelled water technique

5.2.2.1 Theoretical basis of the technique

The DLW technique is a method of indirect calorimetry which can be used to measure the energy expenditure of free-living subjects (Lifson and McClintock 1966). Carbon dioxide production is calculated from the rate of loss of an enriched dose of stable isotopes of oxygen and hydrogen. The calorific equivalent of the volume of carbon dioxide evolved is calculated using the same rationale as in chamber calorimetry (Section 5.2.1.1), except that an estimated RQ must be used.

The DLW technique was developed after the observation that inhaled oxygen rapidly achieved isotopic equilibrium with the oxygen in body water (Lifson *et al* 1949). This is due initially to the combination of inspired oxygen and protons to form water and the breakdown of body water to produce carbon dioxide during the tri-carboxylic acid cycle. The oxygen of carbon dioxide and body water are bought

into complete isotopic equilibrium in the blood by reactions catalysed by carbonic anhydrase. This enzyme ensures that the oxygen of expired carbon dioxide is in isotopic equilibrium with the oxygen of body water (Speakman and Racey 1988b).

The technique involves enrichment of the subject with the stable isotopes ^{18}O and deuterium (^{2}H). A period is allowed for complete equilibration of these isotopes within the body water pool, after which a sample of body fluid is taken. This is normally a blood sample. A subsequent blood sample is taken some multiple of 24 h later.

The principal routes of loss of the ^{18}O label are in respiratory carbon dioxide and water. The deuterium label is used to determine the extent of ^{18}O loss as water so that the amount of ^{18}O lost as carbon dioxide can be calculated from the difference in turnover rates of oxygen and hydrogen label using Equation 5.2 (Lifson and McClintock 1966).

$$rCO_2 = \frac{N}{2.08}(k_O - k_D) - 0.015 k_D N \qquad 5.2$$

where rCO_2 = production rate of carbon dioxide ($mMolh^{-1}$),
2.08 = product of a fractionation factor ($H_2{}^{18}O_{(lq)} \rightarrow C^{16}O^{18}O_{(g)} = 1.04$) and a stoichiometric factor (carbon dioxide has the oxygen equivalent of two molecules of water),
N = volume of the body water pool (mMol) determined from the ^{18}O dilution space at the start of the experiment, or by desiccation, $0.015k_DN$ accounts for fractionation effects of evaporative water loss assuming that this accounts for 50% of total water loss,
k_O = fractional turnover rate of the oxygen label, calculated from Equation 5.3,
k_D = fractional turnover rate of the hydrogen label, calculated from Equation 5.3.

$$k_o = \ln(^{18}O_i - ^{18}O_n) - \ln(^{18}O_f - ^{18}O_n) / \Delta t \qquad 5.3$$

where $^{18}O_i$ = concentration of ^{18}O in initial blood sample (ppm),
$^{18}O_f$ = concentration of ^{18}O in final blood sample (ppm),
$^{18}O_n$ = natural abundance of ^{18}O (ppm),
Δt = interval between initial and final blood samples (h).
For k_D read D instead of O in Equation 5.3.

The rate of carbon dioxide production is used to calculate ADMR (average daily metabolic rate, $cm^3CO_2g^{-1}h^{-1}$) (Equation 5.4).

$$ADMR = rCO_2 \times 22.4 / mass \qquad 5.4$$

where mass = mean of subject mass at start and end of the measurement period (g),
22.4 = conversion factor from mMol to cm^3 of carbon dioxide.

Finally, daily energy expenditure (DEE, kJd^{-1}) is calculated, using an assumed RQ, to determine the calorific equivalent of the volume of carbon dioxide evolved (Equation 5.5).

$$DEE = ADMR \times mass \times 24 \times 26.44 \qquad 5.5$$

where DEE = daily energy expenditure (kJd^{-1}),
26.44 converts cm^3CO_2 to kJ, assuming an RQ of 0.75, typical of mixed lipid and protein metabolism (Brody 1945),
24 converts from h to d.

If more than two blood samples are taken, it is possible to use the multi-point variation upon Equation 5.3, suggested by Schoeller *et al* (1986). This involves calculation of the rate of loss of labelling isotopes (k_O and k_D) by regression of the concentration with time, rather than determination of a gradient between two samples. The multi-point variation in the calculation of energy expenditure by the

DLW technique will be compared with the two-sample method for the samples obtained from male Japanese quail.

5.2.2.2 Assumptions of the doubly labelled water technique

Calculation of metabolic rate from the isotope turnover rates involves several simplifying assumptions which are not normally violated to an extent sufficient to introduce serious errors into the results. The assumptions are listed here for reference during later discussion of possible assumption violations during this study (Section 5.4, Chapter 6).

1. Body water pool, N, is constant during the measurement period.
2. Rates of water flux and carbon dioxide production are constant during the measurement period.
3. Isotopes label only water and carbon dioxide within the body of the subject.
4. Isotopes leave the body only as water and respiratory carbon dioxide.
5. The specific activities of isotopes in water and carbon dioxide leaving the body are the same as in body water.
6. Water or carbon dioxide do not enter the animal across the skin or lung surfaces.

The principles and assumptions of the DLW technique have been discussed in more detail by Lifson and McClintock (1966), Nagy (1980), Speakman and Racey (1988), Tatner and Bryant (1989) and Speakman (1990).

5.2.2.3 Experimental protocol

Each experimental bird was weighed, and the labelling isotopes supplied by intraperitoneal injection. A mixture of 10 ml 20% $H_2{}^{18}O$ and 0.68 g 99.9% D_2O (or 10 ml 15% $H_2{}^{18}O$ and 0.51 g 98 % D_2O) was used to label the birds. The dosage, for 4 days, of 15.3 μlg^{-1} (33% greater for labelled water made up from 15% $H_2{}^{18}O$),

was calculated using the formulae of Tatner and Bryant (1989).

Birds were placed in the calorimeter chambers for 2 hours to allow mixing of the labelling isotopes with the body water pool. A blood sample was then taken from the leg or wing vein using a 0.45x10 mm needle, and 8-10 tubes of approximately 5µl of blood taken up immediately into pre-calibrated glass pipettes (Camlab). The pipettes were flame sealed within 10 min of sample collection. The flow of blood from the vein was stopped using gentle finger pressure to the wound. Each quail was placed in a calorimeter chamber for 4 days during which additional blood samples were taken at multiples of 24 h. Only samples from the first two 24-hour periods were analyzed for all 14 birds, due to the time-consuming nature of the analysis procedure. Samples from the final occasion of sampling, after four 24-hour periods, were analyzed for three birds.

Two additional birds were blood sampled every 20 min for 3 h and at 4 h after injection of the isotope dose. These serial samples were used to determine the period required for mixing of the labelling isotopes through the body water pool. Blood samples were also taken from four unlabelled quail to measure background concentrations of ^{18}O and deuterium.

5.2.2.4 Analysis procedure

Sealed blood samples could be stored at room temperature indefinitely prior to analysis of isotopic composition. The concentrations of ^{18}O and deuterium were measured by isotope ratio mass spectrometry, in the Life Sciences Laboratory at the Scottish Universities Reactor Research Centre on the National Engineering Laboratory campus at East Kilbride.

The concentration of the oxygen label was measured by conversion of the water in the sample to carbon dioxide, via reactions with guanidine chloride and phosphoric acid (Boyer *et al* 1961, Dugan *et al* 1985). The ratio of ^{18}O to ^{16}O in the carbon dioxide was measured using a VG SIRA 10 isotope ratio mass spectrometry.

The concentration of the hydrogen label was determined by reduction of

sample water to hydrogen gas in a uranium furnace at 800°C (Wong and Klein 1986). The hydrogen/deuterium gas was collected on activated carbon for analysis using an X22VG SIRA 9 isotope ratio mass spectrometer. Samples were processed in ascending or descending order of D concentration (determined from the ^{18}O concentration) in order to avoid problems with the "memory" effect in the uranium furnace, which retains a small amount of the previous sample. Details of the procedures used to prepare samples for analysis are given in Tatner and Bryant (1989). Calibration equations used to determine the concentrations (in ppm) of ^{18}O or D from the delta raw values produced by the mass spectrometers are given in Appendix 4.

Two capillaries from each sampling occasion were analyzed consecutively for each labelling isotope. A further replicate was analyzed if duplicate samples did not produce concentrations of the labelling isotope (ppm) within 3% of one another. This was greater than the 2% limit recommended by Tatner and Bryant (1989). However, the majority of replicates differed by less than 1%, and analysis of additional replicates generally did not improve agreement from the sampling occasions with 2-3% differences between replicates. The mean of the closest pair of concentrations from replicate samples was used in Equation 5.3.

5.2.3 Food intake/excreta output + comparative slaughter technique

Energy expenditure can be calculated from Equation 5.6.

$$H = I_E - (F_E + U_E) - R_E \qquad 5.6$$

where H = heat production, or energy expenditure (kJd^{-1}),
I_E = energy content of food intake (kJd^{-1}),
F_E = energy content of faeces (kJd^{-1}),
U_E = energy content of urine (kJd^{-1}),
R_E = energy retained in the body (kJd^{-1}).

The energy content of food and excreta (I_E and F_E+U_E) was measured for each bird (Section 5.2.3.1). R_E was estimated by the comparative slaughter technique and by *in vivo* estimation of individual body condition at the start and end of the experimental period (Section 5.2.3.3).

5.2.3.1 Energy content of food intake and excreta output

The decrease in mass of food in the feeding trough was recorded each day when the calorimeter chambers were opened. Some birds spilt food into the tray used to collect excreta. Split food was separated from excreta and subtracted from the mass of food removed from the feeding trough, to determine daily food consumption. Excreta was collected daily and frozen for storage. Daily excreta samples were pooled for each bird, freeze-dried, weighed (dry mass) and ground. Two samples of the diet were treated in the same way. Weighed sub-samples of ground excreta and diet were combusted in a bomb calorimeter to determine energy content of diet and excreta.

5.2.3.2 Estimation of body composition of live birds

Experimental birds were weighed and measured (Chapter 2) at the start and end of the period of energy expenditure measurement. Control birds were weighed and measured immediately before death. An index of body fat content was made using the pinched thickness of the subalar fat depot (under the wing), where quail are lightly feathered. Fat scores were taken at the start and end of the experimental period. Those made immediately before sacrifice of the bird were compared with carcass fat content, to determine the relationship between fat score and carcass fat content.

Protein content was estimated using an ultrasonic flaw detector (Chapter 2) to measure pectoral muscle thickness at three positions on either side of the keel. Muscle thickness was measured with ultrasound at the start and end of the

experimental period, and shortly after death with both ultrasound and a needle (Chapter 2). This allowed comparison of measurements of muscle thickness made in living and dead birds with those made with a needle. USTHICK, USVOL and USI (three indices of pectoral muscle size defined in Chapter 2) were assessed as measures of carcass protein content.

5.2.3.3 Carcass composition

Birds were slaughtered by intraperitoneal injection of an overdose of Sagatal. Each carcass was weighed and the pectoral muscles removed for separate analysis. Each sample was roughly chopped into small pieces, freeze-dried and weighed (dry mass). Samples were ground and sub-samples used to determine lean dry mass (after extraction of lipid content using petroleum ether in a Soxhlet apparatus) and nitrogen content (Kjeldal process).

Carcass protein content was calculated by multiplication of N content by 6.25 (Brouwer 1965). Carcass lipid and protein content were used to calculate the energy content of each bird using the calorific equivalents of 39.2 kJg^{-1} for lipid and 23.7 kJg^{-1} for protein (Znaniecka 1967).

5.3 Results

5.3.1 Comparison of training and experimental periods in the calorimeter chambers

There were no significant differences in energy expenditure measured by chamber calorimetry between the two days of "training" and the 4 experimental days (paired t-tests $t=1.57$, $p=0.1$ for DEE per bird, $t=1.48$, $p=0.1$ for DEE per $kg^{0.75}$, $n=14$). Birds were heavier during the experimental than during the training period (paired t-test, $t=2.34$, $p=0.04$). This could be accounted for by low food intake when the birds were first placed in the chambers. Daily food intake was significantly greater during the experimental (12.5 gd^{-1}) than the training period (10.2 gd^{-1}) (paired t-test, $t=3.22$, $p=0.008$).

5.3.2 Effect of chamber and experiment number upon energy expenditure

An analysis of variance showed that there were no significant effects of chamber number, experiment number or an interaction between these factors upon energy expenditure measured by any of the three techniques (ANOVA, $p=0.1$, $n=14$).

5.3.3 Body composition of quail

5.3.3.1 Prediction of body composition from *in vivo* measurements

Ultrasound measurements of pectoral muscle thickness of dead quail were highly correlated with those made immediately before slaughter (Pearson correlation, $r=0.89$, $p=0.001$, $n=33$). Measurements taken from live birds were more variable and slightly, but significantly, greater than measurements taken from the same birds after death (paired t-test, $t=3.07$, $p=0.004$, mean thickness measured in live birds=4.29 ultrasound units (sd=0.6), mean thickness in dead birds=4.16 (sd=0.5)). There was a significant relationship between ultrasound measurement of pectoral muscle thickness in live birds and thickness measured with a needle after death (Fig. 5.2). The relationship between pectoral muscle thickness and ultrasound thickness was tighter for females alone ($r^2=0.60$, $n=39$) than for male birds ($r^2=0.50$, $n=120$) or both sexes combined ($r^2=0.53$, $n=159$, Fig. 5.2). Pectoral muscle thicknesses of female birds were measured after those of male birds, when I had more experience of the technique. Unexpectedly, USI, USVOL and USTHICK were not significantly correlated with carcass protein content (all $p>0.1$, $n=14$). Carcass energy content was not significantly correlated with protein content ($r=0.12$, $p=0.4$, $n=37$).

Fat score was highly correlated with both carcass fat content (Fig. 5.3) and carcass energy content (Pearson correlation, $r=0.88$, $p=0.001$, $n=14$). There were highly significant relationships between carcass fat content or bird mass and carcass energy content (Fig. 5.4). In a regression analysis of factors which could be measured in living birds which might predict carcass energy content (mass, fat score, USVOL, USI, USTHICK and structural size measurements), only fat score was

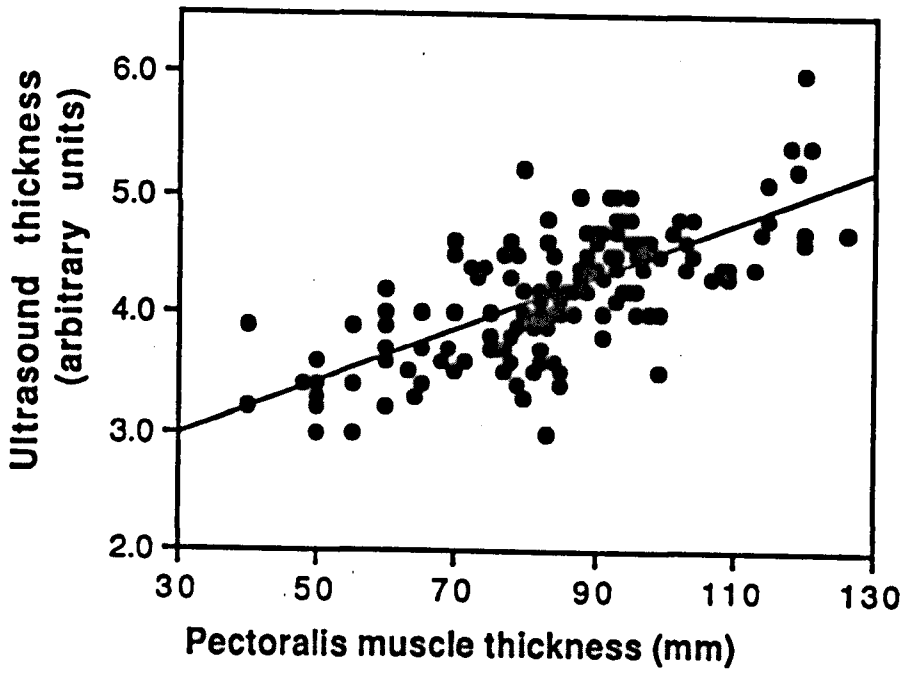


Fig. 5.2 Relationship between pectoralis muscle thickness, measured in live Japanese quail using ultrasound, and thickness measured with a needle on the same birds immediately after death ($y=0.02x + 2.29$, $r^2=0.53$, $p<0.0001$, $n=159$).

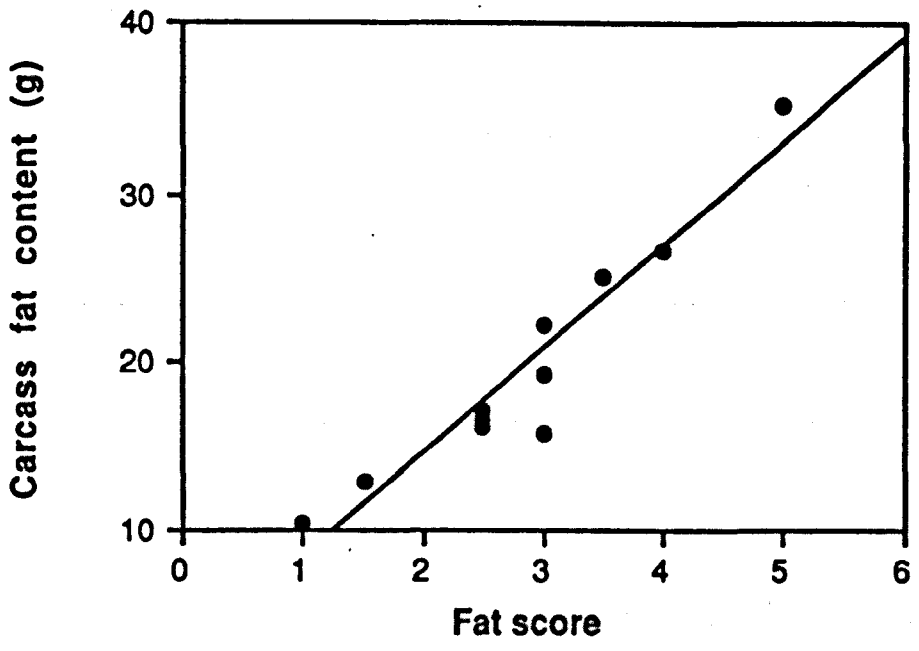


Fig. 5.3 Relationship between carcass fat content of male Japanese quail and fat score (pinched thickness of subalar fat deposit) made from the live bird ($y=6.14x + 2.18$, $r^2=0.91$, $p=0.0001$, $n=11$).

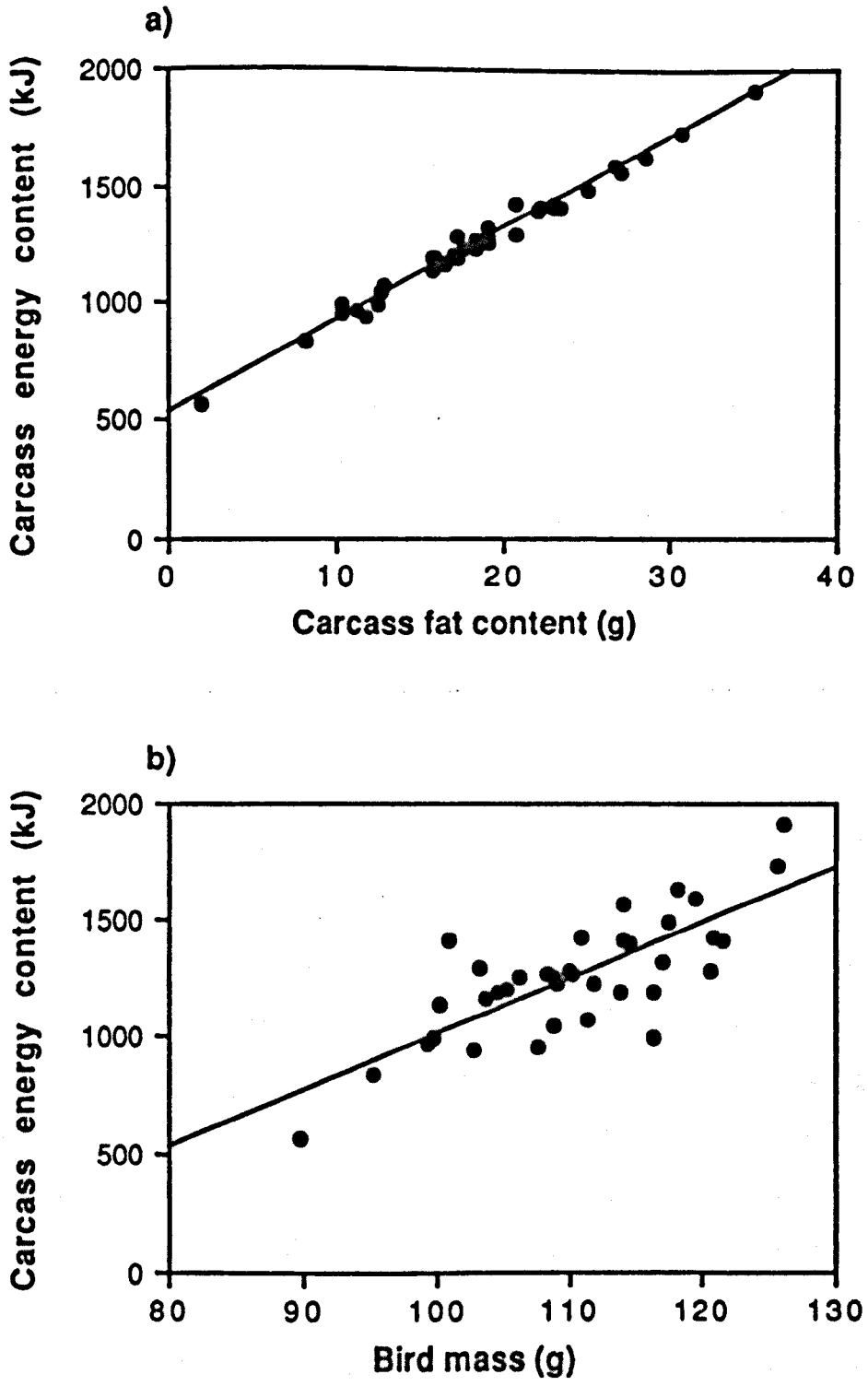


Fig. 5.4 Relationships between carcass energy content of 40 Japanese quail and a) carcass fat content ($y=39.2x + 526.7$, $r^2=0.99$, $p=0.0001$, $n=37$), b) bird mass ($y=24.1x - 1401.6$, $r^2=0.60$, $p=0.0001$).

entered as a significant variable at the 5% level (carcass energy content=225.9 x fat score + 607.8, r^2=0.92, p=0.0001, n=11). Of the measures of body condition which could be made with live birds, only mass and fat score (Fig. 5.4) were therefore useful as predictors of carcass composition. Ultrasound measurements of pectoral muscle thickness and measures of structural size did not contribute significantly to the prediction of carcass composition.

5.3.3.2 Change in body composition during the energy expenditure measurement period

There were no significant differences in mass or fat score between experimental birds at the start and end the of the 4 days of energy expenditure measurement (paired t-tests, p>0.2, n=14). Fat score was the best predictor of carcass energy content, however this was determined only at the start and end of the experimental period, and not after the two days over which DLW and chamber calorimetry measurements were compared. Mass change over these two days was used therefore used to estimate change in R_E (Equation 5.6). Individual mass changes during this period ranged from a loss of 4.0 g to a gain of 2.7 g, equivalent to +3.2 and -2.3% of initial mass. Using the relationship between mass and carcass energy content in Fig. 5.4b, this was equivalent to a change in energy reserves of between +48.1 and -32.5 kJd^{-1}. Mass and carcass energy content of experimental and control birds did not differ significantly (t-tests, p>0.4). This suggested that there was no difference in the energy content of birds during the experimental period. Change in R_E was calculated from the change in mass of individual birds, as this more accurate over a short period for mature birds, than comparison of the two groups by the comparative slaughter technique.

5.3.4 Effect of temperature on energy expenditure

Energy expenditure (measured by each of the three techniques) increased with decreasing temperature (Fig. 5.5). Energy expenditure measured by chamber calorimetry provided the tightest relationship with temperature. This suggested that chamber calorimetry might provide the most consistent measurement of energy expenditure of the three techniques.

Food intake increased as temperature decreased, although there was was not a significant relationship, due to the amount of scatter around the regression line (Fig. 5.6). Birds tended to drink more at higher temperatures although this was not a significant effect (Pearson correlation, $r=0.46$, $p=0.09$). In a stepwise multiple regression analysis of factors with a potential effect upon energy expenditure (temperature, food intake and activity level, mass, structural size and body condition of bird), only temperature was entered as a significant variable at the 5% level (see Fig. 5.5 for regression equations).

5.3.5 Effect of activity on energy expenditure

There were significant relationships between activity count by the Doppler-radar activity meter during the 10 minute sampling period from each chamber and energy expenditure measured by chamber calorimetry for 6 of the 14 experimental birds during the photoperiod and 9 of 14 birds during the scotoperiod. The relationships between energy expenditure and activity for each 10 minute period over the 4 experimental days are shown for one bird in Fig. 5.7. Each unit of activity was more costly during the period when the lights were off than when they were on. A regression equation was constructed for each bird, relating energy expenditure and activity, and the intercept used to calculate energy expenditure predicted for zero activity level. Activity accounted for only 4.7% (sd=4.9, n=14) of daily energy expenditure. This was similar to the 5% of DEE accounted for by activity during calorimetry measurements with domestic fowl (MacLeod *et al* 1982).

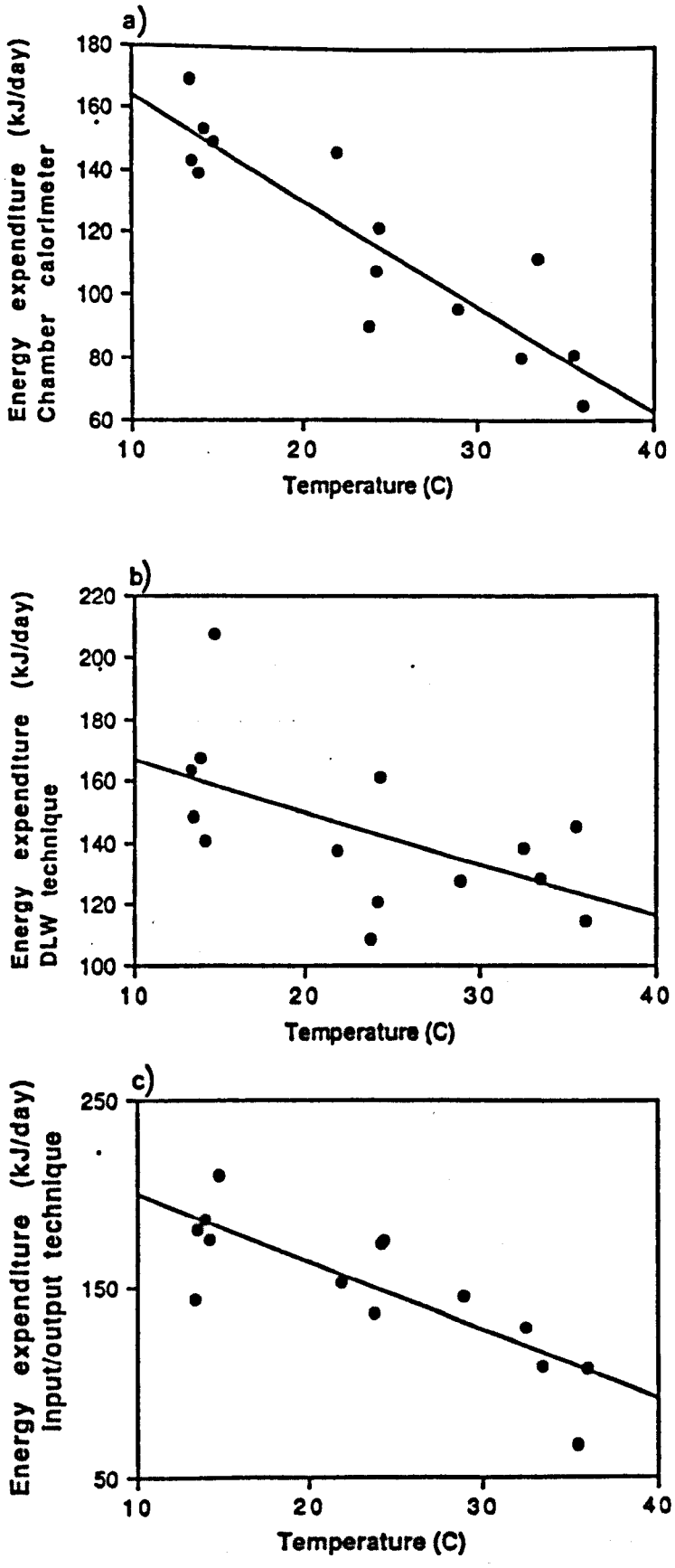


Fig. 5.5 Relationships between temperature and energy expenditure of 14 male Japanese quail measured simultaneously by a) chamber calorimetry ($y=-3.37x + 197.56$, $r^2=0.80$, $p=0.0001$), b) the DLW technique ($y=-1.68x + 183.33$, $r^2=0.32$, $p=0.04$), and c) the input/output technique ($y=-3.15x + 222.31$, $r^2=0.63$, $p=0.0008$).

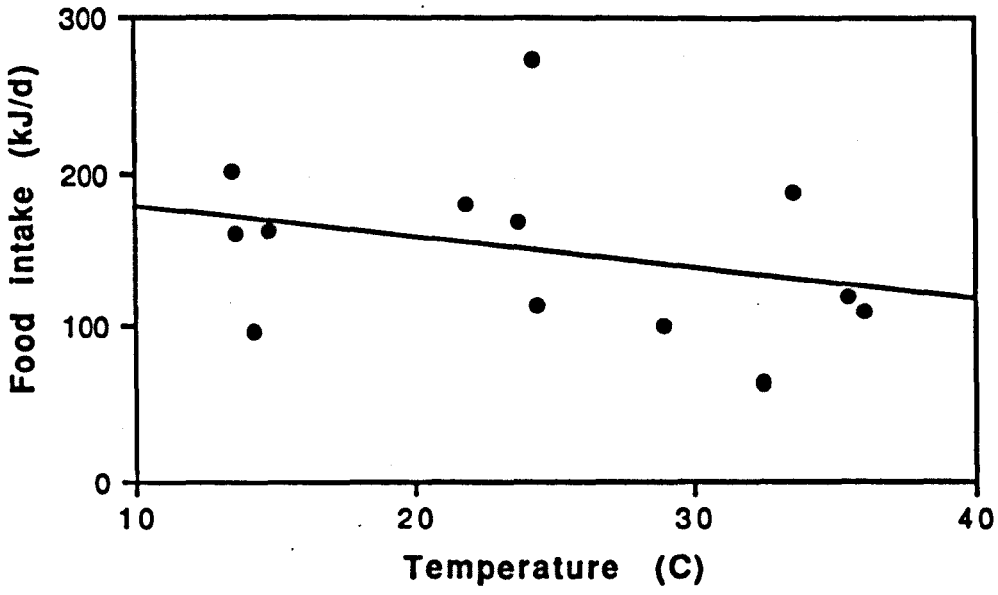


Fig. 5.6 Relationship between temperature and food intake of 14 male Japanese quail, $y=-1.75x + 190.56$, $r^2=0.08$, $p=0.33$. $n=14$

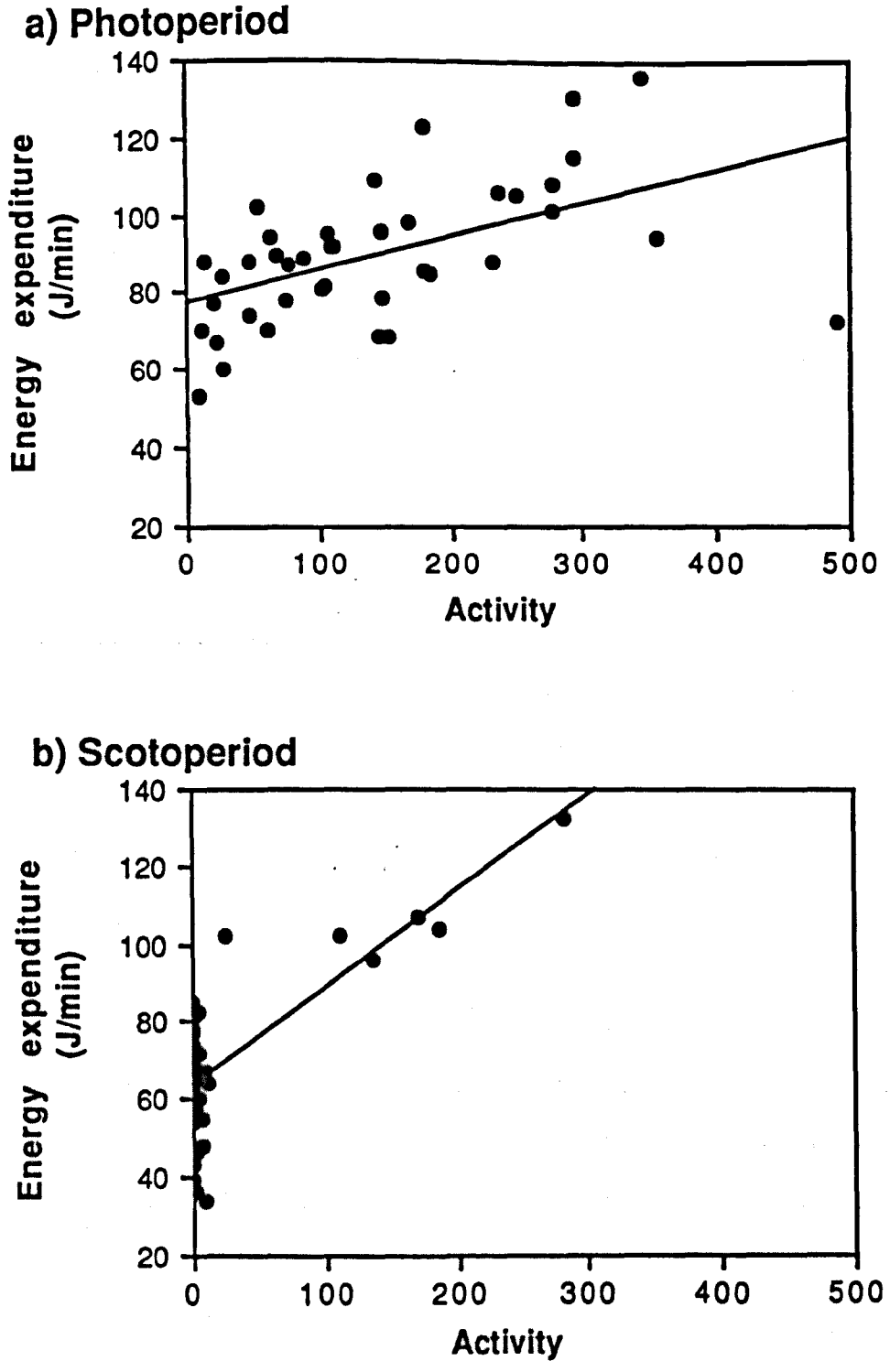


Fig. 5.7 Relationships between energy expenditure and activity count during 10 minute periods over 4 days for a male Japanese quail during a) photoperiod ($y=0.08x + 78.74$, $r^2=0.25$, $p=0.001$, $n=41$), b) scotoperiod ($y=0.25x + 63.11$, $r^2=0.56$, $p=0.0001$, $n=42$).

5.3.6 Equilibration period of ^{18}O and D isotopes in male quail

Two male quail were injected with labelled water and serial blood samples were taken. The labelling isotopes mixed rapidly through the body water pool, reached a peak in concentration after 45-51 minutes, then decreased log-linearly over the next 2 d (Fig. 5.8). Concentrations of both isotopes in both birds showed similar patterns.

5.3.7 Comparison of results of simultaneous energy expenditure measurement by three techniques

Daily energy expenditures, measured by the three techniques over the first two days of the experimental period, are shown in Table 5.1. Algebraic mean differences and mean deviations were similar between chamber calorimetry and either DLW or input/output techniques. The algebraic mean difference between the DLW and input/output techniques was small, although the mean deviation was rather larger.

If geometric mean regressions were performed between the results from pairs of techniques, best agreement was obtained between chamber calorimetry and the input/output technique (gradient not significantly different from 1, r^2=0.49, Fig. 5.9c), followed by chamber calorimetry and the DLW technique (gradient significantly greater than 1, r^2=0.39, Fig. 5.9a). The DLW and input/output techniques showed poorest agreement according to this criterion (gradient significantly less than 1, r^2=0.29, Fig. 5.9b).

Chamber calorimetry gave measurements which were significantly lower than either the DLW or input/output techniques (paired t-tests, t=3.7, p=0.003 calorimeter with DLW technique; t=4.1, p=0.001 calorimeter with input/output technique, n=14). There was no significant difference between the results from the input/output and DLW techniques (t=0.67, p=0.5).

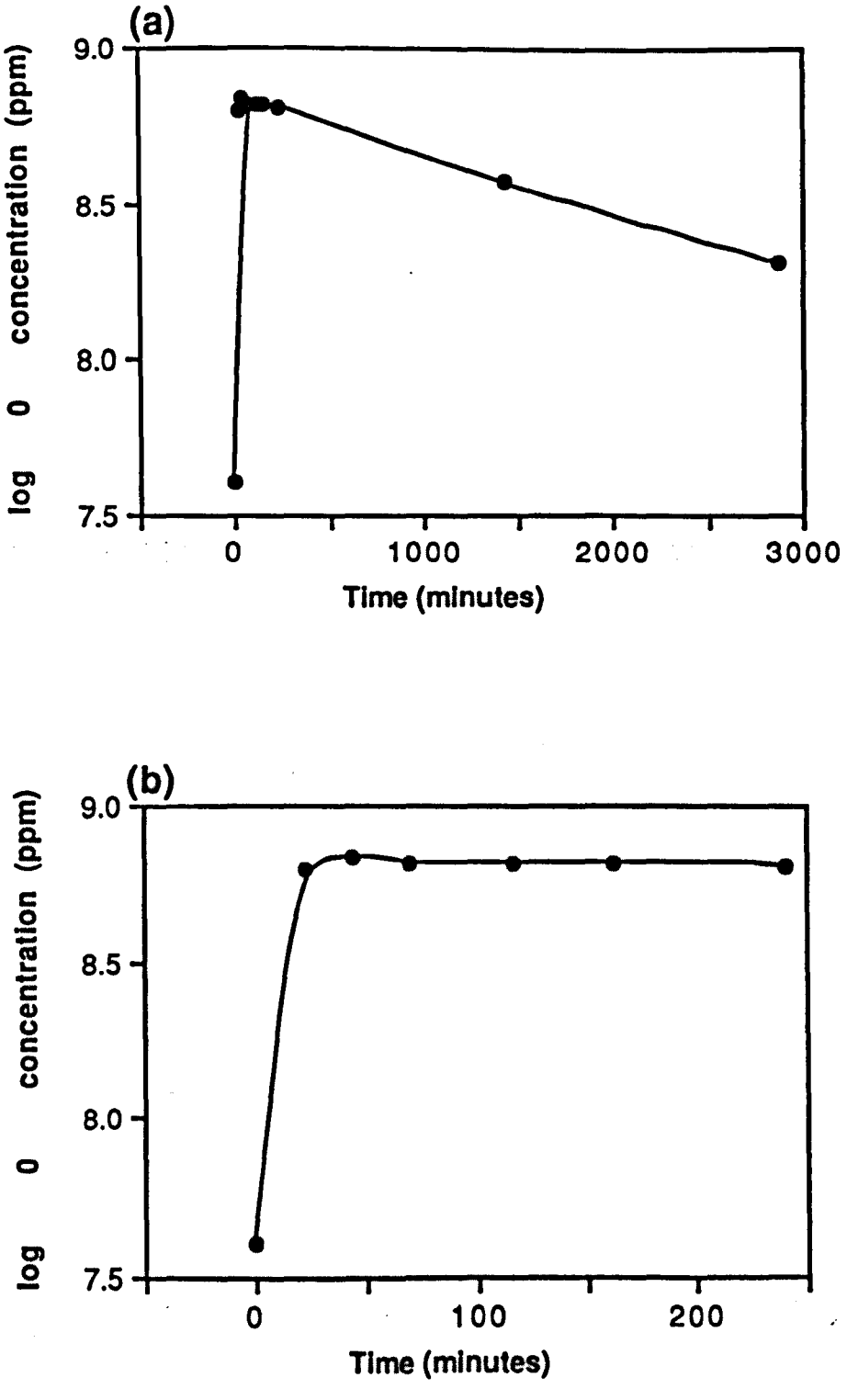


Fig. 5.8 Relationship between $\log_e{}^{18}O$ in the body water pool of a male Japanese quail and time after intraperitoneal injection of 15.3 $\mu l g^{-1}$ of approximately 18.5 ape $H_2{}^{18}O$. Time 0 was the time of the injection with a natural abundance of ^{18}O determined from a blood sample taken shortly before injection of the $H_2{}^{18}O$. a) the rise and subsequent decline in concentration of $H_2{}^{18}O$ over the next two days. b) detail of the first 7 points on graph a), showing the rapid mixing of ^{18}O with the body water pool (c. 1 hour).

Table 5.1 Comparison of the results of three simultaneous methods of measurement of energy expenditure ($kJbird^{-1}d^{-1}$) of male Japanese quail. Mass = mean mass at start and end of 2 days over which measurements of energy expenditure were compared. C = chamber calorimetry, D = doubly labelled water technique and I = food intake/excreta output technique. Algebraic mean is mean for column including the sign of data. Mean deviation is mean for column with all data made positive.

		Energy expenditure techniques			Differences between techniques					
Bird	**Mass**	**C**	**D**	**I**	**%(D-C)/D**	**D-C**	**%(I-C)/I**	**I-A**	**%(D-I)/D**	**D-I**
1	123.8	153.2	140.7	154.2	-8.9	-12.6	0.7	1.0	-9.6	-13.6
2	120.7	112.0	128.5	123.2	12.9	16.5	9.1	11.2	4.1	5.3
3	105.5	169.2	163.8	152.1	-3.3	-5.4	-11.3	-17.1	7.2	11.7
4	107.3	90.0	108.4	134.7	17.0	18.5	33.2	44.8	-24.2	-26.3
5	120.2	80.7	146.0	78.0	44.7	65.3	-3.4	-2.7	46.6	68.0
6	119.7	145.6	137.8	159.6	-5.7	-7.8	8.7	13.9	-15.8	-21.7
7	123.9	143.2	149.1	173.2	4.0	5.9	17.4	30.1	-16.2	-24.1
8	114.0	107.9	120.8	188.4	10.7	12.9	42.8	80.6	-56.0	-67.6
9	130.2	64.3	115.0	108.9	44.1	50.7	41.0	44.6	5.3	6.1
10	117.7	121.7	160.9	160.3	24.3	39.2	24.0	38.6	0.4	0.6
11	106.2	149.3	207.3	206.6	28.0	58.1	27.8	53.4	0.3	0.7
12	114.4	80.4	138.8	117.5	42.1	58.4	31.6	37.1	15.4	21.3
13	102.2	95.6	128.1	136.6	25.5	32.5	30.0	41.0	-6.6	-8.4
14	121.2	139.2	167.2	178.6	16.7	28.0	22.1	39.5	-6.9	-11.4
Mean	116.2	118.0	143.7	148.0						
sd	8.3	22.6	25.5	34.3						
Algebraic mean					18.0	25.7	19.5	30.0	-4.0	-4.2
Mean deviation					20.6	29.4	21.6	32.5	15.3	20.5

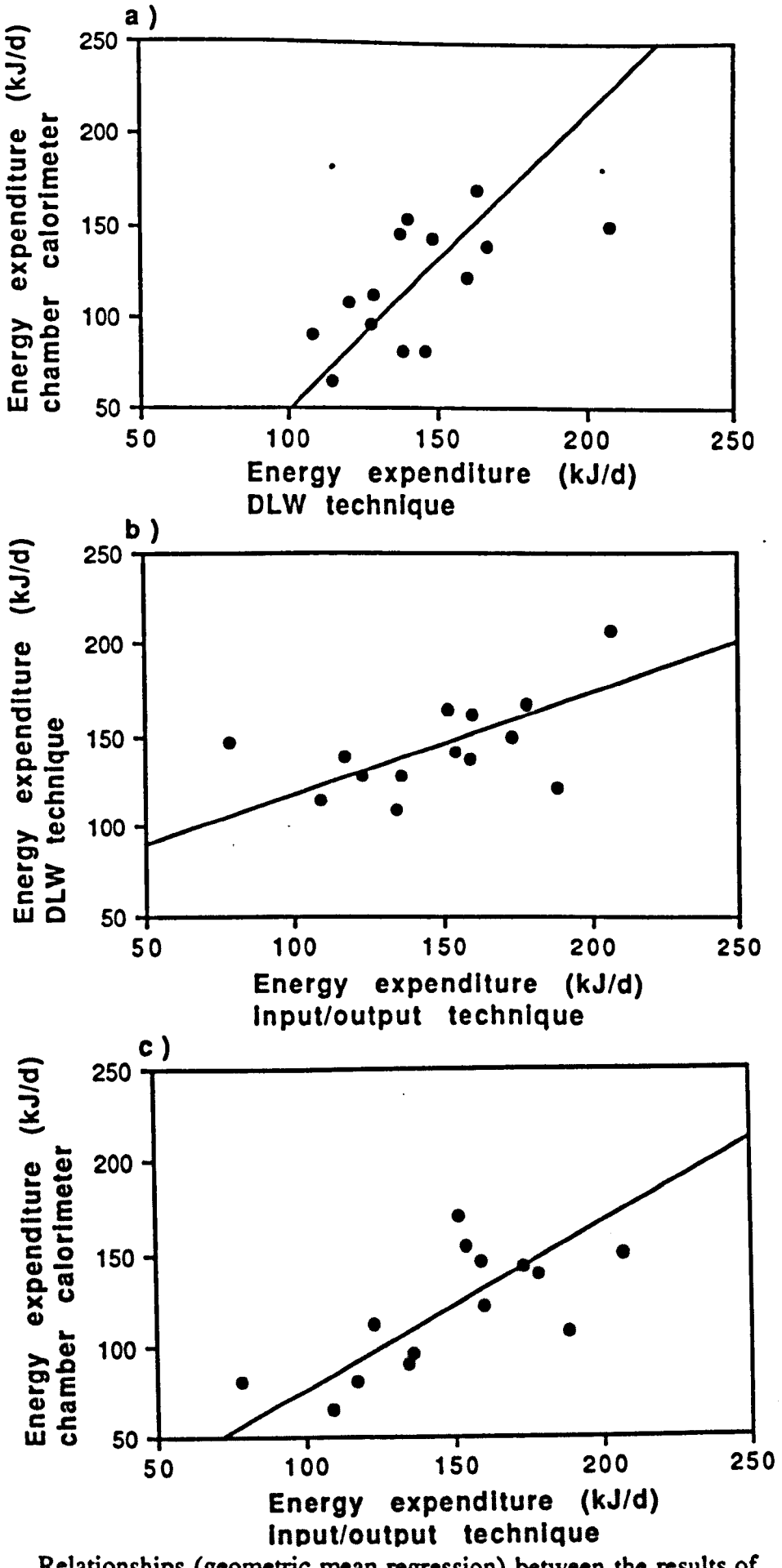


Fig. 5.9 Relationships (geometric mean regression) between the results of energy expenditure measurements of 14 male Japanese quail made simultaneously using chamber calorimetry, the DLW technique and the input/output technique. a) Relationship between chamber calorimetry and DLW measurements, $y=1.631x - 116.460$, $r^2=0.39$, $p=0.02$, $S_b=0.288$, b) the DLW and input/output techniques, $y=0.555x + 61.608$, $r^2=0.28$, $p=0.052$, $S_b=0.183$, c) chamber calorimetry and the input/output technique, $y=0.905x - 15.937$, $r^2=0.49$, $p=0.006$, $S_b=0.197$. $n=14$.

5.3.8 Comparison of the two-sample and multi-point methods for calculation of energy expenditure by the DLW technique

The daily energy expenditure of male quail was measured by the DLW technique using two-sample and multi-point variations of the calculation method. Results of the two calculation methods were highly correlated (Pearson correlation, $r=0.99$, $p=0.001$). Most applications of the multi-point technique have been with humans, when a sequence of samples can be collected readily over 1-2 weeks. It has been considered more appropriate to use the multi-point calculation method when isotope turnover is low, as in large subjects such as humans, and that the two-sample calculation is most suitable for small animals which have more rapid isotope turnover rates (Speakman 1990). Calculation method made virtually no difference to the energy expenditure calculated for male quail. The results from the multi-point method of calculation were an algebraic mean of 0.002% different from those calculated by the two-sample method. The two point method is therefore to be preferred, as this requires analysis of fewer samples, involving fewer interruptions of natural activity regimes in studies of wild birds.

5.4 Discussion

5.4.1 Time required for mixing of $D_2{}^{18}O$ within the body water pool

Two quail which were blood sampled at approximately 20 min intervals after injection of the labelled isotope dose for the DLW technique showed the highest concentrations of the isotopes 45 and 51 minutes after injection. The 2 hour interval between the labelled water injection and the initial blood sample was longer than adequate to allow complete mixing of the ^{18}O and deuterium through the body water pool. Rapid equilibration of the isotopes (30 min to 1 h) was found for serially sampled house martins and starlings (Ricklefs and Williams 1984, Westerterp and Bryant 1984). It follows that 1 h is likely to be an adequate equilibration period for most birds of less than 130 g.

5.4.2 Comparison of the three techniques of measurement of energy expenditure

Agreement between the results of the three techniques of energy expenditure measurement was poor at the individual level (Table 5.1), although the results were significantly correlated (Fig. 5.9). Algebraic mean difference between the DLW and input/output techniques was only -4.0%, within the range of agreement found between the DLW and other techniques found in previous studies (Speakman 1990). Agreement between chamber calorimetry and either the DLW or input/output techniques was poorer (algebraic mean differences: DLW 18.0% greater, and input/output technique 19.5% greater than chamber calorimetry).

Differences between techniques might arise from the cumulative effect of small violations of assumptions of the DLW technique or inherent analytical uncertainties in any of the methods compared. This could introduce both error, in which measurement of energy expenditure by a given technique suffered both positive and negative variations from the true value, and bias, in which the results according to a particular technique would be systematically greater or less than the true level. Possible reasons for the discrepancies between the results of the three techniques are discussed below.

1. Low metabolic rate led to low rates of isotope label depletion, which increased the analytical error in the DLW technique.

A mean of only 60.4% (range 50-70%) of the initial enrichment of the oxygen isotope was lost from the birds after 2 days. An error of >10% in the DLW result would be anticipated if final excess ^{18}O concentration is >50% of initial enrichment (Nagy 1980). The accuracy of the DLW measurements is decreased at low levels of depletion because the ratio of error (inherent in vacuum line preparation of samples and machine error of the mass spectrometers) to the difference between initial and final isotope concentrations measured is high.

Percentage deviation (Table 5.1) between DLW measurements of energy expenditure and chamber calorimetry was significantly negatively related to energy

expenditure (Fig. 5.10). The birds with the lowest energy expenditures had the lowest rates of isotope turnover, and the highest potential analytical error to k_O and k_D ratios. This result was consistent with errors arising in the DLW technique due to low levels of isotope depletion.

To assess whether greater isotope depletion would reduce the discrepancy between DLW and chamber calorimetry measurements of energy expenditure, blood samples taken from three of the birds after the fourth day were analyzed. Differences between the results of the two techniques decreased as the measurement period and the extent of isotope depletion increased (Table 5.2). ^{18}O was depleted to 80-90% of the initial enrichment after 4 days. Despite achieving the desired level of isotope depletion, differences between the two techniques of measurement of energy expenditure remained larger than in previous comparisons of chamber calorimetry and the DLW technique (Speakman and Racey 1988a).

These results suggested that although lack of sufficient isotope turnover after 48 h led to some decrease in accuracy of the DLW technique, this was not the main cause of the discrepancy between results of the different techniques.

2. Reduced precision of the chamber calorimeter at low rates of oxygen consumption and carbon dioxide production.

The calorimetry system was designed for use with domestic fowl, which are 8+ times heavier than Japanese quail. The low flow rate through the tubing connecting the chamber with the gas analyzers, which was required to give an adequate difference between ambient and chamber air, could decrease the accuracy of the calorimeter measurement, particularly for birds with the lowest energy expenditures where there was the smallest difference between ambient and chamber air. A loss of carbon dioxide from the calorimetry system would account for the unexpectedly low RQ values obtained. Physical tests of calorimeter precision, however, suggested that the flow rates used and levels of oxygen depletion and carbon dioxide production observed should have allowed accurate measurements to be obtained (Lundy *et al* 1978).

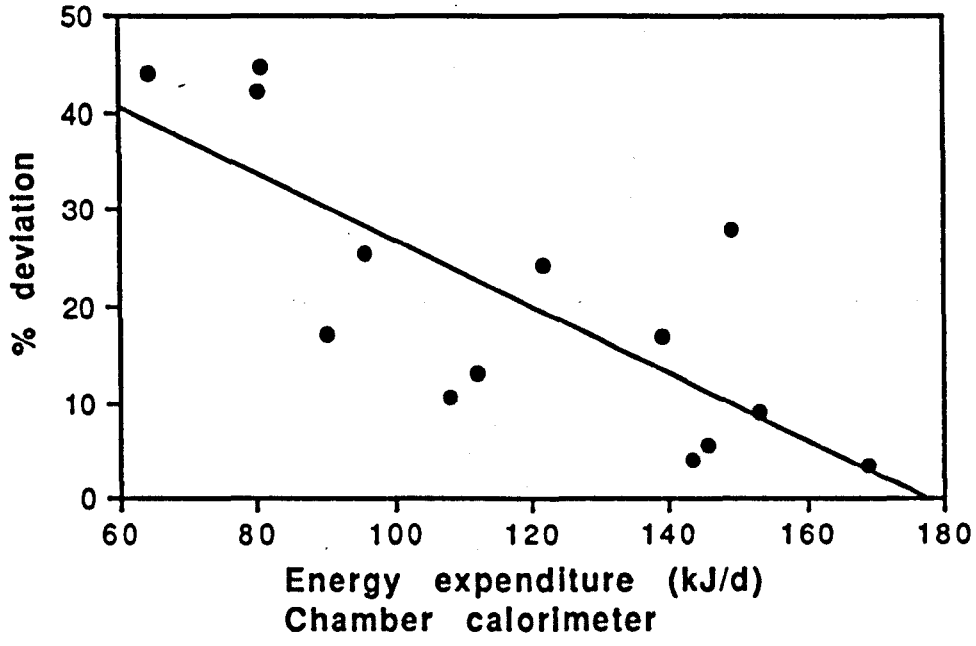


Fig. 5.10 Relationship between the % deviation ((DLW-chamber calorimeter)/DLW) of energy expenditure measurements by the DLW technique and chamber calorimetry and energy expenditure measured by the chamber calorimeter ($y=-0.35x+61.5$, $r^2=0.59$, $p=0.002$, $n=14$).

Table 5.2 Comparison of simultaneous measurement of energy expenditure (kJd^{-1}) of male Japanese quail of 24, 48 and 96 hours. C_{24}, C_{48} and C_{96} = chamber calorimetry over 24, 48 and 96 hours respectively. D_{24}, D_{48} and D_{96} = DLW technique over 24, 48 and 96 hours respectively. (Columns C_{48} and D_{48} are the same as C and D in Table 5.1.)

Bird	C_{24}	D_{24}	$\%(D_{24}-C_{24})/D_{24}$	$D_{24}-C_{24}$	C_{48}	D_{48}	$\%(D_{48}-C_{48})/D_{48}$	$D_{48}-C_{48}$	C_{96}	D_{96}	$\%(D_{96}-C_{96})/D_{96}$	$D_{96}-C_{96}$
5	69.7	138.5	49.7	68.8	80.7	146.0	44.7	65.3	81.0	134.8	40.0	53.9
11	140.7	193.5	27.3	52.8	149.3	207.3	28.0	58.1	145.9	159.1	08.3	13.2
12	75.3	133.6	43.7	58.3	80.4	138.8	42.1	58.4	76.1	124.4	38.9	48.4
Algebraic mean			40.2	60.0			38.3	60.0			29.1	38.5
Mean deviation			40.2	60.0			38.3	60.0			29.1	38.5

Percentage deviation between calorimeter and DLW results (defined as %(DLW-chamber calorimeter)/DLW, made positive) was significantly negatively correlated with calorimeter energy expenditure (Fig. 5.10). Percentage deviation between the calorimeter and input/output technique was non-significantly negatively correlated with calorimeter energy expenditure (Pearson correlation, r=-0.51, p=0.06, n=14). When compared with the other two techniques, the calorimeter underestimated energy expenditure most for the birds with the lowest energy expenditures. This suggests increasing inaccuracy of chamber calorimetry at low respiratory gas concentrations. This would be the pattern expected if the apparatus was operating beyond its capacity with quail showing a low energy expenditure.

3. Decreased accuracy of the DLW technique at high temperatures due to an increase in the proportion of evaporative water loss.

Water lost by evaporation is fractionated with respect to the body water pool of the subject, as the lighter isotopes leave the moist body surfaces more readily. This could violate one of the assumptions in the calculation of energy expenditure from the difference in isotope turnover rates: that 50% of water loss is fractionated due to evaporation.

Percentage deviation between the DLW technique and chamber calorimetry increased significantly with temperature (Fig. 5.11). There was no significant correlation between temperature and percentage deviation between the DLW and input/output techniques (Pearson correlation, r=0.23 p=0.4).

These results could be a consequence of the relationships in Fig. 5.10 and the negative relationship between temperature and energy expenditure (Section 5.3.4). It was not possible to determine whether the cause of the relationships was an increase in fractionated water loss, a decrease in isotope depletion levels, a decrease in accuracy of the chamber calorimeter at low respiratory gas concentrations, or a combination of these factors. The energy expenditure calculated using the DLW technique is relatively insensitive to the extent of isotope fractionation (an error of ± 3% was proposed for plausible variation in the extent of fractionated water loss,

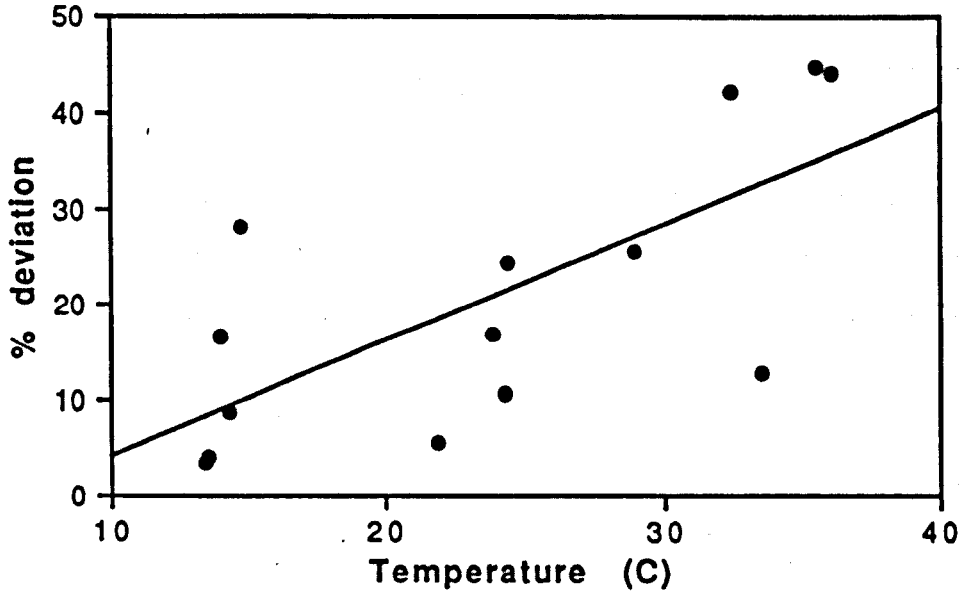


Fig. 5.11 Relationship between the % deviation ((DLW-chamber calorimeter)/DLW) of energy expenditure measurements by the DLW technique and chamber calorimetry and temperature ($y=1.20x - 7.82$, $r^2=0.50$, $p=0.005$, $n=14$).

James *et al* 1988). The proportion of water lost evaporatively could be assessed using triply labelled water (Haggarty *et al* 1988), although this was not attempted in the present study. It was concluded that any change in the proportion of fractionated water loss with temperature would not contribute greatly to the differences observed between techniques.

4. A possible progressive build-up of labelling isotopes in the water collected in the tray below the condenser within the calorimetry chambers.

The purpose of the condenser was to maintain the chamber at a constant level of humidity and assist in temperature regulation. As water accumulated below the condenser each day this might provide a source of the labelling isotopes which could re-enter the bird, violating one of the assumptions of the DLW technique. A further possibility was that a build up of isotopes in condenser water might occur progressively during the experiment. In this case the differences in results between the DLW and the other two techniques should be greatest for birds 10-14, as these were placed in the chambers after the other birds.

Deviation between the DLW technique and the chamber calorimeter measurements increased with experiment number (Fig. 5.12). There were no significant correlations between experiment number and the deviation between the input/output technique and either the DLW or chamber calorimetry results (Pearson correlations, both $p>0.4$). Water was drained from the collection trays daily, so a progressive build up of enriched water was improbable.

As deviation between only the DLW and calorimeter results increased with experiment number, and not between the DLW and input/output technique results, the reason for the discrepancies was unlikely to be one such as isotope re-entry for the later birds, which should have increased the difference between the DLW and both of the other techniques.

5. High concentration of carbon dioxide in the air surrounding the subject could allow cutaneous exchange of carbon dioxide, which would violate an assumption of

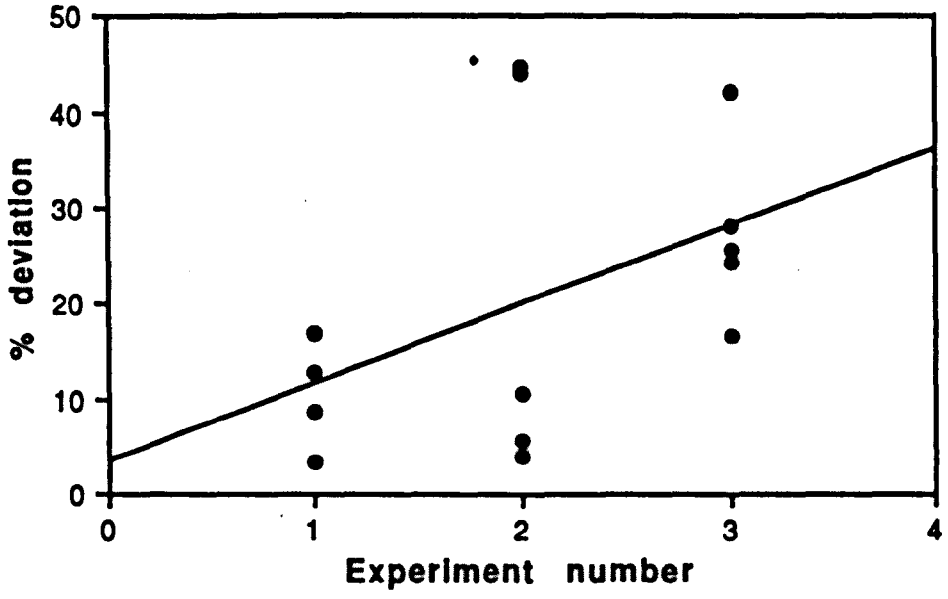


Fig. 5.12 Relationship between the % deviation ((DLW-chamber calorimeter)/DLW) of energy expenditure measurements by the DLW technique and chamber calorimetry and experiment number ($y=8.28x + 3.41$, $r^2=0.22$, $p=0.09$, $n=14$).

the DLW technique.

This may occur under the conditions of chamber calorimetry when carbon dioxide concentrations are greater than in ambient air, however, this should not influence the calculated flux rate as the carbon dioxide in the chamber will be at the same level of enrichment as the bird (Nagy 1980). There was a fan in each chamber, so the air would have been adequately mixed within the chambers.

6. The high initial levels of enrichment used, to label the quail with sufficient isotope for 4 d (rather than the two d normally used for small birds), would increase mass spectrometer imprecision for analysis of initial blood samples.

Mass spectrometer response at high levels of enrichment with ^{18}O is more variable than that at natural abundance (Tatner and Bryant 1989, Speakman *et al* 1990). Since isotope concentrations from the first occasion of sampling fitted the same line as those obtained on subsequent occasions (Section 5.3.8 and Fig. 5.8a), it was concluded that mass spectrometer machine error was not a major source of difference between the results of the DLW and the two other techniques.

7. Food consumption was determined by difference (mass of the trough containing the food at the start and end of each day), but the birds could have selected certain particles of food preferentially.

Dietary selection would not have been taken into account as the samples of the diet analyzed came from the sack of feedstuff and not from the feeding trough of each individual. The accuracy of the input/output technique might have been improved if samples of uneaten food from each trough had also been analyzed, but this would have greatly increased the number of food samples to be analyzed for a probably minimal increase in accuracy of determination of energy intake. It would be possible to provide the food in pellet form, so that birds could not selectively consume certain parts of the diet. The food was homogenous, so it would have been difficult for the birds to select only certain parts.

8. Daily excreta production (F_E+U_E) was calculated over the first two days of a pooled 4 d collection period.

Daily samples of excreta were pooled for analysis, so that possible variation between days was not taken into account. The use of pooled samples would have decreased the accuracy of the measurement of excreta production during the first 48 hours of the collection period.

9. Change in the energy retained (R_E) in the body was estimated from change in mass. Mass changes when the birds eat drink or excrete, so some of the variation in mass recorded would not have been due to change in R_E.

The energy equivalent of mass change was calculated from the mean energy content of experimental and control birds (Section 5.3.3). Variation in R_E which was not proportional to the mean energy content of quail carcasses would not be taken into account. Fat score was a better predictor of the energy content of quail carcasses than mass (Section 5.3.2), but fat scores were not made 48 hours after the start of the experimental period.

10. Although measurement of energy expenditure by the three techniques was simultaneous as far as was possible, the birds were removed from the calorimeter chambers each day. The period during which the birds were removed from the chamber and the hour after they were returned (required for equilibration of respiratory gases) were not included in chamber calorimeter measurement of energy expenditure, but would be included in the DLW and input/output measurements.

The period during and immediately after handling of the birds would presumably involve greater than average energy expenditure, which could partly account for the calorimeter energy expenditure measurements being the lowest of the three techniques (Section 5.3.7). Yet, because handling of the bird lasted only a few minutes, it was thought that this period of presumably greater than average energy expenditure would not contribute much to the daily total, especially since activity accounted for only a few percentage of DEE (Section 5.3.5).

11. Another possible reason for the low calorimetry results is that metabolic rate shows a marked rise shortly after the lights come on each morning (Lundy *et al* 1978). As the calorimeter chambers were sampled in turn, only one of the birds each day would have this peak in energy expenditure included in the calorimetry measurement.

The percentage underestimation of energy expenditure by chamber calorimetry due to this should not be great. If metabolic rate showed, for example, a 50% increase for 15 minutes of a 22 hour measurement period, the error should be less than -1% (22.25 units of energy expenditure rather than 22).

The lack of continuous calorimetry under this experimental protocol (effects of points 10 and 11, above) was thought to be a major reason for the discrepancies between the chamber calorimetry and both DLW and input/output technique results. Exclusion of periods of greater activity from the calorimeter measurements would account for the underestimation of energy expenditure, measured by chamber calorimetry, when compared with the other two techniques. Any "missed" periods of increased energy expenditure would increase in relative importance as energy expenditure decreased. This would account for the greater percentage differences between chamber calorimetry and the other two techniques for birds with the lowest energy expenditures (Fig. 5.10).

This also accounts of the tighter relationship between temperature and energy expenditure measured by the chamber calorimeter than between temperature and the results of the other two techniques. Only temperature had an important influence upon the energy expenditure measured by chamber calorimetry. Additional energy expenditure associated with activity leads to the extra scatter in the relationships between temperature and energy expenditure measured by the DLW and input/output techniques (Fig. 5.5).

It was concluded that all three techniques could be used to measure the energy expenditure of Japanese quail. The chamber calorimeter consistently

underestimated energy expenditure when compared with the other two techniques, particularly for the birds with the lowest metabolic rates. This did not mean that calorimeter measurements were not valid for birds which changed respiratory gas concentrations to the intended levels, rather that some of the birds in this experiment exhibited a metabolic rate below that which the apparatus was designed to measure. Crucially, for the work with female quail in the next chapter, there was no evidence for systematic error in the measurements made by the DLW technique. The accuracy of the input/output technique could be improved with an increase in the number of food and excreta samples analyzed and measures of body condition taken. If this was done agreement between the input/output and DLW techniques should improve for individual birds.

CHAPTER 6

VALIDATION OF THE DOUBLY LABELLED WATER TECHNIQUE FOR MEASUREMENT OF ENERGY EXPENDITURE IN A LAYING BIRD

6.1 Introduction

Egg formation has been proposed as a potentially energetically demanding stage of the reproductive cycle for female birds, and in some species is suggested to be the stage at which reproductive output is limited (e.g., Ricklefs 1974, Jones and Ward 1976, Houston *et al* 1983, Ankney *et al* 1991, Arnold and Rohwer 1991, Drobney 1991, Korpimaki and Hakkarainen 1991). Despite the potential importance of the pre-laying and laying periods, little of the work on the energetics of free-living birds has concentrated upon these stages. This is because many species have been thought to be prone to interrupt ovulation if the DLW technique was used to measure energy expenditure at this time (Bryant and Westerterp 1980). The only other study which used the DLW technique with a laying bird used captive individuals, but was discouraging due to poor agreement between energy expenditure estimated from food balance and that measured by the DLW technique (Meijer *et al* 1989).

There has been extensive study of the energetics of laying domestic fowl, but this has not involved the DLW technique, and data from this species may not be directly applicable to wild species, as egg production rates are much greater than those of wild birds, and food supply and activity levels of domesticated and wild species differ. Energy balance studies using birds such as captive Zebra finch, have the disadvantage that males must be kept with females, so that the energy requirement of the female could not easily be separated from that of her mate (e.g., El-Wailly 1966).

In this chapter, open-circuit chamber calorimetry was used to determine the net energy requirement for egg production for Japanese quail. Although the quail have been in captivity for many generations, they have not been bred selectively, and females laid in the absence of male birds. Energy expenditure was also

measured simultaneously by chamber calorimetry and the DLW technique to determine the accuracy of the DLW technique for a laying bird.

6.2 Methods

6.2.1 Selection of birds

Groups of Japanese quail were hatched at weekly intervals, so that females of the same age from the same group of parents were available at the start of each week. The birds were reared on a 23:1 light:dark regime for 5 weeks when they were transferred to individual cages on a 14:10 light:dark regime. Lights came on at 0700 hours. A daily record was made of egg production by each bird. When the birds were 6-7 weeks old, known layers and non-layers were selected for measurement of energy expenditure. At this age the birds exhibited a range of egg production from zero to daily laying. This could introduce variation in energy expenditure between laying and non-laying birds which was associated with the factor which caused the difference in date of maturity. However, the energy expenditures of non-laying birds lay around the value predicted for zero egg production from a regression equation which predicted energy expenditure for birds which formed different amounts of egg. It therefore seemed that there was no difference, other then egg production, between the energy metabolism of laying and non-laying birds. Use of natural variation in the age at which egg production commenced to produce non-laying birds was preferred to use of ovariectomy or suppression of ovulation by the use of tamaxifen (Dufty 1989), as these techniques might affect metabolic rate. Use of natural variation in the intensity of egg production also produced a range in egg production, rather than two categories (laying or non-laying individuals).

6.2.2 Quantification of daily egg formation

Female quail were fed a gelatin capsule of lipophilic dye each morning during measurement of energy expenditure by chamber calorimetry. This marked a coloured band on the outside of each ovarian follicle undergoing the rapid growth phase (Chapter 3). The birds were sacrificed at the end of the experimental period to allow follicles within the ovary of the bird to be hard boiled and sectioned along with the yolks of the eggs laid during the experiment. The volume of yolk formed during each 24 hour period was calculated by summation of the volumes deposited on each follicle (Equation 6.1).

$$V = \frac{4}{3}\pi\sum_{e=1}^{n}\left[\left(\frac{x}{2}\right)^3 - \left(\frac{y}{2}\right)^3\right] \qquad 6.1$$

where V = volume of yolk deposited (mm^3) in 24 h,
e = follicle or yolk number,
n = number of follicles or yolks containing 2 dye bands,
x = internal diameter of band from first dye feed (mm),
y = internal diameter of band from dye feed supplied 24 h later (mm).

Whether an albumen was formed each day was determined from the position of the dye bands and when the eggs were laid. The difference between the internal volume of the shell and the volume of the yolk was used to calculate albumen volume. Mean energy contents of quail egg yolk and albumen (Table 3.1) were used to determine the energy content of daily egg deposition.

6.2.3 Measurement of daily energy expenditure

The energy expenditure of 35 laying and non-laying female quail was measured by chamber calorimetry (Section 5.2.1). Birds were placed in the chambers for one day of training followed by 3 days of energy expenditure measurement. The temperature of all calorimeter chambers was 25°C. This was the temperature at

which quail were normally kept in captivity. Birds known to be laying or non-laying in the period prior to calorimetry were assigned randomly to chambers.

The metabolic rate of a sample of laying females was measured simultaneously by chamber calorimetry and the DLW technique (Section 5.2.2). The labelled water was given to the birds either by intraperitoneal injection or by intubation at the start of the experimental period. Oral administration of isotopes to laying birds was used to avoid possible damage to developing ova by injection of isotopes. The labelled water dosage was the same as that used for male quail (Section 5.2.2.2), except that 8 g (mean egg mass, to nearest g) was subtracted from the mass of the bird before calculation of the volume of labelled water required for females in which an oviducal egg could be felt.

A blood sample of the body water pool was taken 2 h after administration of the isotopes (Section 5.2.2.2). Further blood samples were taken 24 and 48 hours later when the calorimeter chambers were opened to measure food and water consumption, weigh and feed the lipophilic dye capsules to the birds, and to collect eggs and excreta. Albumen samples were taken from all eggs laid by labelled birds. A sample of yolk was taken from an ovarian follicle of one female after sacrifice at the end of the experiment.

Albumen and blood provided independent routes for sampling the concentration of labelling isotopes in the body water pool of laying females. The rate of loss of labelling isotopes from the bird was calculated using the difference between initial and subsequent blood samples, and between first and subsequent eggs laid. The energy expenditures calculated from these routes of sampling will be termed DLW (blood) and DLW (albumen) results. Blood samples were taken from four unlabelled birds to determine the background concentrations of ^{18}O and deuterium in female quail.

6.3 Results

6.3.1 Comparison of metabolic rates of male, non-laying and laying female quail

The relationship between temperature and energy expenditure (Fig. 5.5a) was used to estimate the energy expenditure of each of the 14 male quail in the experiment described in Chapter 5 at 25°C. Female quail (n=42, 35 birds from the experiment described in Section 6.2.3 plus 7 older birds from a similar preliminary experiment) were divided between those which laid at least one egg during the period which they spent in the calorimeter, and those which did not. Some of the birds classed as non-layers by this criterion did form some yolk during the experimental period or had enlarged oviducts, but the associated energy costs were small enough for there to be a marked difference in metabolic rate between laying and non-laying females.

There were significant differences in energy expenditure between male, non-laying female and laying female quail (t-tests, t=9.98 and t=6.57, p=0.001 for laying females versus males and non-laying females; t=3.31, p=0.003 for non-laying females versus males) (Fig. 6.1). Female quail were heavier than males, and laying females heavier than non-laying birds, which might partly account for the differences between categories in DEE per bird. There were, however, still significant differences if energy expenditure was expressed per $kg^{0.75}$, per g, or as ADMR and whether or not the portion of energy expenditure attributable to activity (Section 5.3.5) was included (t-tests, all $p<0.03$). A difference in metabolic rate between male and laying female birds has also been found for domestic fowl: cockerels had a 30% lower daily energy expenditure per bird and per $kg^{0.75}$ than laying females from a hybrid layer strain (MacLeod *et al* 1979). Laying fowl had a 19 and 45% higher energy expenditure per $kg^{0.75}$ than non-laying or ovariectomized birds respectively (Balnave *et al* 1978).

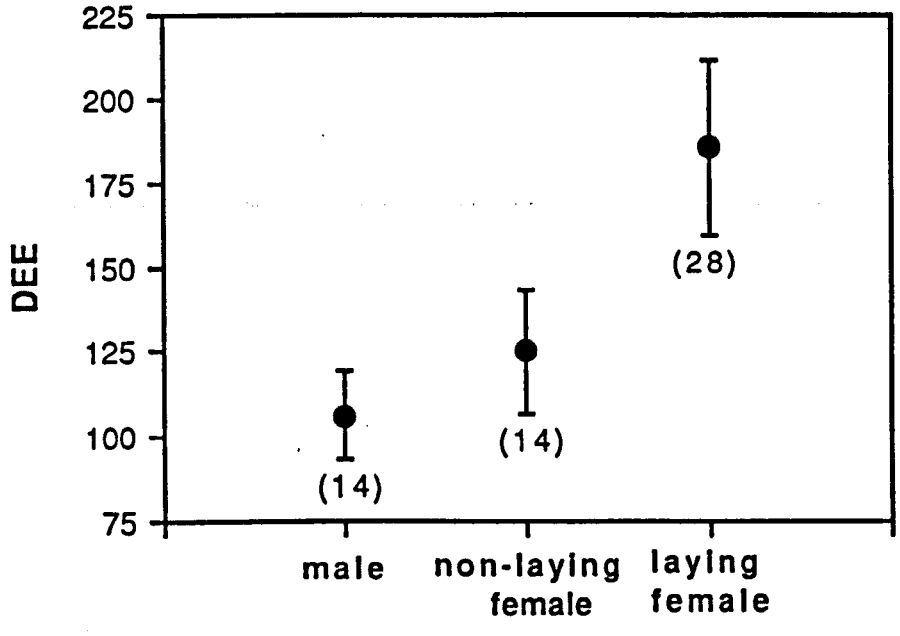


Fig. 6.1 Daily energy expenditure (DEE, kJbird^{-1}d^{-1}) of male, non-laying and laying female Japanese quail including activity costs. Error bars show standard deviation. Sample sizes are shown in brackets. There were significant differences between all groups. Measurements were made at 25°C for female quail and adjusted to 25°C for male birds using the relationship between energy expenditure (measured by the chamber calorimeter) and temperature (Fig. 5.5)

6.3.2 Relationship between egg production and daily energy expenditure

There was a significant positive relationship between mean DEE ($kJbird^{-1}d^{-1}$) of 35 female quail measured using chamber calorimetry over 3 days and mean daily energy content of the egg material formed (Fig. 6.2). The energy content of egg formed comprised up to an additional 26.2% (mean 9.44%, sd=8.23) of DEE.

Bird mass was significantly correlated with energy expenditure (Pearson correlation, r=0.74, p=0.001), and with egg production (r=0.72, p=0.001). Energy expenditure was still significantly correlated with egg production after controlling for bird mass (partial correlation, r=0.46, p=0.004).

Food intake was significantly correlated with energy expenditure (Pearson correlation, r=0.71, p=0.001), but intake was considered to be a consequence of increased energy requirement rather than a causal factor. Average food intake (224.3 kJd^{-1}, sd=104.5, n=35) was somewhat lower than the 260 kJd^{-1} determined by Yamane *et al* (1980) for laying quail.

In a stepwise multiple regression analysis of factors which might be expected to affect energy expenditure only egg production and bird mass, were entered at the 5% level of significance (Table 6.1).

In an analysis of variance, energy expenditure did not vary significantly with calorimeter chamber, experiment number, or an interaction between these factors (ANOVA, all p>0.1).

6.3.3 Comparison of ^{18}O and deuterium concentrations in birds labelled by intubation and intraperitoneal injection

One of the birds for which energy expenditure was measured by the DLW technique (bird 6) was labelled with the ^{18}O and deuterium isotopes by intubation, rather than the intraperitoneal injection normally used to administer the isotopes. Concentrations of the labelling isotopes in the blood sample taken 2 hours later from the intubated bird (5880 ppm ^{18}O and 1350 ppm deuterium) were within the range of

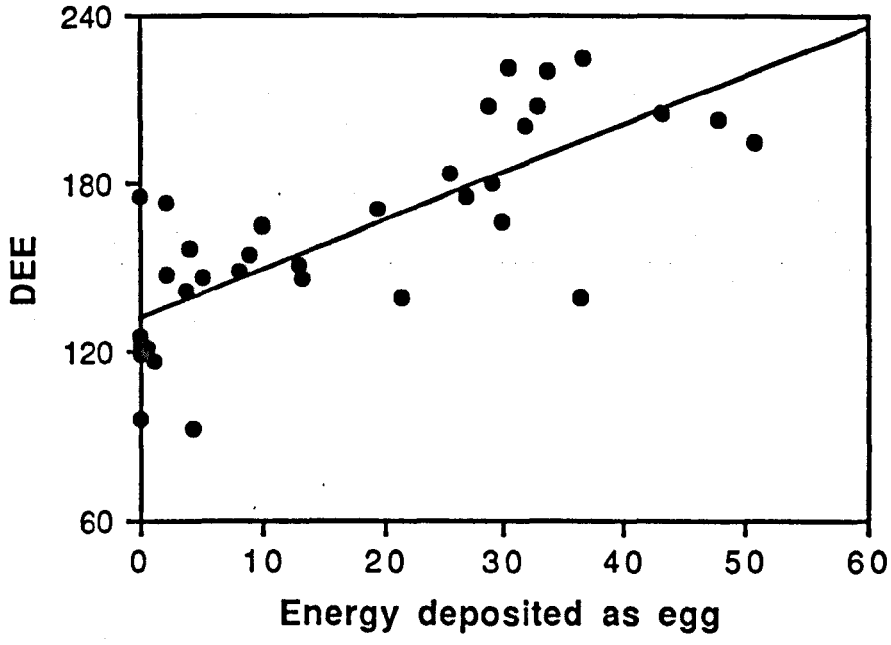


Fig. 6.2 Relationship between daily energy expenditure (DEE, kJbird^{-1}d^{-1}) of female Japanese quail and energy content of daily egg formation (kJ) ($y=1.74x + 132.0$, $r^2=0.59$, $p=0.0001$, $n=35$). Each data point represents the mean of 3 days.

Table 6.1 Stepwise multiple regression analysis of factors affecting energy expenditure ($kJbird^{-1}d^{-1}$) of female Japanese quail. Energy expenditure was measured by chamber calorimetry. Variables are tabulated in the order of inclusion in the regression model. n = 35 birds. Variables not included at the 5% level of significance were food intake, activity count, whether of not energy expenditure was also measured by the DLW technique, and number of eggs laid.

Independent variable	Cumulative r^2	Regression equation	t	p	Beta weight
Egg production ($kJbird^{-1}d^{-1}$)	0.58	$1.10x_1$	3.29	<0.003	0.488
Mass of bird (g)	0.64	$+1.33x_2$ -26.43	-2.63	<0.02	-0.390

the 5 birds labelled by intraperitoneal injection (5855-6343 ppm ^{18}O and 1087-1527 ppm deuterium). Labelling by either route appeared to result in a similar enrichment of the body water pool. This may be a useful alternative method of labelling small birds, where damage to developing ova is a risk.

6.3.4 Is yolk, albumen and the rest of the bird a single water pool?

There was no evidence for fractionation of labelling isotopes between the bird and either egg albumen or ovarian follicles. Albumen samples had ^{18}O and deuterium concentrations which fitted the same mono-exponential decline in labelling isotope concentration as blood samples, (taking into account that the eggs were laid at different times from those at which blood samples were taken) (Fig 6.3, Table 6.2). A sample of yolk from an ovarian follicle, taken immediately after sacrifice of bird 1, also had isotope concentrations close to those in the blood sample taken a few minutes earlier (Fig. 6.3, Table 6.2).

These results suggest that it might be unnecessary to take blood samples from laying birds to sample the body water pool: rates of loss of labelling isotopes could be determined from albumen samples alone.

6.3.5 Validation of the doubly labelled water technique for laying Japanese quail

Six of the nine females labelled to measure energy expenditure by the DLW technique continued to lay during the period of the energy expenditure measurement. The energy expenditure of these birds was measured simultaneously by chamber calorimetry and the DLW technique for 3 days. Only data collected over the first 48 h will be presented here. This was the same period over which the results of the two techniques of energy expenditure measurement were compared for male quail (Chapter 5).

The chamber calorimetry, DLW (blood) and DLW (albumen) measurements of energy expenditure are shown in Table 6.3. Algebraic mean percentage difference

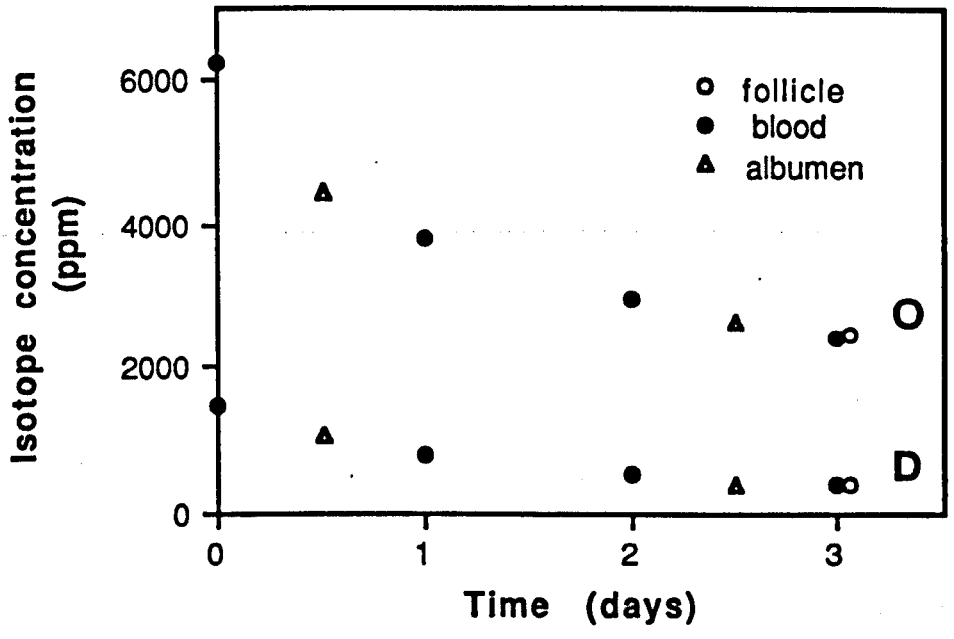


Fig. 6.3 Concentration of ^{18}O and deuterium (D) (ppm) in blood and albumen samples of the body water pool of a female quail, plotted against time. Samples were taken during the period of energy expenditure measurement by the DLW technique and from an ovarian follicle shortly after the bird was killed at the end of the period of energy expenditure measurement. Data are tabulated in Table 6.2.

Table 6.2 Concentration of ^{18}O and deuterium (ppm) in the body water of a female quail, calculated from blood albumen over a period of 3 d, and an ovarian follicle, sampled shortly after the bird was killed at the end of the period of energy expenditure measurement by the DLW technique. Data are shown in Figure 6.3.

Time after labelling (d)	Concentration		Sampling Route
	^{18}O	D	
0	6256	1487	Blood
0.5	4454	1064	Albumen
1	3824	813	Blood
2	2961	534	Blood
2.5	2631	425	Albumen
3	2440	408	Blood
3.05	2458	401	Follicle

Table 6.3 Comparison of energy expenditure ($kJbird^{-1}d^{-1}$) of laying Japanese quail measured simultaneously by chamber calorimetry (C), the DLW technique using blood samples of the body water pool (D), and the DLW technique using albumen samples of the body water pool (A). Mass = mean mass at start and end of 2 d over which measurements of energy expenditure were compared. Algebraic mean = mean for column including sign of data. Mean deviation = mean for column with all data made positive.

Bird		Energy expenditure			Differences between methods					
number	Mass	C	D	A	%(D-C)/C	D-C	%(A-C)/C	A-C	%(D-A)/D	D-A
1	132.2	174.4	139.3	110.8	-20.1	-35.1	-36.5	-63.6	20.4	28.5
2	127.7	211.5	192.6	265.0	-8.9	-18.9	25.3	53.5	-37.6	-72.4
3	142.9	218.0	181.2	192.1	-16.9	-36.8	-11.9	-25.9	-6.0	-10.9
4	141.5	191.1	264.4	214.7	38.3	73.3	12.4	23.6	18.8	49.6
5	126.6	150.4	140.1	162.3	-6.8	-10.3	7.9	11.8	-15.8	-22.1
6	139.6	202.7	219.3	133.3	8.2	16.6	-34.2	-69.4	39.2	86.0
Mean	135.1	191.4	189.5	179.7						
sd	7.2	25.3	48.0	56.3						
Algebraic mean					-1.0	-1.9	-6.2	-11.6	3.2	9.8
Mean deviation					16.5	31.8	21.4	41.3	23.0	44.9

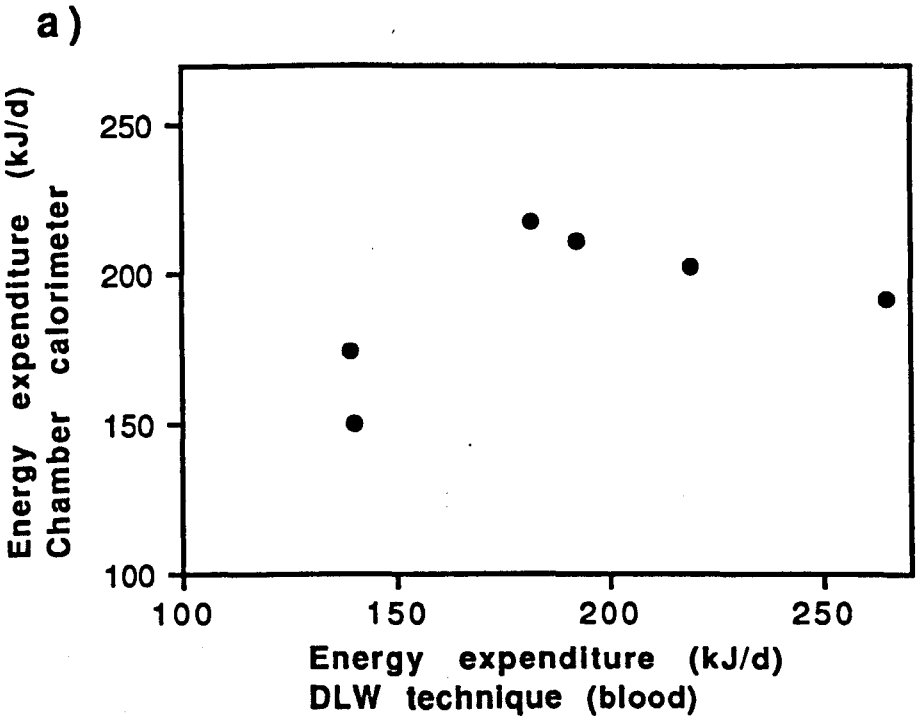
between chamber calorimetry and DLW (blood) was only -1.0% and between chamber calorimetry and DLW (albumen) only -6.2% though agreement between techniques for individual birds was poorer. There were no significant relationships between the results of the three techniques of measurement energy expenditure (Fig. 6.4) although this would be in part due to the small sample size. The slope of the geometric mean regression of energy expenditure measured by chamber calorimetry and that measured by the DLW technique using either route of sampling was significantly less than 1 (Fig. 6.4). The slope of the relationship between DLW (blood) and DLW (albumen) results did not differ significantly from unity.

There were no significant correlations between the energy content of egg formed and energy expenditure measured by the three technique (Spearman correlations, all $r<0.78$, $p>0.1$), probably due to the small sample size, although the birds were a subset of those for which calorimeter energy expenditure was correlated with egg formation (Fig. 6.2).

6.4 Discussion

6.4.1 Net energy requirement for egg formation

The energy expenditure measured by either chamber calorimetry or the DLW technique, did not include energy stored in the egg synthesised during the measurement period. The total daily power output of laying birds is therefore the sum of metabolic energy expenditure and the energy content of egg formed. Females which laid daily had 49% greater DEE than birds which had not begun to lay, and each additional unit of energy deposited in the eggs required 1.74 additional units of DEE (Fig. 6.2). These female quail only deposited 36% of their additional energy output (additional DEE + energy deposited in eggs) as egg, about half the efficiency usually estimated for the domestic fowl (Brody 1945) (36% calculated from 1/2.74, where 1=units of energy in egg and 2.74=additional energy output (1 unit of energy in egg and 1.74 additional units DEE). The low efficiency of egg formation was possibly due to the low calcium content of the diet, as the diet used was formulated



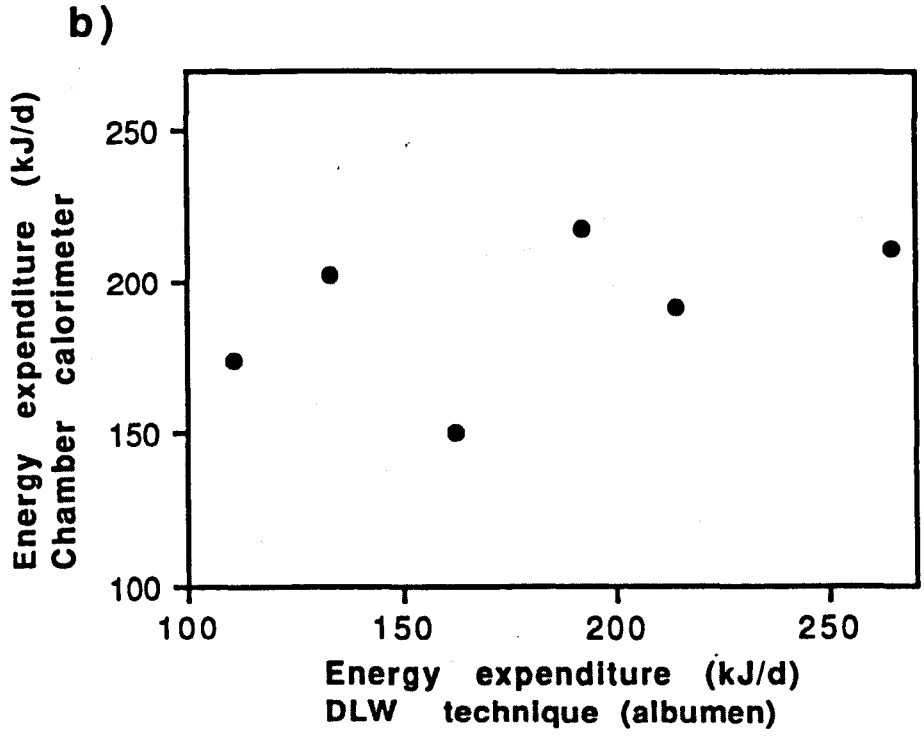


Fig. 6.4 continued overleaf

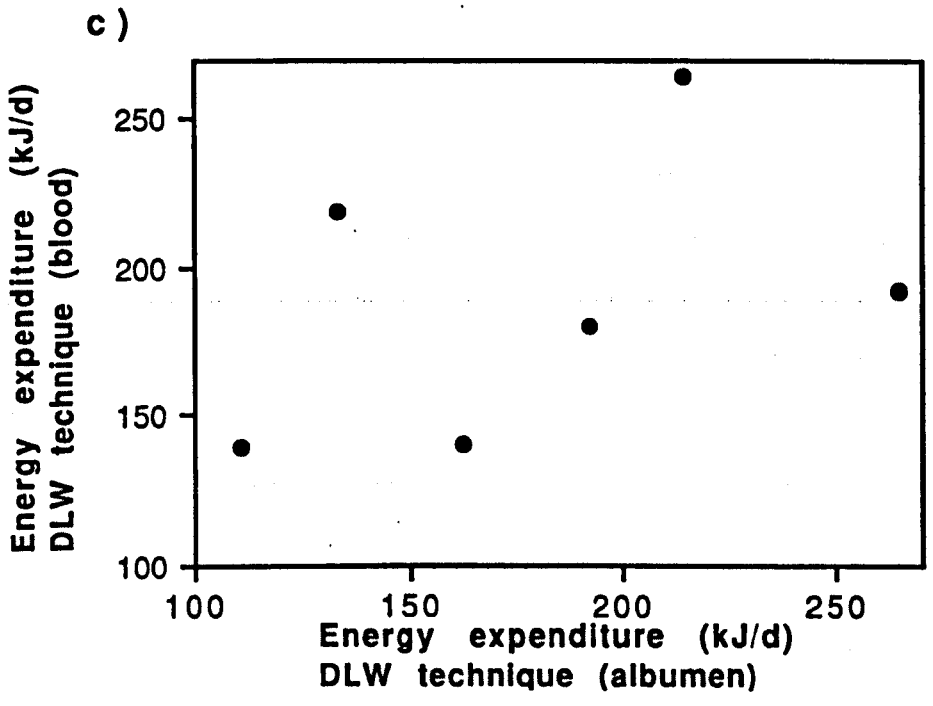


Fig. 6.4 Relationships between results of three techniques of measurement of energy expenditure ($kJbird^{-1}d^{-1}$). a) chamber calorimetry and the DLW (blood) technique: $y=0.278x + 1138.678$, $r^2=0.25$, $p=0.3$, $S_b=0.228$, $n=6$, b) chamber calorimetry and the DLW (albumen) technique: $y=0.202x + 155.010$, $r^2=0.22$, $p=0.3$, $S^b=0.198$. c) DLW (blood) and DLW albumen) techniques: $y=0.727x + 58.760$, $r^2=0.18$, $p=0.4$, $S_b=0.386$.

for growing rather than laying quail. However, it was more likely that a low efficiency of egg formation was typical of quail, as they also had a low efficiency of egg formation when fed the layers' diet.

6.4.2 Validation of the DLW technique for laying birds

Potential reasons for discrepancies between DLW and chamber calorimetry measurements of energy expenditure of male quail (Section 5.4.2) will also apply to laying female quail. As female quail had a greater metabolic rate than males (Fig. 6.1), measurement error associated with low energy expenditure of the subject will be reduced. These included a possible reduced precision of the DLW technique when isotope depletion was small and of the calorimetry system at low respiratory gas concentrations.

There are several potential problems associated with use of the DLW technique which apply specifically to laying birds:

1. Possible fractionation of ^{18}O and deuterium between ovarian follicles or oviducal egg albumen, and the rest of the bird.

This could invalidate an important assumption of the DLW technique: complete mixing of labelling isotopes in the body water pool of the bird. There was no evidence of fractionation of water between ovarian follicles or albumen and the rest of the body (Section 6.3.4), until isolation of the water in the oviducal egg from the rest of the bird some time during egg shell formation. Shell formation requires around 10 hours for Japanese quail (Woodard and Mather 1964), so there will be a period of transition between complete isotopic equilibrium between egg and body water, and isolation of the egg whilst it was within the uterus.

If eggs are laid at approximately 24-hour intervals it is not necessary to know the exact time at which the oviducal egg is separated from the rest of the body water pool. The difference in concentration of the labelling isotopes between one egg and the next can still be used to calculate isotope depletion. Egg albumen would provide

a more accurate means of sampling the body water pool the more quickly and completely the egg was cut off from exchange of water with the rest of the bird. Although the transition may in reality be gradual the good agreement between DLW (albumen) energy expenditures, and those calculated from DLW (blood) samples or chamber calorimetry, suggest that any fractionation is of a limited extent and that egg albumen provides a valid means of sampling the isotopic composition of the body water pool.

2. Body water pool volume does not remain constant during egg production: as each egg is cut off from the rest of the bird the pool size is reduced sharply.

This is clearly different from the assumption that the rate of water flux is constant and that the volume of the body water pool remains constant during measurement of energy expenditure by the DLW technique. This was the reason proposed by Meijer *et al* (1989) for the poor agreement between the DLW technique and a food balance estimate of energy expenditure. Yet, only when body water volume either halves or doubles during the measurement period will the rate of carbon dioxide production be influenced by more than 5% (Nagy 1980). Each egg contains only a mean of 7.3% (n=11) of body water of a female quail so this deviation from the model assumed in the calculation of metabolic rate from the DLW technique is probably unimportant.

3. Less of the water loss from laying birds will be evaporatively fractionated than from birds which do not produce eggs, as water lost in the eggs is unfractionated.

Equation 5.2, used to calculate energy expenditure from isotope turnover rates, assumes that 50% of water loss is fractionated. However, measured metabolic rate is relatively insensitive to the proportion of water loss that is fractionated (± 3% for variation between 0.3 and 0.7 in the proportion of water loss which is fractionated, James *et al* 1988). A decrease in the proportion of fractionated water loss associated with egg production would be unlikely to introduce an important error into the energy expenditure calculated.

4. Incorporation of deuterium label into fat synthesised for deposition as egg.

This would violate the assumption that ^{18}O and deuterium label only the water fraction of the subject. Clearly fat is synthesised during the measurement period, but if the extent of deuterium sequestration to fat was small in comparison with deuterium loss as water, this would not lead to an important departure from the assumption that only the water pool was labelled (Haggarty and McGraw 1988, Speakman 1990).

The DLW technique uses the difference in rates of loss of the ^{18}O and D to calculate energy expenditure the volume of carbon dioxide produced by the subject. If D is lost to fat synthesis as well as in water leaving the subject, this would increase k_D, decrease the difference between k_O and k_D and lead to underestimation of the metabolic rate calculated by the DLW technique (Equation 5.2).

Data from this experiment showed the opposite trend to that expected if increased fat synthesis in birds which formed the most egg lipid (measured by yolk mass) caused underestimation of energy expenditure by the DLW technique. Differences between the DLW (blood) or DLW (albumen) results and chamber calorimetry measurements were non-significantly positively correlated with mass of yolk formed (Table 6.4).

In a comparison of energy expenditure measurements of growing pigs the DLW technique overestimated by 7.58% when compared with simultaneous chamber calorimetry, because of the incorporation of deuterium into fat (Haggarty *et al* 1991). The data from laying quail suggested that the extent of deuterium sequestration to fat was small enough for this to have an unimportant effect on metabolic rates calculated by the DLW technique.

5. Discrepancy between DLW (blood) or chamber calorimetry and DLW (albumen) results due to a difference in the period over which the measurements were taken.

Chamber calorimetry and DLW (blood) results were calculated over 24 h periods running from approximately 0930 each day, whilst the exact time at which the albumen samples became isolated from isotopic exchange with the rest of the

Table 6.4 Spearman correlations between the mass of yolk formed (g) during the period of measurement of energy expenditure (kJd^{-1}) by three techniques: chamber calorimetry (C), DLW using blood samples (D), and DLW using albumen samples (A), and with differences between measurements of energy expenditure made by the 3 techniques. (n = 6 birds.)

Measurement of energy expenditure or difference between measurements of energy expenditure	**r**	**p**
C	-0.03	ns
D	0.71	ns
A	0.77	ns
%(D-C)/C	0.77	ns
D-C	0.71	ns
%(A-C)/C	0.89	*
A-C	0.77	ns
%(D-A)/D	-0.43	ns
D-A	-0.14	ns

body water pool was not known. As egg shell formation requires 10 h (Woodard and Mather 1964), and eggs were laid at around the time at which the lights come on each day (0700 hours), it was likely that the time at which the albumen was separated from the rest of the body water pool was approximately 12 hours out of step with the time at which the blood samples were taken and over which the calorimeter was run. Although calorimeter energy expenditure measurements on consecutive days for birds were significantly correlated (Pearson correlation, r=0.76, p=0.001, n=35), day to day variation in energy expenditure will also account for some of the difference between DLW (albumen) measurements of energy expenditure and the other two methods.

An associated problem was that the interval between laying of consecutive eggs was not always 24 h. The normal interval between eggs is slightly over 24 h (Woodard and Mather 1964). As the calorimetry chambers were sealed for most of the time, the exact time of laying was unknown, and could have been different from the 24 h assumed in the calculation of energy expenditure.

Eggs were removed from the calorimetry chamber each morning, and the albumen sampled around 1000 hours. The time delay between laying of the egg and sampling of the albumen could have allowed fractionated loss of water from the egg.

These three potential problems in the use of albumen to sample the body water pool were either not important, or caused a similar bias in the concentration of labelling isotopes in each egg (so that the difference in concentration between eggs was unaffected), as DLW (albumen) results were in good mean agreement with those from DLW (blood) and chamber calorimetry measurements.

It was concluded that DLW (albumen) and DLW (blood) measurements of daily energy expenditure were in good mean agreement with chamber calorimetry (mean percentage differences of only -1.0 and -6.2%). None of the potential problems discussed above appeared to invalidate the use of the DLW technique with laying birds.

This work also demonstrated a new route of sampling the body water pool:

the albumen of freshly laid eggs. There was a mean percentage difference of only 3.2% between energy expenditure calculated from blood and albumen samples of the body water pool. Use of albumen samples would provide an elegant solution to the problems of recapturing free-living laying birds at a specific time. Albumen samples, conveniently provided by the bird at 24 h intervals, could be used instead of the blood samples normally required.

For birds which lay eggs which are large enough, it should also be possible to remove the small amount of albumen required for isotopic analysis and reseal the egg to allow successful development of the embryo. Deeming (1989) removed up to 5 ml (approximately 8.6% of egg mass, assuming a standard egg mass of 58.0 g, Romanoff and Romanoff 1949) from eggs of the domestic fowl, which went on to develop successfully. A much smaller volume, around 50µl, was required for isotopic analysis. This would represent only around 2.5% of the mass of a swallow egg (1.97 g, Chapter 4), so it might be possible to sample the isotopic composition of the albumen without the death of the embryo. This could be investigated in a future study.

Use of albumen, rather than blood samples, would also remove the requirement to hold the subject for 1-2 hours whilst the labelling isotopes mixed with the body water pool before taking the initial blood sample. A reduction, to around 10 min, in the time spent in captivity would greatly improve the chance that the DLW technique could be used to measure the energy expenditure of a variety of laying birds which might otherwise be too sensitive to disturbance.

CHAPTER 7

ENERGETICS OF LAYING SWALLOWS

7.1 Introduction

Introduction of the doubly labelled water (DLW) technique as a routine method in field ecology has allowed investigation of absolute and relative energy requirements at different stages in the annual and reproductive cycles. The DLW technique provides measurements of metabolic rate which are sufficiently accurate to measure the energetic consequences of differing individual characteristics, environmental factors and levels of reproductive output. Comparisons of this sort have been attempted in earlier work, by calculation of energy expenditure from activity budgets and temperatures recorded in the field combined with laboratory measurements of the costs of thermoregulation and activity (e.g., Holmes *et al* 1979, Ettinger and King 1980). These studies had the twin disadvantages that energy expenditure derived from activity budget models might be very inaccurate (e.g., Weathers and Nagy 1980), and imprecise quantification of potentially costly activities such as flight, diving, egg formation, incubation and moult. This meant that the relative living costs during different parts of the annual cycle could not be assessed without reliance on assumed costs for activities to which the final energy budget was very sensitive. The DLW technique has brought major advances to this field of study, and direct measures of living costs during all stages of the annual cycle have been obtained, with the important exception of laying females. Prior to this study, no direct measurement had been made of the energy expenditure of a normally laying free-living bird.

Previous measurements of energy expenditure using the DLW technique have concentrated on nestling-rearing birds. This was because birds were relatively easy to capture when they returned to the nest and their normal daily routine was unlikely to be disturbed because of the drive to feed the chicks. The ability to recapture birds at a predetermined time and to measure the energy expenditure of normal behaviour are both vital to the success of the DLW technique. The energy expenditure of adults

providing for the peak food demand of their chicks is also of interest as this was thought to be the most demanding stage of the breeding cycle, and therefore the time at which an energetic limitation to reproductive output might be detected (Lack 1954, Bryant and Westerterp 1980, Drent and Daan 1980, Ettinger and King 1980, Krementz and Ankney 1986, Bryant and Tatner 1990).

Egg formation has often been proposed to be potentially demanding for female birds in terms of energy requirement (King 1973, Ricklefs 1974, Walsberg 1983a,b, Blem 1990), but prior to this study, no direct measurement of the energy expenditure of a normally laying free-living bird had been made. The demanding nature of the laying period had been deduced from models of the energy and protein content of eggs, the rate of egg synthesis and the efficiency of egg deposition in captive birds. Yet egg formation by domestic fowl is not a good model for that of a wild passerine because of the generations of selective breeding for egg production in fowl, very low activity and thermoregulatory costs, an artificial diet, and the difference in egg composition between altricial and precocial birds. To add to these problems, different authors have concluded that egg formation has an efficiency of 70 or 75%, based on the same data (King 1973, Ricklefs 1974). This chapter develops an improved model from which to calculate the efficiency of egg formation for any bird for which egg composition is known.

In time budget studies of the annual cycle of energetics, laying females typically have a metabolic rate as high or higher than during nestling-rearing or in midwinter (reviewed by Walsberg 1983a; Bryant and Tatner 1988, Masman *et al* 1988). Only one previous study has attempted to make a direct measurement of energy expenditure for a free-living laying bird (Bryant and Westerterp 1980). Both laying house martins labelled in this study had metabolic rates similar to non-breeding or incubating birds and lower than those of rearing nestlings, but the measurements were considered unrepresentative of laying birds as both birds interrupted laying. Modification of the DLW technique for use with laying birds was considered a high priority (Bryant and Westerterp 1980). Possible alteration of the

laying pattern or nest desertion due to handling during laying has discouraged further attempts to use the DLW technique with free-living laying birds.

In this study, each of the potential barriers to use of the DLW technique with a free-living laying bird has been overcome. The DLW technique was validated for a laying bird by simultaneous measurement of energy expenditure by chamber calorimetry for captive quail (Chapter 6). An appropriate subject, the swallow, was chosen for the first study with a free-living bird, as this species could be captured reliably, was very tolerant of handling during laying and comparative data were available on the energy expenditure of incubating and nestling-rearing females (Westerterp and Bryant 1984, Chapter 9). As refinements, the volume of egg material deposited during the period of energy expenditure measurement could be determined using lipophilic dyes (Chapters 3 and 6), and the turnover rate of labelling isotopes could be determined from albumen samples rather than from blood (Chapter 6). This last variant on the standard operation of the DLW technique provided the possibility of a reduction in handling to the few minutes required to administer the isotope, as there was no need to take blood samples or recapture the subject. Obtaining a mass for the bird at the time at which each egg was laid would require use of a balance which could be used to remotely record mass. This advance promises to make laying the easiest, rather than the most difficult, stage of the annual cycle during which to measure the energy expenditure of a free-living bird using the DLW technique.

7.2 Methods

7.2.1 Measurement of energy expenditure

7.2.1.1 Chamber calorimetry

The resting metabolic rate (RMR) of laying, incubating and nestling-rearing female swallows was measured overnight by chamber calorimetry. Females were caught shortly after dusk during the laying, incubation and nestling-rearing periods. Each bird was weighed, structural size and condition indices recorded (Chapter 2)

before being placed on a perch in the darkened calorimeter chamber between approximately 0000 and 0430 hours. At dawn, birds were re-weighed and released close to their nest site. All birds placed in the calorimeter went on to complete laying, incubation or nestling-rearing.

Metabolic rate was calculated from the rates of oxygen consumption and carbon dioxide production according to the principles described in Section 5.2.1. The calorimetry chamber was enclosed within a darkened incubator. The level of activity was recorded on an arbitrary scale using a Doppler-radar activity meter. Air leaving the chamber was dried by passing over Drierite, prior to analysis by two separate gas analysis systems. The first system consisted of an MSA infrared gas analyzer which measured carbon dioxide concentration and a Beckman OM2 polarographic oxygen analyzer which provided output to chart recorders. Chart output was used to monitor the bird during the run, to make a visual inspection of the data to determine when the lowest metabolic rate was attained and as a backup source of data in case of malfunction in the second analysis system. The second, and newer, analysis system consisted of a VG Quadrapole 200 AMU mass spectrometer with Mediflex software, which determined carbon dioxide and oxygen concentrations in the exhalant stream every 5 min. Metabolic rate could be calculated from the data collected by either analysis system. Only mass spectrometer data were used, as this was digitally recorded and more amenable to analysis by computer, except for bird C4 (Table 7.2), as a fault developed in the mass spectrometer software during the night this bird was in the calorimeter. The gas analysis systems sampled ambient air once every 2 hours, to allow calculation of the difference in concentration of oxygen and carbon dioxide between chamber and ambient air. The total volume of air which passed through the chamber was recorded so that the volumes of oxygen consumed and carbon dioxide produced could be calculated from the differences in concentration between chamber and ambient air. Volumes were converted to STP and resting energy expenditure ($kJbird^{-1}d^{-1}$) calculated from the period of the lowest level of gas exchange, over 1 hour between approximately 0230 and 0400 hours. Resting metabolic rate (RMR, $cm^3CO_2g^{-1}h^{-1}$) was calculated on the basis of the

"metabolising mass" for laying birds (i.e., mean mass during calorimetry minus mass of egg material within a bird at that stage in laying, predicted from Table 8.6).

7.2.1.2 Doubly labelled water technique

The DLW technique (described in Section 5.2.2) was used to measure the energy expenditure of free-living laying female swallows. In the single previous study with free-living laying house martins the operation of the DLW technique disturbed the normal laying pattern (Bryant and Westerterp 1980). Swallows were therefore labelled at a variety of times during the laying period, using several different protocols, as it was anticipated that laying interruptions might occur due to damage to developing ova during injection of the isotopes, that partly formed eggs might be broken if birds were handled close to the time of laying or that behaviour might be altered if laying birds were captured twice within 2 d.

Thirteen laying female swallows were captured at roost, close to or on the nest either after dusk (2230-2400 hours) or before dawn (0215-0315 hours). One bird was caught using a mist net inside a barn during the afternoon. Each bird was weighed and labelled with 10 μlg^{-1} of a mixture of 10ml 20 ape $H_2{}^{18}O$ and 0.68g of 99.9 ape D_2O. The labelled water dose was given either by intraperitoneal injection or orally (birds 10 and 11) together with the beef mince containing lipophilic dye (Section 7.2.2). For birds labelled by injection, care was taken to avoid the part of the abdomen most likely to contain developing ova. The water was injected to the right of a central line, close to the edge of the posterior fat deposit. Initial blood samples from birds captured at dusk were taken either 1 h after the bird was labelled (at approximately midnight), or around 0400 hours, shortly before the bird was released. Initial blood samples from birds captured before dawn were taken 1 h after the bird was labelled. When the period of energy expenditure measurement was from midnight to midnight, the bird spent the remaining few hours of each night on a perch in a perforated cardboard box adjacent to an open window, in order to provide conditions similar to those at the natural roost. Final blood samples were taken 24 or

48 (range ±0.65 h) later, except for birds 7, 9 and 10 for which albumen samples only were taken, and bird 1 which evaded a recapture at roost and was not caught until the following morning, 4.15 h later than intended (Table 7.1). One bird which was labelled before the first egg was laid and recaptured subsequently built a new nest before laying. This bird was excluded from subsequent analyses due to uncertainty of her stage in laying during the measurement period.

Albumen from eggs which were ovulated after the labelled water dose was given to the bird could be used instead of blood samples to determine the isotopic composition of the body water pool (Chapter 6). Albumen samples were taken from eggs within 3 h of laying. Nests were not visited earlier in the morning in order to avoid disturbing birds during oviposition.

There were two advantages of the dawn to dawn measurement period (capture, labelling and recapture shortly before dawn or capture and labelling after dusk but delay of the initial blood sampling until dawn, followed by recapture shortly before dawn). First, the bird spent less time in total, and none of the DLW measurement period, in captivity. Second, if the bird was not recaptured at her roost, there was an opportunity to catch her with a mist net when she returned to the nest after dawn and still obtain a blood sample within 1-2 h of the ideal time. Midnight to midnight measurements, on the other hand, had the advantage that the lipophilic dye could be fed in the evening, which provided clearer dyed layers in yolks (Section 7.2.2).

The volume of the body water pool was calculated as a percentage of mass using the mean body water content of laying swallows determined from carcass analysis (66.53%, sd=7.07, n=3, Chapter 8), where mass=mean of mass at initial capture minus the mass of any oviducal egg (which was not in equilibrium with the rest of the body water pool once the shell was formed), and final mass (this included the ovulated egg, as this did contain labelling isotopes).

Table 7.1 Timing of the period over which energy expenditure was measured by the DLW technique for each laying female swallow. Route = route of sampling body water pool (A = albumen, B = blood). Duration = duration of the period of energy expenditure measurement (h). Max. 1 = whether period of more than 50% of the maximum energy content of daily egg formation occurred during the period over which energy expenditure was measured (calculated from Table 3.8). Max. 2 = whether more than 50% of the maximum energy requirement for egg production occurred during the measurement period (calculated from Table 7.15). Half indicates that more than 50% of the maximum energy content of, or requirement for, egg formation occurred during half the measurement period. Timing of the measurement period was coded:

I = Night before egg 2 was laid till night before egg 4 was laid (i.e., days included in the measurement period were day 1 and day 2, where the first egg was laid on day 0).
II = Night before egg 2 to night before egg 3 (day 1).
III = Afternoon after egg 2 to afternoon after egg 3 (second part of day 1 and first part of day 2).
IV = Night before egg 1 to night before egg 2 (day 0).
V = Night before day -3 to night before day -1 (day -3 and day -2).
VI = Night before day -1 to night before day 0 (day -1).

Ring numbers and nest sites of these birds and the date upon which they were labelled, are given in Appendix 6.

Bird number	Route	Duration	Clutch size	Timing of measurement	Max. 1	Max. 2
1	B	52.15	3	I	No	No
2	B	47.65	4	I	No	Half
2	A	24	4	Egg 3-egg 4	No	No
3	B	23.35	5	II	Yes	Yes
3	A	24	5	Egg 3-egg 4	No	Yes
4	B	23.73	4	II	No	Yes
4	A	24	4	Egg 3-egg 4	No	No
5	B	23.08	4	III	No	Half
6	B	24.65	1	IV	Yes	Yes
7	A	24	4	Egg 3-egg 4	No	No
8	B	47.38	5	V	Yes	Half
9	A	24	4	Egg 3-egg 4	No	No
10	A	24	5	Egg 3-egg 4	No	Yes
11	B	48.33	4	I	No	Half
12	B	48.38	5	I	Half	Yes
13	B	24.12	5	VI	Yes	Yes

7.2.1.3 Activity budget observations

Activity budgets of laying swallows were recorded during DLW measurements of energy expenditure in order to attempt to partition the energy requirements of maintenance, activity and egg formation. Observation periods of 1 to 2 h were distributed over the active day. The duration of each activity was recorded on a Psion Organiser with the "Time and Event" program (Stirling Microsystems, University of Stirling). This allowed each letter key on the Psion to be coded for an activity. When a key was pressed, the time and activity coded for were recorded. The program could be used to calculate the total time spent in each activity during each recording session.

Females were marked on the breast with non-toxic water soluble dye to enable them to be followed with binoculars (10 × 40). Activity was recorded in the following categories:

perched (on wire, gutter, etc.),
flapping flight,
gliding flight,
in farm building (perched at nest),
on ground (collecting grit),
out of sight.

Female swallows were never seen to perch far from the building in which they nested, so birds were assumed to be in flight whilst out of sight away from the farm. The time for which birds were out of sight was divided between flapping and gliding flight, in proportion to the amount of these two types of flight record during the period for which the bird was visible.

Nest-building was complete prior to the start of the periods when energy expenditure was measured for laying swallows, so any costs associated with nest construction (Withers 1977) would not be included in these measurements.

7.2.2 Use of lipophilic dye to determine the energy content of egg formed during DLW measurement of energy expenditure

Lipophilic dye (Sudan B) could be used to stain a layer of newly deposited yolk in each ovarian follicle undergoing the rapid growth phase (Chapter 3). Dye was fed to female swallows when they were caught to inject labelled water or to obtain blood samples. The volume of yolk deposited during the period of energy expenditure measurement was enclosed between the internal diameters of the dyed bands (Plate 7.1). The volume of yolk deposited was calculated using Equation 6.1. The energy content of yolk and albumen deposited was calculated from the mean energy density of swallow eggs (Table 3.2).

7.3 Results

7.3.1 Energy expenditure of laying swallows at rest

Metabolic rate declined during the first 2 to 3 h for which the bird was in the calorimeter chamber to reach a stable low level over the final 1 to 2 h (Figure 7.1). Resting metabolic rate (RMR) was calculated from the rates of oxygen consumption and carbon dioxide production during approximately the final hour of the measurement period (Table 7.2).

Laying birds continued to lay normally after release following chamber calorimetry (at least 2 more eggs laid at 24 hour intervals, and total clutch size at least 4 eggs), so they presumably synthesised egg material during the calorimetry measurement. Given the similarity in activity count and chamber temperatures between laying and non-laying (incubating and nestling-rearing) groups (Mann-Whitney U-test, both $Z<0.8$, $p>0.4$), it was expected that the laying birds would have higher metabolic rates than non-laying females. The difference in energy expenditure between the two groups would represent the net energy requirement for egg synthesis. There was, however, no significant difference between the resting energy expenditure ($kJbird^{-1}d^{-1}$) of laying and non-laying females ($Z=1.22$, $p=0.22$,

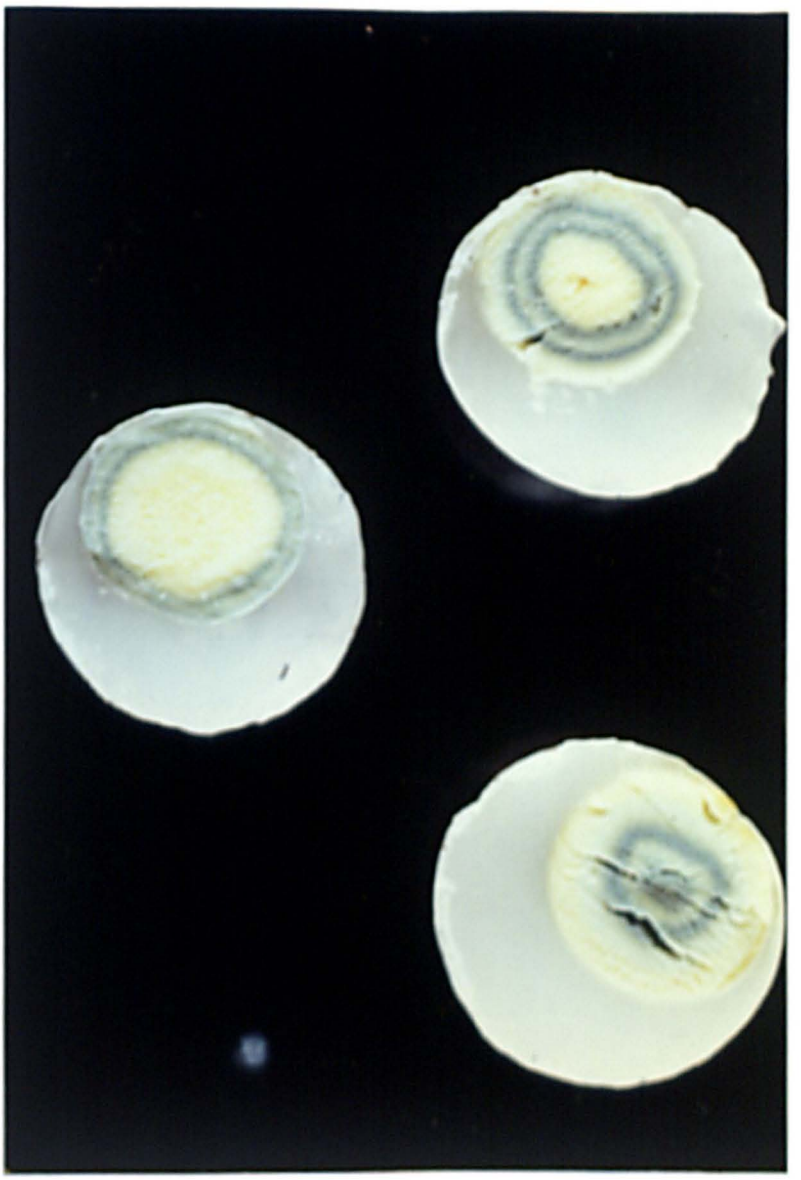


Plate 7.1 Hard boiled, sectioned swallow eggs, showing the bands from two dye feeds, at an interval of 24 hours, of a lipophilic dye which stained the outer layer of each rapidly developing ovarian follicle. The eggs are numbers 1 (left), 2 (upper right) and 3 (lower right) of a clutch of 5 eggs laid by bird 13 during the period for which energy expenditure was measured by the DLW technique. The first dye feed was made at midnight 2 days before the morning on which the first egg was ovulated.

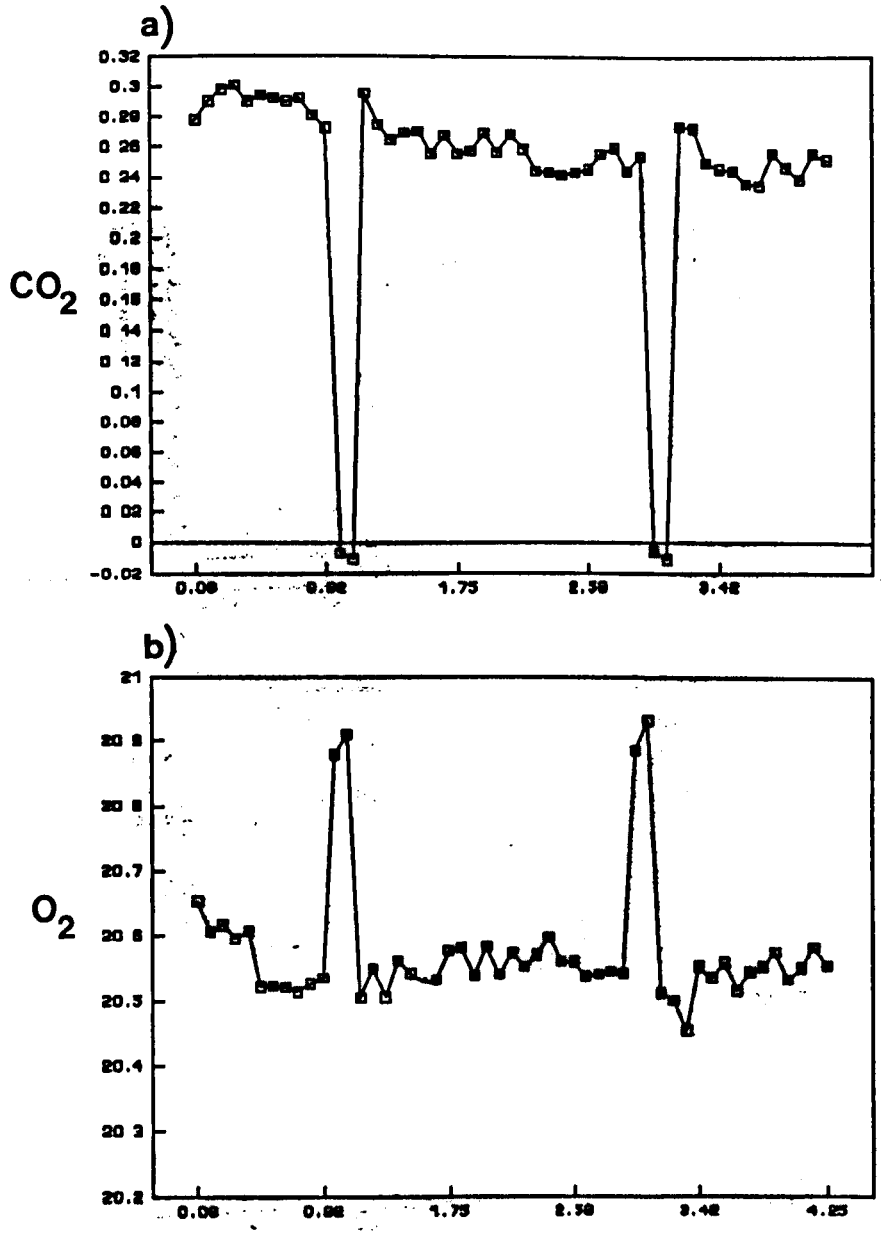


Fig. 7.1 Change in concentration (%) of a) carbon dioxide, b) oxygen during overnight chamber calorimetry with a laying female swallow. Major peaks and troughs show sampling of ambient air. Ambient concentrations of gases were taken from the second peak/trough.

Table 7.2 Resting metabolic rate (RMR, $cm^3CO_2g^{-1}h^{-1}$) and equivalent daily energy expenditure (DEE, $kJbird^{-1}d^{-1}$) of female swallows measured overnight by chamber calorimetry. Activity count (arbitrary units) refers to the entire duration of the period for which the bird was in the chamber, which was longer than the period over which RMR was calculated. RMR was calculated from approximately the final hour of calorimetry (between 0230 and 0400 hours), when oxygen consumption and carbon dioxide production reached a steady low level. Stage refers to the stage of reproduction of each bird (L = laying, I = incubating, N = nestling-rearing). Mass 1 was mean mass during the period of calorimetry. For laying birds, Mass 2 was the metabolising mass, i.e. Mass 1 minus the mass of ovarian follicles and oviducal eggs (calculated from Table 8.6). BMR ($kJbird^{-1}d^{-1}$) was calculated from Aschoff and Pohl (1970) inactive phase ($BMR = 24 \times 0.1326 \times mass^{0.726}$, where mass = Mass 1 for incubating and nestling-rearing birds and Mass 2 for laying birds).

Bird number	Stage	Mass 1	Mass 2	RQ	Activity count	Temp. °C	RMR	DEE	BMR
C1	L	23.10	20.81	0.90	1100	16.4	3.98	45.65	28.83
C2	L	22.30	20.01	0.81	9981	16.25	5.06	60.81	28.02
C3	L	26.50	24.05	0.55	12326	14.29	4.92	101.07	32.02
C4	L	24.00	21.55	0.81	3732	15.49	5.24	67.41	29.57
C5	I	21.10	-	0.96	7695	16.57	6.83	73.34	29.12
C6	I	20.00	-	0.95	8282	12.74	10.45	104.07	28.01
C7	I	19.90	-	0.87	2138	15.54	5.76	64.92	27.91
C8	N	20.60	-	0.85	2215	15.67	6.34	74.89	28.62
C9	N	20.60	-	0.83	1989	15.21	5.69	69.07	28.62
Mean	L	23.97	21.60	0.77	6785	15.6	4.80	68.74	29.61
sd		(1.82)	(1.75)	(0.15)	(5246)	(1.0)	(0.56)	(23.40)	(1.73)
Mean	I+N	20.44	-	0.89	4464	15.15	7.01	77.26	28.46
sd		(0.49)	-	(0.06)	(3225)	(1.44)	(1.98)	(15.48)	(0.50)
Mean	All birds	22.01	-	0.84	5495	15.4	6.03	73.47	28.97
sd		(2.20)	-	(0.12)	(4125)	(1.2)	(1.85)	(18.59)	(1.27)

n=4 laying and 5 non-laying birds, Table 7.2), whilst the resting metabolic rate (RMR, $cm^3CO_2g^{-1}h^{-1}$) was significantly lower for the laying than the non-laying group ($Z=2.45$, $p=0.02$, Table 7.2). The difference in energy expenditure between laying and non-laying females was greater when expressed per gram than when expressed per bird because laying females were heavier than the other birds (even after subtraction of the mass of ovarian follicles and the oviducal egg, as these parts would have minimal metabolic activity).

Neither temperature nor activity count were significantly correlated with energy expenditure or metabolic rate (Spearman correlations, all $r<\pm0.6$, $p>0.09$, $n=9$), although the chamber temperature was slightly lower for bird C6 (Table 7.2) which had the highest metabolic rate. Bird C3 (Table 7.2) had a high energy expenditure, but this was accounted for by her greater mass and her metabolic rate was similar to that of the other birds. The RQ of most of this sample of swallows was higher than would be expected for a bird metabolising lipid (RQ 0.7 to 0.8, Brody 1945).

The RMR determined in this experiment for laying swallows was substantially higher (2.3×BMR) than BMR predicted from the equation of Aschoff and Pohl (1970) (Table 7.2). The chamber temperature was lower than the lower critical temperature for swallows (Kendeigh *et al* 1977), which would account for much of this discrepancy. A longer measurement period might have brought the measured metabolic rate closer to BMR, but as oxygen consumption and carbon dioxide production achieved constant levels in most birds towards the end of the measurement period an increase in the time spent in the calorimeter is unlikely to have lowered metabolic rate substantially. A possible explanation might be that as the ADMR (measured by the DLW technique) of nestling-rearing female swallows was higher than in laying birds, a component of this increase might be a higher BMR (Daan *et al* 1990). Another reason for the low metabolic rate of laying birds at night might be that egg synthesis was not constant throughout the daily cycle. Some evidence for a difference in the composition of yolk formed at night has been suggested by the day/night layers in egg yolk (Grau 1976). Laying birds also have a

lot of non-metabolising fat, so "lean" metabolism would not differ greatly between laying and incubating or nestling-rearing birds.

7.3.2 Energy expenditure of free-living laying swallows

7.3.2.1 Affect of DLW technique upon laying pattern

In the single previous study in which the DLW technique was used to measure the energy expenditure of a free-living laying bird, the female house martins involved interrupted laying during, or after, the measurement period (Bryant and Westerterp 1980). This was accounted for by the trauma caused to the bird by handling during the laying period. In the study with Japanese quail (Chapter 6), it was demonstrated that oral administration of labelling isotopes resulted in a similar enrichment of the body water pool as the conventional intraperitoneal injection. This provided an alternative route of labelling laying swallows which would avoid possible damage of developing ova. Two swallows were labelled orally and 12 by intraperitoneal injection. Birds labelled by either route showed a similar enrichment of labelling isotopes (mean 4902 ppm ^{18}O for oral labelling, within the range 4304 - 5623 ppm, mean=5030, sd=408, for labelling by injection). Neither of the birds labelled orally showed interruption of the normal laying pattern, but only 2 (18%) of the birds labelled by intraperitoneal injection did, and in one of these the interruption did not occur until 2-3 d after the bird was labelled, so route of labelling did not seem to be an important factor in the success of the measurement of energy expenditure.

No blood samples were taken from two of the birds, in order to assess whether taking blood samples had a large effect on energy expenditure or laying pattern (energy expenditure was calculated from albumen samples). Blood sampling of other birds was performed either at midnight or at dawn and one bird was caught in the afternoon, to determine whether labelling of laying birds was only successful at night. None of these different treatments had an obvious affect either on laying pattern or energy expenditure.

Females labelled before the first egg was laid were more likely to stop laying (2 of 4 individuals, although one of these apparently behaved normally until after recapture). The one female which evaded a roost recapture, spent the rest of the night outside the barn and was recaptured in a mist net at dawn, subsequently failed to complete her clutch. Prolonged disturbance of this sort should be avoided in future studies. Otherwise, swallows seemed tolerant of several variants of degree of handling, time and duration of capture, and of operation of the technique. The disturbance caused by operation of the DLW technique was therefore thought unlikely to alter the energy expenditure of laying birds, via an effect on their behaviour or egg production to a greater extent than during other stages in the reproductive cycle.

7.3.2.2 Comparison of metabolic rate determined from blood and albumen samples

There were no significant differences in ADMR or DEE calculated from the rates of isotope depletion between pairs of albumen and blood samples for birds for which both types of sample were available (Wilcoxon matched pairs signed ranks test, all $Z<1.07$, $p>0.28$, $n=3$). Although the sample size in this analysis was small, the conclusion was the same as that for a larger sample of Japanese quail (Chapter 6): either route of sampling could be used to determine the elimination rate of the labelling isotopes. The period over which energy expenditure was measured was different for the blood and albumen sampling regimes, as albumen would be separated from the rest of the body water pool in the evening when the shell began to form (pers. obs.). whilst blood samples were taken between midnight and 0400 hours. The similarity of measurements of energy expenditure taken during overlapping periods as well as between measurements made over 24 and 48 h, suggested that energy expenditure did not vary substantially within the laying period.

Mean metabolic rate and energy expenditure were calculated from blood and albumen sampling regimes for birds for which results were available from both routes of sampling and treated in the same way as results derived either from blood

or albumen samples for the other birds (Table 7.3). The mean DEE of laying swallows was 112.28 $kJbird^{-1}d^{-1}$ (sd=18.33, n=13), equivalent to an ADMR of 8.31 $cm^3CO_2g^{-1}h^{-1}$ (sd=1.49) or 3.85×BMR (sd=0.68). This was 21.4% greater than the 92.5 $kJbird^{-1}d^{-1}$ calculated by Turner (1982) from activity budgets. The mean mass of these birds was 23.74 g (sd=0.93, n=12), of which 3.23 g (sd=0.77) was accounted for by the mass of ovarian follicles, oviducal eggs and oviduct (Table 7.4).

7.3.2.3 Factors affecting energy expenditure of laying swallows

Female swallows deposited egg material with an energy content of up to 9.6% of DEE during the period over which energy expenditure was measured (mean=5.3%, sd=2.7, n=12). Despite the variation in the energy content of egg formation, there were no significant correlations between measures of energy expenditure and clutch size, energy content of yolk, albumen or all egg material formed (Table 7.5). Females which subsequently laid larger clutches formed more yolk and a greater energy content of egg material (Spearman correlations between clutch size and the energy content of yolk and whole egg formed, both $r>0.85$, $p<0.001$, n=12). There were no significant correlations between the energy requirement for egg formation, energy content of yolk, albumen or whole egg formed during the period over which energy expenditure was measured and environmental factors, female mass, condition indices or structural size (all $r<\pm0.48$, $p>0.1$, n=13).

The mean energy content of egg deposited on each day during laying was calculated from Table 3.8, and the energy requirement for egg formation (taking the efficiency of egg formation into account) was calculated by the method shown in Table 7.15. The birds for which energy expenditure was measured during the periods of more than 50% of the maximum energy content of, or requirement for, egg formation, are shown in Table 7.1. The DEE and ADMR of birds for which energy expenditure was measured during the period when more than 50% of the maximum

Table 7.3 Energy expenditure of free-living laying swallows, measured by the DLW technique. Energy expenditure was calculated from the rate of depletion of labelling isotopes in the body water pool, sampled via blood and egg albumen. Mean energy expenditure was calculated from the mean of the blood and albumen results where both types of sample were analyzed for the same bird, or from either route for birds for which only one route of sampling was used. Mass 1 was the mean of initial and final masses. Mass 2 was the mean of initial and final mass from which the volume of the body water pool was calculated (i.e., initial mass minus the mass of any oviducal egg and final mass including oviducal egg mass. ADMR = metabolic rate ($cm^3CO_2g^{-1}h^{-1}$), DEE = daily energy expenditure ($kJbird^{-1}d^{-1}$), M, metabolic intensity = metabolic rate expressed as a multiple of BMR (calculated using metabolising mass (Table 7.4) and the equation of Aschoff and Pohl (1970) inactive phase). Mean BMR was 29.80 kJd^{-1}. - = no data.

Bird			Blood		Albumen		Mean		
number	Mass 1	Mass 2	ADMR	DEE	ADMR	DEE	ADMR	DEE	M
1	22.7	21.2	9.66	129.98	-	-	9.66	129.98	4.45
2	23.5	21.4	7.33	99.45	8.44	107.07	7.88	103.26	3.64
3	23.9	21.5	8.69	118.77	8.05	107.76	8.37	113.26	3.87
4	24.4	22.0	9.51	132.80	6.86	94.17	8.18	113.48	3.81
5	23.7	21.6	6.92	94.71	-	-	6.92	94.89	3.20
6	23.8	22.3	6.69	94.71	-	-	6.69	94.71	3.12
7	25.1	22.8	-	-	10.62	147.07	10.62	147.07	4.98
8	22.1	21.8	8.84	122.41	-	-	6.56	122.41	4.10
9	23.1	20.7	-	-	10.65	123.19	8.12	123.19	4.87
10	25.6	23.2	-	-	6.56	93.23	6.16	93.23	3.10
11	23.3	21.2	8.12	109.42	-	-	9.39	109.42	3.74
12	23.5	21.3	6.16	83.18	-	-	8.31	83.18	2.84
13	23.6	22.1	9.39	131.59	-	-	1.49	131.59	4.37
Mean	23.74	21.78	8.13	111.72	8.53	112.08	6.86	112.28	3.85
sd	0.93	0.69	1.28	17.89	1.77	20.33	1.30	18.33	0.68

Table 7.4 Mean mass of female swallows during measurement of energy expenditure by the DLW technique, with the mean mass of reproductive structures during the measurement period (developing ovarian follicles, oviducal eggs and oviduct, calculated from Table 8.6). Non-reproductive mass was calculated by subtraction of the mass of reproductive structures from the bird mass. n = 12.

Bird number	Whole bird (g)	Reproductive structures (g)	Non-reproductive mass (g)
1	22.70	2.15	20.55
2	23.50	3.44	20.06
3	23.95	3.75	20.21
4	24.40	3.44	20.96
5	23.70	3.78	19.92
7	25.15	3.62	21.53
8	22.15	1.28	20.87
9	23.10	3.26	19.84
10	25.65	3.62	22.03
11	23.35	3.62	19.73
12	23.55	3.86	19.69
13	23.60	2.97	20.63
Mean	23.74	3.23	20.50
sd	0.93	0.77	0.74

Table 7.5 Spearman correlation co-efficients between energy expenditure of laying female swallows (DEE in $kJbird^{-1}d^{-1}$, ADMR in $cm^3CO_2g^{-1}h^{-1}$ and M, metabolic rate expressed as a multiple of BMR), and a) clutch size and the energy contents (kJ) of yolk, albumen or whole egg formed during the measurement period, b) mean environmental factors during the 1 or 2 d of the measurement period (food = $\log_e(V+1)$ where V is insect suction trap volume, temperature in °C and rainfall in mm), and c) structural size of the bird (mm).
* = $p<0.05$, ns = not significant. n = 13.

a)

	Yolk formed	Albumen formed	Egg formed
DEE	0.11 ns	-0.08 ns	-0.09 ns
ADMR	0.15 ns	-0.07 ns	-0.09 ns
M	0.13 ns	-0.07 ns	-0.10 ns

b)

	Food	Max. temp.	Min. temp.	Mean temp.	Rain	Clutch size
DEE	0.04 ns	0.07 ns	-0.25 ns	-0.09 ns	0.50 ns	-0.11 ns
ADMR	0.09 ns	0.13 ns	-0.23 ns	-0.05 ns	0.35 ns	-0.14 ns
M	0.12 ns	0.15 ns	-0.21 ns	-0.02 ns	0.38 ns	-0.14 ns

c)

	Wing	Keel	Head	Tarsus	Outer tail	Second tail	Inner tail
DEE	-0.02 ns	0.61 *	-0.29 ns	-0.62 *	0.47 ns	-0.06 ns	0.19 ns
ADMR	-0.03 ns	0.67 *	-0.22 ns	-0.57 *	0.46 ns	-0.16 ns	0.38 ns
M	-0.03 ns	0.66 *	-0.20 ns	-0.58 *	0.48 ns	-0.12 ns	0.35 ns

energy content of, and energy requirement for, egg formation were not significantly different from the birds on days requiring less than 50% of the maximum energy requirement for, or content of, egg formation (Mann-Whitney U tests, all $Z<1.81$, $p>0.07$).

The energy expenditure of laying female swallows was not significantly correlated with environmental factors (Table 7.5). ADMR, DEE and M were positively correlated with keel length (Table 7.5, Figure 7.2a), although a negative correlation with tarsus length suggested the opposite relationship with structural size (Table 7.5). Fat score 1 at the start of the measurement period was negatively correlated with DEE, ADMR and M (Table 7.6, Figure 7.2b). None of the other indices or change in indices of body condition during the measurement period was correlated significantly with energy expenditure (Table 7.6). These results suggested that birds which had the most fat at the start of the measurement period used it during the period of energy expenditure measurement to supplement daily food intake. This would reduce foraging costs, and hence lower DEE. This suggested that laying swallows might use lipid reserves to supply energy for activity (when flight costs would be great, due the extra mass at this time), rather than for egg production.

Use of lipid reserves was also associated with lower living costs for breeding house martins (Bryant and Westerterp 1983a). Use of lipid reserves would not decrease the overall energy requirement during the breeding season, as any lipid reserve used would have had to be gained on a previous day, which would have increased living costs at that time.

Respiratory energy expenditure, measured by the DLW technique, formed only part of the total energy output of a laying bird, as the energy content of egg synthesised was not included. Total energy output was calculated from DEE plus the energy content of egg material formed. Total energy output was largely composed of DEE, so a high correlation with this variable was expected (Table 7.7). Of the other female characteristics and environmental factors which might effect energy expenditure, only initial fat score 1 and keel length were correlated with total energy

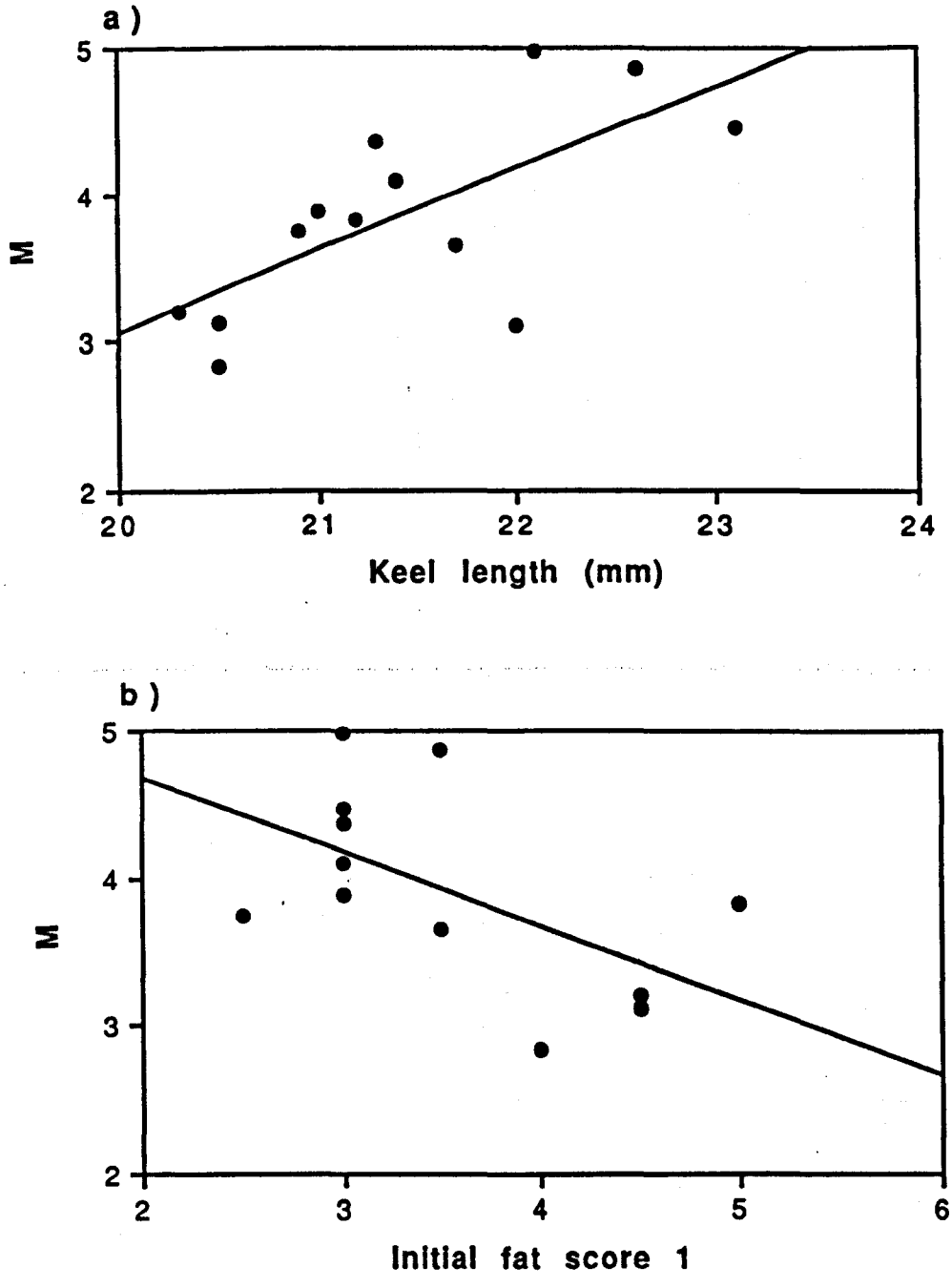


Fig. 7.2 Relationship between metabolic intensity (M) of free-living laying female swallows (measured by the DLW) technique and a) keel length, $y=0.561x - 8.175$, $r^2=0.48$, $p=0.009$, $n=13$, b) initial fat score $y=-0.508x + 5.691$, $r^2=0.35$, $p=0.04$.

Table 7.6 Spearman correlation co-efficients between energy expenditure of laying female swallows (DEE in $kJbird^{-1}d^{-1}$, ADMR in $cm^3CO_2g^{-1}h^{-1}$ and M, metabolic rate expressed as a multiple of BMR), and mass and body condition indices of the bird a) at the start of the measurement period, b) at the end of the measurement period, and c) change in mass and body condition indices of the bird during the measurement period. * = $p<0.05$, ns = not significant. n = 13.

a) Initial	Fat score 1[1]	USVOL[2]	USI[3]	USTHICK[4]	Mass (g)
DEE	-0.56 *	0.39 ns	-0.27 ns	0.06 ns	-0.20 ns
ADMR	-0.55 *	0.37 ns	-0.41 ns	-0.04 ns	-0.11 ns
M	-0.57 *	0.38 ns	-0.41 ns	-0.04 ns	-0.11 ns

b) Final	Fat score 1	USVOL	USI	USTHICK	Mass (g)
DEE	-0.23 ns	0.44 ns	0.25 ns	0.43 ns	-0.05 ns
ADMR	-0.37 ns	0.42 ns	0.17 ns	0.26 ns	-0.21 ns
M	-0.35 ns	0.42 ns	0.17 ns	0.26 ns	-0.15 ns

c) Change	Fat score 1	USVOL	USI	USTHICK	Mass (g)
DEE	-0.40 ns	-0.38 ns	-0.38 ns	0.34 ns	-0.06 ns
ADMR	-0.30 ns	-0.34 ns	-0.36 ns	0.25 ns	0.09 ns
M	-0.30 ns	-0.34 ns	-0.36 ns	0.25 ns	0.07 ns

[1] Fat score 1 is sum of anterior and posterior keel fat scores.
[2] USVOL = sum of pectoral muscle thicknesses (measured by ultrasound) × keel length/1000.
[3] USI = sum of pectoral muscle thicknesses/keel length.
[4] USTHICK = sum of pectoral muscle thicknesses.

Table 7.7 Spearman correlation co-efficients between total energy output of laying female swallows (respiratory DEE plus energy content of egg formed), and a) components of total energy output (energy content of yolk and whole egg formed and DEE ($kJbird^{-1}d^{-1}$)) and clutch size, b) environmental factors during the period of energy expenditure measurement (food = $\log_e(V+1)$ where V is insect suction trap volume, temperature in °C and rainfall in mm) and c) structural size of the bird (all lengths in mm). n = 12. *** = $p<0.001$, * = $p<0.05$, ns = not significant.

a)

	Yolk formed	Egg formed	DEE	Clutch size
Energy	0.21	0.04	0.98	0.00
output	ns	ns	***	ns

b)

	Food	Max. temp.	Min. temp.	Mean temp.	Rain
Energy	-0.04	0.04	-0.32	-0.13	0.54
output	ns	ns	ns	ns	ns

c)

	Wing	Keel	Head	Tarsus	Outer tail	Second tail	Inner tail
Energy	-0.05	0.70	-0.27	-0.57	0.57	0.05	0.30
output	ns	*	ns	ns	ns	ns	ns

output (Tables 7.7 and 7.8).

The proportion of time spent in flight (Appendix 5) (arcsine transformed) was not significantly correlated with energy expenditure, environmental factors, female characteristics or the energy content of egg formed (Spearman correlations, all $r<\pm 0.67$, $p>0.05$). Birds were difficult to keep in sight during observations and none was watched for more than 3 h of her active day. Time budgets were possibly therefore not comprehensive enough to account for the variation in energy expenditure measured by the DLW technique due to differences an activity pattern.

7.3.2.4 Calculation of energy expenditure of laying swallows from the activity budget

The mean activity budget of the laying birds for which energy expenditure was measured by the DLW technique was combined with data on the period spent roosting and at the nest by "balance" birds (Chapter 8), to calculate the energy expenditure of laying birds. Activity patterns varied between birds and times of the day (Table 7.9, Appendix 5) and with environmental factors (Turner 1980). The energy budget calculated here was intended as a comparison with the mean DEE determined by the DLW technique, so only variation in activity during different parts of the day and not that due to environmental factors or differences between birds was taken into account.

The length of the active day of laying swallows was determined from the mean time at which "balance" birds (Chapter 8) left the nest in the morning to the mean time at which they returned to roost in the evening (Table 7.10a). The mean time which females spent on the nest laying, was determined from the same birds (Table 7.10a). The activity budget of a laying female swallow was divided, to the nearest hour, between roosting, activity away from the nest and oviposition (Table 7.10b). The period spent away from the nest was categorised as flapping or gliding flight or perching (Table 7.9). The energy requirements assumed for each activity are shown in Table 7.11. Time spent on the ground collecting grit and

Table 7.8 Spearman correlation co-efficients between total energy output of laying female swallows (respiratory DEE plus energy content of egg formed) and mass and body condition of the bird a) at the start, and b) at the end of the measurement period, and c) change in mass and body condition of the bird during the measurement period. * = $p<0.05$, ns = not significant. n = 12.

a) Initial

	Fat score 1[1]	USVOL[2]	USI[3]	USTHICK[4]	Mass (g)
Energy output	-0.70 *	0.38 ns	-0.41 ns	-0.00 ns	-0.15 ns

b) Final

	Fat score 1	USVOL	USI	USTHICK	Mass (g)
Energy output	-0.30 ns	0.32 ns	0.05 ns	0.18 ns	0.01 ns

c) Change

	Fat score 1	USVOL	USI	USTHICK	Mass (g)
Energy output	-0.45 ns	-0.27 ns	-0.24 ns	0.18 ns	-0.01 ns

[1] Fat score 1 is sum of anterior and posterior keel fat scores.
[2] USVOL = sum of pectoral muscle thicknesses (measured by ultrasound) × keel length/1000.
[3] USI = sum of pectoral muscle thicknesses/keel length.
[4] USTHICK = sum of pectoral muscle thicknesses.

Table 7.9 Time budgets during different parts of the day in laying swallows for which energy expenditure was measured by the DLW technique. Data for individual birds are shown in Appendix 5.

	% flight			
Time	Flapping	Gliding	Total	% perch
Dawn - 1000	68.4	11.5	79.9	20.1
1000 - 1800	46.6	35.2	81.8	18.2
1800 - 2100	53.0	21.2	74.4	25.8

Table 7.10 a) Time at which laying female swallows left the roost in the morning, returned in the evening and time spent on the nest for oviposition, determined for 11 birds (Chapter 8). n = number of evenings/mornings or eggs for which times of arrival and departure were recorded. b) 24-hour time budget for average laying swallow with durations rounded to the nearest hour.

a)

	Mean	Range	n
Time of arrival at roost	2059	2006 - 2244	52
Time of departure from roost	0510	0426 - 0620	49

Time spent at the nest for oviposition, mean = 1h 9min, sd = 0.6, n = 31.

b)

	Duration
Roost	0000 - 0500
Active	0500 - 0600
Oviposition	0600 - 0700
Active	0700 - 2100
Roost	2100 - 2400

Table 7.11 Derivation of the energy requirement for each activity in the time budget of a laying female swallow of mean mass 23.74g and mean metabolising mass of 20.50g (Table 7.4). The cost of flight involving 93% flapping was 0.3063 $kJg^{-1}h^{-1}$, Turner (1982). The energy requirement for 100% flapping flight was calculated proportionally after subtraction of the cost of 7% gliding flight ($0.3063 \times 23.74 - 0.07 \times 3.5774)/0.93$). Resting metabolic rate, at 15°C, was calculated from equations in Kendeigh *et al* (1978) after Turner (1982) as 1.7887 $kJbird^{-1}h^{-1}$.

Activity	Energy requirement ($kJbird^{-1}h^{-1}$)	Source
Flight		
Gliding	2 × resting = 3.5774	Baudinette & Schmidt-Nielsen (1974)
Flapping	7.5496	Turner (1982)
Perching	1.5 × resting = 2.6830	Utter & Lefebvre (1970)
Roosting (15°C)	1.7887	Kendeigh *et al* 1978
Oviposition	1.58 × resting = 2.8261	MacLeod & Jewitt (1985)

Table 7.12 Daily time and energy budget of laying swallows, including sensitivity analysis for effect of activity cost assumptions.

Activity	Duration (hd^{-1})	Energy requirement ($kJbird^{-1}d^{-1}$)	% ΔDEE for 1% Δ in activity cost
Flight			
Gliding	3.91	13.995	0.139
Flapping	8.06	60.850	0.608
Perching	3.03	8.140	0.081
Roosting	8	14.310	0.143
Oviposition	1	2.826	0.028
Total	24.00	100.12 kJ	

perching at the nest were included in the perching category for the purpose of calculation of energy expenditure from the activity budget. Any difference in the energy requirement for perching at the nest or on the ground from perching on wires would have had minimal effect on the DEE calculated, as these activities were recorded for only a few minutes each day and DEE was not sensitive to variation in the cost of perching (Table 7.12).

The daily energy expenditure of an average swallow during laying, derived from the activity budget, was 100.21 $kJbird^{-1}d^{-1}$ (Table 7.12). This was 12.07 kJ, or 10.7% less than determined for the same birds using the DLW technique, but 7.71 kJ (8.3%) greater than determined by Turner (1982) for laying swallows. The difference between this and Turner's result were due to the larger proportion of the day spent in flight (11.97 h) compared to Turner's study in which more individuals were watched across a wider range of environmental conditions (9.22 h in flight). The increase in flight time in this study was accompanied by a reduction in time spent perching. The number of hours spent roosting was the same in both studies. If Turner's (1982) time budget was used with the energy requirements for each activity derived in Table 7.11, the DEE of laying swallows would have been 90.07 $kJbird^{-1}d^{-1}$, close to her result of 92.5 kJd^{-1}. The difference between DEE estimated from the activity budget and that derived from the DLW technique could be accounted for by the cost of egg synthesis. This will be discussed further in Section 7.4.1.3.

7.4 Discussion

7.4.1 Net energy requirement for egg formation by swallows

7.4.1.1 Calculation from a relationship between energy expenditure and the rate of egg formation

The relationship between daily energy expenditure and the energy content of egg material formed allowed calculation of the net energy requirement for egg production for Japanese quail (Chapter 6). There was no significant relationship between DEE and egg production for laying swallows, however, despite a range

between 0 and 9.6% of DEE in the energy content of egg produced (Section 7.3.2.3). The net energy requirement for egg production by swallows could therefore not be calculated in the same way as for quail.

A relationship between the rate of egg production and energy expenditure in captive quail but not in free-living swallows could be explained by the relative importance of the cost of egg formation in the two species: swallows only deposited up to 9.6% of DEE as egg, whilst the energy content of quail egg deposition was up to 26.2% of DEE (Chapter 6). This was because variation in energy requirements for activity and thermoregulation was low in captive quail, whilst in swallows activity levels and environmental factors would also influence DEE.

Female swallows which formed more egg might have compensated for the increased energy requirement for egg formation by a reduction in activity. The proportion of the day spent in flight, however, was not correlated with the amount of egg material formed (Section 7.3.2.3). Laying female swallows did spend a substantially greater proportion of the day perching than during other stages of the reproductive cycle (Turner 1980). This suggests that a reduction in activity may help laying swallows achieve energy balance. The relative importance of food intake, reserves and activity level in balancing the energy budget of a laying swallow are discussed in Chapter 10.

7.4.1.2 Calculation by comparison of the metabolic rate of laying swallows with females at other stages in the reproductive cycle

If the resting metabolic rate of laying female swallows had been greater than that of incubating or nestling-rearing birds, the difference could have been taken to represent the net energy requirement for egg synthesis. Unexpectedly, RMR was lower in laying females than in the incubating/nestling-rearing group, so the energy requirement for egg synthesis could not be calculated in this way (Section 7.3.1). Possible reasons for the higher metabolic rate of incubating and nestling-rearing females include a reduction in the rate of egg formation at night, changes in BMR

between stages of the breeding cycle and the similarity of the lean mass of laying and nestling-rearing/incubating birds (Section 7.3.1).

Measurement of the energy expenditure of laying, incubating and nestling-rearing female swallows using the DLW technique allowed comparison of relative living costs at different stages of the reproductive cycle (Table 7.13). Calculation of the net energy requirement for egg synthesis was not possible from these data either, for although the mean DEE of laying swallows was 3.9% greater than that of nestling-rearing, and 6.5% greater than incubating females, the differences in mass and activity pattern between laying and incubating or nestling-rearing birds would preclude calculation of the net energy requirement for egg formation from a direct comparison of living costs.

The mean ADMR of nestling-rearing females was slightly greater than that of laying birds, due to their lower mass (Table 7.13). Thus depending on whether total or mass-specific energy requirement was considered the more appropriate measure of living costs, either laying or nestling-rearing could be considered the most costly stage in the breeding cycle.

Determination of the net energy requirement for egg formation by comparison of DEE in laying and other birds would require measurement of the energy expenditure of a bird which foraged only for maintenance under the same environmental conditions as laying birds, and which had a similar mass and activity pattern. The energy expenditure of the male partner of a laying female could have been measured over the same period, but he would have been much lighter and might have used additional energy for mate-guarding or seeking extra-pair copulations. A pre- or post-breeding female would be lighter and subject to different environmental conditions, and juvenile birds would be lighter and might have different foraging costs due to inexperience or different aerodynamic properties (because of their shorter wing and tail feathers). These factors would prevent any swallow from being used as a non-laying standard with which the energy expenditure of laying females could be compared to determine the net energy requirement for egg production. This approach was therefore unpromising as a

Table 7.13 Energy expenditure of a) laying, b) incubating and c) nestling-rearing female swallows, measured by the DLW technique, and d) the total energy output of laying swallows (respiratory energy expenditure plus the energy content of egg material formed). Data for laying birds and four of the incubating birds are from this study (this chapter and Chapter 9). Data for nestling-rearing females and the other two incubating birds are from Westerterp and Bryant (1984). 95% CI = 95% confidence interval. 95 % CI overlapped for all stages for all measures of energy expenditure.

a) Laying (n = 13)

	Mean	sd	95% CI
ADMR	8.31	1.49	±0.73
DEE	112.28	18.33	±9.00
M	3.85	0.68	±0.33

b) Incubating (n = 6)

	Mean	sd	95% CI
ADMR	8.03	1.88	±1.49
DEE	105.40	22.89	±18.16
M	3.67	0.84	±0.67

c) Nestling-rearing (n = 8)

	Mean	sd	95% CI
ADMR	8.95	1.56	±1.03
DEE	108.00	19.00	±12.49
M	3.84	0.66	±0.43

d) Laying, total energy output (respiration + energy content of egg formed (n = 12)

	Mean	sd	95% CI
DEE	117.98	19.54	±10.05

method from which to determine the cost of egg formation, although relative living costs at different stages in the reproductive cycle are of also interest (Section 7.4.5).

7.4.1.3 Calculation by comparison of DEE measured by the DLW technique and that calculated from the activity budget

Energy expenditure was measured for laying female swallows using the DLW technique and, simultaneously, calculated from the activity budget (Section 7.3.2). The energy expenditure measured by the DLW technique included all demands for energy, whilst that derived from activity budgets included only activity and thermoregulatory requirements. It should therefore be possible to calculate the energy requirement for egg production from the difference between the DLW and activity budget estimates of energy expenditure. The mean cost of egg synthesis was 12.07 kJd^{-1}, when calculated in this way (Section 7.3.2.4). This suggested that the additional energy requirement for egg formation was 12.04% of DEE for activity and thermoregulation, and that the efficiency of egg formation by swallows was provisionally calculated as 33% (5.95/(12.07+5.95)). This low value was comparable with that determined for quail (36%, Chapter 6).

This method was rather unreliable as a means to calculate the energy requirement for egg production, as the DEE determined from the activity budget varied with the assumed values used to calculate the cost of flight. Other sources of error in the DEE derived from the activity budget, which could be eliminated in a future study, include estimation of the energy requirement for some activities from other species, extrapolation from the relatively short periods of observation to activity budgets for the entire day, and use of data on the duration of the active day determined for other swallows.

Calculation of DEE for a laying swallow from the activity budget was subject to a number of possible problems. The assumed cost of flapping flight might be too high, as the time budget model of daily energy expenditure was most sensitive to variation in the cost of this component (Table 7.12, Turner 1983). The cost of

flapping flight was derived from flight costs of nestling-rearing swallows on a per unit mass basis (Turner 1982). It was possible that the cost of flapping flight did not increase proportionally with mass (b=1), but that this scaled on some other factor, such as b=0.75. The procedure adopted would overestimate flight costs if this were to be the case. However, flight costs rose less rapidly with mass when calculated on a per unit mass basis (0.3063 kJg^{-1}, Turner 1982), than if Pennycuick's (1989) formulae were used (an increase in female swallow mass from 21 g to 24 g would increase the cost of flight by 17% according to Pennycuick 1989, Program 1, for a bird with a wing span of 0.291 m; and only by 14% using Turner's data). Flight costs of laying female swallows increased by around 5% per extra gram of body mass (calculated from the difference between a 21 and 22 g swallow, using Turner's relationship between mass and the cost of flight).

The cost of gliding flight was derived from that of herring gulls in wind tunnels, so again this component was open to re-evaluation, since a gull with a less aerial foraging mode in an laboratory situation would be expected to have a different metabolic rate during gliding than a free-living aerial forager which was not under stress and could take advantage of, or suffer problems due to, air currents.

Accurate estimation of the cost of the most energy demanding activities in the time budget has always been a problem, as activities such as flight or diving are difficult to reproduce in a calorimetry chamber. Estimation of the cost of flight has therefore followed three approaches: experiments in wind tunnels (e.g., Baudinette and Schmidt-Nielsen 1974), mass loss of homing birds which were prevented from feeding (e.g., Lyueeva in Hails 1979), and regression of DEE or ADMR on the percentage time spent in flight during DLW measurement of energy expenditure of a free-living bird. This last method is preferable, and has been used to compute flight costs which vary between the very high 23×BMR for the robin (Tatner and Bryant 1986) which might be associated with anaerobic metabolism, an intermediate 11.9×BMR for the willow tit (Carlson and Moreno 1992) to low values for birds which take or hunt their food from the air (6.3×BMR for the sand martin, Turner 1983; 4.8×BMR for the sooty tern, Flint and Nagy 1984; 8×BMR for the kestrel, Masman and Klassen 1987).

7.4.2 Net energy requirement for egg formation in captive birds

The efficiency of egg formation has been determined for a number of species using the input/output technique or chamber calorimetry. Efficiencies of egg formation (defined in Chapter 1) have been determined as 77%, 65% and 68% for the domestic fowl (Brody 1945, Chwalibog 1982, Kirchgessner 1982), 44-77% for zebra finch (El-Wailly 1966), 53.9-99.2% for bobwhite quail (Case and Robel 1974) and 36% for Japanese quail (Chapter 6). The mean efficiency from these studies (mean of mean from each study) was 63.8% (sd=15.1, n=6). The median efficiency was 66.5%. Calculation of the median efficiency from these studies was thought more representative as this result was not influenced by the single very low efficiency determined for Japanese quail.

These data cannot necessarily be applied directly to swallows for several reasons. The efficiencies of egg production were determined for captive birds, and all but one were precocial species, so they might not be applicable to a free-living altricial bird. There was also considerable variation in the efficiencies of egg production determined in different studies, which might be due to genetic, nutritional or environmental factors (Klein and Hoffmann 1989, Luiting 1990). Another reason for inconsistency in the estimation of efficiency is that egg is composed of protein and lipid in proportions which vary between yolk and albumen, altricial and precocial species and with age in domestic fowl (Chwalibog 1991). Since the efficiencies of protein and lipid deposition differ, when these components are deposited in different proportions the overall efficiency of egg production will change. The efficiency of egg production might therefore be more reliably calculated for altricial species by a factorial method, using the amount of lipid and protein in the eggs and the partial efficiencies of lipid and protein deposition (Section 7.4.3).

7.4.3 Calculation of the net energy requirement for egg formation from the partial efficiencies of lipid and protein deposition by growing animals

7.4.3.1 Partial efficiencies of lipid and protein deposition

Calculation of the efficiency of egg production might be possible using a factorial approach and the partial efficiencies of protein and lipid deposition determined from a variety of growing birds and mammals. This approach has the obvious disadvantage that efficiencies associated with tissue growth might be different from those of egg formation, and that efficiencies determined in one species might not apply to another. However, the energy required to change a given substrate into the appropriate protein or lipid would not differ with the final destination of the product. Any differences in the efficiency of protein or lipid deposition between growth, milk production and egg synthesis would therefore be due to differences in the type of lipid or protein synthesised or in transport to the appropriate site. For protein, there would be the additional complication of possible variation in the rate of turnover after initial synthesis (Van Es 1980). Despite these potential difficulties, the partial efficiencies of protein and lipid deposition in eggs appear to be similar to those in growing animals (Klein and Hoffmann 1989, Chwalibog 1991).

Theoretically, the efficiency of protein synthesis should be 86-88% (Klein and Hoffmann 1989). The efficiency of protein deposition is affected by the rate of turnover, and although turnover costs are unknown, reasonable assumptions suggest an overall efficiency for protein deposition of 61-65% (Van Es 1980). A recent survey of the energy requirement for protein deposition in growing animals found a very large variation in efficiency: 36-83% in non-ruminants (mean=55%, n=55) and 33-53% for ruminants (mean=38%, n=9) (Klein and Hoffmann 1989). In laying domestic fowl, the efficiency of protein deposition as egg also varies widely, with estimates of 44% (Hoffmann and Schiemann 1973, in Klein and Hoffmann 1989), 25-30% (Fisher 1980), 30-87.5% (Van Es 1980), 55-85%, with 85% as the preferred value (Bondi 1982), and 44-60% (Kirchgessner 1982). A partial efficiency of 66%

was determined for protein deposition in Japanese quail eggs (Farrell *et al* 1982).

Partial efficiencies determined for lipid deposition were mostly higher and lay within a narrower range. In their review, Klein and Hoffmann (1989) found a mean partial efficiency of lipid deposition of 81% (range 67-100%, n=57) for ruminant and non-ruminant animals. The mean value from this survey was in good agreement with the theoretical value of 75-85% calculated from the biochemistry of lipid metabolism (Nehring and Schiemann 1966, in Klein and Hoffmann 1989). Studies of growing birds provided a slightly lower efficiency for lipid deposition by the bobolink (58-60%, Gifford and Odum 1965). Partial efficiencies for lipid deposition in eggs were similar to the values in growing animals. Values of 80-90% (Kirchgessner 1982) and 74% (Klein and Hoffmann 1989) have been determined for the efficiency of lipid deposition in domestic fowl eggs, and of 83% for Japanese quail eggs (Farrell *et al* 1982).

The large range in estimates of the partial efficiencies of lipid and protein deposition is partly due to a problem associated with calculation of these efficiencies from multiple regression of protein and lipid deposition on total energy gain. Protein and lipid deposition are often highly correlated which could lead to large errors in the partial efficiencies (e.g., Van Es 1980, Roux *et al* 1982, Klein and Hoffmann 1989).

Recognising these many difficulties, Klein and Hoffmann (1989) recommended use of 55% efficiency for protein deposition and 81% for lipid. These values will be adopted in the subsequent calculation of the efficiency of egg production by swallows (Section 7.4.3.4). First, the potential importance of two more reasons for variation in the efficiency of egg formation will be considered: variation due to the source of nutrients for egg production (body reserves versus daily food intake) (Section 7.4.3.2) and how the efficiency of egg formation might vary when the lipid:protein ratio in the diet was different from that required in the eggs (Section 7.4.3.3).

7.4.3.2 Possible determination of the source of nutrients for egg formation from the efficiency of egg deposition

Efficiencies of lipid and protein deposition are affected by the amount of molecular rearrangement between substrate and product. Milk was produced more efficiently from maternal tissue (85%) than from food intake (70%) by cattle (Blaxter 1989). Similar results were obtained from domestic fowl with egg production efficiencies of 65-68% for utilisation of metabolisable energy and 82-83% when eggs were formed from body tissue (Chwalibog 1982, Kirchgessner 1982).

Knowledge of the source of nutrients for egg production might help in the choice of an appropriate production efficiency as egg formation from body tissue would be more efficient than formation directly from food intake. Equally, it might be possible to determine whether reserves or daily food intake were the source of material for eggs if the efficiency of egg formation were known. An efficiency of over 80% would suggest that eggs were formed from reserves, and one of less than 70% that daily food intake alone was used for egg production. Efficiencies between 70 and 80% would suggest use of a mixture of reserves and daily food intake. According to these criteria, Japanese quail (36%, Chapter 6) formed eggs from daily food intake. The proportion of daily food intake and reserve use varied with temperature for zebra finch (44-77%, El-Wailly 1966) and bobwhite quail (53.9-99.2%, Case and Robel 1974), and domestic fowl normally used a mixture of the daily ration and body reserves (77%, Brody 1945). None of these species formed eggs solely from reserves. It would not be practicable to determine the source of nutrients for egg production in a free-living bird from the efficiency of egg production, as production efficiency would be impossible to determine in the field. This technique might, however, be of use with captive birds.

7.4.3.3 Possible relationship between the efficiency of egg formation and the lipid:protein ratio in the diet

The efficiency of lipid and protein deposition varied with the amount of molecular rearrangement required between substrate and product so the efficiency of egg production might vary with the relative abundances of lipid and protein in eggs and the diet. Efficiency of fattening in cattle was 56% when fed on carbohydrate or fat, but fell to 37% when they were fed on protein (Kleiber 1961). Swallows might suffer an equivalent reduction in efficiency of lipid deposition due to the high protein content of their insect diet, but high lipid content of their eggs.

The protein:lipid ratio in the diet of fattening bobolink was 10.0, and these birds deposited lipid with an efficiency of 58-60% (Gifford and Odum 1965). The swallow diet contained a protein:lipid ratio of 7.5 (Turner 1982) so there was proportionately more lipid than in the bobolink diet. Swallows deposited more lipid each day (up to 0.129 gd^{-1} in eggs alone, compared with 0.07-0.08 gd^{-1} by the bobolink). The swallow diet was therefore deficient in lipid when compared with requirements in relation to the diet of the bobolink.

Swallow eggs contained 8.4% lipid and 10.4% protein (Table 3.2), a lipid to protein ratio of 0.8. The ratio of lipid to protein in egg yolk was even higher (1.9). On all days during egg formation and particularly on days when only yolk was synthesised, protein requirements for egg formation would therefore be met much more quickly than those of lipid, if daily food intake alone were used for egg formation, unless there was rapid transformation of ingested protein to lipid. If conversion of protein to lipid was necessary this would reduce the efficiency of swallow egg formation. This aspect of the efficiency of egg formation will be considered further in Chapter 10, where it will be demonstrated that even birds with exclusively animal diets would not need to transform protein to lipid for egg formation. Swallows would therefore not suffer a reduction in the efficiency of egg formation due to conversion of protein to lipid.

7.4.3.4 Calculation of the efficiency of egg deposition from the partial efficiencies of lipid and protein deposition

Partial efficiencies of 55% for protein and 81% for lipid (Klein and Hoffmann 1989) were used to predict that the efficiency of production of a swallow egg would be 66.7% (Table 7.14). This was close to the median (66.5%) and mean (63.8%) efficiency of egg production determined directly from laying birds (Section 7.4.2), close to the value of 70% adopted by King (1973), but lower than the 75% used by Ricklefs (1974) and Walsberg (1983a), and greater than that provisionally determined for swallows (33%, Section 7.4.1.3).

The eggs of precocial species have a greater lipid content, so using the partial efficiency approach the overall efficiency of egg formation would be predicted to be slightly higher than that of swallows. The efficiency of egg production by the domestic fowl would be 67.9% (at 6.1 g of lipid and 7.0 g of protein per egg, Romanoff and Romanoff (1949), very close to the mean and median values determined directly from laying birds (Section 7.4.2). As the majority of direct studies involved domestic fowl, and the partial efficiency method independently predicted such close agreement, it was concluded that the partial efficiency method was a suitable way to determine the energy requirement for egg production.

The difference in the lipid:protein ratio between egg yolk and albumen would lead to a difference in the efficiency of production predicted for swallow egg yolk and albumen. The efficiency of egg yolk formation would be 72.7% and that of albumen 57.9% (Table 7.15). Albumen deposition was thus predicted to be almost 15% less efficient than that of yolk deposition by swallows.

The partial efficiencies of protein and lipid deposition (Table 7.14) were also used to calculate the mean efficiency of egg formation by the female swallows for which energy expenditure was measured by the DLW technique. Mean protein and lipid contents of egg formed during the period of energy expenditure measurement were used to determine an efficiency of egg formation of 63.6% (Table 7.15). This was less than the efficiency for deposition of whole egg, as most of the birds were

Table 7.14 Calculation of a) the efficiency of egg production by swallows, and b) the energy requirement for the synthesis of one swallow egg as a % of BMR and DEE. The energy contents of lipid and protein were taken from Znaniecka (1967) (39.2 kJg^{-1} for lipid and 23.7 kJg^{-1} for protein.) Lipid and protein contents of swallow egg were taken from Table 3.2. DEE and BMR are from Table 7.3.

a) Partial efficiency of protein deposition

55% (range 83 - 36%, n = 55) Klein & Hoffman 1989

Partial efficiency of lipid deposition

81% (range 100 - 67%, n = 57) Klein & Hoffman 1989

Protein content of one swallow egg = 0.1924 (g) × 23.7 (kJg^{-1}) = 4.55 kJ

Cost to deposit = 4.55/0.55 = 8.28 kJ

Lipid content of one swallow egg = 0.1404 (g) × 39.2 (kJg^{-1}) = 5.50 kJ

Cost to deposit = 5.50/0.81 = 6.79 kJ

Cost to deposit protein + lipid = 8.28 + 6.79 = 15.07 kJ

Energy content of egg = 10.06 kJ

Overall efficiency of egg production = (10.06/15.07) = 66.7%

b) Energy content of 1 swallow egg as a percentage of DEE = 10.06/112.28 = 9.0%

Energy requirement for synthesis of 1 egg as a percentage of DEE

= 15.07/112.28 = 13.4%

Energy requirement for synthesis of 1 egg as a percentage of BMR

= 15.07/29.80 = 50.6%

Table 7.15 Calculation of net energy requirement for egg production by swallows during measurement of energy expenditure by the DLW technique. n = 13 birds. Calculation method follows Table 7.14.

Net energy requirement for:

Lipid deposition = 2.50/0.81 = 3.09 kJ

Protein deposition = 3.45/0.55 = 6.27 kJ

Total = 9.36 kJ

Energy requirement for egg synthesis as a percentage of DEE

= 9.36/112.28 = 8.3%

Energy requirement for egg synthesis as a percentage of BMR

= 9.36/29.80 = 31.4%

Efficiency of egg production = 5.95/9.36 = 63.6%

labelled during the period when a greater proportion of albumen than yolk was formed than was present in a single egg. Assuming the partial efficiency method accurately predicted the energy requirement for egg synthesis, the mean net energy requirement for egg production would have been 9.36 kJ for the birds for which DEE was measured by the DLW technique (Table 7.16). This was 8.3% of mean DEE or 31.4% of BMR. The energy requirement for formation of one swallow egg, which was also the peak energy requirement for egg formation (on day -1), would be 15.07 kJ, equivalent to 13.4% of DEE or 50.6% of BMR (Table 7.14).

Partial efficiencies of protein and lipid deposition were used to predict the net energy requirement for egg formation each day during the formation of a 5 egg clutch by a female swallow. Maximum energy requirement for egg synthesis occurred on the day before the first egg was laid (day -1). This was predicted to be 14.38 kJ (12.81% of DEE or 48.26% of BMR) (Table 7.16, Figure 7.3). The period of more than 50% of the maximum daily energy requirement for egg formation was different to that which would have been predicted from the daily energy content of egg deposition and a fixed efficiency of egg formation (Table 7.15).

The present approach to calculation of the energy requirement for egg formation had the advantage that the efficiency of formation of egg yolk and albumen could be separated. This allowed a more accurate quantification of the distribution of the cost of egg formation than would have been possible with a single efficiency for egg formation (Fig. 7.3, Table 7.16). No study to date has been able to separate the efficiencies of yolk and albumen production, although it would be possible in a future study to compare the production efficiency of females which formed only yolk with that of birds which formed both yolk and albumen. Separation of the efficiency of yolk and albumen deposition is important to allow more accurate prediction of the energy requirement for egg synthesis on each day of egg formation, as swallows never formed yolk and albumen in the proportion that they were found in whole egg.

Table 7.16 Energy content and efficiency of egg deposition, the net energy requirement for egg synthesis and the percentage of DEE and BMR that this represented on each day during the formation of a clutch of 5 swallow eggs. Values shown in bold are more than 50% of the maximum value for this variable (4.81 kJ for energy content and 7.24 kJ for energy requirement of egg formation). Only yolk was formed between days -6 and -2, both yolk and albumen between days -1 and 2, and albumen alone on day 3. The final egg was laid in the morning on day 4.

Day	Energy content (kJ)[1]	Effic. (%)[2]	Net energy req.[3] (kJd^{-1})	% DEE[4]	% BMR[4]
-6	0.07	72.7	0.10	0.09	0.32
-5	0.53	72.7	0.73	0.65	2.45
-4	2.47	72.7	3.40	3.03	11.40
-3	**4.99**	72.7	6.86	6.11	23.03
-2	**6.12**	72.7	**8.42**	7.50	28.25
-1	**9.55**	66.4	**14.38**	12.81	48.26
0	**9.06**	66.1	**13.71**	12.21	46.15
1	**7.15**	64.5	**11.09**	9.88	37.20
2	4.63	60.9	**7.60**	6.77	25.50
3	3.50	57.9	6.04	5.38	20.28
4	0	0	0	0	0

[1] energy content of egg yolk and albumen deposited, summed over all eggs of the clutch (from Table 3.8)

[2] efficiency of egg formation, calculated from the partial efficiencies of lipid and protein deposition (Tables 3.2, 3.9 and 7.14)

[3] net energy requirement for formation of egg material

[4] net energy requirement for egg formation, expressed as a percentage of DEE of laying swallows determined by the DLW technique (112.28 $kJbird^{-1}d^{-1}$) and of BMR (29.80 $kJbird^{-1}d^{-1}$, determined from Aschoff and Pohl (1970), inactive phase, for a bird of 21.78g - the mean metabolising mass of the laying swallows for which energy expenditure was measured by the DLW technique, Table 7.3).

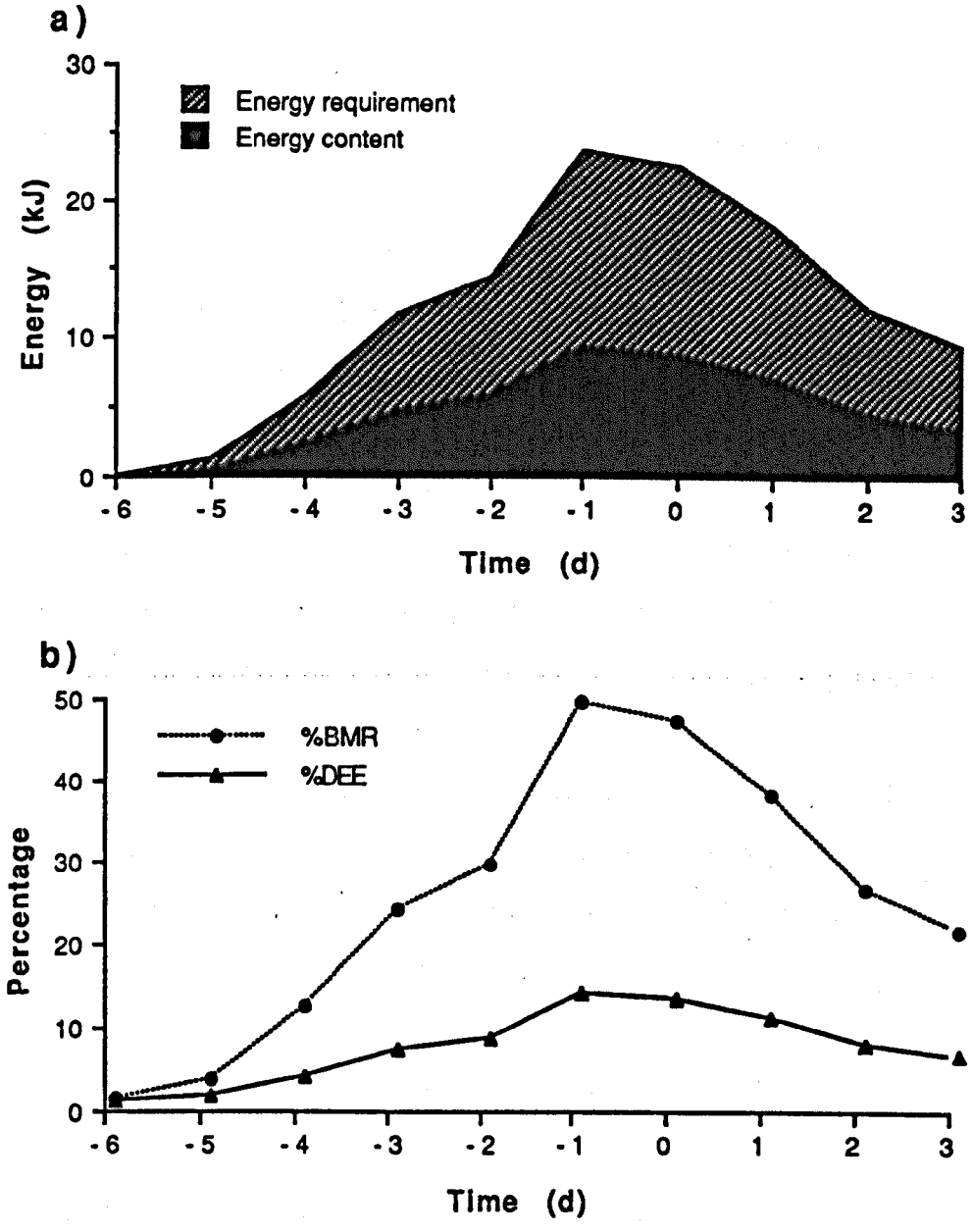


Fig. 7.3 Pattern of energy demand upon a female swallow laying a clutch of 5 eggs. a) daily energy content and energy requirement for egg formation, b) the percentage of DEE and BMR that this represented. Derivation of the daily energy requirement for egg formation is shown in Table 7.16. The first egg was laid on day 0.

7.4.4 Comparison of the metabolic intensity of laying swallows with house martins which suspended egg formation during measurement of energy expenditure

The metabolic intensity of laying swallows was 3.18×BMR, slightly greater than the 2.7×BMR for the laying house martins which did not continue with normal egg production during the measurement period (Bryant and Westerterp 1980). The difference between these two metabolic intensities for swallows which proceeded with laying and house martins which did not suggested that the cost of egg formation was 3.18-2.7=0.48×BMR, very close to the 50.6% of BMR predicted by the partial efficiency method in swallows (Table 7.14). Again, this was consistent with the view that the energy requirement for egg formation could be predicted accurately from the partial efficiencies of protein and lipid deposition. Nestling-rearing female swallows and house martins worked at 3.84 and 3.86×BMR respectively, so calculation of the net energy requirement for egg production by comparison of these two species was unlikely to be greatly influenced by the differences in living costs inevitable in intraspecific comparisons.

7.4.5 Factors affecting energy expenditure of laying swallows

Neither clutch size nor the amount of egg formed during the period of energy expenditure measurement had a detectable influence upon the energy expenditure of laying female swallows, even though birds which laid larger clutches synthesised more yolk. Environmental factors and observed activity were apparently not important influences on energy expenditure. Only three individual attributes were correlated with energy expenditure: females with longer keels, shorter tarsi and lower fat score 1 at the start of the measurement period had greater metabolic rates and daily energy expenditures (Section 7.3.2.3).

More importance was given to the positive correlation between energy expenditure and keel length than to the negative one with tarsus, because keel was a better measure of the structural size of swallows than was tarsus (Chapter 2). Thus

larger birds had greater energy expenditures, metabolic rates and metabolic intensities than small individuals. This was the opposite result to that found for another aerial forager, the house martin, which also used keel length as a size measure (Bryant and Westerterp 1982, Bryant and Westerterp 1983a,b). The ADMR of house martins was positively correlated with food abundance, temperature, rainfall and % time in flight, and negatively correlated with mass, keel length, wind speed and rainfall (Bryant and Westerterp 1980). The higher metabolic rate of nestling-rearing birds in favourable weather conditions was due to their more active foraging mode when returns were higher (Bryant and Westerterp 1983a). Lack of correlation between environmental factors and the energy expenditure of laying swallows (n=13) might be due to the much smaller sample of swallows labelled than of house martins (n=55). An increase in sample size would increase the probability of detection of a significant correlation in two ways: a larger sample size in itself has this effect, and the effects of a larger range of environmental conditions would be likely to be measured.

The negative correlation between initial fat score and all three measures of energy expenditure might be an important indicator of the relative importance of daily food intake, use of reserves and reduction in activity in the different ways in which energy balance might be achieved by laying birds. Birds with a large lipid reserve at the start of the measurement period could subsidise daily food intake with energy from reserves, reducing activity costs and energy expenditure (Bryant and Westerterp 1983a). A portion of the reduction in energy expenditure might also be attributable to increased efficiency of egg formation if lipid was taken from reserves rather than from daily food intake (Section 7.4.3.3). Female swallows with high fat scores were generally those closest to the peak in energy requirement for egg production (Chapter 8), and which might be expected to have a higher total energy output (respiratory DEE plus energy content of egg formed). Reduction in respiratory DEE at this time by birds with the largest lipid reserves would smooth the peak in dietary energy requirement for egg formation. Birds which lose weight during measurement of energy expenditure by the DLW technique typically have lower

DEE than those which maintain or gain weight (Bryant and Westerterp 1983a, Bryant and Tatner 1988, Chapter 9). Here, there were no significant relationships between energy expenditure and change in body condition indices, although there was a (non-significant) negative correlation between decrease in fat score 1 and energy expenditure, which was consistent with the results of Bryant and Westerterp (1983a).

7.4.6 Comparison of energy expenditure of laying with that of other birds at other stages in the annual cycle

Egg formation has frequently been proposed to be a demanding process for birds (King 1973, Ricklefs 1974, Walsberg 1983a,b, Blem 1990) and that energy or nutrient availability during laying may impose proximate limitations to clutch size and laying date (Perrins 1970, Jones and Ward 1976, Murphy 1978, Drent and Daan 1980, Houston *et al* 1983, Bancroft 1984, Järvinen and Väisänen 1984, Hatchwell and Pellatt 1990).

This study demonstrated for the first time that the laying period entailed a DEE which was slightly greater than during incubation and the nestling-rearing period (Table 7.13). Similar conclusions concerning relative living costs during laying and other stages in the annual cycle have been reached in studies based on time budgets (reviewed by Walsberg 1983a). Many of the earlier studies of the energetics of the reproductive cycle contained potentially large errors in estimation of DEE inherent in studies based on time budgets alone. The efficiency of egg production derived in this study by the partial efficiency method suggested that studies which have followed Ricklefs (1974) and Walsberg (1983a) with an assumption of 75% efficiency of egg production have used an efficiency which was too high. The resulting underestimate in DEE was probably so small (it would have been less than 1 kJd^{-1} for the swallow) that it was insignificant in comparison with other errors in estimation of energy expenditure from activity budgets. In effect, therefore, the present study confirmed the conclusions of earlier studies which

assigned a slightly higher DEE to laying than to later stages in the breeding cycle.

A recent study of the annual cycle of energetics in the dipper used the DLW technique to check the accuracy of the estimated living costs calculated from time budgets (Bryant and Tatner 1988). DEE was highest during laying for a dipper, although this was not measured directly using DLW. The combined energy requirement of a female dipper plus half of the brood was substantially greater during the nestling-rearing period than while laying, so the energy gain from foraging would be greater for a nestling-rearing than for a laying bird.

CHAPTER 8

BODY CONDITION OF LAYING SWALLOWS

8.1 Introduction

There has been considerable debate about whether daily food intake or body reserves are the source of nutrients for egg formation, and if levels of food and reserves influence egg and clutch size or laying date. Drent and Daan (1980) proposed two models to describe the source of nutrients for egg formation: "income" species which formed eggs from daily food intake, whilst "capital" species used reserves for egg synthesis. There are a few examples of species which form eggs entirely from "capital", such as penguins which move onto the ice at the start of the period of rapid follicular growth (Grau 1982, Astheimer and Grau 1985), eiders and some arctic nesting geese (Korschgen 1977, Ankney and MacInnes 1978, Parker and Holm 1990). There is controversy over the importance of nutrient reserves for temperate waterfowl, with different interpretations suggesting a protein (Drobney and Fredrickson 1985), lipid (Ankney and Afton 1988), or no (Arnold and Rohwer 1991) nutrient constraint to clutch size.

The importance of reserves to passerines during laying also varies between studies, with apparent use of a protein reserve during egg production in some species (Jones and Ward 1976, Fogden and Fogden 1979, Young 1989), but not others (Hails and Turner 1985, Krementz and Ankney 1988). A number of species used lipid reserves during laying (Jones and Ward 1976, Fogden and Fogden 1979, Schifferli 1980, Ojanen 1983, Krementz and Ankney 1988). The brown-headed cowbird showed no change in nutrient reserve level during egg formation, but this species was unusual in that it was a nest parasite (Ankney and Scott 1980).

Changes in the body composition of female birds during the laying period have been investigated by carcass analysis of females killed before, during and after laying. This approach has several disadvantages: first, a substantial sample of birds must be killed at all stages of the laying cycle. Sufficiently extensive sampling has normally only been possible amongst pest species such as the quelea (Jones and

Ward 1976) or lesser black-backed gull (Houston *et al* 1983), or from very large colonies (Hails and Turner 1985). One recent study has been criticised for attempting to draw conclusions concerning nutrient dynamics of laying females from too small a sample of birds (Alisauskas *et al* 1987, Murphy 1987). A second disadvantage with carcass analysis studies is that the analysis process is time consuming and expensive, particularly for larger birds. Thirdly, it was often not possible to match the birds sampled with their nests, so that the stage in the laying cycle had to be determined from examination of the carcass or from the rest of the population (e.g., Krapu 1974). This would reduce the accuracy with which the female could be placed in her laying cycle. These three problems could be overcome by choice of an appropriate study species or increased research effort. The fourth, and most serious, difficulty could not be resolved without a new approach, for once a bird was killed for carcass analysis, the effect of her body condition upon subsequent breeding performance could not be determined. The status of pre-breeding birds has been difficult to determine (would they have bred, when and how many eggs would they have laid?, Murphy 1987). The clutch size of females killed during laying could not be determined accurately either, as not all follicles which would be ovulated might have entered the rapid growth phase and some enlarged follicles might suffer atresia rather than ovulation (Ankney and MacInnes 1978, Krementz and Ankney 1988).

In the present study, carcass analysis was used to quantify changes in body composition during laying which were assessed primarily using mass and condition indices of living female swallows. Four methods were used to monitor changes in mass or body condition of female swallows during laying. The mass of individual birds was recorded over a series of days using electronic balances placed under swallow nests (Section 8.2.1). Mass and condition indices were recorded each time a female swallow was caught. The size of the protein reserve was assessed using an ultrasound device to measure pectoral muscle thickness. Scoring of visible sub-cutaneous fat was used as an index of lipid reserves (Chapter 2). Carcass analysis of female swallows (Section 8.2.2) was used to interpret the changes in mass and condition indices of living birds as changes in body composition during the laying period.

8.2 Methods

8.2.1 Use of automated electronic balance system at swallow nests

8.2.1.1 Procedures used to place swallow nests on balances

The stage in the laying cycle at which swallow nests were moved onto balances was critical to the successful weighing of the pair. If the nest was moved too long before laying was due to begin, the birds often built a new nest on an adjacent rafter. Nests could be moved onto balances without causing nest desertion after the first egg was laid, but this meant that no weighings were obtained during the period of rapid follicular growth. Nests were therefore moved onto balances at a variety of times between completion of the nest rim and the day the first egg was laid, in order to obtain as many weighings as possible from a range of stages in the laying cycle.

Swallow nests were placed on balances in 3 ways. The most straightforward way, used by Jones (1985) and Thompson (1992), was to remove the nest from the rafter, stick it to a hardboard support and place this on the balance. The nest and balance were replaced as close as possible to the original position of the nest. It was thought that such large changes around the nest might make laying birds more likely to desert, so the majority of nests were stuck to hardboard supports which were attached to the tops of 1-1.5 m vertical wooden poles (1×2 cm) which rested on the balance. A few nests were suspended from balances through the floor of the room above the nest: this was the least obtrusive method, but could only be used when the birds nested in a suitable building.

8.2.1.2 Details of balance systems

Two types of electronic balance system were used in this study: the printer balance system, originally used by Jones (1985), and the computer balance system, developed at Stirling University by Thompson (1992) in conjunction with the Microcomputer and Media Technology group. The printer balance system consisted

of a 2000 g Mettler balance which was prompted (manually or at predetermined intervals) to record nest mass on a Mettler printer (Plate 8.1). Nest mass was recorded at 2 min intervals during the laying period and at 10 min intervals during incubation. The manual weighing prompt was used during daily checks of the accuracy of masses obtained, when 10, 20 and 30 g weights were placed in the nest. These masses were weighed with an accuracy of ±0.05 g.

The computer balance system was a 1600 g Mettler balance linked to a BBC B+ computer and disc drive (Plate 8.2). The BBC ran a program which compared the nest mass transmitted from the balance at time *t* and with that transmitted *x* seconds later (where *x* was a variable, set to 5 s during this study). If the mass at time *t+x* was more than a threshold level (10 g in this study) different from the mass at time *t*, the program ran the "weight changed" routine. The "weight changed" routine stored the time at which the bird landed on or left the nest together with three masses taken at 5 s intervals. The program then returned to the *x* second comparisons of mass.

If the "weight changed" routine was not run for more than *y* min (a variable set to 15 min in this study), the current mass on the balance was recorded. This meant that when a bird landed or left the nest a base weight had always been recorded within the previous 15 min. All times and masses were recorded on disc and transferred to the University mainframe computer for analysis.

8.2.1.3 Analysis of balance data

The list of times and masses collected at each nest was converted to a sequence of weighings for each member of the pair each time they landed. For birds weighed by the computer balance system, the mean of the three "nest only" and "bird+nest" weighings was used to calculate bird mass upon arrival at the nest. If the range of either of these three weighings was greater than 0.1 g, the mass was considered not accurate enough to be included in subsequent analyses. Bird mass when leaving the nest was only used at dawn after departure from the roost. Printer

Plate 8.1 Printer balance system, showing the balance which was placed under the nest, cables which led to the timer and printer which were placed at a distance from the nest. The balance and printer were powered by mains electricity and the timer by a 9V battery.

Plate 8.2 Computer balance system, showing the balance which was placed under the nest and cables leading to the connection box (light grey) which converted voltage to digital output suitable for the BBC computer which was placed at some distance from the nest. Balance and computer were powered by mains electricity.

balance data were converted to a list of times and masses by subtraction of the previous "nest only" weight each time a "bird+nest" weight was recorded. Weighings collected by both balance systems were then scanned to remove aberrant weighings. Apparent bird masses of less than 17 g and between 27 and 40 g were excluded, as no hand-caught birds had masses in these ranges. Masses greater than 40 g were recorded when both members of a pair landed on the nest. Weighings which caused an apparent mass gain followed by loss (or loss and gain in quick succession) of more than 0.83 gh^{-1} were excluded on the basis that these weighings were probably affected by the factors listed below. A rate of mass change of 0.83 gh^{-1} was more than double the maximum sustained rate of mass gain during the period 0800 to 1600, six times the overnight rate of mass loss and would not have included mass losses due to defecation as mean swallow faecal mass was 0.397 ±0.160 g (Jones 1987b). The spurious weighings excluded by these procedures were attributed to the effects of wind, or birds moving, taking off or landing during weighings and to the extra mass of nesting material brought to the nest on some visits.

Female swallows were 1-3 g heavier than males during the laying period, so the masses of pair members could be separated easily. Male swallows landed on the nest much less frequently than females (Fig. 8.1), so any male weighings classified as female when the masses of pair members were similar (early in egg formation and after the start of incubation) would have little effect upon the pattern of mass change recorded for females.

8.2.2 Carcass analysis of female swallows

Two female swallows were killed (by chloroform inhalation, under licence from the NCC) during the night after they laid the first egg of their second clutches in July 1991. A starving female swallow was found trapped in fishing line which had been used as nest lining. Her brood was about 20 days old when she was killed in July 1991. The first two birds were weighed, measured and their fat scores and pectoral muscle thickness (Chapter 2) recorded immediately before death (less than

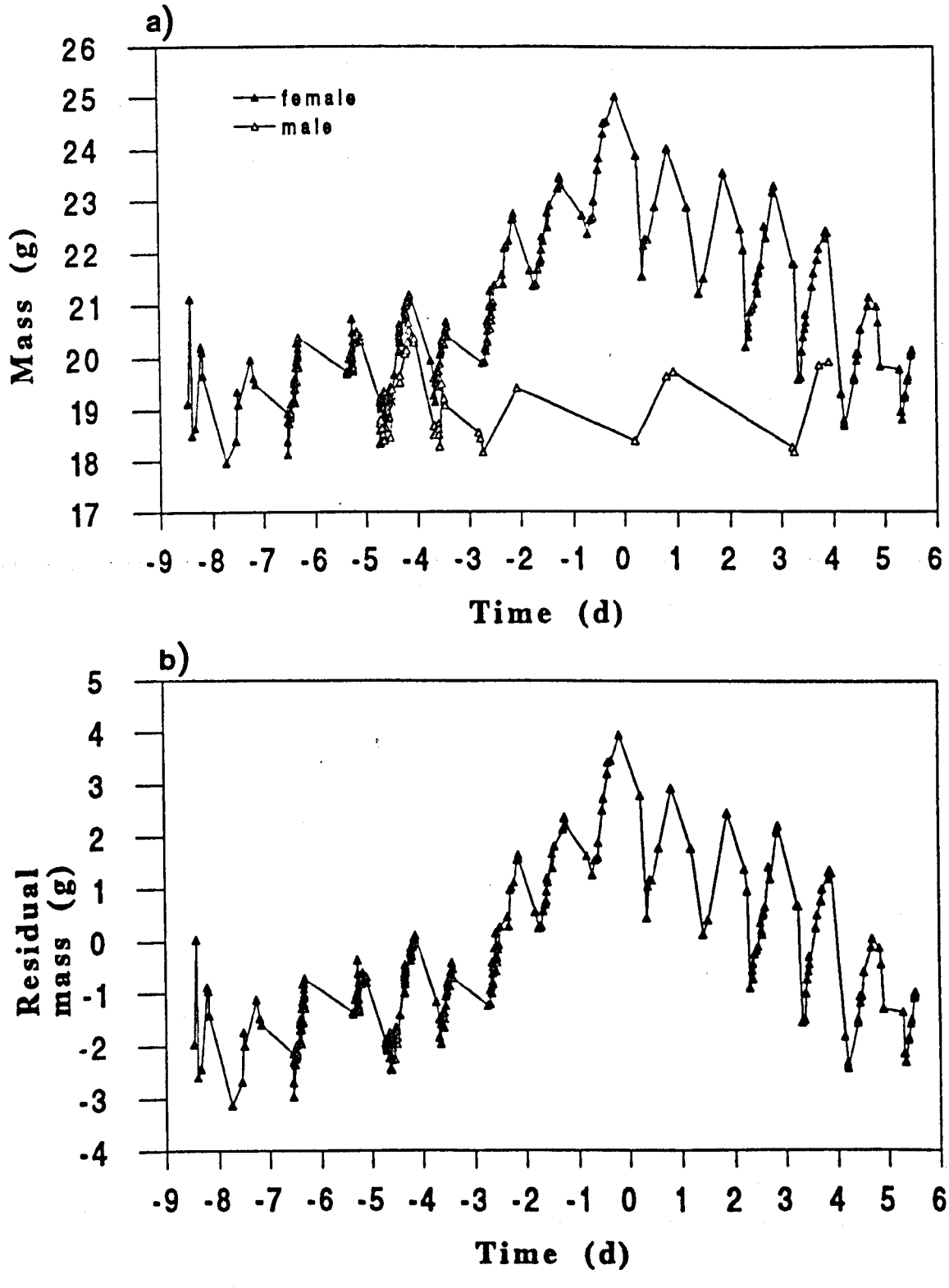


Fig. 8.1 Changes in a) mass of a pair of swallows, and b) residual mass of the female, during laying of a 4 egg clutch. The eggs were laid in the morning on days 0, 1, 2 and 3. Residual mass was calculated from mass minus incubation mass predicted from structural size (21.09 g, Chapter 2). Masses were obtained using an electronic balance from which the nest was suspended through the ceiling above the nest. Data from this female are also shown in Fig. 8.7.

30 min after capture). All birds were weighed after death and frozen for storage.

Each bird was defrosted, weighed, plumage and fat deposits photographed (Plate 2.1), wing profile drawn and pectoral muscle thickness measured using ultrasound and a needle, before dissection into the following components:

wing feathers (primaries and secondaries),

tail feathers,

skin and feathers (excluding wing and tail feathers above),

head (cut at base of skull),

neck,

wings (cut at shoulder),

legs (cut at hip parallel to body),

pectoralis major,

pectoralis minor,

heart,

lungs,

liver,

gizzard and oesophagus,

intestine,

ovary,

each developing follicle (if present),

oviducal egg (if present),

oviduct,

kidneys,

body shell (remainder of carcass after above components were removed).

Each component was weighed shortly after dissection (wet mass), after freeze-drying for 10 d (dry mass) and after extraction of lipid in Soxhlet apparatus using 5:1 dimethyl ether:chloroform solvent and freeze-drying for a further 3 days (lean dry mass). All weighings were made to 0.0001 g using an Oertling balance.

Pectoral muscle lean dry mass (LDM) was predicted from the relationship

between keel length and pectoral muscle derived for female swallows by Thompson (1992) (Equation 8.1).

$$Pectoralis\ LDM(g) = -3.2124 + 0.19934 \times keel\ length(mm) \qquad 8.1$$

(r^2=0.890, n=11, laying and starving birds excluded.)

Carcass lipid content was predicted from anterior fat score using Equation 8.2 (Thompson 1992).

$$Carcass\ lipid\ content = -0.87530 + 0.50648 \times fat\ score \qquad 8.2$$

(r^2=0.749, n=9),
where fat score = anterior fat score × 2.

The regression of lipid content upon a combined anterior and posterior fat score explained no more of the variation in lipid content (Thompson 1992). Lipid and water indices were calculated from Equations 8.3 and 8.4.

$$Lipid\ index = 100 \times fat\ content(g) / lean\ dry\ mass(g) \qquad 8.3$$

$$Water\ index = 100 \times water\ content(g) / lean\ dry\ mass(g) \qquad 8.4$$

Lipid and water indices described lipid and water content, controlling for differences in structural size between birds (as measured by lean dry mass). Protein content was calculated on the assumption that 13% of the lean dry component was ash and that the remainder was protein (Ricklefs 1974). The carbohydrate component was assumed to be negligable (Ricklefs 1974). Carcass analysis followed procedures used by Jones (1985) and Thompson (1992) to allow comparison of data between studies.

8.3 Results

8.3.1 Comparison of body composition of laying and incubating female swallows

Carcass analysis data from the present study (Appendix 7) were pooled with that from Jones (1985) and Thompson (1992). This allowed comparison of the body composition of laying female swallows which were killed (n=3) or found dead (n=2), with that of females killed during incubation (n=4) and a single starved nestling-rearing female (Tables 8.1 and 8.2). The number of carcasses analyzed and the range of stages in laying were not sufficient for a detailed study of the changes in body composition using data from carcass analysis alone. Instead, these data were used to allow interpretation of changes in mass and condition indices for the much larger sample of living birds (Section 8.4).

Female swallows killed the evening after they laid the first egg of a clutch (day 0) were 2.68 g heavier and had 1.45 g more fat than incubating females (Table 8.2). This was partly due to the enlarged ovarian follicles and oviducal egg within laying birds. If egg material was excluded, females were still heavier and had more fat on day 0 of laying than during incubation. Exclusion of egg material and oviduct from females on day 0 gave a fresh mass close to that of incubating birds, however dry mass and lipid content were still 0.44 and 1.08 g greater on day 0. The female killed on day 4 of laying was unusually heavy for this stage (see Fig. 8.2a for range in mass on this day) however her lipid content was intermediate between birds killed on day 0 and during incubation. This suggested that the extra lipid present at the start of the albumen formation period was used before the end of laying.

Females which died during laying had the lowest body lipid content (1.40 g), which was reduced to only 1.29 g if the lipid content of eggs and oviduct were excluded. This was less that the starving nestling-rearing female (1.45 g fat), which possessed no visible lipid deposits. The lipid content of the females which died during laying was 8.4% of fresh mass, or 24.8% of lean dry mass. These values were considerably higher than the quantities of structural lipid found in yellow 52-

Table 8.1 Body composition of laying female swallows. Masses are in g. Lipid index = 100 × lipid mass / lean dry mass. Water index = 100 × water mass / lean dry mass. Protein content was calculated on the assumption that 13% lean dry mass was ash and the remainder protein (Ricklefs 1974). Birds 1 and 2 had laid one egg, had one oviducal egg and three rapidly developing ovarian follicles. Bird 4 had laid 3 eggs and had one oviducal egg and one enlarged ovarian follicle. Birds 5 and 6 were both found dead below their nests having laid three eggs. Bird 5 had just ovulated her final enlarged ovarian follicle and bird 6 had one enlarged ovarian follicle but no oviducal egg. Bird 5 had been partially consumed by a predator/scavenger so fresh mass was not recorded; carcass composition was calculated by doubling the mass of components from the undamaged half of the bird. Bird 6 had been dead for perhaps 24 hours before collection and so would have been subject to some desiccation prior to analysis. Birds 1 and 2 were analyzed during this study, birds 4 and 5 by Thompson (1992) and bird 6 by Jones (1985). - = data not available.

	Day 0 of laying		Day 4 of laying	Died during laying	
Bird number	**1**	**2**	**4**	**5**	**6**
Wet mass	22.40	24.30	25.00	-	20.70
Dry mass	8.6410	9.3704	9.1024	6.1165	7.8400
Lean dry mass	6.1023	6.1763	7.3665	5.0516	6.0961
Water, mass	13.759	18.1237	15.8976	-	12.860
% fresh mass	61.4	74.6	63.6	-	64.62
Index	225.5	293.4	215.8	-	210.90
Lipid, mass	2.5387	3.1941	1.7359	1.0649	1.7439
% fresh mass	11.3	13.1	6.9	-	8.4
Index	41.6	51.7	23.6	21.1	28.6
Protein, mass	5.3090	5.3734	6.4089	4.3949	5.3037
% fresh mass	23.7	22.1	25.6	_	25.6

Table 8.2 Body composition of laying, incubating and of a starving nestling-rearing female swallow. Mean composition excluding ovarian follicles and oviducal egg (= minus eggs) and excluding ovarian follicles, oviducal egg and oviduct (= minus eggs and oviduct) are shown. Data are from laying and incubating birds analyzed during this study, by Thompson (1992) and Jones (1985). Indices and the stage of laying of each bird are explained in Table 8.1

	Day 0 of laying (Birds 1 & 2)			Day 4 of laying (Bird 4)		
	Whole bird	**Minus eggs**	**Minus eggs and oviduct**	**Whole bird**	**Minus eggs**	**Minus eggs and oviduct**
Wet mass	23.35	21.32	20.22	25.00	24.134	23.034
Dry mass	9.0057	8.3090	8.0173	9.1024	8.6236	8.3490
Lean dry mass	6.1393	5.7429	5.5125	7.3665	7.1005	6.8505
Water						
Mass	15.941	11.1256	10.3196	15.8976	15.5104	14.685
% fresh mass	68.0	57.3	56.3	63.6	64.3	63.8
Index	259.4	193.0	187.3	215.8	218.4	214.4
Lipid						
Mass	2.8664	2.5435	2.5048	1.7359	1.5231	1.4985
% fresh mass	12.2	13.1	13.6	6.9	6.3	6.5
Index	46.6	44.1	45.4	23.6	21.5	21.9
Protein						
Mass	5.3412	5.0159	4.7959	6.4089	6.1774	5.9600
% fresh mass	22.9	25.8	23.8	25.6	25.6	25.9

Table 8.2 continued

	Died during laying (Birds 5 & 6)		Incubating (n = 4)	Starving nestling-rearing (Bird 3)
	Whole bird	Minus eggs and oviduct	Whole bird	Whole bird
Wet mass	20.70	19.60	20.67	16.38
Dry mass	6.9782	6.5622	7.5794	6.5722
Lean dry mass	5.5738	5.2703	6.1594	5.1175
Water				
Mass	12.860	12.3018	12.8695	9.8078
% fresh mass	64.62	62.7	62.4	59.9
Index	210.95	217.0	204.7	197.7
Lipid				
Mass	1.4044	1.2919	1.4200	1.448
% fresh mass	8.4	8.5	7.0	8.8
Index	24.8	24.2	22.3	28.3
Protein				
Mass	4.8493	4.5852	5.3404	4.4522
% fresh mass	25.6	25.8	26.6	27.2

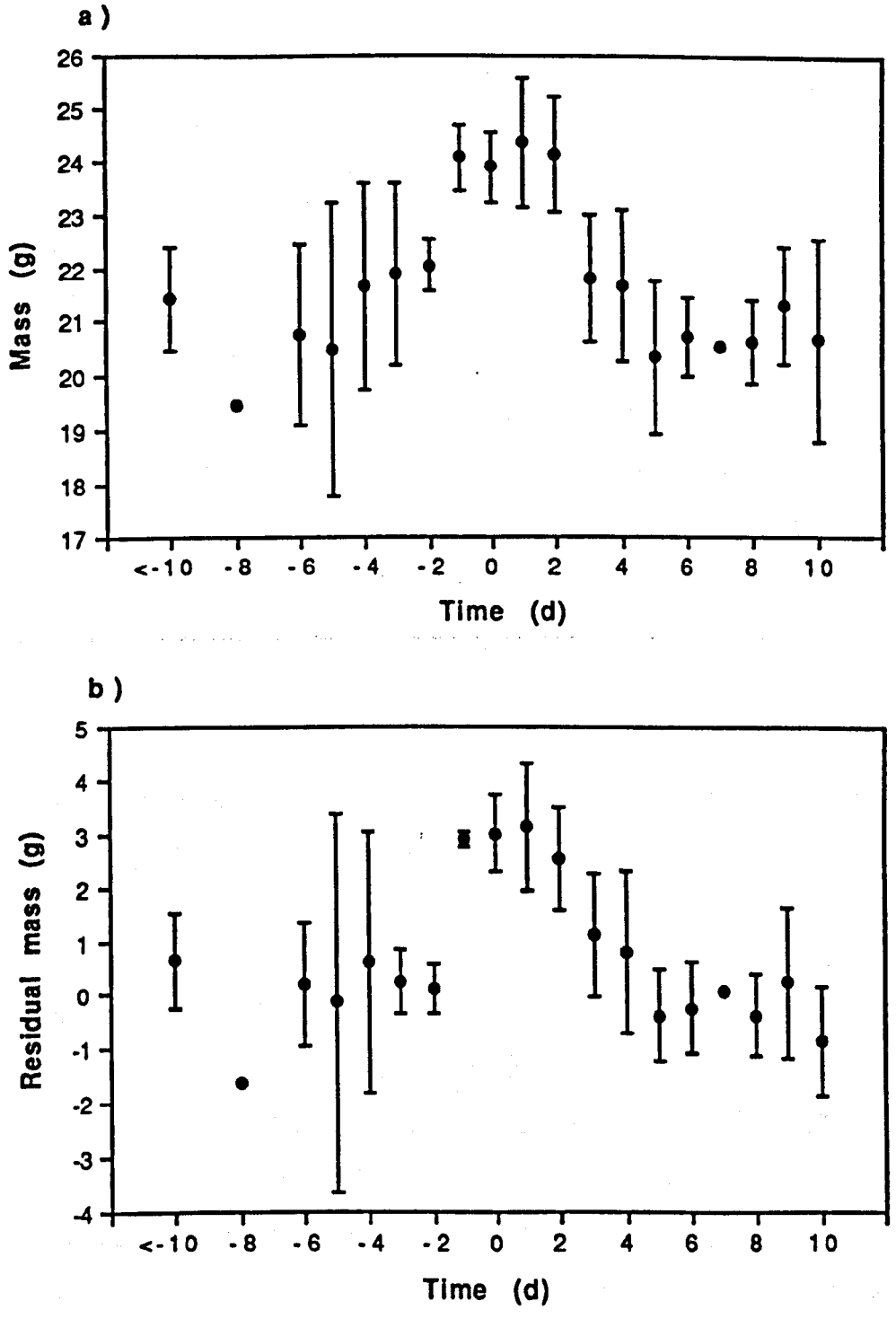


Fig. 8.2 Changes in a) mass, b) residual mass (mass minus incubation mass predicted from structural size) of female swallows weighed during laying (mean, with error bars showing sd). Mass was adjusted to midnight at the start of each day (using a mean mass loss of 0.15 gh^{-1} during the night, and no adjustment for the time of weighing during the day, Chapter 2). Only the first time each female which laid a clutch of 3 to 6 eggs was weighed during each breeding attempt was included in this analysis. Sample sizes, starting with day <-10, were 4, 1, 2, 3, 5, 2, 3, 2, 5, 15, 5, 3, 7, 6, 4, 1, 4, 3 and 3 females on each day. The first egg was laid on day 0. Birds weighed by balances were excluded from this analysis, to allow comparison between this and Figs. 8.10 and 8.12.

vented bulbuls (1.3-1.4% fresh mass, Ward 1969) or in camaroptera (5-7% lean dry mass, Fogden and Fogden 1979) which had starved to death. Either swallows required a higher level of structural lipid, the laying birds had not died from starvation, or they had died due to inability to metabolise their reserves sufficiently rapidly.

There was only 0.01 g more protein in female swallows on day 0 than during incubation (Table 8.2). If the protein content of egg material and oviduct were excluded, females on day 0 had 0.54 g less protein than incubating birds. Females which died during laying and the starving nestling-rearing female had the lowest protein contents. Changes in protein content of female swallows during laying appeared to be small.

The lipid content of laying female swallows was compared with that predicted from anterior fat scores for females from other stages in the reproductive cycle (Table 8.3a). Both females killed on day 0 had a greater lipid content than predicted from fat score, whilst the bird killed on day 4 of laying had a lower lipid content. The lean dry mass of the pectoralis muscles of laying females only differed slightly from that predicted for a bird of that keel length at other stages in the reproductive cycle (Table 8.3b).

There were no significant differences in whole body wet mass, dry mass, lean dry mass or lipid content between laying and incubating females (Table 8.4). Lack of a statistically significant difference was due to the small sample sizes (n=3 laying and 4 incubating females), as laying female swallows in general were significantly heavier than incubating birds (Table 8.7). The 1.08 g utilised by female swallows during the last three days of laying could be of great importance in the energy budget of laying swallows (Section 8.4).

The high wet mass of laying birds was partly due to the presence of the oviduct (mean mass of 1.10 g, Table 8.5). The oviduct was so small in incubating birds that it was not dissected as a separate component. Essentially the entire protein and lipid content of the oviduct were therefore available to supplement daily food intake during or shortly after laying. Other organs which differed significantly in

Table 8.3 Comparison of a) total lipid content and b) protein content of the pectoralis muscles, of laying female swallows with that predicted from females analyzed at other stages in the reproductive cycle by Thompson (1992) using measures which could be made on living birds. Total lipid content was predicted from: lipid (g) = -0.87530 + 0.50648 fat score (where fat score = 2 × anterior fat score), r^2 = 0.749, n = 9. Pectoralis lean dry mass (LDM) was predicted from: pectoralis LDM = -3.2124 + 0.19934 × keel length (mm), r^2 = 0.890, n = 11 (where keel length, measured by SW = keel length measured by MT - 0.084mm, Chapter 2).

a)

Bird number	Anterior fat score × 2	Total extractable lipid		
		Predicted	Actual	Difference
1 (day 0 of laying)	6	2.16350	2.5387	+0.3752
2 (day 0 of laying)	8	3.17654	3.1941	+0.0176
4 (day 4 of laying)	7	2.67006	1.7359	-0.9342

b)

Bird number	Keel length (mm)	Pectoralis LDM		
		Predicted	Actual	Difference
1 (day 0 of laying)	20.6 - 0.084	0.87726	0.8449	-0.0324
2 (day 0 of laying)	20.0 - 0.084	0.75766	0.8164	+0.0587
4 (day 4 of laying)	21.5	1.0734	1.0993	+0.0259

Table 8.4 Differences in dry mass, lean dry mass and lipid content of whole birds and of each body component of laying and incubating female swallows (Mann-Whitney U-tests). The oviduct was so small in incubating females that it was not dissected as a separate component. * = $p<0.05$, ns = not significant. n = 3 laying and 4 incubating females.

Component	Dry mass Z	Signif.	Lean dry mass Z	Signif.	Lipid mass Z	Signif.
Whole bird	1.77	ns	1.41	ns	1.06	ns
Skin & feathers	0.00	ns	1.77	ns	0.35	ns
Wing & tail feathers	0.00	ns	1.41	ns	1.76	ns
Head	0.00	ns	1.76	ns	1.76	ns
Neck	0.35	ns	0.00	ns	1.06	ns
Pectoralis major	0.00	ns	0.71	ns	2.12	*[1]
Pectoralis minor	0.00	ns	0.71	ns	1.76	ns
Wings	1.41	ns	0.71	ns	1.06	ns
Legs	1.41	ns	0.71	ns	1.41	ns
Gizzard & oesophagus	1.77	ns	1.06	ns	2.12	*[1]
Liver	0.71	ns	0.35	ns	0.35	ns
Heart	0.35	ns	0.35	ns	2.12	*[1]
Intestine	0.35	ns	1.06	ns	1.06	ns
Kidneys	1.76	ns	2.12	*[2]	0.62	ns
Lungs	2.12	*[1]	1.06	ns	2.12	*[1]
Body shell	0.71	ns	0.35	ns	1.41	ns
Oviduct	*[1]		*[1]		*[1]	

*[1] Component of greater mass in laying than incubating females.

*[2] Component of greater mass in incubating than laying females.

Table 8.5 Mass and composition of the oviduct of laying female swallows. The lipid component of the oviduct of bird 5 was much higher than that of the other birds as part of an ovulated ovarian follicle was included due to damage caused by a predator/scavenger. This oviduct was not included in calculation of mean oviducal mass and composition. Energy content was calculated from lipid and protein contents, assuming that ash formed 13% of lean dry mass (Ricklefs 1974) and that the energy contents of lipid and protein were 39.2 and 23.7 kJg^{-1} respectively (Znaniecka 1967). Birds 1 and 2 had laid one egg, had one oviducal egg and three rapidly developing ovarian follicles. Bird 4 had laid 3 eggs and had one oviducal egg and one enlarged ovarian follicle. Birds 5 and 6 were both found dead below their nests having laid three eggs. Bird 5 had ovulated her final enlarged ovarian follicle and bird 6 had one enlarged ovarian follicle but no oviducal egg. Birds 1 and 2 were analyzed during this study, birds 4 and 5 by Thompson (1992) and bird 6 by Jones (1985). Lipid index = 100×lipid mass/lean dry mass. - = data not available.

	Day 0 of laying		End of laying	Died during laying		Birds 1, 2, 4 and 6		
Bird number	**1**	**2**	**4**	**5**	**6**	**Mean**	**sd**	**n**
Wet mass (g)	1.1442	1.0509	-	-	-	1.0975	0.06	2
Dry mass (g)	0.3043	0.2790	0.2746	0.2902	0.3603	0.3045	0.04	4
Lean dry mass (g)	0.2641	0.2418	0.2500	0.1867	0.3279	0.2709	0.04	4
Protein (%wet mass)	20.1	20.0	-	-	-	20.05	0.07	2
Lipid mass (g)	0.0402	0.0372	0.0246	0.1035	0.0324	0.0336	0.007	4
Lipid (% wet mass)	3.5	3.5	-	-	-	3.5	0.00	2
Lipid index	15.2	15.4	9.8	55.4	9.9	12.6	3.15	4
Energy content (kJ)	7.021	6.444	6.119	7.907	8.031	6.904	0.84	4

composition between laying and incubating birds were the pectoralis major, heart, lungs, gizzard and oesophagus, all of which had a greater lipid content in laying females (Table 8.4). Lipid depots were not dissected as separate components, so it was likely that anterior and posterior deposits were included with the pectoralis major and that abdominal fat was included partly with each of the heart, lungs, gizzard and oesophagus. The lean dry mass of each body component did not differ significantly between laying and incubating females, except that the kidneys of incubating birds were heavier (Table 8.4).

In summary, the difference in wet mass between laying and incubating female swallows killed for carcass analysis could be accounted for by the mass of egg material already deposited and oviduct. The dry mass of laying birds was still greater than that of incubating females, even if the mass of reproductive material was excluded. This was due to the greater lipid content of laying birds. Changes in body lipid during laying could complicate inferences about changes in body condition from the changes in mass recorded in living birds. The difference in lipid content between females on day 0 and during incubation was therefore used as the best estimate of the rate of use of lipid reserves during the period of albumen formation, although data from females taken for carcass analysis thoughout the laying cycle would be preferable. There was no change in lean dry mass between laying and incubation, other than that incubating birds had heavier kidneys.

8.3.2 Mass and body condition of laying female swallows

8.3.2.1 Changes female swallow mass during laying

The mass of pairs of swallows was measured during laying and early incubation using electronic balances placed under nests (Section 8.2.1). Data were collected at 17 nests from 15 pairs of swallows (two pairs were weighed in more than one year). The balances were deployed at 29 nests altogether, but a number of problems meant that no useful data were collected at 12 of these (desertion of the nest when it was moved onto the balance at 6 nests, insufficiently accurate

properly at 5 nests and equipment failure at 1 nest).

Two pairs of swallows weighed by the balance systems had interruptions in laying, one for 2 days after the first egg in a clutch of 4 and the other of 1 day after the second egg of a clutch of 5. One pair laid only one egg, but this female failed to lay a complete clutch during the entire season, so the unusually small clutch was probably a result of the abnormal behaviour of this pair rather than due to disturbance caused by the balance system. The remaining birds laid clutches of 6 (1 pair), 5 (9 pairs) and 4 eggs (3 pairs). The clutch sizes laid by balance females were not significantly different from the clutch sizes laid by the rest of the population (t-test, $t=1.52$, $p=0.14$, 260 d.f.). All females which accepted the initial movement of the nest onto the balance (except the bird which laid only 1 egg) went on to incubate their clutches. The behaviour and pattern of mass of these pairs was therefore thought not to have been altered by the balance system. This was confirmed by the similarity of the pattern of mass change recorded for individual females by the balances and from the population in general using females caught once per breeding attempt (Figs. 8.1a and 8.2a).

Female swallow mass began to increase 4-5 d before the first egg was laid, typically reaching a peak of around 25 g on the night before the first egg was laid (Figs. 8.1a and 8.2a). During each 24-hour cycle, mass decreased overnight, fell during a short flight at dawn, presumably due to defecation, fell by around 2 g if an egg was laid and then increased progressively during foraging over the rest of the day. Peak daily mass increased during the period of rapid follicular growth, and decreased as the eggs were laid. After the final egg was laid, mass returned to a level similar to that before the initiation of rapid follicular growth (20-21 g), and remained around this level until the end of incubation (Chapter 2). Male swallow mass fluctuated little during the laying period (18-20 g) (Fig. 8.1a). The pattern of mass change of laying swallows was similar to that recorded for curlew using balances placed under the nest (Mulder and Swaan 1992).

The mass of laying female swallows was expressed as residual mass (mass minus incubation mass predicted from structural size, Chapter 2) to adjust for

minus incubation mass predicted from structural size, Chapter 2) to adjust for differences in mass between birds attributable to structural size. For an individual female, changes in residual mass during laying were the same as changes in mass, except that the scale differed on the vertical axis (Fig. 8.1). For the population, the pattern of residual mass change during laying was slightly different to that of mass (Fig. 8.2).

Mass and residual mass were significantly lower at the end than at the start of the period of albumen formation (Mann-Whitney U-tests, both $Z>2.52$, $p<0.012$, for differences between mass and residual mass on days 1 and 4, and days 0 and 4, $n=5$ on day 0, 15 on day 1 and 7 on day 4). In a regression analysis, mass and residual mass decreased significantly between days 0 and 4 (Fig. 8.3). Mass and residual mass on the night before and the night after the day the first egg was laid were not correlated with laying date (Pearson correlations, all $r<0.26$, all $p<0.2$, $n=26$ on the night after the first eggs was laid, and 12 on the night before the first egg was laid).

Carcass analysis demonstrated that the difference in mass between laying and incubating swallows was largely accounted for by the mass of reproductive structures (Section 8.3.1). To determine whether mass changes of living birds during the laying cycle could be explained in the same way, the predicted mass of ovarian follicles, oviducal eggs and oviduct was calculated for each day during the laying period for females which laid clutches of different sizes (Table 8.6). The mass of the oviduct was calculated using the assumption that the swallow oviduct enlarged according to the same pattern as that of the bank swallow (Petersen 1955), and decreased in mass during the period of albumen formation to 37.5% of maximum mass when the final egg was laid (Table 8.6). The basis for these assumptions is discussed in Section 8.4.2.

Residual non-egg mass was calculated by subtraction of the predicted mass of ovarian follicles and oviducal egg from residual mass (where residual mass=mass minus incubation mass predicted from structural size, Chapter 2). Residual non-reproductive mass was calculated by subtraction of the predicted mass of

Table 8.6 a) Mean mass (mg) of each swallow ovarian follicle or oviducal egg at midnight at the start of each day during egg formation, with oviducal egg masses given in italics (from Chapter 3). b) Total mass of egg formed at midnight at the start of each day for clutches of different sizes. c) Mass of the oviduct at the start of each day assuming growth according to the pattern of the sand martin (Petersen 1955) and degradation during the period of albumen formation at a rate dependent upon clutch size (Appendix 3). d) Total mass of ovarian follicles, oviducal egg and oviduct at the start of each day. The first egg was laid in the morning on day 0.

Day	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**
a) Mean mass of ovarian follicles and oviducal eggs at midnight at the start of each day												
Egg 1	0.03	4.65	34.65	159.93	322.39	395.58	*1970.00*					
Egg 2		0.03	4.65	34.65	159.33	322.39	395.58	*1970.00*				
Egg 3			0.03	4.65	34.65	159.33	322.39	395.58	*1970.00*			
Egg 4				0.03	4.65	34.65	159.33	322.39	395.58	*1970.00*		
Egg 5					0.03	4.65	34.65	159.33	322.39	395.58	*1970.00*	
Egg 6						0.03	4.65	34.65	159.33	322.39	395.58	*1970.00*
b) Total mass of egg material at the start of each day for clutches of different sizes												
Clutch = 3	0.03	4.7	39.3	199.2	516.4	887.3	2688.0	2365.6	1970.0			
Clutch = 4	0.03	4.7	39.3	199.3	521.0	912.0	2847.3	2688.0	2365.6	1970.0		
Clutch = 5	0.03	4.7	39.3	199.3	521.1	916.6	2882.0	2847.3	2688.0	2365.6	1970.0	
Clutch = 6	0.03	4.7	39.3	199.3	521.1	916.6	2886.6	2882.0	2847.3	2688.0	2365.6	1970.0
c) Mass of oviduct at midnight at the start of each day for clutches of different sizes												
Clutch = 3	131	207	284	436	677	1092	1223	829	436	218	218	218
Clutch = 4	131	207	284	436	677	1092	1223	961	698	436	218	218
Clutch = 5	131	207	284	436	677	1092	1223	1027	830	633	436	218
Clutch = 6	131	207	284	436	677	1092	1223	1066	909	752	594	436
d) Total extra mass carried at midnight at the start of each day for clutches of different sizes												
Clutch = 3	131	212	323	635	1193	1979	3911	3195	2406	218	218	218
Clutch = 4	131	212	323	635	1198	2004	4070	3649	3064	2406	218	218
Clutch = 5	131	212	323	635	1198	2009	4105	3874	3518	2999	2406	218
Clutch = 6	131	212	323	635	1198	2009	4110	3948	3756	3440	2960	2406

ovarian follicles, oviducal egg and oviduct from residual mass. The significant decrease in mass and residual mass of laying swallows between days 0 and 4 (b less than 0, Fig. 8.3) was accounted for by the reduction in mass of reproductive material. Residual non-egg mass and residual non-reproductive mass also declined between days 0 and 4 (b less than zero, Fig. 8.4), although residual non-reproductive mass was not fitted significantly by a regression equation.

Examination of residual mass, residual non-egg mass and residual non-reproductive mass for females weighed during the laying period showed that residual mass increased during the period of rapid follicular growth, and decreased as the eggs were laid, whilst this pattern was not discernable for residual non-egg or non-reproductive mass either for the population (Figs. 8.5, 8.6) or for an individual (Fig. 8.7).

Residual mass was significantly greater than predicted incubation mass only on days -1 to 4 (Mann-Whitney U-tests, all $Z>2.37$, $p<0.02$, sample sizes as Fig. 8.5). Residual non-egg mass did not differ significantly from predicted incubation mass on any day during laying (all $Z<1.86$, $p>0.06$). Residual non-reproductive mass was not significantly different from predicted incubation mass on any day during rapid follicular growth (all $Z<0.94$, $p>0.34$), but was significantly lower than predicted incubation mass between days 0 and 4 (all $Z>2.33$, $p<0.02$).

Analysis of variance showed mass and residual mass were significantly different among females weighed during the periods of rapid follicular growth, albumen formation, incubating and nestling rearing (Table 8.7). There were significant differences in mass and residual mass between all four stages, other than between incubation and rapid follicular growth. There were no significant differences in residual non-egg mass or residual non-reproductive mass other than that nestling-rearing females were significantly lighter than females at other stages in the reproductive cycle.

The co-efficients of variation of female swallow mass did not differ significantly between females weighed during rapid follicular growth, albumen formation and incubation (ANOVA, $p>0.26$, 23 d.f. (days)). This suggested that

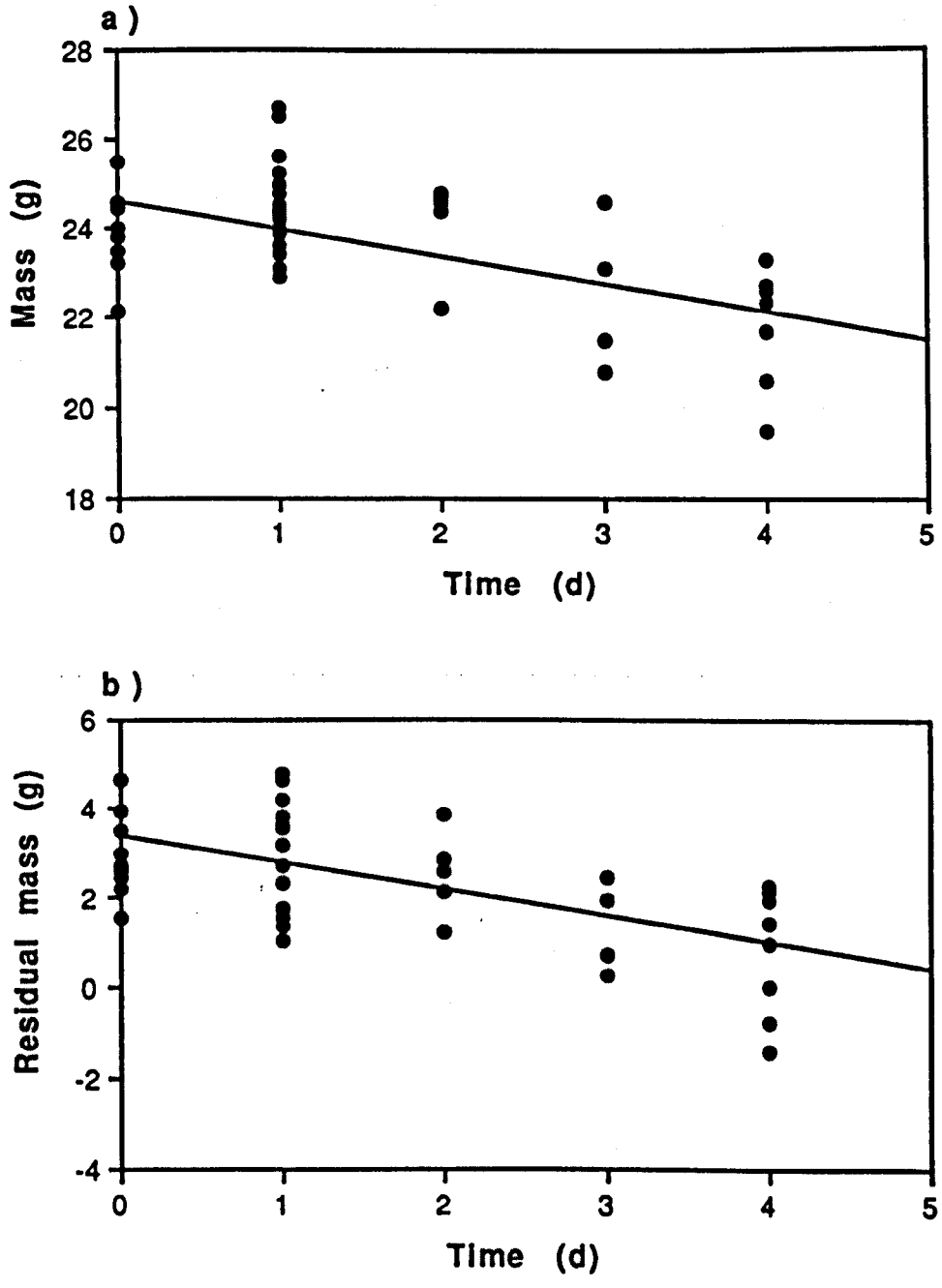


Fig. 8.3 Change in a) mass and b) residual mass of female swallows during the period of albumen formation. Residual mass was mass minus incubation mass predicted from structural size (Chapter 2). Data were the same as used in Fig. 8.2 (each female which laid a clutch of 3 to 6 eggs was included the first time she was caught per breeding attempt). The first egg was laid on day 0. Regression equations were a) $y=-0.613x + 24.537$, $r^2=0.32$, $S_b=0.132$, $p=0.0001$, b) $y=-0.593x + 3.345$, $r^2=0.37$, $S_b=0.116$, $p=0.0001$. $n=46$.

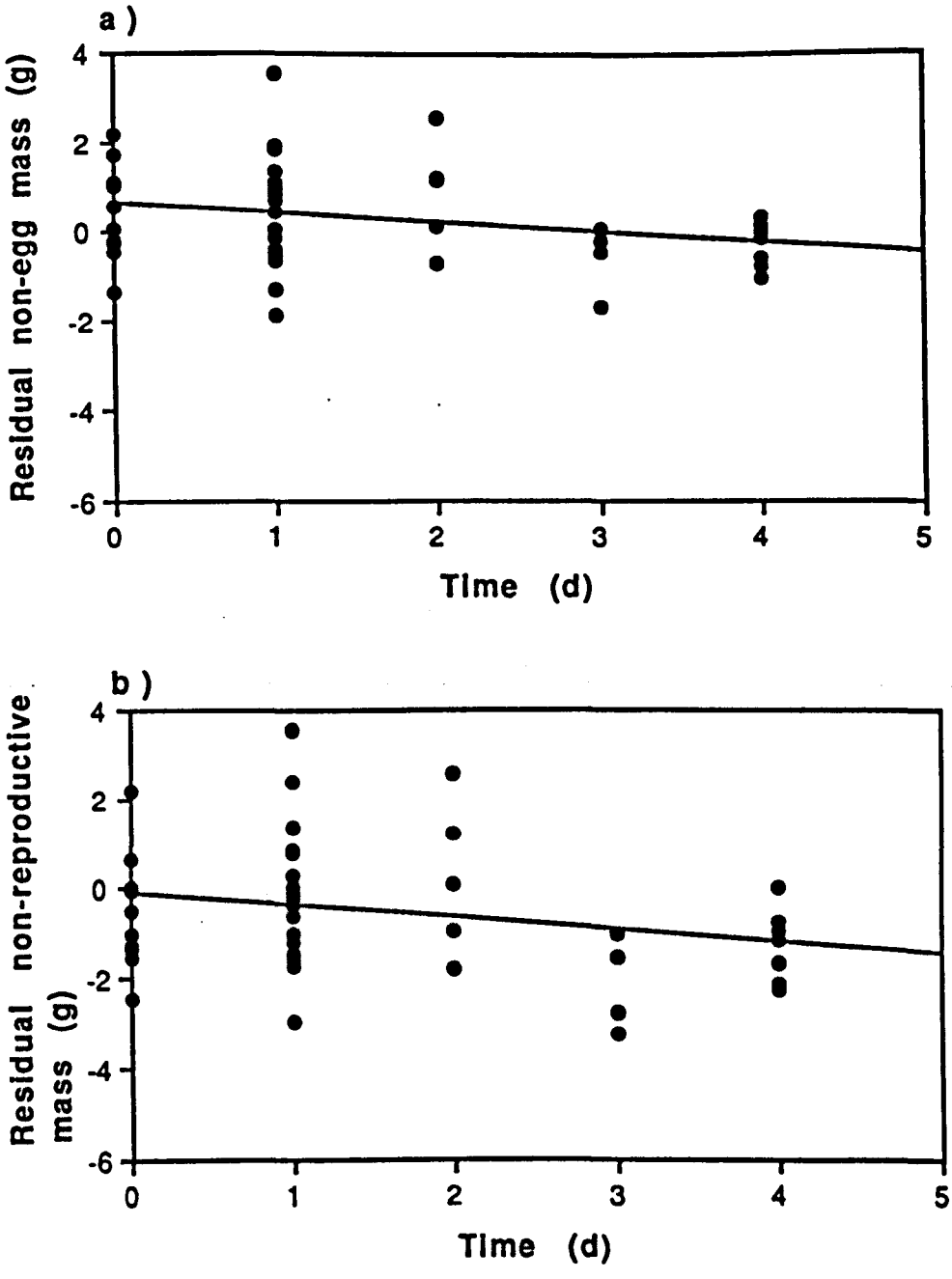


Fig. 8.4 Change in a) residual non-egg mass and b) residual non-reproductive mass of female swallows during the period of albumen formation. Residual non-egg mass was mass minus incubation mass predicted from structural size (Chapter 2) minus mass of ovarian follicles and oviducal egg (Table 8.6). Residual non-reproductive mass was residual non-egg mass minus the mass of the oviduct (Table 8.6). Data were for the same females as in Fig. 8.3 (each female which laid a clutch of 3 to 6 eggs was included the first time she was caught per breeding attempt). The first egg was laid on day 0. Regression equations were a) $y=-0.279x + 0.686$, $r^2=0.11$, $S_b=0.132$, $p=0.03$, b) $y=-0.237 - 0.3045$, $r^2=0.06$, $S_b=0.122$, $p=0.11$. $n=46$.

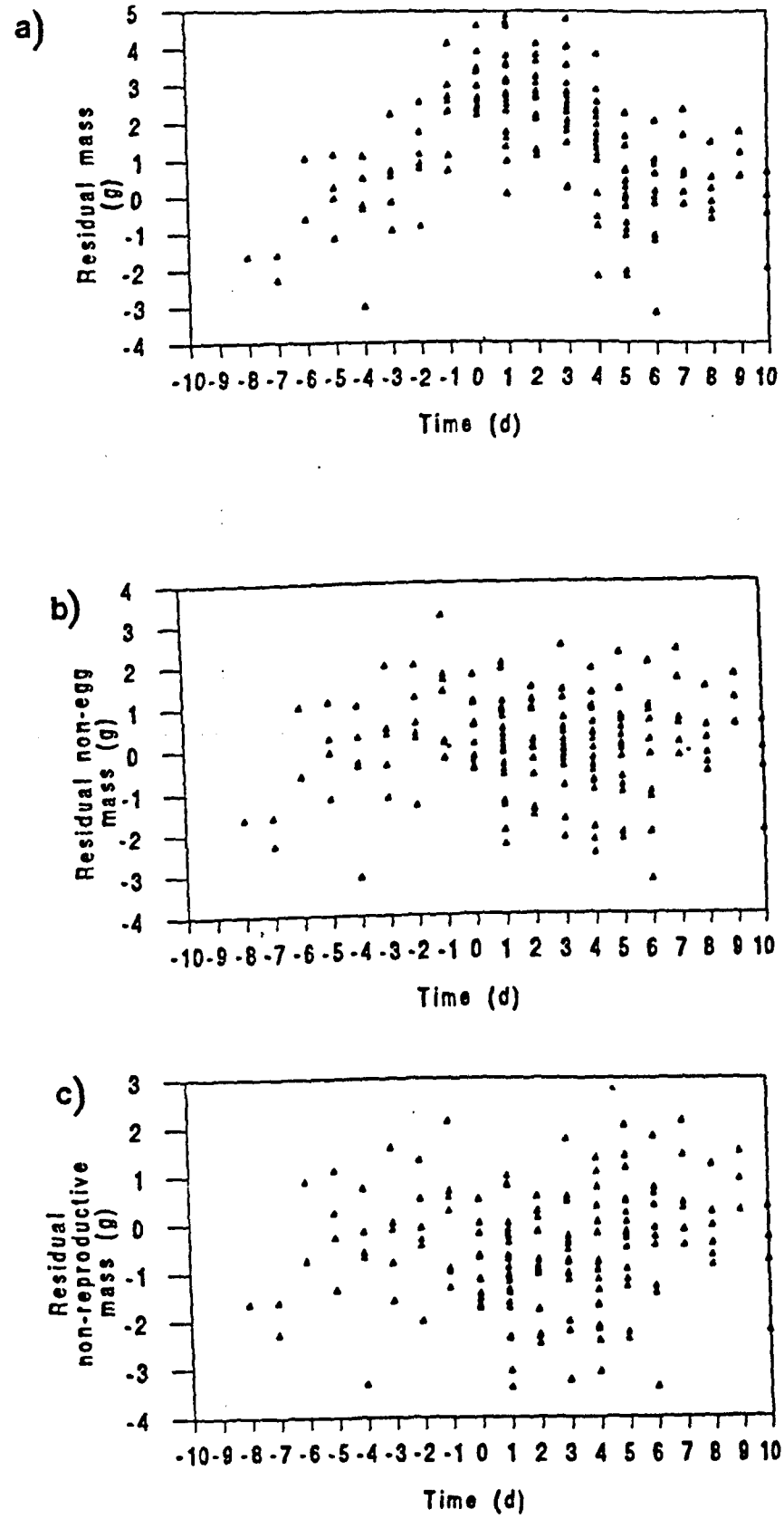


Fig. 8.5 Change in a) residual mass, b) residual non-egg mass and c) residual non-reproductive mass of female swallows during the laying period and at the start of incubation. Residual mass was mass minus incubation mass predicted from structural size (Chapter 2). Residual non-egg mass was residual mass minus mass of ovarian follicles and oviducal egg (predicted from Table 8.6). Residual non-reproductive mass was residual non-egg mass minus the mass of the oviduct (predicted from Table 8.6). The first egg was laid on day 0. Data included females weighed on balances and hand caught birds, with mass adjusted to midnight at the start of each day using an overnight rate of mass loss of 0.15 gh^{-1} (Chapter 2). Each female which laid a clutch of 3-6 eggs was included the first time she was weighed during each breeding attempt.

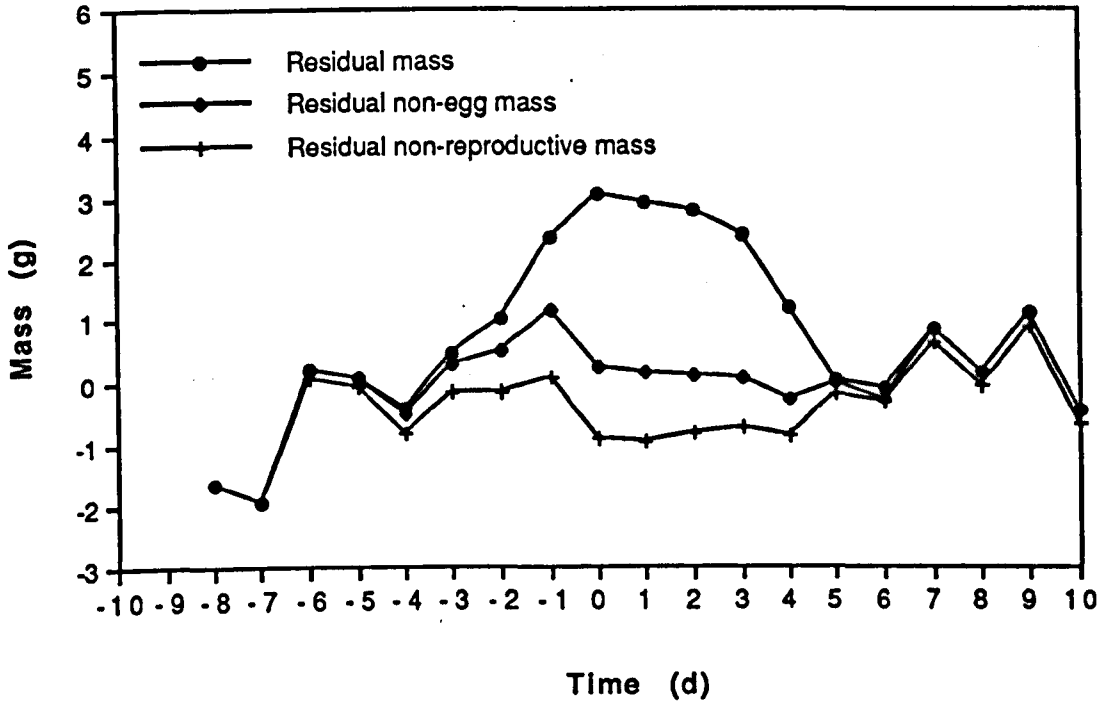


Fig. 8.6 Change in mean residual mass, residual non-egg mass and residual non-reproductive mass of female swallows during the laying period and at the start of incubation. Residual mass was mass minus incubation mass predicted from structural size (Chapter 2). Residual non-egg mass was residual mass minus predicted mass of ovarian follicles and oviducal egg (predicted from Table 8.6). Residual non-reproductive mass was residual non-egg mass minus the mass of the oviduct (predicted from Table 8.6). The first egg was laid on day 0. Data are the means for each day of those shown in Fig. 8.5.

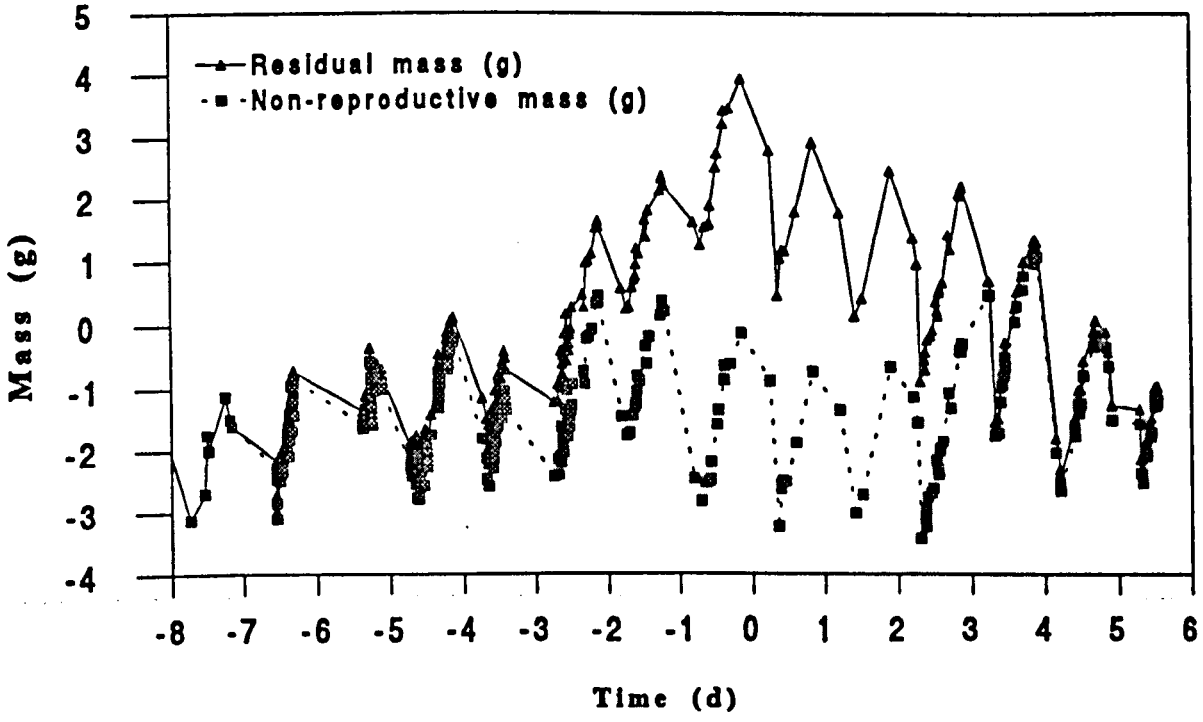


Fig. 8.7 Change in residual mass and residual non-reproductive mass of one female swallow during laying. Residual mass was mass minus incubation mass predicted from structural size (Chapter 2). Residual non-reproductive mass was residual mass minus the mass of ovarian follicles, oviducal egg and oviduct (predicted from Table 8.6). A clutch of 4 eggs was laid on days 0, 1, 2 and 3. Data from this female are also shown in Fig. 8.1.

Table 8.7 Analysis of variance of a) mass (g), b) residual mass (mass minus incubation mass predicted from size, Chapter 2), c) residual non-egg mass (residual mass for nestling-rearing and incubating birds, residual mass minus the mass of ovarian follicles and oviducal egg predicted from Table 8.6 for laying birds), d) residual non-reproductive mass (residual mass for nestling-rearing and incubating birds, residual non-egg mass minus the mass of the oviduct (Table 8.6) for laying birds). Only the first time a female was weighed per breeding attempt was included in this analysis. RFG = period of rapid follicular growth (day -6 to -2), alb. fmn. = period of albumen formation (day -1 till the day before the final egg was laid: day 3 to 5, depending upon clutch size), incub. = incubation period (from the day the final egg was laid till the eggs hatched), nestl. = nestling-rearing period (from the day the eggs hatched till the chicks fledged), * = groups which were significantly different at $p<0.05$.

a) Mass

	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	205.46	68.49	29.79	3	<0.0001
Within groups	264.39	2.30		115	
Total	469.85			118	

Scheffe multiple range test.

Mean	Group	Nestl.	Incub.	RFG	Alb. fmn.
19.50	**Nestl.**				
20.98	**Incub.**	*			
21.87	**RFG**	*			
23.59	**Alb. fmn.**	*	*	*	

b) Residual mass

	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	204.84	68.28	33.29	3	<0.0001
Within groups	229.72	2.05		112	
Total	434.56			115	

Scheffe multiple range test.

Mean	Group	Nestl.	Incub.	RFG	Alb. fmn.
-1.59	**Nestl.**				
-0.11	**Incub.**	*			
0.75	**RFG**	*			
2.60	**Alb. fmn.**	*	*	*	

Table 8.7 continued

c) Residual non-egg mass

	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	45.34	15.11	8.04	3	<0.0001
Within groups	210.53	1.88		112	
Total	255.89			115	

Scheffe multiple range test.

Mean	Group	Nestl.	Incub.	RFG	Alb. fmn.
-1.59	**Nestl.**				
-0.12	**Incub.**	*			
-0.02	**RFG**	*			
0.70	**Alb. fmn.**	*			

d) Residual non-reproductive mass

	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	22.39	7.46	3.41	3	<0.02
Within groups	245.44	2.19		112	
Total	267.83			115	

Scheffe multiple range test.

Mean	Group	Nestl.	Incub.	RFG	Alb. fmn.
-1.59	**Nestl.**				
-0.14	**Incub.**	*			
-0.02	**RFG**				
0.70	**Alb. fmn.**	*			

other stages of the reproductive cycle. Female swallows did not therefore appear either to successfully select only particularly favourable weather for laying, or to regulate their mass during this period to a greater extent than at other times.

One female swallow (the Bankend bird, Tables 4.14, 4.15 and 4.16) which was weighed by a nest balance did not show the normal pattern of mass change during the laying period. This bird laid her first clutch very late in the season, and experienced a spell of poor weather at the end of the period of rapid follicular growth. She weighed only 2 g more than her predicted incubation mass at the end of day -1 (Fig. 8.8), whilst most females at this stage weighed 3-4 g more than during incubation (Fig. 8.2). There were low temperatures and low insect suction trap catches, and heavy rainfall on the final three days before the first egg was laid. Laying stopped for two days after the first egg was laid, and did not recommence until after the weather had improved (Table 4.16). This bird had a small clutch and an interruption in laying. Her daily food intake was presumably inadequate to build up a lipid reserve during the period of rapid follicular growth, so that continued poor weather during the period of albumen formation meant that a laying interruption could not be avoided, as the bird had no reserves with which to supplement daily food intake.

8.3.2.2 Relationship between mass and condition of laying swallows

Female swallow mass on the night after the first egg of a clutch was laid was not significantly correlated with any of the indices of body condition (Table 8.8). This demonstrated that mass and body condition could vary independently. Residual mass (mass minus incubation mass predicted from structural size, Chapter 2) was positively correlated with all measures of body condition, although significantly so only with fat score 1 (sum of anterior and posterior fat scores). Correlations between mass and condition indices were not improved by subtraction of the mass of ovarian follicles and oviducal eggs (to obtain residual non-egg mass), although reserves of protein or fat should be most closely correlated with mass after body size, ovarian

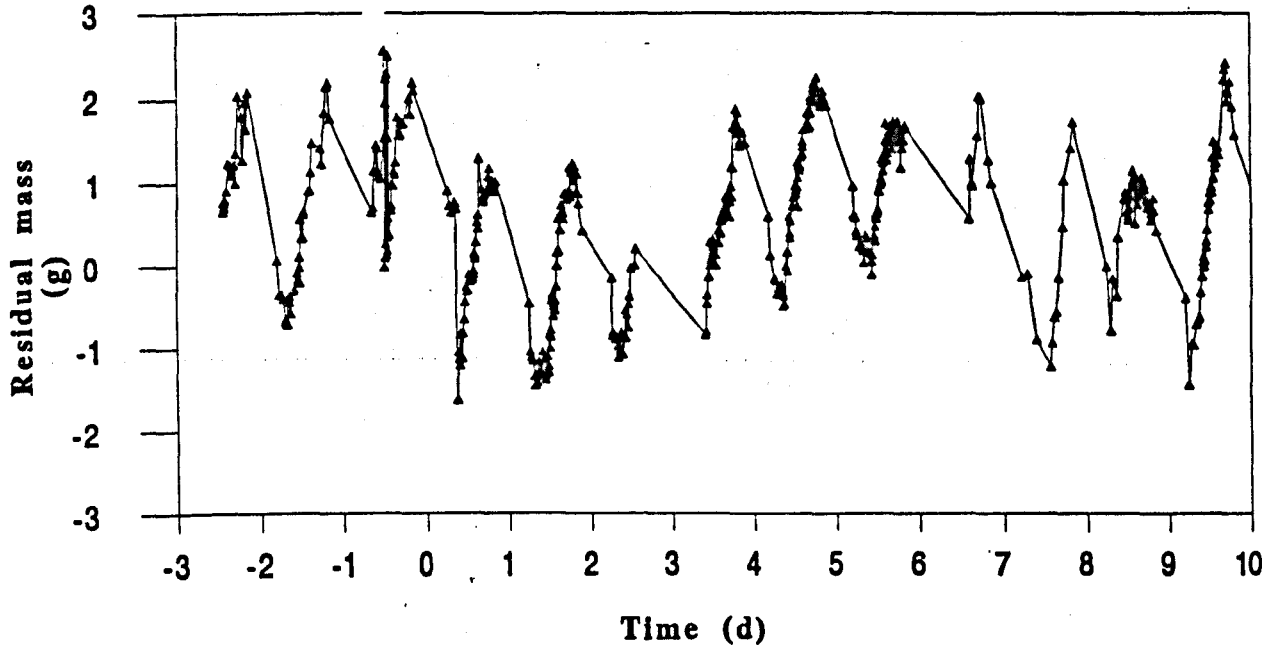


Fig. 8.8 Residual mass of a female swallow during laying and at the start of incubation, for a bird which had an interruption in laying of 2 d after she laid the first egg of her clutch. Eggs were laid on days 0, 3, 4 and 5. Residual mass was mass minus incubation mass predicted from structural size (Chapter 2).

Table 8.8 Pearson correlation co-efficients between mass and condition of female swallows during the night after the first egg of a clutch was laid. Residual mass was mass minus incubation mass predicted from structural size (Chapter 2), residual non-egg mass was residual mass minus the mass of ovarian follicles and oviducal egg (Table 8.6). Measures of condition were: fat score 1 (sum of the scores from the anterior and posterior deposits), fat score 2 (percentage cover of abdominal fat, arcsine transformed), USTHICK (sum of the ultrasound measurements of pectoral muscle thickness), USI (sum of the ultrasound measurements/keel length) and USVOL (sum of the ultrasound measurements × keel length/1000). * = $p<0.05$, ns = not significant.

	Fat score 1	Fat score 2	USTHICK	USI	USVOL
n	14	6	12	12	12
Mass	0.45 ns	0.51 ns	0.05 ns	-0.03 ns	0.15 ns
Residual mass	0.60 *	0.81 ns	0.44 ns	0.46 ns	0.17 ns
Residual non-egg mass	0.58 *	0.64 ns	0.44 ns	0.44 ns	0.21 ns

follicle and oviducal egg mass were taken into account. Sample sizes in this analysis were small, so conclusions need confirmation in a future study.

Too few females were caught during the night before the first egg of a clutch was laid to allow analysis of the relationship between body mass and condition at this time. It was likely that the relationship between mass and condition would be broadly similar to that on the night after the first egg was laid, when a larger sample of birds was caught.

8.3.2.3 Changes in female swallow body condition during laying

Condition indices of laying female swallows were determined for females caught once per breeding attempt, and for a few individuals which were caught twice during the laying period. The changes in residual mass of female swallows caught twice during the laying period were consistent with the results in Sections 8.3.1 and 8.3.2.1. Fat score 1 changed in a similar way to mass (Fig. 8.9b), whilst indices of pectoral muscle condition showed no consistent change during laying (Fig. 8.9c,d). Bird C (Fig. 8.9) was lighter than expected on day 1, and had a low fat score, although indices of pectoral muscle condition were similar to those in the other birds. Female swallows recaptured after measurement of energy expenditure using the DLW technique (Chapter 7) were not included in this analysis.

Similar changes in condition indices were found for the sample of birds caught once per breeding attempt. Fat score 1 (the sum of anterior and posterior fat scores) and fat score 2 (arcsine % cover of abdominal fat) showed patterns of change which paralleled those of mass (Figs. 8.2 and 8.10). Fat scores were variable throughout the laying period, but the mean was low in birds caught more than 2 d before the first egg was laid, high on day 0, declined between days 0 and 5, and increased after the start of incubation (Fig. 8.10). In a regression analysis, fat score 1 declined significantly between days 0 and 4 (b significantly less than 0, Fig. 8.11).

An analysis of variance showed that fat score 1 was slightly higher during incubation than during the period of albumen formation, but was significantly greater

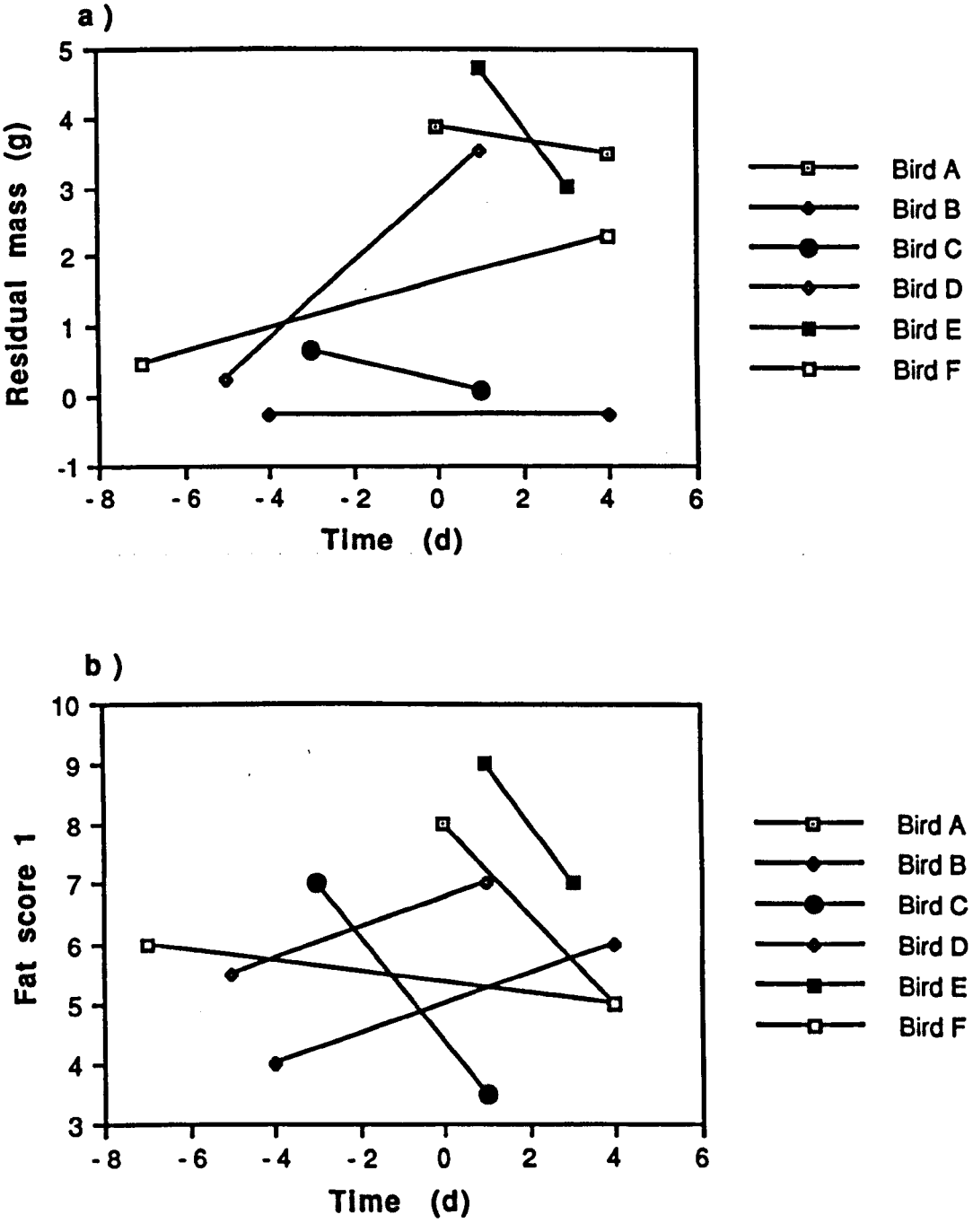
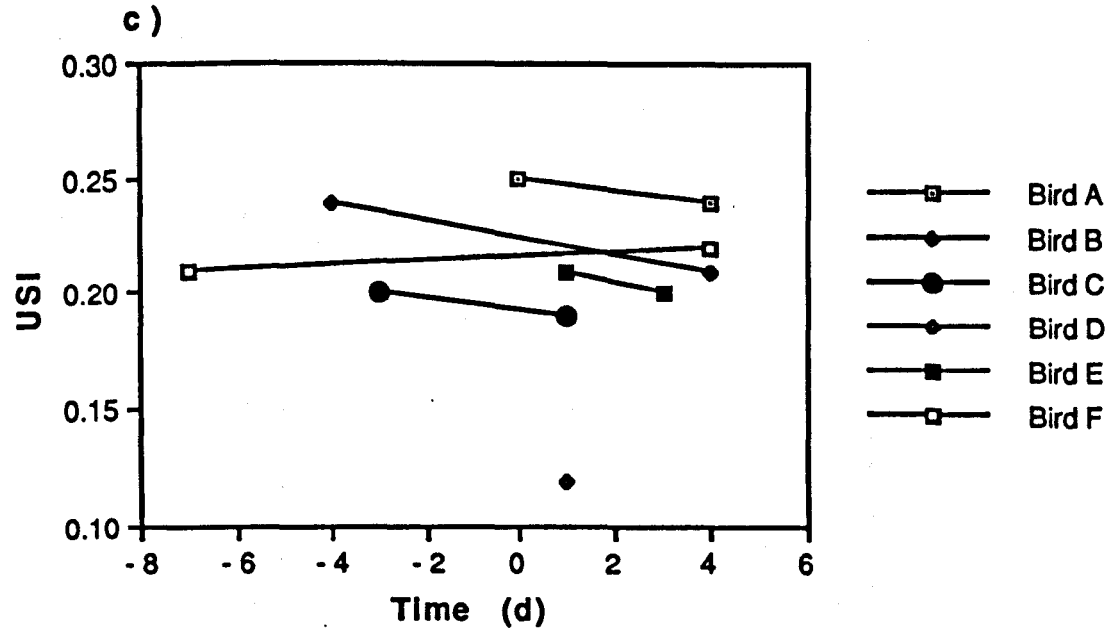


Fig. 8.9 Changes in residual mass and condition indices of female swallows caught twice during the laying period: a) residual mass (mass minus incubation mass predicted from structural size, Chapter 2), b) fat score (sum of the anterior and posterior fat scores), c) USI (ultrasound index) was the sum the ultrasound measures of pectoral muscle thickness/keel length, d) USVOL (ultrasound volume) was the sum of ultrasound measurements × keel length/1000. See Chapter 2 for a more detailed explanation of condition indices.



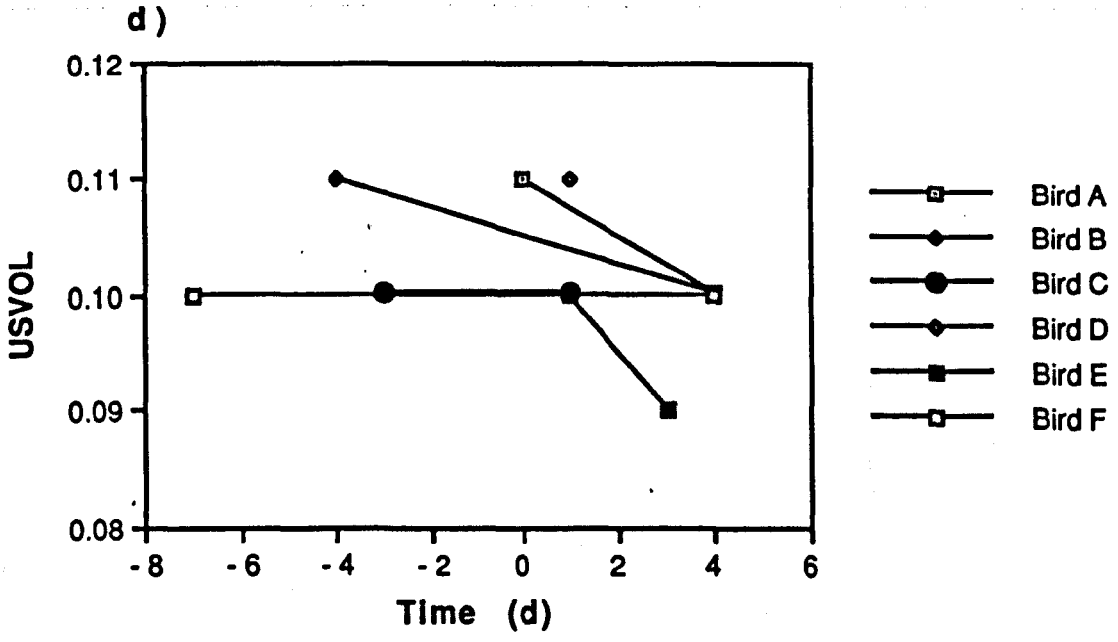


Fig. 8.9 continued

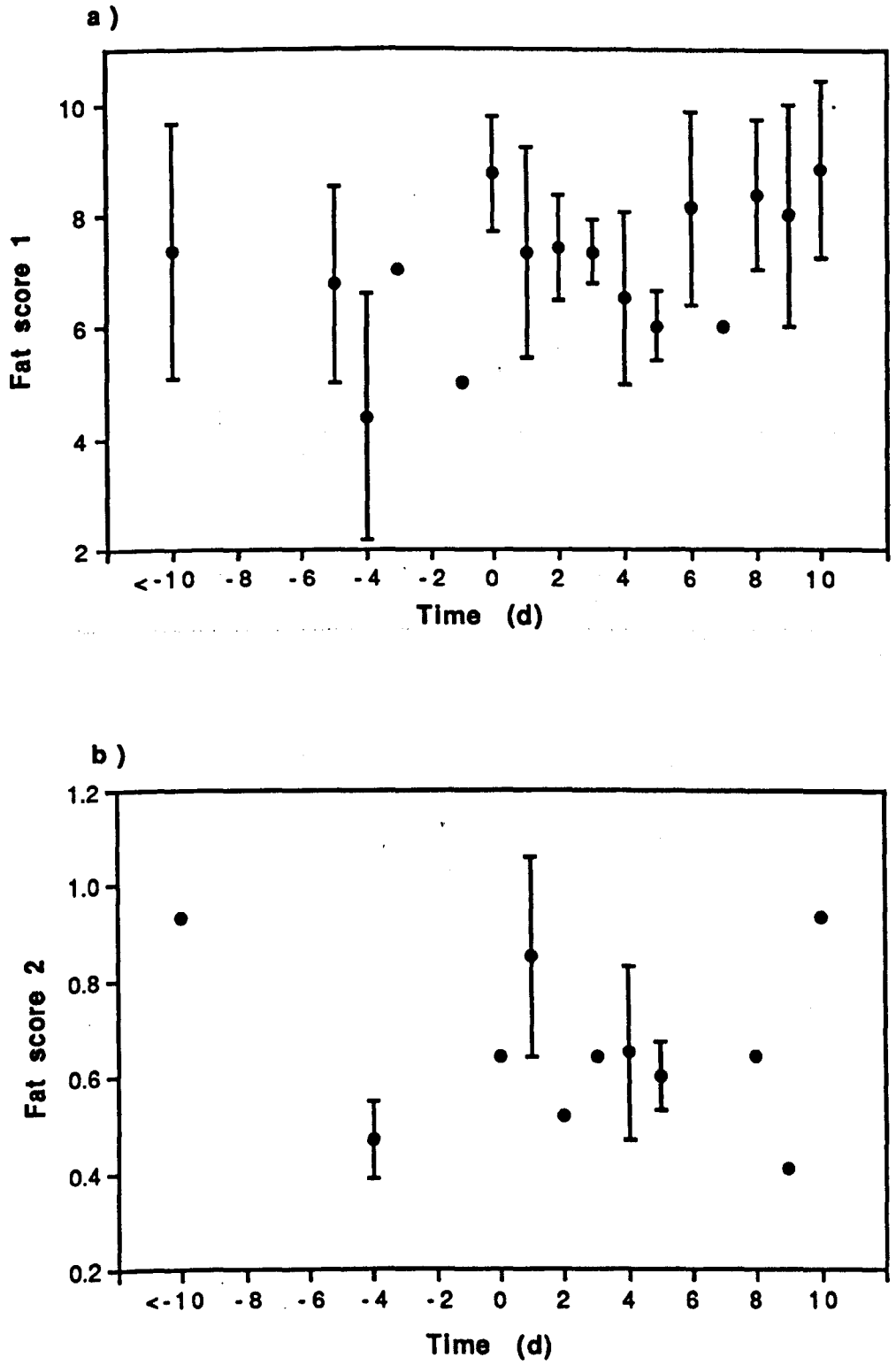


Fig. 8.10 Visual fat scores of female swallows during the laying period (mean, with error bars showing sd): a) fat score 1 (the sum of the anterior and posterior fat scores), b) fat score 2 (the percentage cover of abdominal fat, arcsine transformed). Only the first occasion each female which laid a clutch of 3 to 6 eggs was caught during each breeding attempt was included in this analysis (the same birds as in Fig. 8.2). Sample sizes, starting with day <-10, were 4, 2, 4, 1, 1, 2, 12, 5, 3, 7, 6, 4, 1, 4, 3 and 3 for fat score 1 and 1, 2, 1, 5, 1, 1, 2, 3, 1, 1 and 1 for fat score 2. The first egg was laid on day 0.

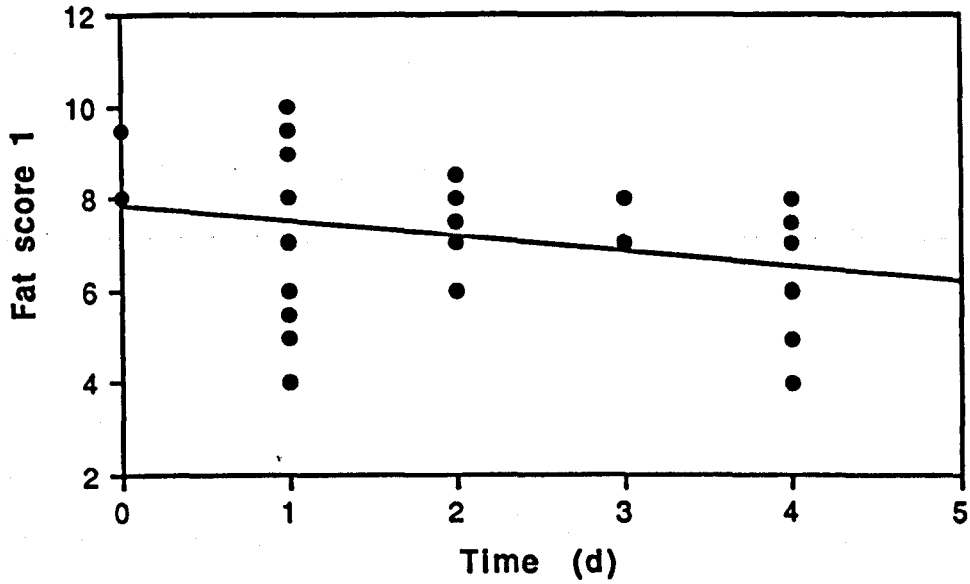


Fig. 8.11 Change in fat score 1 (the sum of the anterior and posterior fat scores) of female swallows during the albumen formation period. Each female which laid a clutch of 3 to 6 eggs included the first time she was caught per breeding attempt. The first egg was laid on day 0. The regression equation was $y=-0.350x + 7.848$, $r^2=0.02$, $n=38$.

during both albumen formation and incubation than during nestling-rearing or the period of rapid follicular growth (Table 8.9). There were no significant differences in fat score 1 between birds caught on days 1 and 4 or on days 0 and 4 (Mann-Whitney U-tests, both $Z<1.56$, $p>0.11$, $n=2$ on day 0, 12 on day 1 and 7 on day 4). The co-efficients of variation of fat scores 1 and 2 did not differ significantly between incubation and the periods of rapid follicular growth or albumen formation (ANOVA, $p=0.26$, 23 d.f. (days)). Fat score 1 was significantly more variable during rapid follicular growth than during albumen formation (ANOVA, $p<0.04$, 19 d.f.).

Indices of pectoral muscle condition derived from the ultrasound measurements of pectoral muscle thickness did not show the pattern of rise and fall shown by mass and fat score during the laying period (Fig. 8.12). In an analysis of variance, there were no significant differences between indices of pectoral muscle condition during the periods of rapid follicular growth, albumen formation, incubation and nestling rearing (Table 8.9). Indices of pectoral muscle condition did not differ significantly between days 0 or 1 and day 4 (Mann-Whitney U-tests, both $Z<1.76$, $p>0.08$, $n=2$ on day 0, 12 on day 1 and 7 on day 4). No significant regression equation could be fitted to the indices of pectoral muscle condition between days 0 and 4 ($p=0.6$, $n=26$), when most egg protein was deposited. This was, however, after the period during which quelea protein reserves declined (Jones and Ward 1976). The co-efficients of variation of indices of female swallow pectoral muscle condition were not significantly different between the period of rapid follicular growth, albumen formation, incubation and nestling rearing (ANOVA, $p=0.26$, 23 d.f. (days)). These results could be due to a lack of change in pectoral muscle thickness during laying, or if the ultrasound device were not capable of detecting small, but real, differences in thickness.

Table 8.9 Analysis of variance of a) fat score 1 (sum of anterior and posterior fat scores), b) USTHICK (sum of the ultrasound measurements of muscle thickness), c) USI (sum of the ultrasound measurements of muscle thickness/keel length), and d) USVOL (sum of the ultrasound measurements of muscle thickness × keel length/1000). Only data from the first time a female was caught per breeding attempt was included in this analysis. RFG = birds caught during the period of rapid follicular growth (day -6 to -2), alb. fmn. = period of albumen formation (day -1 till the day before the final egg was laid: day 3 to 5, depending upon clutch size), incub. = incubation period (from the day the final egg was laid till the eggs hatched), nestl. = nestling-rearing period (from the day the eggs hatched till the chicks fledged), * = groups significantly different at $p<0.05$.

a) Fat score 1

	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	76.51	25.50	8.59	3	<0.0001
Within groups	293.83	2.97		99	
Total	370.34			102	

Scheffe multiple range test.

Mean	Group	RFG	Nestl.	Alb. fmn.	Incub.
5.15	RFG				
5.73	Nestl.				
7.44	Alb. fmn.	*	*		
7.56	Incub.	*	*		

b) USTHICK

	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	0.46	0.15	2.26	3	0.09
Within groups	4.16	0.07		61	
Total	4.63			64	

Scheffe multiple range test: no two groups were different at the 0.05 level.

c) USI

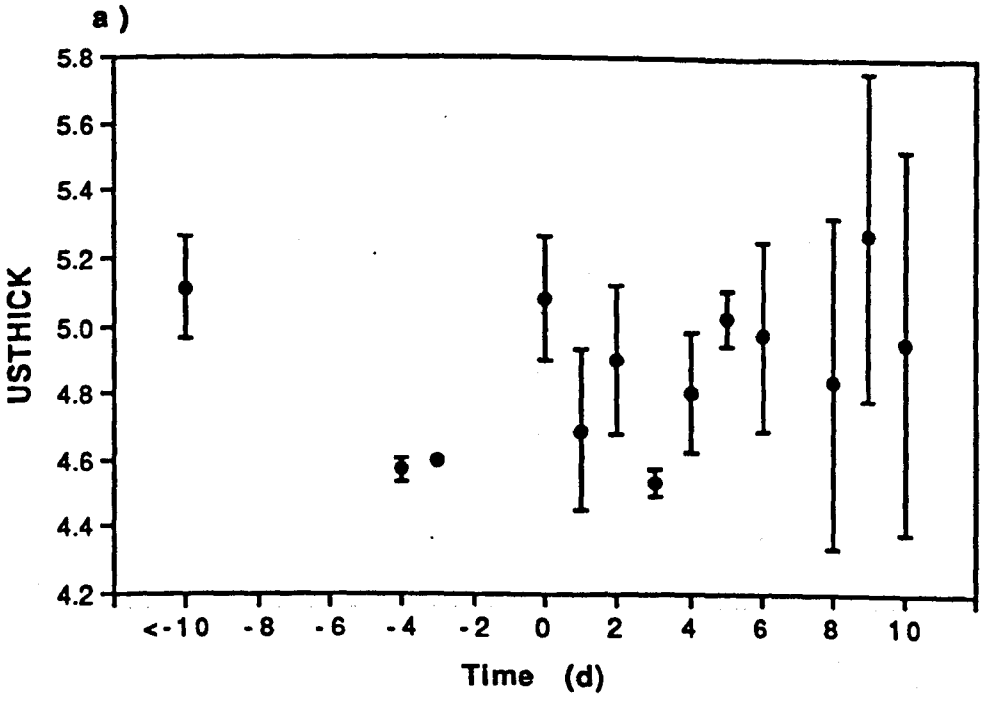
	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	0.0012	0.0004	1.8980	3	1.90
Within groups	0.0130	0.0002		61	
Total	0.0142			64	

Scheffe multiple range test: no two groups were different at the 0.05 level.

d) USVOL

	Sum of squares	Mean squares	F	d.f.	Signif.
Between groups	0.0002	0.0001	2.19	3	0.09
Within groups	0.0021	0.0000		61	
Total	0.0023			64	

Scheffe multiple range test: no two groups were different at the 0.05 level.



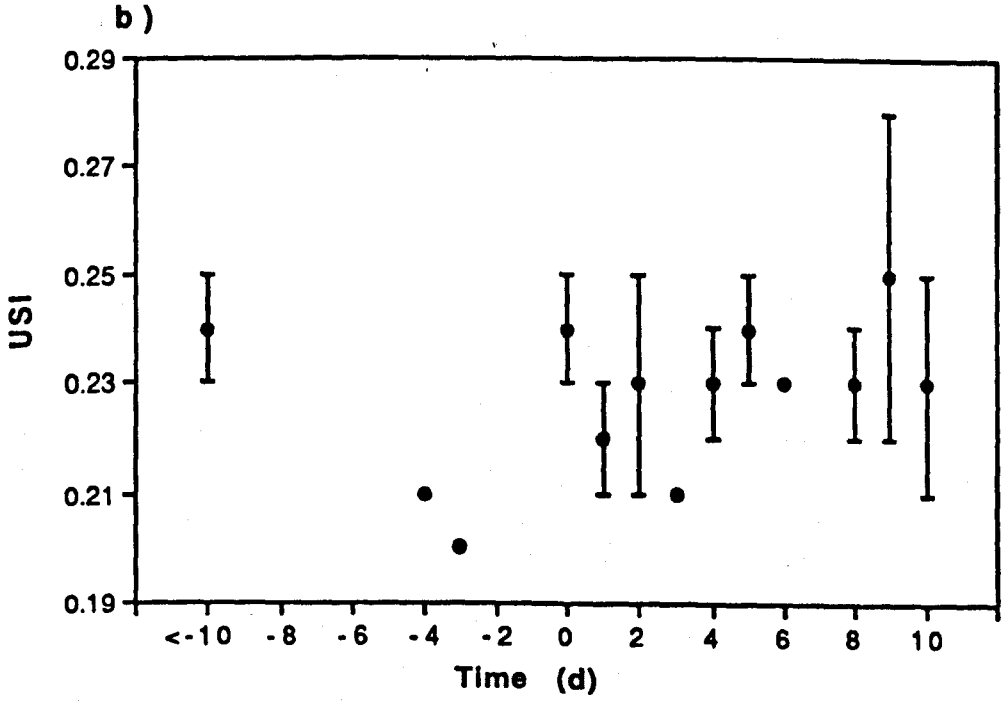


Fig. 8.12 Continued overleaf

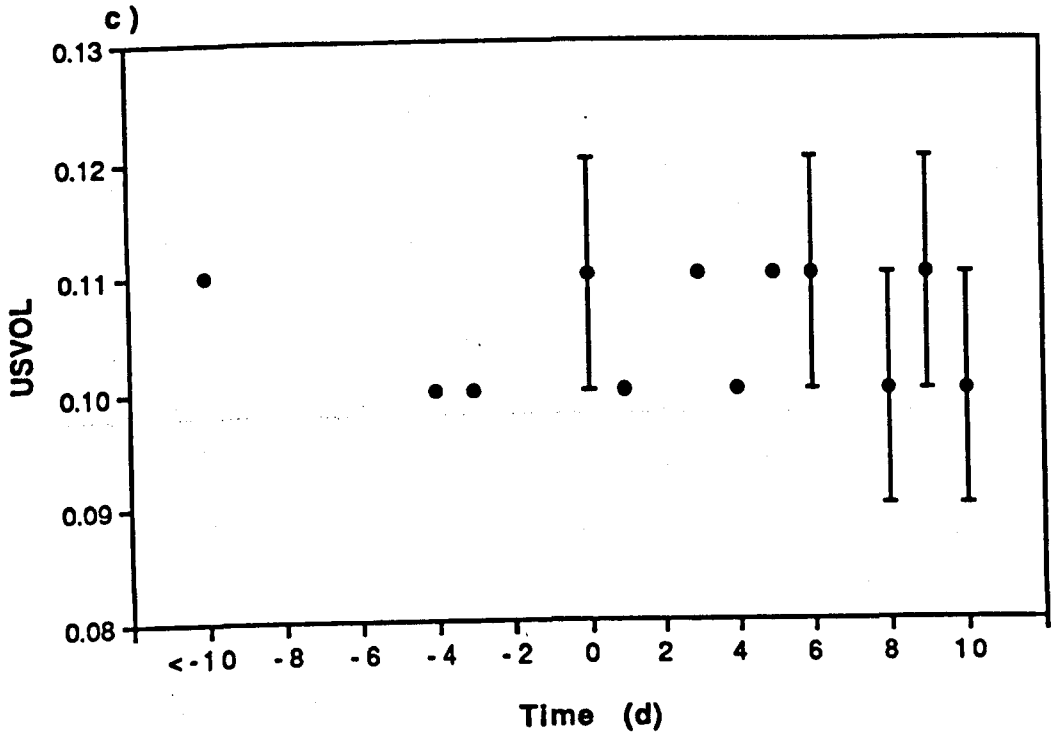


Fig. 8.12 Pectoral muscle condition indices of female swallows during the laying period (mean, with error bars showing sd): a) USTHICK (ultrasound thickness, the sum of the ultrasound measurement of pectoral muscle thickness), b) USI (ultrasound index, the sum the ultrasound measures of pectoral muscle thickness/keel length), d) USVOL (ultrasound volume, the sum of ultrasound measurements × keel length/1000). Only the first occasion each female which laid a clutch of 3 to 6 eggs was caught during each breeding attempt was included in this analysis. Sample sizes, starting with day <-10, were 4, 2, 1, 2, 8, 3, 2, 7, 3, 3, 4, 2, and 2. The first egg was laid on day 0. The mass of these birds was shown in Fig. 8.2 and indices of lipid content in Fig. 8.10.

8.3.3 Factors related to the mass and condition of laying swallows

8.3.3.1 Clutch number

There were no significant differences between the mass or condition indices of female swallows laying first and second clutches (t-tests, $t<1.65$, $p>0.12$, n=21 first clutch and 19 second clutch females for mass, 7 and 8 females for condition indices, albumen formation period only). The mean mass of females laying first clutches was 24.2 g, 0.6 g heavier than those laying second clutches. In an analysis of a larger number of females, first clutch females were significantly heavier than those laying second clutches (Thompson 1992). This sort of analysis was potentially subject to bias, as mass decreased as the eggs were laid, and neither clutch size nor the number of eggs already laid was controlled for in this analysis. There were insufficient weighings for a comparison between most single days during laying, but on the night after the first egg was laid, there was no significant difference in mass between first and second clutch females ($t<0.85$, $p>0.4$, n=14 first clutch and 11 second clutch birds) although the mean mass of first clutch females was 0.42 g greater. Condition indices on the night after the first egg was laid did not differ significantly between first and second clutches (all $t<0.39$, $p>0.7$, n=5-8 first and 2-6 second clutch females). The greater mass of females during laying of first clutches might be due to their carrying a larger nutrient reserve as an insurance against poor feeding conditions, as food supply was less predictable during laying of first the second clutches (Chapter 4), or it could be accounted for by birds which laid larger clutches being heavier during laying (first clutches were significantly larger than second, Chapter 4).

8.3.3.2 Egg and clutch size

Possible relationships between mass and condition indices of female swallows at the start of laying and their subsequent egg and clutch sizes would ideally be investigated from data before the first egg was laid. As it was more difficult to

predict when laying would start than to catch birds during the night after the first egg was laid, analyses performed with mass and egg or clutch size on the night before the first egg was laid were repeated for the night after the first egg was laid, with a larger sample of birds.

Egg size, clutch size and clutch mass were not correlated with measures of mass or condition of female swallows on the nights before or after the first egg was laid (Tables 8.10 and 8.11). There were no significant differences in mass or condition indices on the nights before or after the first egg of a clutch was laid between birds which subsequently laid clutches of 3 to 6 eggs (analyses of variance, all $p>0.08$, $n=12$, mass only, on night before the first egg was laid; sample sizes as Table 8.11 for the night after the first egg was laid). No relationship was apparent between female body condition and subsequent clutch size in quelea either (Jones and Ward 1976).

If significance criteria were relaxed to $p<0.10$, residual mass and residual non-egg mass on the night before the first egg was laid and fat score 2 on the night after the first egg was laid would have been positively correlated with clutch size (Tables 8.10 and 8.11). A relationship between mass and subsequent clutch size would not be expected merely on the basis that the additional mass of females about to lay larger clutches would contain an extra rapidly developing ovarian follicle, since the additional mass of reproductive material was predicted to be small (Table 8.6). These results suggested that reserve size at the start of laying was related to subsequent clutch size, but that the relationship was rather weak.

The correlation between fat score 2 and clutch size could indicate a genuine relationship between fat reserves and subsequent clutch size, however there was the complication that birds developing more follicles would have a more swollen abdomen and consequently might show a larger area of abdominal fat which would however be thinner than that of birds about to lay smaller clutches. Anterior and posterior fat scores have been shown to be highly correlated with carcass fat content, whilst a relationship with abdominal fat cover and body lipid content has not been validated by carcass analysis. These results were consistent with the hypothesis that

Table 8.10 Pearson correlation co-efficients between clutch size (number of eggs), clutch mass (mass of clutch) and mean egg mass, and the mass of female swallows during the night **before** they laid the first egg of a clutch (midnight at the start of day 0). Residual mass was mass minus incubation mass predicted from structural size (Chapter 2), residual non-egg mass was residual mass minus the mass of ovarian follicles and oviducal egg (Table 8.6). ns = not significant. n = 12.

	Clutch size	**Clutch mass**	**Mean egg mass**
Mass	0.40 ns	0.46 ns	-0.39 ns
Residual mass	0.51[1] ns	0.52 ns	-0.33 ns
Residual non-egg mass	0.51[1] ns	0.51 ns	-0.33 ns

[1] would have been significant at $p<0.10$

Table 8.11 Pearson correlation co-efficients between clutch size (number of eggs), clutch mass (mass of clutch) and mean egg mass, and mass and condition indices of female swallows during the night **after** the day upon which they laid the first egg of a clutch (midnight at the start of day 1). Residual mass was mass minus incubation mass predicted from structural size (Chapter 2), residual non-egg mass was residual mass minus the mass of ovarian follicles and oviducal egg (Table 8.6). Fat score 1 was the sum of the fat scores from the anterior and posterior deposits, fat score 2 was the percentage cover of abdominal fat (arcsine transformed), USTHICK was the sum of the ultrasound measurements of pectoral muscle thickness, USI the sum of the ultrasound measurements/keel length and USVOL the sum of the ultrasound measurements × keel length/1000. ns = not significant.

	Clutch size	Clutch mass	Mean egg mass
Mass	-0.02	-0.28	-0.11
(n = 25)	ns	ns	ns
Residual mass	0.17	-0.23	-0.01
(n = 25)	ns	ns	ns
Residual non-egg mass (n = 25)	0.05 ns	0.31 ns	0.00 ns
Fat score 1	0.30	-0.09	0.08
(n = 14)	ns	ns	ns
Fat score 2	0.77	0.64	0.07
(n = 6)	ns[1]	ns	ns
USTHICK	0.22	-0.30	0.11
(n = 12)	ns	ns	ns
USI	0.36	-0.24	0.06
(n = 12)	ns	ns	ns
USVOL	-0.14	-0.25	0.14
(n = 12)	ns	ns	ns

[1] would have been significant at $p<0.10$.

the bulk of the energy and material for egg formation in swallows came from food intake on the day that the egg material was deposited.

8.3.3.3 Environmental factors

Mass and condition indices of female swallows on the night after the first egg of a clutch was laid were significantly correlated with environmental factors and food supply during the previous 1 to 5 d (Table 8.12). Mass was positively correlated with minimum temperature on the previous day and on the mean of the previous 2 to 5 d. Residual mass and residual non-egg mass were positively correlated with temperature and negatively correlated with rainfall. Fat score 1 was positively correlated with maximum temperature and insect suction trap catch on the previous day. There were no significant correlations between abdominal fat cover and environmental factors. USTHICK and USI were positively correlated with temperature and negatively correlated with rainfall, but USVOL was not significantly correlated with any of the environmental factors. The number of significant correlations between mass or condition indices and environmental factors decreased when a longer period before the birds were caught and examined was included (12 significant correlations with environmental factors on the previous day, but only 7 with the mean of the previous 5 d, Table 8.12).

The only significant correlations between environmental factors over the previous 1-5 days and measures of female mass on the night before the first egg was laid, were with rainfall on the previous 2 to 5 days (all $r<-0.58$, $p<0.05$, $n=12$). This was consistent with the results shown in Table 8.12. The reason for the low number of correlations was probably the smaller sample size.

In a regression analyses none of the environmental factors on the previous five days, condition indices, egg or clutch size was a significant predictor of the residual mass of females weighed on the night after they laid the first egg of a clutch ($p>0.05$). For the same birds, clutch size was not predicted by environmental factors either before or after the day the first egg was weighed, or by female mass or

Table 8.12 Pearson correlation co-efficients between mass or condition indices of female swallows at midnight on the night after they laid the first egg of a clutch and environmental factors on, a) the previous day, b) the mean of the previous 2 d, c) the mean of the previous 3 d, d) the mean of the previous 4 d, and e) the mean of the previous 5 d. Measures of female mass and condition were: mass (g), residual mass (mass minus incubation mass predicted from structural size, Chapter 2), residual non-egg mass (residual mass minus the mass of ovarian follicles and oviducal egg, predicted from Table 8.6), fat score 1 (the sum of the scores from the anterior and posterior deposits), fat score 2 (the percentage of abdominal fat, arcsine transformed), USTHICK (the sum of the ultrasound measurements of pectoral muscle thickness), USI (the sum of the ultrasound measurements/keel length), USVOL (the sum of the ultrasound measurements × keel length/1000). Measures of environmental variables were: mean t. = mean temperature (°C), max. t. = maximum temperature (°C), min. t. = minimum temperature (°C), rain = rainfall (mm) and food=$\log_e(V+1)$ where V=insect suction trap volume. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, ns = not significant. Sample sizes are shown in brackets. - = correlation could not be computed (all values the same for one variable).

a) Environmental factors on previous day

	Mean t.	**Max. t.**	**Min. t.**	**Food**	**Rain**
Mass (n = 25)	0.39 ns	0.29 ns	0.42 *	-0.03 ns	-0.27 ns
Residual mass (n = 25)	0.45 *	0.39 *	0.42 *	0.10 ns	-0.51 **
Residual non-egg mass (n = 25)	0.45 *	0.39 *	0.42 *	0.10 ns	-0.51 **
Fat score 1 (n = 14)	0.49 ns	0.62 *	0.22 ns	0.54 *	-0.49 ns
Fat score 2 (n = 6)	-0.29 ns	0.02 ns	-0.46 ns	-0.45 ns	-
USTHICK (n = 12)	0.57 ns	0.78 **	0.20 ns	0.24 ns	-0.46 ns
USI (n = 12)	0.66 *	0.83 ***	0.28 ns	0.31 ns	-0.53 ns
USVOL (n = 12)	0.14 ns	0.28 ns	-0.03 ns	-0.01 ns	-0.11 ns

Table 8.12 continued

b) Environmental factors on previous 2 d

	Mean t.	Max. t.	Min. t.	Food	Rain
Mass	0.39 ns	0.27 ns	0.48 *	0.17 ns	-0.25 ns
Residual mass	0.42 *	0.35 ns	0.44 *	0.23 ns	-0.44 *
Residual non-egg mass	0.42 *	0.35 ns	0.44 *	0.23 ns	-0.44 *
Fat score 1	0.40 ns	0.45 ns	0.24 ns	0.40 ns	-0.47 ns
Fat score 2	-0.11 ns	-0.04 ns	-0.13 ns	-0.38 ns	0.26 ns
USTHICK	0.49 ns	0.66 *	0.17 ns	0.19 ns	-0.45 ns
USI	0.63 *	0.81 **	0.25 ns	0.16 ns	-0.52 ns
USVOL	0.02 ns	0.09 ns	-0.06 ns	0.15 ns	-0.11 ns

c) Environmental factors on previous 3 d

	Mean t.	Max. t.	Min. t.	Food	Rain
Mass	0.35 ns	0.20 ns	0.46 *	0.26 ns	-0.45 *
Residual mass	0.39 ns	0.28 ns	0.44 *	0.34 ns	-0.54 **
Residual non-egg mass	0.39 ns	0.28 ns	0.44 *	0.34 ns	-0.54 **
Fat score 1	0.32 ns	0.41 ns	0.13 ns	0.44 ns	-0.50 ns
Fat score 2	-0.12 ns	-0.21 ns	0.44 ns	-0.16 ns	0.26 ns
USTHICK	0.42 ns	0.67 *	0.06 ns	0.32 ns	-0.53 ns
USI	0.60 *	0.82 ***	0.21 ns	0.23 ns	-0.64 *
USVOL	-0.08 ns	0.08 ns	-0.20 ns	0.32 ns	-0.07 ns

Table 8.12 continued

d) Environmental factors on previous 4 d

	Mean t.	Max. t.	Min. t.	Food	Rain
Mass	0.31 ns	0.16 ns	0.41 *	0.22 ns	-0.31 ns
Residual mass	0.35 ns	0.22 ns	0.43 *	0.32 ns	-0.46 *
Residual non-egg mass	0.35 ns	0.22 ns	0.43 *	0.32 ns	-0.46 *
Fat score 1	0.22 ns	0.34 ns	0.03 ns	0.52 ns	-0.40 ns
Fat score 2	-0.42 ns	-0.40 ns	-0.44 ns	-0.45 ns	-0.32 ns
USTHICK	0.34 ns	0.66 *	0.05 ns	0.47 ns	-0.38 ns
USI	0.51 ns	0.77 **	0.22 ns	0.37 ns	-0.47 ns
USVOL	-0.10 ns	0.16 ns	-0.24 ns	0.34 ns	-0.03 ns

e) Environmental factors on previous 5 d

	Mean t.	Max. t.	Min. t.	Food	Rain
Mass	0.31 ns	0.16 ns	0.43 *	0.23 ns	-0.28 ns
Residual mass	0.35 ns	0.22 ns	0.43 *	0.32 ns	-0.45 *
Residual non-egg mass	0.35 ns	0.22 ns	0.43 *	0.32 ns	-0.45 *
Fat score 1	0.20 ns	0.30 ns	0.03 ns	0.54 *	-0.42 ns
Fat score 2	-0.34 ns	-0.33 ns	-0.36 ns	-0.27 ns	-0.32 ns
USTHICK	0.24 ns	0.53 ns	0.02 ns	0.43 ns	-0.36 ns
USI	0.38 ns	0.61 *	0.16 ns	0.34 ns	-0.45 ns
USVOL	-0.09 ns	0.13 ns	-0.22 ns	0.36 ns	-0.04 ns

condition indices (regression analyses, all $p>0.05$).

The correlations between clutch size and mass or fat score 2 were weaker than those between clutch size and the effects of environmental factors during the previous 1 to 5 days (Section 8.3.3.3). As the effect of mass and condition indices upon subsequent clutch size was weak, and swallows were heavier and in better condition following favourable weather conditions and, furthermore, larger clutches were laid when the weather was favourable during laying, the direct effect of environmental factors upon both female condition and clutch size was probably indirectly responsible for the weak relationships between mass or fat score 2 and clutch size.

8.3.3.4 Daily rate of mass gain and peak mass of laying swallows

Balances placed under swallow nests were used to record the mass of individual female swallows during laying and early incubation. The rate of mass gain between 0800 or when the female left the nest after laying and 1900 hours was determined by linear regression for 14 females which laid clutches of 4 or 5 eggs.

The mean rate of mass gain of laying birds during the period of albumen formation was 0.20 gh^{-1} (mean of means for each bird between day -1 and the end of laying, sd=0.03, n=14). The rate of mass gain during albumen formation was greater, although not significantly so, than the rates of mass gain during rapid follicular growth (mean=0.1967, sd=0.024, n=7) and incubation (mean=0.1885, sd=0.67, n=14) (ANOVA, $p=0.09$, 2 d.f.). The rate of mass gain did not differ significantly between females which laid clutches of 4 or 5 eggs on any day during rapid follicular growth, albumen formation or incubation (t-tests, all $p>0.3$).

Pearson correlations were calculated between the rates of mass gain and peak mass on each day during the laying period and measures of bird size, clutch size, egg size, temperature, suction trap catch and rainfall on that day. There were few significant correlations and neither rates of mass gain nor peak mass were consistently correlated with any environmental factor.

Female swallows showed a large range in the rate of mass gain during the laying period (Fig. 8.13), with no clear relationship between the stage of laying and rate of mass gain. Each bird achieved a peak mass which was greater around the day when the first egg was laid than at the end of laying (Wilcoxon matched pairs tests: day 0 vs 4, $Z=1.78$ $p=0.07$, $n=6$; day 1 vs 4, $Z=2.03$, $p=0.04$, $n=7$). It was anticipated that females might gain mass more rapidly on days when they attained greater masses, although the rate of mass gain and peak mass were significantly correlated only on day -1 (Spearman rank correlations, $r=0.71$, $p=0.04$, $n=7$ on day -1; $r<\pm0.70$, $p>0.08$, $5<n<14$ on days -3 to 8). The mean rate of mass gain tended to decline as the eggs were laid (Fig. 8.13), but there were no significant differences between rates of mass gain on day 0 or 1 and day 4 (both $Z<1.3$, $p<0.20$, $n=5$ for day 0 vs 4, $n=7$ for day 1 vs 4) and regression analysis did not provide a significant fit between rate of mass gain during the period day 0 to 4 ($p>0.05$).

8.4 Discussion

8.4.1 Components of mass change of female swallows during laying

Female swallow mass increased during the period of rapid follicular growth and decreased as the eggs were laid. This might be due to the changes in mass of three body components:

1. An increase in the mass of egg material within the body during the period of rapid follicular growth, and when the albumen and shell were formed for each egg, which was then lost when the eggs were laid.
2. Increase in mass of the oviduct (and bird) as the oviduct enlarged during the period of rapid follicular growth, and a possible decrease as nutrients stored in the oviduct were used at the time of, and probably for, albumen formation.
3. Lipid or protein storage in reserves during the period of rapid follicular growth and release during the period of albumen formation.

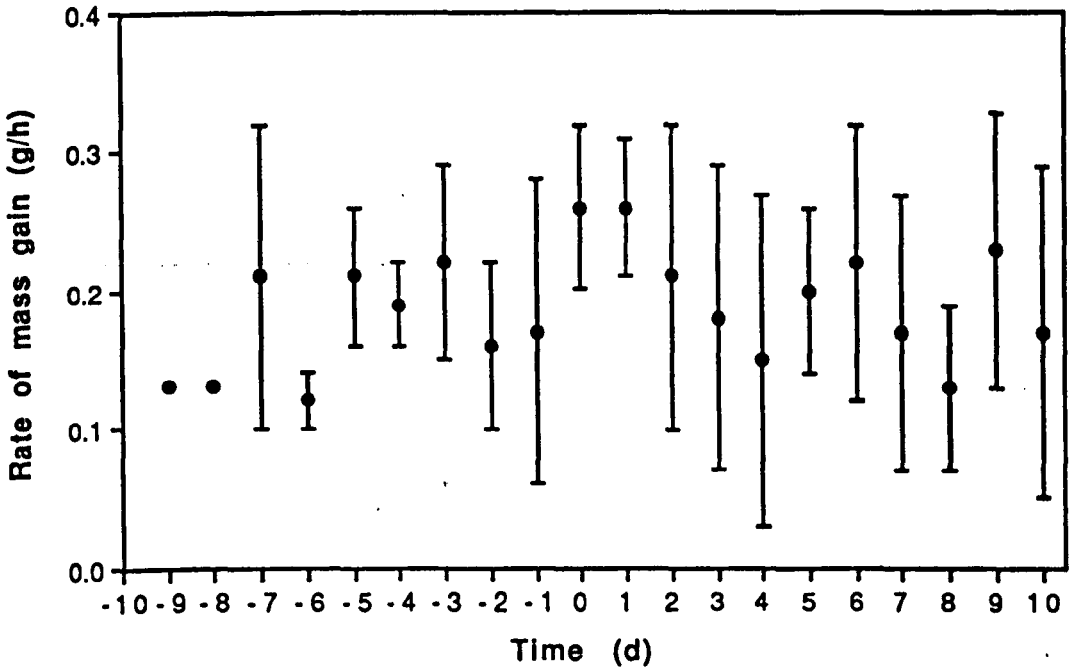


Fig. 8.13 Rate of mass gain (mean, with error bars showing sd) of female swallows between 0800 and 1900 hours each day during laying and early incubation. The first egg was laid on day 0. Sample sizes, starting day <-10, were 1, 1, 2, 2, 3, 3, 6, 6, 7, 9, 10, 14, 14, 12, 9, 11, 8, 5, 4, 3. Each bird was included on every day for which a rate of mass gain was calculated.

A possible fourth option, a build up of reserves prior to initiation of rapid follicular growth and use of these to form eggs, was not supported by the changes in swallow mass and condition indices (Section 8.3.2), although more data would be required to rule this out entirely. There was no increase in mass between days -24 and -12 (where the first egg of the first clutch was laid on day 0) in a sample of pre-breeding females (Thompson 1992), so consideration only of birds from day -10 onwards in this study did not miss a period of mass change.

Use of day -10 as the base level for analysis of changes in mass and condition of laying swallows was also justified from the length of the inter-brood interval. Thompson (1992) found that swallows with unmanipulated brood sizes had a mean interval of 34 d between hatching of the first brood and laying of the second clutch. The inter-brood interval was 4 d less for pairs with an experimentally reduced first brood size. Chicks remained in the nest for 20 d, followed by possibly 4 d of post-fledging care by the female (calculated on the basis that no female post-fledging care would have been required for reduced brood sizes). This allowed a mean interval of 10 d between completion of care for the first brood and the day the first egg of the second clutch was laid. This was the same period as found before a repeat breeding attempt for a female swallow whose first clutch was removed on the day that incubation began (pers. obs.). Starlings could produce replacement clutches after an even shorter interval (5.4 to 10.1 d, Meijer 1992).

Ten d therefore appeared to be the normal period over which any build-up of reserves would be possible for first, second or repeat clutches. This was only 3 d longer than the period of rapid follicular growth and oviducal recrudescence. The lipid reserve present at the start of the period of albumen formation appeared to be built up only over the 4 previous days, when mass and fat score increased (Figs. 8.2 and 8.10), rather than over the entire 10 d period.

The mass of laying females began to increase from day -5 (Figs. 8.1 and 8.2), the start of the period of significant oviducal and rapid ovarian follicular growth (Table 8.6). The increase in mass prior to laying the first egg could be accounted for entirely by the extra mass of ovarian follicles and oviduct (Figs. 8.6

and 8.7). This appeared to be in conflict with an increase in lipid content which occurred at the same time (Fig. 8.10). However, the components of body composition did not remain in a fixed proportion during laying. Females at the start of laying had more lipid than incubating or pre-laying birds (Table 8.2, Fig. 8.10), but the non-reproductive water content of laying birds was lower (Table 8.2). A reduction in body water content as lipid content increased would account for the lack of change in non-reproductive mass during the period of rapid follicular growth, even though body lipid content increased.

As the eggs were laid, female swallow mass reached a progressively lower peak each evening (Fig. 8.1). This could be accounted for by the lower mass of ovarian follicles within the bird as the eggs were laid (Fig. 8.6). Again, the change in mass did not reflect the changes in body composition, as the lipid content of laying females fell during the period of albumen formation, whilst water and protein content rose (Figs. 8.10 and 8.11, Table 8.2).

Consideration only of mass changes of female swallows would lead to the erroneous conclusion that the non-reproductive portion of female swallows remained at the incubation level throughout the laying period, as mass change could be accounted for entirely by eggs and oviduct. Measurement of the mass of female swallows during laying could not provide a complete picture of changes in nutrient reserves, as the proportions of lipid, protein and water in the non-reproductive portion of the body changed. The body lipid content of non-reproductive tissue of female swallows increased during the period of rapid follicular growth, and decreased during albumen formation. This pattern was also followed by laying quelea (Jones and Ward 1976). Water content followed the opposite pattern. There were no obvious trends in indices of pectoral muscle size, or in total body protein content during laying. These trends might would be preferably confirmed with more data. The extent and potential importance of the changes in lipid reserves during the laying period will be discussed in Sections 8.4.3 and 8.4.4.

8.4.2 Potential use of the oviduct for short term nutrient storage by laying swallows

Changes in oviduct mass during the laying period were of interest not only because these accounted for changes in bird mass, but because the oviduct might be used as a source of nutrients during the period of albumen formation.

Albumen synthesis occurs from polypeptide precursors stored in the oviduct (Siva-Sanker and Theis 1959). Oviduct mass decreases as albumen is secreted to each ovum, although this is replaced in domestic fowl oviducts prior to ovulation of the next egg (Edwards *et al* 1976). In determinate layers, it would be plausible that a mass equivalent to an egg albumen was not replaced following ovulation of each egg. Krementz and Ankney (1986) pointed out that use of the oviduct for short term storage of protein for albumen formation would be more sensible than use of pectoral muscle, as they considered that birds could least afford to catabolize flight muscles during laying, as proposed by Kendall *et al* (1973) and Jones and Ward (1976). This might be especially important for aerial foragers. The potential use of the oviduct as a short term nutrient store by swallows was therefore investigated.

Data from other species has shown that the oviduct increased in mass during the 5-6 d before the first egg was ovulated, with the majority of mass gained in the last 3 d (bank swallow, Petersen 1955; canary, Hinde 1967). Less attention has been given to any change in oviduct mass during the period of albumen formation. This could be because oviducts did not alter in mass during the albumen formation period, and so the point has not been mentioned specifically in many papers. The mass of pied flycatcher oviduct did not decrease until after the end of laying (Ojanen 1983).

Oviduct mass decreased during the period of albumen formation in three other passerines. In the bank swallow, oviduct mass declined during the period of albumen formation, although there was considerable variation between birds (Petersen 1955, Appendix 3). The house sparrow oviduct weighed 30% of the initial mass when the final egg was laid (Krementz and Ankney 1986) and starling oviducts were 51% of the initial mass when the final or penultimate egg was laid (Ricklefs

and Hussell 1984).

Carcass analysis of a limited number of laying swallows indicated that there was little change in oviduct mass or protein content during albumen formation, although the lipid content fell by 36% (Table 8.5). This comparison was not necessarily representative of the changes which might occur in an average swallow, as the single female killed at the end of laying was especially heavy for this stage, and so might have had a heavier oviduct than most birds.

Four possible patterns of change in swallow oviduct mass were therefore considered:

1. The oviduct remained at the maximum size during the period of albumen formation, as this was the size required for maintenance of mechanical function and regression occurred rapidly only at the start of incubation. This pattern was followed by some swallows (Table 8.5), and suggested for others by the increase in residual non-reproductive mass predicted at the end of laying if oviducal degradation during albumen production were assumed (Fig. 8.7).

2. The oviduct decreased in mass during the period of albumen formation at the rate described for the bank swallow (Petersen 1955), adjusted for the difference in oviduct mass between swallows and bank swallows. This hypothesis had the disadvantage that oviduct would have been reduced to only 17.8% of maximum mass when the final egg of a clutch of 6 was ovulated (Appendix 3). A clutch as large as 6 eggs was rare among the bank swallows in Petersen's study. This pattern seemed unlikely as the oviduct would presumably have become too small for proper function, and was lower than in starling or house sparrows at the end of laying (Ricklefs and Hussell 1984, Krementz and Ankney 1986).

3. The oviduct decreased in mass during the period of albumen formation, but at a rate which varied so that the oviduct reached the same mass at the end of laying irrespective of clutch size. The oviduct mass reached at the end of laying could not

be determined directly for swallows, so a figure of 35.7% of the maximum mass was used. This was the mean percentage of maximum mass of a bank swallow oviduct after laying a clutch of 4 eggs (Petersen 1955, Appendix 3), and was close to the 30% determined for the house sparrow (Krementz and Ankney 1986). The actual proportion of the swallow oviduct which remained when the last egg was ovulated might differ from this assumed value, but variation in the proportion of oviduct left would not generate a different prediction of the importance of the oviduct as a short term store of nutrients (Section 8.4.4).

4. The oviduct decreased in mass during the period of albumen formation at a rate which varied according to food abundance. Nutrients from the oviduct used for albumen synthesis would not be replaced on days when the food supply was low. Thus swallow oviducts might only lose mass during periods of albumen formation under poor foraging conditions.

The size of the oviduct when the first egg was laid could depend upon the predictability of the food supply during laying. Species with variable food supplies might have relatively larger oviducts than those with a more reliable food supply if the oviduct were a nutrient source which might be used as an insurance against poor foraging conditions later in laying. Female swallows laying first clutches might have larger oviducts than those laying second clutches, as the food supply was less predictable during laying of first than second clutches (Chapters 4 and 10, Turner 1982). Testing these predictions would be an area for future work. The relatively greater mass of the bank swallow than the swallow oviduct (Appendix 3), was consistent with this hypothesis, due to the higher probability of unfavourable feeding conditions during laying for the early nesting sand martin than for the swallow (Turner 1982).

The preferred model of change in oviducal mass, if the oviduct reduced in mass at all during the period of albumen formation, would depend upon whether

reserves were viewed primarily as an insurance against temporary poor feeding conditions, or as a necessary requirement for egg production. The limited data available suggested that swallow oviducts did not decrease in mass during albumen formation, consistent with model 1, above, but in order to assess the importance of the contribution which the swallow oviduct might make as a source of nutrients for albumen production, the nutrient release according to models 3 and 4 was also calculated (Section 8.4.4).

8.4.3 Quantification of changes in body lipid content of laying swallows

The mass of reserve lipid built up by laying swallows during the period of rapid follicular growth, and used during the period of albumen formation, would ideally be calculated for individual birds which laid clutches of different sizes under different conditions, using measures made on living birds. However, the lipid content of laying swallows could not be predicted from the relationship between visual fat score and lipid content determined by Thompson (1992) for birds during subsequent stages in the reproductive cycle (Table 8.3), and the sample of laying birds for which carcass analysis was performed was too small to allow derivation of a separate relationship for laying females.

Visual fat score was a good predictor of the lipid content of swallows during other stages of the reproductive cycle (Thompson 1992), so it was assumed that this would also be true for laying birds, although scoring might be more complicated as abdominal lipid, as well as anterior and posterior deposits, would need to be incorporated. Visual fat scores of laying birds were therefore only of value as a qualitative assessment of body lipid content, rather than an index which would allow calculation of the calorific value of changes in body lipid content during laying. The changes in lipid reserve level for an average laying swallow was therefore calculated from the body composition of birds killed for carcass analysis (Table 8.2), with the timing of changes in reserve levels taken from data on living birds (Fig. 8.10).

Changes in mass and fat score were similar in swallows to those of sand

martins (Jones 1987c). In sand martins, fat score was a good measure of body lipid content, except in females during the period of rapid follicular growth, when lipid content was greater than would have been predicted from fat score (Jones 1987c). No swallow carcasses were taken during the period of rapid follicular growth, so the results of the sand martin study were used as an indicator of possible changes in body lipid content during this period. Although the fat score of swallows began to rise only 4 d before the first egg was laid (Fig. 8.10), an increase in body lipid content from 10 d before the first egg was laid was also considered in calculation the rate of lipid reserve deposition.

If it was assumed that lipid mass was linearly related to fat score 1 during the period of albumen formation, and that the average bird possessed an additional 1.08 g of lipid at the end of day 0 (Table 8.2), the decline in fat score 1 (Fig. 8.11) would represent a mean rate of lipid reserve use of 0.36 gd^{-1} during the period of albumen formation. Extrapolation back to the start of day 0 (when fat score and mass peaked, Figs. 8.2 and 8.10) allowed the prediction that a lipid reserve of 1.45 g would be held at the start of laying. If the initial and final lipid reserve levels were the same for birds which laid clutches of 4, 5 and 6 eggs, birds which laid these clutches would use reserve lipid at 0.48, 0.36 and 0.29 gd^{-1} respectively.

If the lipid reserve was built up over 4 d (as suggested in Fig. 8.10), a rate of deposition of 0.36 gd^{-1} would be required. This would be reduced to 0.15 gd^{-1}, if the lipid reserve were instead formed over 10 d. More exact quantification of changes in the body composition of laying swallows in a future study would require carcass analysis of a larger number of birds before and during the laying period. The importance of the contribution of lipid reserves to the daily energy budget of a laying swallow are discussed in Section 8.4.4.

8.4.4 Build up and use of reserves in relation to the daily requirements of female swallows during laying

Minimum and maximum daily contributions from reserves, for a swallow laying a clutch of 5 eggs, were calculated from the assumptions that either body lipid reserves alone, or lipid reserves plus 62.5% of the oviduct (Section 8.4.2) were available for use during the period of albumen formation.

8.4.4.1 Potential contribution from lipid reserves

If the oviduct was not used as a nutrient store, a swallow laying a clutch of 5 eggs could release up to 0.36 gd^{-1} on days 0, 1, 2 and 3 of laying. If body lipid were transferred to the eggs with an efficiency of 82% (Chwalibog 1982), reserves could provide 0.30 g of lipid each day to the eggs. This was equivalent to between 2.5 times (on day 0) and 21.4 times (on day 3) the daily lipid requirement for egg formation (Table 3.9). The entire lipid requirement for egg formation could clearly be readily supplied from lipid reserves during the period of albumen formation. If the same lipid reserve was used over an additional day by a bird laying a clutch of 6 eggs, lipid from reserves could still have provided at least 1.85 times the requirement for egg formation. However, the timing of lipid deposition and catabolism did not match the period when most lipid was required for egg formation, suggesting that the primary function of the lipid reserve of laying birds was not to supply lipid for translocation to the eggs.

If lipid reserves were used as an energy source, rather than for egg production, again assuming an efficiency of 82%, they could provide 11.62 kJd^{-1} during albumen formation, for a bird which laid a clutch of 5 eggs. This would be 9.8% of the energy output of a laying swallow (where energy output=DEE + energy content of egg formed, 117.89 kJd^{-1}, Chapter 7).

Lipid reserves would therefore provide the equivalent of the entire lipid requirement for egg formation, with between 6.95 (on day -1) and 11.08 kJd^{-1} (on

day 3) (5.9 to 9.4% of energy output) for activity and maintenance during the period of albumen formation.

8.4.4.2 Potential contribution from the oviduct

The maximum sustained daily contribution of the swallow oviduct as a source of nutrients during the albumen formation period would arise from its degradation to 37.5% of the initial mass when the final egg was laid (Section 8.4.2). This would have supplied $0.62 \times 0.04 = 0.02$ g of lipid and $0.62 \times 0.22 = 0.14$ g of protein during the period of albumen formation (where 0.04 g lipid and 0.22 g protein were the mean lipid and protein contents of the swallow oviduct at the start of laying, Table 8.5; and 100 - 37.5 = 62.5% of the oviduct was available for degradation). Lipid and protein released from the oviduct would presumably be used directly for albumen synthesis, as this was the site of albumen formation (Siva-Sanker and Theis 1959). The lipid and protein potentially released from the oviduct during the entire albumen formation period were equivalent to only 143% of the lipid and 90% of the protein requirement for the albumen of one egg (Table 3.2 for swallow egg composition, assuming a conversion efficiency of 82%, Chwalibog 1982). For a swallow which laid a clutch of 5 eggs, the daily contribution from the oviduct would be 28.6% of the lipid and 18.0% of the protein for each albumen. These were considerable proportions of the requirements for albumen formation, but were relatively unimportant as they represented less than 1% of energy output.

The oviduct was therefore unimportant as a potential energy store for a laying swallow. As a store of nutrients for albumen formation, use of the oviduct could be important as an insurance against poor feeding conditions during the albumen formation period, as almost an entire albumen could be formed from the energy content of the oviduct. The oviduct could equally make a daily contribution to albumen formation, but this was relatively small in relation to the daily energy requirement. Routine use of the oviduct as a nutrient source would only be important if assimilation and conversion of food to albumen would have otherwise been too

slow, or to allow collection of specific amino acids over the whole laying period (12-14 d) rather than only upon the (4-6 d) upon albumen was deposited. Both these factors were likely to be unimportant, as domestic fowl and Japanese quail which lay eggs almost continuously, must be able to assimilate and produce sufficient albumen for each egg on a single day indicating that this was not necessarily a problem for birds in general. Shortage of specific amino acids seemed unlikely for birds such as swallows, which have an insect diet with a high protein content, although this possibility remains, and would be an area for future study.

8.4.5 Effect of build-up and use of reserves on the energy budget of laying swallows

Build up and mobilisation of reserves during laying increased the total energy requirement for egg formation by swallows, if compared with the energy requirement for egg and oviduct synthesis directly from food intake (Table 8.13). This was because energy was lost when reserves were formed and mobilised, and there would be a cost to carrying the extra mass of the reserves. This would also be the case in other species, although reserve use might still provide a mechanism to distribute the cost of egg synthesis over a longer period, in order to reduce the peak daily requirement. In the swallow, the peak daily lipid, protein and energy requirements were actually greater for a bird which built up and used reserves than for one which did not, due to the higher energy requirement for reserve synthesis during the period of rapid follicular growth (Table 8.13, Figs. 8.14 and 8.15). Even if lipid reserves were formed over 10 (rather than 4) days, synthesis would involve an energy requirement of 6.91 kJd^{-1}, and the peak daily requirement would still be greater than if eggs were formed from daily food intake. The diet did contain sufficient lipid to supply the peak requirement for egg formation (Chapter 10). In the swallow at least, reserves were not useful to reduce the peak daily lipid, protein or energy requirement for egg formation. Hence reserves were more likely to be of use as an insurance against poor feeding conditions during the period of albumen formation. Female swallows which had just experienced favourable conditions were

Table 8.13 Daily energy content of lipid and protein required for egg formation, with concurrent requirements and savings (negative requirements) due to build up and use of oviduct and reserves for a swallow which laid a clutch of 5 eggs. Requirements for egg formation were calculated from Table 3.9. and for oviduct formation from Table 8.5 and Appendix 3 (using a rate of degradation during laying according to model 3, Section 8.4.2). The requirement for lipid reserve formation was calculated from Table 8.2 and Figs. 8.9 and 8.10 (lipid reserve built up during the 4 d before the first egg was laid and used at a rate of 0.3616 gd^{-1} during albumen formation). Conversion efficiencies were 81% for dietary lipid to egg, oviduct or reserve lipid, 55% for dietary protein to eggs or oviduct, and 82% for conversion of lipid reserves or oviduct to eggs (Chwalibog 1982, Klein and Hoffmann 1989).

	Lipid requirement (J)				Protein requirement (J)			Total energy requirement (J)	
Day	Egg	Oviduct	Reserve	Total	Egg	Oviduct	Total	Eggs and oviduct	Eggs, oviduct and reserves
-7	0.5	55.9	0	56.4	0	285.1	285.1	341.5	341.5
-6	65.8	128.7	0	194.5	31.0	656.6	687.6	882.1	882.1
-5	489.8	130.4	0	620.2	231.4	665.3	896.7	1516.9	1516.9
-4	2260.0	257.5	17500	20017.5	1068.2	1313.2	2381.4	4898.9	22389.9
-3	4555.9	408.2	17500	22464.1	2153.3	2082.2	4235.5	9199.6	26699.6
-2	5589.6	702.9	17500	23792.5	2614.9	3585.5	6200.4	12492.9	29992.9
-1	6195.1	221.9	17500	23917.0	7992.9	1131.8	9124.7	15541.7	33041.7
0	5771.1	-220.5	-11481	-5930.4	7792.6	763.7	7028.9	12579.4	1098.5
1	4000.8	-221.6	-11481	-7701.8	6955.7	767.6	6188.1	9967.3	-1513.7
2	1705.0	-221.6	-11481	-9997.6	5870.7	767.6	5103.1	6586.6	-4894.5
3	670.8	-221.6	-11481	-11031.8	5382.1	767.6	4614.5	5063.7	-6417.3
4	0	0	0	0	0	0	0	0	0
Total (days -7 to 4)								79010.6	103137.6

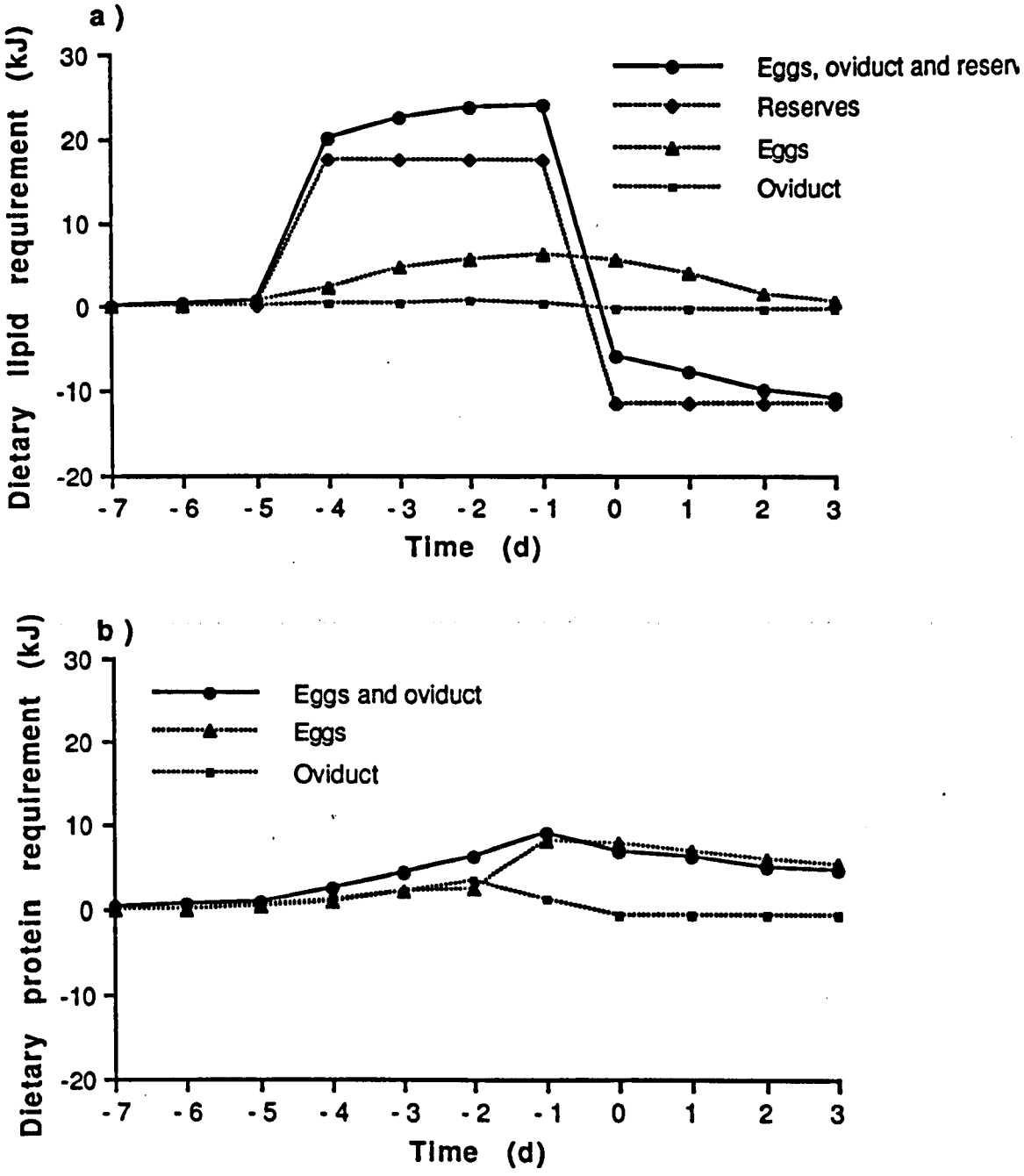


Fig. 8.14 Daily requirement from the diet for a) lipid, b) protein for a female swallow which laid a clutch of 5 eggs. The first egg was laid on day 0. Positive values represent daily requirement for egg or tissue synthesis and negative values were due to energy released from reserves. The energy requirement for oviducal recrudescence was small in comparison with that for egg synthesis, and the energy stored in and released from reserves was much greater than that involved in egg or oviduct formation. Derivation of data is explained in Table 8.13.

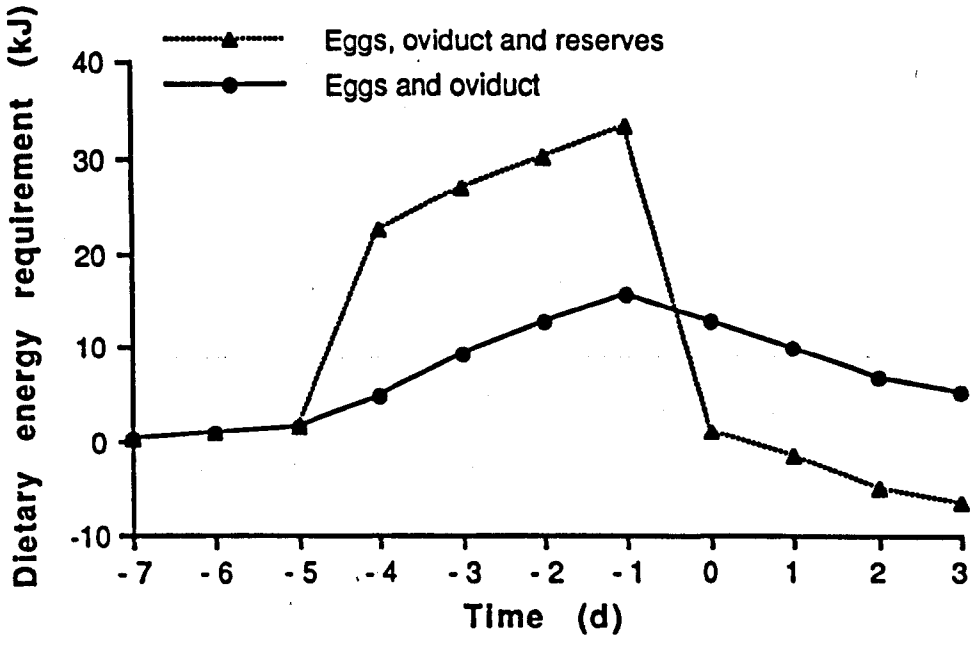


Fig. 8.15 Comparison of the daily energy requirement for a female swallow which formed an oviduct and a clutch of 5 eggs directly from food intake, with that if the cost of lipid reserve synthesis and degradation were included as well as egg and oviduct formation. Derivation of data is explained in Table 8.13.

heavier and had higher fat scores at the start of laying than females which laid in poorer weather (Table 8.12). One female swallow which laid during very poor weather and was unable to build up a lipid reserve during the period of rapid follicular growth, had an interruption in laying and a small clutch. Build-up and use of reserves would reduce the probability that a swallow would suffer the problems experienced by the Bankend bird. Under good conditions, egg synthesis and reserve formation could occur together, whilst in poorer conditions, reserves were not built up during the period of rapid follicular growth or could be used to supplement food intake during the period of albumen formation.

The relative importance of reserves and daily food intake for egg formation in birds in general, and whether the size of the nutrient reserve was important as a determinant of clutch size (or whether females amassed a reserve of an appropriate size before laying their clutch), will be discussed in Chapter 10.

CHAPTER 9

TIME AND ENERGY BUDGETS OF INCUBATING BIRDS

9.1 Introduction

Birds which are uniparental incubators must balance the conflicting demands of warming the eggs and self-feeding. Eggs must be maintained above 25-27°C to allow embryonic development, preferably at 35-37°C, but as the bird must leave the nest to feed, the eggs will cool and will require rewarming at the start of the next incubation session.

There has been much controversy over whether warming the clutch during incubation sessions involves an additional energy requirement for a bird (Kendeigh 1973, King 1973). Much of this has been due to difference in the non-incubating standard with which incubating birds have been compared. The doubly-labelled water (DLW) technique has allowed a synthesis of the DEE of birds incubating clutches of different sizes under a variety of environmental conditions, and a comparison with living costs at other stages in the reproductive cycle (Bryant and Tatner 1988, Tatner 1990, Moreno *et al* 1991). The specific question of the energy requirement for maintenance of clutch temperature during incubation sessions remains unresolved. In general, incubating birds have proved to have a lower DEE than during nestling-rearing (references cited above), but the reduction in time available for foraging has led to the suggestion that incubation could be the time at which energy balance was most difficult to maintain (Yom-Tov and Hilborn 1981, Walsberg 1983b).

The relative importance of energy reserves and daily food intake in balancing energy requirements during incubation has also received attention. Some species, such as arctic nesting geese, rely almost the entirely upon reserves during incubation (Lessells *et al* 1979, Aldrich and Raveling 1983). At the other extreme, small passerines such as the swallow showed no consistent loss in mass during incubation (Jones 1987b), whilst the pied flycatcher increased in mass as incubation proceeded (Askenmo 1982). Males of some species also play an important role in maintenance

of the energy balance of the incubating female, by feeding the female on the nest (Royama 1966, Nisbet 1977, Lyon and Montgomerie 1985, Nilsson and Smith 1988).

In this study, the metabolic rates of free-living incubating female swallows and dippers were measured using the DLW technique simultaneously with activity budget observations and measurement of nest temperature. The effects upon metabolic rate of variation in environmental variables and, for dippers, clutch size manipulation, were examined. Activity budgets were converted to energy budgets using cost of activity whilst off the nest, thermoregulation and reheating the clutch after recesses which have been derived in previous studies. The difference between DEE measured by the DLW technique and energy expenditure accounted for by the activity budget should represent the net energy requirement for incubation (i.e., keeping the clutch warm during incubation sessions). This was compared with the net cost of incubation calculated from the equation given by Kendeigh (1963).

9.2 Methods

The DLW technique (Section 5.2.2) was used to measure the energy expenditure of 4 incubating swallows in 1990, and of 10 incubating dippers in 1990-1991. Swallows incubated their natural clutch sizes (5 eggs), whilst dippers incubated either their natural clutch or one manipulated within the naturally occurring range (3-6 eggs). Eggs were moved temporarily between nests for 2-3 d during clutch manipulations. These data were supplemented by DLW measurements of energy expenditure of incubating females from the same populations on swallows and dippers studied by Westerterp and Bryant (1984) and Bryant and Tatner (1988).

9.2.1 Activity budgets of incubating dippers and swallows

9.2.1.1 Observation of activity during recesses

The activities of each incubating female dipper and swallow were observed

during the period during which energy expenditure was measured by the DLW technique. Each labelled bird was marked on the breast with red water-soluble dye, to allow rapid identification at a distance. This lasted for 2-3 d on dippers and 2-3 weeks on swallows. Observation periods were distributed over each bird's active day so that the activity budget should not be biased by activities common only at one time. Observations also provided a check on the timing of incubation sessions and recesses recorded by the nest temperature monitor (Section 9.2.1.2).

Dippers were observed through binoculars from 20-100 m. Each observation session began at a bank or tree overlooking the nest site, from which the nest was watched to record the number of feeds provided by the male and to wait for the female to leave the nest. Dipper activities during incubation recesses were recorded (after Bryant and Tatner 1988) as:

resting (standing and preening),
foraging (walking activity either fully exposed to the air or in shallow water),
diving (subsurface feeding or swimming on the surface of deep water),
flying,
or out of sight.

Dippers left the nest for around 10 min in each hour, and often moved out of sight for some of the recess period. This meant that it was difficult to avoid biasing the observations towards the "resting" category as birds could be observed easily when resting or preening adjacent to the nest, whilst time spent foraging often involved the bird moving out of sight. In order to overcome this potential bias, time for which the bird was temporarily out of sight was divided between the "foraging" and "diving" categories, in proportion to the time for which these activities were recorded for that bird. This had the disadvantage that brief periods of resting between foraging bouts would not be taken into account, however this would only lead to a slight underestimation of the time spent resting.

Swallows were observed by watching the entrance to the building containing the nest from a car. An attempt was made to record the activities of incubating

swallows during recesses, however the bird often flew out of sight behind farm buildings as soon as she left the nest. The shorter recesses (4-5 minutes) of swallows meant that it was difficult to relocate the bird before it was time for her to return to the nest. Swallow activities were recorded in the same categories as laying birds (Section 8.2.1). Due to difficulties in observation, and following Turner (1982), the whole of the recess time was assumed to be spent in flight for the purposes of energy budget construction. Inclusion of time spent perching whilst away from the nest would have had a minimal effect on the energy budget.

9.2.1.2 Use of the nest temperature monitor to determine the time budget of incubating birds

Seven thermistors were used to measure temperatures within and around dipper and swallow nests. The nest temperature monitor was set up 1 d before the start of the DLW measurement period, in order to accustom the birds to its presence. Thermistors (1.5 mm diameter) were placed in the nest lining, below the eggs, on top of the eggs, on the nest rim, in the nest wall and roof (for dipper nests) and in the open about 20 cm from the nest. The thermistors were connected to a battery powered data-logger which recorded the voltage from each thermistor once each minute. The memory of the data-logger was large enough to store 22.5 h of data, which could be down-loaded directly to the University mainframe computer or to a portable Psion Organiser (programmed by the Microcomputer and Media Technology group, Stirling University). Down-loading to the Psion in the field required about 20 min and meant that the monitor could be used to collect data almost continuously. It was assumed that behaviour followed the same pattern as the rest of the day during the period whilst the nest temperature monitor was down-loaded as the incubating bird was not normally disturbed. Data were transferred to the University mainframe computer for analysis.

Nest temperature monitor data were recorded in a compact form as voltages in hexadecimal notation, to reduce the amount of memory required in the

data-logger. This output was converted to temperature (°C) using a formula obtained from calibration of the thermistors against a quartz digital thermometer accurate to 0.01°C (Appendix 8).

The thermistor probe placed on top of the eggs recorded a rapid change in temperature of around 10°C, when incubation sessions or recesses began (Fig. 9.1). The temperature at the thermistor on top of the eggs was used to calculate the total duration of incubation sessions and recesses during DLW measurements. The period between release of the bird after labelling and its return to the nest to resume incubation could also be determined.

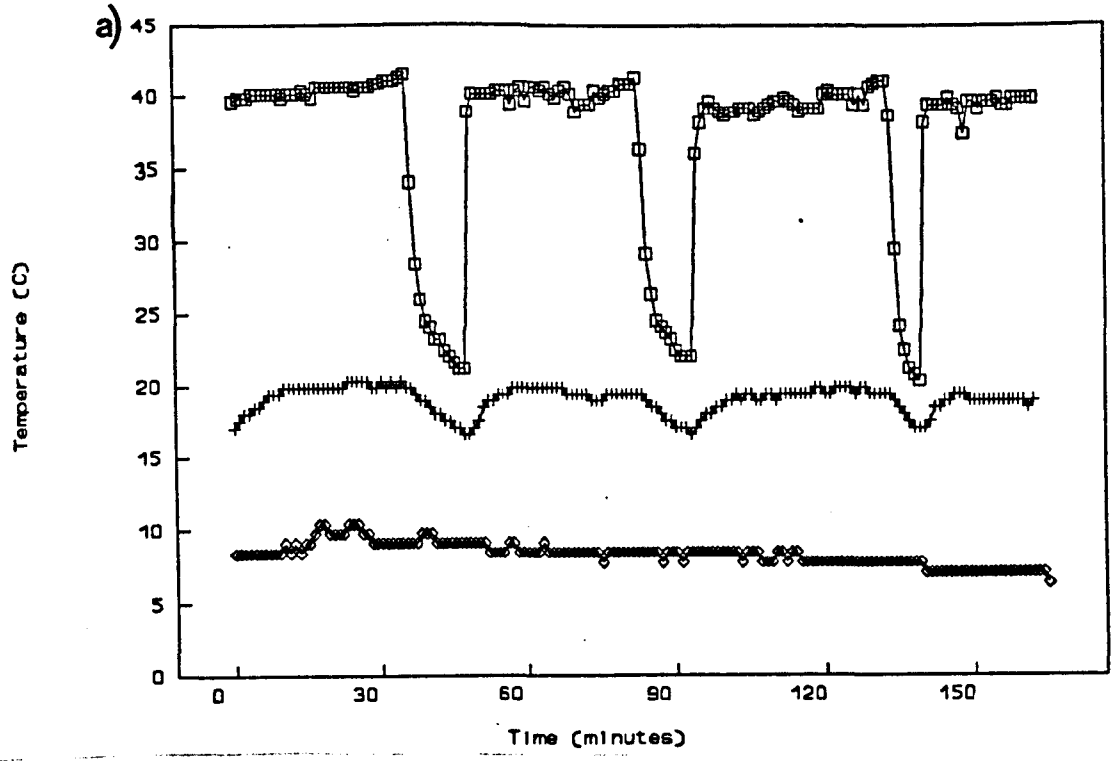
9.2.2 Calculation of clutch reheating costs

9.2.2.1 Egg cooling rates

The temperature inside a dipper egg during incubation was measured in 2 nests in 1990. One of the nest temperature monitor thermistors was sealed inside an unincubated dipper egg (from a deserted clutch) using Evostik adhesive. The thermistor egg was placed in the nest among the other eggs. The other thermistors were arranged within and around the nest. Mean dipper egg cooling rate was calculated from data collected by the thermistor inside the egg.

The cooling rate of swallow eggs was calculated from the nest air temperature measured during each recess and data collected by Jones (1985). Egg cooling rates were fundamentally non-linear, for cooling rate decreased as egg temperature approached nest air temperature. However egg cooling rate was approximately linear for the first 10 min of a recess (Jones 1985) and most incubation recesses recorded during DLW measurements lasted less than 10 min, so a linear cooling rate was assumed for the purpose of calculation of the heat lost by eggs during incubation recesses.

At nest air temperatures greater than or equal to 25°C, swallow eggs cooled at 0.183°Cmin^{-1} (Jones 1985). At temperatures below 25°C, egg cooling rate was calculated from data given by Jones 1985 for 5 egg clutches (Equation 9.1).



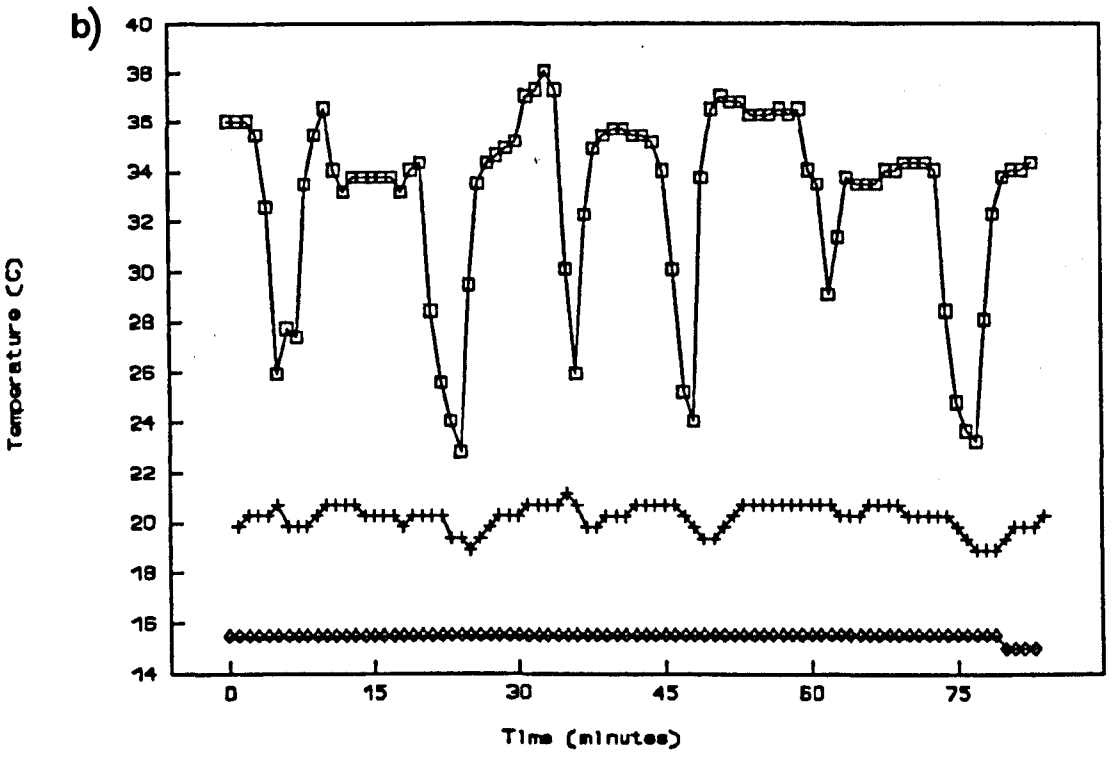


Fig. 9.1 Typical temperatures measured within and around a) dipper b) swallow nests using the nest temperature monitor during the incubation period. Temperatures in the air next to the eggs are shown by squares, under the eggs by crosses and ambient temperature by diamonds. Temperature in the air next to the eggs fell at the start of each incubation recess and rose again at the start of incubation sessions. The positions of the probes are shown in Fig. 9.3.

$$Cooling\ rate\ (°C min^{-1}) = -0.029 \times T + 0.887 \qquad 9.1$$

where T = nest air temperature (°C).

Egg temperature during incubation sessions (T_s) was taken as 35.7°C for swallows (Turner 1983) and 34.4°C for dippers (this study). Egg temperature at the end of each recess (T_r) was calculated from Equation 9.2.

$$T_r = T_s - (cooling\ rate\ of\ eggs\ (°C min^{-1}) \times duration\ of\ recess\ (min)) \qquad 9.2$$

Embryonic heat production during incubation recesses would decrease egg cooling rates from those calculated here, as swallow and dipper eggs used in the cooling rate experiments were fresh, unincubated eggs. Embryonic heat production increases exponentially during incubation (Vleck *et al* 1979), but would probably only be important in the energy budget of precocial species (Drent 1970, Ricklefs 1974).

9.2.2.2 Clutch reheating costs

The daily energy requirement for reheating the clutch after incubation recesses was calculated from Equation 9.3.

$$E = \sum_{x=1}^{n} \frac{(T_s - T_r) \times 3.3 \times m}{1000} \qquad 9.3$$

where E = daily energy requirement for clutch reheating (kJ),
T_s = egg temperature during incubation sessions,
T_r = egg temperature after each incubation recess (calculated from Equation 9.2),
m = mass of clutch (g),
n = number of incubation recesses per day,
3.3 = the specific heat capacity of eggs ($Jg^{-1}°C^{-1}$).

9.2.3 Measurement of energy expenditure using the doubly labelled water technique

Incubating female dippers were caught on the nest using a hand held net usually during the late afternoon. Each bird was weighed and given an intraperitoneal injection of labelled water (10 μlg^{-1} of a mixture of 10 ml 20 ape $H_2{}^{18}O$ and 0.68 g of 99.9 ape D_2O). Structural size was recorded (Chapter 2). After isotope equilibration (1 h), a blood sample was taken from the leg vein and the bird released around 100 m from the nest. Birds were recaptured 24 or 48 h later for a second blood sample, re-weighing and scoring of body condition.

Incubating female swallows were caught on the nest using a hand-held mist net at sites where the female could not see out of the nest easily, or an hour before dawn and treated in the same way as dippers, except that condition indices (Chapter 2) were also recorded.

9.2.4 Measurement of mass change during the incubation period

A sample of female swallows and dippers was caught on the nest once during the incubation period, weighed and measured. Ideally, the extent of mass change during incubation would have been established by catching each bird at the start and the end of incubation. However, as an alternative, mass change for the population during incubation was assessed by determining whether there was a significant relationship between mass (adjusted for size) and day of incubation upon which the individual was weighed.

9.3 Results

9.3.1 Activity budgets

9.3.1.1 Dippers

The mean duration of incubation sessions of labelled dippers was 41.4 min (sd=20.0, n=273 sessions by 10 birds), and mean recess duration was 10.6 min

(sd=5.1, n=295 recesses). There was a significant decrease in mean session and recess duration decreased with increasing clutch size (Fig. 9.2). There were no significant relationships between ambient temperature and clutch size, session or recess duration (Spearman correlations, all $r<\pm0.36$, $p>0.12$, $n=12$).

Incubation sessions occupied 79.2-86.0% of the 24-hour day of female dippers during DLW measurements of energy expenditure (Table 9.1). There were no significant relationships between clutch size and the (arcsine transformed) percentage of the 24-hour day or of the active day (defined as the period between the start of the first recess in the morning and the end of the last recess in the evening) which the bird spent in sessions or recesses (Spearman correlations, all $r<\pm0.2$, $p>0.3$, $n=10$).

Each bird was observed during a sample of incubation recesses, to determine the amount of time spent resting, foraging, diving and in flight (Table 9.2). Bird D6 proved unexpectedly difficult to observe during recesses, and was never seen to feed, so a representative activity budget could not be constructed for this individual.

9.3.1.2 Swallows

Birds S2, S3 and S4 adopted a normal incubation schedule, spending 68.5 to 86.1% of the 24-hour day on the nest (Table 9.3). This was within the range of time spent at the nest by undisturbed birds (Turner 1980). The energy expenditure of bird S1 was measured on a day of very heavy rain and strong wind, and she left the nest unattended for unusually long periods during the afternoon. This bird deserted her clutch after the completion of the DLW measurement period, as did at least one other (undisturbed) bird in the study population. A similar alteration in swallow incubation pattern during poor weather was recorded by Jones (1987b), although in that case, the weather improved so that the bird did not reach the point at which she had to desert the clutch in order to maintain energy balance.

The mean duration of swallow incubation sessions was 10.7 min (sd=9.2, n=247 sessions, 4 birds). The three birds which showed a normal incubation pattern

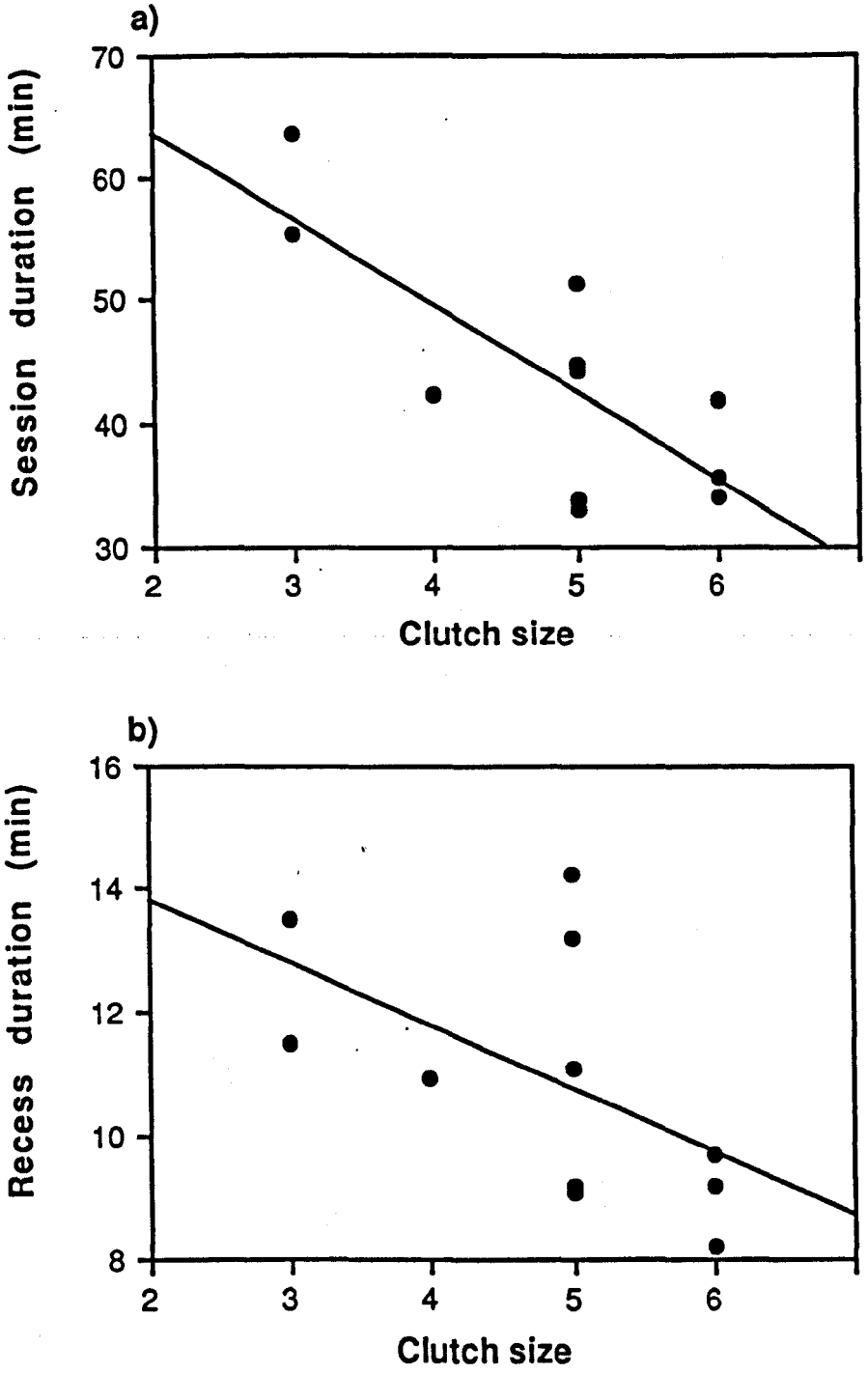


Fig. 9.2 Relationship between the duration of a) incubation sessions and b) incubation recesses of female dippers and clutch size. The regression equations were session duration=6.70 x clutch size +75.5, r^2=0.57, p=0.005 and recess duration=-0.99 x clutch size+ 15.6, r^2=0.29, p=0.07), n=12.

Table 9.1 Activity budget (%24-hour day) and duration of measurement period of incubating female dippers for which energy expenditure was measured by the DLW technique. Activity during incubation recesses is described in Table 9.2. [a] after first session after release. [b] after first and before second blood sample.

Bird		**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
Session	**- day**	44.5	41.2	37.4	32.4	47.3	24.0	40.5	43.2	45.6	44.6
	- night	40.3	39.8	41.8	48.9	38.7	56.9	46.9	39.9	39.9	39.8
	Total	84.8	81.0	79.2	81.3	86.0	80.9	87.4	83.1	85.6	84.4
Recess	**- normal[a]**	12.5	14.2	11.6	12.2	11.1	4.4	9.1	12.2	9.5	10.2
	- after release	1.8	4.1	6.9	5.4	1.8	13.9	2.7	3.5	3.8	4.5
	Total	14.3	18.3	18.5	17.7	13.5	18.3	11.8	15.8	13.3	14.7
Held to obtain blood samples[b]		0.9	0.7	2.3	1.0	0.6	0.9	0.8	1.2	1.1	0.8
Duration of measurement period (h)		48.00	48.07	24.22	48.10	48.32	48.12	48.22	48.43	48.23	48.27

Table 9.2 Activity budget of incubating female dippers during incubation recesses during measurement of energy expenditure by the DLW technique. (- = insufficient data collected to partition time budget for this bird.)

Bird	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Rest	52.4	86.9	31.2	82.7	76.9	-	18.9	43.9	46.9	18.9
Forage	43.2	7.6	56.5	14.8	14.4	-	3.2	46.5	33.6	60.9
Dive	0.0	1.6	6.8	0.0	5.1	-	72.3	3.0	14.0	15.1
Fly	4.4	3.9	5.4	2.6	3.6	-	6.2	6.6	5.5	5.1
Duration of observation (min.)	32.1	52.6	38.3	22.9	43.6	-	76.2	35.7	38.4	43.6

Table 9.3 Activity budget (%24-hour day) and duration of measurement period of incubating female dippers for which energy expenditure was measured by the DLW technique. [a] After first session after release. [b] After first and before second blood sample.

Bird		S1	S2	S3	S4
Session	- day	25.5	40.7	56.6	34.1
	- night	37.9	30.0	29.5	34.4
	Total	63.4	70.7	86.1	68.5
Recess	- normal[a]	25.5	20.1	12.6	25.4
	- after release	10.0	8.4	0.4	2.1
	Total	35.5	28.5	13.0	27.5
Held to obtain blood samples[b]		1.1	0.8	1.0	4.1
Duration of measurement period (h)		23.88	47.32	47.58	48.22

had shorter and less variable incubation session duration than bird S1, however this was not a significant difference (95% confidence intervals overlapped: mean session duration=16.0 min, sd=12.8, n=20, 95% C.I.=±4.94 for bird S1, mean duration=10.2 min, sd=8.7, n=227, 95% C.I.=±0.95 for the other three birds). The mean duration of swallow recesses was 6.14 min (sd=10.5, n=244 recesses, 4 birds). Bird S1 left her nest unattended for up to 60 min, however her incubation recesses were not significantly longer than those of the other 3 birds (95% confidence intervals overlapped: mean recess duration=17.0 min, sd=31.4, n=20, 95% C.I.=±12.11 for bird S1; mean recess duration=5.2 min, sd=5.0, n=224, 95% C.I.=±0.53 for the other 3 birds). Recess duration was slightly longer than for undisturbed birds in the same study area (mean recess duration=4.12±2.62 min, Jones 1989).

All swallows in this study incubated their natural clutch size of 5 eggs, so incubation behaviour could not be examined in relation to clutch size with this sample of birds. A previous study on the same population showed that female swallows spent significantly longer incubating manipulated clutches of 8 than of 2 eggs (Jones 1987b).

The mean durations of swallow incubation sessions and recesses were not significantly correlated with temperature (Spearman correlations, both $r>-0.8$, $p<0.1$, n=4 birds). These results were consistent with the significant negative relationship between temperature and session/recess duration, for a larger samples of undisturbed swallows in the same study area (Turner 1980, Jones 1985). There was no significant correlation between the mean percentage of time spent in incubation sessions or recesses (arcsine transformed) and mean temperature (Spearman correlations $r<\pm0.2$, $p>0.8$, n=4 birds).

9.3.2 Energy budgets

9.3.2.1 Egg cooling rates in dippers and swallows

Internal egg temperature was measured at two dipper nests using one of the thermistor probes from the nest temperature monitor. Temperatures recorded within

and around the nest during an overnight incubation session are shown in Table 9.4. Internal egg temperature varied between 32.29 and 37.34°C, presumably due to egg turning. There was a larger variation in the temperature measured by the probe placed on top of the eggs, as this would cool more rapidly than an egg when the bird altered position in the nest. The mean incubation temperature recorded for dipper eggs was 34.4°C.

Egg cooling rate was measured during incubation recesses. Mean cooling rate of the thermistor inside a dipper egg was 0.906°Cmin^{-1} (n=4, sd=0.11). A typical cooling curve for the dipper egg containing the thermistor was shown in Fig. 9.4. Egg cooling rate decreased asymptotically with time, however, cooling rate could be described adequately by a linear relationship during the first 10-15 min. Insufficient data were collected to determine the effects of clutch size or ambient temperature upon cooling rate, however the temperature inside dipper nests during recesses was similar for different ambient temperatures and clutch sizes, so use of a single egg cooling rate should not introduce a substantial error to calculation of clutch reheating costs.

Swallow incubation temperature and egg cooling rates were taken from data in Turner (1980) and Jones (1985) as described in Section 9.2.2.1.

Internal egg temperature at the end of recesses was calculated as 24-28°C for dippers and 32-34°C for swallows. This meant that swallow eggs would never, and dipper eggs only rarely, fall below the minimum temperature required for embryonic development (25-27°C, Drent (1970)) during the normal incubation schedule. The swallow which altered her incubation schedule due to bad weather allowed her eggs to cool repeatedly to ambient temperature (10-11°C), during recesses of 30-60 min. As she subsequently deserted the clutch it was uncertain whether this schedule would have allowed normal embryonic development. However, another swallow which adopted a similar strategy during bad weather did successfully hatch her clutch (Jones 1987b).

Table 9.4 Temperatures (°C) within and around a dipper nest and inside a dipper egg during an incubation overnight session (n = 718 temperature readings at 1 min. intervals). The nest was in a sheltered culvert behind a waterfall in Alva Glen. The positions of the probes are shown in Fig. 9.3.

	Mean	sd	Minimum	Maximum
In egg	34.4	1.0	32.3	37.3
Air next to eggs	28.1	2.4	21.2	34.6
Under eggs	16.9	0.8	13.9	18.5
In nest wall	9.2	0.5	8.4	10.4
Ambient	7.6	0.6	6.3	9.1

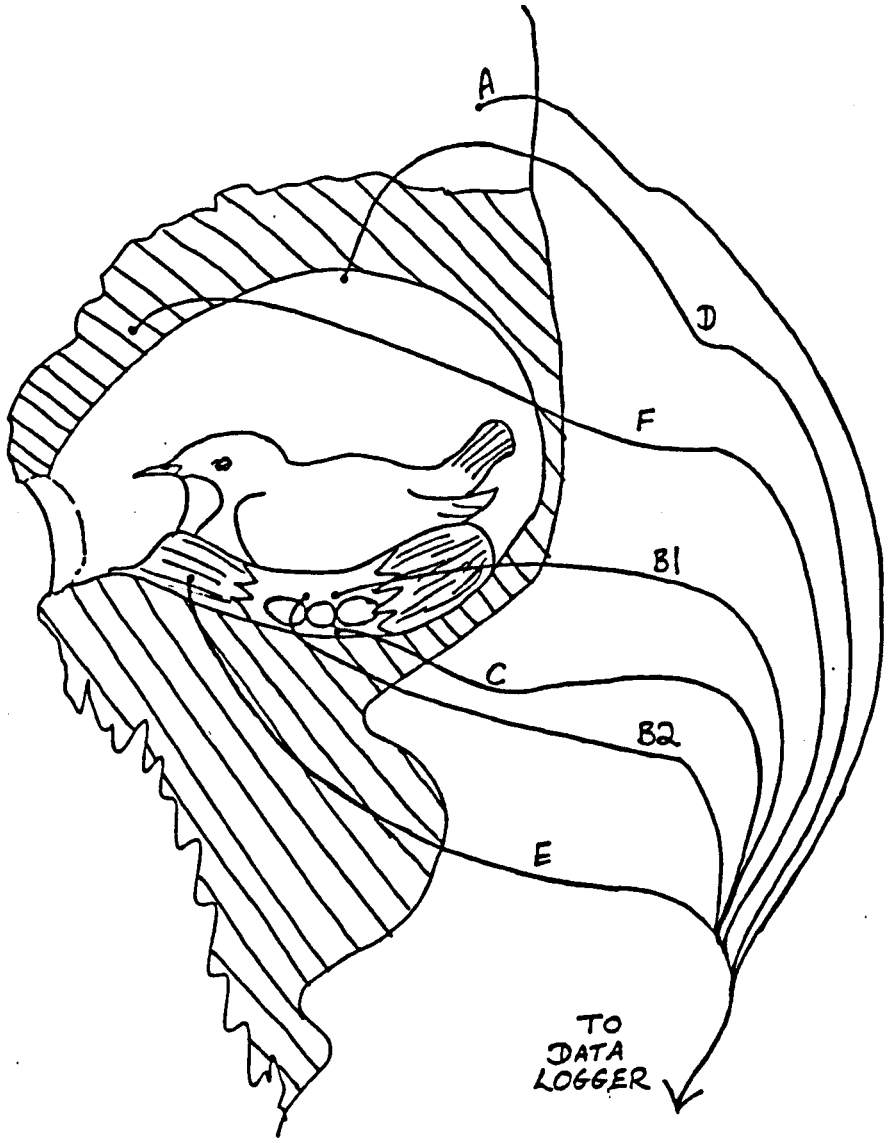


Fig. 9.3 Cross section through a dipper's nest, showing the incubating female and a cut-away portion of the nest cup to shown some of the eggs and the positions of the thermistor probes. Thermistor probes are labelled:

A	ambient
B1	air next to the eggs
B2	air next to the eggs
C	under eggs
D	nest air
E	nest lining
F	nest wall

Two thermistor probes were placed in the air next to the eggs, as the it was important to record the change in temperature at this position, as these probes were used to time incubation sessions and recesses, and the birds sometimes moved one of the probes.

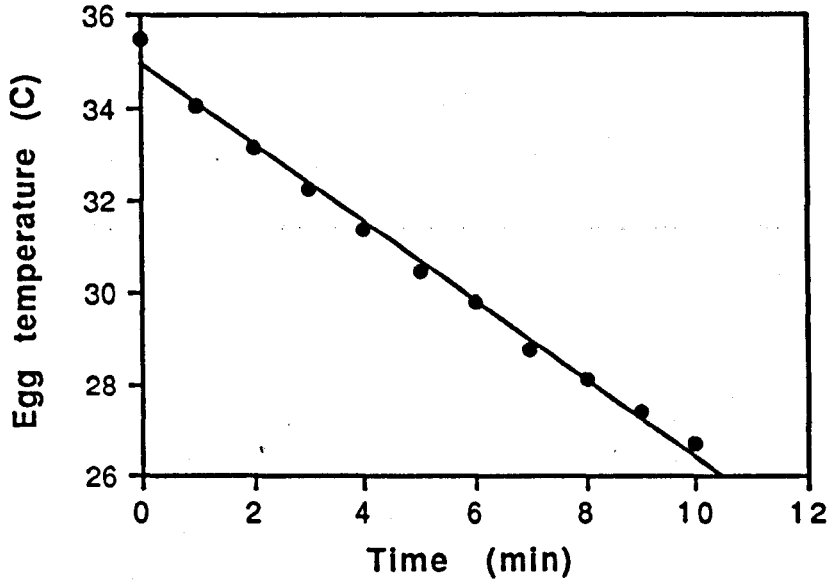


Fig. 9.4 Cooling curve of a fresh dipper egg during a typical incubation recess, measured using the nest temperature monitor. Internal egg temperature= -0.86 × time + 35.0, r^2=0.994.

9.3.2.2 Energy expenditure of incubating dippers

The mean DEE of 18 incubating female dippers was 224.38 kJd^{-1} (sd=50.08), equivalent to an ADMR of 5.69 $cm^3CO_2g^{-1}h^{-1}$ (sd=1.35), or 3.54×BMR (sd=0.82) (Table 9.5). The two birds which incubated artificially enlarged clutches of 6 eggs had significantly greater metabolic rates than the other birds (Mann-Whitney U-test, Z=2.25, p=0.025), and these were the only birds which exceeded a metabolic intensity of 5×BMR (Table 9.5, Fig. 9.5). A more representative metabolic rate for incubating dippers might be obtained if these two birds were excluded, on the basis that although clutches of 6 did occur naturally during the study, they are unusual (only 3 such clutches found in 3 years). If these birds were excluded, the mean DEE of an incubating female dipper would be 209.26 kJd^{-1} (sd=25.40), equivalent to an ADMR of 5.29 $cm^3CO_2g^{-1}h^{-1}$ (sd=0.71, n=16), or 3.30×BMR (sd=0.42).

The only other dipper which exceeded a metabolic intensity of 4×BMR was bird D7 (Table 9.5, Fig. 9.5), which nested near an unusually deep part of the river. This meant that she spent most of her foraging time diving, an energetically costly activity, whilst the other birds fed mainly by wading in shallow water (Table 9.2).

There were no significant correlations between temperature, mean mass or mass change during the period of energy expenditure measurement, structural size (wing, keel or tarsus length), age, or (arcsine transformed) percentage of the 24-hour day spent in incubation sessions or recesses or different activities during incubation recesses and measures of energy expenditure (Pearson correlations, all $r<\pm0.43$, $p>0.15$, n=18, except for correlations with measures of activity, where n=10).

The results were, however, consistent with trends in energy expenditure demonstrated for larger samples of dippers (Bryant *et al* 1985, Bryant and Tatner 1988). Energy expenditure increased with percentage recess time spent diving or flying, and decreased with time spent resting and foraging (Fig. 9.5). Incubating birds spent a large proportion of their time on the nest, so that a very large variation in energy expenditure during the relatively short recess periods would be required to have a significant effect upon daily energy expenditure. Only bird D7 spent a large

Table 9.5 Mass, clutch size and energy expenditure calculated by the DLW technique of incubating female dippers. Birds D1 to D10 were labelled during this study. Birds D11 to D18 are data collected from the same population by Bryant and Tatner (1988). M, metabolic intensity = metabolic rate expressed as a multiple of BMR (calculated from Aschoff and Pohl (1970) inactive phase). Details of ring numbers and nest sites of birds D1 - D10, and the dates upon which the measurements were made, are given in Appendix 6.

Bird number	Mean mass (g)	Clutch size	ADMR $cm^3CO_2g^{-1}h^{-1}$	DEE $kJbird^{-1}d^{-1}$	M
D1	62.0	5	4.45	177.00	2.78
D2	59.8	4	5.58	214.06	3.45
D3	59.9	5	4.77	183.14	3.95
D4	57.5	5	6.31	232.76	3.86
D5	64.5	4	5.25	217.07	3.31
D6	62.6	5	5.51	221.28	3.45
D7	58.3	3	7.31	273.16	4.49
D8	60.8	6	8.84	344.52	5.49
D9	60.1	6	8.99	346.32	5.57
D10	61.7	3	4.88	193.00	3.04
D11	59.7	5	5.93	188.65	3.05
D12	59.1	4	4.87	184.48	3.00
D13	63.2	4	4.67	189.34	2.93
D14	63.2	5	4.96	201.10	3.11
D15	62.3	5	4.88	195.04	3.05
D16	60.8	5	5.47	213.35	3.40
D17	68.5	5	5.18	227.63	3.32
D18	66.5	4	5.56	237.02	3.54
Mean	61.67	4.61	5.69	224.38	3.54
sd	2.81	0.85	1.35	50.08	0.82

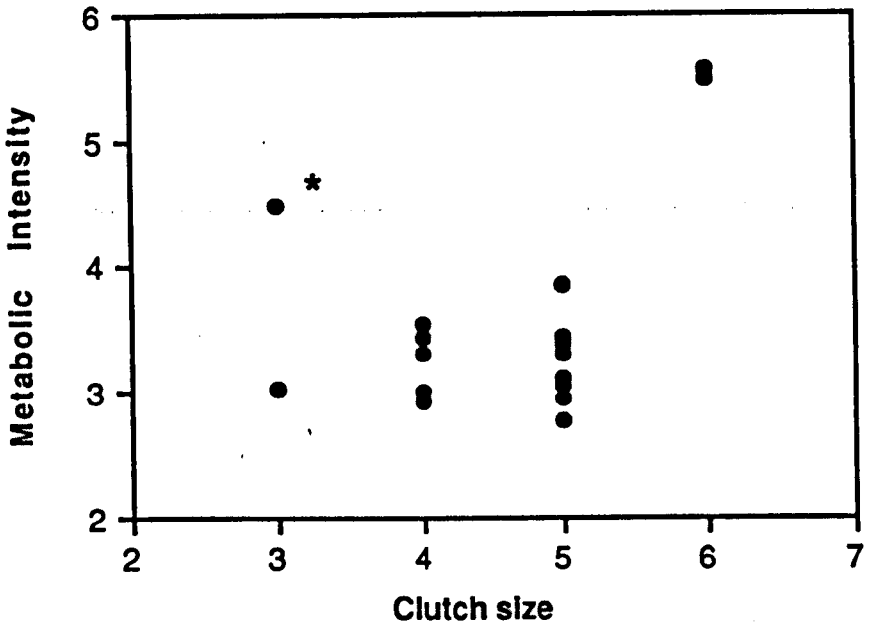


Fig. 9.5 Relationship between clutch size and metabolic intensity of incubating female dippers. Metabolic intensity was metabolic rate (measured using the DLW technique) expressed as the number of multiples of BMR (calculated from mass using the equation of Aschoff and Pohl (1971), inactive phase). The bird marked with a * used an unusual amount of diving during her incubation recesses, probably causing a relatively high energy expenditure for this clutch size.

proportion of her recess time diving, and her energy expenditure was indeed greater than that of other birds incubating clutches of less than 6 eggs. Trends were consistent with the positive correlation between DEE and body size, and the negative relationship with temperature found by Bryant and Tatner (1988).

Daily energy expenditure was calculated independently from the activity budget during the DLW measurement period. The energy requirement assigned to each activity followed Bryant and Tatner (1988) (Table 9.6). Thermoregulation was taken to be additional to the costs of all activities other than flight (Bryant and Tatner 1988). Nest air and ambient temperature were determined from nest temperature monitor data. Clutch reheating costs were calculated using the method described in Section 9.2.2.2, and added to thermoregulatory and activity costs to give a DEE which included all costs except the energy requirement for keeping the clutch warm during incubation sessions (Table 9.7). The net energy requirement for warming the clutch during incubation sessions was calculated from the difference between DEE calculated from the activity budget and that measured using the DLW technique.

The two birds with clutch size 6 were considered separately, as they had an unusually high net energy requirement for incubation (Table 9.7). Mean DEE measured by the DLW technique for the other 7 birds was 212.90 kJd^{-1} (sd=33.19), but only 187.25 kJd^{-1} (sd=16.21) by calculation from the activity budget. The mean net energy requirement to maintain egg temperature during incubation sessions was 25.65 kJd^{-1}, very close to that calculated using Kendeigh's (1963) equation (21.38 kJd^{-1}). This good agreement suggested that both techniques could provide a good estimate of the cost of incubation.

Although the mean DEE of incubating female dippers calculated from the activity budget (if this included the cost of incubation calculated from Kendeigh's equation), was in good agreement with the DLW result, the activity budget method did not accurately predict individual variation in energy expenditure. Predictions of DEE using the two techniques were not correlated for individuals (Spearman correlation coefficient=0.18, p=0.7, n=9) although there was no significant difference

Table 9.6 Net metabolic costs ($Jg^{-1}h^{-1}$) for female dipper thermoregulation and activity. Activities with the symbols +TR required addition of a thermoregulatory component, TR_N for night-time and TR_D for daytime costs. T_{aN} and T_{aD} denote mean night and day ambient temperature (°C) respectively. Metabolic costs follow Bryant and Tatner 1988.

	Symbol	Net metabolic costs ($Jg^{-1}h^{-1}$)	Method
Basal (night)	M_{bN}	43.7	Aschoff and Pohl (1970)
Basal (day)	M_{bD}	57.2	Aschoff and Pohl (1970)
Resting (day)	M_{re}	$31.9+TR_D$	Bryant *et al* (1985)
Foraging	M_{fo}	$63.8+TR_D$	Bryant *et al* (1985)
Diving	M_{di}	$262.2+TR_D$	Bryant and Tatner (1988)
Flying	M_{fl}	386.7	Hails (1979)
Thermoregulation (night)	TR_N	$45.14 - 1.646\ T_{aN}$	Bryant *et al* (1985)
Thermoregulation (day)	TR_D	$68.50 - 2.630\ T_{aD}$	Bryant *et al* (1985)

Table 9.7 Energy expenditure (kJd^{-1}) of incubating female dippers, determined simultaneously by the DLW technique and from the activity budget. The energy expenditure calculated from activity budget did not include the energy required for keeping the eggs warm during incubation sessions. The difference between the two measurements (DLW-Act.) should be the net energy requirement for keeping the eggs warm during incubation sessions. For comparison, Kendeigh cost (kJd^{-1}), the cost of keeping the eggs warm calculated from Kendeigh's (1963) equation (cost of incubation (kJd^{-1}) = clutch mass × 0.8 × cooling rate × (egg temperature - nest air temperature) × 24 (1 - 0.25 × 0.8) × 4.187 × 0.001), was also shown. Mean 1 was mean of data for all birds (n = 9). Mean 2 was mean of data for all birds with a clutch size of less than 6 eggs (n = 7).

	Energy expenditure					
Bird number	**DLW**	**Activity budget**	**Difference (DLW-Act.)**	**%(DLW-Act.)/DLW**	**Clutch size**	**Kendeigh cost**
D1	177.04	190.63	-13.59	-7.68	5	25.5
D2	213.90	161.41	52.48	24.54	4	20.1
D3	183.20	187.70	-4.50	-2.46	5	26.6
D4	232.78	171.57	61.20	26.29	5	25.6
D5	217.14	196.39	20.75	9.56	4	21.3
D7	273.19	210.47	62.72	22.96	3	15.1
D8	344.63	202.32	142.31	41.29	6	31.6
D9	346.37	188.71	157.66	45.52	6	30.4
D10	193.06	192.55	0.50	0.26	3	15.0
Mean 1	242.37	189.08	53.28	17.81	4.56	23.52
sd	65.16	14.90	61.87	19.04	1.13	6.02
Mean 2	212.90	187.25	25.65	10.50	4.14	21.38
sd	33.19	16.21	32.82	14.18	0.90	4.91

between the results of the two techniques (Wilcoxon matched-pairs signed ranks test: Z=0.34, p=0.7).

The two dippers which incubated artificially enlarged clutches of 6 eggs had much greater net incubation costs than predicted by the Kendeigh equation (Table 9.7), averaging 7.4 kJh^{-1} during incubation sessions. This extremely high cost was difficult to account for. If the bird was only able to cover 5 eggs at a time, so that one egg constantly cooled at the cooling rate of eggs during recesses ($0.906°Cmin^{-1}$), less than 1 kJh^{-1} should be required in reheating costs. Possibly these birds attempted to keep all the eggs warm all the time, and as the brood patch was not large enough to cover the whole clutch, heat loss from the entire body was increased. Another explanation might be that activity costs were underestimated for these two females, but this was unlikely as activity budgets were no less accurate for these individuals than for the other birds.

9.3.2.3 Energy expenditure of incubating swallows

Mean ADMR of incubating female swallows was 8.03 $cm^3CO_2g^{-1}h^{-1}$ (sd=1.88, n=6 birds), mean DEE was 105.40 kJd^{-1} (sd=22.99), equivalent to 3.67×BMR (sd=0.84). This was higher than the 2.71×BMR calculated by Turner (1982) from activity budgets. While two of these birds did not adopt a normal incubation schedule (birds S1 and S5, Table 9.8), they did not have energy expenditures outside the range of the other four individuals.

There were no significant correlations between measures of energy expenditure of incubating swallows and activity pattern or structural size (n=4), or with environmental factors, mass or mass change during the measurement period (n=6) (Spearman correlations, all $r \leq \pm 0.80$, $p > 0.17$). There was a positive correlation between ADMR and clutch reheating cost (r=1.00, p=0.00, n=4), although clutch reheating was calculated to be only a small (2-6%) proportion of daily energy expenditure. This suggested that clutch reheating costs may have been underestimated, possibly due to inefficient transfer of heat between the bird and

Table 9.8 Mass and energy expenditure (measured by the DLW technique) of incubating female swallows. Birds S1 to S4 were labelled during this study. Birds S5 and S6 are data collected from the same population by Westerterp and Bryant (1984). All birds incubated an unmanipulated clutch of 5 eggs. ADMR = metabolic rate ($cm^3CO_2g^1h^{-1}$). DEE = energy expenditure $kJ^{-1}d^{-1}$. M, metabolic intensity, = metabolic rate expressed as a multiple of BMR (calculated from Aschoff and Pohl (1970) inactive phase). Details of ring numbers and nest sites of birds S1 - S4, and the dates upon which the measurements were made, are given in Appendix 6.

Bird number	Mean mass (g)	ADMR	DEE	M
S1	19.85	9.83	123.82	4.44
S2	20.65	9.79	128.28	4.47
S3	21.95	7.32	101.96	3.40
S4	21.35	5.28	71.53	2.44
S5	20.25	6.71	86.22	3.05
S6	20.50	9.27	120.59	4.23
Mean	20.76	8.03	105.40	3.67
sd	0.77	1.85	22.89	0.84

clutch (this was assumed to be 100% efficient). It was also possible that the relationship between clutch reheating costs and energy expenditure was due to the more continuous effect of the cost of clutch warming during incubation sessions, since both clutch reheating costs and the energy requirement for maintenance of clutch temperature will vary with temperature. There was a negative, but non-significant, relationship between energy expenditure and temperature.

An activity budget was constructed for incubating swallows in order to make an independent calculation of DEE during the period of energy expenditure measurement by the DLW technique. This included thermoregulatory, activity and clutch reheating costs. The difference between DEE calculated from the activity budget and the DLW technique should represent the net cost of maintaining clutch temperature during incubation sessions (Table 9.9). Mean DEE determined by the DLW technique was 106.38 kJd^{-1} (sd=25.92, n=4), and from simultaneous calculation from the activity budget 69.71 kJd^{-1} (sd=6.43). There was a mean difference of 36.67 kJd^{-1} (sd=20.86) between these two estimates, which suggested that the net cost of incubation was rather greater than calculated from Kendeigh's (1963) equation (20.3 kJd^{-1}, sd=4.1) (Table 9.9).

9.3.3 Mass changes during incubation

9.3.3.1 Dippers

In a regression analysis of factors effecting the mass of incubating dippers, 19% of mass variation could be explained by wing and tarsus length (Table 9.10). Residual mass (mass minus incubation mass predicted from structural size, Table 9.10) decreased significantly during the incubation period (Fig. 9.6). The maximum contribution of this reserve use to the daily energy budget would be if the entire mass loss during incubation (4.44 g) was of lipid. If the energy content of lipid was 39.2 kJg^{-1}, this would be equivalent to 174 kJ during incubation, or 10.89 kJd^{-1} (4.8% DEE).

Table 9.9 Energy expenditure of incubating female swallows (all clutches = 5 eggs) measured over the same period by the DLW technique and from the activity budget. Energy expenditure calculated from the activity budget included thermoregulation, activity and clutch reheating after recesses. The difference between the two measurements (DLW-Act.) should be the net energy requirement for keeping the eggs warm during incubation sessions. For comparison, Kendeigh cost (kJd^{-1}), the cost of incubation calculated from the Kendeigh (1963) equation, is also shown.

	Energy expenditure (kJd^{-1})				
Bird number	**DLW**	**Act. method**	**Difference (DLW-Act.)**	**%(DLW-Act.)/DLW**	**Kendeigh cost (kJd^{-1})**
S1	123.81	77.15	46.66	37.69	25.09
S2	128.23	69.83	58.40	45.55	20.89
S3	101.95	70.40	31.55	30.95	20.30
S4	71.52	61.44	10.08	14.09	15.00
Mean	106.38	69.71	36.67	32.07	20.32
sd	25.92	6.43	20.86	13.39	4.14

Table 9.10 Stepwise multiple regression analysis of the effect of body size on the mass of incubating female dippers during incubation. Independent variables not included at the 5% level of significance were other measures of structural size, n=40. The multiple regression equation was used to predict the incubation mass of female dippers from structural size.

Independent variable	Cumulative r^2	Regression equation	t	p	Beta weight
Wing	0.11	$0.424x_1$	2.428	<0.02	0.338
Tarsus	0.19	$1.217x_2$ -20.943	2.249	<0.03	0.313

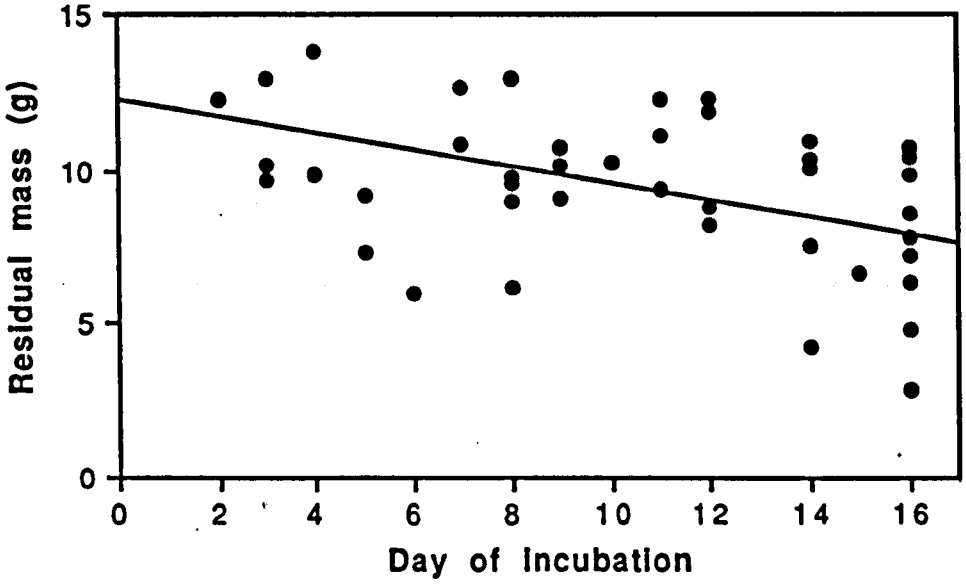


Fig. 9.6 Relationship between female dipper residual mass (after adjustment for bird size using the regression equation in Table 9.10) and day of incubation upon which the bird was weighed ($y=-0.278x + 12.371$, $r^2=0.21$, $p=0.002$, $n=44$). Residual mass was calculated from the difference between mass measured in the field and mass predicted for a bird of that size from a multiple regression equation which calculated bird mass from wing and tarsus length (Table 9.10).

9.3.3.2 Swallows

There was no significant change in the mass of female swallows with day of incubation (Chapter 2). In a multiple regression model, day of incubation was not entered as a significant predictor of mass or residual mass (mass minus incubation mass predicted from structural size, Chapter 2). Temperature, rainfall and food supply during the previous 3 days, and date had a significant effect on bird mass (Table 9.11). These results were consistent with those of Jones (1987b), working in the same study area: mass during the incubation period was not related to day of incubation, but instead was correlated with food supply and temperature.

9.4 Discussion

9.4.1 Use of DLW technique with incubating birds

In order for the energy expenditure measurement obtained using the DLW technique to be relevant, subjects must return to their normal routine shortly after release. Most birds returned to their nests within an hour, although one dipper (bird 6) stayed away for over 6 h (Tables 9.2 and 9.4). Birds which were watched spent the time between release and return to the nest resting and preening. The periods between release and resumption of normal activity were considered a sufficiently low percentage (normally less than 5%) of the DLW measurement period for the results to be representative of energy expenditure during normal incubation. Birds also had to use extra energy to rewarm a clutch which had cooled to ambient temperature when they did return to the nest. The greatest such reheating cost was calculated to be 2.5 kJ for a dipper reheating a clutch of 6 eggs from 6°C (1.1% DEE). Swallows used 0.6 to 0.7 kJ for clutch reheating (0.7% DEE). Activity budgets recorded by the nest temperature monitor showed that the behaviour of labelled individuals was otherwise similar to that of undisturbed birds.

The advantage of using a 48-hour rather than a 24-hour period for the DLW measurement is demonstrated in Tables 9.2 and 9.4. The percentage of measurement

Table 9.11 Stepwise multiple regression analysis of factors affecting a) mass and b) residual mass (mass minus incubation mass was predicted from structural size, Chapter 2) of incubating female swallows. Food supply is $\log_e(V+1)$ where V = insect suction trap volume. (d), (d-1) and (d-2) indicate measurements made on the day the bird was weighed, and one and two days before. Date is coded as the number of days after 1st April. Variables are listed in the order of inclusion in the regression model. Day of incubation, other environmental factors, condition indices and other measures of structural size (Chapter 2) were not included at the 5% level of significance. n = 68 birds.

a) Mass

Independent variable	Cumulative r^2	Regression equation	t	p	Beta weight
Head	0.09	$0.808x_1$	3.62	<0.001	0.364
Rain (d-2)	0.18	$-0.112x_2$	-2.13	<0.04	-0.221
Date	0.24	$-0.019x_3$	-3.27	<0.002	-0.368
Maximum temp. (°C) (d-2)	0.29	$+0.131x_4$	2.67	<0.01	0.297
Food supply (d-1)	0.33	$+0.604x_5$ -5.222	2.11	<0.04	0.211

b) Residual mass

Independent variable	Cumulative r^2	Regression equation	t	p	Beta weight
Rain (d-2)	0.08	$-0.129x_1$	-0.27	<0.009	-0.281
Date	0.12	$-0.028x_2$	-4.04	<0.0001	-0.579
Minimum temp. (°C) (d)	0.20	$+0.232x_3$	3.48	<0.001	0.604
Maximum temp. (°C) (d)	0.24	$-0.213x_4$	-3.40	<0.002	-0.513
Food supply (d-1)	0.28	$+0.619x_5$	2.30	<0.03	0.237
Maximum temp. (°C) (d-1)	0.32	$+0.120x_6$ +1.17	2.11	<0.04	0.312

time spent processing the bird (when energy expenditure will probably be greater than normal) and the time taken before return to the normal incubation schedule (energy expenditure lower than normal) both doubled for the two birds measured over 24 rather than 48 h. This would double any biases in the measurement, although as these biases were of opposite sign, their effects would tend to cancel one another.

The differences between labelled and undisturbed birds acted over only a small part of the measurement period or could account for only a small proportion of DEE. It was therefore concluded that the energy expenditures measured would essentially not differ from those of undisturbed birds.

9.4.2 Significance of mass loss during incubation

Dippers lost mass during the incubation period, but on average this accounted for only 4.8% of DEE (Section 9.3.3.1). Swallows showed no trend in mass as incubation progressed (Chapter 2), and their mass was instead related to temperature, rainfall, date and food supply. Swallows did not use their reserves systematically during incubation, but instead used them during times of low food availability.

Freed (1981) and Norberg (1981) proposed that mass loss at the end of incubation could be viewed as an adaptive preparation for increased activity levels during nestling-rearing, when it would be advantageous to decrease the power requirement for flight. Programmed anorexia was also suggested as the explanation for the 2g loss of mass of female swallows between the incubation and nestling-rearing periods (Jones 1987a). Thus mass loss during incubation was not necessarily an indicator of a reproductive cost, or that it was a crucial part of the energy budget, although any mass loss would be used to supplement the energy gain through daily food intake.

It was therefore concluded that reserves contributed little to the overall energy requirement of incubating dippers and swallows. However possession of lipid reserves might still serve as an insurance against poor conditions, even if these

reserves were only needed on rare occasions. Dippers lost an unimportant amount of mass during incubation whilst swallow mass fluctuated according to foraging conditions.

9.4.3 Clutch reheating costs

Clutch reheating costs were calculated to be only 2-6% of daily energy expenditure measured by the DLW technique. However, this did not take into account the possible inefficiency of clutch rewarming. If a bird increases heat production to warm eggs, heat loss by other avenues may also increase (Vleck 1981).

Biebach (1986) suggested that heat generated by activity during the recess period may be stored and transferred to the clutch, so that clutch reheating after recesses would not be an additional energy cost. This possible source of "waste" heat is more likely to benefit swallows than dippers, as swallows flew throughout the recess period whilst dippers often returned to the nest after a period of preening, during which less excess heat would be produced than during flight.

The heat production of the embryo has been omitted from the calculations of rate of heat loss of the clutch during incubation recesses as egg cooling rates were measured from fresh eggs. Oxygen consumption of embryos increases exponentially during incubation (Vleck *et al* 1979), however this source of heat has been considered to be thermally unimportant as it is very small for altricial eggs when compared with the rate of heat loss due to convection during recesses (Webb and King 1983).

9.4.4 Comparison of swallow and dipper energy expenditure during the incubation period

Dippers (60 g) will clearly have a greater absolute DEE than 20g swallows, so the metabolic intensity of the two species was compared (Fig. 9.7). The modal

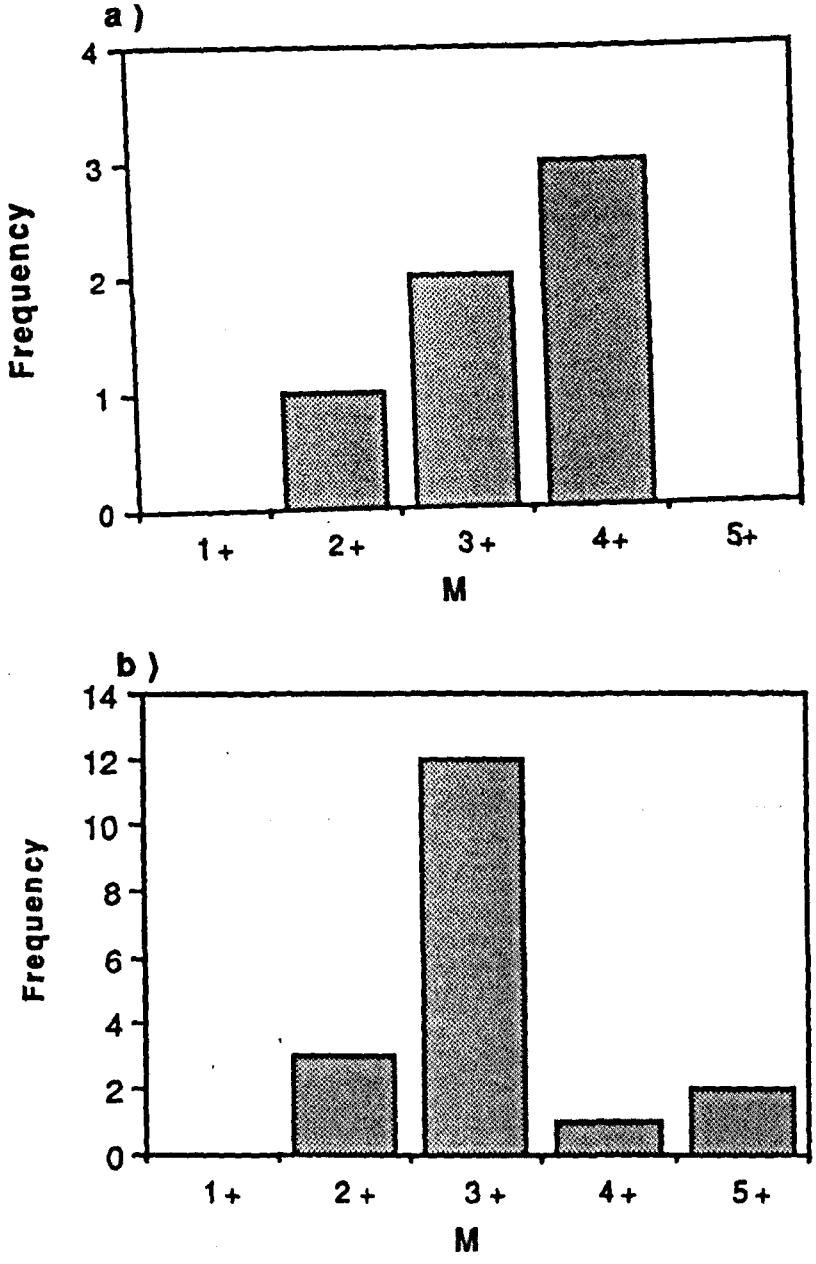


Fig. 9.7 Comparison of the frequency of each integer category of metabolic intensity (M) of a) incubating swallows (n=6) and b) incubating dippers (n=18). Metabolic intensity was metabolic rate (measured using the DLW technique) expressed as the number of multiples of BMR (BMR calculated from mass using the equation of Aschoff and Pohl (1971), inactive phase).

category for the metabolic intensity of incubating dippers was 3+×BMR. Dippers with higher metabolic intensities were "unusual" as they either had a manipulated clutch size of 6 eggs or used a high proportion of diving whilst foraging. In contrast, incubating swallows operated at 3+ or 4+×BMR, with no indication that the birds with the higher metabolic rates were incubating under unusual circumstances. The swallow which worked at 2+×BMR was the only bird which experienced a high daytime ambient temperature at the nest (25°C). This high temperature would reduce thermoregulatory costs and decrease the energy required for clutch warming. The metabolic intensity of incubating swallows was significantly greater than that of incubating dippers (Chi-square test, χ^2=19.8, p=0.001, 3 d.f.).

The difference between metabolic intensity of swallows and dippers during incubation could be due to several factors.

1. Dippers make some systematic use of energy reserves during incubation, and are occasionally fed by their mates.

Reserve use could reduce energy requirement from food intake by 4.8% each day. Feeding of the female on the nest by male dippers was unlikely to be of significant benefit to females, as only 4 definite and 6 possible male feeds were seen during 57 h of observations at 12 nests. At most this could amount to 3 feeds per day, and this was assumed to make only a minor contribution to the daily energy requirement of incubating female dippers. Male feeds have been shown to be of importance in the energy budget of incubating females in other species (e.g., common tern, Nisbet 1977, marsh tit, Nilsson and Smith 1988, and snow bunting, Lyon and Montgomerie 1985).

2. Despite incubating at lower ambient temperatures, dippers have extremely thick downy plumage and sit within an enclosed nest where the nest air temperature is higher, relative to ambient temperature, than for swallows on open nests. The favourable nest microclimate would reduce thermoregulatory energy requirements during incubation sessions which made up 70-80% of the 24-hour day.

3. Swallows spent their recess time in flight at an estimated metabolic rate of 307 $Jg^{-1}h^{-1}$ (Turner 1983) whilst dippers spent an average of 50% (Table 9.2) of recess time resting, at 75 $Jg^{-1}h^{-1}$ (Bryant *et al* 1985). Both species obtained the energy necessary for their requirements, but swallows consumed and expended relatively more. This might be expected both from the difference in mass between the two species (smaller animals have a higher mass-specific metabolic rate, Calder 1984), and from the difference in foraging mode (aerial foragers expend 13-38% more energy than species with alternative foraging modes, Williams 1987).

9.4.5 The net cost of incubation for the dipper and swallow

The net cost of incubation was calculated from the difference between DLW measurements and activity budget estimates of energy expenditure. For swallows, the mean difference between the DLW and activity budget estimates was 34.5% of DEE measured by the DLW technique, whilst for dippers, the mean difference was 12.1% of DEE (Tables 9.7 and 9.9).

These estimates of the cost of incubation were similar to those calculated from Kendeigh's (1963) equation for dippers but were higher than Kendeigh's prediction for swallows. The greater net cost of incubation for the swallow could be accounted for by the open nest of this species, which provided less insulation than the enclosed nest of the dipper.

Calculation of the cost of incubation by difference between the DLW and activity budget measurements of DEE was subject to errors arising from both techniques, but particularly from the activity budget (Weathers and Nagy 1980). Despite problems with the accuracy of the activity budget estimate of DEE, subtraction of known thermoregulatory and activity costs from DEE measured simultaneously by the DLW technique seems the most promising way to determine the net energy requirement for incubation. This approach was used by Masman *et al* (1988) to calculate a net cost of incubation of 23% for a female kestrel. This figure was a close match with the mean of the results for swallows and dippers

(34.5+12.1)/2=23.3%. An incubation cost of 20-25% of DEE has also been derived for birds in general from the use of biophysical models (reviewed by Ricklefs 1974).

9.4.6 Influence of clutch size upon incubation costs

DEE was much greater for dippers which incubated an enlarged clutch of 6 eggs than birds incubating either unmanipulated clutches of 4 or 5, or reduced clutches of 3 eggs. Dippers decreased the duration of both incubation sessions and recesses as clutch size increased (Fig. 9.2), but this did not result in a different proportion of time spent on and off the nest. There was no significant difference in activity pattern of the birds incubating larger clutches whilst off the nest, and no increase in metabolic rate for birds incubating clutches of 5 compared with those with 3 or 4 eggs, despite the trend in session and recess duration. This implied that increased energy costs for birds incubating 6 eggs occurred during incubation sessions rather than recesses or as a result of clutch reheating costs after recesses.

Lack of an increase in DEE with increasing clutch size up to a certain level, followed by a sharp rise in costs upon a further increase in clutch size has also been found for starlings by nest box calorimetry (Biebach 1986). Biebach proposed that the brood patch produced the same amount of heat irrespective of clutch size, but as it was only large enough to cover a certain number of eggs (6 for the starling) larger clutches would require constant rearrangement of the eggs and a continuous clutch reheating cost.

Clutch size manipulations affected collared flycatcher energy expenditure in the same way (Moreno *et al* 1991). Energy expenditure increased for birds incubating enlarged clutches, did not decrease for reduced clutch sizes, and was associated with greater costs per unit time spent incubating and not from different activity patterns. The cost of incubation also increased with clutch size for the great tit (Mertens 1977), zebra finch (Vleck 1981), blue tit (Haftorn and Reinertsen 1985), canary (Weathers 1985) and Bengalese finches (Coleman and Whittall 1988). This supports suggestions such as those of Yom-Tov and Hilborn (1981) and Walsberg

(1983b) that clutch size may be proximately limited by the incubating capacity of the female due to the potential for an energetic bottleneck during incubation.

Indirect evidence also suggests difficulty in incubating clutches larger than those actually laid by the bird. These studies are important as they show that there are consequences for reproductive success of factors which have been measured over only a few days in energetics studies when clutch size increases and temperatures or food availability decrease during incubation. Smith (1989) showed that blue tits take longer to incubate enlarged clutches, which would be disadvantageous since fledgling survival decreases toward the end of the breeding season (Perrins 1965, Kluyver *et al* 1977). Wheatear and pied flycatchers (Moreno 1989b, Moreno and Carlson 1989) but not collared flycatchers (Moreno *et al* 1991) also took longer to incubate larger clutches. This may be because clutches which are incubated at a lower temperature take longer to hatch (White and Kinney 1974, Boersma 1982). Larger clutches are associated with a decrease in nest attentiveness in the swallow (Jones 1987b) and the dipper (this study), although no effect of clutch size was found upon attentiveness of collared flycatchers (Murphy 1978, Moreno *et al* 1991). Other indications of an increased difficulty of incubating larger clutches are reduced hatching success (Moreno *et al* 1991) and increased hatching asynchrony (Moreno and Carlson 1989).

It was concluded that incubation costs increased with clutch size but the nature of relationship differed between species. Incubation cost seemed to be constant up to a threshold for dippers, whilst Biebach (1984) found a linear increase in energy expenditure with clutch size in starlings. Merten's (1977) model suggested that incubation costs might increase with a decelerating curve as eggs in larger clutches insulate each other, although the need to rewarm eggs not in contact with the brood patch may counteract this (Biebach 1984). A more detailed investigation into the form of the relationship between clutch size and the cost of incubation would be an area for future work.

9.4.7 Influence of temperature upon incubation costs

Swallow clutches cooled more quickly in an incubator at lower temperatures, when they were in smaller clutches and when insulation was decreased by removal of the nest lining (Jones 1985). The energetic consequences for the female of clutch reheating were demonstrated for starlings using nest box calorimetry (Biebach 1986). Energy expenditure increased more when females returned to the nest after long recesses than short ones, and was greater when the nest box had been cooled to 10°C than 25°C. These studies showed that clutch cooling rates do increase as temperature decreases and that this imposes measurable increases in energy expenditure for the incubating female. However, although the dippers in this study incubated over a 11°C range of mean ambient temperature, there was no affect upon DEE. This suggested that, at least for this species, clutch reheating costs were an unimportant fraction of total energy requirements. In contrast, the one swallow which incubated at a high ambient temperature had a markedly low DEE. This could, in part, be accounted for by decreased clutch reheating costs and thermoregulatory energy requirements during incubation sessions. Lower foraging costs (due to an increase in food abundance, Chapter 2) would perhaps also decrease DEE via a reduction in activity costs, although nestling-rearing house martins actually worked harder when the energy gain from foraging was greater (Bryant and Westerterp 1983b).

Energy expenditure during incubation sessions responded to manipulation of temperature in nest box calorimetry studies (El-Wailly 1966, Mertens 1977, Biebach 1984, Haftorn and Reinertsen 1985). Experiments which involved heating or cooling of the clutch showed that the sitting bird responds directly to clutch temperature when determining her energy expenditure. A zebra finch gamely adjusted her metabolic rate to try to return a copper clutch supplied with a flow of hot or cold water to the correct incubation temperature (Vleck 1981).

These experiments show that temperature can have an important effect upon the energy expenditure of an incubating bird, however the fluctuations used in nest box calorimetry studies are more extreme than those experienced by birds incubating

in most natural situations. This probably accounted for the lack of a significant effect of temperature upon the DEE of incubating swallows and dippers in this study.

CHAPTER 10

GENERAL DISCUSSION

10.1 A model of the energy and nutrient intake and requirements of a laying swallow

10.1.1 Potential daily lipid, protein and energy intake of a laying swallow

A model was developed to describe the daily lipid and protein intake of a laying female swallow over a range of foraging conditions, to show if energy and nutrient requirements for maintenance and egg production could be met from daily food intake. This allowed the importance of reserves as a supply of energy or nutrients to a laying swallow to be assessed.

The potential foraging rate of a laying swallow was assumed to change with temperature according to the relationship described by Turner (1982) for swallows feeding nestlings (see Table 10.1). The composition of the diet of laying swallows was known to be similar to that of nestling-rearing birds (Turner 1982). Laying swallows spent a mean of 15 h away from the nest each day (Chapter 7), but only the period of flapping flight (8.06 h) was assumed to be spent foraging (Møller 1987b). Some insects would probably be captured during gliding flight, so potential food intake could be somewhat greater than that calculated in Table 10.1. There would also be individual variation in foraging rates.

Data on insect composition and the assimilation efficiency of swallows given by Turner (1982) was used to calculate rates of lipid and protein gain (Table 10.1). Swallows assimilated 70% of the energy content of insects (22.833 kJg^{-1} dry mass, determined by bomb calorimetry), so lipid and protein must have been assimilated more efficiently as they accounted for 18.621 kJg^{-1}, only 81% of the calorific value of insects determined by bomb calorimetry. The additional energy content of insects was accounted for by chitin, which was included in the bomb calorimetry measurement, but not digested by swallows. The carbohydrate content of insects was small enough to be ignored for the purposes of this model (Turner 1980). The assimilable energy content of insects was $0.70 \times 22.83 = 15.98$ kJg^{-1} dry mass,

Table 10.1 Calculation of potential daily lipid, protein and energy intake of a laying swallow.

Period[1]	0500-0600 0700-1000	1000-1800	1800-2100
Duration (h)	4	8	3
Flapping flight[2]			
%	68.4	46.6	53.0
Duration (h)	2.74	3.73	1.59
Mean temp. (°C)[3]	9.89	15.44	12.36
Foraging rate			
Assimilable kJh^{-1}[4]	13.99	43.27	27.02
Total dry mass (gh^{-1})[5]	0.88	2.71	1.69
Total lipid (gh^{-1})[6]	0.076	0.234	0.146
Total protein (gh^{-1})[6]	0.566	1.743	1.087

Potential daily intake (assimilation efficiency not taken into account)

Lipid (g) $2.74 \times 0.076 + 3.73 \times 0.234 + 1.59 \times 0.146 = 1.31$
Protein (g) $2.74 \times 0.566 + 3.73 \times 1.743 + 1.59 \times 1.087 = 9.78$
Energy (kJ) $39.2 \times 1.31 + 23.7 \times 9.79 = 283.28$

[1] The activity budget was divided between the three parts of the day used in Chapter 7.
[2] The proportion of time spent in flapping flight was used to calculate the duration of foraging.
[3] The mean temperature during each part of the day was calculated by proportion from the mean maximum temperature during measurement energy expenditure of laying swallows by the DLW technique (Chapter 7), using the daily fluctuation in temperature at swallow nests recorded by the nest temperature monitor (Chapter 9).
[4] The rate of assimilable energy gain was based upon Turner's (1982) relationship between ambient temperature and the foraging rate of a nestling rearing swallow (rate of assimilable energy gain (kcalmin^{-1}) = 0.021 T (°C) - 0.152, multiplied by 60×4.187 to convert from kcalmin^{-1} to kJh^{-1}).
[5] The rate of dry mass gain was calculated using an energy content of 15.983 kJg^{-1} dry mass (derived from 22.833 (energy content of insects, determined by bomb calorimetry) $\times$ 0.70 (assimilation efficiency), Turner 1982).
[6] Total lipid and protein intake were calculated from their dry mass contents in insects (8.62 % lipid and 64.3 % protein, from Turner 1982). Energy intake was calculated using an energy content of 39.2 kJg^{-1} for lipid and 23.7 kJg^{-1} for protein (Znaniecka 1967).

equivalent to an assimilation efficiency of 85.8% (15.98/18.62) for the lipid and protein components of insects. The mean rate of energy gain by foraging swallows (488.7 $Jmin^{-1}$, Bryant and Turner 1982) would have been achieved at 12.80°C, according to this model. Swallow food delivery rate has also been recorded by Jones (1987e) using balances placed under swallow nests. The peak food delivery rate, of 8 gh^{-1} wet mass, or 38.92 kJh^{-1}, would have been supplied at a mean day time temperature of 14.6°C according to the model developed above. The broad agreement between the foraging rate of swallows in other studies, and that predicted here, suggested that the model was realistic.

The lipid:protein ratio was much lower in insects than in swallow eggs (Chapter 7), so it was possible that lipid was the limiting nutrient for egg formation, and might therefore be assimilated more efficiently than protein. If lipid was assimilated with 100% efficiency, this would provide 3.379 kJg^{-1} dry mass of insects, leaving 15.983 - 3.379 = 12.604 kJ to be obtained from the protein component, with an efficiency of 82.7%. Accordingly, the potential daily lipid, protein and energy assimilated by a laying swallow were calculated assuming either 100% assimilation efficiency for lipid and 82.7% for protein, or 85.8% for both components (Table 10.2). Daily lipid intake would be greater if this component was assimilated with 100% efficiency, whereas protein and energy intakes would alter only slightly. As it was not possible to determine which assumption of assimilation efficiency was the more realistic, both of the potential intakes of lipid, protein and energy derived in Table 10.2 were used in subsequent calculations.

Laying swallows had a mean energy output (respiratory energy expenditure plus energy content of egg material deposited) of 117.98 kJd^{-1} (95% CI=±10.05) (Chapter 7). Potential energy intake was 243.07 - 243.18 kJd^{-1} (Table 10.2). The energy requirement of a laying female swallow was therefore much lower than could be gained by foraging. Under normal conditions, a laying swallow would have no difficulty in gathering sufficient food for maintenance, egg and oviduct formation from daily food intake. The large excess foraging capacity in normal feeding conditions would also serve to ensure that adequate food could still be obtained

Table 10.2 Effect of variation in the assimilation efficiency of lipid and protein upon the potential lipid, protein and energy assimilated by a laying swallow. Total daily intakes were calculated in Table 10.1.

a) Potential assimilable daily intake (assuming 100% assimilation efficiency for lipid and 82.7% for protein)

Lipid (g) $1 \times 1.31 = 1.31$

Protein (g) $0.827 \times 9.78 = 8.09$

Energy (kJ) $39.2 \times 1.31 + 23.7 \times 8.09 = 243.18$

b) Potential assimilable daily intake (assuming an assimilation efficiency of 85.8% for lipid and protein)

Lipid (g) $0.858 \times 1.31 = 1.13$

Protein (g) $0.858 \times 9.78 = 8.39$

Energy (kJ) $39.2 \times 1.13 + 23.7 \times 8.39 = 243.07$

during periods of poorer weather when foraging rates might fall.

Grit was readily available on the ground in farmyards (pres. obs., Turner 1982), and was also ingested in the insect diet (Turner 1982). Calcium was therefore thought unlikely to limit egg production by the swallow, although grit would occupy gut space and so potentially reduce nutrient uptake.

10.1.2 Would energy, lipid or protein intake limit egg formation, if swallow eggs were formed from daily food intake alone?

Energy flow through a laying swallow can be described by Equation 10.1:

10.1

Food intake + tissue catabolism = DEE + tissue production + excretion

where DEE was measured by the DLW technique (Chapter 7) (kJd^{-1}),
tissue catabolism/production = energy used from or deposited in body reserves, or that deposited as egg (kJd^{-1}),
excretion = 30% of food intake (follows from 70% energy assimilation efficiency, Turner 1982), (kJd^{-1}).

The only unknown variables in Equation 10.1 were food intake, reserve use and reserve production. Changes in the level of reserves might be determined from a combination of condition indices and carcass analysis, but if it was assumed that reserve use = reserve production over a 24-hour period (as would be the case if food intake alone were used for maintenance and egg production), Equation 10.1 could be used to determine the daily food intake of a laying swallow (Table 10.3).

A range in daily energy and nutrient intake for a laying swallow was generated from Equation 10.1 using the 95% confidence intervals of energy output, and the rates of lipid and protein intake for a given energy output (Table 10.3). The maximum daily lipid requirement for egg and oviduct formation was only 26-35% of intake (Table 10.3). If a lipid reserve was built up in 4 days, as suggested in Chapter 8, the maximum daily lipid requirement would have been 74-102% of intake. No conversion from dietary protein to lipid would be necessary to supply the

Table 10.3 Comparison of daily lipid and protein intake and requirements of a laying swallow, a) calculation of assimilable intake, b) maximum lipid requirement (on day -1) in relation to intake, c) maximum protein requirement (on day -1) in relation to intake.

a) Daily energy output (respiratory DEE + energy content of egg formed)
= 117.98 kJ (95% CI, range 107.93 - 128.03, Table 7.13)

Minimum daily lipid assimilation[1] = 1.13 × 107.93/243.07 = 0.50g
Maximum daily lipid assimilation[1] = 1.31 × 128.07/243.18 = 0.69g
Minimum daily protein assimilation[1] = 8.39 × 107.93/243.07 = 3.73g
Maximum daily protein assimilation[1] = 8.09 × 128.03/243.18 = 4.26g

[1] calculated by proportion from Table 10.2

b) Maximum daily lipid content of egg formed (Table 3.9) = 0.129g
Maximum daily lipid requirement for egg formation
(at 81% efficiency, Chapter 7) = 0.159g
Maximum daily lipid content of oviduct formed
(Appendix 3 and Chapter 8) = 0.014g
Maximum daily lipid requirement for oviduct formation
(at 81% efficiency) = 0.018g
Maximum daily lipid content of reserves formed
(if built up over 4 days, Chapter 8) = 0.271g
Maximum daily lipid requirement for reserve formation
(at 81% efficiency) = 0.335g

Maximum daily lipid requirement for egg and oviduct formation = 0.177g
Intake would be 282 - 390% of requirement
Requirement would be 26 - 35% of intake

Maximum daily lipid content of egg, oviduct and lipid reserve formed
= 0.512g
Intake would be 98 - 135% of requirement
Requirement would be 74 - 102% of intake

c) Maximum daily protein content of egg formed (Table 3.9) = 0.186g
Maximum daily protein requirement for egg formation
(at 55% efficiency, Chapter 7) = 0.338g
Maximum daily protein content of oviduct formed
(Appendix 3 and Chapter 8) = 0.083g
Maximum daily protein requirement for oviduct formation
(at 55% efficiency) = 0.151g

Maximum protein requirement for egg and oviduct formation = 0.489g
Intake would be 761 - 870% of requirement
Requirement would be 11 - 13% of intake

peak requirement for reserve, egg and oviduct deposition on day -1. Lipid from daily food intake would cover the requirements for egg, oviduct and reserve formation whether swallows assimilated lipid with 100% or 85.8% efficiency from the diet. There would be no need to reduce the efficiency of egg formation by conversion of protein to lipid as had been proposed in Chapter 7. The maximum daily protein requirement for egg and oviduct represented only 11-13% of intake (Table 10.3), so protein was not a limiting nutrient for laying swallows.

In conclusion, swallows would be able to obtain all the lipid and protein necessary for maintenance, egg and oviduct production directly from food intake, so reserves would not be required under normal environmental conditions.

10.1.3 Temperature thresholds for energy and nutrient intake of a laying swallow for maintenance and egg production

The relationships between mean daytime temperature and the potential daily lipid, protein and energy intake of a laying swallow (Tables 10.1 and 10.2) allowed prediction of daily intake over a range of temperatures (Table 10.4, Fig. 10.1). For each 1°C change in mean day-time temperature, daily protein intake would change by 1.47 g, lipid intake by 0.20 g and energy intake by 42.50 kJ (Fig. 10.1). The critical temperatures below which daily intake would be less than requirements for maintenance and egg production were 7.57°C for protein, 8.14°C for lipid and 10.01°C for energy (Fig. 10.1). A laying swallow would be constrained by daily energy intake at a higher temperature than was necessary for her to achieve the lipid or protein requirements for egg and oviduct formation. Laying swallows would therefore be limited by total energy intake, rather than availability of lipid or protein.

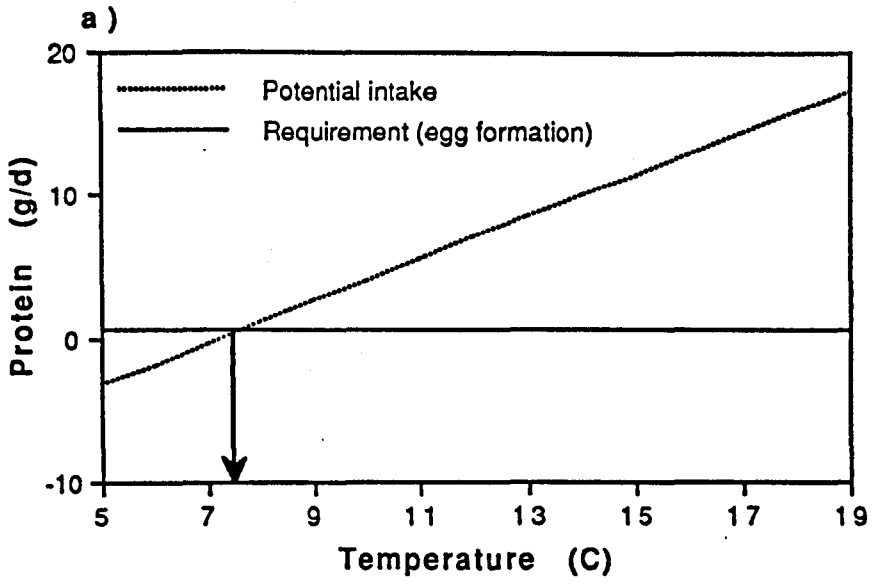
The critical temperature below which the energy requirements of a swallow could not be supplied from daily food intake varied little with changes in energy output (Table 10.4, Fig. 10.2a). If mean daytime temperature fell below 10.01°C (the temperature at which daily food intake would just match requirements for egg and oviduct formation), a laying swallow would have several options. She could stop egg

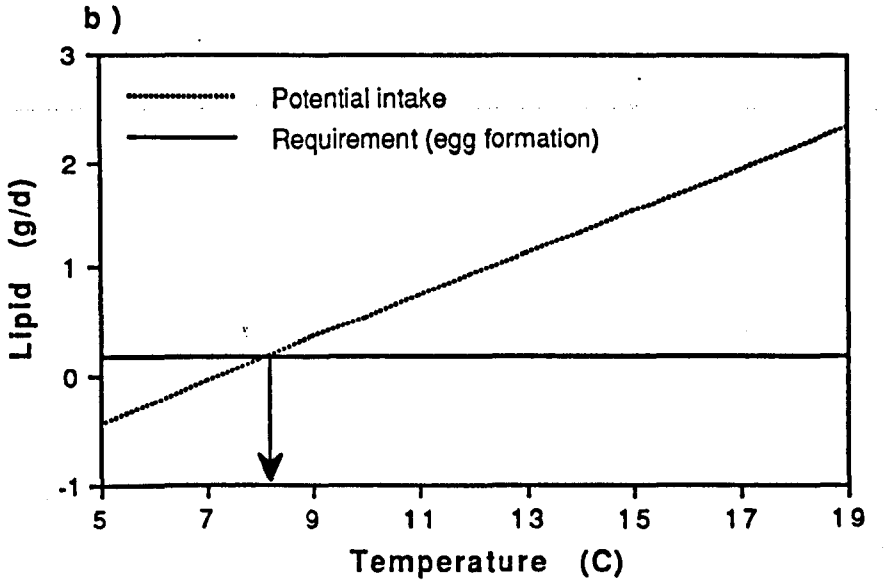
Table 10.4 Potential daily intake of lipid, protein and energy by a laying swallow over a range of mean day time temperatures. Intakes were calculated from the model in Tables 10.1 and 10.2. Data are shown graphically in Fig. 10.1.

Daily protein intake = 1.468 × temperature (°C) - 10.622
Daily lipid intake = 0.197 × temperature (°C) - 1.423
Daily energy intake = 42.499 × temperature (°C) - 307.610

	Predicted intake		
Temperature °C	**Lipid (gd^{-1})**	**Protein (gd^{-1})**	**Energy (kJd^{-1})**
5	-0.44	-3.28	-95.12
6	-0.24	-1.82	-52.62
7	-0.05	-0.35	-10.12
8	0.15	1.12	32.28
9	0.35	2.59	74.88
10	0.54	4.05	117.38
11	0.74	5.52	159.88
12	0.94	6.99	202.37
13	1.13	8.46	244.87
14	1.33	9.92	287.37
15	1.53	11.39	329.87
16	1.72	12.86	372.37
17	1.92	14.33	414.87
18	2.12	15.80	457.37
19	2.31	17.26	499.86

Fig. 10.1 Relationships between mean day time temperature and the potential intake of a) protein, b) lipid and c) energy of a laying swallow (dotted lines). The maximum lipid and protein requirement for egg and oviduct formation, and the mean energy requirement for maintenance, plus egg and oviduct formation are shown by the solid lines. The maximum daily requirements for egg and oviduct formation were 0.177 g lipid and 0.4895 g protein (Table 10.3). The mean energy requirement for maintenance, egg and oviduct formation was 117.98 kJd^{-1} (Chapter 7). The relationships between temperature and assimilated daily intake were described by the equations a) daily protein intake = $1.4676 \times T(°C) - 10.6223$, b) daily lipid intake = $0.1966 \times T - 1.4227$, and daily energy intake = $42.4987 \times T - 307.6101$.


a)
Potential intake
Requirement (egg formation)
Protein (g/d)
20
10
0
-10
5
7
9
11
13
15
17
19
Temperature (C)


b)
Potential intake
Requirement (egg formation)
Lipid (g/d)
3
2
1
0
-1
5
7
9
11
13
15
17
19
Temperature (C)

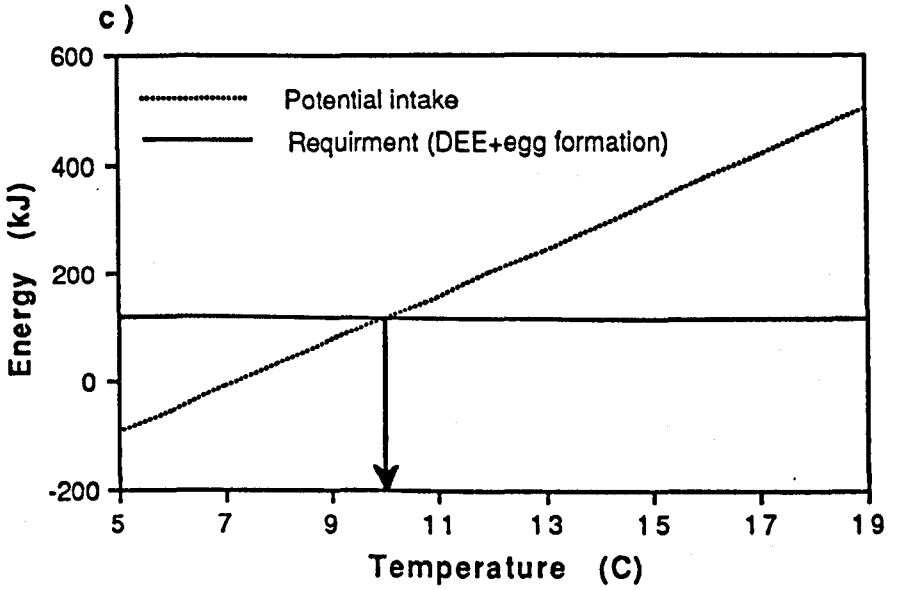
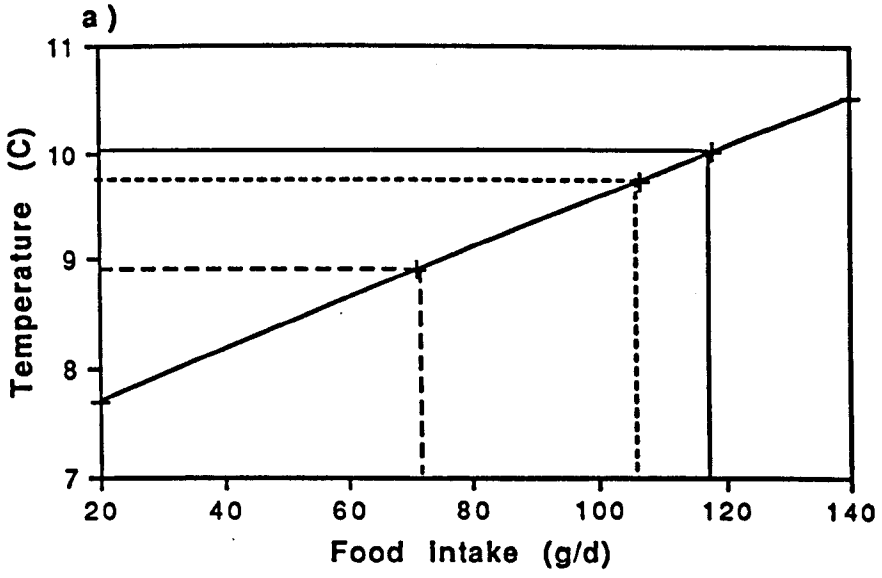
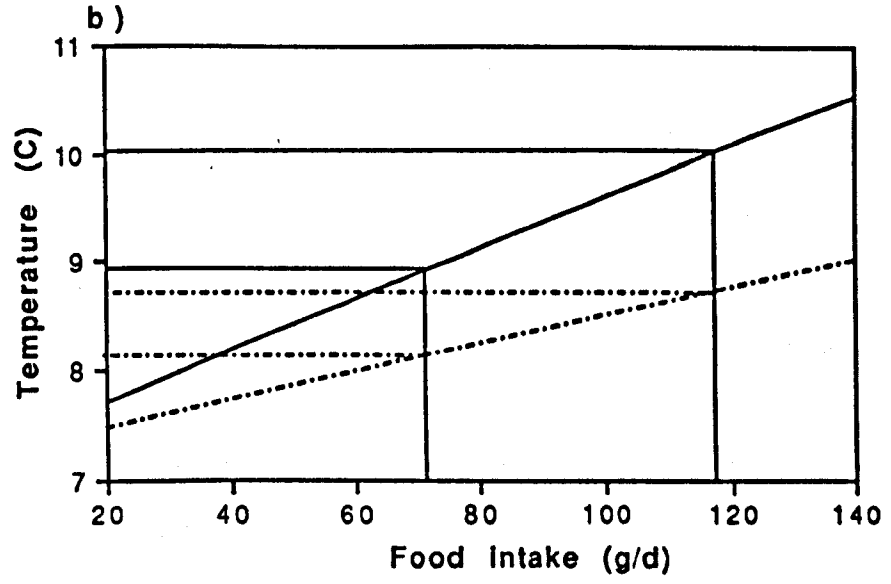

c)
Potential intake
Requirment (DEE+egg formation)
Energy (kJ)
600
400
200
0
-200
5
7
9
11
13
15
17
19
Temperature (C)

Fig. 10.2 Minimum temperatures necessary for a female swallow to achieve the required daily food intake for egg and oviduct formation if a) food intake alone, food intake plus an equal part of the lipid reserve, or food intake plus the whole lipid reserve and oviduct were used on a single day. The mean daily energy requirement for maintenance, egg and oviduct formation was 117.98 kJd^{-1} (Chapter 7). This could be supplied from food intake alone at temperatures down to 10.01°C (dotted line). If 11.48 kJ were supplied from lipid reserves, the remaining 106.50 kJ could be supplied from daily food intake at temperatures down to 9.74°C (medium dashed line). If 46.81 kJ were supplied from the entire lipid and oviduct reserve, the remaining 71.17 kJ could be supplied from daily food intake at temperatures down to 8.91°C (long dashed line). b) Reduction in the minimum temperature at which daily food intake could supply the requirements for egg production if a laying swallow foraged for 15 h, rather than 8.06 hd^{-1}. If the entire daily energy requirement for maintenance, egg and oviduct formation (117.98 kJ) were met from daily food intake, swallows could maintain energy balance down to 10.01°C (solid lines). If foraging time was increased, the minimum temperature for energy balance would be 8.73°C (dotted/dashed line). If a swallow used all her lipid and oviduct reserves on a single day and increased her foraging time, she could continue to lay eggs down to a temperature of 8.14°C (dotted/dashed line).


a)
11
10
9
8
7
Temperature (C)
20
40
60
80
100
120
140
Food Intake (g/d)


b)
11
10
9
8
7
Temperature (C)
20
40
60
80
100
120
140
Food Intake (g/d)

production to reduce her total energy output from 117.98 to 109.43 kJd^{-1} (109.43 derived from mean DEE of laying swallows minus the cost of egg synthesis, i.e., 112.28-(5.7/0.667), Chapter 7). This would allow her to achieve energy balance at temperatures down to 9.81°C, only 0.20° less than the temperature at which she could have continued laying. She could continue egg production if lipid reserves were used to supplement daily energy intake during the period of albumen formation (or if lipid was not stored during poor foraging conditions during the period of rapid follicular growth). This would allow a reduction in the required energy intake by 11.48 kJd^{-1}, for a swallow which used an equal part of the lipid reserve on each day during the period of albumen formation (Table 8.13). The energy requirement from daily food intake would then be reduced to 106.50 kJ and the lowest temperature at which a swallow could continue to lay would be 9.74°C (Fig. 10.2a).

If the temperature fell below 9.74°C a swallow could still continue to lay if she used all her lipid and oviduct reserves in a single day (46.81 kJ, Table 8.13). Her daily energy requirement from food intake would be reduced to 71.17 kJd^{-1} and the mean daytime temperature could drop to 8.91°C (Fig. 10.2a), before she would have to stop egg production. This would be a risky strategy, however, as a female swallow which used her entire reserve one day would depend on an improvement in feeding conditions the next day to survive.

A laying swallow could increase the proportion of the day which she spent foraging as temperature fell, rather than relying upon reserves. The mean period spent foraging was assumed to be only 8.06 hd^{-1} (Table 10.1), but if the full 15 hd^{-1} spent away from the nest was spent feeding (and it was assumed that the rate of food intake did not change, other than with temperature), the potential daily food intake at each temperature would be increased by a factor of 1.86 (15/8.06). The minimum temperature at which a laying swallow could support maintenance, egg and oviduct formation from daily food intake alone would be reduced to 8.73°C (Fig. 10.2b). At the other extreme, the minimum temperature at which a swallow could continue to lay if she used her entire lipid and oviduct reserve on a single day and foraged for 15 h would be 8.14°C, 1.87°C lower than the minimum temperature

required by a bird which foraged for only 8.06 hd^{-1} and used no reserves (Fig. 10.2b). The threshold temperature levels at which these factors would constrain egg production by swallows are summarised in Table 10.5.

The temperature below which daily food intake would be inadequate to supply sufficient energy for maintenance and egg formation (8.14°C) would therefore always be greater than or equal to the critical temperature for adequate dietary lipid or protein intake for egg and oviduct formation (8.14 and 7.57°C). Laying swallows would therefore always be limited by total energy intake rather than by shortage of crude lipid or protein for egg formation.

10.1.4 Probability that environmental conditions would lead to an inadequate food intake for a laying swallow

The probability that the mean daytime temperature would be lower than 10.01°C (the lowest temperature at which a laying swallow could achieve the necessary food intake for maintenance, egg and oviduct production from daily food intake alone, Section 10.1.3) was calculated for each week between the beginning of April and the end of August (Table 10.6). Only one female initiated egg formation in April, when there was more than a 50% probability that maintenance, egg and oviduct production could not be supplied from daily food intake alone. The majority of birds did not begin to form eggs until mid-May, when only 1 day per week was likely to be too cold to allow them to obtain the energy requirement for maintenance, egg and oviduct production from daily food intake alone (column (a), Table 10.6). If a swallow used an equal part of her lipid reserves on each day of albumen formation (or did not accumulate reserves during rapid follicular growth) this would reduce the probability that temperature would be too low for a swallow to continue laying (compare columns (a) and (b), Table 10.6). A situation where the temperature would be too low for a swallow to continue egg formation if all reserves were used and the time spent foraging was increased, would be very rare (column (c), Table 10.6). The lipid reserve was therefore important for a swallow

Table 10.5 Threshold mean day-time temperatures (°C) at which a laying swallow would need to adopt different strategies in order to continue egg production.

Temperature	Threshold for a laying swallow to obtain
10.01	total energy output (i.e DEE plus energy for egg formation) from daily food intake by foraging for 8.06h
9.81	DEE from daily food intake if egg production was stopped by foraging for 8.06h
9.74	energy output by foraging for 8.06h with use of 11.48kJ from reserves (an equal part of the lipid reserve used during each day of albumen formation)
8.91	energy output by foraging for 8.06h with use of 46.81kJ from reserves (use of entire lipid and oviduct reserve in 1 day)
8.73	total energy output from daily food intake by foraging for 15h
8.14	energy output by foraging for 15h with use of 46.81kJ from reserves (use of entire lipid and oviduct reserve in 1 day) ALSO lipid required for egg formation matched by crude lipid assimilated from daily food intake
7.57	protein required for egg formation matched by crude protein assimilated from daily food intake

Table 10.6 Weekly probability that the daily food intake of a female swallow would provide sufficient energy for maintenance and egg formation under 3 assumptions a) to c), and the probability that swallows which had not yet laid would initiate first clutch egg formation.

Week[1]	Month	Probability of bad weather[2] (a)	(b)	(c)	Number of 1st clutches initiated[3]	Probability of initiation of 1st clutch[4]
1	April	0.84	0.80	0.54	0	0
2		0.85	0.82	0.54	0	0
3		0.72	0.64	0.30	0	0
4		0.57	0.49	0.27	1	0.01
5	May	0.43	0.39	0.22	5	0.04
6		0.27	0.25	0.08	14	0.11
7		0.21	0.19	0.08	32	0.29
8		0.14	0.10	0.02	45	0.58
9	June	0.09	0.07	0.06	20	0.63
10		0.12	0.08	0.01	7	0.58
11		0.00	0.00	0.00	2	0.40
12		0.04	0.04	0.03	1	0.33
13	July	0.03	0.03	0.01	2	1.00
14		0.00	0.00	0.00	-	-
15		0.08	0.08	0.08	-	-
16		0.09	0.09	0.09	-	-
17	August	0.08	0.08	0.08	-	-
18		0.06	0.06	0.06	-	-
19		0.08	0.08	0.08	-	-
20		0.08	0.08	0.08	-	-

[1] Week 1 began on 1st April.

[2] The probability that the weather would be too poor for daily food intake to match requirements (called "bad weather" above) was calculated from the model in Tables 10.1 to 10.3 and Fig. 10.1. This predicted that swallows required a mean temperature of a) 10.01°C if daily food intake alone were used for maintenance and egg formation and the bird foraged for $8.06hd^{-1}$, b) 9.74°C if daily food intake was supplemented by an equal part of the lipid reserve on each day of albumen formation (or if lipid was not stored during rapid follicular growth), c) 8.14°C if the bird used her whole lipid and oviduct reserve on 1 day and foraged for 15h. Critical temperatures were taken to be 2°C greater if there was more than 2mm of rainfall (determined arbitrarily after Turner 1982). Weather data were for the years 1989-1991.

[3] Clutch initiation data (n = 129 clutches) were combined for 1989, 1990 and 1991. The date of clutch initiation was 7 d before the first egg was laid (Chapter 3).

[4] The probability of initiation of egg formation during each week was the probability that a swallow which had not already initiated egg formation would do so during that week. All first clutches were laid by week 13.

laying her first clutch, as it would reduce the probability that she would be unable to continue to form eggs and almost eliminate it from mid-May onwards. A bird which made no use of lipid reserves would need to wait until July before laying in order to achieve the same level of stability in energy supply. A delay of laying until July would have meant that only one, rather than two, clutches could be laid each year.

This analysis supported the hypothesis and conclusions of Bryant (1975a) and Turner (1982), that aerial foragers delayed laying until the food supply reached a high level of abundance and stability. This could be termed the "probabilistic threshold response model" (PTRM). This was a development of Perrins' (1970) hypothesis that birds laid when there was sufficient food available to form eggs. Perrins' model applied to birds where food supply did not fluctuate, or did so only within a narrow range, as only the level, and not the stability of the food supply was considered. This was the "deterministic threshold response model" (DTRM). The PTRM model demonstrated that reserves were not important as a supplement to daily food intake under normal foraging conditions, but that they were of great importance as an insurance which allowed swallows to lay early in the season, when the probability of poor foraging conditions would otherwise have been high. Reserves were predicted to be of much greater importance as an insurance during laying of first than second clutches as the probability of daily food intake falling below the requirement was essentially zero during laying of second clutches (July).

The conclusions drawn from the PTRM model would also apply to all other insectivorous birds: egg formation would be constrained by total energy rather than lipid or protein intake, and use of reserves to supplement daily food intake during laying would not be necessary under normal feeding conditions. Birds which relied on a plant-based diet would be more likely to suffer a protein limitation to egg formation. Identification of a lipid or protein limitation to egg formation in other species would require modelling of the rate of energy, lipid and protein gain from different diets, in a way similar to that done above for insectivores, and comparison with the requirements of laying females.

10.1.5 Limitations of the probabilistic threshold response model

The model of energy and nutrient requirements for a laying swallow was based upon the mean foraging rate of a nestling-rearing swallow, determined by Turner (1982), and the mean activity budget and energy output of a laying bird (Chapter 7). Individual foraging performance and energy expenditure would be expected to differ from the mean values since energy expenditure shows much variability (Bryant and Westerterp 1982, Bryant and Tatner 1991), and this seems likely to be mirrored by variation in foraging efficiency. However, in the equivalent of a sensitivity analysis, substantial changes in input values for foraging time and daily energy requirement made only small differences to the minimum temperature at which swallows could obtain adequate food from foraging (Section 10.1.3). The conclusions thus appear robust to variation in energy loss or gain rates.

Several factors were not taken into account which might have an unknown, but presumed to be minor, effect on the model. The most serious of these was that no account was taken of a difference in foraging capacity between laying and nestling-rearing swallows, due to the greater mass and possibly decreased manoeuvrability of laying females, and the bill-full of food held by nestling-rearing birds. The relationship between temperature and foraging rate was assumed to be the same throughout the breeding season, although insect abundance varied with date independently of temperature (Chapter 2), so that foraging early in the season would be more difficult than inferred from temperature alone. The model did not consider the potential importance of reserve use during energy deficiencies within a day. Reserves might be used to maintain a constant rate of egg synthesis during short periods of reduction in energy intake related to nest building or mating. Under these circumstances use of the lipid reserve might have been important to maintain egg production, even if the overall daily energy budget would have balanced on a daily basis using food intake alone.

Courtship feeding might increase the daily food intake of female birds, and might be particularly important when foraging conditions were poor. Courtship feeding does not occur in the swallow (Cramp 1988, pers. obs.), so this additional source of food would not be available to laying birds in this species, although courtship feeding has been demonstrated to be important in other species (Nisbet 1977, Nilsson and Smith 1988, Lyon and Montgomerie 1985).

10.2 Is reproductive output determined by constraints during laying?

10.2.1 Strategies of nutrient acquisition for egg formation

Egg production represents an additional energy requirement of 13-70% DEE (King 1973), and an additional protein requirement of 86-232% above maintenance levels (Robbins 1981, 1983). All materials for egg formation must originally come from food intake, but there are three strategies by which a female bird might provide the nutrients for egg formation during the laying period. Nutrient reserves stored within the body could be mobilised, daily food intake could be increased, or activity costs could be reduced so that nutrients from normal levels of daily food intake or reserve use could be diverted to egg production.

Drent and Daan (1980) distinguished only two alternative models of the nutrient source for egg production. Birds could either use reserves stored in the body (the "capital" strategy) or egg could be synthesised directly from daily food intake (the "income" strategy). Reduction in activity costs to increase the energy available for egg formation does not count as a separate strategy in this context. Subsequent studies have demonstrated that strategies for egg formation are more complicated than this, so that a more realistic view would place species upon a continuum from the "income" to the "capital" models (Thomas 1989). Different tactics may also be used simultaneously by the same bird, if the sources of lipid and protein for egg formation are considered separately (Fig 10.3). Strategies of nutrient acquisition can also vary between yearling and adult females (Ankney and Alisauskas 1991), between birds laying their first and second or replacement clutches of the season

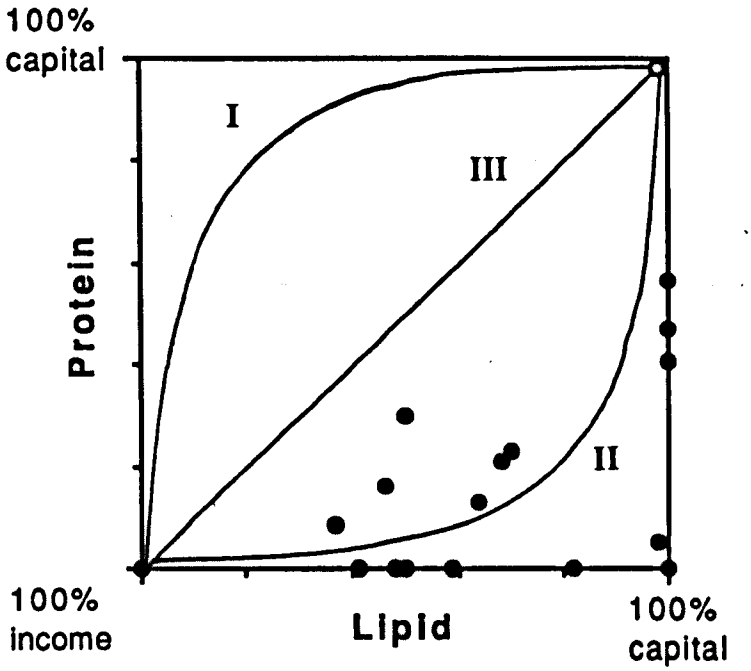


Fig. 10.3 Three possible patterns of reserve use by female birds during the laying period.
I - "protein reserve" strategy
II - "lipid reserve" strategy
III - "mixed lipid and protein reserve" strategy
Each solid point represents data for one or two species from Table 10.7, and the open point 3 species which fasted during the laying period. Only species listed in Table 10.7 for which the percentage of both reserve lipid and protein were calculated are included (n=23). If birds followed the "income" strategy of Drent and Daan (1980) they would be grouped in the bottom left hand corner. Those which followed the "capital" strategy would be the top right hand corner. The data fit model II, the lipid reserve strategy.

(Krapu 1981, Rohwer 1986 in Ankney *et al* 1991, Barzen and Serie 1990, Chapter 8), and between breeding areas (Mainguy and Thomas 1985).

Variation in strategies of nutrient acquisition for egg formation within and between species, have led to a number of conflicting theories regarding the relative importance of daily food intake and body reserves as sources of different nutrients for egg formation (Section 10.2.2). Almost every study has placed the species in question at a different position in resource acquisition framework (Fig. 10.3), so generalisations regarding the source of nutrients for egg synthesis have been difficult to make, as exceptions have been found to most proposals.

Three possible patterns of nutrient reserve use by laying birds are proposed: the "lipid reserve", "protein reserve" and "mixed lipid and protein reserve" strategies (Fig. 10.3). According to the protein reserve strategy, a high proportion of the protein, but low proportion of the lipid, for egg formation would originate from reserves. The lipid reserve strategy would require that a high proportion of the lipid, and a low proportion of the protein, for egg formation would come from reserves. According to the mixed reserve strategy, laying birds would draw an equal proportion of the lipid and protein for egg formation from reserves.

Data on the proportion of egg lipid and protein which could come from reserves for the selection of birds in Table 10.7 were used to test which of these strategies was followed by laying birds: laying birds clearly followed the lipid reserve strategy (Fig. 10.3). The significance of use of a lipid reserve by laying females will be discussed in Section 10.2.2.

10.2.2 Relative importance of reserves, daily food intake and reduction in activity costs in provision of nutrients for egg formation

For most species, daily food intake makes a much greater contribution to the nutrient requirement for egg formation than do reserves (Table 10.7). If studies in which females fasted during laying were excluded (as this was an unusual situation) reserves accounted for 64.3%, sd=31.8 (n=21) of the lipid requirement and 12.9%,

Table 10.7 The periods of use and storage of reserves, the percentage contribution of lipid and protein from reserves to egg formation (% egg), the mass and diet of each species for which the contribution of reserves to egg formation could be calculated. The percentage contribution of reserves was calculated on the basis of the mass of lipid or protein lost from reserves and the proportion of the clutch formed over the period when reserves declined. Lipid and protein requirements for oviducal growth were not included, as the necessary data was presented in too few studies. A conversion efficiency of 82% was assumed for transfer of lipid or protein from reserves to eggs (Chwalibog 1982). Where egg composition was not given in original papers, this was taken from Sotherland & Rahn 1987 to allow calculation of the percentage contribution from reserves to the lipid or protein required for egg formation. Mass (g) was mean female mass outside the laying period. - = could not be determined from the data available. (No) = No storage/usage of lipid according to original paper, although there was a non-significant trend for build-up or use of reserve. Min. = minimal. RFG = period of rapid follicular growth.

Table 10.7 continued

Species	Mass (g)	Timing of lipid storage	Timing of lipid use	% egg	Timing of protein storage	Timing of protein use	% egg	Diet	Reference
Ruddy duck *Oxyura jamaicensis*	546	3-4 weeks before laying	RFG & albumen formation	37	-	RFG & albumen formation	8	Animal	Tome 1984
Lesser snow goose *Chen caerulescens caerulescens*	1900	Before arrival at breeding area	RFG & albumen formation	100	Before arrival at breeding area	RFG & albumen formation	100	Fasting	Ankney & MacInnes 1978
Canada goose *Branta canadensis maxima*	3558	-	RFG	100	-	RFG	47	Plant	Mainguy & Thomas 1985
Canada goose *Branta canadensis minima*	1287	Final 2 weeks before migration to breeding grounds	RFG & albumen formation	100	Final 2 weeks before migration to breeding grounds	RFG & albumen formation	41	Plant	Raveling 1979
Brant *Branta bernicla*	1202	Before arrival at breeding area	RFG & albumen formation	100	Before arrival at breeding area	RFG & albumen formation	57	Plant	Ankney 1984
Wood duck *Aix sponsa*	618	Just before laying (40%) RFG (60%)	Albumen formation	82	No	No	0	Plant & animal	Drobney 1980, 1982 Drobney & Fredrickson 1985

Table 10.7 continued

Species	Mass (g)	Timing of lipid storage	Timing of lipid use	% egg	Timing of protein storage	Timing of protein use	% egg	Diet	Reference
Ring-necked duck *Aythya collaris*	588	Before & after arrival at breeding area	RFG & albumen formation	39	End of albumen formation	Start of albumen formation	-	Animal & plant	Alisauskas *et al* 1990
Lesser scaup *Aythya affinis*	576	Before arrival at breeding area	RFG & albumen formation	41	RFG	No	0	Animal	Afton & Ankney 1991
Eider *Somateria mollissima bolrealis*	2025	4-6 weeks before laying area	Albumen formation	100	4-6 weeks before laying area	Albumen formation	100	Fasting	Parker & Holm 1990
American eider *Somateria mollissima dresseri*	1800	Before RFG	RFG & albumen formation	100	Before RFG	RFG & albumen formation	100	Fasting	Korschgen 1977
White-bellied swiftlet *Collocalia esculenta*	8.5	-	RFG & albumen formation	100	No	No	0	Insect	Hails & Turner 1985
American coot *Fulica americana*	-	Before arrival at breeding area	RFG & albumen formation	70	Before arrival at breeding area	RFG & albumen formation	23	Plant & animal	Alisauskas & Ankney 1985
Herring gull *Larus argentatus*	900	3-5 weeks before laying median & start of laying	Not known if RFG or albumen formation	-	No	No	0	Plant & animal	Hario *et al* 1991

Table 10.7 continued

Species	Mass (g)	Timing of lipid storage	Timing of lipid use	% egg	Timing of protein storage	Timing of protein use	% egg	Diet	Reference
Lesser black-backed gull *Larus fuscus*	-	No	(No)	-	Shortly before laying	(? RFG) albumen formation	-	Animal & plant	Houston *et al* 1983
Common crow *Corvus brachyrhynchos*	-	RFG	Albumen formation	-	RFG	Albumen formation	-	Plant & animal	Young 1989
Pied flycatcher *Ficedula hypoleuca*	12.9	-	(RFG?) & albumen formation	98	-	RFG	5	Insect	Ojanen 1983
Starling *Sturnus vulgaris*	76.3	No	No	0	No	No	0	Animal & plant	Ricklefs & Hussell 1984
Sand martin *Riparia riparia*	13.2	-	Albumen formation	-	-	Albumen formation	-	Insect	Jones 1987c
Swallow *Hirundo rustica*	19.5	RFG	Albumen formation	48	No	No	0	Insect	This study
Grey-backed camaroptera *Camaroptera brevicaudata*	-	RFG	Albumen formation	46	-	RFG	16	Insect	Fogden & Fogden 1979
House sparrow *Passer domesticus*	27.2	RFG	Albumen formation (when clutch=6)	-	-	Albumen formation	-	Plant & animal	Pinowska 1976 (in Schifferli 1980 & Ojanen 1983)

Table 10.7 continued

Species	Mass (g)	Timing of lipid storage	Timing of lipid use	% egg	Timing of protein storage	Timing of protein use	% egg	Diet	Reference
House sparrow *Passer domesticus*	29.4	Immediately before RFG	RFG & albumen formation	98	-	-	-	Plant & animal	Schifferli 1980
House sparrow *Passer domesticus*	28.3	RFG	Albumen formation	50	No	No	0	Plant & animal	Krementz & Ankney 1988
House sparrow *Passer domesticus*	28.3	-	-	-	-	RFG & albumen formation	10	Plant & animal	Jones 1990
Red-billed quelea *Quelea quelea*	-	RFG	Albumen formation	50	-	RFG & albumen formation	30	Seed & insect	Jones & Ward 1976
Brown-headed cowbird *Molothrus ater*	40	No	No	0	No	No	0	Plant (Insect during laying)	Ankney & Scott 1980

sd=17.5 of the protein requirement for egg synthesis (data in Table 10.7). Eggs were formed primarily from daily food intake, but this did not mean that the nutrients from reserves should be considered unimportant, as the level of reserves available to supplement food intake might determine egg quality or clutch size, and hence affect reproductive output. The relationship between reserve size at the start of laying, and reproductive output will be considered further in Section 10.2.4. A second conclusion from Table 10.7 was that lipid reserves were stored and used more frequently (19 of 21 species) as well as more extensively than those of protein. Ankney *et al* (1991) suggested that only use of a particular nutrient from reserves could indicate that this nutrient would otherwise normally limit egg production. Insectivorous birds were therefore predicted to store lipid, as egg production was proposed to be lipid limited and herbivorous species should store protein, because their normal diet was relatively lower in protein than were eggs (Afton and Ankney 1991, Ankney and Alisauskas 1991, Ankney *et al* 1991).

The majority of species studied to date used lipid reserves during laying (Table 10.7). Those few which did not were either unusual cases (renesting mallard, and the cowbird, a nest parasite), or use of a lipid reserve was neither demonstrated nor excluded due to small sample sizes (lesser black-backed gull, Houston *et al* 1983, and crow, Young 1989). This did not necessarily imply a widespread shortage of lipid in the diet of laying females as there were a number of advantages to storage of lipid rather than protein. Lipid has a greater energy content and lower density than protein and a protein reserve would also have a maintenance cost, due to replacement of denatured amino acids (Waterlow *et al* 1978). Use of lipid reserves during laying might therefore indicate a shortfall in dietary lipid, protein or energy intake.

Use of a lipid store during laying might indicate a shortage of lipid in the diet, as has been proposed for the white-bellied swiftlet, another aerial insectivore (Hails and Turner 1985). The proposal that energy rather than lipid was the limiting requirement for laying swallows (Section 10.1) suggests that the same would apply to the white-bellied swiftlet.

Lipid reserves could be used to supplement the daily energy intake of those herbivorous species, which increased the invertebrate content of their diet to obtain protein necessary for egg formation (Jones and Ward 1976, Drobney 1980, 1991, Krapu 1981, Drobney and Fredrickson 1985, Krementz and Ankney 1988). In this situation, the lipid reserve would be used due to a shortage of protein in the normal diet, and a reduction in the rate of energy gain when feeding on invertebrates, so that daily energy intake was lower while on the modified diet. Thus herbivorous species might store lipid rather than protein even though egg production was limited by shortage of protein in the normal diet.

Only those birds which required protein reserves for egg formation would be predicted to store protein, as storage of reserve protein would be more costly than storage of lipid (see above). However, reduction in protein content during laying, without a clearly defined period of storage, could be due to a reduction in the rate of replacement of denatured proteins because of diversion of the supply of amino acids to the oviduct, rather than because stored proteins were used directly for egg formation. This type of reduction in self-maintenance has been demonstrated in quelea, which become anaemic during laying (Jones 1983). Storage and use of a lipid reserve would therefore be more likely if a female was likely to experience an overall energy deficit during laying, or stored energy as an insurance against poor feeding conditions, rather than due to a requirement for egg production. This will be discussed in more detail in Section 10.2.3.

The potential constraints of lipid, protein and total energy supply would occur at the same time for a laying female. None of these could normally be isolated as the limiting nutrient since an increase in supply of any of these components would allow an increase in egg production. The only exception to this would result from a shortage of certain amino acids which could not be formed from other substances, but protein for egg formation would be available in abundance from an invertebrate diet (Section 10.1). An absolute shortage of this component would be very unlikely if enough energy was available, even for a normally herbivorous species. Arguments about whether lipid or protein was the limiting nutrient in egg production (Drobney

and Fredrickson 1985, Afton and Ankney 1991, Ankney and Afton 1988, Ankney *et al* 1991, Ankney and Alisauskas 1991, Drobney 1991) would therefore not be relevant, due to an overall energy limitation, which could be relieved if either more lipid or more protein were available.

Large birds have greater potential for nutrient storage than smaller species (Calder 1984). It might therefore be expected that the reliance upon reserves during egg formation would increase with mass, if egg production was under energetic constraint due to a limitation in energy intake. A high correlation between mass and the proportion of the energy content of the clutch derived from reserves would then imply a widespread requirement for reserves to supplement daily food intake during egg production. A low correlation between mass and reserve use would suggest that egg production was not under energetic constraint. Use of energy as the currency in this comparison was justified as energy rather than lipid or protein availability will always limit egg production in birds. A Spearman correlation between female mass and the (arcsine transformed) proportion of the energy content of the clutch produced a non-significant relationship ($r=0.45$, $p=0.9$, $n=12$). This suggested that while larger birds showed a slight tendency to use more energy from reserves during egg formation, mass was not the only factor which determined the extent of reserve storage and use.

Less attention has been given in the literature to the importance of energy savings from a reduction in activity level, which might allow laying birds to divert energy to egg production. Reduced levels of activity, and particularly of flight, during laying have been noted in several studies (e.g., Korschegen 1977, Ankney and MacInnes 1978, Masman *et al* 1988, Mulder and Swaan 1992). Both house martins and swallows reduced the time spent in flight and the proportion of time in flapping flight (Bryant and Westerterp 1980, Turner 1980). Energy savings from reduction in activity might play an important part in diversion of energy to egg production, particularly as activity costs would be greater for females carrying the extra mass of reproductive material and reserves.

10.2.3 Are nutrient reserves necessary for egg formation or are they an insurance against a temporary drop in daily food intake?

For species with constant access to a predictable food supply which was adequate for the peak demand for egg formation, clutch size could not be constrained by food supply during laying as the peak daily energy and nutrient requirement for egg formation would only increase slightly, if at all, with clutch size (King 1973, Bryant 1975a, Chapter 3). In these species, clutch size could only be limited by the extent of nutrient release from reserves, if there was an energetic limitation on clutch size during laying. However, it might be that few free-living species could rely on such a food supply. While aerial insectivores such as swallows have a food supply which fluctuates unpredictably with weather conditions, laying females of most other species might have unpredictable access to food due to other factors such as social interference (Hails and Turner 1985) or predators. The pattern of build-up and use of reserves by swallows was compatible with the hypothesis that lipid reserves functioned primarily as an insurance against a temporary reduction in daily food intake during laying.

A build up of reserves before or during the period of rapid follicular growth, followed by partial or complete use of these reserves during the laying period could be necessary for four reasons. Extra abdominal lipid might be needed for physical support of reproductive structures; energy or nutrient availability during laying might always otherwise limit egg production; energy or nutrient availability could otherwise sometimes limit egg production; or possession of reserves may be necessary prior to laying because they were needed during incubation. Abdominal lipid was unlikely to be necessary for physical support of reproductive structures, as some birds laid without possession of a large lipid reserve (Section 10.2.1). The other three reasons for the possession of reserves by laying birds need more careful consideration.

Reserve use during laying might be necessary for egg production if daily food intake was inadequate to meet the energy necessary for maintenance and egg

production, if the diet lacked the lipid or protein required for egg synthesis, or if it was not possible to assimilate sufficient food on a single day to supply the requirements for maintenance and egg synthesis. None of these was applicable to the swallow (Sections 10.1, 10.2 and Chapter 8).

A few swallows laid eggs without accumulation of a substantial lipid reserve (Chapter 8), however, this was a risky strategy for a swallow, as the breeding attempt was not buffered against a temporary food shortage. The benefits gained by the majority of females which did build up and use lipid reserves during laying can be assessed by examination of the adverse consequences for some birds which laid without a nutrient reserve. Females caught during an interruption in laying were light and had small fat reserves. If they had continued to lay they would have become even lighter, and might not have survived (Bryant 1979). Swallows which reach an exceptionally low mass have a lower probability of survival than well nourished individuals (Thompson 1992). One female swallow (Chapter 8) which experienced a period of poor weather during the final few days of the period of rapid follicular growth, did not increase in mass prior to laying her first egg. This bird had a laying interruption and a small clutch size (Chapter 8). Clutch size was also reduced in some swallows if there was poor weather on day -1, when the peak energy demand for egg production would occur (Chapter 4). Possession of reserves would mean that laying swallows would be less likely to encounter an energy deficit on this day. The costs associated with a build up of reserves by a laying swallow were presumably worthwhile, in order to avoid the problems encountered by this bird. The consequences of a short laying interruption would not seem to be either serious or long term, although delay in fledging of the first clutch would reduce the probability that a second clutch would be laid (Bryant 1979). Food shortage during laying might also reduce reproductive output by a reduction in clutch size. The apparently quite small benefits to be gained from the lipid reserve of laying swallows might therefore be expected to be associated with an equivalently small cost to the bird for lipid reserve accumulation.

Possession of a nutrient reserve would be costly because the energy

requirement for flight increases with mass, the risk of predation increases if flight was slower or the bird less manoeuvrable, or the foraging success of aerial foragers decreases for heavier, less agile birds. Laying swallows would be likely to have increased flight costs and decreased manoeuvrability due to the mass of eggs and oviduct, but they avoided an additional cost of carrying a lipid reserve by a reduction in the water content and in water index (Chapter 8).

Carrying the extra mass of a nutrient reserve is potentially costly, so it would be expected that birds should adjust lipid reserves to the minimum necessary size. Incubating swallows with larger clutches had greater masses (Chapter 9), possibly so that they would have an equivalent level of insurance against a spell of poor weather, as incubating larger clutches involved a greater energy requirement. Alternatively, these might have been "better quality" individuals, and this might be demonstrated both by their larger clutches and their ability to build up a greater lipid reserve. Female swallows lost mass when the chicks hatched, which provided energy, although with the main purpose of allowing a reduction in flight costs at a time when the female became much more active (Freed, 1981, Norberg 1981, Jones 1987a). A similar reasoning has been used to explain the storage of reserves by female sparrowhawks prior to laying. These reserves could be used by the female for maintenance during the nestling-rearing period, when all the food provided by the male could be fed to the chicks if necessary (Newton *et al* 1983). During the nestling rearing period, hirundines build up a lipid reserve in their chicks rather than in the female, so that the chicks could survive temporary food shortages (Bryant and Gardiner 1979). Thus the lipid reserve of breeding swallows was always regulated, although it also fluctuated according to environmental conditions (Jones 1987b, Chapters 8 and 9), and either the female or the nestlings carried a lipid reserve so that the breeding attempt was normally buffered against a temporary food shortage (Bryant and Gardiner 1979). This suggested that female swallows should also be capable of regulating the size of their lipid reserve during laying.

The lipid reserve was built up in the final days of rapid follicular growth, and used during the albumen formation period, because this allowed swallows to gain the

maximum benefit from the reserve as an insurance against poor feeding conditions during laying, without increasing flight costs over a long period. Possession of a substantial lipid reserve would also have been most valuable on day -1, as this was the single day when poor environmental conditions were most likely to reduce clutch size (Chapter 4). This pattern would not be consistent with use of lipid reserves because of shortage of lipid or energy in the diet, as reserves were not used during the period of peak demand for egg and oviduct formation and the maximum daily lipid and energy requirement would be increased (Chapter 8), except in species in which the albumen contained more protein than in swallows. Use of lipid reserves only during the period of albumen formation would not seem necessary to supplement daily energy intake, nor whilst herbivorous birds foraged for protein rich foods, as the requirement for dietary protein would only be slightly higher during the period of albumen formation than during that of rapid follicular growth (Chapter 8). The lipid reserve was more likely to decrease in size during the period of albumen formation as the potential demand upon reserves for subsequent egg formation declined as each egg was laid.

The hypothesis that lipid reserves served primarily as an insurance against poor foraging conditions during laying is most applicable to birds with an unpredictable food supply, such as aerial foragers or seabirds, although females from many species might have unpredictable access to food during laying due to social interference and other factors. An analogous idea, the "migrational uncertainty hypothesis", has been proposed for waterfowl in North America (Rohwer 1986 in Ankney and Alisauskas 1991): lipid reserves might be carried to the breeding grounds as an insurance against poor weather upon arrival. As females bred shortly after arrival, the excess lipid was used for egg formation. Ankney and Alisauskas (1991), however, pointed out that this hypothesis could not account for the pattern of nutrient storage by all species of waterfowl, as other species stored lipid after arrival at their breeding grounds (Alisauskas *et al* 1990, Barzen and Serie 1990, Afton and Ankney 1991, Ankney and Alisauskas 1991).

In summary, use of a lipid store during laying might serve a different

purpose for different groups of birds. Species with an unpredictable food supply, or unpredictable access to food, might use lipid reserves as an insurance, to allow continuation of egg production during short periods when they were unable to forage adequately. Herbivorous species could use a lipid reserve as an energy store which would allow foraging for protein rich foods. The few species which did not forage during laying, would need a lipid reserve for both egg formation and energy requirements. Birds which began incubation after the first egg was laid might also need to use reserves to help to complete egg formation. Use of a protein reserve could indicate that protein, or possibly a particular amino acid, was deficient in the diet. The extent of protein storage was always minor, and usually only occurred in herbivorous species (Table 10.7). Use of a protein reserve by species with a protein-rich diet could not be explained in terms of an energetic or nutrient constraint.

In some species, it has been demonstrated that birds could form eggs without use of a previously accumulated lipid reserve, but this was a risk prone strategy, as the breeding attempt was not buffered against a temporary food shortage. Presumably laying interruptions were disadvantageous for all species, and use of a lipid reserve during laying would help to prevent this, for the only species in Table 10.7 which made no use of reserves was a nest parasite (brown-headed cowbird). As this bird did not need to lay eggs as a clutch, a laying interruption would be unimportant, and so reserve use was not necessary in order to guard against this possibility or guard against clutch size reduction.

10.2.4 Does reserve size limit clutch size, or clutch size determine reserve size?

Relationships between clutch size and reserve levels have been found for a number of species (Jones and Ward 1976, Ankney and MacInnes 1978, Houston *et al* 1983, Newton *et al* 1983, Ankney and Afton 1988). This supported the idea that nutrient reserves were necessary for egg formation in these species. Yet this does not necessarily demonstrate that nutrient reserves limited clutch size, as it

might equally be argued that if clutch size was determined by another factor, females could accumulate reserves appropriate for the clutch size to be laid. This would seem plausible for swallows at least, given the evidence for regulation of body condition throughout the breeding season (Section 10.2.3). A third interpretation of a correlation between clutch size and food supply during laying would not involve an energetic constraint to egg production, for the level of nutrition during laying might be associated with the clutch or brood size which could be cared for later in the reproductive cycle (Drent and Daan 1980).

Arnold and Rohwer (1991) proposed three criteria by which to assess whether nutrient reserves limited clutch size:

1. Are stored nutrients used during egg formation?
2. Are stored nutrients required for egg formation?
3. Does the level of stored nutrients limit clutch size?

Swallows, along with many other birds, used stored nutrients during laying (Table 10.7). However, use of nutrients was not necessary for swallows which laid during normal environmental conditions (Sections 10.1 and 10.2), and was not always necessary for other species (Section 10.2.1). The question of whether use of stored lipid limited clutch size would be much harder to answer, and no study to date has been able to resolve this point. An energy or nutrient limitation to reproductive output was implied in studies where provision of supplementary food increased egg or clutch size (Chapter 4). The correlations between environmental factors (i.e., food supply) during laying and swallow clutch size also suggested that food intake might be important in determination of reproductive output, although the same result would emerge if swallows used the level of food abundance during laying as a cue to likely future conditions which would indicate the number of offspring for which they could care.

If clutch size was to be limited for the majority of birds by factors acting during the laying period, these must be non-energetic factors, such as those discussed in Section 10.2.5. Hence, clutch size must normally be determined by restraint, rather than constraint, mainly by factors acting later in the breeding cycle.

10.2.5 Other factors which might limit clutch size during laying

The reproductive value of the eggs already laid might decrease if the start of incubation was delayed (whilst more were laid) because of the risk of predation and because eggs lose viability as they sit unincubated (Arnold *et al* 1987). The combined effects of predation and egg mortality might set the upper limit to clutch size. The predation rate was low for both swallow and dipper clutches before and during incubation (pers. obs.), and swallows and dippers had much smaller clutch sizes than waterfowl, so that the eggs were not routinely exposed for long periods before the start of incubation. Furthermore, one swallow egg which was not incubated for 19 days after it was laid, hatched and fledged successfully (Chapter 4). The egg viability hypothesis was therefore thought unlikely to limit the clutch size of passerine birds. This hypothesis has also been dismissed for waterfowl, on the grounds that the duration of egg viability was more likely to evolve in relation to clutch size, than visa versa (Ankney *et al* 1991).

10.3 Is reproductive output determined by constraints during incubation?

Uniparental incubating females suffer a great reduction in the time available for foraging during the incubation period. This has led to the proposal that incubation might be an energetic bottleneck (Yom-Tov and Hilborn 1981), as the maximum possible rate of energy gain from foraging would only just provide the energy requirement of the female, or reserves would be required to supplement food intake. Females of many species use reserves during the incubation period (e.g., Ankney and MacInnes 1978, Ankney 1984, Ricklefs and Hussell 1984, Tome 1984, Mainguy and Thomas 1985, Barzen and Serie 1990, Parker and Holm 1990), and successful completion of incubation might depend upon the size of the reserve at the end of laying (Ankney and Alisauskas 1991). Extensive use of reserves during incubation is only common amongst larger birds, and many small passerines maintained, or even increased in mass during incubation (Askenmo 1982, Moreno

1989a, Chapter 2), although some such as the dipper (Chapter 9) decreased slightly in mass during incubation. Incubating females have been found to desert their nests due to lack of a sufficient reserve or food intake (Ankney and MacInnes 1978, Chapter 4), and females in general might be close to energy imbalance.

Increases in clutch size have been demonstrated to increase the energy expenditure of the incubating bird in a number of studies (Mertens 1977, Biebach 1984, Haftorn and Reinertsen 1985, Weathers 1985, Biebach 1986, Moreno *et al* 1991, Chapter 9). Other adverse consequences of an increased clutch size include a reduction in hatching success (Moreno *et al* 1991), an increase in hatching asynchrony (Moreno and Carlson 1989) and a longer incubation period (Smith 1989). Clutch size manipulations could clearly alter the cost of incubation and influence the success of the breeding attempt. Several studies showed no change in the cost of incubation over the normal range in clutch size, but an abrupt increase in costs when an extra egg was added (Biebach 1986, Moreno *et al* 1991, Chapter 9), presumably because the female was unable to cover the entire clutch at once and had to continuously rewarm the egg which had just been exposed. This would also account for the longer incubation period of larger clutches, as eggs which were incubated at lower temperatures took longer to hatch (White and Kinney 1974). Energetic constraints could therefore explain the upper limit to clutch size for each species, but would be unlikely to explain variation in clutch size between individuals because body size varies much less than clutch volume.

10.4 The energetics approach to investigation of constraints on reproductive output

A problem with the energetics approach to investigation of possible constraints upon reproductive output is that although energy expenditure and food intake can be quantified, potential foraging rate cannot. It is the difference between the actual and potential rates of energy gain from foraging, rather than the absolute level of either, which is of interest in addressing the question of whether birds operate under energetic constraint. The measures which are available are therefore

only presumed correlates of this difference between actual and potential foraging rate. In order to show that reproductive output was under energetic constraint, it would be necessary to demonstrate that provisioning more offspring would exceed birds' maximum potential foraging capacity.

It has been demonstrated that larger clutches or broods, or greater nestling feeding rates require a greater parental energy expenditure (reviewed by Bryant and Tatner 1990, Bryant and Tatner 1991 for nestling-rearing birds, and in Chapter 9 for incubating birds) and this has led to the proposal that parental energy expenditure might be used to compare the costs of raising different numbers of offspring. However, increased energy expenditure might be compensated by a greater food intake or more favourable conditions, so that birds with a higher energy expenditure would not necessarily be closer to energy imbalance (Bryant 1988a). In this case a higher energy expenditure could not easily be interpreted as a greater parental investment. A greater energy expenditure might affect parental survival if birds which spent a longer period foraging were at greater risk from predators, diseases or accidents, but this would be difficult to quantify (Bryant 1988a).

If it could be demonstrated that a higher energy expenditure did indeed bring a bird closer to energy imbalance, this would suggest that a reproductive cost might be incurred by the parent, as there would be a greater probability of starvation, or a lower rate of tissue repair which could increase the susceptibility to disease or lower the efficiency of tissue function. This would require measurement of actual and potential food intake, rather than energy expenditure alone, although measurements of energy expenditure could be used in the calculation of actual food intake rate (Equation 10.1). Techniques available at present cannot measure changes in body composition of living birds sufficiently accurately for food intake to be calculated from Equation 10.1, but this difficulty might be resolved by future technical advances. An alternative approach would be to determine food intake directly from the turnover rate of ^{22}Na. This method has been used concurrently with the DLW technique to determine the food intake and energy expenditure of penguins (Gales 1989, Green *et al* 1989).

Even if difficulties in quantification of changes in body energy content for living birds were overcome so that food intake could be calculated from Equation 10.1, or if food intake were measured directly using the turnover rate of ^{22}Na, or some other technique, the problem of assessment of the potential rate of energy gain would remain. Energy imbalance would only be imposed upon a bird if the required foraging rate was greater than the potential rate of energy gain. Any mass loss would indicate that a bird had not maintained energy balance, but unless an assessment of the potential foraging rate was possible, it would could not be determined whether mass loss should be interpreted as an inability to gather enough food (a reproductive cost), or an adaptive measure which reduced flight costs (Freed 1981, Norberg 1981, Jones 1987a) or brought other benefits (Lima 1986).

Even without knowledge of potential energy intake, mass losses and decreases in body condition indices can be given a tentative interpretation. Female mass loss at the start of the nestling rearing period would be likely to be due to a reallocation of the nutrient reserve store from the female to the chicks, whilst mass loss later in the nestling rearing period could be interpreted more confidently as an energy imbalance imposed by the demands of reproduction (Jones 1987a, Bryant 1988a). One study has demonstrated the link between mass loss during breeding and reduced fitness: female blue tits which lost most mass were least likely to survive until the following year (Nur 1984).

A complete picture of the timing and extent of any energetic constraint upon reproduction would require determination of the potential as well as the actual rate of energy gain. This could theoretically be done using a more sophisticated version of the model in Section 10.1, but determination of potential foraging rate for each individual in every set of environmental conditions, and for all stages in the breeding cycle, would be an formidable task. Determination of an average maximum foraging capacity might be achieved by enlargement of brood sizes or by placing a new group of hungry chicks in the nest each time the parents satisfied the demands of the brood (manipulation of energy output), or by altering the food supply and hence the potential rate of energy gain whilst foraging, to stimulate birds to forage at

the maximum rate. Even if this were to be achieved, it would still not be possible to determine how close to their maximum capacity the manipulated birds had worked. Instead, only the difference between energy gain rates under different circumstances could be assessed, so that it would be possible to state at which stage in the reproductive cycle birds came closest to their maximum potential foraging capacity, and hence closest to energetic constraint of offspring number. It might well be found that no stage in the reproductive cycle was more demanding than another, in terms of how close actual energy gain rate approached the maximum potential, for the demands of each stages can be partially offset by increased parental energy input at other times (e.g., egg size and composition are inversely related to the requirement for brooding and feeding of nestlings). Birds would achieve the greatest fitness by as wide a distribution of the energy demands of reproduction as possible, rather than operating well below their maximum capacity for some of the time only to have reproductive output limited by an energetic bottleneck at another stage.

Although the energetics approach cannot satisfactorily answer the question of how close birds would get to energy imbalance at different levels of reproductive output, this approach could determine the relative importance of particular factors in the energy or nutrient budget. For example, clutch reheating costs were suggested to lead to a high energy expenditure for single-sex incubating birds (Westerterp and Bryant 1984). This suggestion was made on the basis of a small number of energy expenditure measurements in the swallow. The increased sample of birds included in this study showed that the original results represented extremes of the variation for this species, and that the mean metabolic rate during incubation was similar to that of nestling-rearing birds (Chapter 9). A model of the energy budget of an incubating swallow was also used to demonstrate that reheating costs, and changes in these with clutch size, were only a minor part of the energy requirement of an incubating female and therefore unlikely to affect energy balance or reproductive output during incubation.

The energetics approach also allows assessment of "sources" and "sinks" in the energy and nutrient budget of laying birds. Loss of oviduct mass during the

period of albumen formation led to the proposal that starlings might use the oviduct as a short term nutrient store (Ricklefs and Hussell 1984). Consideration of the potential for lipid and protein release by the swallow oviduct demonstrated that it could not provide an important daily contribution to the energy or nutrient requirement of a laying bird, but that it might serve as a source of nutrients during a temporary food shortage (Chapter 8).

The energy output of laying swallows was only slightly greater than that of incubating or nestling-rearing birds (Chapter 7). This suggested that an energy limitation to egg production in swallows was unlikely, as laying occurred only a short time before they were fully able to provide the energy for incubation or nestling rearing. A similar conclusion was reached for the white-bellied swiftlet (Hails and Turner 1985). These conclusions were confirmed by the model developed in Section 10.1, which showed that there would be no energetic or nutrient limitation to swallow egg production under normal foraging conditions, that the supply of energy rather than lipid or protein would limit egg production during periods of food shortage, and that these conclusions would also apply to all other birds which were able to continue to eat during egg production. In particular, the importance of protein as a limitation on egg production has often been overemphasised (e.g., Robbins 1981, 1983, Blem 1990).

Lipid reserves would not be required for egg production by swallows, but might be of use as an insurance against temporary food shortages. Herbivorous birds which switched to an insect diet during egg formation would also be able to supply the protein and lipid requirement for egg production from food intake, but use of a lipid reserves might be necessary to supply some of the energy required for maintenance and activity.

This study demonstrated that total energy output would be the factor which limited egg production, if egg production were under energetic or nutrient constraint. Egg production was apparently not constrained during normal foraging conditions, but a reduction in food supply could lead to a drop in reproductive output. There might be an energetic constraint to offspring number during incubation or nestling-rearing, but this cannot be determined from the energetics approach at its present level of development.

SUMMARY AND CONCLUSIONS

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1. Many authors have suggested that clutch size may be limited by the ability of females to lay eggs, or that incubation may be an energetic bottleneck which limits clutch size. These claims have been difficult either to substantiate or refute, as the relative living costs for birds at different stages of the reproductive cycle were unknown, as the energetics of laying and incubation by birds had received little attention compared with that of nestling-rearers.

This study uses the doubly labelled water (DLW) technique to make the first measurement of the energy expenditure of a free-living laying bird, the swallow, and compares this with the energy expenditure of incubating and nestling-rearing swallows. The affects of clutch size upon incubation costs were investigated for the dipper.

The nutrient dynamics of laying swallows were investigated by placing balances under swallow nests, to record the mass changes of individual females during the laying cycle. Body condition indices, validated by carcass analysis, were used to assess changes in the size of nutrient reserves during laying. The relative importance of nutrient reserves and food supply during laying in the determination of egg and clutch size was examined.

2. Variation in the body size of incubating female swallows was best explained by a regression model which incorporated keel and head+bill lengths.

3. Larger swallow eggs contained a significantly greater proportion of albumen (wet mass) and a lower proportion of yolk, lipid and water, although larger eggs contained absolutely more of all components. This was accompanied by a slight (and non-significant) increase in the lipid and lean dry components.

4. Swallow egg size and composition were related to environmental conditions during (but not before) the periods of rapid follicular growth and albumen formation.

Yolks formed more quickly when the temperature was higher, and had a greater lipid and lean dry content when food was more abundant and rainfall lower. The effects of environmental conditions on albumen content were less important. Eggs were heavier if they were formed during favourable environmental conditions. Larger (heavier) eggs contained proportionally less water, yolk and yolk lipid, and proportionally more albumen. This suggested that poor environmental conditions affected the total energy available for egg formation, rather than a specific limitation to albumen synthesis via a shortage of protein.

5. Lipophilic dyes were used to mark ovarian follicles undergoing rapid follicular growth at the time of the dye feed. Layers could be seen in the yolks if the eggs were hard boiled and sectioned. This allowed non-invasive determination of the number of days required for egg formation, and of the volume of egg deposited each day. Japanese quail took 7 days to form the yolk and a further day to deposit the albumen and shell of each egg. The egg was laid in the morning on the ninth day after the start of rapid follicular growth. Swallows required 6 days to form the yolk and 1 day to form the albumen and shell of each egg. Only a small volume of yolk was deposited on the first 2 days of rapid follicular growth by either quail or swallows. The bulk of follicular growth therefore occurred during the final 5 days before ovulation for quail, and the final 4 days for swallows.

6. Swallows deposited the peak energy content of egg material on the day before the first egg of a clutch was laid. The peak energy requirement varied little between clutch sizes of 4-6 eggs, but the duration of egg formation increased by one day for each egg laid. The entire energy content of an egg would need to be deposited every day by a continuously laying quail.

7. The diameter of the airspace at the blunt end of eggs increased, due to water loss, after eggs were laid. Airspace diameter could be used to determine the laying order of unincubated swallow and dipper eggs, and to predict the hatching date (±2 d) of partly incubated eggs.

8. Swallow eggs were hatched in an incubator, so that hatchlings could be matched with their eggs. Larger eggs hatched larger, heavier chicks which had more yolk. A review of the literature revealed a general relationship between egg size and chick size, growth rate and survival. This suggested that egg size was a measure of parental fitness, although other factors might also be involved.

9. There was no effect of egg size on the hatching success of swallow or dipper eggs. Neither of these species, nor birds in general, laid eggs which were too small to be viable.

10. Swallow egg size was not affected by year, clutch number, clutch size, laying order, nest site, female age or female body size. Most of the variation in egg size occurred between females, rather than between eggs laid by the same female. Egg size was highly repeatable for individual females.

There was a lower correlation between mean first clutch swallow egg size than between second clutch egg sizes (for swallows which laid in more than one year of the study). This was an indication that the lower and more variable level of food availability at the time of year when first clutches were laid had an effect on egg size.

The effect of environmental conditions upon egg size was confirmed by the correlation between egg size and environmental factors during different parts of the laying cycle. Larger eggs were laid when the temperature was higher during the period of albumen formation and on the days when the maximum energy content of egg was deposited. Environmental factors before and during rapid follicular growth did not have an important effect on egg size. This supported the hypothesis that eggs

were formed directly from food intake, and that egg synthesis was under energetic constraint. Temperature could affect resource availability for egg synthesis by a decrease in food intake (insect supply was highly correlated with temperature), or via increased thermoregulatory or foraging costs for the female.

11. Swallow clutch size declined through the breeding season, in common with many other passerines. Older swallows laid significantly earlier and so had larger clutches than first year birds. Older swallows were also more likely to rear two broods. Swallow egg size, female characteristics and territory were not related to clutch size, other than via a relationship between age and clutch size which could be accounted for by the effect of age and laying date.

Insect abundance and temperature increased during the breeding season, so the decline in clutch size through the season could not be explained by an energetic constraint upon egg production. Effects of resource availability upon clutch size were therefore more likely to be manifest during laying of first than second clutches. There were indeed more correlations between environmental factors and clutch size for first than second clutches. Larger first clutches were more likely to be laid when food supply and temperature were high on the day when rapid follicular growth would be initiated for the final egg of the clutch. Temperature on the day of maximum demand for egg production (the day before the first egg was laid) was most highly correlated with clutch size. This suggested that resource availability during egg production was important in the determination of clutch size and that clutch size was sometimes under energetic constraint. The correlation between clutch size and temperature on the days when laying females built up their lipid reserve (days -3 to -1) suggested that the size of the lipid reserve also had an effect on clutch size.

12. There were no significant correlations between dipper egg size and year, clutch size, laying order, nest site, female age or female body size. Dipper clutch size was not correlated with female characteristics, but birds with lowland territories laid a

fortnight earlier than pairs which nested on upland burns. This suggested that dippers laid when environmental conditions became favourable, and that this occurred later in the year at higher altitude.

13. Swallows stopped egg production during periods of bad weather, when foraging success would be low. The delay caused by this tactic would have an unimportant effect on reproductive output, when compared with the advantage of laying a larger clutch as opposed to terminating laying at the start of the period of bad weather, laying very small eggs, and not entering a potentially lethal state of energy imbalance.

14. The energy expenditure of fourteen captive-bred male Japanese quail was measured simultaneously by three techniques over a range of temperature (14-36°C). Energy expenditure decreased at higher temperatures. The best agreement between results of methods of energy expenditure measurement was obtained between the DLW and input/output techniques. The energy expenditure measured by chamber calorimetry was consistently lower than that measured by the other two techniques. Close agreement between techniques was not achieved for individual birds.

Possible reasons for discrepancies between techniques were discussed, but no single factor was considered responsible. Contributory factors might be: a reduction in accuracy of the DLW technique at low levels of isotope depletion, increasing inaccuracy of chamber calorimetry at low respiratory gas concentrations, an increase in evaporative water loss at high temperature, possible isotope re-entry, mass spectrometer imprecision, and inaccuracy in quantification of the energy content of food intake, excreta output or change in body composition. The main reason for underestimation of energy expenditure by chamber calorimetry, when compared with the DLW or input/output techniques, was probably that the measurement periods were not strictly comparable. Short periods of particularly high energy expenditure were excluded from the chamber calorimeter measurements, but would be included by the other two techniques.

All three techniques were valid methods by which to measure the energy expenditure of Japanese Quail.

15. The energy expenditure of 35 captive-bred female quail was measured by chamber calorimetry at 25°C. The birds displayed a range in egg production between zero and daily laying. This was achieved by monitoring the egg production of individually caged birds prior to calorimetry, so that birds which had, or had not, begun to lay could be selected. There was a natural range in the date at which quail started to lay (age 5-6 weeks).

The energy content of yolk deposited during the period of energy expenditure measurement was determined from the volume of yolk between rings from lipophilic dye feeds at the start and end of the measurement period. The efficiency of egg formation by quail was only 36%, about half the figure usually determined for domestic fowl.

16. The energy expenditure of 6 captive-bred laying female Japanese quail was measured simultaneously by the DLW technique and chamber calorimetry at 25°C. The results of the two techniques were in close agreement.

A new route of administering the labelling isotopes in birds - by intubation rather than intraperitoneal injection - was demonstrated to result in an equivalent enrichment of the labelling isotopes in the body water pool as achieved by intraperitoneal injection (the normal route of isotope administration). This may be a useful alternative method with small birds, where damage to developing ova is a risk.

There was no evidence for fractionation of labelling isotopes between body water and egg albumen or ovarian follicles. This suggested a novel route of sampling the body water pool of a laying bird. Albumen samples from freshly laid eggs could be used determine the rate of isotope depletion. The albumen samples from consecutive eggs allowed calculation of an energy expenditure only 3.2% greater than that by the DLW technique using blood samples.

Eggs are laid by birds at approximately 24 hour intervals, and measurements of energy expenditure are normally made over some multiple of this period. Use of albumen to sample the isotopic composition of the body water pool would avoid the need to take blood samples from a laying bird. This would be a great advantage for a free-living species as the bird could be released immediately after labelling, and need not be recaptured. The reduction in handling time, from around 1½ hours to 10-15 minutes, would mean that the DLW technique could be used with a number of species which might previously have been thought to be too prone to nest desertion if an attempt was made to measure energy expenditure during laying. If birds were not to be recaptured, a technique would be required by which they could be weighed remotely, as bird mass at the end, as well as at the start, of the measurement period, is important for calculation of energy expenditure by the DLW technique, and for interpretation of the results. This was achieved for swallows, by placing the nest on a balance, so that the female was weighed each time she landed on the nest (see below).

These developments should make laying the easiest, rather than the most difficult, stage in the annual cycle during which to measure the energy expenditure of a free-living bird using the DLW technique.

17. The energy expenditure of 13 laying female swallows was measured using the DLW technique. Mean DEE was 112.28 kJd^{-1} (ADMR=8.31 $cm^3CO_2g^{-1}h^{-1}$, M=3.85). DEE was slightly greater during laying than for incubating or nestling-rearing female swallows. The ADMR and M of laying birds were greater than those of incubating, but less than nestling-rearing females, due to the lower mass of nestling-rearing birds. The foraging rate of a nestling-rearing female would be much greater than that of laying bird if the requirements of half the brood as well as her own metabolism, were taken into account.

Birds which formed more egg material during the measurement period did not have a greater energy expenditure, but larger birds (keel length) had higher DEE whilst those with a higher initial fat score had a lower energy expenditure.

18. Swallows were tolerant of a number of variants in the procedure of energy expenditure measurement by the DLW technique (labelling orally, or by intraperitoneal injection; capture at dusk, dawn or in the afternoon; labelling over 24 or 48 hours; and whether or not blood samples were taken). The turnover rate of labelling isotopes was determined both from blood samples and from egg albumen: energy expenditure calculated from albumen samples was not significantly different from that calculated using blood samples from the same bird.

19. Energy expenditure was estimated from the activity budget of laying swallows during DLW measurement of energy expenditure. Mean DEE calculated by this method was 100.21 kJd^{-1}. The result was most sensitive to the assumed cost of flapping flight. The mean cost of egg production, calculated from the difference between the DLW and activity budget results, was 12.07 kJd^{-1}. This would be an efficiency of egg production of 33%, but this was not a reliable estimate, as calculation of energy expenditure from the activity budget would be prone to error.

20. An improved method was proposed for calculation of the efficiency of egg production, based on the partial efficiency of lipid (81%) and protein (55%) deposition by animals. This was more accurate than previous methods, which assumed a fixed cost to egg biosynthesis, as the efficiency of egg formation would vary between altricial and precocial birds and between egg yolk and albumen (66.7% for swallow whole egg but 67.9% for whole egg of domestic fowl; 57.9% for swallow albumen but 72.7% for swallow egg yolk). The efficiency of whole egg production was close to the median of studies where this was determined by chamber calorimetry or food balance (66.5%). The partial efficiency method of calculation of the efficiency of egg production produced a figure close to the 70% recommended by King (1973), but rather lower than the 75% proposed by Ricklefs (1974).

The mean energy requirement for egg formation was predicted to be 15.07 kJd^{-1} (50.6% BMR or 13.4% DEE) for swallows for which energy expenditure was

measured by the DLW technique (partial efficiency method). This was close to the difference (48% BMR) between the metabolic intensity of house martins which did not continue egg production during the period over which energy expenditure was measured (Bryant and Westerterp 1980) and swallows which did continue egg synthesis.

21. Swallow nests were moved onto electronic balances so that the mass of laying birds could be remotely logged. Changes in mass and body condition indices during laying were also assessed for swallows using data from females caught once per breeding attempt.

The mass of female swallows began to increase from day -5 (where the first egg was laid on day 0), rose to a peak of around 25 g on the night before the first egg was laid, and decreased toward the pre-laying level (20-21 g) as the eggs were laid. Mass fluctuated during each diurnal cycle: mass declined overnight, dropped during a short flight at dawn (presumably due to defecation), was reduced by 2 g when the egg was laid, then rose during foraging through the rest of the day.

22. The mass of egg formed each day during laying was determined from the position of dyed layers from lipophilic dye feeds in swallows egg yolks. Carcass analysis of laying and incubating swallows revealed that the increase in mass of a female swallow during laying could be accounted for entirely by the additional mass of eggs and oviduct carried by the birds at this time.

Examination of mass changes alone would have led to the erroneous conclusion that the non-reproductive portion of swallow remained at the incubation level throughout laying. Carcass analysis, and visual fat scoring of living swallows, revealed a build-up of around 1.45 g of lipid during the final 4 days of the period of rapid follicular growth. This was catabolized during the period of albumen formation. Body water content (in the non-reproductive part of the bird) decreased during rapid follicular growth and increased during albumen formation, so that overall non-reproductive mass remained roughly constant.

23. The potential of the oviduct for short-term nutrient storage was explored for the swallow. There was no evidence that routine catabolism of the oviduct occurred during the period of albumen formation, and this could have provided only a small contribution to albumen formation. Use of all nutrients which might be derived from the oviduct in a single day would have made an important contribution to the nutrient requirement for albumen synthesis. The oviduct was therefore likely to be a potential source of nutrients for albumen formation which could be used to continue egg production during a period of poor foraging conditions.

24. Lipid from reserves could provide swallows with all they required for egg synthesis and 5.9-9.4% of the energy required for activity, maintenance and egg production, if a bird which laid a clutch of 5 eggs used an equal portion of her lipid reserve on each day during the period of albumen formation. This would make an important contribution to the daily requirements during laying. However, build-up and catabolism of the lipid reserve was unlikely to be for this purpose, as lipid was stored during the 4 days before the first egg was laid, when the lipid demand for oviduct and egg formation was as high as when reserves were catabolized. Build-up of lipid reserves increased the total energy requirement for egg synthesis, as would be expected due to the costs of biosynthesis and carrying extra mass. The peak daily energy requirement for biosynthesis by a laying bird was also substantially increased by lipid reserve deposition. Build-up of reserves during the period of rapid follicular growth was therefore proposed to be as an insurance against a deterioration in feeding conditions during the albumen formation period.

One female swallow studied in detail did not increase in mass during rapid follicular growth due to a period of poor weather at this time. The weather deteriorated further on the day the first egg was ovulated, and this was followed by a laying interruption and a small clutch size. It was proposed that the lipid reserve which was built up by the majority of females meant that they could avoid the adverse consequences experienced by this individual, during brief periods of reduced food intake.

25. Mass and condition indices of female swallows at the start of laying did not differ significantly between birds which subsequently laid clutches of different sizes, although there were weak trends for heavier birds with higher fat scores to lay more eggs. Females were heavier and had higher fat scores at the start of laying if this was preceded by a period of high temperature and insect abundance, and low rainfall.

26. Mean DEE of 18 incubating female dippers was 224.38 kJd^{-1} (equivalent to an ADMR of 5.69 $cm^3CO_2g^{-1}h^{-1}$ or 3.54xBMR). Two of these birds incubated a manipulated clutch size of 6 eggs and they had a significantly greater energy expenditure than birds which incubated clutches of 3, 4 or 5. This was attributed to the increased cost of maintaining egg temperature during incubation sessions. If the two birds which incubated manipulated clutches of 6 eggs were excluded on the basis that although clutches of 6 eggs were within the natural range, they were very rare, mean DEE was reduced to 209.26 kJd^{-1} (ADMR=5.29, 3.30xBMR).

27. Mean DEE of 6 swallows which incubated their natural clutch size of 5 eggs was 105.40 kJd^{-1} (equivalent to an ADMR of 8.03 $cm^3CO_2g^{-1}h^{-1}$, or 3.67xM). The metabolic intensity of incubating swallows was significantly greater than that of dippers. This was attributed to the smaller size, more active foraging mode and open nest of the swallow, with some contribution made by systematic catabolism of reserves by incubating dippers which were also fed on the nest by the male.

28. Energy expenditure was calculated from the activity budget during DLW measurements of incubating swallows and dippers. The net energy requirement for incubation was calculated from the difference between DEE measured by the DLW technique and that accounted for by activity, thermoregulation and reheating the clutch after incubation recesses. Clutch reheating costs were small (2-6% DEE).

The net cost of incubation was 17.8% DEE for the dipper and 32.1% DEE for the swallow. The cost of incubation was similar for the dipper to that calculated

from a biophysical model (Kendeigh 63), but greater for the swallow. This difference might be a real one, due to the more favourable nest microclimate and insulative plumage of the dipper, or may be due to inaccuracy in estimation of DEE from the activity budget.

29. A model was developed which showed that a laying swallow could always provide the crude lipid and protein requirements for egg formation from daily food intake under foraging conditions which allowed her to maintain energy balance. If food intake fell below the level at which energy balance could be maintained from the normal foraging time of a laying swallow, she could catabolize lipid or oviduct reserves, increase the time spent foraging, or suspend egg production until food supply increased. Total energy intake, rather than a specific requirement for lipid or protein would constrain egg production under these circumstances. This conclusion would apply to all other insectivorous birds, and to those which generally ate plant material, but modified their diet to contain more insects during laying. Arguments as to whether egg production was constrained by the lipid or protein content of the diet would not be relevant, for if any dietary component was available in greater abundance, more resources would be available for egg production.

30. A review of the literature revealed that a non-random selection of birds derived only 12.9% of the protein for egg formation from daily food intake, whilst 64.3% of the lipid for egg formation derived from reserves. Lipid reserves could be used as a source of energy as well as for conversion to egg lipid. This might be particularly important for birds which modified their diet during laying so that it included adequate protein for egg formation. These birds might be less efficient when foraging for invertebrates and would need to rely on energy from reserves.

Birds with an unpredictable food supply, or with unpredictable access to food, might use a lipid reserve at the start of laying as an insurance against poor foraging conditions.

31. A problem with the energetics approach to investigation of possible constraints upon reproductive output is that although energy expenditure and food intake can be quantified, potential energy gain from foraging cannot. Living costs at different stages of the reproductive cycle, or of birds which raise different numbers of offspring can be compared, but at no time can the difference between actual and maximum potential rates of energy gain be determined. This presents a fundamental problem, as it is the magnitude of the difference between the actual and maximum potential rates of energy gain which would be required in order to assess directly whether there was an energetic constraint upon reproductive output. Determination of energy expenditure and requirements at different stages of the reproductive cycle can, however, provide insight into the relative importance of a given factor in the energy or nutrient budget.

The energetics approach to laying and incubation led to the conclusions that swallows formed eggs primarily from food intake, but used reserves as an insurance which would allow them to continue egg production during poor weather when foraging success would be reduced. Clutch size was reduced for some birds which experienced poor foraging conditions on the day of the maximum energy requirement for egg production (the day before the first egg was laid). The DEE of laying swallows, however, was not related to the energy content of egg formed, so egg formation was presumably a relatively minor component of the energy budget, under normal circumstances.

It was concluded that the energy requirement for warming the clutch during incubation might set the upper limit to clutch size for uniparental incubating birds. The energy requirements for laying and incubating eggs both made a contribution to the determination of clutch size, although other factors, acting during nestling-rearing or after the end of the breeding season would also affect reproductive output.

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APPENDICES

Appendix 1

List of common and latin bird names in the text and tables:

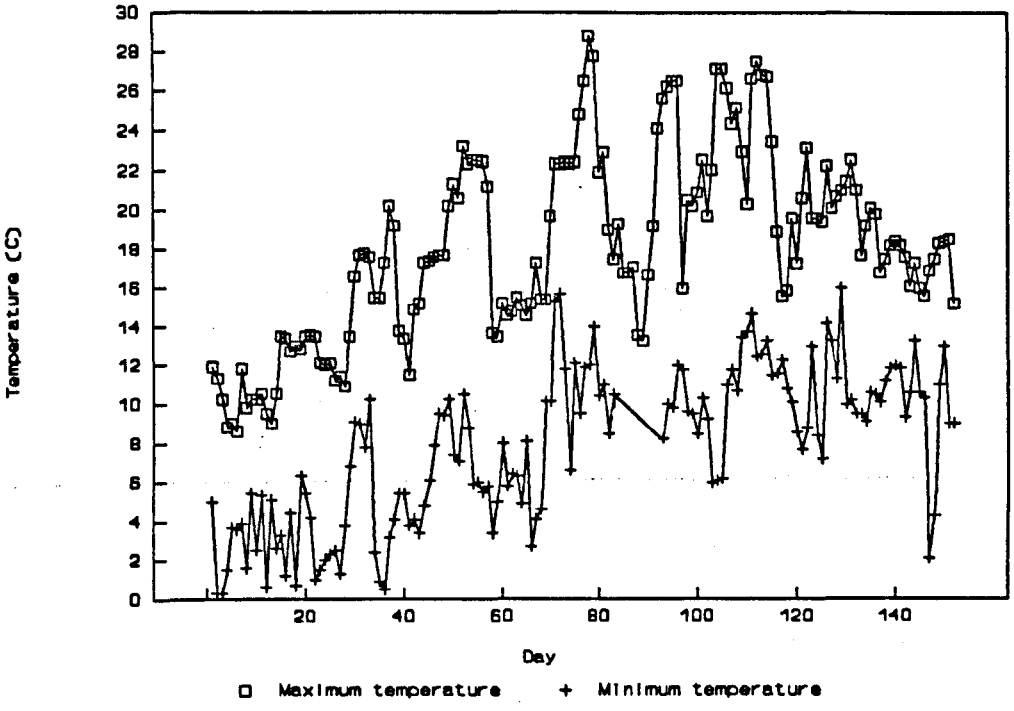
Adélie penguin	*Pygoscelis adeliae*
American oystercatcher	*Haematopus longirostris*
American coot	*Fulica americana*
American eider	*Somateria mollissima dresseri*
Bank swallow	*Riparia riparia*
Barn swallow	*Hirundo rustica*
Bengalese finch	*Lonchura striata domestica*
Black-headed gull	*Larus ridibundus*
Blue tit	*Parus caeruleus*
Bobolink	*Dolichonyx oryzivorus*
Bobwhite quail	*Colinus virginianus*
Brant	*Branta bernicla*
Brewer's sparrow	*Spizella breweri*
Brown-headed cowbird	*Molothrus ater*
Brünnich's guillemot	*Uria lomvia*
Californian quail	*Callipepla californica*
Canada goose	*Branta canadinis maxima*
Canada goose	*Branta canadinis minima*
Canvasback	*Aythya valisineria*
Collared flycatcher	*Ficedula albicollis*
Common crow	*Corvus brachyrhynohos*
Common guillemot	*Uria aalge*
Common tern	*Sterna hirundo*
Crow	*Corvus corone*
Curlew	*Numenius arquata*
Dipper	*Cinclus cinclus*
Domestic fowl	*Gallus domesticus*
Eastern kingbirds	*Tyrannus tyrannus*
Eider	*Somateria mollissima*
Eider	*Somateria mollissima bolrealis*
Fork-tailed storm-petrel	*Oceanodorma furcata*
Gadwall	*Anas strepera*
Great skua	*Stercorarius skua*
Great tit	*Parus major*
Grey-backed camaroptera	*Camaroptera brevicaudata*
Herring gull	*Larus argentatus*
Hooded crow	*Corvus corvus cornix*
House martin	*Delichon urbica*
House sparrow	*Passer domesticus*
Japanese quail	*Coturnix coturnix japonica*
Kestrel	*Falco tinnunculus*
Lapwing	*Vanellus vanellus*
Lesser black-backed gull	*Larus fuscus*
Lesser scaup	*Aythya affinis*
Lesser snow goose	*Anser caerulescens*

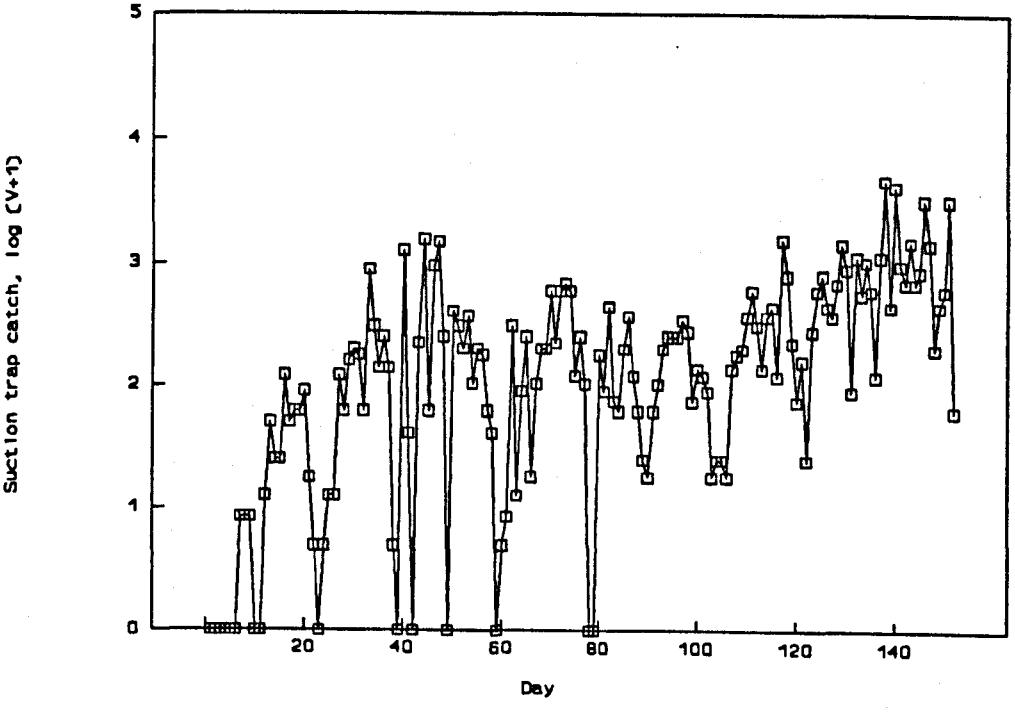
Magpie	*Pica pica*
Mallard	*Anas platyrhynchos*
Marsh tit	*Parus palustris*
Northern shoveler	*Anas clypeata*
Pied flycatcher	*Ficedula hypoleuca*
Pintail	*Anas acuta*
Puffin	*Fratercula arctica*
Quail	*Coturnix coturnix japonica*
Quelea	*Quelea quelea*
Razorbill	*Alca torda*
Red-billed quelea	*Quelea quelea*
Red grouse	*Lagopus lagopus scoticus*
Redshank	*Tringa totanus*
Ring-billed gull	*Larus delawarensis*
Ring-necked duck	*Aythya collaris*
Robin	*Erithacus rubecula*
Rook	*Corvus frugilegus*
Roseate tern	*Sterna dougallii*
Ruddy duck	*Oxyura jamaicensis*
Sand martin	*Riparia riparia*
Shag	*Phalacrocorax aristotelis*
Silver gull	*Larus novaehollandiae*
Snow bunting	*Plectrophenax nivalis*
Snow goose	*Anser caerulescens*
Song sparrow	*Melospiza melodia*
Sooty tern	*Sterna fuscata*
Sparrowhawk	*Accipiter nisus*
Spruce grouse	*Dendragapus canadensis*
Starling	*Sturnus vulgaris*
Swallow	*Hirundo rustica*
Swift	*Apus apus*
Thick-billed murre	*Uria lomvia*
Tree swallow	*Tachycineta bicolor*
Wheatear	*Oenanthe oenanthe*
Whimbrel	*Numenius phaeopus*
White-bellied swiftlet	*Collocalia esculenta*
Willow tit	*Parus montanus*
Wood duck	*Aix sponsa*
Yellow-vented bulbul	*Pycnonotus goiavier*
Zebra finch	*Taeniopygia guttata*

Appendix 2

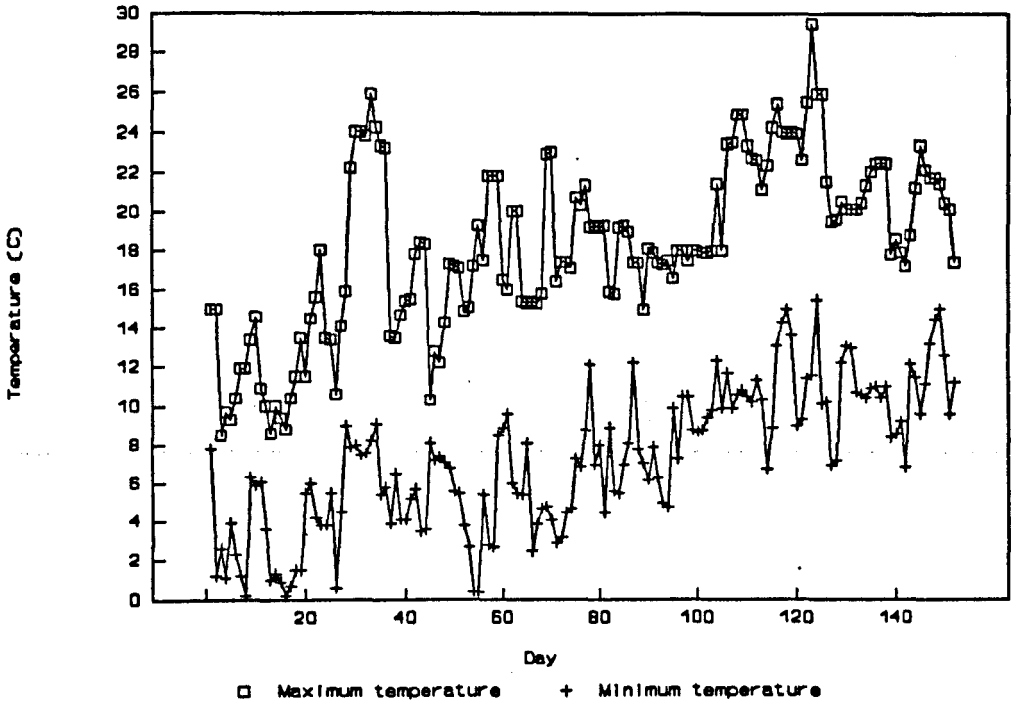
Daily maximum and minimum temperatures (°C) and insect suction trap catch ($\log_e(V+1)$) in a) 1989 (Pearson correlation coefficients, r=0.37, suction trap catch with maximum temperature, r=0.47 with minimum temperature, p=0.0001), b) 1990 (r=0.52 suction trap catch with maximum temperature, r=0.49 with minimum temperature, p=0.0001), and c) 1991 (r=0.57 with maximum temperature, r=0.49 with minimum temperature, p=0.0001).

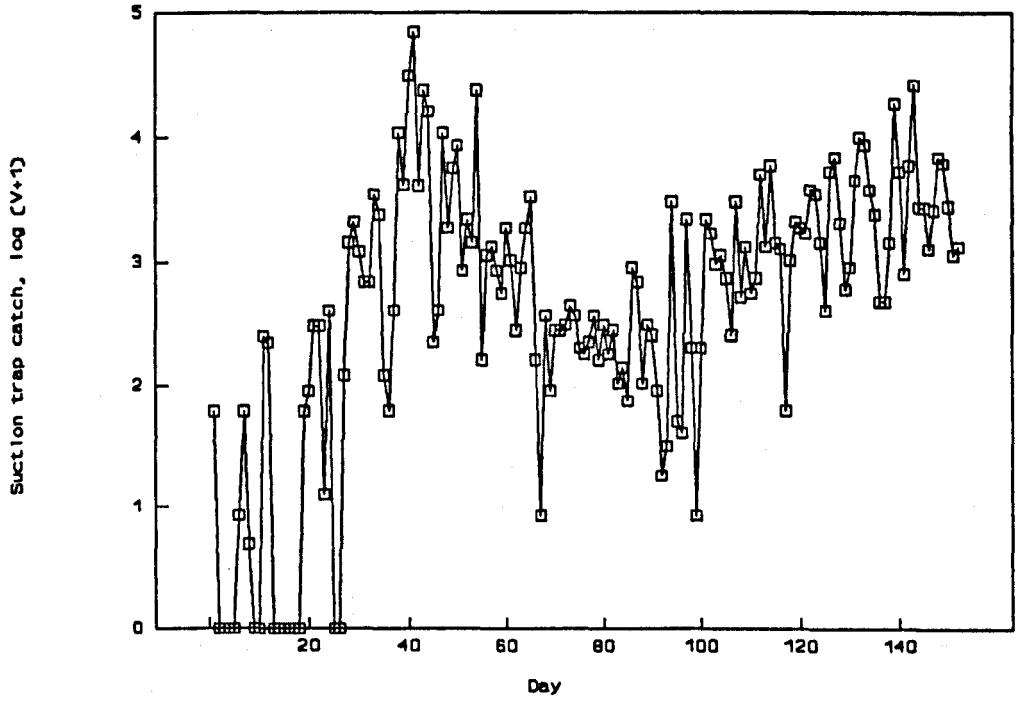
a) 1989


Temperature (C)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
20
40
60
80
100
120
140
Day
Maximum temperature
Minimum temperature

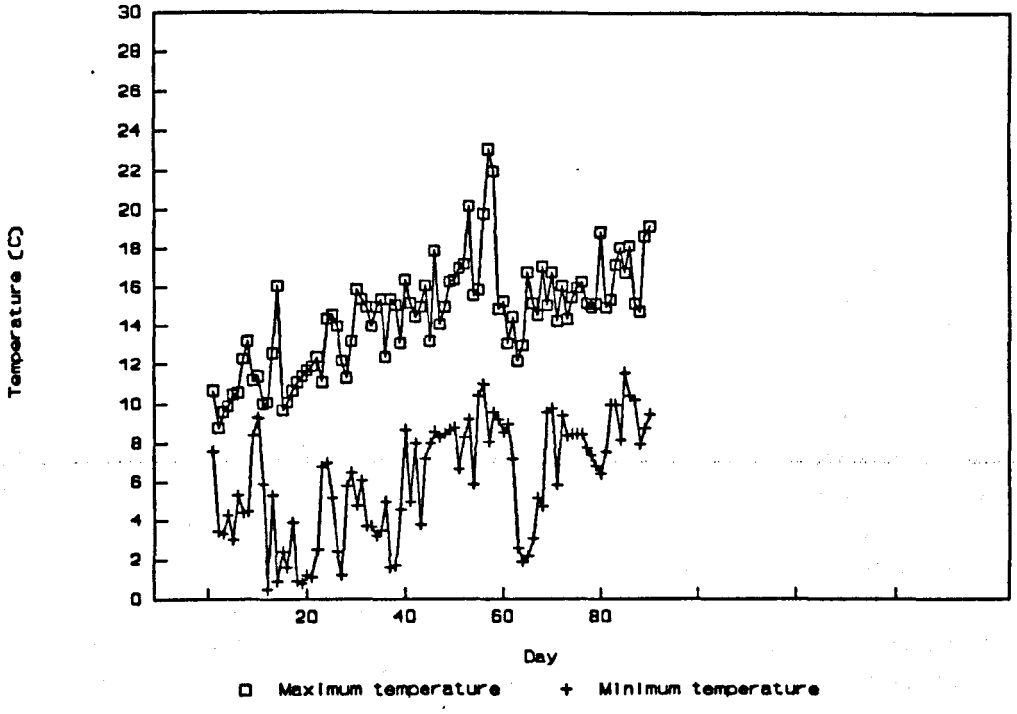

Suction trap catch, log (V+1)
5
4
3
2
1
0
20
40
60
80
100
120
140
Day

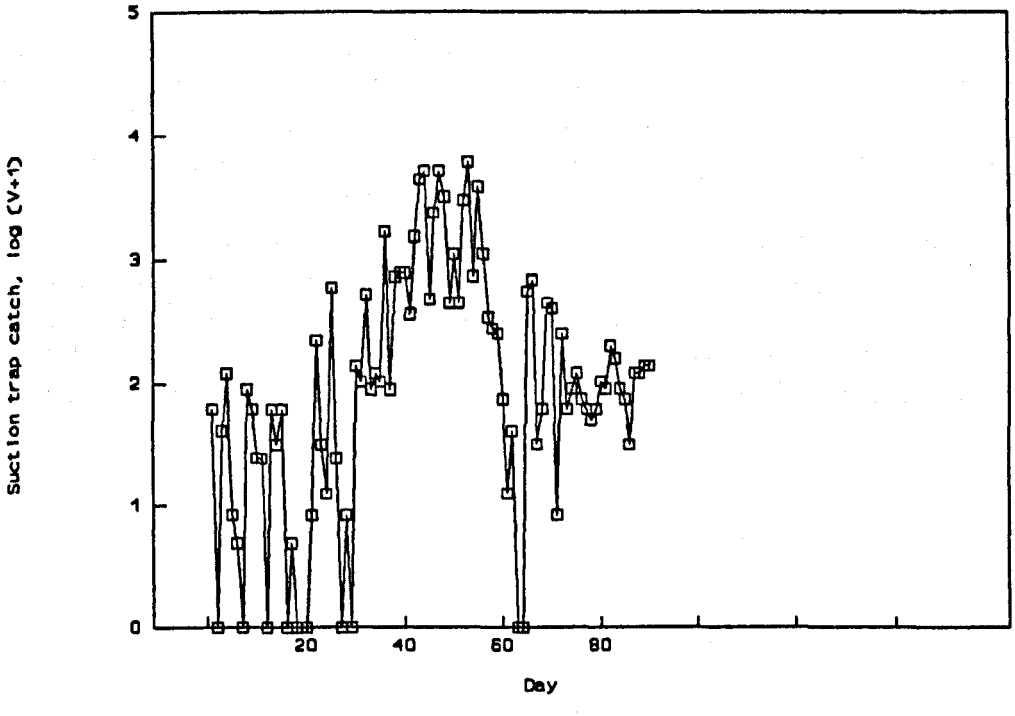
b) 1990


Temperature (C)
Day
Maximum temperature
Minimum temperature


Suction trap catch, log (V+1)
Day

c) 1991


Temperature (C)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
20
40
60
80
Day
Maximum temperature
Minimum temperature


Suction trap catch, log (V+1)
5
4
3
2
1
0
20
40
60
80
Day

Appendix 3

Mean mass of bank swallow *Riparia riparia* oviducts during enlargement prior to laying, from Petersen (1955), and conversion (in proportion with the difference in oviduct mass between the two species) to the pattern for swallow *Hirundo rustica* oviducts.

Although laying bank swallows were lighter than swallows on the night between laying of the first and second eggs (mean female bank swallow mass 17.5g, n = 3 (Petersen 1955); mean swallow mass 24.04g, sd = 1.27, n = 25 this study), the wet mass of the swallow oviduct was less than that of the bank swallow (mean wet mass of swallow oviduct = 1097mg (Table 8.5), n = 2; wet mass of bank swallow oviduct = 1322mg). The predicted mass of a swallow oviduct was calculated each day by multiplication of the mass of the bank swallow oviduct by 1097/1322 (the ratio of the wet masses of oviducts of the two species at the start of day 1). The mass of the swallow oviduct was assumed a) to follow the same pattern as that of the bank swallow in Petersen (1955), with degradation during laying; b) not to be degraded until the end of laying; or c) to be degraded linearly during the laying at a rate which depended upon clutch size, so that the oviduct was 35.7% of maximum mass (526mg) when the final egg was laid. Masses given are for midnight at the start of each day. The first egg was laid on day 0. Daily energy requirements and savings associated with oviducal growth and degradation were calculated from the mean energy content of swallow oviduct (6.904J/mg Table 8.5) and a conversion efficiency of 70% from food intake to tissue formation, (Turner 1982) and an efficiency of 82% for conversion of the oviduct to egg albumen (Chwalibog 1982). 1mg of oviduct therefore required 9.863 J for synthesis and released 5.661 J upon degradation.

Appendix 3 continued

			a) Assuming degradation during laying			b) Assuming no degradation during laying		
	Bank swallow oviduct mass		Swallow oviduct	Change during day		Swallow oviduct	Change during day	
Day	(mg)	% max. mass	Mass (mg)	mg	J	Mass (mg)	mg	J
-7	118	8.0	98	33	352	98	33	352
-6	158	10.7	131	76	750	131	76	750
-5	250	17.0	207	77	759	207	77	759
-4	342	23.2	284	152	1499	284	152	1499
-3	526	35.7	436	241	2377	436	241	2377
-2	816	55.4	677	415	4093	677	415	4093
-1	1316	89.3	1092	131	1292	1092	5	59
0	1474	100.0	1223	-126	-713	1097	0	0
1	1322	89.7	1097	-233	-1319	1097	0	0
2	1053	71.4	874	-438	-2480	1097	0	0
3	526	35.7	436	-218	-1234	1097	0	0
4	-	-	-	-	-	1097	0	0
5	-	-	-	-	-	1097	0	0
Incubation	263	17.8	218	218	0	218	-879	-4976

Appendix 3 continued

c) Assuming degradation during laying at a rate dependent upon clutch size

	Clutch = 3			Clutch = 4			Clutch = 5			Clutch = 6		
	Mass	Change during day		Mass	Change during day		Mass	Change during day		Mass	Change during day	
Day	mg	mg	J	mg	mg	J	mg	mg	J	mg	mg	J
-7	98	33	325	98	33	325	98	33	325	98	33	325
-6	131	76	750	131	76	750	131	76	750	131	76	750
-5	207	77	759	207	77	759	207	77	759	207	77	759
-4	284	152	1499	284	152	1449	284	152	1499	284	152	1499
-3	436	241	2377	436	241	2377	436	241	2377	436	241	2377
-2	677	415	4093	677	415	4093	677	415	4093	677	415	4093
-1	1092	131	1292	1092	131	1292	1092	131	1292	1092	131	1292
0	1223	-394	-2230	1223	-262	-1493	1223	-196	-1110	1223	-157	-889
1	829	-393	-2225	961	-263	-1489	1027	-197	-1115	1066	-157	-889
2	436	-218	-1234	698	-262	-1493	830	-197	-1115	909	-157	-889
3	-	-	-	436	-218	-1234	653	-197	-1115	752	-157	-889
4	-	-	-	-	-	-	436	-218	-1234	594	-157	-894
5	-	-	-	-	-	-	-	-	-	436	-218	-1234
Incubation	218	0	0	218	0	0	218	0	0	218	0	0

Appendix 4

Calibration equations were used to convert the delta raw values given by the mass spectrometers to a value in ppm (parts per million) for ^{18}O or deuterium concentration. Points in Figs. 1 and 2 are the mean of 2 samples at each concentration of standard. Replicate standard samples were analyzed till two delta raws agreed to within 1%. Concentration of the standards was determined empirically by dilution of a known enriched standard.

Consistent response of the SIRA 10 which was used to measure ^{18}O concentration was checked daily against two reference gases. The SIRA 9 (used to measure deuterium concentration) was checked weekly with standard samples. This machine was used to run fewer samples than the SIRA 10.

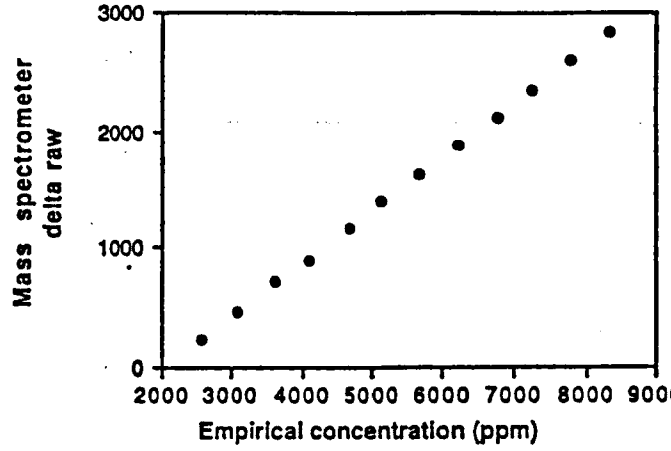


Fig. 1. SIRA 9 calibration a) using reference gas with a concentration of 140 ppm which was used in 1989 (delta raw=7.337 × empirical ppm - 586.807, r^2=.999) b) using a new cylinder of reference gas, used in 1990 and 1991, with a concentration of 99 ppm (delta raw=7.749 × empirical ppm - 548.150, r^2=.999).

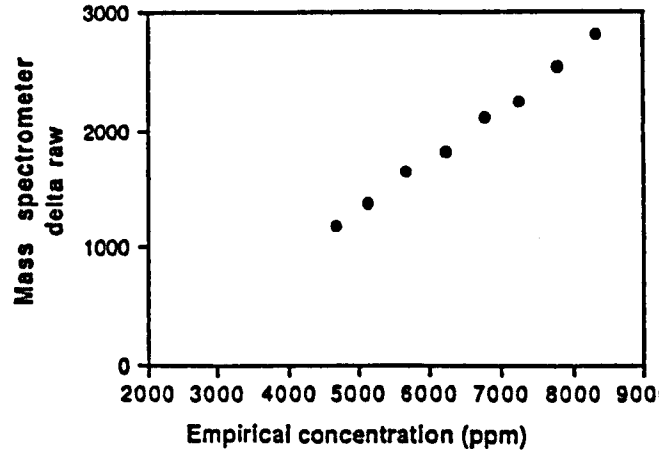


Fig. 2. SIRA 10 calibration a) in 1989-90 (delta raw=.456 × empirical ppm - 956.299, r^2=.999) b) in 1991 (delta raw=.442 × empirical ppm - 896.166, r^2=.997) when machine response had altered slightly.

Appendix 5

Time budgets of laying swallows, observed during daytime in periods of measurement of energy expenditure by the DLW technique (Chapter 7.). - = no data collected for this bird.

Bird number	Duration of observations (minutes)	% flap	% glide	% flight	% perch
1	-	-	-	-	-
2	-	-	-	-	-
3	124	68.6	18.4	87.0	13.0
4	87	59.8	32.6	92.4	7.6
5	40	41.6	35.9	77.5	22.5
6	14	46.3	34.2	80.5	19.5
7	119	62.8	26.3	89.1	10.8
8	108	45.2	19.2	64.4	35.6
9	49	52.0	22.7	74.7	25.3
10	-	-	-	-	-
11	66	55.8	22.5	78.3	21.6
12	177	65.4	16.7	82.1	17.8
13	67	41.7	8.3	50.0	50.0

Appendix 6

Nest sites, ring numbers, blood sample codes and date of labelling of swallows and dippers for which energy expenditure was measured using the doubly labelled water technique. Bird number (the first column) is the identification used for each bird in this thesis.

Laying swallows

Bird number	Farm	Site	Ring number	Sample code	Date labelled
1	Redhall	straw	F646848	SY 5	23.6.90
2	White House	-	F145642	SY 7	4.7.90
3	Powis Cottage	-	F145743	SY 8	11.7.90
4	Mains	upstairs	F646649	SY 9	14.7.90
5	Redhall	upstairs	F781588	SY10	19.7.90
6	Old Keir	Petrovore	F646850	SY11	20.7.90
7	West Drip	CB door	F781508	SY15	27.7.90
8	Manor Steps	upstairs	F646694	SY16	24.5.91
9	Mid Lecropt	doorway	H227501	SY17	28.5.91
10	Glenhead	bat arch	H227505	SY18	28.5.91
11	Westwood Lane	-	H227511	SY19	29.5.91
12	Mains	HM	H227513	SY20	29.5.91
13	Mid Lecropt	skylight	E649538	SY22	1.6.91

Incubating swallows

Bird number	Farm	Site	Ring number	Sample code	Date labelled
1	Old Keir	car	F646856	SY 1	5.6.90
2	Glenhead	passage	F781535	SY 2	13.6.90
3	Inverardach	A	F145629	SY 3	20.6.90
4	Westwood Lane	-	F139036	SY12	23.7.90

Incubating dippers

Bird number	Site	Grid reference	Ring number	Sample code	Date labelled
1	Blackford	NN892093	RB93651	SAL 1	24.4.90
2	Greenhill	NN840054	XR68715	SAL 2	30.4.90
3	Wharry	NN823013	XR68572	SAL 3	3.5.90
4	Gannel	NS912982	XR68830	SAL 6	11.5.90
5	Merryhills	NT018999	XR68808	SAL 7	15.5.90
6	Doune Castle	NN729011	XR68590	SAL10	26.3.91
7	Cromlix	NN792056	XR68810	SAL11	16.4.91
8	Auld Dalbrack	NN749053	XR68814	SAL12	21.4.91
9	Ardoch hide	NN750049	RS03029	SAL13	24.4.91
10	Alva 5th bridge	NS888973	XR68821	SAL14	30.4.91

Appendix 7

Structural size, body condition and body composition determined by carcass analysis of female swallows analyzed during this study. Birds 1 and 2 were killed by chloroform inhalation (under licence from the NCC) on the evening of the day upon which they laid the first egg of their second clutch (day 0), in July 1991. Bird 3 was found trapped in fishing line which had been used as nest lining when her brood was about 20 days old, in August 1991. This bird was thought to be close to death from starvation when she was killed.

a) Structural size (mm)

Bird	**1**	**2**	**3**
Wing length	124	123	125
Outer tail	80	87	93
Second tail	58	60	60
Inner tail	47	45	46
Keel	20.6	20.0	18.6
Head and bill	29.1	29.4	29.8
Tarsus	11.7	11.6	11.3

b) Body condition

Bird	**1**	**2**	**3**
Live mass (g)	22.40	24.30	-
Mass after death (g)	22.30	24.81	-
Fat score			
Upper keel	3	4	0
Lower keel	3	4	0
% abdominal	70	70	0
Pectoralis thickness - live (ultrasound, arbitrary ultrasound units)			
Upper right	2.7	2.7	-
Lower right	2.0	1.8	-
Upper left	2.8	2.7	-
Lower left	2.1	1.95	-
Pectoralis thickness - thawed (ultrasound, arbitrary ultrasound units)			
Upper right	2.7	2.7	1.9
Lower right	2.0	2.0	1.5
Upper left	2.8	2.6	2.1
Lower left	1.8	2.0	1.5
Pectoralis thickness - thawed (needle, mm)			
Upper right	5.0	5.2	4.4
Lower right	4.5	4.9	3.2
Upper left	5.8	5.9	4.6
Lower left	4.4	3.2	3.6

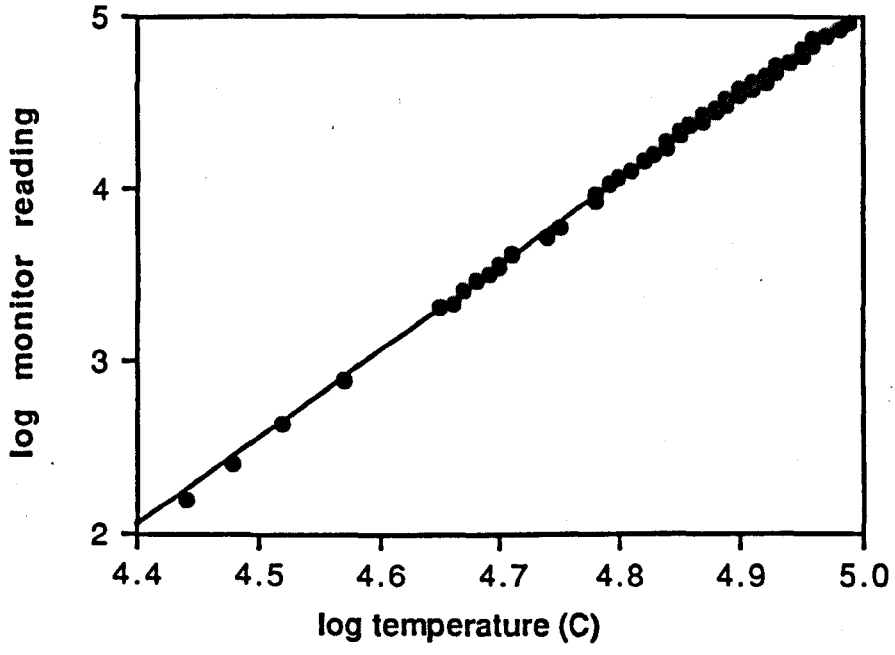
c) Composition of body components

	Bird 1			Bird 2			Bird 3		
Component	**WM**	**DM**	**LDM**	**WM**	**DM**	**LDM**	**WM**	**DM**	**LDM**
Skin & feathers	2128.6	1482.6	962.3	2317.0	1579.0	952.6	2391.5	1433.5	1108.32
Wing feathers	505.9	493.8	451.0	521.7	513.6	500.2	510.1	488.5	460.0
Tail feathers	110.8	103.3	88.1	104.0	103.5	85.4	107.9	104.8	90.1
Head	1898.1	542.4	406.9	1893.4	546.2	407.5	1858.2	527.6	399.3
Neck	528.9	192.5	147.6	454.6	170.4	121.6	643.2	221.3	165.1
Pectoralis major	3060.8	935.5	781.9	2988.4	929.7	776.0	2589.9	790.6	662.4
Pectoralis minor	243.3	74.1	63.0	153.4	48.8	40.4	232.8	71.9	62.3
Wings	1606.7	819.6	593.8	1559.7	821.9	579.2	1735.0	820.8	533.8
Legs	907.7	454.0	271.5	978.6	524.5	102.0	880.1	335.9	233.6
Gizzard & oesophagus	1150.6	301.1	234.3	783.4	478.9	408.1	555.8	193.4	180.5
Liver	1040.2	356.2	257.6	1201.5	377.3	293.8	709.9	227.3	196.7
Oviduct	1144.2	304.3	264.1	1050.9	279.0	241.8	-	-	-
Heart	353.5	108.2	87.2	348.7	106.8	84.2	316.2	97.9	86.4
Intestine	861.7	252.6	170.6	1286.0	370.8	251.6	703.8	189.1	130.5
Kidneys	218.7	64.0	51.9	345.5	92.4	77.6	248.8	79.0	66.9
Lungs	611.2	173.4	136.7	342.5	116.9	75.9	271.3	75.3	63.1
Body shell	2779.8	1247.8	726.5	3389.3	1652.9	838.0	2252.7	921.3	684.5
Oviducal egg	1767.5	414.2	267.7	1008.9	315.9	192.3	-	-	-
Ovary	55.1	16.0	13.1	44.5	13.0	9.1	-	-	-
Ovarian follicle 1	350.2	193.0	80.4	346.3	184.8	79.5	-	-	-
Ovarian follicle 2	173.4	93.9	37.8	220.6	117.2	48.9	-	-	-
Ovarian follicle 3	39.2	18.5	8.3	56.8	26.9	10.6	-	-	-
Total	21.5361	8.6410	6.1023	21.3955	9.3704	6.1763	16.0012	6.5722	5.1175

WM = Wet mass (mg), DM = Dry mass (mg), LDM = Lean dry mass (mg). - = Not possible to dissect as a separate component. Ovarian follicles 1, 2 and 3 are rapidly developing ovarian follicles.

Appendix 8

Nest temperature monitor calibration curve, obtained by placing the thermistors of the nest temperature monitor inside an darkened incubator for at least an hour at each temperature before thermistor output and a quartz digital thermometer (accurate to 0.01°C) readings were recorded. A log-log relationship between temperature and monitor reading was described by the equation ($\log_e$ monitor reading=5.0 × log temperature - 20.0, r^2=0.99.



ENERGY METABOLISM OF FARM ANIMALS

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DOUBLY LABELLED WATER MEASUREMENTS OF THE ENERGY METABOLISM OF AN AVIAN SPECIES UNDER DIFFERENT AMBIENT TEMPERATURES AND REPRODUCTIVE STATES

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Summary

The doubly labelled water (DLW) technique can be used to measure the energy expenditure of an unrestricted subject, without confinement in a chamber or encumbrance by a mask. In this study energy expenditure of Japanese quail (*Coturnix coturnix*) was measured simultaneously by the DLW technique and by indirect chamber calorimetry. there was a mean difference between paired measurements of 13.4% for 6 laying females at 25°C and 26.1% for 15 male birds over a temperature range of 15-33°C. The DLW measurement was greater than the calorimeter result for both males and females. Energy expenditure increased with decreasing temperature (males) or increasing egg production (females), but there was no effect of temperature or egg production on the agreement between the two techniques. This is the first time that the DLW technique has been used either in this species or with a laying bird.

Keywords: *Coturnix coturnix,* doubly labelled water, egg production, energy expenditure, heat production, Japanese quail

Introduction

Important simplifying assumptions of the DLW technique are that the labelling isotopes disperse only through the body water pool, not other body components, and that water leaving the body has the same isotope ratio as that remaining. These assumptions would be violated during egg production if there was fractionation between body water and egg water or if significant amounts of the label were incorporated into egg materials. Temperature changes might also cause an error in DLW measurement of energy expenditure because of changes in fractionation associated with increased evaporative water loss at high temperatures. The energy expenditure of male and female quail was measured simultaneously using the DLW technique and indirect calorimetry, to determine whether agreement between the two techniques was affected by temperature or egg production.

Materials and methods

Ten-week-old male and seven-week-old female quail from a randomly bred captive stock at IAPGR, Roslin, were used in this experiment. At these ages, the males had reached adult size and the females had begun to lay. Birds were placed singly in calorimetric chambers for 1 or 2 days of training, followed by 2d of measurement of energy expenditure. Food and water were provided *ad libitum.* The lighting pattern was 14 light:10 dark. Energy expenditure was measured by indirect calorimetry, using the equipment and methods described by MacLeod *et al.* (1985). The DLW measurement of energy expenditure was made by the procedure described by Tatner and Bryant (1989). After a 2h equilibration period, ten 5µl blood samples were flame-sealed in calibrated pipettes. The bird was placed in the calorimeter until subsequent blood sampling 24 and 48h later. Samples were also taken from the albumen of eggs laid shortly before females were removed from the calorimeter for the 24 and 48h blood samples. Energy expenditure was calculated from isotope turnover

rate using the equation of Lifson and McClintock (1965). The body water pool was calculated from the ^{18}O isotope dilution space. The background level of the heavy isotopes was measured in blood samples taken from quail from the same hatch as the experimental birds.

The volume of yolk formed during the experiment was calculated by marking the diameter of each ovarian follicle at the begining and end of the experimental period by feeding a gelatin capsule of lipophilic dye (Sudan B). Then dye stained a distinct band in follicles undergoing the rapid growth phase (Gilbert 1972). The yolks of eggs laid and the follicles within the ovary were examined at the end of the experiment and used to calculate the total volume and energy content of yolk formed.

Results and discussion

Two independent estimates of energy expenditure of laying female quail were calculated using the DLW technique. Either blood or egg albumen could be used to sample the body water pool to calculate the rate of loss of the isotope label. The ^{18}O and ^{2}H enrichments of the albumen samples were only 80-365ppm greater than those of the blood samples taken 1-2h after the eggs were laid (Table 1). This suggests that water in the egg albumen was in isotopic equilibrium with the body water pool until the egg was isolated from the bird by shell formation shortly before laying. Samples taken from the yolk of ovarian follicles immediately after slaughter of the quail, following the final blood sample, were also in isotopic equilibrium with the rest of the body. there was a mean difference of 2.0% between energy expenditure calculated using blood and albumen samples (Table 2). As there is no evidence of fractionation of isotopes between egg water and body water, which would have violated one of the assumptions of the DLW technique, albumen samples can be used as an alternative to blood samples to determine the isotopic composition of body water. This alternative method of handling would be preferable if handling of the subject is to be minimised or, in the case of free-living subjects, when recapture is difficult.

Table 1. Enrichment (ppm) of ^{18}O and ^{2}H in albumen and blood samples of the body water of female quail no. 2.

^{18}O				^{2}H			
egg1 albumen	24h blood	egg2 albumen	48h blood	egg1 albumen	24h blood	egg2 albumen	48h blood
4121	3757	2978	2897	759	675	493	484

The DLW and calorimeter measurements of energy expenditure for each female are shown in Table 2. The DLW measurements using using blood and egg samples were 13.4 and 10.4%, respectively, greater than the calorimeter measurement. These results are within the range of agreement between the two techniques found in previous studies (Speakman and Racey, 1988). There was no significant difference between the two DLW measurements of energy expenditure ($Z=0.73$, $p=0.46$, Wilcoxon matched-pairs signed ranks test) or between the DLW measurement calculated from egg samples and the calorimeter ($Z=1.99$, $p=0.05$). There was no significant difference between the two techniques and the volume of white, yolk or total egg energy. Energy expenditure, measured by either technique, increased with increasing egg production although the relationship was not significant. There was a highly significant relationship between egg production and energy expenditure in a larger sample of females which included these birds ($r^2=0.59$, $p<0.001$, $n=35$).

Table 2. Comparison of energy expenditure (kJ d^{-1}) of female quail measured simultaneously by indirect calorimetry and the DLW technique.

Bird no.	1	2	3	4	5	6
A Indirect calorimetry	174	212	218	191	150	203
B DLW (blood)	179	210	242	277	175	216
C DLW (albumen)	181	247	225	225	171	186
% difference						
100(B-A)/A	3	-1	11	45	16	7
100(C-A)/A	4	17	3	33	14	-8
100(C-B)/B	1	18	-7	-8	-2	-14
Egg deposition kJ d^{-1}	31	56	45	78	52	53

The energy expenditure of 14 males was manipulated over a 3-fold range by measurements at temperatures between 15 and 33°C. Both chamber and DLW measurements of energy expenditure were negatively correlated with temperature ($r=-0.89$, $p<0.001$, and $r=-0.47$, $p=0.09$ respectively). The agreement between the DLW technique and chamber results was much poorer than for the female quail. There was a mean difference of 26.2% between the DLW and calorimeter measurements (mean of absolute differences=32.5%, range: DLW 22.1% less than, to 95.9% greater than, calorimeter). The DLW measurements were significantly greater than those of the calorimeter ($t=2.8$, $p<0.02$, paired t-test). There was a significant correlation between the results of the two techniques ($r=0.57$, $p<0.04$).

No single cause was identified which could account for the discrepancy between the calorimeter and DLW measurements of energy expenditure. Difference between the two measurements was not correlated with temperature, energy expenditure, amount of egg material formed or change in mass or subcutaneous fat of the bird during the measurement. The calorimeter was thought to give a more accurate measurement because these results showed a much tighter relationship with temperature, which confirmed apparatus precision with a small bird. Physical tests of precision are described by Lundy *et al.* (1978). Measurement of isotope enrichments varied slightly between replicate samples which will contribute to the error in the DLW measurement. This could be due to differences in sample fractionation before or during sealing of the pipettes, fractionation during preparation of samples for analysis, or machine error of the mass spectrometer itself. The mean enrichment of a replicate pair of samples was used if they differed by less than 3%, rather more than advised by Tatner and Bryant (1989), although most differed by less than 1%. Errors which may have been introduced to the DLW results due to these problems were, however, small compared with the differences between calorimeter and DLW results. There was good agreement between DLW measurements calculated independently form egg and blood samples for the female quail and between estimates for the male quail using the two-point and multi-point calculation methods. This suggests that the discrepancy between the DLW and calorimeter is not due to errors in the measurement of isotope ratios.

One difference between the two measurement techniques is that the DLW measurement is taken over 24h while the calorimeter does not measure the energy expenditure of the bird during handling for blood sampling. Yet, because handling of the bird

only lasted a few minutes, it was not thought that this period of presumably greater than average metabolic rate could contribute much to the daily total. The most likely cause of the large difference between DLW and calorimeter results was the low metabolic rate of the quail and related low turnover of isotope labels. The decrease in the enrichment of the oxygen isotope was only 34-48% over 48h in the male quail, and 48-58% in the females, whereas an error of >10% is expected unless final ^{18}O concentration is less than 50% of initial (Nagy, 1980). Smaller or more active subjects have a faster turnover of isotopes and should yield a more accurate result over a 48h period.

The DLW technique gave measurements of energy expenditure which were consistent between different routes of sampling the body water pool and between the two-point and multi-point methods for calculation of the rate of isotope loss. Agreement between individual DLW and calorimeter measurements was reasonable for female but poor for male birds. Energy expenditure measured by either technique showed the same trends with temperature and egg production, but with a greater scatter in the case of the DLW method. The DLW technique can be used with laying birds as there appears to be no fractionation of water transferred to the egg. Under captive conditions, where activity levels and metabolic rate are low, a sufficient depletion of isotope levels must be achieved for the potential of the DLW method to be realised.

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Energy cost of egg formation in quail. By S.WARD, *Department of Molecular and Biological Science, University of Stirling, Stirling, FK9 4LA* and M.G. MacLEOD, *AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, EH25 9PS.*

Little information is available on the additional energy requirement for egg production in birds. In this study the daily energy expenditure (DEE) of 7-week-old female Japanese quail was measured using indirect calorimetry (MacLeod *et al* 1985). At this age individuals showed a range of egg production from zero to daily laying.

Birds were placed singly in calorimetric chambers for one day of training followed by 3 experimental days. Food and water were provided ad libitum. The diameter of each ovarian follicle was marked at the start of the experiment by feeding a gelatin capsule of lipophilic dye (Sudan B) which stained a distinct band in follicles undergoing the rapid growth phase (Gilbert 1972). The yolks of those eggs laid and the follicles still within the ovary were examined at the end of the experiment. The increase in volume of each yolk was used to calculate the total energy content of yolk formed. The energy content of any albumen formed was added to that of the yolk, to calculate mean daily energy content of egg formed.

These measurements showed that females which laid daily had 49% greater DEE than birds which had not begun to lay, and that each unit of energy deposited in the eggs required 1.75 additional units of DEE. The birds in this experiment only deposited 36% of their additional energy output (additional DEE + energy deposited in the egg) as egg, about half of the efficiency usually estimated for the domestic fowl.

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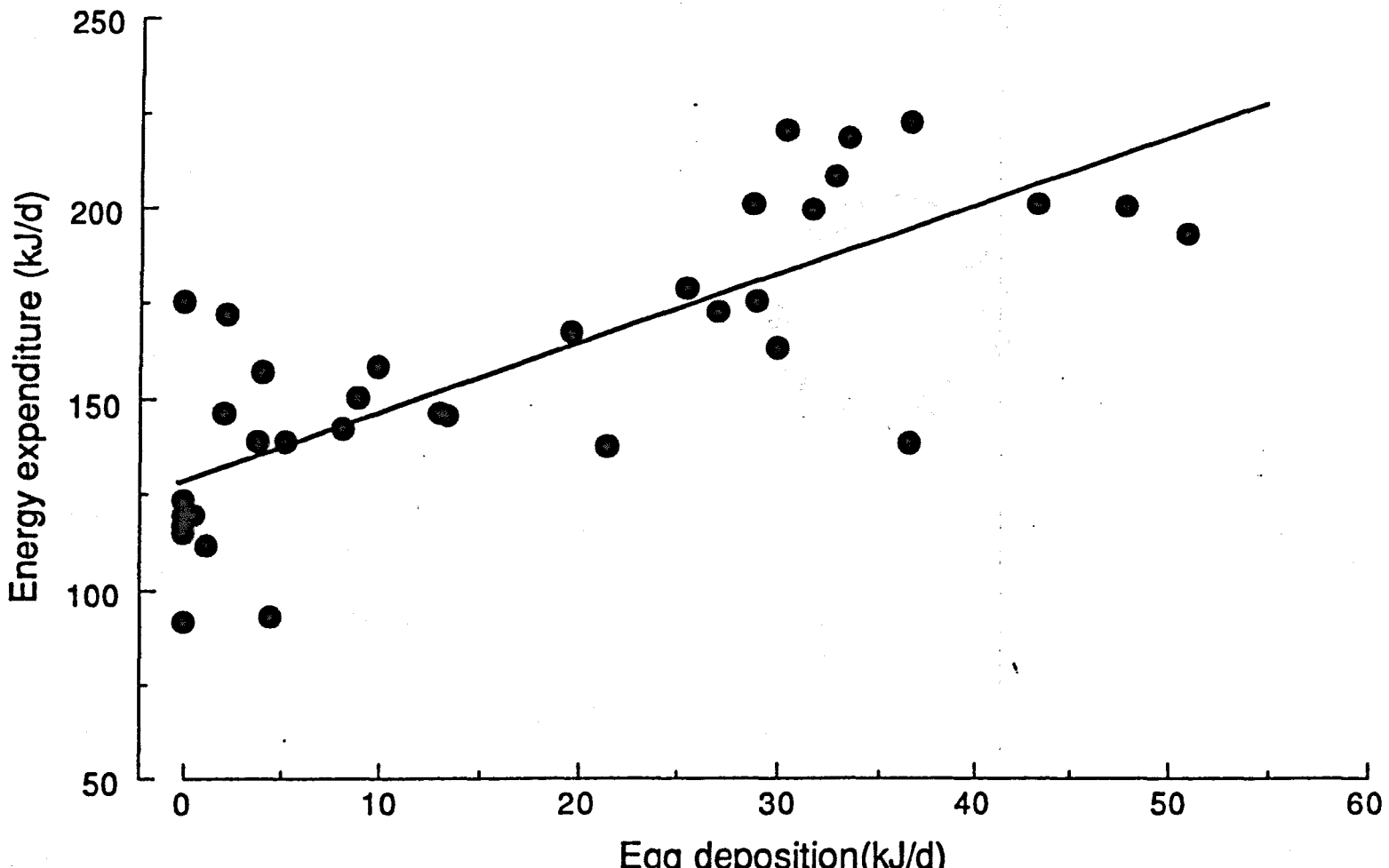

Fig. 1. Increase in daily energy expenditure of quail with daily energy content of egg deposited ($y=1.75x + 129$, $r^2=0.59$, $p<.0001$, $n=35$ birds).