# EGG PRODUCTION IN LESSER BLACK-BACKED GULLS:

# AN EXPERIMENTAL APPROACH

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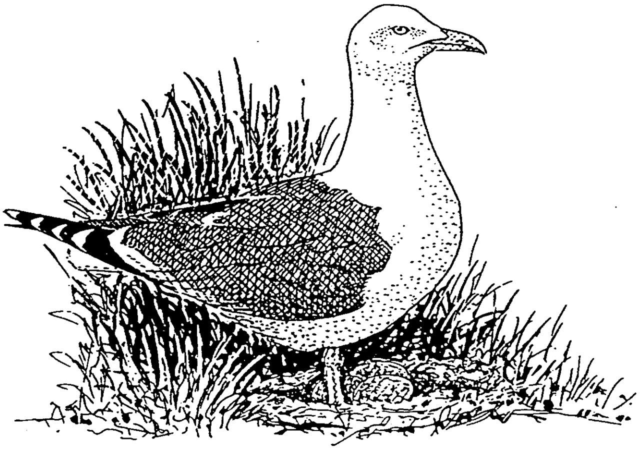

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# CANDIDATE'S DECLARATION

I declare that the work recorded in this thesis is entirely my own unless otherwise stated and that it is of my own composition. No part of this work has been submitted for any other degree.

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# SUMMARY

1. A new technique to estimate pectoral muscle protein condition from body measurements which can be taken from live lesser black-backed gulls (*Larus fuscus*) is described. The method uses the profile of the pectoral muscles over the keel to estimate the maximal muscle cross sectional area. The product of cross sectional area and muscle length provides an index of muscle volume, which, together with body weight provides an accurate predictor of actual pectoral muscle protein (muscle lean dry weight) determined by carcass analysis, in a stepwise regression procedure. The regression model was validated using a second, independent sample. Different methods of controlling for body size to calculate protein condition from measures of total protein were considered.

2. The consequences of variation in egg size for offspring survival to fledging in lesser black-backed gulls, was examined using a clutch transfer experiment. The experiment tests the hypothesis that parents producing large eggs also provide better parental care for their offspring than those which lay small eggs, which explains part of the correlation between egg size and offspring survival in non-manipulative studies. Clutches of large eggs were cross fostered with clutches of small eggs in order to examine the egg size and parental effects independently. Large eggs produced chicks which were not only skeletally larger (tarsus length) but also heavier for their size than those from smaller eggs, both of which could contribute to increased chances of survival. However, logistic regression analysis showed that the parental effect was more important in determining chick survival to fledging, though egg size does have a separate, independent, but smaller effect. This appears to be mediated by differences in chick skeletal size rather than condition. Since large egg size is advantageous, selection for increasing egg size is implied. The likely counterbalancing forces are discussed.

3. Experimental evidence is provided to demonstrate that a reduction in clutch size and egg size among unfed lesser black-backed gulls in one year was the result of food limitation of egg production. The average clutch size of unfed birds was among the smallest recorded for the species, and significantly lower than in previous years at the same colony. Pairs provided with supplementary food in the same year produced significantly larger clutches and larger eggs than controls, which were typical for the species. Provision of additional food at a second colony, in a different year, where egg production of unfed birds was normal, had no effect on either clutch size or egg size, suggesting that food limitation of egg production is not the cause of the normal limit to clutch size at three eggs.

4. The nutritional requirements of egg formation were examined through a series of pairwise feeding experiments. Provision of either fish or fat supplements to different experimental groups in the year of depressed egg production showed that the reduced clutch and egg sizes of unfed birds were the result of limited protein, not energy intake. Supplements of two different protein types (fish and egg) in a further year, when egg production of controls was good, demonstrated that egg production (in this case egg size only) could be limited by availability of specific nutrients, possibly certain essential amino acids, provided by the egg but not the fish protein supplements.

5. The role of female body condition in providing a causal link between food supply and egg production was investigated using the pectoral profile method outlined above. There was a significant correlation between pectoral muscle protein condition at the start of laying and the number of eggs laid among females. There was no relationship between body condition and either clutch size or egg size among males. In addition, a treatment-based analysis showed

that in the year of food limitation, unfed and fat-fed females, which laid small clutches, were in poorer condition at the start of laying, than those receiving the protein supplement, which laid larger clutches. In the following year, when there were no differences in clutch size according to prelaying feeding regime (though egg-fed birds laid eggs 10% larger than controls), there were no differences in body condition at the start of laying between treatments. These results suggest a causal role of flight muscle protein condition in the determination of clutch size but not egg size.

6. The current selection pressures involved in the usual truncation of the clutch at three eggs were investigated using a series of clutch size manipulations. The costs of egg laying and egg rearing were independently manipulated to determine whether the limit to clutch size is currently influenced by egg production costs, incubation capacity or chick rearing ability. Females which laid a clutch of further three eggs following the removal of their first egg tended to rear fewer chicks to fledging than those laying unmanipulated clutches of three eggs, but the differences did not quite reach significance. Incubation efficiency was not reduced in four egg clutches which were produced by adding an extra egg to recently completed clutches, and there was no indication of reduced hatchability among last-laid eggs of enlarged clutches, compared with controls. Broods of four were not more productive than control broods of three. The upper limit to clutch size may be determined by brood rearing capacity, which could act in conjunction with the suggested effects of increased egg production. Four chick broods did not produce more fledged young than control broods of three due to increased chick mortality early in life. It is suggested that the increase in hatching asynchrony which inevitably accompanies the production of a four chick brood may be the cause of the increased mortality, and may set the upper limit to clutch size at three eggs.

# CHAPTER 1

## INTRODUCTION

### *Determination of egg production - levels of explanation*

In examining the factors which are involved in the determination of avian clutch size, it is essential to distinguish between ultimate and proximate levels of explanation, as emphasised by Winkler & Walters (1983). At the proximate level, one seeks causal explanations in terms of the control mechanisms. At the ultimate level, the question of why birds lay a given number of eggs is answered in functional terms, relating to the adaptive significance of clutch size and the selection pressures which have operated over the course of evolutionary history to determine the optimal number of eggs to lay. Clearly there is considerable interaction between these two levels of explanation: any hypothesised ultimate causation must be compatible with known proximate mechanisms and in addition, some hypotheses may function on both levels - a factor which acts as a proximate limit on clutch size, such as food availability, may also determine clutch size in an ultimate sense.

The first formal explanation of the ultimate factors which have shaped clutch size was provided by David Lack (1947, 1948), who suggested that the characteristic clutch size of each species was that which produced the maximum number of surviving offspring. His theory has gained general acceptance (Klomp 1970, Ricklefs 1977), although there have been several important subsequent modifications.

Williams (1966), Perrins (1970) and Charnov & Krebs (1974) have emphasised the importance of life history considerations, since natural selection will act to maximise *lifetime* rather than annual reproductive success. Charnov & Krebs (1974) have demonstrated that if adult survival is inversely related to the number of offspring raised in a season, then the clutch size which maximises the number of young produced over the individual's lifetime will be smaller than that which maximises annual reproductive success. Clearly, such considerations will be particularly relevant for long-lived (k-selected) species. A further modification of Lack's original hypothesis, initially proposed by Perrins & Moss (1975) and highlighted by Drent & Daan (1980) is that the optimal clutch size for each individual female/pair, will not necessarily be the same as that for the species as a whole. If females differ in their ability to raise young, then the optimal clutch size for poor quality individuals will be lower than that of higher quality birds. Variation in clutch size between pairs may therefore represent adaptive differences related to variation in individual foraging ability or breeding experience.

### *Clutch size of lesser black-backed gulls*

Within the spectrum of life history strategies, gulls are generally considered as relatively k-selected. Adults typically have low annual mortality (for example between 5 and 15 % per year; Coulson & White 1959, Coulson & Butterfield 1986), with the highest mortality amongst adults occurring during the breeding season (Coulson *et al.* 1983), suggesting that reproduction is a comparatively costly event and carries survival consequences. Annual reproductive effort would therefore be expected to be relatively low, to avoid lowering the probability of survival to the following breeding season. In line with this, gulls lay small average clutches (2-3 eggs) in comparison with more r-selected species, such as many passerines, where annual adult mortality is typically much higher.

## *Ultimate determination of clutch size*

Although the principles of Lack's original hypothesis with its subsequent modifications, are generally accepted, a variety of specific hypotheses have been advanced to explain the evolution of clutch size in different species. Given that the average clutch size for the species is that which maximises lifetime reproductive success, the question that must then be answered is at what stage in the breeding cycle does the ultimate limit on clutch size operate ? For precocial species, such as gulls, it is generally considered that there are three alternative hypotheses (Winkler & Walters 1983):

### *1. Chick rearing capacity.*

Lack (1947, 1948) considered that for most species the ultimate upper limit to clutch size is set by the ability of the parents to provide food for the chicks. Experimental brood manipulations on a variety of seabird species have produced disparate results. Addition of a second chick to single-egg species such as albatrosses and shearwaters (Rice & Keynon 1962, Harris 1966) suggest that in such species, parents are unable to provide sufficient food to rear more chicks in a season, than they would normally produce. In contrast, there is some evidence from similar experiments on gulls which lay a normal clutch of three eggs, that some larids are capable of providing sufficient food to rear up to five chicks (lesser black-backed gull, Harris & Plumb 1965, western gull *L. occidentalis* Coulter 1973b, glaucous-winged gull *L. glaucescens* Ward 1973, herring gull *L. argentatus* Haymes & Morris 1977). However, whilst such studies may provide a useful insight into the current selection pressures maintaining or limiting clutch size, they are open to various criticisms and provide little information concerning past selection pressures involved in the evolution of clutch size. Lack (1968) has commented that the current ability of gulls to rear supernumerary young may be attributable to artificially increased food supplies provided by refuse dumps and commercial fisheries in recent years, and gives little indication of past selection pressures. In addition no attempt was made in these studies to measure possible lifetime fitness costs incurred by the increased seasonal reproductive effort involved in raising an enlarged brood. Furthermore, these brood enlargements were produced by merely adding a newly hatched chick to a recently hatched brood. This procedure fails to consider the cost of increased egg formation which producing an enlarged brood would entail, or the increased hatching spread which would accompany a four-chick brood. Whilst adults may be able to meet the increased costs of chick rearing, and successfully fledge artificially enlarged broods, they may be unable to meet the full costs of producing and incubating an extra egg as well as rearing an additional chick.

### 2. *Incubation capacity.*

The truncated frequency distribution of clutch size in gulls has been attributed to the inability to incubate clutches larger than three eggs. Although experiments on passerine species suggest that some birds can effectively incubate artificially enlarged clutches, in one of his earliest papers on clutch size, Lack (1947, 1948) appreciated that gulls may be a special case and incubation efficiency may set an upper limit on clutch size. Gulls lay unusually large eggs, which may be related to their developmental precocity (see Martin 1987), and have only three brood patches, which may limit clutch size to three eggs. The suggestion is that the pre-gull ancestor may have laid only three eggs because food supply could not support more than this, which led to evolution of three brood patches, which now imposes selection against clutches of more than three eggs (Coulter 1987). This hypothesis begs the question of adaptation, since other groups of birds can successfully incubate clutches of more than three eggs, and one might expect behavioural (increased nest insulation or frequency of egg turning) or physiological (greater vascularisation of the brood patch and heat transfer) adaptations in the absence of morphological plasticity.

Although the hypothesis that gulls with three brood patches cannot effectively incubate enlarged clutches is easily tested, there are few unequivocal published data for larids. Reid (1987) added eggs to newly completed clutches to create clutch sizes ranging from 1 to 5 eggs in glaucous-winged gulls. The data he presents indicate that the enlarged clutches did not hatch significantly more eggs than the three egg clutches, and the proportion of eggs hatched was highest for the three egg clutches. However, the failure of many of the eggs in the enlarged broods was due to the tendency of pairs to stop incubating viable (even pipping) eggs after three or four eggs had already hatched. The failure to successfully hatch enlarged broods may not therefore be due a failure to successfully maintain egg viability during incubation but rather a failure to continue to incubate unhatched eggs after several chicks have hatched and require food.

### *3. Egg formation ability.*

Traditionally the female's ability to produce eggs has been largely disregarded as a likely selection pressure in the evolution of clutch size, since many bird species, including gulls, are able to lay replacement eggs if eggs are removed from the nest soon after laying. This may seem good evidence for the lack of any constraints on the ability to produce eggs. However, in herring gulls these replacement eggs are not laid in a continual sequence of one egg every other day, as in a normal clutch. Instead the pattern of laying more closely resembles the laying of several discrete "clutches" of three or four eggs with a period of about 10 - 15 days between successive "clutches" (Parsons 1976). This indicates that not all the material required for egg formation can be obtained from "daily surplus intake" (*sensu* Perrins & Birkhead 1983) and that females need to acquire reserves before laying further replacement "clutches". Further, the ability to lay replacements if eggs are removed within one day of laying does not provide adequate evidence that females have the resources to produce a fourth egg under normal circumstances. Eggs are usually laid at two day intervals and incubation begins on the day the first egg is laid. The necessity to start incubation may impose time constraints on foraging opportunity which could potentially limit the females' ability to acquire the resources necessary to produce a fourth egg. This potential time constraint is relieved under an experimental egg removal procedure. In addition, theoretical calculations of the protein requirements of egg formation in different species (Robbins 1981) suggest high egg formation costs for larids, and a number of experimental studies have indicated that the ability to produce eggs may limit either clutch or egg size in some gulls species (Winkler 1985, Pierotti & Bellrose 1986, Reid 1987).

### *Variation in clutch size*

Avian clutch size is known to vary in response to a variety of factors. Variation between years or breeding locality may be related to differences in food supply (Hogstedt 1980, Hornfeldt & Eklund 1989, Korpimaki & Hakkarainen 1991, Meijer, Daan & Dijkstra 1988), or population density (Arcese & Smith 1988). Among gulls, clutch size is known to increase to an upper limit according to age and breeding experience (Coulson 1966, Mills 1973, Reid 1988, Sydeman *et al* 1991) and most gull species show the seasonal decline in clutch size which characterises many avian breeding systems (Brown 1967, Davis & Dunn 1976, Mills 1979, Sydeman *et al* 1991). Part of the explanation for this decline with laying date is due to the effect of age on clutch size, since experienced birds which lay large clutches tend to lay in the early part of the breeding season. However, both Coulson (1966) and Mills (1973) have been able to demonstrate independent effects of both age and timing of laying on clutch size, for kittiwakes (*Rissa tridactyla*) and red-billed gulls (*Larus novaehollandiae*) respectively. The reasons for the effect of laying date on clutch size are unclear, but may be related to poorer survival of chicks fledged late in the season (Parsons, Chabrzyk & Duncan 1975).

### *Proximate control of egg production*

Since the early work of Jones & Ward (1976), there has accumulated a growing body of evidence for the role of body protein reserves (especially flight muscle protein) in the proximate control of the timing of breeding and/or regulation of clutch size. Correlative studies on a wide range of avian species, including Arctic nesting geese, gulls, weaver finches and warblers have suggested that the accumulation of threshold levels of body protein provides the trigger for breeding or laying and the amount of protein accumulated may determine clutch size (Jones & Ward 1976, Fogden & Fogden 1979, Ankney & MacInnes 1978, Houston, Jones & Sibly 1983, Jones 1991). However, it must be emphasised that there is likely to be large interspecific variation in the extent to which females draw on body protein rather than daily food intake to provide nutrients for egg formation, and some species which have a particularly protein-rich diet may be able to acquire all the nutrients for egg synthesis from their daily food intake. Studies on the body composition of pied flycatchers (*Ficedula hypoleuca*, Ojanen 1983) and sand martins (*Riparia riparia* Jones 1987), failed to detect a decline in body protein condition during the period of egg laying. Similarly, investigations into the reserve dynamics of two species of duck have suggested that the timing of laying and clutch size may be determined by the level of lipid reserves, and that the protein requirements of egg formation are met from exogenous sources (Hohman 1986, Alisauskas, Eberhardt & Ankney 1990). Meijer, Daan & Hall (1980) have highlighted that not all species will solve the problem of how to regulate clutch size, in the same way. An extensive series of studies into the control of egg production in the kestrel (*Falco tinnunculus*), has suggested that female body condition plays a minimal role in the determination of clutch size, and instead the number of eggs laid is a function of the timing of laying. They have also emphasised that correlative analyses do not provide good evidence of the causal role of protein condition in breeding, but rather that an experimental approach is required.

### *The significance of egg size*

Comparisons between species indicate that relative egg size (egg size/female body mass) increases with precocity; precocial species lay proportionally larger eggs that produce chicks which are larger and more developed at hatching (see review in Martin 1987). It is generally accepted that a similar relationship is also found within species. Chicks hatching from large eggs are larger, have greater yolk reserves, grow faster and survive better than chicks from small eggs (review in Martin 1987). However, the confounding effects of parental quality have seldom been examined, and some studies have found that these effects of egg size can be overridden by variation in parental care (Ricklefs 1984, Amundsen & Stokland 1990).

### *Study species*

The lesser black-backed gull was chosen as the study species for this investigation for a variety of reasons. Robbins (1981) has demonstrated that the daily protein requirements of egg formation are particularly large for gulls, in comparison with other species. He calculated the protein requirement of egg formation, per egg, as a percentage of the daily maintenance protein requirements. Of the six groups studied (passerines, galliformes, raptors, ducks, shorebirds and gulls and terns), larids had the highest protein demands of egg formation, in relation to maintainence requirements (232%, compared with figures of 228% for ducks and 165% for passerines), although no account was taken of clutch size. In addition, there is evidence of the role of pectoral muscle protein as a source of nutrients for egg formation in lesser black-backed gulls: Houston, Jones & Sibly (1983) have demonstrated that flight muscle protein levels decline sharply during the period of egg formation, and there is a positive relationship between protein levels and the number of eggs laid.

The lesser black-backed gull is an ideal study species for an experimental investigation into the role of food supply in the determination of clutch size. They typically nest in large, accessible colonies, and will readily consume a variety of different food supplements, which can be targeted at specific pairs. They are remarkably tolerant of disturbance; nests may be visited daily, and egg manipulations performed without risk of desertion. Adults may be trapped on the nest soon after the start of laying (even repeatedly) with no observable effect on subsequent behaviour or breeding performance.

### *Study sites*

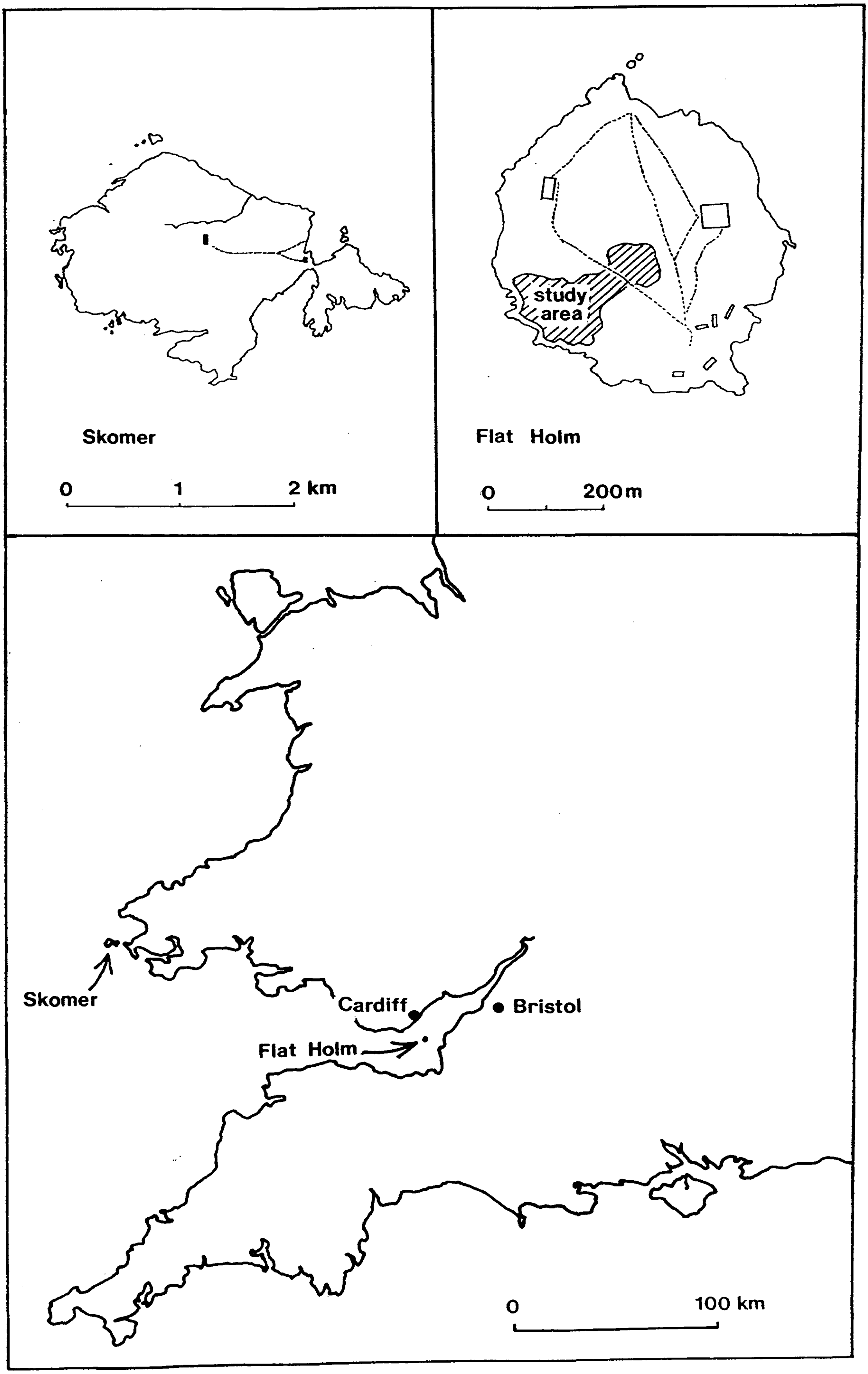
The fieldwork for this study was carried out on Flat Holm Island (South Glamorgan U.K. $51^{o}$ 45'N. $5^{o}$ 21'E., Figure 1) between March 1989 and August 1990. In addition, data collected by Louise Hiom from a supplementary feeding experiment on Skomer Island (Pembrokeshire, $51^{o}$20'N. $3^{o}$10'E.) between April and July 1988 are also included (chapter 2).

Flat Holm is composed of Carboniferous limestone and covers approximately 23 hectares. The vegetation is dominated by nettle (*Urtica dioica*), ragwort (*Senecio jacobaea*) and bracken (*Pteridium aquilinum*), with smaller areas of elder (*Sambucus nigra*) and bramble (*Rubus* spp.) scrub. The island was first colonised by lesser black-backed gulls in 1957, and the population increased steadily to a peak of approximately 4055 pairs in 1974 (Mudge 1978). Since then the number of breeding pairs has declined to around 1500 pairs and has remained apparently stable since 1986 (P. Ferns *pers. comm.*).

### *Controlling for the effects of age and laying date*

In view of the large number of variables known to influence the number of eggs laid by gulls, in particular the effects of age, the studies described here have adopted an experimental approach. Whilst the age of most of the individuals was unknown, it is assumed that there were no differences in age structure between treatments. In the establishment of study plots, and the allocation of pairs to treatments, care was taken to ensure that pairs were either allocated at random, or, in the case of the feeding experiments where treatments were grouped, that treatments were as similar as possible in terms of nesting density and distance from the edge of the colony and were located in contiguous areas of the subcolony. The possible effects of laying date on clutch size were investigated by comparison of laying dates between treatments, and although some differences did exist, in no case could they account for the differences observed between experimental groups.

**Figure 1.** Location of study sites; Skomer and Flat Holm Islands (note differences in scale).


Skomer
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study area
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Cardiff
Bristol
Flat Holm
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## *Aims*

The focus of this study is on the factors involved in the proximate regulation of both clutch size and egg size, in particular the roles of food supply, food quality and body condition. Body condition was assessed on the basis of flight muscle protein levels, rather than merely gross body weight, since the flight muscles have been implicated as a possible source of protein for egg synthesis in a number of avian species (Jones & Ward 1976, Fogden & Fogden 1979, Jones 1991). Chapter 2 details an improved method of determining flight muscle protein levels on the basis of body measurements which can be taken from live gulls, which is based on the earlier work of Sibly, Jones & Houston (1987).

The consequences of variation in egg size for chick survival was examined using a cross fostering experiment, described in chapter 3. This investigation tests the hypothesis that parents which lay large eggs provide better parental care than those laying smaller eggs, leading to a correlation between egg size and chick survival, which is independent of a direct egg size effect. The relative contributions of the parental effect and egg size are examined using a logistic model of the type proposed by Martin (1987).

The roles of food availability and food quality in determining both clutch size and egg size are investigated through a series of supplementary feeding experiments, described in chapters 4 and 5. By providing food supplements which differ in nutritional composition, but not calorific value, the hypothesis that egg production may be limited by protein supply during the prelaying period is tested. The precise protein requirements are further examined by providing supplements of different protein types in a further experiment, to investigate the hypothesis that egg synthesis demands specific amino acids which are scarce in the normal dietary intake. The role of flight muscle protein condition in the regulation of clutch and egg size is examined in Chapter 6, by relating the protein condition of females at the start of laying to their supplementary feeding regime, and the number and size of eggs laid.

Finally, a series of clutch enlargement experiments is used to investigate the current limits to clutch size in lesser black-backed gulls, independently manipulating the costs of egg production, incubation and chick rearing. This experimental approach examines whether the usual truncation of clutch size at three eggs is a result of incubation capacity, chick rearing ability, or the costs associated with production of a fourth egg.

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# CHAPTER 2

# AN IMPROVED TECHNIQUE FOR ESTIMATING PECTORAL MUSCLE PROTEIN CONDITION FROM BODY MEASUREMENTS OF LIVE GULLS

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## SUMMARY

Body measurements, which could be taken from live birds, were used to estimate total pectoral muscle protein in lesser black-backed gulls. The maximum cross-sectional area of the flight muscles was measured from the profile of the muscle surface over the keel, and this was used in conjunction with the length of the flight muscle to estimate muscle volume. The estimate of muscle volume was then used with fresh body weight to estimate total flight muscle protein. A highly significant correlation was found between the estimated values and actual pectoral muscle protein mass determined by carcass analysis. The model developed from the source group was then validated using a second independent sample, in which flight muscle protein was estimated from the model. Carcass analysis again demonstrated a good correlation between estimated and actual total protein. Different methods of controlling for body size to calculate protein condition from measures of total protein were considered. The technique described here provides a simple and reliable method of estimating pectoral muscle protein condition in live gulls which could be applied to a wide range of studies of body condition in other species.

# INTRODUCTION

The assessment of body condition is an important aspect of a wide variety of avian studies. However, any rigid definition of the term "body condition" makes assumptions about the partitioning and utilisation of resources. Studies of condition usually quantify the amount of either fat or protein which may be available for specific activities such as fuel for migration, or to provide material for egg production. Whilst fat levels may be built up and "set aside" for a specific purpose, muscle protein is continually being broken down and replaced so the term "reserve" is less applicable when considering protein condition. A general definition of protein condition may simply be an assessment of the protein content of the body, relative to body size, which may change in relation to food intake and metabolic demand and which has consequences for the survival and reproductive potential of an individual.

Determination of the whole body protein content of an individual can be time consuming and costly, so pectoral muscle protein content is commonly used as an index of body protein instead (for example Evans and Smith 1975; Houston 1977). This muscle block is discrete, can easily be dissected and is the largest muscle block in most bird species, representing some 15% of total body weight in lesser black-backed gulls. Furthermore, the importance of the pectoral muscles as a possible source of protein for egg production has been demonstrated in a wide range of species, such as red-billed quelea *Quelea quelea* (Jones & Ward 1976), lesser snow goose *Chen caerulescens caerulescens* (Ankney & McInnes 1978), lesser black-backed gull (Houston *et al.* 1983). In all these species there is a decline in pectoral muscle protein associated with egg formation, and potential clutch size has been shown to be positively correlated with flight muscle condition in pre-laying female lesser black-backed gulls (Houston *et al* 1983).

The pectoral muscles are therefore a logical starting point for investigations into body protein condition.

Traditionally body protein and fat content, and hence condition, have been determined by soxhlet extraction of lipids from whole carcasses (e.g. Jones & Ward 1976, Fogden & Fogden 1979, Houston *et al.* 1983, Hails & Turner 1985) or from aliquots following carcass homogenisation (eg. Hohman & Taylor 1986). These methods are not only time consuming but also suffer the major drawback that they cannot be used on live animals. Attempts have therefore been made to estimate body condition from live body measurements and some degree of success has been achieved in predicting the fat reserves of a number of avian species (e.g. Wishart 1979, Gauthier & Bedard 1985, Perdeck 1985, Sibly *et al.* 1987). However some doubt has been cast on the general accuracy of these techniques, since most have large errors of estimation associated with them (see Perdeck 1985). In comparing protein and fat content between individuals it is necessary to take into account variation resulting from differences in body size, rather than condition. For example, Sibly *et al.* (1987) used stepwise regression to assess body size in gulls and were able to predict fat content accurately, but were unable to estimate protein condition from body measures.

This paper presents a study of body measurements of lesser black-backed gulls in an attempt to provide a more accurate technique for estimation of pectoral muscle protein condition in live birds. Here differences in the profile of the pectoral muscles over the keel are used, together with other body measures to estimate pectoral muscle protein condition. It was possible to validate the technique by carcass analysis of a sample of dead birds available from a cull which had been carried out as part of a population control programme.

## METHODS

### *Carcass analysis*

A number of lesser black-backed gull corpses (16 male  30 female) were available from a cull of breeding birds at Abbeystead in Lancashire by the North-West Water Authority for public health reasons in  May 1988. The gulls had been killed by placing bait containing alpha-chloralose on the breeding territories. The corpses were collected 2 - 3 hours after death and fresh body weight was recorded. Each bird was individually wrapped in a plastic bag, taking care not to deform the shape of the pectoral region, and frozen until analysis.

After thawing overnight at room temperature, the following biometrics were taken: Total head and bill length, wing-length, tarsus-length, keel-length, and the length from the mid-point of the posterior end of the sternum to the distal end of either clavicle (the effective length of the Pectoralis major), which is referred to here as the sternum diagonal. Both right and left pectoral muscles (Pectoralis major and Supracoracoideus) were dissected from the sternum and weighed, dried at 60'C to constant mass and reweighed. Lipid was then removed by soxhlet extraction using chloroform solvent and the muscles were again dried at 60'C to constant weight and reweighed to determine pectoral  muscle lean dry weight (PMLDW). Weights used were the sum of the left and right muscle blocks.  After the pectoral muscles had been dissected from the sternum, the maximum keel height (about 1.5cm. from the anterior end) was measured.

### *Measurement of the pectoral muscle profile*

The pectoral muscle profile was recorded perpendicular to the keel 1.5cm. from its anterior end. In order to measure the profile it was necessary to prepare a band about 0.5cm wide clear of feathers. This could be achieved without removing feathers, but by carefully moistening and parting them. A 20cm length of 5-core solder wire was then pressed firmly and evenly across the pectoral muscles starting at the keel and working laterally towards the dorsal surface, perpendicular to the keel, (Figure 1), pressing down both sides simultaneously. Adequate preparation of the pectoral area was essential to obtain a good trace. Two traces were taken from each bird and a permanent record of each was made by placing the wire flat on a piece of paper and spraying over with aerosol paint thereby leaving an imprint.

The two profile traces from each bird were analysed separately and the following measures were taken, as shown in Figure 2:

1. The angle subtended by the surface of the muscle blocks across the keel.

2. Three different measures of the cross sectional area of the pectoral muscles were obtained from each trace, in order to establish the best measuring technique.

The first (Area i) was taken at a depth of 2 cm. from the apex and the second (Area ii) at a depth of 3 cm. The third (Area iii) was measured at a variable depth, $h$, where $h$ was an estimate of the actual keel height. It is necessary to estimate $h$ from other biometics because it cannot be measured directly on live birds. The value of $h$ was obtained from the regression of keel height on keel length (Equation (1); Keel height = 0.213 x keel length + 6.711 $r_{44} = 0.679$ $P<0.0001$) The prediction of keel height could not be improved by adding further biometric variables. Covariance analysis demonstrated that there was no significant effect of sex on either the slope or the intercept indicating that the regression was not significantly different between males and females.

**Figure 1.** Ventral view of lesser black-backed gull, showing method used to obtain a trace of the pectoral profile. The feathers were parted in a line perpendicular to the keel, then a length of solder wire was carefully pressed over the keel and muscle blocks.

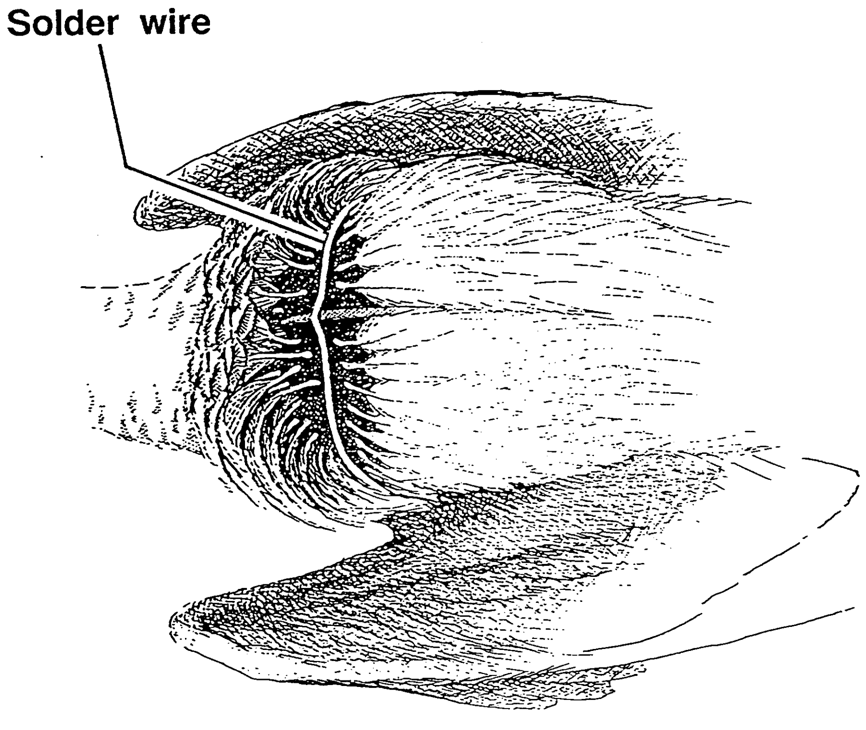
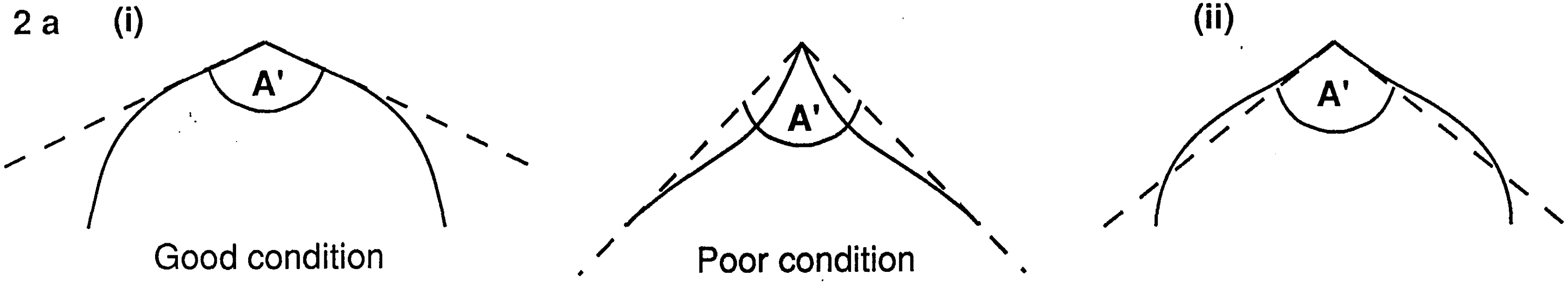

Solder wire

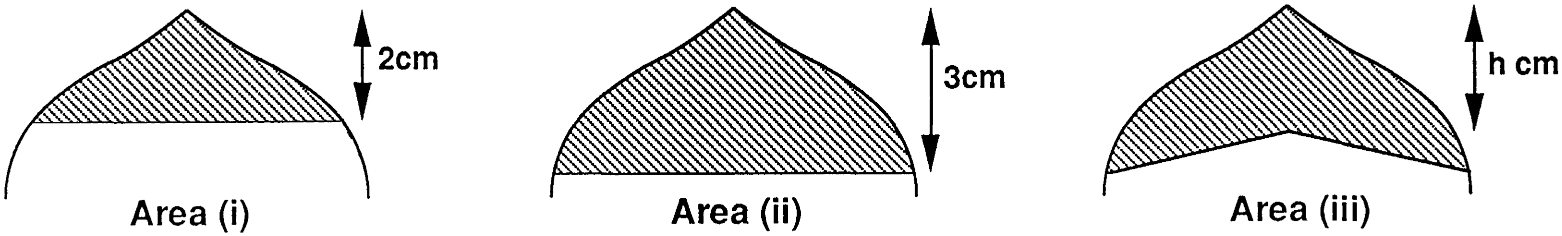
**Figure 2. a.** (i) Method used to determine "pectoral angle" from the profile trace in this study. N.B. The measurement of the pectoral angle is different from that used by Sibly *et al.* (1987) which is shown in (ii).

**b.** Measurement of the three areas i-iii from the profile trace.

2 a


(i)
A'
Good condition
A'
Poor condition
(ii)
A'

2 b


2cm
Area (i)
3cm
Area (ii)
h cm
Area (iii)

Areas (i) and (ii) were bounded by a line perpendicular to the keel, whereas for area (iii) the lower limit was at an angle of 105' to the lower edge of the keel, (this being the mean angle subtended between the keel and the sternum) on either side, as shown in Figure 2.

Each of these measures of pectoral cross-sectional area was separately multiplied by sternum diagonal (total muscle length) or keel length (length of the major part of the muscle) to produce six separate estimates of total muscle volume, as follows;

A. Area(i) x sternum diagonal
B. Area(ii) x sternum diagonal
C. Area(iii) x sternum diagonal
D. Area(i) x keel length
E. Area(ii) x keel length
F. Area(iii) x keel length

Throughout this paper means are given +/- one standard deviation.

### *Assessment of body size*

Since pectoral muscle mass will vary in relation to body size, it is necessary to take size into account when making comparisons in condition between individuals or groups from differing size classes. Various authors have used a number of different body measurements to estimate body size, not all of which can be taken on live birds (for example, Fogden and Fogden 1979, Jones 1987, Piersma *et al.* 1984). Freeman and Jackson (1990) have recently shown that univariate biometrics may be inadequate as a measure of overall body size, since some univariate measurements explain only a relatively small proportion of the total variance in overall body size. Both Freeman and Jackson (1990) and Rising and Somers (1989) suggest that a more accurate assessment of body size is obtained from principal component analysis, which reflects variance in a larger number of body measurements. Principal component analysis of five body measures (total head and bill length, tarsus-length, wing-length, keel-length and sternum-diagonal) was used to produce a single "body size" factor. Only one factor was extracted by the analysis, with high loadings (>0.88) on all five body measures. However, whilst principal component analysis may provide a more accurate measure of general body size, the more appropriate measure for the purpose of this study might be the use of a variable which is more directly related to the size of the sternum, to which the pectoral muscles are attached, such as sternum length, or sternum diagonal. In order to determine which of these three possible measures was more appropriate, the simple correlations of each with pectoral muscle lean dry weight were examined.

### *Calculation of condition*

Any attempt to measure body condition must first determine total mass of the body component in question, and then allow for variance due to differences in body size. In previous studies of body condition, the most common method to control for body size is to divide the total mass of the body component by some coefficient of body size (eg. Hails & Turner 1985). However, Packard and Boardman (1988) have cast doubt on the use of such ratios to scale data that vary allometrically with body size. A more accurate size correction may be obtained using the regression of the total mass of the body component on body size to give a predicted component mass for an individual of a given size. The relative "condition" of an individual is then derived from the deviation ($d$), of the actual component mass from that predicted ($r$) from the regression equation, (i.e. the size of the residual), as shown in Figure 3. There are then two possible ways of using *d* as an index of condition. It may be expressed as an absolute value, which would give equal condition status to all individuals having the same *d* value, regardless of their body size. This method enables direct computations of the amount of material which may be available for egg production, or use as fuel etc. However, a given amount of protein will be of greater value to a small individual than to a larger one, because of size related differences in metabolic costs. To take this into account, *d* may be expressed as a proportion of the predicted component mass (*r*). This gives greater condition status to small individuals compared with large ones having the same *d* value, and facilitates direct comparisons between individuals of different size, since condition is expressed relative to size. This latter method is the one chosen for this study, with condition being expressed as a positive or negative percentage of the regression prediction (*r*). However, it should be emphasised that the most appropriate method for calculating condition from *d* will vary according to the nature of the study.

### *Estimation of pectoral muscle protein condition from body measures*

There are two approaches to estimating pectoral muscle protein condition from pectoral profile. The first relates the pectoral muscle angle (Figure 2.) directly to the condition index derived from Figure 3. The second uses the estimate of muscle volume, in combination with other body measures, to estimate total PMLDW. From this an estimate of condition is derived, following the method outlined in Figure 3, using estimated PMLDW in place of actual PMLDW.

In all the following regression analyses, covariance analysis was applied to determine the effect of sex. In no case was sex found to have a significant effect on the slope or intercept, so data from males and females were pooled for all

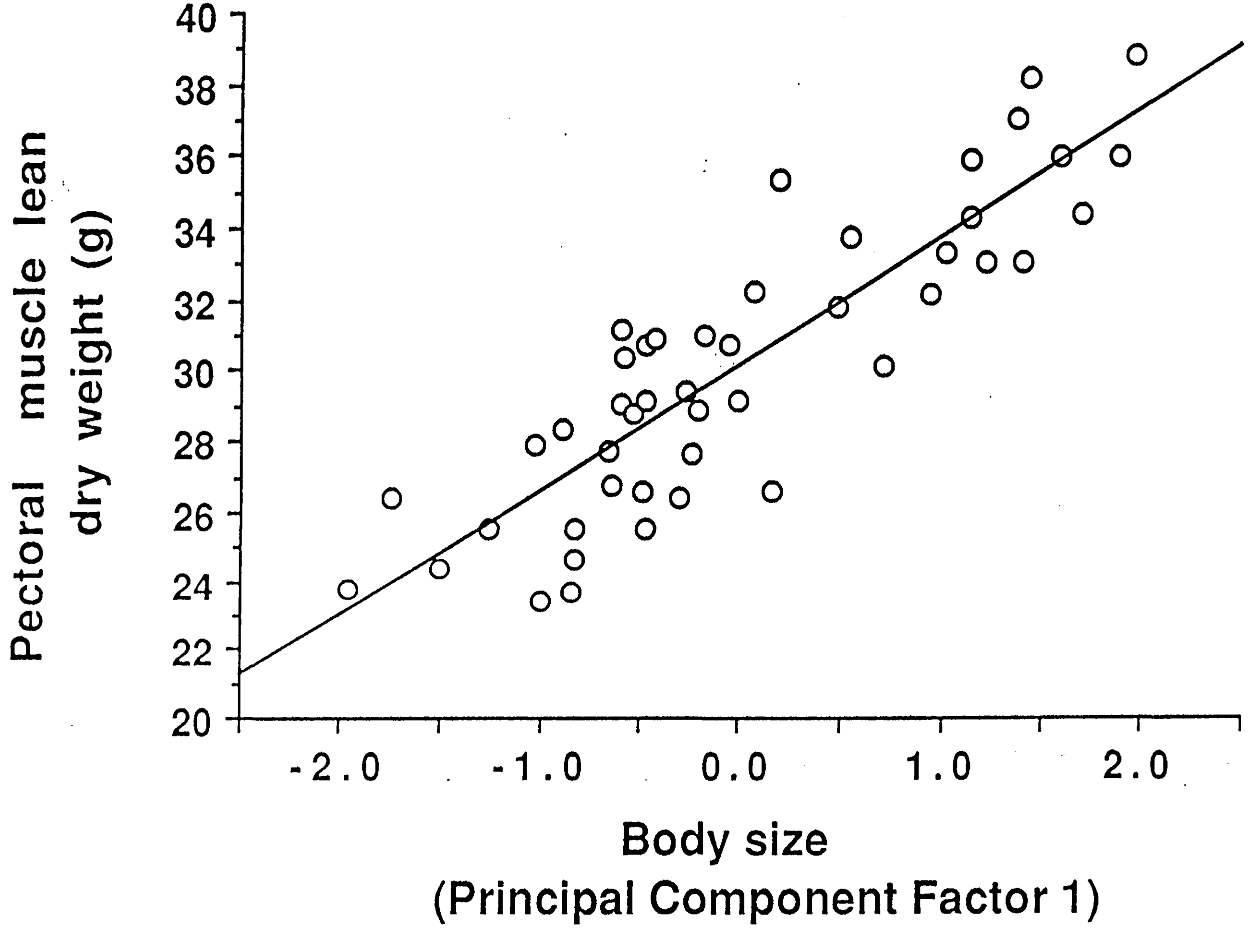
**Figure 3.** Derivation of an individual's actual condition from the deviation of total mass (PMLDW) from that predicted from the regression equation with body size. In this study, to make comparisons between large and small individuals, the deviation is expressed as a percentage of the total mass expected from the regression.

Condition Index = $100(d/r)$

$r$=regression estimate of PMLDW

$d$=deviation from regression estimate

Regression equation: PMLDW = 3.558(body size) - 30.132


Pectoral muscle lean dry weight (g)
40
38
36
34
32
30
28
26
24
22
20
-2.0
-1.0
0.0
1.0
2.0
Body size
(Principal Component Factor 1)

regressions and the resulting equations were applied to both sexes.

## RESULTS

### *Most appropriate measure of body size*

The measure of body size which showed the strongest correlation with PMLDW was the multivariate Principal Component Factor 1, rather than sternum length or sternum diagonal (Pearson's $r_{44}=0.87$ $P<0.001$ ; $r_{44}=0.73$ $P<0.001$ and $r_{44}=0.79$ $P<0.001$ respectively). This is presumably because variation in flight muscle mass is more closely related to variance in overall body size rather than simply sternum size. PC Factor 1 has therefore been used as a measure of body size in the following analyses.

### *Precision of the method of recording muscle profile.*

In order to determine the level of precision associated with recording the pectoral muscle profile, the difference in pectoral angle and area between repeated traces from each individual was calculated. These were then expressed as a proportion of the mean of the two traces. The difference in area was on average 0.031 of the mean area, (s.d.=0.024, N=46), and the difference in recorded angle was on average 0.023 of the mean angle (s.d.=0.016, N=46) which indicate that highly consistent measures of the pectoral profile were obtained.

### *Variation in lipid content of the flight muscles.*

Since the method described here is unable to discriminate between differences in pectoral muscle volume due to variation in fat or protein content of the pectoral muscles, it is important to determine the extent of variation in lipid content of the fresh muscle, since large variation in lipid would prevent accurate determination of flight muscle protein. Lipid removed by soxhlet extraction comprised between 0.020 and 0.057 of the total wet muscle weight (mean=0.039 s.d.=0.009 N=46), which is considered sufficiently small to permit reliable estimation of protein content from the muscle profile.

### *Estimation of pectoral muscle condition from the pectoral angle*

The pectoral angle may be considered to be a direct measure of muscle condition, being independent of body size (Pearson's correlation with PC Factor 1; For females; $r_{28}=0.081$ $P>0.05$ For males: $r_{14}=0.256$ $P>0.05$). The accuracy with which the pectoral angle estimates condition can be determined from the strength of its correlation with the condition index. Figure 4 shows that this correlation, though statistically significant, is rather weak, only 14% of the variance in condition being related to pectoral angle. Therefore pectoral angle is considered to be only a poor predictor of flight muscle condition.

### *Estimation of pectoral muscle condition from muscle volume*

PMLDW was estimated separately from each of the variables A - F (different measures of muscle area multiplied by muscle length). The strength of the correlations between each of these estimates and actual PMLDW are given in Table 1.

**Figure 4.** The relationship between pectoral angle and pectoral muscle condition ($r_{44} = 0.379$ $P<0.005$).

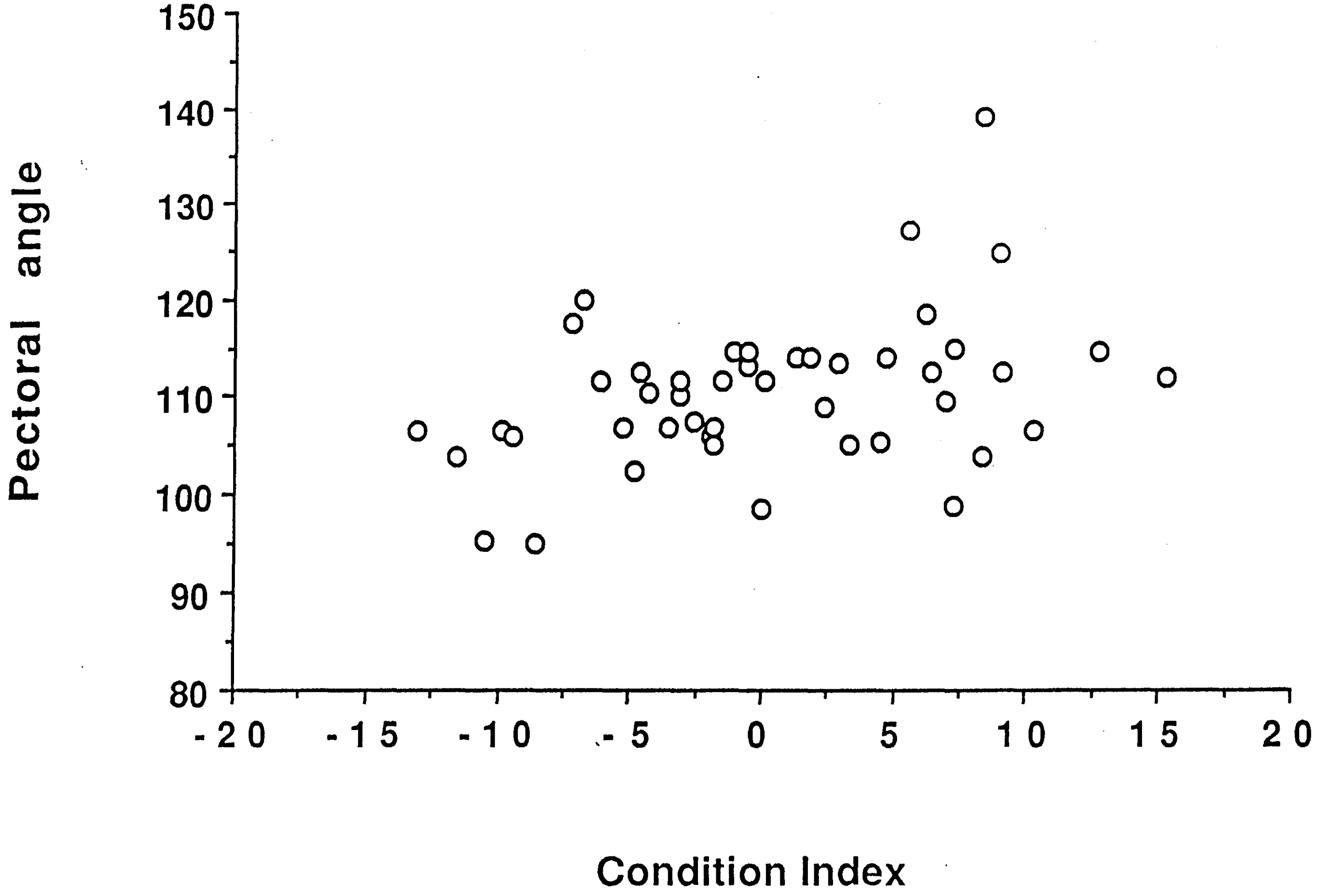

150
140
130
120
110
100
90
80
-20
-15
-10
-5
0
5
10
15
20
Pectoral angle
Condition Index

Table 1. Pearson correlations with actual PMLDW for each of six estimates of pectoral muscle volume from body measures.

| Variable | r | N | sig. |
|---|---|---|---|
| A. Area(i) x stern. diag. | 0.707 | 46 | 0.00001 |
| B. Area(ii) x stern. diag. | 0.775 | 46 | 0.00001 |
| C. Area(iii) x stern. diag. | 0.753 | 46 | 0.00001 |
| D. Area(i) x keel length | 0.675 | 46 | 0.00001 |
| E. Area(ii) x keel length | 0.747 | 46 | 0.00001 |
| F. Area(iii) x keel length | 0.704 | 46 | 0.00001 |

Fresh body weight was then added as a second variable to each of these in a stepwise regression procedure in an attempt to increase the accuracy of the estimate further. The strongest correlation with actual PMLDW was given by regression of PMLDW on variable C (area(iii) x sternum diagonal) and fresh body weight, as given below:

**Step 1.** variable C entered, multiple $r=0.753$ adjusted $r^2=0.557$

$F_{44}=57.67$ $P<0.0001$

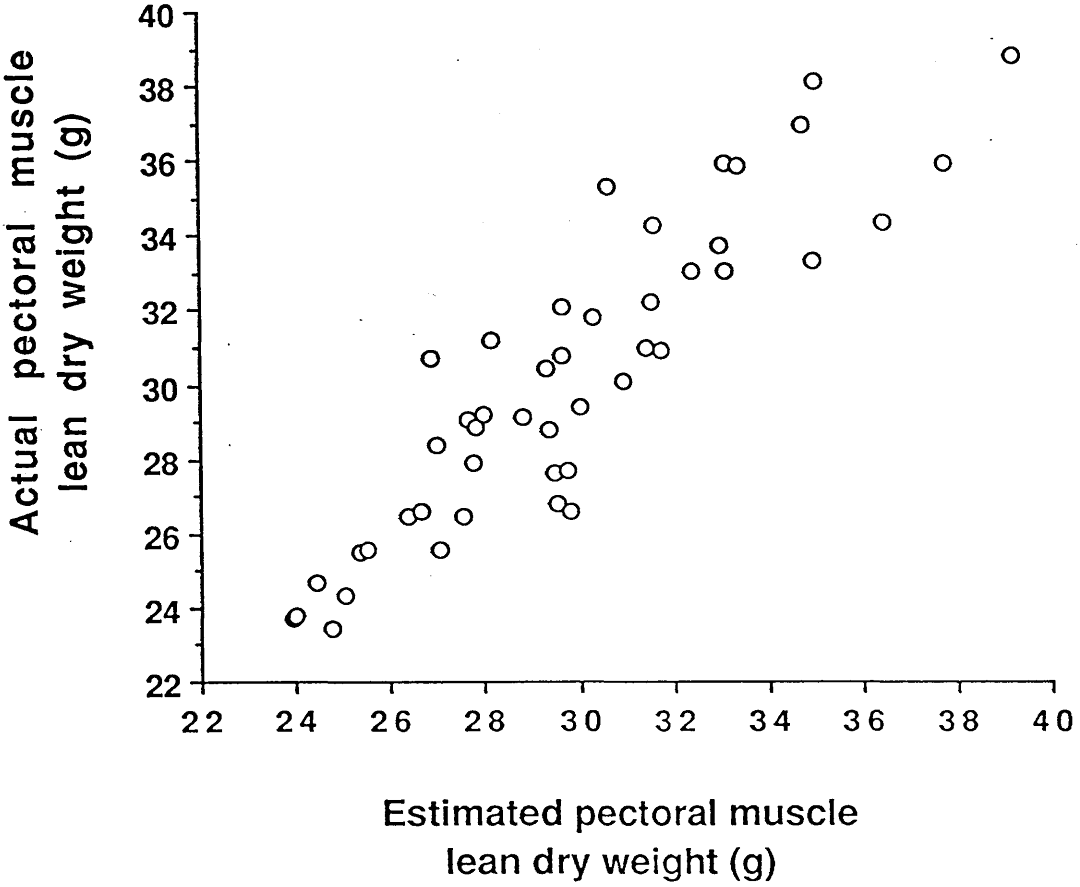
**Step 2.** fresh body weight entered, multiple $r=0.901$ adjusted $r^2=0.804$

$F_{44}=93.11$ $P<0.0001$

Regression equation: $\text{PMLDW} = 0.008(\text{variable C}) + 0.024(\text{body weight}) + 1.160$ (2)

The relationship between actual and estimated PMLDW, (derived from the above equation) is shown in Figure 5. A measure of the accuracy of the estimate of total PMLDW is obtained from the difference between the estimated and actual values, expressed as a proportion of the actual value. The mean difference

**Figure 5.** The relationship between the best estimate of PMLDW (derived from stepwise regression using estimated pectoral muscle volume and fresh body weight) and actual PMLDW ($r_{44} = 0.901$ $P<0.0001$).


40
38
36
34
32
30
28
26
24
22
22
24
26
28
30
32
34
36
38
40
Actual pectoral muscle lean dry weight (g)
Estimated pectoral muscle lean dry weight (g)

was 0.045 of the actual value (s.d.=0.035, N=46) indicating that an accurate estimate of total flight muscle protein had been obtained.

From the estimate of total PMLDW it is possible to derive an estimate of condition, (following the second procedure outlined in the methods), where the estimated value of total PMLDW (as derived above) was used in place of actual PMLDW. The correlation coefficient between the actual condition index, derived from actual PMLDW and the estimated condition index was $r_{44} = 0.614$, $P<0.0001$. The mean difference between estimated and actual values was 4.37%, (s.d.=3.49 N=46) compared with a range in condition of -13.24% to 14.78%. Unlike the discrepancy between estimated and actual PMLDW above, it is not meaningful to express the discrepancy between estimated and actual condition as a proportion of actual condition, as most birds have actual values around zero ("average" condition). This means that the value of such a proportion will vary widely , not due to the size of the error of estimation, but depending on how close actual condition is to zero, as any difference expressed as a proportion of zero will tend to infinity.

### *Independent assessment of the accuracy of predicting PMLDW*

In order to determine the accuracy of the model to predict pectoral muscle condition it was applied to an independent sample. A further sample of 12 carcasses (6 males, 6 females) was available, which had not been used in the original analysis from which the model had been derived. These were analysed in the same manner and estimated PMLDW and condition were calculated from the model. This was then compared with the actual PMLDW determined from soxhlet extraction of lipid. There were significant correlations between estimated and actual PMLDW ($r_{10}=0.908$ $P<0.001$) and between estimated and actual pectoral muscle condition ($r_{10}=0.639$ $P<0.02$). The mean difference between estimated and actual PMLDW, expressed as a proportion of the actual value was 0.041 (s.d.=0.034 N=12). The mean difference between estimated and actual condition was 3.70% (s.d.=2.20 N=12), compared with a total range in actual condition of -7.23% to 8.71%.

## DISCUSSION

### *Accuracy of estimating PMLDW and pectoral muscle condition*

The correlations between actual and estimated flight muscle protein were highly significant for both the source and test groups while the relatively small discrepancies between estimated and actual PMLDW indicated that the estimates were sufficiently accurate to be useful for estimating condition. By allowing for body size, flight muscle protein condition was then calculated for both groups from both actual and estimated PMLDW. The decrease in accuracy when estimating condition in comparison with estimations of PMLDW was because the discrepancy between actual and estimated values of total PMLDW, though small, became more significant when considering the size of the *residuals* in the regression on body size, rather than merely the absolute values of PMLDW. This highlights the need for highly accurate estimates of total PMLDW in order to obtain reasonably accurate estimates of protein condition which allows for body size.

Examination of the accuracy of estimating total PMLDW in both the source and test group shows that for *individual* birds the estimation may be subject to errors of up to +/-13% of the actual value. However, the mean errors in the estimation of both total PMLDW and condition in samples of 46 (source group) and 12 (test group) were acceptably low and the accuracy was no lower in the smaller group compared with the larger one. This indicates that whilst estimates for individual birds may be unreliable, comparisons between groups with sample sizes of 10 - 15 should provide reliable estimates of average condition for the group.

### *Comparison with other methods of determining protein condition in live animals*

There are currently a number of methods available to determine whole body and pectoral muscle protein condition in live animals. An accurate measure of total body lean mass can be obtained from the electrical conductivity of the body tissues using equipment developed for the meat industry. This method has been used with considerable success on live animals covering a range of species of rodents and birds (Walsberg 1988). For birds, the maximum value of $r^2$ between estimated and actual whole body protein was 0.988, in an analysis across 15 species ranging from 14.6g to 170g. However, Walsberg presents no data relating to the accuracy of estimating total body protein over smaller ranges within species, so direct comparison with the results obtained in this study is not possible. A major drawback of the electrical conductivity technique is that its use is limited to relatively small animals; although a number of body composition analysis machines are commercially available, those for use on animals weighing more than about 400 g. are very expensive and are not portable.

Several studies have shown that the size of body reserves can be estimated using ultrasound meters developed initially for measurement of the thickness of inert materials in industry, (Baldasarre 1980, Sears 1988). Sears (1988) used ultrasound to measure the thickness of the breast muscle over the sternum in mute swans *Cygnus olor*. She demonstrated that ultrasound measurements of muscle thickness are both repeatable and accurate. The mean variance of repeat measurements of muscle thickness was 3% of their mean, (a similar value to that obtained in this study for the precision of measuring the pectoral muscle area). The measurements of muscle thickness showed a highly significant correlation with pectoral muscle lean dry weight ($r=0.929$ $n=27$ $p<0.001$, a figure which is very similar to that obtained for lesser black-backed gulls here). Sears made no attempt to calculate condition for individuals by controlling for body size, so it is not possible to make direct comparisons of the accuracy of estimating condition. However, the figures given above indicate that the level of accuracy achieved by the ultrasound technique applied to mute swans is rather similar to that of the technique described here, although the two species considered are very different in size and probably also in sternum structure.

### *General applicability of the profile technique*

The results presented here indicate that accurate determination of total flight muscle protein is possible from careful measurement of the profile of the pectoral muscles over the keel, together with other body measures. The estimates of total protein thus obtained are sufficiently accurate to allow the calculation of condition by allowing for body size. Whilst the estimates of condition of individuals is subject to some error, the technique allows accurate assessment of the average condition of samples of 10 - 15 birds.

The method presented here is intended as a model for others workers to follow in developing a set of equations from which to determine pectoral muscle condition. For fellow gull workers, it is suggested that the equations given here may suffice for estimating condition of other larid species, though carcass analysis of species of different body size would be helpful. Workers studying other avian groups should derive their own equations, following these methods, which will necessitate carcass analysis. The steps to be followed to calculate condition from the equations presented here are;

1. Calculate keel height from equation (1).
2. Using this estimate of keel height, measure area (iii) (figure 2) from the profile trace.
3. Compute the estimate of flight muscle volume (Variable C) from the product of area (iii) and sternum diagonal.
4. Calculate total PMLDW from equation (2).
5. Use a principal component analysis of the body measures in the sample to generate an overall body size score. Alternatively a single variable such as tarsus-length could be used, but this is subject to the limitations discussed above.
6. Pectoral muscle condition is then derived from the regression of calculated calculated PMLDW (step 4) on body size.
7. Condition is then calculated from the regression residuals. These may either be expressed directly, in terms of their absolute magnitude and sign, or alternatively they may be expressed relative to the predicted PMLDW on the basis of body size, as discussed in the methods.

One potential problem with the technique is the failure to discriminate between protein and fat overlying and within the muscle tissue. This problem may be overcome if it can be demonstrated that variation in lipid content lies within reasonable limits. When birds lay down fat reserves they generally accumulate as localised deposits such as the great omentum and in the tracheal pit, rather that being dispersed within the musculature. It may therefore be generally true that variations in level of lipid associated with the pectoral muscles lie within tolerable limits. However, species which spend a considerable amount of time on water will undoubtedly lay down significant amounts of subcutaneous fat over the pectoral region in winter, partly for insulation (Piersma 1984). It may be possible to control for this, since a number of studies have shown that estimation of body lipid reserves may be possible on the basis of body measures. If there is a good correlation between total body lipid and lipid associated with the pectoral region then it may be possible to control for differences in pectoral lipid when estimating flight muscle protein.

Given that variations in lipid levels will lie within reasonable limits in many situations, the method presented here provides a simple and accurate method of estimating total pectoral muscle protein and pectoral muscle condition in live gulls, which should be applicable to a wide range of studies of body condition in other species.

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# CHAPTER 3

# DETERMINANTS OF CHICK SURVIVAL IN THE LESSER BLACK-BACKED GULL: RELATIVE CONTRIBUTIONS OF EGG SIZE AND PARENTAL QUALITY

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# SUMMARY

Avian egg size is commonly held to be an important index of egg "quality" since it reflects the quantity of yolk reserves available to the chick during embryonic development and on hatching. The assumption that egg size may therefore play an important part in determining chick prefledging survival overlooks the confounding variable of parental quality which may influence both egg size and fledging success. In order to test the hypothesis that the abilities to produce large eggs and to rear chicks successfully may be positively related, and to investigate the relative contributions of parental quality (ability to produce large eggs) and egg size *per se* to chick fledging success, a clutch transfer experiment was performed in which clutches of large eggs were cross fostered with clutches of small eggs.

Large eggs produced chicks which were not only skeletally larger (tarsus length) but also heavier for their size than those from smaller eggs, both of which could contribute to increased chances of survival. Logistic regression analysis showed that both egg size and the quality of the foster parents contributed significantly to the probability of fledging. Parental quality was more important than egg size in determining chick survival, which suggests that correlations between survival and egg size found by other workers in non-experimental situations may be due, in part, to the confounding effect of parental quality.

The contribution of large egg size to chick prefledging survival in the lesser black-backed gull, appears to be mediated via advantages of increased skeletal size, rather than body condition, on hatching. Since large egg size is advantageous, selection for increasing egg size is implied. The likely counterbalancing forces are discussed.

# INTRODUCTION

It is commonly believed that egg quality, as measured by egg size, is an important parameter in determining chick survival in many bird species. Several authors have demonstrated that egg size is correlated with chick hatching weight, hatching size, growth rate, survival and fledging weight (Parsons 1970, Schifferli 1973, Nisbet 1978, Lundberg & Vaisanen 1979, Moss *et al.* 1981, Birkhead & Nettleship 1982, Furness 1983). Parsons (1970) demonstrated that herring gull chicks from large eggs hatched with more yolk reserves, and suggested that these reserves were crucial for survival during the first week of life.

Much of this work has recently been reviewed by Mueller (1990), who challenged the assertion that chicks from large eggs have a greater probability of survival than those from small eggs. An important criticism, highlighted by Birkhead & Nettleship (1982), and Amundsen & Stokland (1990), is that previous studies either fail to consider the possible confounding effect of parental quality on both egg production and chick survival, or make no attempt to quantify its importance relative to the direct egg size effect. Parsons (1975) performed within clutch manipulations on the herring gull in order to control for parental quality and to demonstrate the significance of egg size on chick survival. However, this method provides no information concerning the magnitude of the direct effect of egg size in relation to the possible effect of parental quality. The hypothesis to be tested here is that there is a positive correlation between the capacity of parent gulls to produce large eggs and successfully rear chicks. If this hypothesis is correct, parents that produce large eggs will also provision their young well and have high fledging success compared with parents laying smaller eggs. This will result in a positive relationship between egg size and chick survival, mediated largely by differences in parental quality, rather than a direct effect of egg size.

This hypothesis is tested here through an egg transfer experiment in the lesser black-backed gull in which clutches of large eggs were cross fostered with clutches of small eggs. The ability of the foster parents to produce large eggs was determined from the total clutch volume of their original three-egg clutch.

For simplicity, those birds which laid clutches of large eggs are referred to hereafter as "high quality", and those laying clutches of small eggs as "low quality" parents. It will be appreciated therefore, that the usage of the term "parental quality" applied in this chapter refers only to the ability of the adults to produce large eggs in the year of the study (following Amundsen & Stokland 1990), and not their lifetime reproductive success. "Quality" as used here is thus a measure of ability of a pair to produce large eggs, relative to others breeding in the same conditions. This ability, in any particular year, is likely to be influenced by many factors, which may include the age of the female (Coulson 1966, Davis 1975 and Mills 1979), the extent of previous breeding experience, the number of previous breeding attempts with the current mate, the ability of the male to provide courtship food (Nisbet 1973), colony density and possibly body size (Perrins 1979, Murphy 1986). Growth and survival of each chick was then examined in relation to both the size of egg from which it had hatched (the direct egg size effect), and also the quality of it's foster parents, assessed from their ability to lay large eggs.

## METHODS

Lesser black-backed gulls lay a modal clutch size of three eggs, and clutches exceeding this are rare. The young are semi-precocial and fledge at 30 to 40 days (Cramp 1983). Forty-four nests containing clutches of three eggs were marked on Flat Holm Island between 16 and 19 June. Laying sequence was determined from the order of hatching, and the first, second and third laid eggs are referred to as a-, b- and c-eggs respectively. Each egg was individually marked with indelible ink and measured to the nearest 0.1mm with callipers. Egg volume ($cm^3$) was calculated from the equation (Harris 1964):

$$\text{Volume} = 0.000476 \times \text{length} \times \text{width}^2$$

Following Amundsen & Stokland (1990), parental quality and egg size were evaluated on the basis of total clutch volume. Clutches were assigned to either "large-egg" or "small-egg" categories according to which side they fell of the modal value (195 $cm^3$). This provides a satisfactory method of classifying the size of individual eggs since the size of the three eggs within a clutch show highly significant positive correlations (a-egg with b-egg Pearson's $r_{42}=0.848$, $P<0.001$; a-egg with c-egg $r_{42}=0.670$, $P<0.001$; b-egg with c-egg $r_{42}=0.729$, $P<0.001$) and the between-clutch component accounts for 74% of the total variance in egg size. Complete clutches were cross-fostered, in order to maintain the degree of hatching asynchrony, which is likely to play an important role in chick survival (Parsons 1975). Clutch transfers were carried out just before hatching to produce four treatments;

1. High quality adults rearing large-egg chicks (n=12).
2. High quality adults rearing small-egg chicks (n=11).
3. Low quality adults rearing large-egg chicks (n=11).
4. Low quality adults rearing small-egg chicks (n=10).

Eggs classified as large were on average 13.8% bigger than those classified as small (69.3 cm compared with 60.9 cm) a difference which was statistically significant ($t_{42}=8.60$, $P<0.01$). There were no significant differences in clutch volume between treatments 1 and 3, or 2 and 4 ($t_{21}=1.57$, $P>0.05$ and $t_{19}=0.681$,

$P>0.05$ respectively).

All nests were visited daily and surrounded with a chicken wire enclosure (approximately $3m^2$, 45cm high) within two days of hatching, to facilitate the location of chicks. Each territory was also provided with a wooden shelter, since nest enclosure denied chicks access to thick vegetation or rabbit burrows in which to shelter from rain or hot sun. All chicks were marked on the day of hatching with insulation tape around the tarsus, weighed (using a digital pan balance) and measured (tarsus length). Chick hatching condition was calculated using regression analysis to control for differences in body size. Hatching weight was regressed on tarsus length, and the residuals, expressed as a proportion of the predicted value, were used as a measure of chick condition on hatching. Tarsus length was used as a measure of skeletal size, rather than $(\text{tarsus length})^3$, since the former showed a stronger correlation with weight. Chicks were measured every three days until 16 days of age and then at six day intervals until 40 days of age, when they were judged to have fledged.

Since brood size may affect chick growth and fledging rates, this variable was held constant in all broods for the first two weeks by replacing chicks which died with chicks of similar age (assessed from tarsus length). Introduced chicks which were older than this were not accepted into the brood, so chicks which died after two weeks were not replaced. The subsequent growth and fledging success of replacement chicks was not recorded, so data presented for growth and mortality refer only to the original foster brood.

Attempts to fit conventional equations (Logistic, Gompertz, von Bertalanffy) to the tarsus and weight growth curves were considered inappropriate, since these methods require an estimate of the asymptote, and cannot therefore be applied to chicks which do not survive to fledging. Growth data were therefore analysed by regression during the linear phase between day four and twenty two. For each chick, weight was regressed against age and the slopes of the individual regressions were used to compare mean growth rates between groups. A similar analysis was carried out for tarsus length with age. Although this method allows an assessment of growth of chicks which do not survive to fledging, in order to fit a regression line to the data it was necessary to obtain at least four measures from each chick. It therefore excludes those individuals which die during the first two weeks of life, which might be expected to show lower growth rates. In order to examine the importance of egg size on chick survival during the first two weeks, I also compared egg size, hatching size (tarsus), hatching weight and hatching condition of chicks which survived the first 10 days with those that died during this period. The hatching success (proportion of eggs which hatched) and fledging success (proportion of chicks which hatched that subsequently fledged) of the three original foster chicks was recorded for each nest. Due to non-independence of data from chicks of the same brood, in making statistical comparisons between treatments, the degrees of freedom were reduced from relating to the number of chicks, to relate to the number of broods. Throughout this paper means are given ± one standard error.

# RESULTS

## *Hatching*

There were no significant differences in the mean a-egg hatching date or in hatching success between the four treatments (Table 1, one-way ANOVA: hatching date; $F_{3,36}=0.370$, $P>0.05$; hatching success Kruskal Wallis one-way ANOVA $H_3=2.956$, $P>0.05$).

Table 1. A-egg hatching dates and hatching success (proportion of eggs which hatched) and fledging success (proportion of chicks which fledged) by treatment (mean ± S.E. (N)).

| Treatment | A-egg hatching date | Hatching success | Fledging success |
|---|---|---|---|
| 1 | 3/7 ± 2.0 days<br>(11) | 0.75 ± 0.09<br>(12) | 0.33 ± 0.13<br>(11) |
| 2 | 3/7 ± 2.6 days<br>(10) | 0.79 ± 0.09<br>(11) | 0.38 ± 0.13<br>(10) |
| 3 | 1/7 ± 1.1 days<br>(10) | 0.91 ± 0.05<br>(11) | 0.27 ± 0.08<br>(11) |
| 4 | 4/7 ± 1.8 days<br>(9) | 0.73 ± 0.08<br>(10) | 0.00 ± 0.00<br>(10) |

However, there were significant differences in the hatching size, weight and condition of chicks according to treatment as shown in Table 2. Two-way analysis of variance showed that egg size, but not parental quality, had a significant effect on both tarsus length and hatching weight, (tarsus length: egg size $F_{1,36}=18.541$, $P<0.01$; parental quality $F_{1,36}=1.661$, $P>0.05$, no significant interaction; Weight: egg size $F_{1,36}=31.879$, $P<0.01$; parental quality $F_{1,36}=0.159$, $P>0.05$, no significant interaction). A similar two-way ANOVA of hatching condition showed that, in addition to their larger skeletal size, chicks hatching from large eggs were also in better condition than those from small eggs; as expected, the quality of the foster parents had no significant effect on hatching condition (two-way ANOVA; egg size $F_{1,36}=9.257$, $P<0.01$ parental quality $F_{1,36}=0.666$, $P>0.05$, no interaction). In addition, an across-treatment analysis showed significant correlations between egg size and hatching size (tarsus), weight, and condition (Pearson's $r_{38}=0.626$, $P<0.001$; $r_{38}=0.829$, $P<0.001$; $r_{38}=0.560$, $P<0.001$ respectively).

Table 2. Hatching size, weight and condition (weight allowing for skeletal size) of chicks analysed by egg size and parental quality. (means ± S.E. (N)).

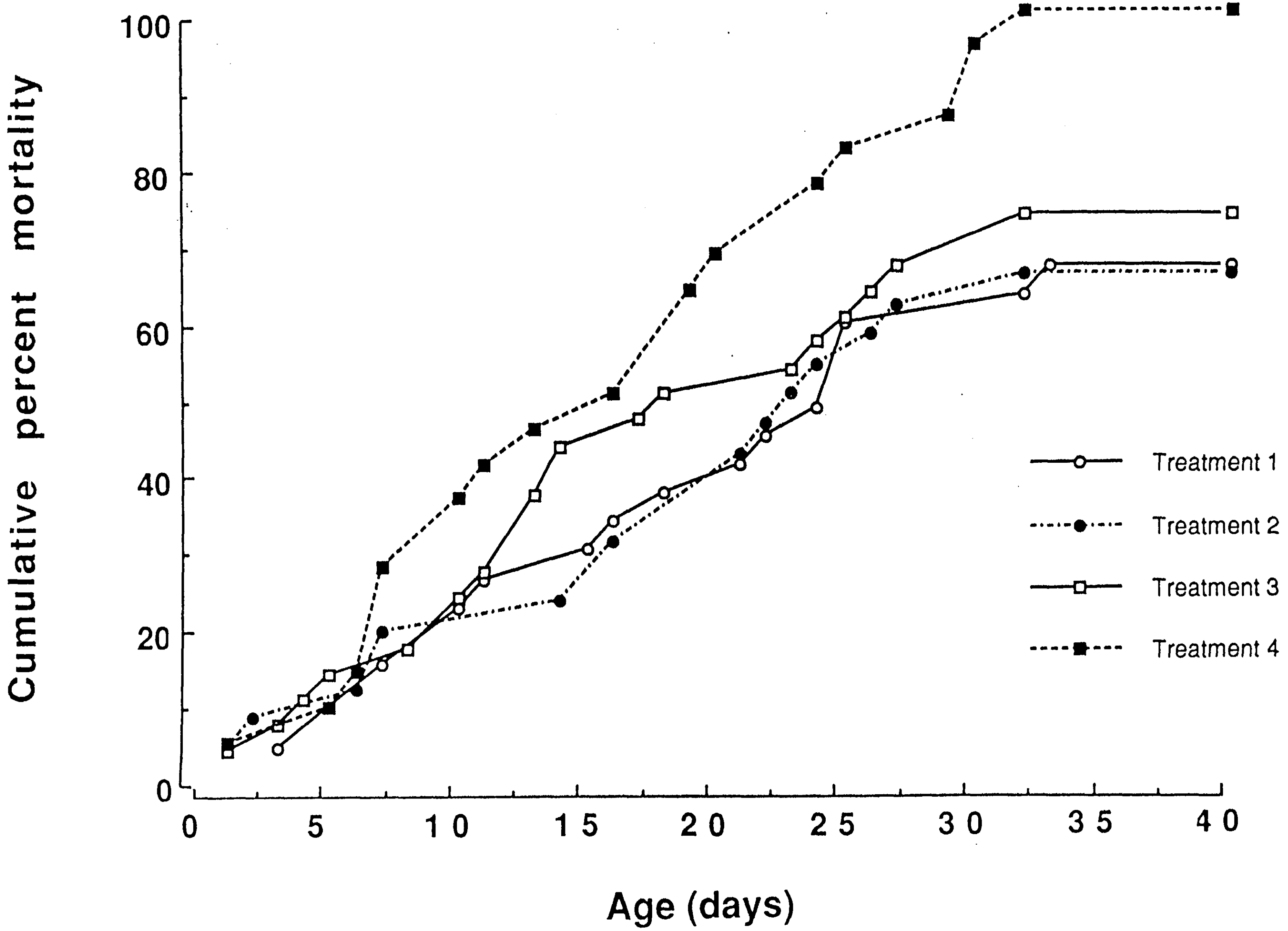
| | Treatment | | Tarsus length (mm) | Weight (g) | Condition |
|---|---|---|---|---|---|
| | Parental quality | Egg size | | | |
| 1. | High | Large | 25.87 ± 0.24<br>(19) | 53.26 ± 1.40<br>(19) | 1.51 ± 2.18<br>(19) |
| 2. | High | Small | 24.92 ± 0.22<br>(25) | 47.08 ± 1.01<br>(25) | -3.00 ± 1.31<br>(25) |
| 3. | Low | Large | 25.60 ± 0.23<br>(23) | 53.39 ± 1.27<br>(23) | 3.92 ± 1.85<br>(23) |
| 4. | Low | Small | 24.60 ± 0.20<br>(18) | 46.00 ± 1.04<br>(18) | -2.49 ± 1.82<br>(18) |

### *Chick growth and mortality*

Figure 1 shows the cumulative percentage chick mortality with age for each of the four treatments. The Kolmogorov-Smirnov two sample test shows that there were significant differences in the pattern of chick mortality between treatments 1 and 4 ($D=0.370$, $P<0.05$).

An across-treatment comparison showed that chicks which fledged successfully increased weight at significantly higher rates than those which died before fledging (16.7 ± 1.2 g/day compared with 12.4 ± 1.4 g/day, Mann Whitney $U=226.0$, $n_1=29$

**Figure 1.** Cumulative percent chick mortality with age (see text for treatment definitions).


Cumulative percent mortality
0
20
40
60
80
100
0
5
10
15
20
25
30
35
40
Age (days)
Treatment 1
Treatment 2
Treatment 3
Treatment 4

$n_2$=24, $P<0.03$), whereas there was no difference in tarsus growth rate between chicks which fledged and those that died (Mann-Whitney U=294.0, $n_1$=29 $n_2$=24, $P>0.05$).

Table 3 shows the tarsus and weight growth rates of chicks in each treatment. There were no significant differences in the rates of weight increase between treatments (Kruskal Wallis one-way ANOVA: $H_3$=6.961, $P>0.05$) and although the overall differences in tarsus growth rates were significant (Kruskal Wallis one-way ANOVA: $H_3$=8.410, $P<0.04$), nonparametric range tests (Siegel and Castellan 1988) failed to pinpoint a significant difference between any two treatments ($R_1$-$R_2$=11.01 critical difference ($D_c$)=14.64 $P>0.05$; $R_1$-$R_3$=11.21, $D_c$=14.64 $P>0.05$; $R_1$-$R_4$=3.62, $D_c$=18.46 $P>0.05$; $R_2$-$R_3$=0.20 $D_c$=14.88 $P>0.05$; $R_2$-$R_4$=14.63, $D_c$=18.65 $P>0.05$; $R_3$-$R_4$=14.83 $D_c$=18.65 $P>0.05$).

Table 3. Tarsus and weight growth rates during the linear growth phase (day 4 - 22) analysed by treatment (mean ± S.E. (N)).

| Treatment<br>Parental quality | <br>Egg size | Tarsus (mm/day) | Weight (g/day) |
|---|---|---|---|
| 1. High | Large | 1.03 ± 0.10 (16) | 13.30 ± 1.83 (16) |
| 2. High | Small | 1.34 ± 0.07 (15) | 17.18 ± 1.52 (15) |
| 3. Low | Large | 1.31 ± 0.11 (15) | 15.08 ± 1.96 (15) |
| 4. Low | Small | 0.90 ± 0.18 (7) | 8.91 ± 2.53 (7) |

A comparison was made of the egg size, chick hatching size (tarsus), weight and condition of chicks which survived the first 10 days, and those that died during this period, as shown in Table 4. This comparison showed that chicks which died during the first 10 days hatched from significantly smaller eggs, and were skeletally smaller than those that survived this period (Mann Whitney; egg size, U=691.5, $n_1$=25

$n_2=80$, $P<0.03$; tarsus $U=431.0$, $n_1=20$ $n_2=65$, $P<0.03$). The differences in the hatching weight of chicks surviving or dying in the first 10 days were just significant ($U=465.0$, $n_1=20$ $n_2=65$, $P=0.05$), and when hatching condition (i.e. weight corrected for size) was examined, there were no significant differences ($U=634.0$, $n_1=20$ $n_2=65$, $P>0.05$).

Table 4. Egg size, chick hatching size (tarsus length), weight and condition (weight corrected for size), of chicks which survived and died during the first 10 days (mean ± S.E. (N)).

| | Chicks which died | Chicks which survived |
|---|---|---|
| Egg volume (cc) | 63.2 ± 1.4 (25) | 66.7 ± 0.8 (80) |
| Tarsus length (mm) | 24.7 ± 0.2 (20) | 25.4 ± 0.1 (65) |
| Weight (g) | 47.6 ± 1.4 (20) | 50.7 ± 0.8 (65) |
| Condition | -0.239 ± 1.87 (20) | 0.059 ± 1.07 (65) |

Although these results indicate that chicks from small eggs suffered a higher mortality during the first 10 days than those from large eggs, this differential mortality may be attributable to the sequence of hatching, rather than egg size itself, since the smallest egg of the clutch is normally the last to hatch. Analysis of chick survival during the first 10 days in relation to egg order indicates that there is indeed a significant effect of hatching sequence on mortality, with 15% of 40 a-chicks, 19% of 36 b-chicks and 41% of 29 c-chicks not surviving to 10 days ($X^2_2$ $P<0.03$). The effects of hatching order and chick hatching size on survival from day 10 to fledging (day 40) was also investigated. There was not a significant effect of egg sequence on survival over this period; of those chicks alive at day 10, 65% of 34 a-chicks, 62% of 29 b-chicks and 82% of 17 c-chicks died before fledging

($X^2_2=2.220$ $P>0.05$). However, mortality from day 10 to fledging was significantly related to chick size on hatching (mean tarsus length of those which died = 25.2 ± 0.17, those which survived = 25.9 ± 0.21, $t_{63}=2.39$, $P<0.02$).

### *Fledging weights and fledging success.*

There were no significant differences in fledging weight according to treatment among those chicks that fledged (Kruskal Wallis one-way ANOVA $H_2=4.421$, $P>0.05$), but there were significant differences in fledging success (proportion of hatched chicks which fledged per pair) between treatments (Table 1, Kruskal Wallis ANOVA $H_3=8.322$, $P<0.04$).

In the preceding analysis, egg size and parental quality have been quantified on an ordinal scale (large/high or small/low). Whilst this method has certain precedents (for example Amundsen and Stokland 1990) and is helpful in revealing coarse grained differences between these categories, it provides little insight into the interaction between these variables in determining chick growth and survival, on a finer scale. In order to examine these interactions in more detail, the fledging success data from all four treatments were pooled and analysed using stepwise logistical regression with egg size and parental quality as continuous independent variables. There was no effect of egg sequence on survival from day 1 to fledging ($X^2_2=4.47$, $P>0.05$) so this variable was not considered for entry. (If egg sequence is available for entry it is not selected, and the result is unchanged). Parental quality was quantified as the total clutch volume of the original three-egg clutch produced by the foster parents, and is therefore measured in the same units as egg size. The logistic regression procedure assesses the importance of one or more independent variables in predicting a particular outcome on a binary dependent variable (see Engleman 1985). The predicted probability of fledging follows the logistic model:

$$\text{Probability of fledging} = \exp(U)/[1 + \exp(U)]$$

where U is a linear function of the independent variables.

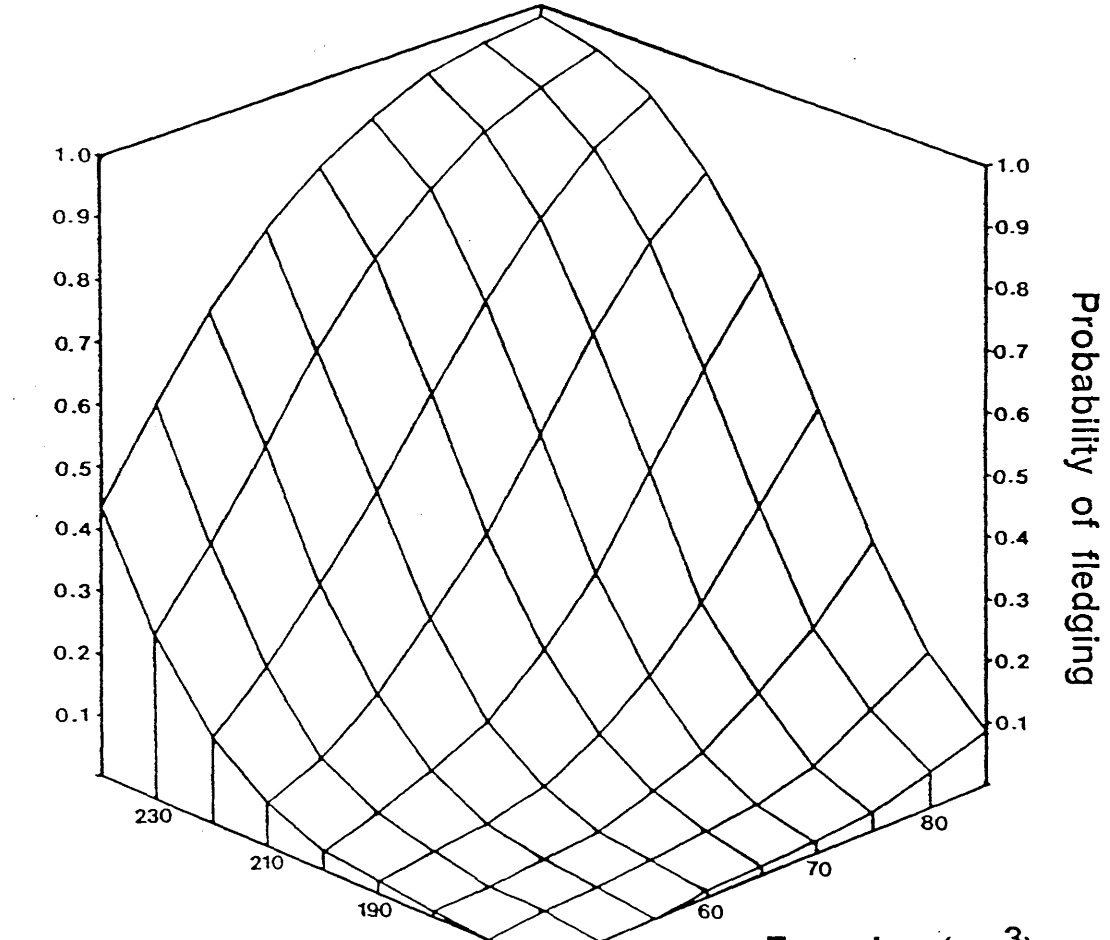
The analysis showed that both egg size and quality of the foster parents were significant predictors of the probability of fledging (parental quality $F_{1,38}=15.55$ $P<0.01$, egg size $F_{1,38}=6.15$ $P<0.05$). Parental quality was selected for entry on the first step of the procedure, (improvement $X^2_1=15.45$, $P<0.001$) indicating its greater contribution to chick survival. However, egg size selected on step 2 significantly increased the predictive power of the model (improvement $X^2_1=6.58$, $P<0.01$). Figure 2 illustrates the model graphically and is generated from the logistic equation, where:

$$U = 0.104 \times \text{Egg size} + 0.077 \times \text{parental quality} - 23.401$$

(The standard errors of the slopes are 0.043 for egg size and 0.020 for parental quality).

Figure 2 shows that for any given value of foster parent quality, increasing egg size results in an increase in chick survival, and similarly for any given egg size increasing parental quality increases the chances of survival.

**Figure 2.** Logistic regression of the probability of fledging on egg size and quality of the foster parents (assessed from their ability to produce large eggs).



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Probability of fledging
230
210
190
170
50
60
70
80
Foster parent quality
(original clutch volume (cm3))
Egg size (cm3)

## DISCUSSION

While several workers investigating the contribution of egg size to chick survival have appreciated the importance of controlling for parental quality, the relative contribution of this variable has not been quantified. Although Schifferli (1973) cross fostered great tit *Parus major*, hatchlings to randomly assigned foster parents and Nisbet (1978) cross fostered clutches of the smallest tern eggs with the largest eggs, neither author attempted to assess chick survival in relation to parental quality. Other authors (Davis 1975, O'Connor 1975, Lundberg & Vaisanen 1979, Williams 1979, Moss *et al.* 1981, Birkhead & Nettleship 1982) apparently made no attempt to separate the direct effect of egg size from the possible confounding influence of parental effects. Parsons (1970) transferred eggs between clutches and related chick survival during the first week to laying sequence and egg size. Although he states that he performed "egg transfer experiments" he provides no further details, and makes no attempt to relate chick survival to parental quality.

Despite this, the criticisms of Mueller (1990) are not wholly justified. His claim that these studies merely made comparisons between years or sites or within clutches, is not valid. Whilst it is correct that Parson's (1970, 1975) work on herring gulls examined the importance of egg sequence, he also showed how egg size affected survival for a- b- and c-eggs separately (1970, Figure 1). Similarly, although Nisbet's nine egg transfer studies spanned six years and two colonies, he related egg size to survival for each study separately, not across years. Mueller's interpretation that "mortality of chicks may be due to the same environmental factors (e.g. food scarcity) that may have an effect on egg size" cannot account for the data. Parsons (1970) found a positive correlation between egg size and yolk content in the herring gull and proposed that large eggs produce more viable chicks since they hatch with larger yolk reserves. This energy store is important in ensuring the survival during the first few days of life, when high mortality often occurs. Several authors have demonstrated a similar relationship between egg size and yolk content in a variety of avian species (Ricklefs, Hahn & Montevecchi 1978, Nisbet 1978, Bancroft 1985, Alisauskas 1986, Meathrel & Ryder 1987, Meathrel, Ryder & Termaat 1987, Hepp *et al.* 1987), although several (including Parsons 1970, Nisbet 1978, Ricklefs 1984, Meathrel & Ryder 1987, Meathrel, Ryder & Termaat 1987) found that the yolk fraction did not increase isometrically with egg size, so large eggs contained *proportionally* less yolk than small ones. Alisauskas (1986) and Hepp *et al.* (1987) have also investigated the relationship between the lipid content of the hatchling and egg size (in American coots *Fulica americana* and wood ducks *Aix sponsa* respectively). Both studies found that although neonate lipid content increased in direct proportion to egg mass, the correlation was weak: variation in egg mass accounted for only 30% and 19% of the variation in lipid, respectively).

This study has shown that lesser black-backed gull chicks from large eggs are heavier for their skeletal size than those from small eggs, suggesting that they hatch with larger yolk reserves to draw upon. However, chick condition on hatching was not significantly related to chick survival during the first 10 days in this study. The importance of yolk reserves may depend on factors such as food availability and weather conditions on the days immediately after hatching. In this study, chick mortality during the first two weeks was relatively low. There was a significant relationship between survival during the first 10 days and hatching size (tarsus), indicating that skeletal size, rather than yolk reserves may be important. The low survival rates of chicks from small eggs is related to hatching sequence, since the last hatching c-egg is generally smaller than the first two. The data presented here do not permit an analysis of whether last hatching c-chicks suffer high mortality during the first week of life because they hatch from small eggs or because they hatch after their sibs. However, in an experimental investigation of this in the herring gull, Parsons (1970) was able to evaluate the relative importance of these two variables using an egg transfer experiment. He showed the importance of hatching asynchrony on survival: first hatching c-eggs suffered significantly lower mortality (a minimum of 58.5% survived the first week) than last hatching c-eggs of approximately the same size (a minimum of 41.3% survived). In addition, the data also suggest an effect of egg size on chick survival: last hatching a-chicks survived better than last hatching c-chicks (minima of 49.3% and 41.3% respectively), although the effect was small in relation to the effect of hatching sequence mentioned above. Although hatching sequence appears to be an important factor in determining chick survival during the first week of life, it did not have a significant effect on survival to fledging in the present study. Mortality between day 10 and day 40 was significantly related to the skeletal size of the chicks at hatching, rather than hatching order.

In addition to the direct effect of egg size, it has been shown that the ability of the foster parents to produce large eggs, contributes to chick survival and is more important than egg size *per se* in determining fledging success. Parental quality would therefore act as a confounding variable in studies where no clutch manipulations were carried out. It has been shown for a number of larids that egg size and breeding success are related to female age (eg. Coulson 1966, Davis 1975). However, the relationship between age and egg size is not a simple linear function, and Davis (1975) has shown that in herring gulls egg size tends to increase with age up to 8 years followed by a decline. It is difficult to speculate on the age composition of the groups of birds laying large and small eggs, since whilst the largest eggs may be laid by females of intermediate age, those producing small eggs may either be very inexperienced or very experienced. Despite this, the results presented here clearly indicate that the ability to produce large eggs is positively related to chick rearing ability, and a primary determinant of fledging success; pairs laying clutches of small eggs were less able to rear chicks, hatching from eggs of any size, to fledging. The ability to produce large eggs therefore represents a useful measure of parental quality.

As Davis (1975) pointed out, experimental results which indicate advantages of large egg size imply selection for increased egg size. Davis suggested that such selection did not occur because the suggested advantages of large egg size were not real, but merely the result of changing age structure of the populations studied. However, this interpretation has been questioned (see Lundberg & Vaisanen 1979), and the data presented here indicate that the advantages of large egg size are real and can act independently of parental quality, which implies selection for large eggs. Furness (1983) postulated two selective pressures which could oppose increased egg size; adults laying smaller eggs may be able to lay earlier, which may increase breeding success. Alternatively, small chicks may require less food than larger ones, and so survive better if food availability was low. In addition to these explanations, I suggest that such selection may be counterbalanced by physiological and ecological constraints on egg production.

Studies on larids have demonstrated that the difference between large and small eggs of the same sequence lies mainly in the albumen component, rather than the yolk. The equations presented by Meathrel, Ryder & Termaat (1987), relating the mass of egg constituents to whole egg mass in the herring gull can be used to estimate the difference in the mass of the yolk and albumen fractions between the largest and smallest a-eggs in this study, assuming similar relationships between egg size and composition in lesser black-backed gulls. The 25g increase in fresh egg mass between the smallest and largest a-eggs in this study would result in a 5.3g increase in yolk (1.1g lipid, 0.8g non-lipid dry and 3.4g water) and a 17.2g increase in albumen. Since albumen is composed almost wholly of protein and water, then differences between large and small eggs must lie mainly in the protein and/or water content, rather than lipid.

Houston, Jones & Sibly (1983) have indicated that for lesser black-backed gulls, at least some of the protein requirements for egg formation may be provided by depletion of pectoral muscle protein, However, this can only account for some 15% of the total requirements of a three-egg clutch, and they found correlations between the extent of muscle depletion and protein content of the yolk, suggesting that endogenous proteins were used in follicle development rather than albumen formation. A large proportion of albumen protein must therefore be supplied from exogenous sources. I suggest that one of the primary counterbalancing forces on egg size is likely to be the acquisition of exogenous protein for albumen formation as the egg is passing through the oviduct. If the rate of albumen secretion is at or close to the physiological upper limit, increased albumen production could only be achieved by prolonging the duration of albumen secretion. It is likely that this would lead to a deleterious increase in laying and therefore hatching asynchrony (see Amundsen and Stokland 1988).

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# CHAPTER 4

# EXPERIMENTAL EVIDENCE FOR FOOD LIMITATION OF EGG PRODUCTION IN GULLS

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THE DATA PRESENTED IN THIS CHAPTER FORM THE BASIS OF A PAPER PUBLISHED IN

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# SUMMARY

Lesser black-back gulls nesting on Flat Holm Island in 1989 laid significantly smaller clutches than birds nesting in the same colony in the years 1983-86. Provisioning territories with supplementary food (fish) in 1989 during the prelaying period significantly increased clutch size and egg size compared with unfed controls. Similar experiments carried out on nearby Skomer Island in 1988, where egg production by unfed birds was typical of the species in Britain, did not increase clutch or egg size. The results clearly indicate that egg production can be limited by poor food supply during the laying period, but suggest that such food limitation is not a proximate mechanism for the restriction of the normal clutch to three.

## INTRODUCTION

For bird species in which the young are fed by their parents, the parents' ability to adequately provision the brood is generally held to be an important selection pressure in the evolution of clutch size. However, where the cost of egg production is high, clutch size may ultimately be limited by energetic and/or nutritional constraints on the female's capacity for egg production (see Drent & Daan 1980, Winkler & Walters 1983 and Martin 1987 for reviews). This hypothesis has been used to explain why some species, such as gulls, appear to be able to rear more young per annum than the maximum brood size which their usual clutch size permits (see review in Salzer & Larkin 1990), though lifetime fitness considerations are also likely to be important (Reid 1987a). Food supply can also act on clutch size in a proximate manner. In gulls, differences in the clutch size produced by individuals may arise directly from differences in food availability to females during egg formation, resulting from differences in female foraging ability, in the capacity of males to provide courtship food, or from local variations in food supply (e.g. Winkler 1985, Reid 1987a and 1987b, Pierotti & Annett 1987, Salzer & Larkin 1990). Supplementary feeding experiments can be used to demonstrate the proximate influence of food supply on egg production since, when food availability is low, the provision of additional food should produce either earlier laying and/or an increase in egg or clutch size in comparison with unfed controls (Arcese & Smith 1988).

In this chapter, details of two such experiments on lesser black-backed gulls are described. One in a colony where the clutch size of the unfed controls indicated normal food availability and the other in a colony where the low clutch size of the control birds suggested poor food availability.

## STUDY SITES & METHODS

Supplementary feeding experiments were carried out on Skomer Island (Pembrokeshire, Wales) in May 1988 and on Flat Holm Island (South Glamorgan, Wales) in May 1989. Observations (2-4 hours) were made daily from a hide to assess pair formation. At each site provisioning of pairs was commenced when a territory was established and nest building had begun. This was usually three to four weeks before the first egg was produced, and food was provided on individual territories daily until the clutch was complete. While the average duration of provisioning was slightly less on Flat Holm than on Skomer, there was no significant difference between the two; experimental nests were provisioned for an average of 29.4 days (± 1.5, N=22) on Skomer and 24.5 days (± 3.1, N=21) on Flat Holm (Mann-Whitney U=155.5, $P>0.05$).

Experimental territories were provisioned with chopped scad (*Trachurus trachurus*, 200g/day) after dark, between 00.00 hours and 01.00 hours, by placing the fish next to the nest scrape. Territories were provisioned at night to reduce the risk of food stealing by neighbouring pairs. (A watch after provisioning confirmed that there was little movement of birds between territories). The experimental design used was similar to that of Arcese and Smith (1988). Experimental (fed) and control (unfed) nests were located in the same area of the colonies, with the nests in each treatment being separated by a buffer zone of 10 - 20 m containing unallocated nests to minimise the possibility of food being stolen across treatments. All nests were checked daily and laying dates and clutch sizes were recorded for each nesting attempt. Eggs were measured (with callipers, length and breadth ± 0.1mm) on the day of laying, and marked according to laying sequence. Egg volume was estimated from the equation: $\text{Volume}=0.476 \times \text{egg length} \times \text{egg width}^2$ (Harris 1964).

Data on frequency distribution of clutch sizes were collected for lesser black-backed gulls breeding on Flat Holm from 1983-86 based on individually marked nests followed from the initiation of laying through to clutch completion.

## RESULTS

### *Clutch size of control females*

Figure 1 shows that there was a significant difference in mean clutch sizes between years on Flat Holm (analysis of variance; $F_{4,512}=4.12$ $P<0.01$). The clutch sizes of unfed control females in 1989 were markedly lower than those produced in the same colony from 1983-86 . In contrast the average clutch size of unfed birds nesting on Skomer in 1988 was typical of lesser black-backed gulls (Cramp 1983) and did not differ significantly from the 1983-86 clutch sizes on Flat Holm (analysis of variance; $F_{4,520}=1.38$ $P>0.05$).

### *Effect of Supplementary food*

Table 1 shows the frequency distribution of clutch sizes laid by artificially provisioned and unfed females at both sites. These data show that supplementary food had a significant effect on clutch size on Flat Holm, but not on Skomer (for Flat Holm, $X^2_1=4.195$ $P<0.05$, aggregating clutches <3 eggs for analysis; for Skomer $X^2_2=3.708$ $P>0.05$, aggregating clutches <3 and also >3 eggs for analysis).

Egg size may be a more sensitive measure of egg production than clutch size, since food limitation is likely to operate initially on egg volume rather than egg number (Martin 1987). In gulls which lay a three egg clutch, the third egg is generally smaller than the first two (e.g. Harris 1964, Parsons 1972, Davis 1975).

**Figure 1.** Clutch sizes of unfed lesser black-backed gulls on Flat Holm 1983-86 and 1989, and on Skomer 1988.

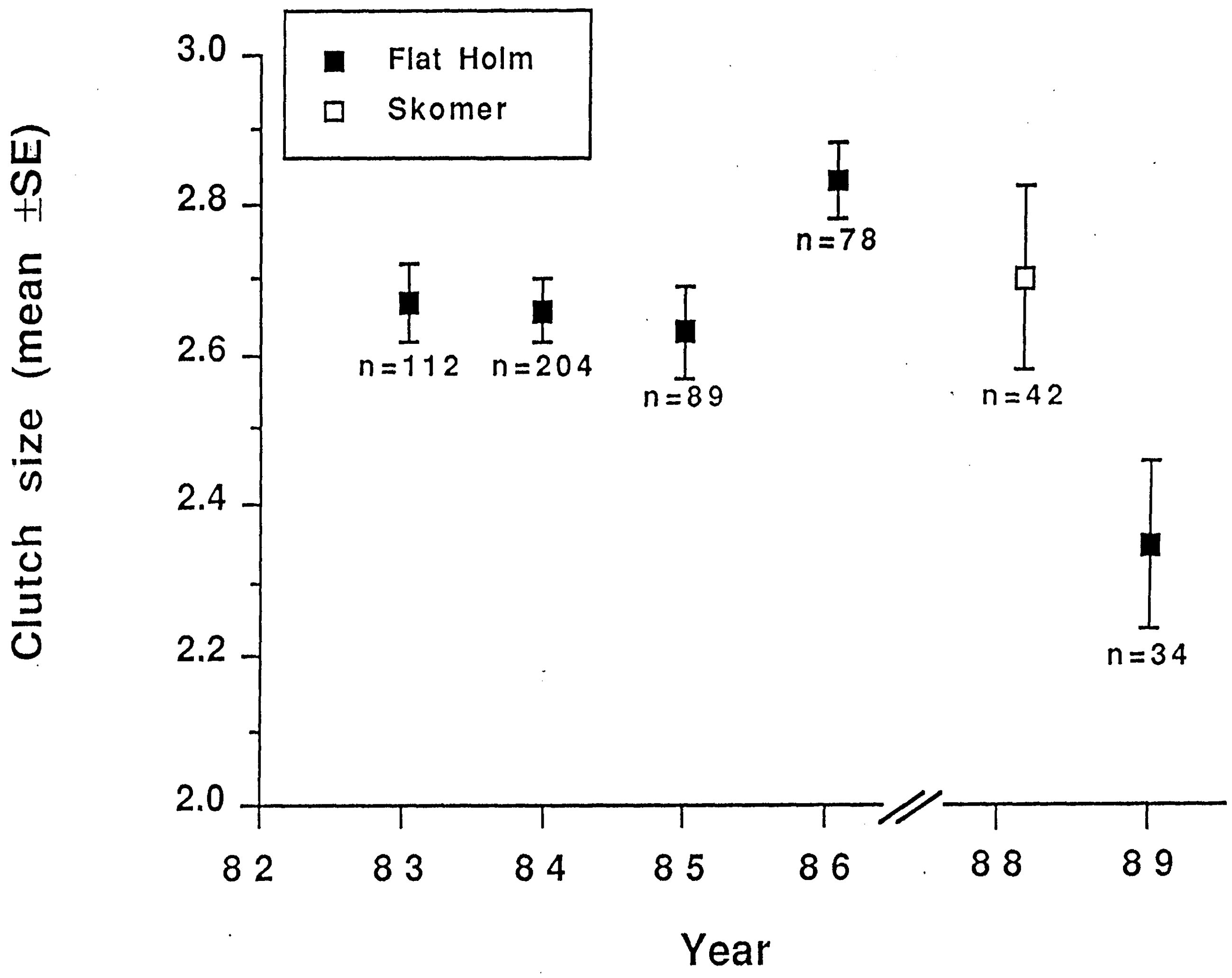
Flat Holm
Skomer
3.0
2.8
2.6
2.4
2.2
2.0
Clutch size (mean ±SE)
n=112
n=204
n=89
n=78
n=42
n=34
82
83
84
85
86
88
89
Year

Table 1 Frequency distribution of clutch sizes laid by fed and unfed female lesser black-backed gulls on Skomer 1988 and Flat Holm 1989.

| | Skomer | | Flat Holm | |
|---|---|---|---|---|
| Clutch size | Control | Fed | Control | Fed |
| 1 | 4 | 4 | 3 | 1 |
| 2 | 9 | 3 | 16 | 4 |
| 3 | 24 | 9 | 15 | 16 |
| 4 | 5 | 6 | 0 | 0 |
| 5 | 0 | 1 | 0 | 0 |
| Mean | 2.71 | 2.87 | 2.35 | 2.71 |
| S.E. | 0.12 | 0.24 | 0.11 | 0.12 |
| N | 42 | 23 | 34 | 21 |

Table 2 shows the effect of supplementary food on egg size for each of the three eggs in 3-egg clutches on Skomer and Flat Holm. (There were insufficient two egg clutches in the experimental groups for examination of egg size effects, see Table 1).

Table 2. Mean volume of a- b- and c-eggs in 3-egg clutches of fed and control lesser black-backed gulls on Skomer and Flat Holm (mean ± S.E. (N)). Also given is the average volume of the c-egg as a proportion of the volume of the a-egg in the same clutch.

| | Skomer | | Flat Holm | |
|---|---|---|---|---|
| | Control | Fed | Control | Fed |
| a-egg | 69.85 ±1.22<br>(24) | 69.89 ±1.88<br>(9) | 68.52 ±1.31<br>(15) | 71.68 ±1.04<br>(16) |
| b-egg | 70.32 ±1.22<br>(23) | 71.11 ±2.06<br>(9) | 65.94 ±1.09<br>(14) | 71.39 ±1.26<br>(16) |
| c-egg | 64.26 ±1.26<br>(24) | 64.70 ±1.33<br>(9) | 58.88 ±1.07<br>(14) | 65.53 ±1.34<br>(16) |
| c-egg/ a-egg | 0.922 ±0.014<br>(24) | 0.928 ±0.015<br>(9) | 0.868 ± 0.014<br>(14) | 0.914 ±0.011<br>(16) |

For birds nesting on Skomer, there was the typical effect of laying order on egg volume in both fed and unfed birds, but there was no effect of supplementary food on egg size (Table 2, repeated measures 2-way ANOVA; laying order: Wilk's $F_{2,30}=30.85$ $P<0.001$; treatment: $F_{1,31}=0.06$ $P>0.05$; no significant interaction). However, for birds on Flat Holm, not only was there the expected relationship between egg size and laying order in both treatments, but there was also a significant increase in egg volume in the birds provided with supplementary food (repeated measures 2-way ANOVA; laying order: $F_{2,26}=63.07$ $P<0.001$; treatment: $F_{1,27}=11.91$ $P<0.002$; no significant interaction). The smaller c egg may be expected to be the most sensitive to food availability (Reid 1987b, Salzer and Larkin 1990). The average size of the c-egg relative to the a-egg in the same clutch is shown in Table 2 for all groups. There was no difference in the extent of the reduction in c-egg size between fed and control birds on Skomer, ($t_{31}=-0.25$ $P>0.05$), whereas for birds breeding on Flat Holm the relative decrease in c-egg size was significantly greater in control birds compared with birds which received supplementary food ($t_{28}=-2.59$ $P<0.02$).

### *Laying date*

A further effect of supplementary feeding can be to advance the laying date instead of, or in addition to, any effect on clutch size (see reviews in Martin 1987 and Arcese & Smith 1988). Furthermore, since in gulls both clutch size and egg size decrease through the laying season (Brown 1967, Davis 1975, Davis & Dunn 1976, Parsons 1972), it is also important to examine the extent to which differences in clutch and egg size observed in this study might be explained by differences in laying date. Table 3 gives the mean first egg dates of females in each treatment from both sites.

Table 3. First egg dates of control and supplementary fed females on Skomer and Flat Holm.

| Site | Treatment | First egg date (mean ± S.E.) | N |
|---|---|---|---|
| Skomer | Unfed | 21 May ± 1.2 days | 42 |
| | Fed | 21 May ± 1.5 days | 23 |
| Flat Holm | Unfed | 14 May ± 2.5 days | 34 |
| | Fed | 14 May ± 2.7 days | 21 |

There was a significant difference in laying dates between sites, but not between fed and unfed birds at each site (2-way ANOVA, site: $F_{1,107}=9.84$ $P<0.002$; treatment: $F_{1,107}=0.06$ $P>0.05$; no significant interaction). Supplementary feeding thus had no effect on average laying date at either site. Since the birds on Flat Holm actually laid on average one week earlier than the birds on Skomer, the observed differences in egg production between sites are unlikely to be explained by laying date.

## DISCUSSION

Several authors have suggested both proximate and ultimate relationships between egg production and food supply in Larids, mediated either through courtship feeding rates or regional differences in food supplies (see reviews in Winkler & Walters 1983 and Salzer & Larkin 1990). The experiments on lesser black-backed gulls in this study address the potential proximate relationships. Control birds on Skomer produced normal sized eggs and clutches whereas those on Flat Holm produced reduced clutches of relatively small eggs in 1989, suggesting that food supply was low in the latter, as confirmed by the results of supplementary feeding. On Skomer, supplementary feeding had no effect on egg production. The results demonstrate that, in a situation where food is apparently not in short supply, as on Skomer, the provision of supplementary food prior to and during laying has no effect on laying date, egg size or clutch size. In a similar situation Reid (1987b) found that the provision of supplementary food prior to the onset of laying in the glaucous-winged gull did not affect egg production. However, Reid (1987b) also found that provision of supplementary food during the laying period itself resulted in an increase in the size of the c-egg. This was not the case in this study. On the other hand, in a situation where clutch and egg sizes were depressed, as on Flat Holm, the provision of supplementary food restored both egg and clutch size to their normal values. This demonstrated that shortage of food had indeed limited egg production in the control birds.

The effect of supplementary food on egg and clutch size in conditions of low food availability are similar to those reported by Arcese & Smith (1988) for the song sparrow (*Melospiza melodia*), in which clutch and egg size increased with provisioning in conditions of high breeding density. However, in contrast to the song sparrow results, and to many similar experiments, mainly on passerine birds (see Drent & Daan 1980 and Arcese & Smith 1988), laying date did not appear to be affected in this study. The increase in clutch size is not therefore simply a function of the general relationship between clutch size and laying date in gulls which has been demonstrated by Brown (1967). The lack of any advancement of laying date in the experimental compared with the control birds on Flat Holm may be the outcome of the kind of trade off between the production of a high quality clutch and timing of laying outlined by Drent & Daan (1980). However, since earlier laying is advantageous in gulls (e.g Davis 1975), one would have predicted an advancement of laying date in the Skomer experiment. This was not the case. Similarly, Reid (1987b) found no effect of supplementary feeding on laying date in glaucous-winged gulls and Neihbur (1981) found no relationship between courtship feeding rate and laying date in herring gulls.

The Flat Holm data clearly demonstrate that a shortage of food during the prelaying and laying periods can depress egg production in gulls. The data from Skomer however also suggest that food limitation per se may not be the proximate cause of the truncation of the normal clutch at three.

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# CHAPTER 5

# NUTRITIONAL CONSTRAINTS ON EGG FORMATION IN THE LESSER BLACK-BACKED GULL: AN EXPERIMENTAL STUDY

**M. Bolton, D.C. Houston, & P. Monaghan**

THIS CHAPTER FORMS THE BASIS OF A PAPER SUBMITTED TO

JOURNAL OF ANIMAL ECOLOGY

# SUMMARY

The use of supplementary feeding experiments to investigate the relationship between food supply and clutch and egg size in birds has yielded disparate results, some authors showing an advancement in laying date and/or increased clutch size in response to additional food whereas other studies have found no effect. Here the nutritional requirements of egg formation in lesser black-backed gulls are investigated through a series of pairwise feeding experiments providing additional food of different quality.

Provision of additional food on the nesting territories did not result in a decrease in the amount of time spent away from the colony by females, although fed males did spend more time on territory during the early part of the prelaying period in a year of normal food supply. Courtship feeding rates were very low in comparison with other studies and were not influenced by provision of additional food.

Providing either supplementary fish or fat during the prelaying period demonstrated that in a year of apparently low food availability, clutch size was depressed by protein, but not energy, limitation. A second experiment, supplying either fish or egg to prelaying birds, suggested that in addition to these general protein requirements, egg production may further be limited by the supply of specific nutrients, contained in the egg, but not the fish supplement. The possible identity of these specific nutrient is discussed.

There was no advancement in laying dates of fed birds compared with controls in the same year. This contrasts with studies on other species and suggests that clutch size may not be primarily determined by laying date in lesser black-backed gulls.

Chicks hatching from the eggs laid by females receiving an egg supplement prior to laying tended to be skeletally larger and heavier on hatching, but any advantages of hatching from larger eggs were short lived. There was no difference in subsequent growth or survival of chicks of egg-fed parents compared with controls.

## INTRODUCTION

The production of eggs is widely recognised as a demanding process for many birds. However, while the costs are frequently assumed to be most extreme in small passerines laying large clutches, the nutritive and energetic demands of egg production have been shown to be relatively high in larids, despite their comparatively large body size (Ricklefs 1974, Robbins 1981, Houston, Jones & Sibly 1983, Salzer & Larkin 1990). The importance of food supply during the pre-laying and egg-laying period as a proximate determinant of egg production has been examined in a variety of birds, for example several passerine and raptor species (see reviews in Martin 1987, Arcese & Smith 1988 and Boutin 1990), owls (Korpimaki 1987, 1989, Korpimaki & Hakkarainen 1991) and larids (Winkler 1985, Pierotti & Annett 1987). Experimental studies of the effect of supplementary feeding on egg laying have usually found an advancement of laying date and, less frequently, an increase in clutch size; effects on egg size and weight are less well documented (Arcese & Smith 1988). As pointed out by Nisbet (1978) and Ewald & Rohwer (1982), the quality of the food provided is likely to play an important role in the outcome of these experiments; differences in the protein and energy content of the supplementary food may explain some of the inconsistencies in the results (Arcese & Smith 1988). Furthermore, since egg proteins are particularly rich in sulphur and phosphorus (Grau 1984, O'Connor 1984), the kind of protein supplied may also be important. However, the effect of the provision of specific types of food on egg production has hitherto not been tested experimentally.

The data presented in chapter 4 demonstrated experimentally that, in a year of apparent food shortage, the provision of supplementary food in the form of fish resulted in an increase in both clutch size and egg size in the lesser black-backed gull. This chapter reports the results of further feeding experiments to examine the extent to which specific nutrient requirements constrain egg production. By the provision of different types of food supplement during the prelaying and laying period to lesser black-backed gulls, I examine:

1) whether the influence of food supply on egg production is mediated through constraints during the pre-laying period on energy or on protein supplies,

2) whether the nature of the supplementary protein provided influences egg production,

3) the effects of particular food supplements on egg composition and on the subsequent growth and pre-fledging survival of the chicks.

## METHODS

### *Provision of supplementary food*

#### *Energy or protein limitations on egg production ?*

In order to determine whether the increased egg production of fish-fed lesser black-backed gulls reported earlier (chapter 4) was the result of a protein requirement or a more general energy deficit, an additional supplementary feeding experiment was conducted on Flat Holm in the same year (1989) in which an experimental group received a food supplement composed of animal fat (120g/pair/day) of similar calorific value to the fish supplement. (Calorific values were estimated from figures given in Ricklefs 1974). The food was provided after a territory had been established and nest building had begun (see Hiom *et al.* 1991, chapter 4 for details). A buffer zone 10 - 20m wide of unallocated territories was left between treatments. The food was placed beside the nest at night (between 00.00 and 01.00 hrs), which reduced the risk of it being stolen by neighbouring pairs. At each nest provisioning ceased five days after the a-egg was laid. The fat was provided for an average of 19.4 days prior to laying (S.E. ± 1.7 n=34), and did not differ from a mean provisioning period of 24.5 days for fish-fed birds (S.E. ± 3.1 n=21 Mann-Whitney U=272.5 P>0.05). Egg production of the fat-fed group was compared with that of unfed controls which were subjected to the same degree of human disturbance.

*Requirements for general or specific amino acids ?*

The hypothesis that egg synthesis demands specific amino acids, which may be scarce in the diet and the supply of which may limit egg production, was examined in 1990 by providing a food supplement composed of either fish (scad 200g/pair/day) or eggs of approximately the same calorific value (140g/pair/day, calorific values measured by bomb calorimetry). It is likely that any specific amino acids required for egg synthesis would be concentrated in the egg supplement. To provide the egg supplement in a form which could be eaten easily by gulls but would not encourage intraspecific egg predation, eggs were shelled, beaten and cooked in a microwave for a few minutes to form an omelette of cake-like consistency. During the early part of the provisioning period, free range hens eggs were used. However, gull eggs were used once laying had begun in another part of the colony, where eggs were being removed by the reserve warden as part of a population control programme. The mean provisioning periods (± S.E.) were 22.1 ± 2.3 (n=26) and 16.3 ± 2.0 (n=28) days prior to laying for fish and egg-fed groups respectively (Mann Whitney U=255.5 P>0.05). The food was distributed in the same manner as in the previous experiment. A third group which received no additional food and was subject to a similar level of disturbance acted as a control.

### *Territory attendance and courtship feeding*

It is important to assess the effect of providing supplementary food on the normal pattern of foraging and therefore the likely effect on total intake. The increased food availability at the breeding colony for experimental birds may simply result in a reduction in the amount of foraging effort rather than an overall increase in food intake. Although gulls breeding on Flat Holm do occasionally kill rabbits *Oryctolagus cuniculus* and small passerine birds on the island (pers obs), most food is obtained away from the breeding colony at estuaries, refuse tips, freshwater or agricultural areas on the mainland (Mudge and Ferns 1982, *pers. obs.*). Whilst it was not possible to record the amount of time devoted to active foraging by specific individuals, measurements of attendance at the territory were used to provide an indication of the amount of time spent away from the territory which was available for foraging.

Territory attendance of specific individuals was assessed by daily observations from a hide situated 20 m from the nearest and 100 m from the furthest territories, using 10x binoculars and a 20-45x telescope. A minimum of two one-hour hide watches were conducted daily in 1989 and two two-hour watches in 1990. Scans were made every 30 minutes and the territories of all individually recognisable birds were checked and males and females recorded as present or absent separately. Individuals were recognised of the basis of variation in bill markings and plumage features, especially the size and extent of wear on the primary spots. Adults were sexed on the basis of behavioural and size differences when both members of the pair were present. Data from 1989 indicated that there were significant diurnal variations in the pattern of territory attendance, which could potentially obscure treatment differences. In order to avoid this, in 1990 observation sessions were rotated throughout the day, covering most of the daylight hours (0600-2000 hrs) in a four day cycle.

In order to make comparisons between treatments of the amount of time gulls spent on the territory, the prelaying period was divided into two phases; the phase of rapid follicle growth (Phase 2), from 10 days prior to laying (d. -10) until the day before laying (d. -1) (Brown 1967) and the 10 days preceding this (Phase 1), during which resources may be acquired for egg formation. For each individual, the proportion of the total number of scans during each phase for which it was present was calculated.

The supplementary feeding protocol did not permit the specific targeting of females, but rather made additional food available to both males and females of experimental pairs. In order to determine whether this influenced the courtship feeding rate, in 1989 the number of such feeds observed during the course of each daily hide watch was recorded. An equal amount of time was devoted to observing pairs in each treatment during each session. Courtship feeding rates were calculated in terms of the number of feeds per hour when both members of the pair were present.

### *Egg production*

In order to obtain as accurate data as possible on egg production in a species where intraspecific egg predation is often common, nests were visited each morning, usually before 09.00 hrs, since laying normally occurs during the early morning. On the day of laying, each egg was measured (with callipers, length and maximum width to ± 0.1mm) and marked according to laying sequence (a-, b- or c-egg etc) with indelible ink. Clutch sizes (total number of eggs laid per nest) and laying dates were recorded for each nest.

### *Egg quality*

It is not clear how accurately egg lipid or protein content reflect true egg quality, which may more meaningfully be measured in terms of hatchability, hatchling size and weight, growth and survival. Unfortunately, conventional methods of egg composition analysis (soxhlet extraction of lipid) are incompatible with normal egg development, preventing the measurement of lipid or protein content of eggs which are allowed to develop to hatching. Therefore egg composition was measured in 1989, but eggs were allowed to develop naturally in 1990.

### *Egg composition*

In 1989 egg quality was assessed in terms of lipid and protein content. All eggs were removed from the nest on the day they were laid and replaced with dummy eggs made of plaster. The eggs were hard boiled and frozen for subsequent laboratory analysis of egg composition. For analysis, each egg was separated into its shell, albumen and yolk fractions and the three egg components were dried to constant weight at 75 $^{o}$C (± 0.01g). (Since the water content of the egg may change as a result of boiling, the fresh weights of these components were not determined). The chloroform-soluble lipid fraction of the yolks was extracted using soxhlet apparatus and the yolk lipid-free dry weight measured after redrying to constant weight. The lipid-free dry weight was taken as a measure of protein content (Meathrel & Ryder 1987). For each egg the yolk ratio was calculated as the the dry weight of the whole yolk, divided by the albumen dry weight, and the yolk lipid index was calculated as the yolk lipid weight divided by the yolk lipid-free dry weight.

### *Hatching success, chick size, growth and survival*

In 1990, egg quality was assessed in terms of hatchability, the skeletal size and weight of the chicks produced, and the subsequent growth and survival of these chicks. Each nest was visited daily during incubation and any egg losses were noted. The date of hatching and total number of eggs hatched per nest was recorded and the hatching success (proportion of eggs which hatched) was calculated for each clutch. Eggs which were lost through predation (one egg only) or to trampling by grazing stock (four complete clutches lost, totalling 11 eggs) were excluded from the analysis of hatching success. The assessment of hatchability therefore includes only those eggs which failed to hatch due to infertility or embryo death.

Each nest was surrounded with a chicken wire enclosure (measuring approximately $3m^2$ and 45cm high) just before hatching, to facilitate the location of the chicks. A wooden shelter (approximately 30cm x 30cm x 45cm) was also placed beside each nest, since the enclosure denied chicks access to extensive thick vegetation or rabbit burrows in which to shelter from rain or hot sun. Observations from a hide confirmed that most adults resumed incubation within a few minutes of returning to the nest site after erection of the fences.

On the day of hatching, each chick was marked according to hatching sequence with coloured insulation tape around the tarsus and weighed (using a digital pan balance to ± 1g) and measured (tarsus length, using callipers to ± 0.1mm). The condition (weight allowing for skeletal size) of the chicks on hatching was calculated from linear regression of weight on tarsus length. The residual, expressed as a proportion of the predicted value for each case, was taken as a measure of condition. Subsequently, chicks were weighed and measured every three days until 16 days old and at 6 day intervals thereafter, until 34 days old when they were judged to have fledged. At least 39 (35%) chicks were capable of flight soon after 34 days and could not be measured at 40 days.

Weight and tarsus growth rates during the first 13 days were calculated separately for each chick. Plots of $\log_{10}$(tarsus) and $\log_{10}$(weight) with age produced linear relationships during the first 13 days, the slope of which was taken as a measure of exponential growth. The calculation of growth rates was limited to chicks for which at least five sets of measurements had been obtained during this period. Fledging success (proportion of chicks which survived to at least 34 days) was calculated for each brood.

### *Data analysis and presentation*

Most data were examined using non-parametric analysis of variance (laying dates, territory attendance, courtship feeding, hatching success and chick size and weight). If a significant difference between the three treatments was found this was then followed by non-parametric range tests to determine which groups were different. Data which were analysed by other methods are indicated in the text. Due to the large number of statistical tests applied in this paper, those which are significant only at the 0.05 level should be regarded with caution. In general, full statistics are only presented for analyses which indicated significant differences between treatments.

## RESULTS

### *Experiment 1 - energy or protein limitation on egg production ?*

*Territory attendance*

The mean proportion of time spent on the territory by males and females during the period of rapid follicle growth (Phase 2) and during the 10 preceding days (Phase 1), is shown in Figure 1. These data were examined using Kruskal Wallis analysis of variance, which showed that there were no differences between treatments for either sex in the amount of time spent away from the territory, during either phase (in all cases $P>0.05$). This suggests that the provision of supplementary food did not result in a decrease in the amount of time spent foraging away from the colony.

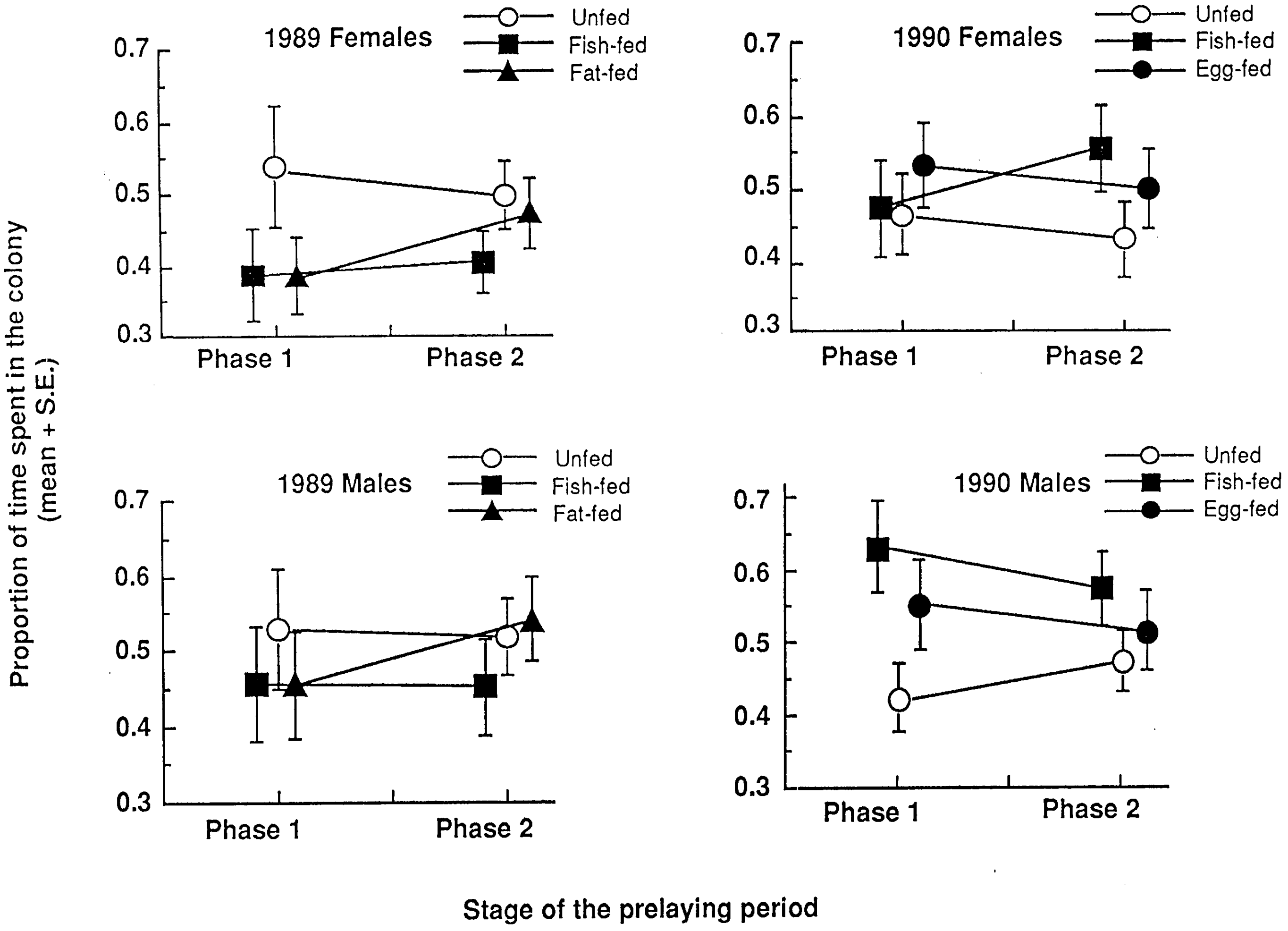
*Courtship feeding*

The recorded levels of courtship feeding were very low (a total of 24 feeds were recorded in 797 bird-hours when both members of the pair were present). Kruskal Wallis analysis of variance indicated that there were no significant differences in the rate of courtship feeding between treatments (mean number of feeds per hour that both birds were present ± S.E. (N): control 0.052 ± 0.029 (19), fish-fed 0.028 ± 0.012 (15), fat-fed 0.040 ± 0.024 (22), $P>0.05$).

*Laying dates*

There were no significant differences in laying date between treatments, which indicated that any differences in clutch and egg size between treatments would be due to differences in feeding regime, rather than differences in laying date *per se* (mean first egg dates ± S.E. (N): control 14 May ± 2.5 days (34), fish-fed 14

**Figure 1.** Proportion of time spent at the territory by male and female lesser black-backed gulls during Phase 1 and Phase 2 of the prelaying period, according to supplementary feeding regime (see text for definitions, mean ± S.E.). There were no significant differences between treatments in either year during either phase except for males during Phase 1 in 1990 (Kruskal Wallis Anova $H_2=6.266$ $P<0.05$, non-parametric range tests $R_{control}-R_{fish\text{-}fed} = 11.16$ $D_{crit} = 9.856$ $P<0.05$, $R_{control}-R_{fat\text{-}fed} = 11.38$ $D_{crit} = 9.487$ $P<0.05$)


1989 Females
Unfed
Fish-fed
Fat-fed
1990 Females
Unfed
Fish-fed
Egg-fed
1989 Males
Unfed
Fish-fed
Fat-fed
1990 Males
Unfed
Fish-fed
Egg-fed
0.7
0.6
0.5
0.4
0.3
Phase 1
Phase 2
Proportion of time spent in the colony
(mean + S.E.)
Stage of the prelaying period

May ± 2.7 days (21) fat-fed 15 May ± 2.1 days (34), $P>0.05$).

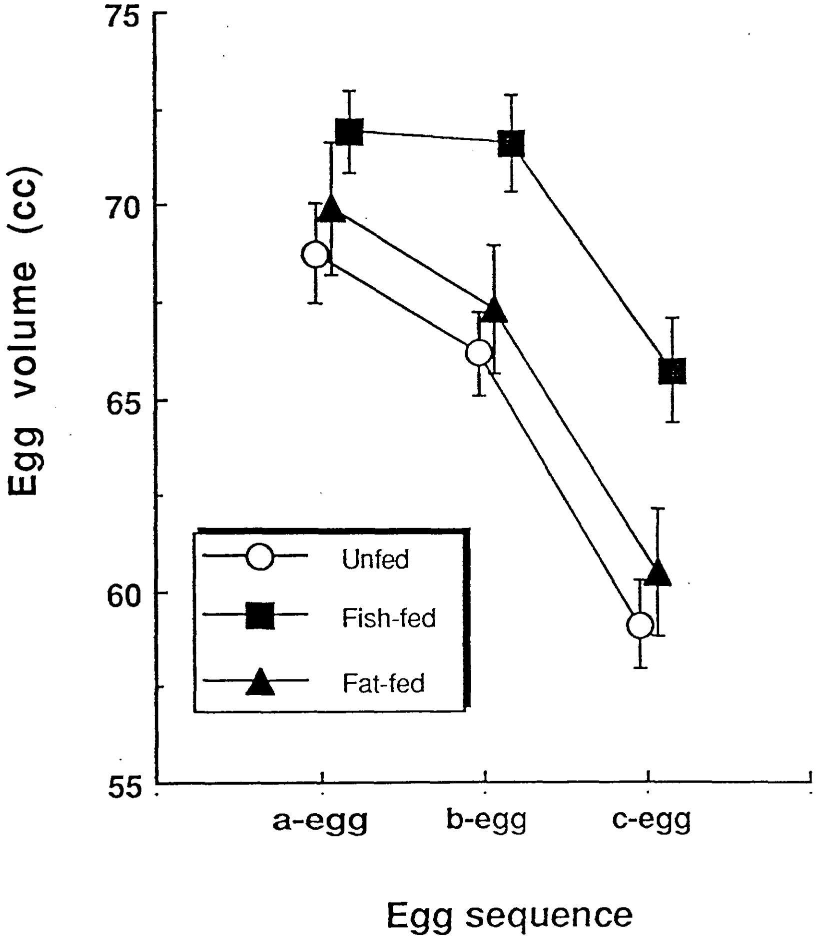
*Egg production*

In a year when egg production of control birds was depressed (1989), provision of supplementary fish led to a significant increase in both clutch and egg size (Hiom *et al.* 1991, chapter 4). However, as shown here (Table 1), the provision of a fat supplement in the same year did not lead to a significant change in the relative proportions of each clutch size ($X^2_1=0.529$ $P>0.05$, clutch sizes $\leq 2$ combined and those $\geq 3$ combined for analysis). Also, in contrast to the effect of supplementary fish on egg size in 1989, provision of a fat supplement had no significant effect on egg size (Figure 2), although the expected effect of laying sequence on egg size (Parsons 1972, Davis 1975) was found.

Table 1. Frequency distributions of clutch sizes produced by control and supplementary fed lesser black-backed gulls on Flat Holm in 1989 and 1990.

| | 1989 | | | 1990 | | |
|---|---|---|---|---|---|---|
| Clutch size | Control | Fish fed | Fat fed | Control | Fish fed | Egg fed |
| 1 | 3 | 1 | 1 | 2 | 2 | 1 |
| 2 | 16 | 4 | 14 | 5 | 2 | 8 |
| 3 | 15 | 16 | 18 | 25 | 22 | 18 |
| 4 | 0 | 0 | 1 | 1 | 0 | 1 |
| mean | 2.35 | 2.71 | 2.56 | 2.76 | 2.77 | 2.68 |
| S.E. | 0.11 | 0.12 | 0.11 | 0.11 | 0.12 | 0.12 |
| N | 34 | 21 | 34 | 33 | 26 | 28 |

**Figure 2.** Mean volume ($cm^3$, ± S.E.) of eggs from three-egg clutches, laid by control, fish-fed and fat-fed gulls in 1989 (the data referring to eggs laid by fish-fed gulls are taken from Hiom *et al.* 1991). Repeated measures Anova comparing control vs fat-fed; effect of egg sequence: Wilkes $F_{2,28}=64.50$ $P<0.001$, effect of feeding treatment: $F_{1,29}=0.57$ $P>0.05$, interaction not significant.


75
70
65
60
55
Egg volume (cc)
Unfed
Fish-fed
Fat-fed
a-egg
b-egg
c-egg
Egg sequence

### *Egg quality - lipid and protein content*

The dry weights of each egg component (shell, albumen, yolk lipid-free and yolk lipid) are given in Appendix 1, separately for a-, b- and c-eggs of three-egg clutches in each treatment. The ratio of dry (whole) yolk to dry albumen (yolk ratio), and the ratio of yolk lipid to yolk lipid-free dry weight (yolk lipid index) are also given. The effect of within-clutch factors (laying sequence) on egg composition were examined using repeated measures analysis of variance, and the between-clutch factors (treatment effects) were investigated by one-way analysis of variance, followed by Tukey tests if significant, to locate differences between groups. The Bonferroni adjustment was applied to the significance criteria; only values <0.02 were considered significant. Full statistics are presented in Appendix 2.

Although there were differences in the albumen content of eggs according to laying order (Appendix 2, repeated measures ANOVA), there was no effect of feeding regime on albumen dry weight among eggs of the same sequence. In contrast, there were significant differences in shell and (whole) yolk dry weight among c-eggs according to treatment; c-eggs laid by fish-fed females contained significantly heavier yolks and shells than those of control females (there were no differences in yolk mass between a-, b- or c-eggs of fat-fed and control females). Investigating the increase in yolk content further, both the lipid and protein content of the yolk were examined, to determine which component of the yolk was increased by the fish supplement. Among c-eggs, there were significant differences in yolk protein, but not in yolk lipid, between eggs of fish-fed and both fat-fed and control gulls.

### *Experiment 2. General or specific protein limitations on egg production; comparison of fish and egg proteins*

#### *Laying date*

As in the previous experiment, Kruskal Wallis analysis of variance indicated that there were no significant differences in laying date between treatments, indicating that any differences in egg production were attributable to feeding regime rather than timing of laying (mean first egg dates ± S.E. (N): control 6 May ± 1.7 days (33), fish-fed 8 May ± 1.9 days (26), egg-fed 7 May ± 1.8 days (28), $P>0.05$).

#### *Territory attendance*

The mean proportion of time spent on the breeding territory during phases 1 and 2 of the prelaying period of this experiment (1990) are given in Figure 1. Kruskal Wallis analysis of variance of these data shows that among females there were no significant differences between treatments in the recorded proportion of time present on territory for either phase ($P>0.05$). However, among males there was a significant difference in the level of territory attendance during phase 1 ($P<0.05$); both fish-fed and fat-fed birds spent more time at the territory than control males. There were no significant differences among males during phase 2 of the prelaying period ($P>0.05$).

#### *Egg production*

In 1990, clutch sizes of unfed gulls on Flat Holm were larger on average than in the previous year (Table 1), and typical for the species. This indicates that, in contrast to the situation in 1989, there was no apparent local food shortage. In this case, as expected from the results of earlier feeding experiments (Hiom *et al.* 1991, chapter 4), provision of either a fish or egg supplement had no effect on the frequency distribution of clutch sizes ($X^2_2$=2.233 P>0.05, clutch sizes <2 aggregated and >3 aggregated for analysis). However, pairs which received the egg supplement laid eggs which were on average 10% larger than those laid by control females, as shown in Figure 3. The smaller size of the c-egg has been interpreted as a result of limited food supply during the prelaying period (Pierotti and Bellrose 1986) and it may be expected to be the most sensitive to variation in food availability or quality. However, there were no significant differences between treatments in the size of the c-egg relative to the a-egg of the same clutch, which indicated an overall increase in egg size within a clutch (ratio of c-egg size to a-egg size, three-egg clutches only, control: 0.867 ± 0.018 (23) fish-fed: 0.903 ± 0.017 (21) egg-fed: 0.903 ± 0.020 (16); 1-way ANOVA $F_{2,57}$=1.44 P>0.05).

*Egg quality - hatching success*

In 1990, there were significant differences in hatching success (proportion of eggs hatched per clutch) in relation to clutch size but not laying sequence (three-egg clutches only) when data from all treatments were pooled (hatching success by clutch size, Kruskal Wallis ANOVA $H_2$=7.864 P<0.02 N=82 (aggregating clutch sizes $\geq$ 3); number hatched or failed by laying order $X^2_2$=1.890 P>0.05 N=165). Clutches laid by egg-fed females tended to have higher hatching success and tended to produce more chicks per clutch than those of control females (Table 2), but these differences were not significant.

**Figure 3.** Mean volume ($cm^3$, ± S.E.) of eggs from three-egg clutches, laid by control, fish-fed and egg-fed gulls in 1990. Repeated measures Anova; effect of laying sequence Wilkes $F_{2,59}=111.61$ $P<0.001$; effect of feeding treatment $F_{2,60}=10.86$ $P<0.001$, interaction not significant.

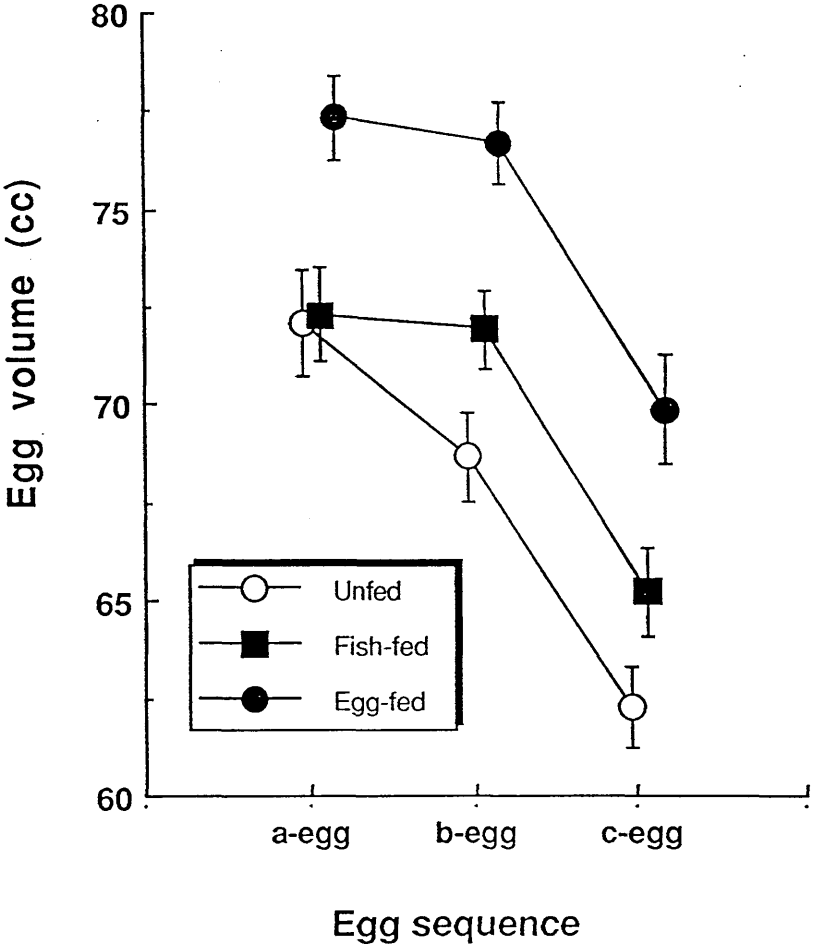

80
75
70
65
60
Egg volume (cc)
Unfed
Fish-fed
Egg-fed
a-egg
b-egg
c-egg
Egg sequence

Table 2. Average number of eggs hatched per clutch (± S.E. (N)), hatching success (proportion of eggs hatched per clutch), number of chicks fledged per pair and fledging success (proportion of chicks fledged per brood) for each treatment in 1990. Eggs lost during incubation to predators or trampled by stock are excluded.

| | Control | Fish-fed | Egg-fed | Sig. |
|---|---|---|---|---|
| Mean number of eggs hatched | 2.25 ± 0.18<br>(32) | 2.26 ± 0.22<br>(23) | 2.37 ± 0.17<br>(27) | H=0.024<br>P>0.05 |
| Hatching success | 0.779 ± 0.055<br>(32) | 0.775 ± 0.072<br>(23) | 0.883 ± 0.049<br>(27) | H=2.746<br>P>0.05 |
| Number of chicks fledged per pair | 1.62 ± 0.20<br>(29) | 1.37 ± 0.23<br>(19) | 1.73 ± 0.20<br>(26) | H=1.306<br>P>0.05 |
| Fledging success | 0.655 ± 0.075<br>(29) | 0.544 ± 0.088<br>(19) | 0.689 ± 0.074<br>(26) | H=2.056<br>P>0.05 |

### *Chick hatching size, weight and condition*

Figures 4 and 5 show that the chicks which hatched from eggs produced by egg-fed females tended initially to be larger skeletally, and also heavier, than those produced by unfed females, although the differences were only significant for b-chicks (statistics presented in Appendix 3). There were no significant differences in condition (weight controlling for skeletal size), suggesting that larger eggs produced chicks which were heavier simply because they were skeletally bigger, rather than carrying proportionally greater yolk reserves. There were no significant differences in size, weight or condition of the chicks of fish-fed gulls compared with controls.

**Figure 4.** Tarsus length of a-, b- and c-chicks at hatching, day 13 and day 34 (fledging), according to prelaying feeding regime of their parents (in mm, means ± S.E.).

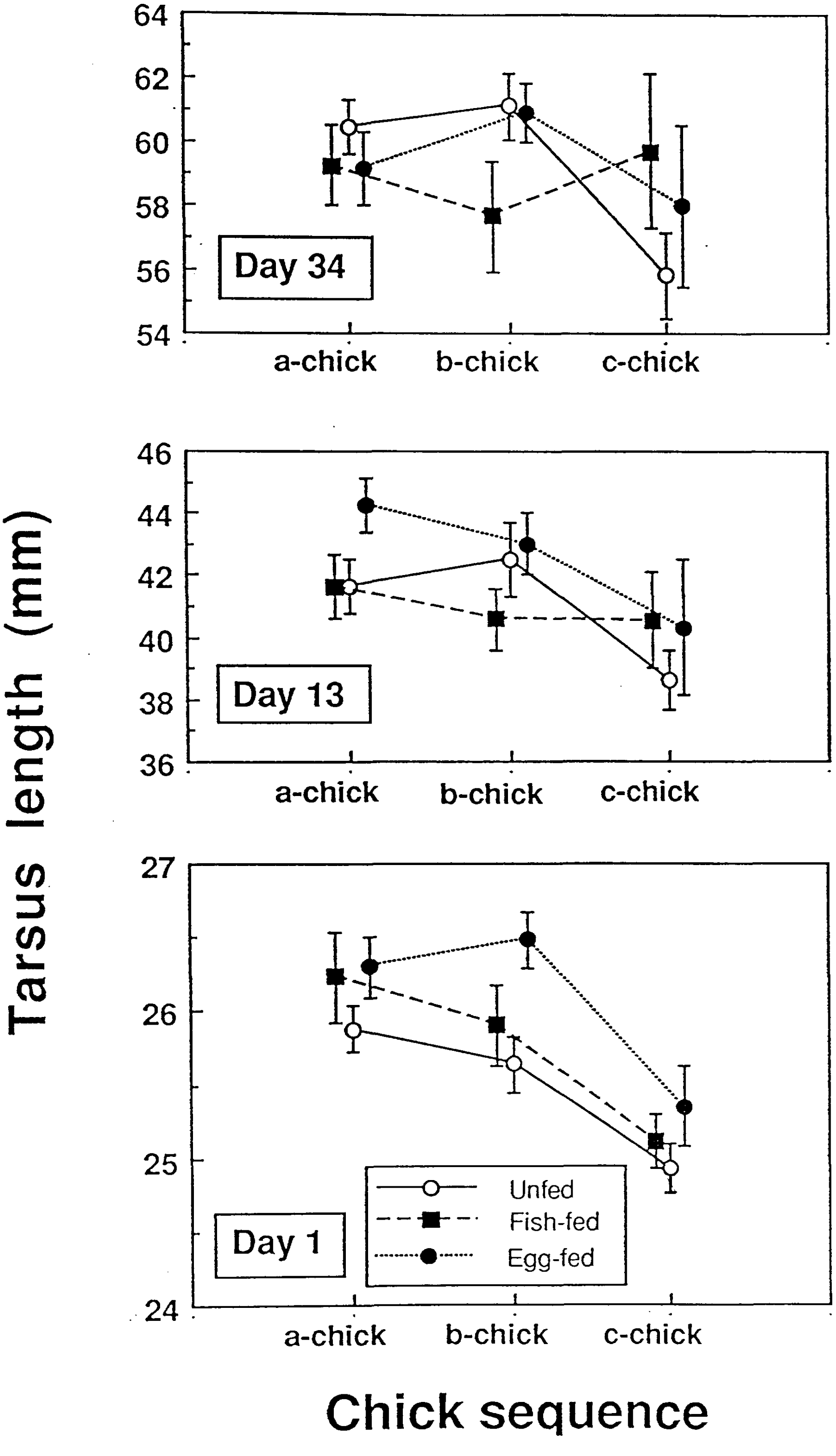
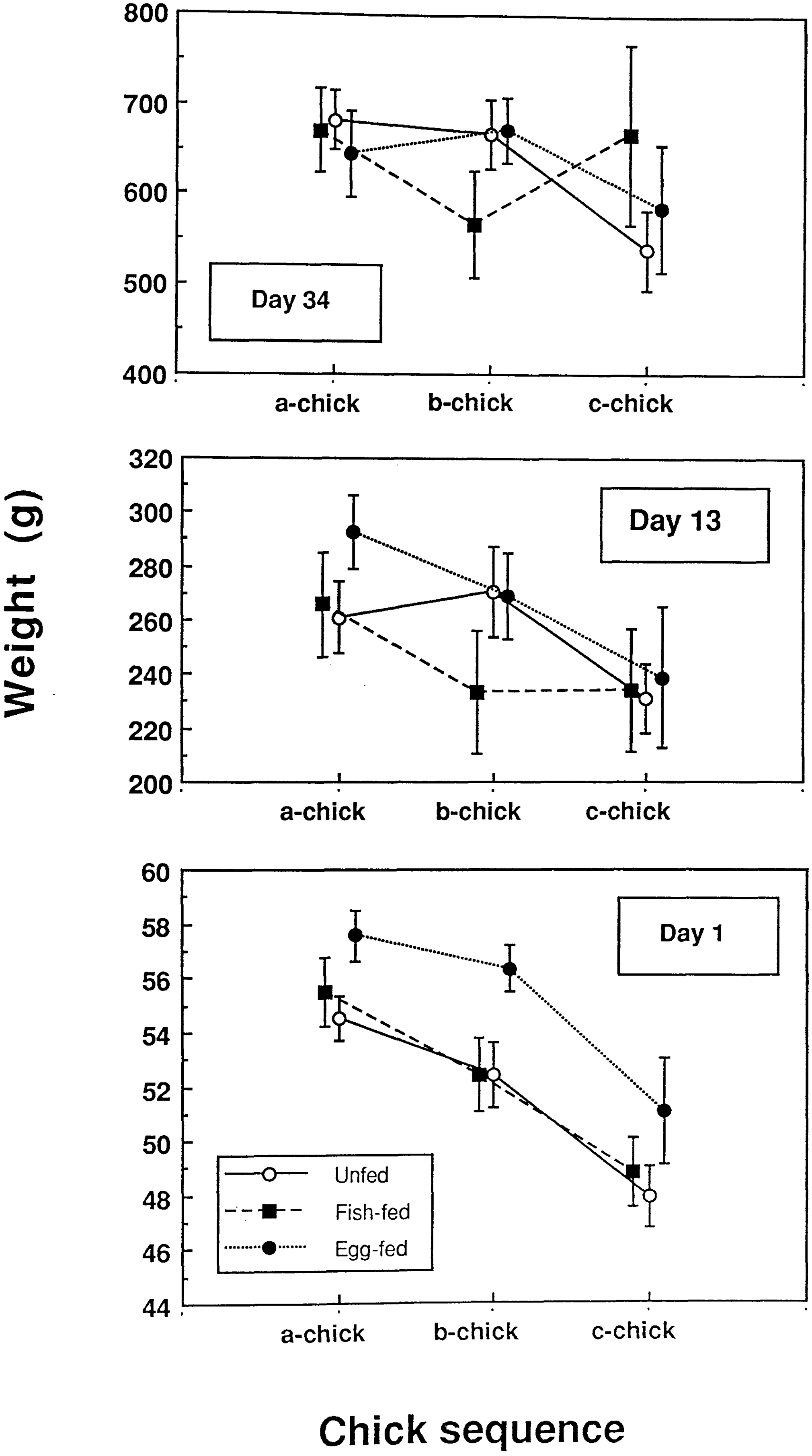

64
62
60
58
56
54
Day 34
a-chick
b-chick
c-chick
46
44
42
40
38
36
Day 13
a-chick
b-chick
c-chick
Tarsus length (mm)
27
26
25
24
Day 1
Unfed
Fish-fed
Egg-fed
a-chick
b-chick
c-chick
Chick sequence

**Figure 5.** Weight of a-, b- and c-chicks at hatching, day 13 and day 34, according to prelaying feeding regime of their parents (g, means ± S.E.).


Day 34
800
700
600
500
400
a-chick
b-chick
c-chick
Day 13
320
300
280
260
240
220
200
a-chick
b-chick
c-chick
Day 1
60
58
56
54
52
50
48
46
44
Unfed
Fish-fed
Egg-fed
a-chick
b-chick
c-chick
Weight (g)
Chick sequence

*Chick growth and survival*

There were no significant differences in the exponential tarsus or weight growth during the first two weeks, for either a- b- or c-chicks between treatments (Table 3). The data presented in Figures 4 and 5 show that there were no differences in tarsus length or weight of either a-, b- or c-chicks between treatments at day 13, or at fledging (day 34). The Kolmogorov-Smirnov two-sample test indicated that there were also no differences in the pattern of mortality for either a-, b- or c-chicks between either fish-fed or egg-fed parents and the control group (in all cases $P>0.05$). There were no significant differences in fledging success (proportion of chicks which fledged per brood) or in the number of chicks reared per pair (Table 2).

Table 3. Exponential tarsus and weight growth rates of a-, b- and c-chicks, according to treatment (mean ± S.E. (N), for method of calculation see text).

| | Tarsus growth rate ($\log_{10}$(tarsus)/day, $x10^{-3}$) | Weight growth rate ($\log_{10}$(weight)/day, $x10^{-3}$) |
|---|---|---|
| a-chicks | | |
| control | 17.18 ± 0.88 (18) | 55.77 ± 2.68 (18) |
| fish-fed | 16.95 ± 0.99 (10) | 56.02 ± 3.64 (10) |
| egg-fed | 18.90 ± 0.87 (15) | 60.33 ± 2.04 (15) |
| Kruskal Wallis | $H_2=4.000$ $P>0.05$ | $H_2=1.795$ $P>0.05$ |
| b-chicks | | |
| control | 17.58 ± 1.12 (19) | 60.15 ± 3.15 (18) |
| fish-fed | 17.39 ± 1.15 (8) | 59.41 ± 2.12 (8) |
| egg-fed | 17.85 ± 1.21 (13) | 56.69 ± 3.18 (13) |
| Kruskal Wallis | $H_2=0.209$ $P>0.05$ | $H_2=2.107$ $P>0.05$ |
| c-chicks | | |
| control | 16.53 ± 1.03 (11) | 58.36 ± 2.22 (11) |
| fish-fed | 14.40 ± 3.73 (4) | 60.30 ± 4.43 (3) |
| egg-fed | 17.03 ± 1.89 (7) | 57.53 ± 3.60 (7) |
| Kruskal Wallis | $H_2=0.678$ $P>0.05$ | $H_2=0.044$ $P>0.05$ |

## DISCUSSION

### *Effect of food supplementation on foraging behaviour.*

The addition of food on the breeding territory had no observable effect on the amount of time females spent away from the territory during the prelaying period in either year. This suggests that the food supplement was taken in addition to, and not in place of, normal foraging away from the colony. Among males there was similarly no demonstrable change in the pattern of territory attendance during the first experiment, when local feeding conditions were apparently relatively poor (Hiom *et al.* 1991, chapter 4). However, in the second year, when food availability was higher, both fish-fed and egg-fed males spent less time away from the territory than unfed males from 20 to 11 days before laying began. This suggests that the provision of the food supplement to the pair enabled these males to devote more time to colony-centered activities such as territory defence during this period. However there were no differences between fed and control males in the amount of time spent in the territory during the 10 days immediately preceding laying.

### *Courtship feeding in relation to provision of supplementary food*

Although several authors have suggested that courtship feeding in gulls may provide a valuable source of energy and nutrients for egg formation (for example Salzer & Larkin 1990), and Spaans (cited in Drent & Daan 1980) was able to increase the level of courtship feeding by provision of supplementary food, it was not possible to demonstrate any increase in the rate of courtship feeding on territories provided with additional food. The mean levels of courtship feeding recorded in this study were very low in comparison with those reported by Brown (1967) for lesser black-backed gulls (0.1 rising to 0.7 feeds/pair/hour during the 20 days prior to laying), and Salzer & Larkin (1990) for glaucous-winged gulls (0.09 rising to 0.18 feeds/pair/hour over the same period). This may be a reflection of the apparently poor food supply in 1989, though there are no data available on the level of courtship feeding away from the breeding colony, which may have been substantially higher.

### *Variation in egg size and composition in relation to supplementary food*

The differences in size between eggs of fish-fed and those of control gulls in 1989 lay in the yolk protein content of the c-eggs rather than in the a- or b-eggs. There were no differences in the mass of yolk fat or albumen among a-, b- or c-eggs between treatments. Parsons (1976) has shown that the reduced size of the c-egg in relation to the a- and b-egg of the same clutch in unfed herring gulls, is due to a reduction in albumen rather than yolk content. This is not surprising, since Parsons (1970) has also shown that the survival of chicks during the first few days of life is correlated with the level of yolk reserves which they possess on hatching, so reduction in yolk content of c-eggs would probably lead to a decrease in the survival probability of the c-chick. The results of the first experiment showed that poor protein supply during the prelaying and laying period can result in lowered yolk content of the c-egg, which may have a deleterious affect on the survival of c-chicks. The results of the second experiment described here suggest that the reduced size of the third egg is adaptive, and not the result of food limitation under normal food conditions. Birds provided with supplementary egg-protein produced eggs which averaged

10% bigger than controls, yet the size differential between a- and c-eggs was maintained.

### *Effect of prelaying food supply on chick hatching condition and survival*

In the second feeding experiment egg quality was measured in terms of viability (hatching success) and the size, condition, growth and survival of the chicks which were produced, since several authors have demonstrated that egg size has implications for chick size, growth and probability of fledging (Schifferli 1973, Nisbet 1978, Bolton 1991, chapter 3), which may act independently of parental quality. The results presented here show that the larger eggs produced by the egg-fed gulls produced chicks which were, on average both skeletally larger and heavier than those from eggs laid by unfed birds, though the differences were only significant for b-chicks. However, there were no differences in subsequent exponential growth nor in the size or weight of any chicks at 13 days. Nor were there significant differences in survival through to fledging of either a, b or c chicks according to treatment. These results appear to contradict those of Furness (1983), who has demonstrated that Great Skua *(Catharacta skua* Brunnich), chicks hatching from large eggs remain larger throughout development, and Parsons (1970) and Nisbet (1978), who have shown higher survival of large-egg chicks compared with those from small eggs (in herring gulls and terns respectively). However, it is important to control for parental quality in such studies. The data presented in chapter 3 show that parents which lay large eggs have greater success in rearing chicks hatching from eggs of any given size, compared with those laying small eggs. Furthermore, the effect of parental quality is more important than egg size in determining chick survival, as expected for a species with a comparatively long fledging period, in which the young are fed by the parents. In the experiment outlined here, provision of supplementary nutrients for egg formation allowed females (which presumably varied in their ability to produce large eggs) to increase the size of eggs which they laid. This may have conferred a slight advantage on the chicks which hatched from these larger eggs, in terms of both skeletal size and yolk reserves at hatching. However, such advantages were apparently short lived, since the egg-fed parents were unable to maintain the larger size and weight of their offspring during chick growth.

### *Limitation of egg production by availability of specific proteins*

The results of the first experiment strongly suggest that egg production of unfed birds was limited by a protein rather than an energy deficit; the fish protein supplement significantly increased both clutch and egg size, whilst a fat supplement of equivalent calorific value had no effect. That the need for specific nutrients also has a limiting effect on egg production is illustrated by the results of the second experiment. In a year in which no local food shortage was apparent since control birds produced the typical clutch size, the provision of food in the form of eggs, while not affecting the number of eggs produced, did result in an increase in egg size. Provision of a general protein supplement (fish) had no effect. Both the identity and destination (yolk or albumen) of these specific nutrients are unknown, and since the subsequent fate of the chicks hatched from eggs laid by egg-fed females was examined, these eggs could not be subjected to composition analysis.

Studies on poultry have shown that egg production can be improved by increased methionine, lysine or tryptophan intake (Fisher 1976). The experimental results presented here clearly demonstrate that the egg supplement provided some nutrient required for egg formation, not provided in sufficient quantities by the fish, which suggests that the frequent practice of egg robbing in gulls, if carried out at the time of egg formation, may serve to increase the supply of these specific nutrients, rather than being purely opportunistic.

Several recent reviews (Meijer, Daan and Hall 1980, Martin 1987, Arcese and Smith 1988) emphasise that the provision of supplementary food during the prelaying period may have variable results on egg production. Of 27 studies (Appendix 4), 7 reported an advancement in laying date and an increase in clutch size, 10 had an effect on laying date only and 2 increased clutch size independent of timing of laying. The remaining 8 studies found no effect on either clutch size or laying date. Disappointingly, the effect on egg size is often not reported, though as shown here, an effect on egg size may be demonstrable even when clutch size is unaffected. As Martin points out, if resources for egg formation are limited, egg size may be reduced before clutch size since the latter represents a quantum reduction in reproductive potential, whereas reduction of egg size may have lesser consequences on fitness (Bolton 1991, chapter 3).

There are various reasons for the disparate nature of the results obtained from feeding trials and the results of this study demonstrate two important points regarding the interpretation of effects of supplementary feeding experiments:

1. The quality of the food provided is important; not all food supplements will necessarily have an equivalent effect on egg production. This is the first known study to examine the effects of more than one food type in a single year. An "energetic" approach to understanding the requirements and control of egg formation may not be appropriate, since the data presented here have demonstrated the existence of protein requirements, which are not met by an

increased energy intake. In addition to these general protein requirements, these data show that that egg production can be limited by the availability of specific nutrients, possibly essential amino acids, which are not provided in significant quantities by all protein rich foods.

2. The demonstration of a significant increase in egg production of fed birds, compared with unfed controls, is dependent on the performance of the control group in the year of the study. Here it has been shown that the provision of fish can lead to a significant increase in both clutch and egg size, compared with unfed controls (experiment 1), yet in the same colony, in the following year, an identical supplement had no effect on either variable (experiment 2). These results confirm the findings of Hiom *et al.* (1991) who also demonstrated that provision of food to lesser black-backed gulls at a colony (Skomer) where clutch size of unfed birds was normal caused no further increase in egg production. Unfortunately authors of feeding experiments often fail to report whether egg production of control birds in the year of the study is typical for the species. Martin (1987) suggests that one reason for the failure of many experimental studies to detect an influence of food supply on clutch size is that they are typically conducted over just one or two years, and so are more likely to include average years when clutch size is at, or close to, that which produces the maximum number of chicks that the parents are capable of rearing. This contrasts with the non-manipulative approach which has often demonstrated the importance of prelaying food supply on clutch size (for example Jarvinen and Vaisanen 1984, Hussell and Quinney 1986, Korpimaki and Hakkarainen 1991). Studies relating natural food abundance to clutch size are typically conducted over several years and are therefore more likely to include years of low food availability when clutch size may be reduced.

The failure to detect an advancement of laying date among fed birds, despite an obvious effect on egg number and quality, contrasts with most other studies. However, it is in accordance with other supplementation studies on gulls (Reid 1987) and may provide an insight into the relationship between food supply, the timing of laying and clutch size determination. Extensive work on Kestrels (Meijer, Daan & Hall 1980, Beukeboom *et al.* 1988, Meijer, Daan and Dijkstra 1988) has provided a model for these relationships. In this species clutch size is determined by the timing of rapid follicle development, which may be triggered once the female's body reserves cross a threshold level. Once triggered, the number of follicles which are ovulated appears to be determined by the date of follicle development, rather than the level of body reserves. Provision of food advances laying, but does not lead to an increase clutch size above that predicted from the laying date; thus clutch size is not increased independent of laying date, females are merely advanced up the preprogrammed regression slope relating clutch size to laying date. This effect contrasts markedly with the results presented here for lesser black-backed gulls, where additional food has been shown to affect clutch size independent of the timing of laying, despite the existence of a similar inverse relationship between clutch size and laying date in gulls (Brown 1967, Davis & Dunn 1976). This suggests that clutch size may not be primarily determined by the timing of laying, as it appears to be in kestrels, but possibly by the level of the prelaying body reserves (Houston, Jones & Sibly 1983). A possible reason for such a difference may lie in the unpredictable nature of fluctuations in food supply. In species such as the kestrel, where food supply varies in a predictable hyperbolic fashion, the timing of crossing a certain threshold may provide a good indication of food availability later that season, and therefore be a meaningful cue to optimal clutch size. No data are available on the pattern of fluctuations in food availability for lesser black-backed gulls, but for a such species which is characterised by opportunist foraging such a mechanism may not provide a reliable indication of food availability later in the season. It is more likely that lesser black-backed gulls lay as many eggs as they can (within the upper limit set by incubation and/or brood rearing capacity), and opt for brood reduction later in the season if food supplies fail.

*Appendix 1. Dry mass (g, mean + S.E. (N)) of components of three egg clutches, by laying sequence and treatment.*

| | Control | Fish-fed | Fat-fed |
|---|---|---|---|
| A-eggs | | | |
| Shell | 4.92 ± 0.14 (12) | 5.23 ± 0.12 (14) | 5.11 ± 0.13 (16) |
| Albumen | 5.81 ± 0.19 (12) | 5.80 ± 0.30 (14) | 6.12 ± 0.14 (16) |
| Yolk (whole) | 9.88 ± 0.26 (12) | 10.32 ± 0.13 (14) | 10.44 ± 0.34 (16) |
| Yolk protein | 3.58 ± 0.15 (12) | 3.82 ± 0.16 (14) | 3.68 ± 0.10 (16) |
| Yolk lipid | 6.30 ± 0.17 (12) | 6.50 ± 0.15 (14) | 6.76 ± 0.24 (16) |
| Yolk ratio | 1.72 ± 0.06 (12) | 1.88 ± 0.15 (14) | 1.72 ± 0.07 (16) |
| Yolk lipid index | 1.78 ± 0.07 (12) | 1.74 ± 0.08 (14) | 1.84 ± 0.03 (16) |
| B-eggs | | | |
| Shell | 4.60 ± 0.10 (10) | 5.12 ± 0.14 (16) | 4.83 ± 0.16 (17) |
| Albumen | 5.48 ± 0.16 (10) | 5.92 ± 0.23 (16) | 5.93 ± 0.14 (17) |
| Yolk (whole) | 9.54 ± 0.33 (10) | 10.33 ± 0.18 (16) | 10.02 ± 0.28 (17) |
| Yolk protein | 3.46 ± 0.11 (10) | 3.71 ± 0.09 (16) | 3.55 ± 0.13 (17) |
| Yolk lipid | 6.08 ± 0.24 (10) | 6.62 ± 0.12 (16) | 6.47 ± 0.19 (17) |
| Yolk ratio | 1.75 ± 0.07 (10) | 1.79 ± 0.08 (16) | 1.70 ± 0.06 (17) |
| Yolk lipid index | 1.76 ± 0.05 (10) | 1.79 ± 0.04 (16) | 1.83 ± 0.04 (17) |
| C-eggs | | | |
| Shell | 4.24 ± 0.09 (14) | 4.68 ± 0.13 (16) | 4.27 ± 0.11 (16) |
| Albumen | 5.05 ± 0.14 (14) | 5.50 ± 0.16 (16) | 5.26 ± 0.18 (16) |
| Yolk (whole) | 8.45 ± 0.18 (14) | 9.59 ± 0.19 (16) | 8.73 ± 0.37 (16) |
| Yolk protein | 3.03 ± 0.08 (14) | 3.56 ± 0.11 (16) | 3.02 ± 0.10 (16) |
| Yolk lipid | 5.42 ± 0.14 (14) | 6.03 ± 0.14 (16) | 5.71 ± 0.28 (16) |
| Yolk ratio | 1.69 ± 0.05 (14) | 1.76 ± 0.05 (16) | 1.69 ± 0.09 (16) |
| Yolk lipid index | 1.80 ± 0.06 (14) | 1.72 ± 0.06 (16) | 1.89 ± 0.05 (16) |

*Appendix 2. Statistical analysis of egg composition data.*

| Egg component | Egg | one-way ANOVA (effect of treatment) | | repeated measures ANOVA (effect of sequence) | |
|---|---|---|---|---|---|
| | | F | Sig. | Wilkes F | Sig. |
| Shell dry | a | $F_{2,39}=1.32$ | $P>0.02$ | $F_{2,35}=54.37$ | $P<0.001$ |
| | b | $F_{2,40}=2.86$ | $P>0.02$ | | |
| | c | $F_{2,43}=4.72$ | $P<0.02$** | | |
| Albumen protein | a | $F_{2,39}=0.71$ | $P>0.02$ | $F_{2,35}=19.99$ | $P<0.001$ |
| | b | $F_{2,40}=1.49$ | $P>0.02$ | | |
| | c | $F_{2,43}=1.87$ | $P>0.02$ | | |
| Yolk (whole) | a | $H=3.736$ | $P>0.05$ $n=42$[1] | sig. differences in dispersion matrices | |
| | b | $F_{2,40}=1.99$ | $P>0.02$ | | |
| | c | $F_{2,43}=4.97$ | $P<0.02$* | | |
| Yolk protein | a | $F_{2,39}=0.72$ | $P>0.02$ | $F_{2,35}=22.91$ | $P<0.001$ |
| | b | $F_{2,40}=1.20$ | $P>0.02$ | | |
| | c | $F_{2,43}=9.46$ | $P<0.0001$** | | |
| Yolk lipid | a | $F_{2,39}=1.35$ | $P>0.02$ | $F_{2,35}=17.18$ | $P<0.001$ |
| | b | $F_{2,40}=1.95$ | $P>0.02$ | | |
| | c | $F_{2,43}=2.30$ | $P>0.02$ | | |

There were no interactions between egg sequence and treatment in any group.

[1] Homogeneity of variance assumption violated, Kruskal Wallis ANOVA applied.

* Tukey test: Fish-fed group significantly greater than control group.

** Tukey test: Fish-fed group significantly greater than both control and fat-fed groups.

## *Appendix 3. Statistical analysis of tarsus, weight and condition of chicks on hatching, at 13 and 34 days, according to treatment.*

| | Tarsus length | | Weight | | Condition | |
|---|---|---|---|---|---|---|
| | H | sig. | H | sig. | H | sig. |
| Hatching[1] | | | | | | |
| a-chicks | $F_{2,60}=1.20$ | N.S. | $F_{2,60}=2.74$ | N.S. | $F_{2,60}=1.02$ | N.S. |
| b-chicks | $F_{2,60}=4.51$ | $P<0.02$ | $F_{2,60}=4.20$ | $P<0.02$ | $F_{2,60}=0.74$ | N.S. |
| c-chicks | $F_{2,49}=1.02$ | N.S. | $F_{2,49}=1.25$ | N.S. | $F_{2,49}=0.50$ | N.S. |
| Day 13[2] | | | | | | |
| a-chicks | $H_2=4.36$ | N.S. | $H_2=2.03$ | N.S. | $H_2=0.45$ | N.S. |
| b-chicks | $H_2=2.88$ | N.S. | $H_2=3.22$ | N.S. | $H_2=0.38$ | N.S. |
| c-chicks | $H_2=1.33$ | N.S. | $H_2=0.72$ | N.S. | $H_2=2.25$ | N.S. |
| Day 34[2] | | | | | | |
| a-chicks | $H_2=0.33$ | N.S. | $H_2=0.78$ | N.S. | $H_2=0.52$ | N.S. |
| b-chicks | $H_2=3.27$ | N.S. | $H_2=1.85$ | N.S. | $H_2=0.31$ | N.S. |
| c-chicks | $H_2=2.16$ | N.S. | $H_2=1.61$ | N.S. | $H_2=0.26$ | N.S. |

[1] One-way ANOVA
[2] Kruskal Wallis ANOVA

*Appendix 4. Studies of the effect of food supplementation on egg production.*

| Study | Species | Laying date | Clutch size | Egg size |
|---|---|---|---|---|
| Krebs 1971 | *Parus ater* | N | N | - |
| Jones 1973 | *Parus major* | N | N | - |
| Kallander 1974 | *P. major* | A | I | - |
| Yom-Tov 1974 | *Corvus corone* | A | N | - |
| Von Bromssen & Jansson 1980 | *Parus cristatus* | A | N | - |
| Von Bromssen & Jansson 1980 | *Parus montanus* | A | N | - |
| Smith *et al.* 1980 | *Melospiza melodia* | A | N | - |
| Hogstedt 1981 | *Pica pica* | A | I | I |
| Newton & Marquiss 1981 | *Accipter nisus* | A | I | - |
| Ewald & Rohwer 1982 | *Agelaius phoeniceus* | A | N | - |
| Harper 1984 | *Erithacus rubecula* | N | I | - |
| Reese & Kadlec 1984 | *Pica pica* | - | N | - |
| Davies & Lundberg 1985 | *Prunella modularis* | A | N | - |
| Poole 1985 | *Pandion haliaetus* | N | N | - |
| Hochachka & Boag 1987 | *Pica pica* | A | N | N |
| Reid 1987 | *Larus glaucescens* | N | - | I* |
| Arcese & Smith 1988 | *Melospiza melodia* | A | I | N |
| Knight 1988 | *Pica pica* | A | N | - |
| Meijer Daan & Dijkstra 1988 | *Falco tinnunculus* | A | I | - |
| Hochachka 1988 | *Pica pica* | A | N | I |
| Korpimaki 1989 | *Aegolius funereus* | A | I | N |
| Hornfeldt & Eklund 1990 | *Aegolius funereus* | A | I | - |
| Hiom *et al.* 1991 (Skomer) | *Larus fuscus* | N | N | N |
| Hiom *et al.* 1991 (Flat Holm) | *Larus fuscus* | N | I | I |
| Dhindsa & Boag 1990 | *Pica pica* | A | N | - |
| This study (fish) | *Larus fuscus* | N | N | N |
| (egg) | *Larus fuscus* | N | N | I |

* Last-laid (third) egg only.

N = No significant effect of supplementary food.
A = Advancement in laying date among birds fed supplementary food.
I = Increase in clutch or egg size among birds fed supplemeary food.

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# CHAPTER 6

# PROXIMATE DETERMINATION OF CLUTCH SIZE IN LESSER BLACK-BACKED GULLS:THE ROLES OF FOOD SUPPLY AND BODY CONDITION

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THE DATA PRESENTED IN THIS CHAPTER FORM THE BASIS OF A PAPER TO BE SUBMITTED TO

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# SUMMARY

Whilst many authors have demonstrated an adaptive relationship between food supply or territory quality and clutch size, the proximate mechanism by which clutch size is determined remains largely unknown. Here, a series of supplementary feeding experiments are used in conjunction with a new technique to measure pectoral muscle protein levels in live birds (chapter 2), in order to investigate the relationships among food supply, body condition and clutch size. Across treatment comparisons revealed a positive relationship between levels of flight muscle protein at the start of laying and clutch size, but not egg size. In a year when the clutch sizes of unfed birds were reduced, birds provided with a protein supplement had higher flight muscle protein levels at the start of laying and laid larger clutches than birds which received no food supplement. In contrast, gulls provided with an additional energy source (fat) did not attain higher protein levels, or lay larger clutches than unfed controls. In a second year, when unfed gulls laid larger clutches, which were typical for the species, supplements of two different protein types (fish and egg) had no effect on clutch size, though egg-fed females laid considerably (10%) larger eggs. However, there were no differences in condition between treatments at the start of laying, suggesting that whilst flight muscle protein levels may be of importance in determining clutch size, they may be of lesser significance for egg size, which may instead be influenced by dietary intake.

# INTRODUCTION

## *Food supply and clutch size*

Variation in reproductive effort both among years and among individuals is generally considered to be an adaptive response to variation in food supply and/or individual foraging capacity (Drent & Daan 1980). Clutch size is one aspect of avian reproduction that may be adjusted to tailor reproductive effort to individual capacity and local conditions, thereby maximising lifetime reproductive success. Many authors have demonstrated a correlation between average clutch size and food supply across years, (for example Bryant 1975, Dijkstra *et al.* 1982, Jarvinen & Vaisanen 1984, Hornfeldt & Eklund 1990, and Sydeman *et al.* 1991) or related individual variation in clutch size to differences in territory quality, individual foraging ability , or body condition in a single year (Nisbet 1973, Ankney & MacInnes 1978, Hogstedt 1980, Houston Jones & Sibly 1983, Dijkstra *et al.* 1988 Korpimaki 1989, Sydeman *et al.* 1991). However, the proximate mechanism by which clutch size is adjusted remains poorly understood.

## *The role of body reserves*

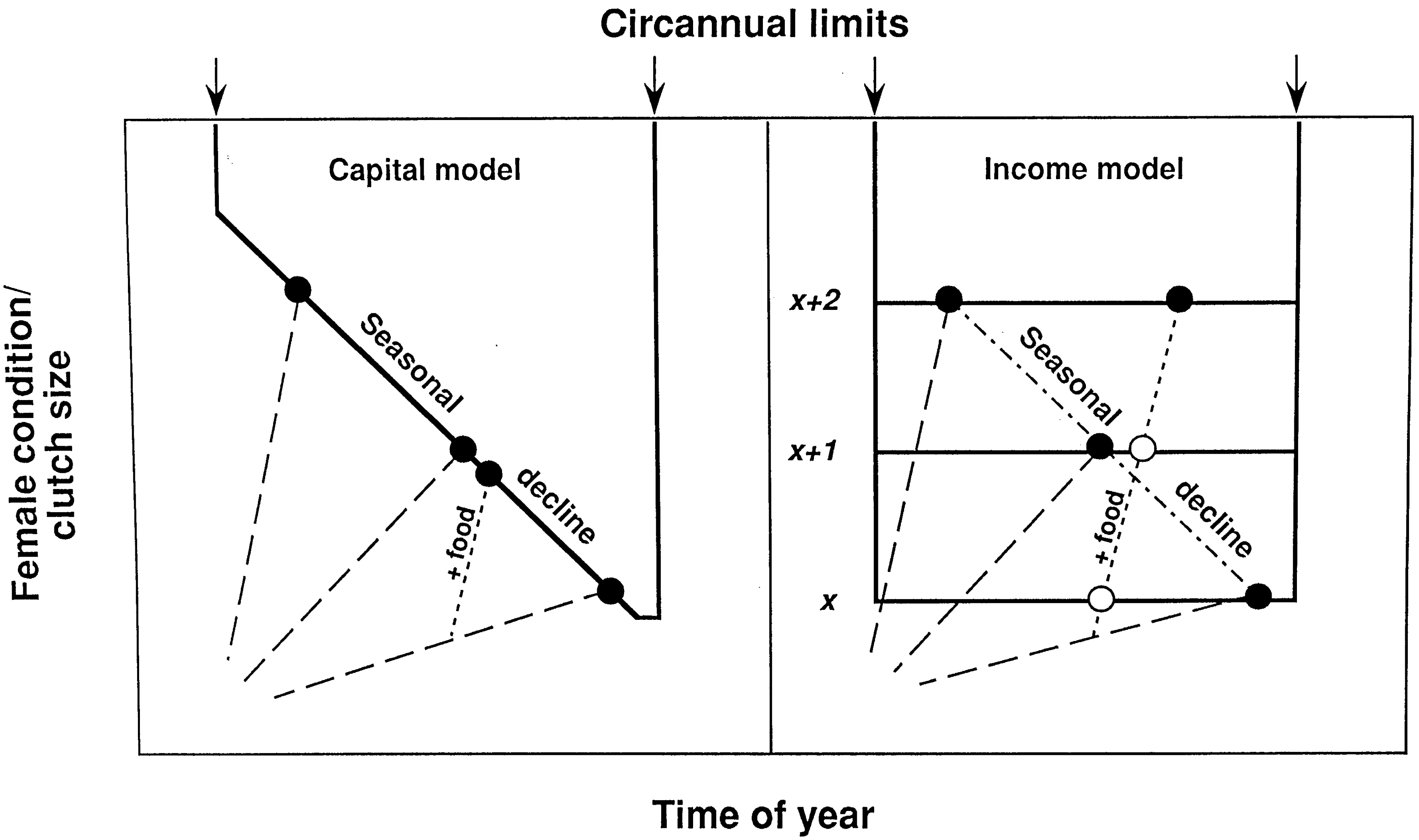
Studies on the changes in flight muscle protein during the prelaying and laying periods have suggested that the accumulation of a threshold level of protein reserves may provide the proximate stimulus to trigger rapid follicle development and determine the number of follicles ovulated (Jones & Ward 1976, Fogden & Fogden 1979, Houston, Jones & Sibly 1983, Jones 1991). Thus body protein condition may provide the causal link between food supply and egg production. However, Meijer, Daan & Dijstra (1988) have emphasised that correlative analyses cannot prove such a causal mechanism; covariance between body condition and egg production may be the result of a third variable, such as breeding experience or competitive ability. In order to demonstrate a causal role of body condition an experimental approach is required. More convincing evidence that protein reserves provide the proximate mechanism through which clutch size is regulated, would be provided by the experimental demonstration that:

1. increased food supply produces an increase in body condition at the start of laying in comparison with a group of unfed controls.
2. the experimentally fed birds, having accumulated greater body reserves, lay significantly larger clutches, on average, than the control group.

### *Models of clutch size determination*

Drent & Daan (1980) have proposed two alternative models to describe the way in which the accumulation of body reserves might be monitored in order to determine the appropriate clutch size, and produce the seasonal decline in clutch size which is characteristic of many avian species. Under the first ("capital") model (Figure 1), it is proposed that the timing of laying and clutch size are determined by the date at which an individual accumulates a threshold level of reserves. The decline in clutch size with laying date results from a seasonal decline in the threshold reserve level which initiates laying. Under the alternative ("income") model, the decisions of when and how many eggs to lay are based on the *rate* of accumulation of reserves rather than on acquisition of a given quantity at a particular time. Individuals which accumulate reserves at a high rate lay larger clutches than those accumulating reserves more slowly. The number of eggs laid and the timing of laying are determined by the rate of change of reserves relative to a number of fixed thresholds. An individual reaching the threshold for laying $x$ eggs whilst accumulating reserves at a high rate responds by continuing to the threshold for $x+1$ eggs. Individuals

**Figure 1.** "Capital" and "income" models of the proximate regulation of laying date and clutch size in relation to female body condition (after Drent & Daan 1980). Broken lines: body condition of females of varying quality, or under differing conditions of food supply. Dotted lines: predicted change in body condition of late laying birds given supplementary food. The capital model predicts late laying fed birds should produce the appropriate clutch size for the laying date. The income model predicts larger clutches in late laying fed birds compared with unfed controls laying at the same time. Closed circles represent clutch sizes laid, open circles represent thresholds for laying which are passed by individuals going on to lay larger clutches. The characteristic seasonal decline in clutch size of birds not receiving supplementary food is shown.


Circannual limits
Capital model
Seasonal
decline
+ food
Income model
x+2
x+1
x
Seasonal
decline
+ food
Female condition/
clutch size
Time of year

accumulating reserves more slowly (due to lower competitive ability or poor food supply) do not continue to the next threshold but rather opt for laying smaller clutches.

Both models predict a positive correlation between the level of reserves in birds about to lay and the clutch size produced; Drent & Daan (1980) have shown that a critical test between these hypotheses is provided by experimentally providing extra food to determine whether clutch size can be increased independently of laying date. The capital model predicts an advancement in laying date but no increase in clutch size of late laying fed birds compared to unfed birds laying at the same time, while the income model predicts larger clutches in the late laying fed birds, compared with unfed birds laying at the same time (dotted lines in Figure 1).

### *Experimental manipulations of food supply*

Experimentally increasing food supply has led to an increase in clutch size in several avian species (reviews in Martin 1987, Arcese & Smith 1988, Meijer, Daan & Hall 1980) and clutch size in lesser black-backed gulls has been shown to increase in response to provision of a protein supplement (chapters 4 and 5). Here further data from the same feeding experiments are presented, examining the body condition of fed and control birds. The data are examined to determine whether experimentally increased protein supply, which led to an increase in egg production in one year, was accompanied by an increase in body condition. The body condition of females given supplements of other food types, but which did not lay increased clutches, is also examined. In addition the data on clutch sizes of fed birds laying at the end of the season are analysed to discriminate between the competing hypotheses of Drent and Daan (1980).

## METHODS

### *Study site and experimental provision of food*

This study was conducted on Flat Holm Island, from April to August in 1989 and 1990. Food was provided to individual pairs for three to four weeks prior to laying and continued until five days after the first egg was laid. The food supplement was placed next to the nest scrape at night (between 00.00 and 01.00 hrs) to reduce the risk of stealing by neighbouring pairs (full details of the artificial provisioning procedure are given in chapter 4). In 1989 two alternative supplements were provided; one experimental group received chopped fish (200 g/day/pair) and a second received chopped fat (120 g/day/pair) of similar energy value. In the following year two alternative supplements were again provided; one group received fish as before and a second group received cooked eggs, in the form of an omelette (140 g/day/pair, see Chapter 5 for further details).

### *Capture of birds and assessment of flight muscle protein condition*

Data on follicle growth from Brown (1967) suggest that the maximum daily protein requirements of egg formation of a gull producing a three-egg clutch will occur the day before the first egg is laid. However, it was difficult to predict when females were about to begin laying, so capture of birds on the day before the first egg was laid was not possible. However, on the day the first egg was laid, adults could be caught easily on the nest using walk in traps made of wire mesh. Once caught, each bird was weighed (spring balance to ± 10 g) and measured (total head and bill, keel length, caudal end of keel to the distal end of the humerus (the effective length of the pectoral muscle), tarsus and wing length, all measurements to ± 0.1 mm, except wing, to ± 1 mm). In addition a recording was made of the profile of the flight muscles over the keel, from which pectoral muscle protein was assessed using the method of Bolton, Monaghan & Houston (1991, chapter 2). Most birds were individually recognisable on the basis of variation in plumage features (the size and wear of the primary spots) and bill markings. Observations were made daily from a hide and individuals were sexed on differences in behaviour and size when both members of the pair were present for direct comparison. All individuals were marked with colour rings and/or plumage dye when first captured. Birds were classified as at the "start of laying" if caught within one day of laying their first egg and as at the "end of laying" if caught within 2 days after laying their last egg. A total of 149 captures were made (64 in 1989 and 85 in 1990) at these two stages, involving 127 individuals. The sample of birds from each treatment caught at the start of laying was considered to be random (in all cases there were no differences between the median clutch size of the sample and the treatment as a whole) and independent of the second sample (a total of 22 individuals were caught twice). There was no change in the likelihood of trapping a bird at the end of laying if it had been captured previously (observed proportion retrapped = 27%, expected proportion, (number of birds caught previously, at the start of laying/total number of birds) = 19% $X^2_1$=2.745 P>0.05). However, among birds caught at the end of laying, there was a significant bias towards those which had laid a clutch of three eggs, since it was not possible to predict which birds were going to lay a clutch of two eggs only. Once it had become clear that no third egg was to be laid (i.e. two to three days after the second egg), it was not possible to catch birds under the criteria outlined above for the end of laying (within two days of the last egg).

### *Controlling for variation in body size*

In making comparisons of condition between individuals, it is necessary to control for differences in body size, since body size explains approximately 76%

of the variance in flight muscle protein (see Bolton, Monaghan & Houston 1991, chapter 2). To do this, principle component analysis of the five linear measures given above was used to produce a single variable representing body size. Only one factor was extracted, with high (>0.79) loadings on each of the independent variables.

Regression analysis of pectoral muscle protein on this body size variable (PC Factor 1) was applied in order to control for differences in body size. For each individual in the regression, condition was determined from the size and sign of the residual, which provided a measure of the deviation in pectoral muscle protein from that expected on the basis of body size. The use of this method means that the calculated condition value of any individual is relative to the other individuals in the sample. It is important therefore to examine the effects of sex and year on the relationship to determine whether calculation of condition for within-sex and within-year analyses can be made on the basis of a single regression using data from both sexes and years. Covariance analysis of the data from both years of birds caught at the both stages of laying showed that there were no differences in the relationship between pectoral muscle protein and body size according to either year or sex, so it was possible to pool data for calculation of condition (year: $F_{1,146}=1.91$ $P>0.05$, sex: $F_{1,146}=0.16$ $P>0.05$, interactions not significant).

There are two approaches to computing condition from the residuals obtained from the regressions of muscle protein on body size described above; the residuals may either be expressed in absolute terms (positive or negative) or as a proportion, relative to the predicted value. The former method provides an index of the protein mass which may be available for egg formation. The latter method expresses this deviation in mass as a proportion of the expected mass and thereby enables comparisons to be made of the relative condition of individuals of different body size, since a given mass of protein may be of more value to a small individual, compared to a large one (Blem 1990). The former method was chosen in this study since both large and small females would need to acquire similar absolute amounts of protein to produce a clutch of a given size. In fact, the correlations between clutch size and the two measures of condition were virtually identical and using the relative measure of condition would not alter any of the following results.

### *Clutch size, egg size and laying dates*

Data on clutch size, egg size and laying dates were obtained by checking all nests daily (usually before 09.00 hrs) from the beginning of nest building through to clutch completion. Eggs were measured (length and breadth) on the day of laying, and egg volume was calculated from the equation:

$$\text{volume} = 0.476 \times \text{length} \times \text{breadth}^2$$

Mean egg size was determined for three-egg clutches from the total clutch volume. In both years the clutch size of fed birds was compared with that of unfed controls which were subjected to the same degree of human disturbance. These comparisons indicated that in 1989 the availability of food for egg production was low, whereas in 1990 food supply was higher (full details in chapters 4 and 5).

### *Statistical analysis and presentation*

In view of the relatively large number of statistical comparisons, especially of the data presented in Figure 4, those which are significant at only the 0.05 level should be regarded with caution.

## RESULTS

### *Body size, body condition and egg production*

Pooling data from all females caught in both years, at both stages and from all treatments, there was no relationship between female body size and either clutch size or body condition (clutch size; Spearman's $r=0.026$ $N=117$ $P>0.05$, body condition; Pearson's $r=0.026$ $N=117$ $P>0.05$). Plotting the residuals from the regression of condition at the start of laying on clutch size, against body size, showed no correlation (Pearson's $r=-0.091$ $N=54$ $P>0.05$), indicating that there was no tendency for larger females producing a clutch of any given size to be in poorer condition than smaller individuals laying the same number of eggs. However, there was a significant positive correlation between body size and egg size (clutch volume of three egg clutches) for both years (1989: Pearson's $r=0.421$ $N=26$ $P<0.04$, 1990: $r=0.446$ $N=44$ $P<0.002$). The relationship between body size and egg size was examined further, using a treatment-based approach. For each year, there was no significant correlation between egg size and body size among females which were provided with a supplement which enabled them to lay larger eggs than controls (fish-fed in 1989: $r=0.365$ $N=10$ $P>0.05$, and egg-fed in 1990: $r=0.271$ $N=11$ $P>0.05$, see chapter 5). However, among the individuals which were laying relatively smaller eggs in each year there was such a correlation (unfed and fat-fed gulls in 1989: $r=0.681$ $N=14$ $P<0.008$, and unfed and fish-fed gulls in 1990: $r=0.420$ $N=33$ $P<0.02$).

### *Body condition, clutch size and egg size*

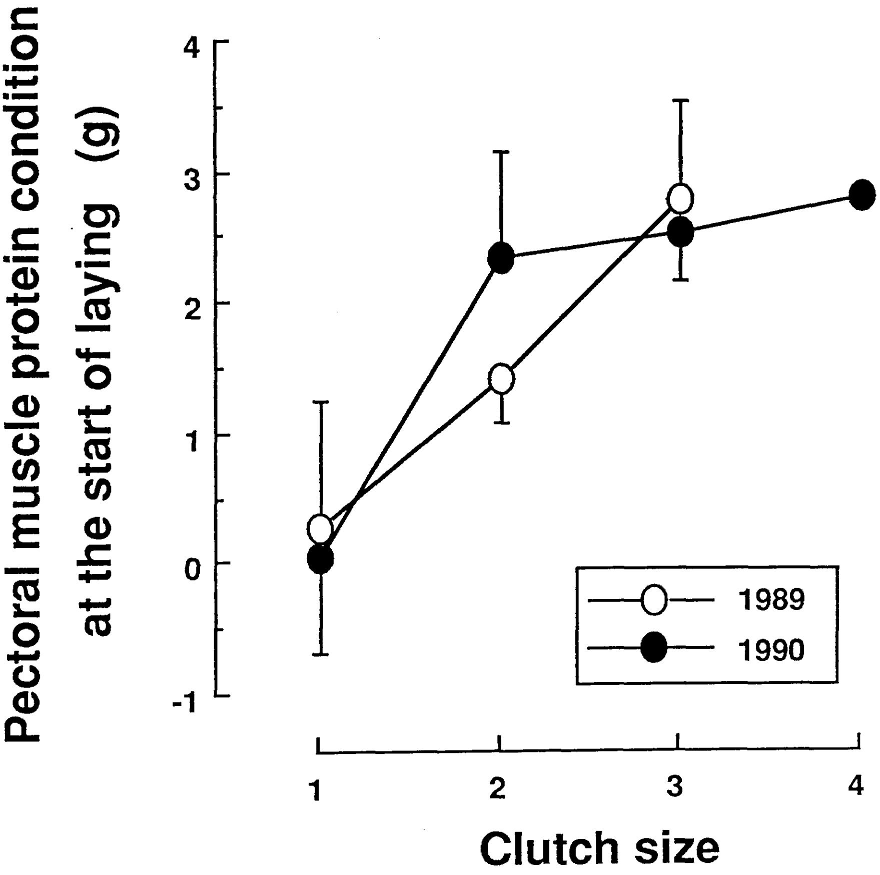
Combining data from both years and all treatments, there was a significant relationship between female pectoral muscle protein condition at the start of laying and clutch size among females (Figure 2, Spearman's $r=0.464$ $N=54$ $P<0.002$). In contrast, there was no indication of a relationship between condition of males at the start of laying and the number of eggs laid by their mates (Spearman's $r=-0.190$ $N=13$ $P>0.05$). Among females which laid clutches of three eggs, the relationship between condition at the start of laying and total clutch volume was not significant (Pearson's $r=0.340$ $N=32$ $P>0.05$).

The relationship between clutch size and condition was examined further, applying a two-way analysis of variance to determine whether there were differences in the relationship between condition and clutch size between years. Although there were significant differences in the condition of females at the start of laying according to the size of clutch produced, there were no differences according to year (effect of clutch size: $F_{2,47}=6.62$ $P<0.003$ (clutches $\geq 3$ aggregated for analysis), effect of year: $F_{1,47}=0.00$ $P>0.05$, interaction not significant).

### *Body condition of experimentally fed and unfed birds*

The correlative analyses given above do not prove a causal relationship between body reserves and clutch size. In order to demonstrate such a causal relationship, it is necessary to effect an experimental increase in both body reserves and egg production as a result of increased food availability. In the first feeding experiment (1989), when clutch sizes of unfed birds were reduced, provision of a protein (fish) supplement led to a significant increase in clutch size (chapters 4 and 5), whereas an energy (fat) supplement in the same year, though eaten, did not lead to an increase in clutch size (chapter 5). In the following year (1990), egg production of unfed birds was normal and there was no further increase in clutch size in response to either additional fish or egg protein (chapter 5), although egg-fed birds laid considerably larger eggs than unfed controls. The variation in the level of flight muscle protein acquired at the start of laying was

**Figure 2.** Relationship between clutch size and condition at the start of laying among females in 1989 and 1990.


Pectoral muscle protein condition at the start of laying (g)
4
3
2
1
0
-1
1
2
3
4
Clutch size
1989
1990

examined in response to these different feeding regimes. Figure 3 clearly shows that gulls which laid enlarged clutches in response to a protein supplement in 1989 attained, on average, higher levels of flight muscle protein than either control females or those receiving the fat supplement, which laid smaller clutches. In contrast, there were no differences in body condition at the start of laying between treatments in the following year, when there were no differences in clutch size according to feeding regime, though egg fed females laid considerably larger eggs than controls. These results indicate that whilst flight muscle protein condition may be of importance in the regulation of clutch size, it may be of lesser significance in the determination of egg size.

The changes in flight muscle protein during the period of egg laying were then examined (Figure 4). In control (unfed) females in both years, there was a significant decline in flight muscle protein (averaging 2.82g in 1989 and 3.61g in 1990). Among males however, there was no change in the protein condition during the laying period in either year. The body condition of females at the end laying was also examined according to feeding regime. There were no differences in condition of females at the end of laying, between feeding treatments in either year, suggesting that the increased protein reserves of fish fed birds in 1989, compared with controls, had been used to produce their larger clutches.

### *Capital vs. income models of clutch size determination*

It is possible to examine the two models proposed by Drent & Daan (1980) to explain the way in which body reserves may determine clutch size: the capital model predicts that fed birds should lay earlier than unfed controls, and produce the appropriate clutch size for the laying date. The income model makes no firm prediction regarding the timing of laying, but predicts that fed birds should lay

**Figure 3.** Clutch sizes (mean ± S.E.) and flight muscle protein condition (g, + S.E.) at the start of laying, of lesser black-backed gulls under different supplementary feeding regimes (condition: one-way ANOVAs 1989; $F_{2,20}=5.77$ $P<0.01$, 1990; $F_{2.28}=0.551$ $P>0.05$, data on clutch sizes from chapter 5). Tukey tests indicate that in 1989 the only difference between treatments lies between the control and fish-fed groups.

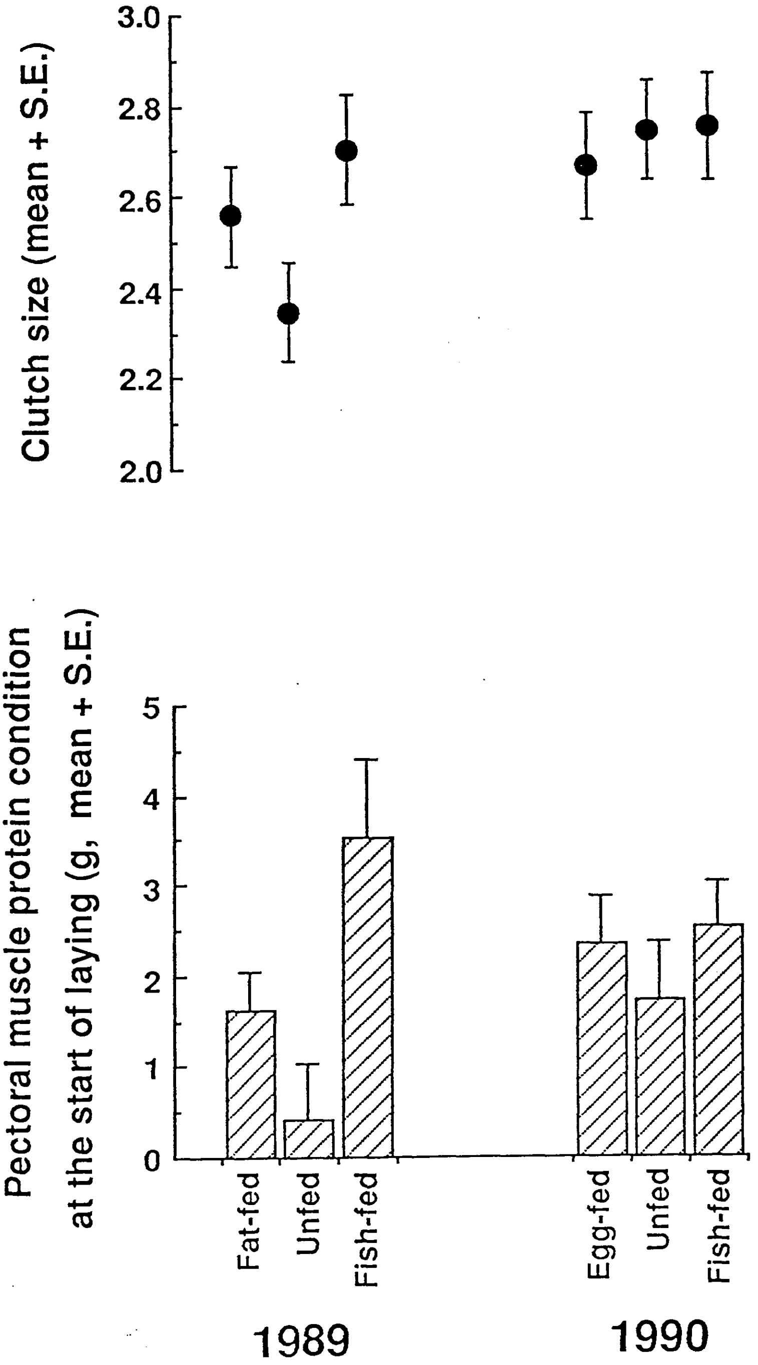
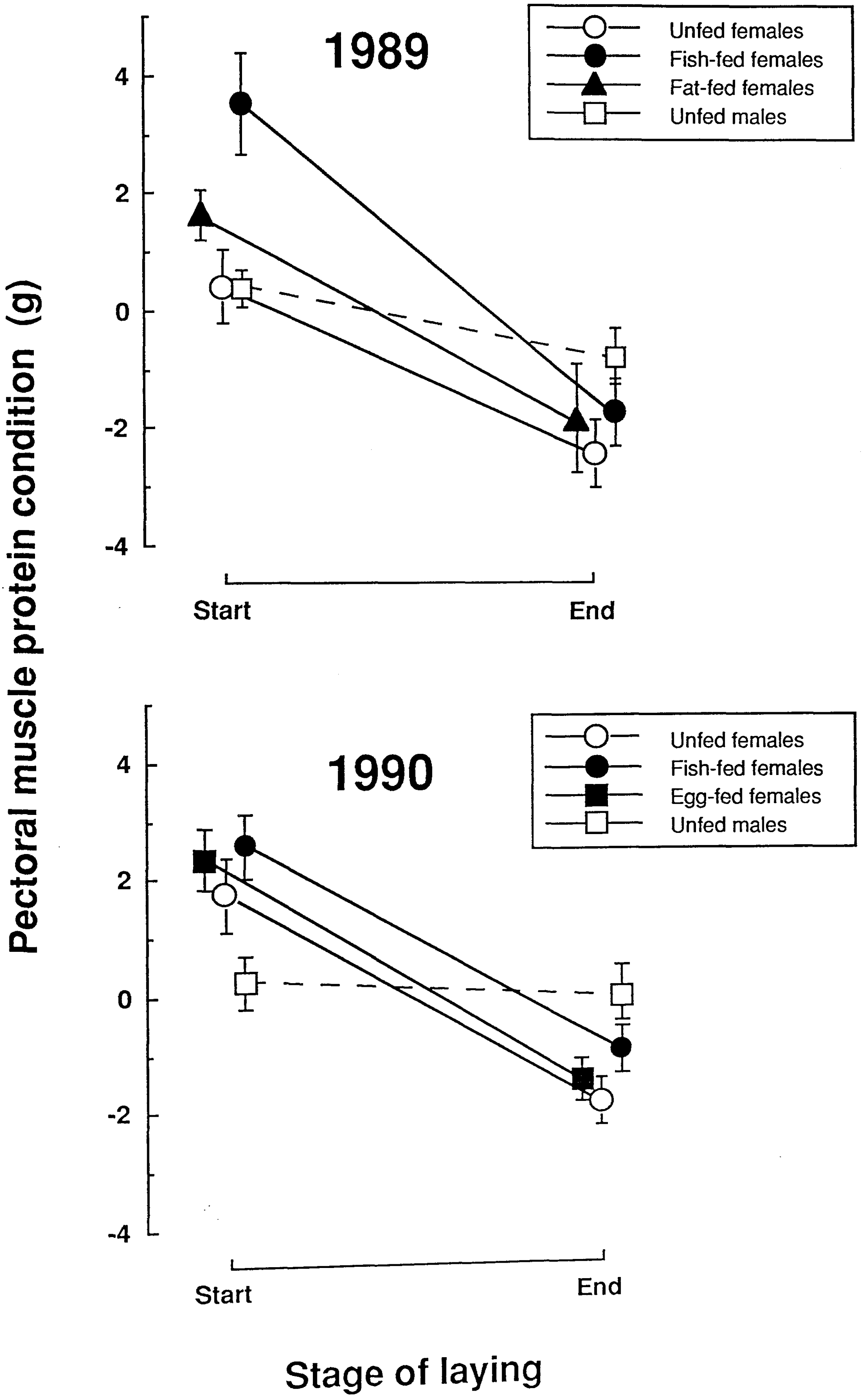
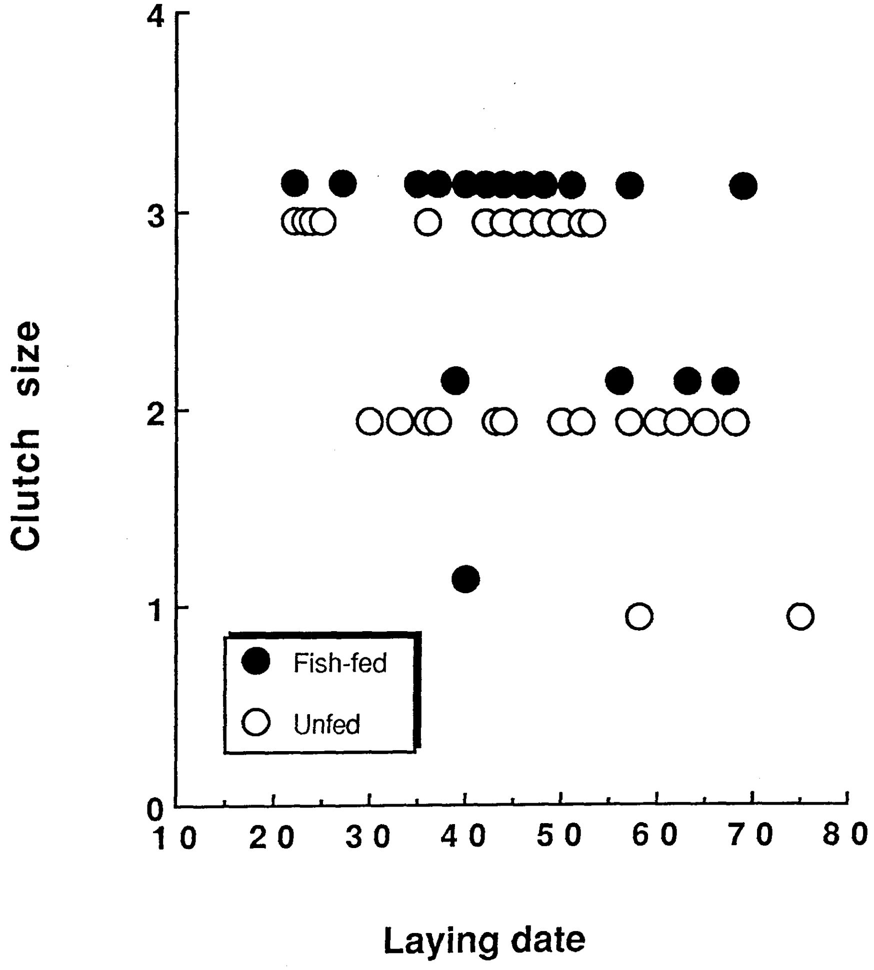

Clutch size (mean + S.E.)
3.0
2.8
2.6
2.4
2.2
2.0
Pectoral muscle protein condition at the start of laying (g, mean + S.E.)
5
4
3
2
1
0
Fat-fed
Unfed
Fish-fed
Egg-fed
Unfed
Fish-fed
1989
1990

**Figure 4.** Changes in pectoral muscle protein condition during laying in 1989 and 1990. Decline significant for all females (1989: control; $t_{14}=3.32$ $P<0.005$, fish-fed; $t_{14}=5.17$ $P<0.001$, fat-fed; $t_{17}=3.18$ $P<0.005$, 1990: control; $t_{23}=4.88$ $P<0.001$, fish-fed; $t_{20}=5.32$ $P<0.001$, egg-fed; $t_{17}=5.68$ $P<0.001$). Decline in males not significant (1989: $t_{11}=1.54$ $P>0.05$, 1990: $t_{17}=0.35$ $P>0.05$). Condition of females at the end of laying one-way ANOVAs: 1989; $F_{2,25}=0.253$ $P>0.05$, 1990; $F_{2,32}=1.37$ $P>0.05$.



Pectoral muscle protein condition (g)
1989
Unfed females
Fish-fed females
Fat-fed females
Unfed males
4
2
0
-2
-4
Start
End
1990
Unfed females
Fish-fed females
Egg-fed females
Unfed males
4
2
0
-2
-4
Start
End
Stage of laying

**Figure 5.** Dependence of clutch size on laying date (1 = 1st April) for unfed and fish-fed lesser black-backed gulls in 1989.


Clutch size
4
3
2
1
0
10
20
30
40
50
60
70
80
Laying date
Fish-fed
Unfed

larger clutches than controls laying at the same date predicts.

Provision of supplementary fish in 1989 led to an increase in clutch size independent of laying date (chapter 4). Among unfed birds, clutch size declined as the season progressed (Figure 5, Spearman's $r=-0.541$ $P<0.001$ $N=34$). However, among fed birds, there was no significant decrease in clutch size with season (Spearman's $r=-0.232$ $P>0.05$ $N=21$). These results show that birds receiving the food supplement did not show the seasonal decline in clutch size indicating that the clutch sizes of late laying fed birds were larger than unfed controls. These results provide support for the "income" model of clutch size determination.

## DISCUSSION

Many authors have demonstrated a correlation between female condition (usually body mass) and clutch size, both between years and between individuals. Whilst some have accepted this as good evidence of a causal link between body condition and egg production, others have highlighted the limitations of such an approach, and the need for a manipulative procedure (for example Meijer, Daan & Dijkstra 1988). In this study, body condition has been determined not merely from gross differences in body mass, but rather on the basis of levels of flight muscle protein, which has been implicated as a source of proteins for egg formation (Jones & Ward 1976, Fogden & Fogden 1979, Jones 1991). It has been shown that there was a positive correlation between the level of flight muscle protein acquired at the start of laying and the number of eggs laid, and also that flight muscle protein declined sharply in females during egg laying, whereas there was no decline in males over the same period. However, there was not a significant relationship between female protein condition at the start of laying and egg size, indicating that the females' protein status may be of less importance for egg size than clutch size. Most importantly it has been shown that provision of supplementary fish protein, which led to an increase in clutch size in a year of apparent food shortage (Hiom *et al.* 1991, chapter 4), was accompanied by a significant increase in flight muscle protein acquired at the start of laying. By the end of laying there were no significant differences in protein levels between treatments, indicating that the fish-fed females had lost more muscle protein than control gulls, which may have been used for egg synthesis, thereby enabling them to produce larger clutches. However, provision of a fat supplement in the same year did not lead to an increase in either body condition (this chapter) or egg production (chapter 5). In the following year, when the availability of food for egg formation was apparently good, neither fish nor egg supplements had any impact on clutch size, though egg size was increased in response to an egg-protein supplement. There were no differences in flight muscle protein condition at the start of laying between fed and control gulls, which provides further evidence that whilst body condition may influence clutch size, it may be of less significance in determining egg size. Egg size was influenced however by female body size. In both years there was a general correlation between skeletal size and clutch volume of three egg clutches. Examining the importance of female body size in relation to food supply revealed that in each year, body size effects were only significant for those treatments in which females laid relatively small eggs. Body size was not correlated with egg size among those females which received a supplement which enabled them to lay larger eggs. These results are consistent with the hypothesis that flight muscle protein levels determine the number of follicles ovulated, and hence clutch size, but egg size is influenced by dietary intake during the period of egg (especially albumen) formation. If nutrients required for albumen synthesis are in limited supply, larger females, being probably competitively superior to smaller individuals, are able to acquire greater quantities of such nutrients and thereby produce larger eggs. Under the supplementary feeding regimes, the provision of fish and egg supplements on the breeding territories increased the availability of nutrients with which to form larger eggs and therefore reduced the competitive advantage of larger females. The correlation between body size and egg size which may be apparent in most non-manipulated situations therefore disappeared, as smaller individuals had greater access to nutrients for egg formation.

The predictions of the "capital" and "income" hypotheses of Drent & Daan (1980) explaining the possible relationship between food supply, body condition and clutch size, were also examined. Whilst provision of additional protein led to an increase in clutch size, it did not advance laying, and in addition, the seasonal decline in the clutch sizes of unfed birds in that year was absent in birds receiving the protein supplement. These results provide support for the "income" model, and they are interpreted as further evidence of a causal link between body condition and clutch size, which may provide the physiological mechanism by which clutch size is tuned to environmental food supply and individual foraging capacity.

These results are in accord with those obtained by Korpimakki (1987, 1989) and Korpimakki & Hakkarainen (1991) for Tengmalm's owl (*Aegolius funereus*). On the basis of natural variation in food supply, body mass and clutch size, as well as experimental manipulations of food availability, these studies concluded that in Tengmalm's owl, clutch size is proximately determined by food supply during the prelaying period, mediated by female body condition. They found that whilst laying date was correlated most strongly with winter vole (the main food) abundance, clutch size was related to food supply during spring. In addition, experimentally increasing food availability led to an increase in clutch size independent of laying date. Korpimakki and Hakkarainen interpreted these findings as evidence in support of the "income" model of clutch size determination.

In contrast to these findings, extensive studies on the roles of food supply laying date and body condition in the determination of clutch size in kestrels have suggested, in that species, that clutch size is determined primarily by laying date (Meijer, Daan & Dijkstra 1988). The effect of laying date is mediated via an increasing tendency in later breeders to incubate soon after clutch initiation which inhibits further follicle development, thereby reducing clutch size (Beukeboom *et al.* 1988). The role of female body condition appears to be minimal, although it may set the threshold which must be reached in order to trigger follicle development, thereby determining laying date.

As suggested by Meijer, Daan & Hall (1980) it is not surprising that different mechanisms of clutch size determination appear to be operative in different species, due to interspecific variation in temporal and spatial fluctuations in food supply. It is clear too that in gull species there tends to be less variation in clutch size compared with many other species, including birds of prey, such as those studied by Dijkstra *et al.* (1988) and Korpimakki & Hakkarainen (1991). In gulls, clutches exceeding three eggs are very uncommon, and supplementary feeding experiments have shown that the normal truncation of the clutch at three eggs is unlikely to be the result of food limitation (chapter 4). When egg production is normal (mean clutch size about 2.7 eggs) provision of additional food does not result in a further increase in clutch size. This may be the result of smaller fluctuations in food availability for breeding, and/or it may reflect a life history strategy. The lesser black-backed gull is generally a long lived species, and the modal clutch of three eggs may represent the clutch size which produces the maximum number of chicks which may be raised in a season without incurring deleterious lifetime fitness costs, given the normal patterns of fluctuations in food availability.

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# CHAPTER 7

# THE UPPER LIMIT TO CLUTCH SIZE IN LESSER BLACK-BACKED GULLS: COSTS OF EGG PRODUCTION, INCUBATION CAPACITY AND CHICK REARING ABILITY

# SUMMARY

The limit to clutch size in lesser black-backed gulls may potentially be determined by either the costs associated with the production of additional eggs, by incubation capacity or by chick rearing ability. This study uses a series of clutch manipulations to independently increase the costs associated with egg production, incubation and chick rearing. Breeding performance, measured in terms of hatching success, the number, size and weight of chicks reared to fledging was related to these experimental manipulations, in order to examine the potential limits to clutch size.

The costs of laying were experimentally increased by removal of the first laid egg, which led to an increase in the total number of eggs laid per pair. There was an indication that females which laid a further three eggs after the removal of their first egg reared fewer chicks than those rearing an unmanipulated clutch of three. There were no differences in the fledging size or weight of those chicks which survived in comparison with controls.

Pairs which were given a fourth egg to incubate, hatched more eggs than control gulls laying unmanipulated clutches of three, suggesting that clutch size is not restricted to three by incubation capacity. However, these broods of four suffered lower fledging success and did not produce more fledged chicks than control broods of three, although there were no differences in size and weight at fledging of those chicks which survived.

These results suggest that incubation capacity does not limit clutch size, but rather the limit to clutch size may be set by the inability of the parents to rear more than three chicks, if the normal pattern of hatching asynchrony, which

would inevitably accompany the unmanipulated production of a clutch of four eggs, is maintained. The data also suggested that experimentally increased egg production may result in a decrease in subsequent breeding performance. The mechanism by which increased egg production may impair productivity is unclear, but may be related to the competitive ability of chicks soon after hatching, rather than parental body condition.

# INTRODUCTION

Clutch size is considered to be an important, adaptive component of reproductive effort in birds. Variation in the number of eggs laid among individuals and among years has been shown to be related to the availability food for breeding, which may act either at the stage of egg production or chick rearing.

Whilst many avian genera show a wide variation in clutch size, most species of seabird exhibit much less variation in the number of eggs laid each breeding attempt. For lesser black-backed gulls, in common with many gull species, the modal clutch size is three eggs (accounting for 75% of all clutches in some years) and clutches exceeding this are rare (approximately 2% of clutches, *pers. obs.*). Whilst clutch size may be reduced in some years in response to low availability of protein during the prelaying period, supplementary feeding experiments in years when egg production is normal, suggest that food limitation of egg production is not the cause of the usual truncation of the clutch at three (Hiom *et al.* 1991, chapter 4; chapter 5).

When Lack (1947, 1948) proposed the first formal hypothesis to explain species variation in clutch size, he considered that for many birds the limit to clutch size was likely to determined by food supply for chick rearing. However, brood enlargement experiments have suggested that several gull species (including lesser black-backed gulls) are capable of rearing larger broods (four or five chicks) than their usual clutch size permits (Harris & Plumb 1965, Ward 1973).

Lack further suggested that, whilst food supply may determine clutch size in many species, for gulls which lay relatively large eggs, and have three discrete brood patches, the current limit to clutch size may be set by incubation capacity. The suggestion is that in evolutionary history, clutch size was set at three eggs by food limits on chick rearing, and gulls secondarily became highly adapted to care for three eggs, with the evolution of three brood patches. These adaptations currently exert selection pressure maintaining clutch size at three eggs (see Coulter 1987).

Here a series of clutch manipulation experiments are used to examine whether the upper limit to clutch size in lesser black-backed gulls is currently limited to three eggs by the costs of egg production, by incubation capacity, or by the inability of adults to rear more than three chicks. The approach adopted here is therefore different to that used in other clutch and brood manipulation studies, which have only considered the costs involved in incubation and chick rearing. Previous experiments on the incubation and brood rearing capacities of seabirds (for example herring gull Haymes & Morris 1977, lesser black-backed gull, Harris & Plumb 1965, glaucous-winged gull Ward 1973, western gull Coulter 1973a, long-tailed skua *Stercorarius longicaudus* Andersson 1976, glaucous-winged gull Reid 1987), have all been effected by simply adding an extra egg to recently completed clutches, or by adding a newly hatched chick to recently hatched broods. Such an approach fails to consider the possible effects of increased egg production on incubation and brood rearing capacity which would accompany the non-experimental production of a supernormal brood. By independently manipulating the number of eggs laid and the clutch size incubated and reared, it is possible to isolate and examine the effects of each of these factors on breeding performance separately.

## METHODS

The study was conducted on Flat Holm Island (S. Glamorgan, U.K.) between May and August 1990. Observations were carried out from a raised hide during territory establishment and forty nest scrapes were individually marked during nest construction. All nests were visited daily (usually before 09.00 hrs) during the prelaying period and randomly assigned to a treatment group when the first egg was laid.

### *Manipulations*

In order to imitate the increased costs of egg production which would accompany the unmanipulated production of a clutch of four eggs, clutch enlargements were produced by removing the first laid egg from the nest on the day it was laid. It is well known that gulls will usually replace eggs which are lost on the day of laying (Paludan 1951, Parsons 1976) and that they normally develop more follicles than they eventually ovulate (Houston *et al.* 1983). It was assumed that egg removal was normally followed by replacement of the lost egg, followed by laying of the rest of the clutch. Eggs which were removed were placed in a warm room (15-22'C) until the remainder of the clutch had been laid.

In order to examine the effects of these experimentally induced increased costs of egg production on later incubation and chick rearing performance, a series of four treatments were produced in which the size of the clutch laid and the size of the clutch incubated (and reared) were independently manipulated (Table 1).

In the first treatment, which served as a control, neither the size of the clutch produced, nor the number of eggs incubated were manipulated. In the second group, (termed "laying costs only") the size of the clutch laid was increased by

Table 1. Establishment of four treatments, independently manipulating clutch size and the costs of increased egg production.

| Treatment | Clutch produced | Clutch incubated |
|---|---|---|
| 1. "control" | Unmanipulated | Unmanipulated |
| 2. "laying costs only" | Increased | Unmanipulated |
| 3. "rearing costs only" | Unmanipulated | Increased |
| 4. "Laying & rearing costs" | Increased | Increased |

egg removal, but the first-laid egg was not returned to the nest after clutch completion, so the 'normal' clutch size was incubated. In the third group, (termed "rearing costs only") egg production was not experimentally increased, but an extra egg was added to the clutch two days after completion (to maintain the normal pattern of laying). Finally, a fourth treatment (termed "laying and rearing costs") was produced in which both the costs of egg production, and also the size of the clutch incubated were increased. The first egg was removed on the day of laying, and returned to the nest after clutch completion.

Table 2. Frequency distributions of clutch sizes laid according to treatment.

| Clutch size | TREATMENT | | | |
|---|---|---|---|---|
| laid | 1 | 2 | 3 | 4 |
| 1 | 2 | 0 | 1 | 0 |
| 2 | 3 | 1 | 4 | 0 |
| 3 | 15 | 6 | 15 | 8 |
| 4 | 0 | 13 | 0 | 12 |
| mean | 2.65 | 3.60 | 2.70 | 3.60 |
| S.E. | 0.15 | 0.13 | 0.13 | 0.11 |
| N | 20 | 20 | 20 | 20 |

Using this egg removal procedure, it was possible experimentally induce females to lay enlarged clutches. There were significant differences in the frequency distribution of clutch sizes between gulls which laid an unmanipulated clutch, and those which had their first egg removed (Table 2, $X^2_2=54.46$ $P<0.01$, clutch sizes of 1 & 2 eggs aggregated for analysis), with the majority of the latter tending to lay an additional egg. As a result of the manipulations outlined above, there were also significant differences between treatments in the number of eggs incubated (Table 3).

### *Nest histories*

Nests were visited daily throughout the entire breeding season (laying, incubation, hatching and chick growth). Each egg was marked with indelible ink on the day of laying, according to laying sequence (a-, b-, c-egg etc) and laying dates, manipulated and incubated clutch sizes were recorded for each pair. (For treatments 2 and 4 the manipulated clutch size was the total number of eggs produced under the egg removal procedure). Hatching success was measured as the proportion of incubated eggs which hatched (chicks were able to break free from the egg completely). Unfortunately, eggs which were removed on the day of laying, and then returned to the nest after clutch completion (treatment 4) did not remain viable, probably due to the low humidity of the storage conditions. All these eggs failed to hatch and showed no sign of embryo development. For this group, hatching success was therefore calculated as the proportion of the remaining eggs which hatched. It was not possible to predict beforehand if clutch manipulation would affect hatching success, which could potentially nullify the enlargement procedure. Therefore it was decided to replace those eggs which failed to hatch with chicks known to have hatched on the day expected for the failed eggs (it was possible to predict which eggs had failed since they showed no sign of pipping, usually visible up to two days before hatching). Thus it was possible to record the hatching success of the incubated clutch, and also maintain the clutch enlargements, established at laying, until after hatching. Four complete clutches were eaten by a predator partway through incubation and a further three complete clutches failed to hatch due to infertility and were not replaced with newly hatched chicks. For each nest, the duration of hatching was calculated as the delay, in days, between the hatching of the first and last eggs of the clutch.

Nests were fenced with wire enclosures within two days of hatching to facilitate location of chicks, and each enclosure was provided with a wooden shelter, since the enclosure denied chicks access to thick vegetation or burrows in which to shelter.

Within each brood, all chicks were individually marked with insulation tape around the tarsus on the day of hatching. Nests were visited daily and all chick losses were recorded. All chicks which survived to 34 days were considered to have fledged since some were capable of flight a few days later and were able to escape from their enclosures. Chicks were weighed (± 1g) and measured (tarsus length, ± 0.1mm) at 34 days and the number of chicks fledged successfully from each brood was recorded.

### *Body condition*

To determine whether the increased egg production of females in treatments 2 and 4 resulted in a demonstrable cost in terms of body condition, a sample of females from each treatment was trapped within two days of completing their clutches and their flight muscle protein condition was measured (Bolton, Monaghan & Houston 1991, chapter 2). Condition was assessed from the residuals from the regression of total flight muscle protein on body size (principal component analysis of five univariate body measurements). Both males and females were also trapped during the final week of incubation to determine whether gulls incubating experimentally enlarged clutches were in poorer condition than those incubating normal clutches, and whether there was an inverse relationship between clutch size and condition during incubation.

### *Data analysis*

It was anticipated that each of the breeding parameters outlined above (number of eggs hatched, chicks fledged etc), could be affected by both clutch size (within treatments) and also by the experimental clutch enlargement procedure (i.e. treatment effects). This study is primarily concerned with the factors involved in determining the upper limit to clutch size, and is therefore mainly restricted to comparisons of breeding performance of pairs laying control clutches of three eggs with those laying or rearing enlarged clutches of four eggs. In addition, small sample sizes generally prohibit statistical analysis of data from clutches of less than three eggs. Chi-squared tests have been conducted following recommendations of

Snedecor & Cochran (1967), to aggregate categories only if expected frequencies fall below 1, rather than 5. These authors have shown that reducing the number of categories severely impairs the sensitivity of the chi-squared test, whereas acceptance of expected frequencies between 1 and 5 does not have serious effects. Throughout this chapter means are presented ± one standard error.

## RESULTS

The overall breeding performances of pairs in each of the four treatments are given in Table 3. These data clearly show that there were no differences in either laying date, or hatching success between treatments as a whole. Gulls with enlarged clutches (treatment 3 only, due to non-viability of removed a-eggs in treatment 4) therefore hatched significantly more chicks, per clutch, than those with normal sized clutches. However, there were no differences in the number of chicks fledged per brood, despite significant differences in the original brood sizes (treatment 4 included since all unhatched eggs in all treatments were replaced with wet chicks). The number of chicks fledged per brood are shown, according to both brood size and treatment in Figure 1. These data suggest that both the costs of increased egg production, and chick rearing ability may limit clutch size to three eggs. These effects are analysed in more detail below, with respect to variation in egg and chick production costs.

**Figure 1.** Number of chicks fledged from control and experimentally enlarged broods, by initial brood size (for treatment definitions see text, mean ± S.E.).

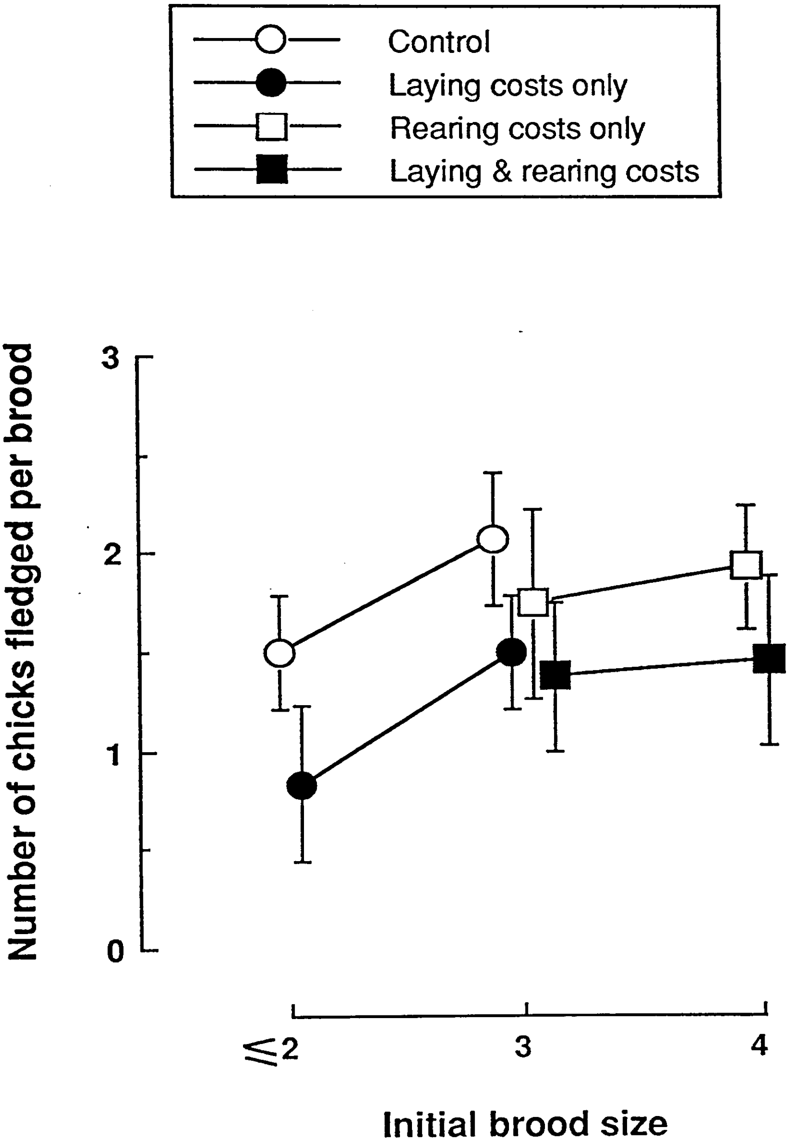

Control
Laying costs only
Rearing costs only
Laying & rearing costs
Number of chicks fledged per brood
3
2
1
0
≤2
3
4
Initial brood size

Table 3. Laying date, clutch size laid and incubated, hatching success and number of chicks fledged according to treatment (mean ± S.E., N=20, except where shown).

| | TREATMENT | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Sig.[1] |
| Laying date | 15/5±1.8 | 18/5±2.1 | 17/5±2.1 | 19/5±2.1 | $F_{3,76}=0.625$<br>N.S. |
| Clutch laid | 2.65±0.15 | 3.60±0.13 | 2.70±0.13 | 3.60±0.11 | $H_3=35.75$<br>$P<0.0001$ |
| Clutch incubated | 2.65±0.15 | 2.60±0.13 | 3.70±0.13 | 3.60±0.11 | $H_3=40.29$<br>$P<0.0001$ |
| Hatching success[2] | .775±.067 | .733±.088 | .750±.065 | (.700±.071) | $H_3=0.903$<br>N.S. |
| No. eggs hatched[3] | 2.10±0.20 | 1.90±0.26 | 2.85±0.26 | (1.80±0.19) | $H_3=7.26$<br>$P<0.03$ |
| Fledging success[4] | 0.73±0.09<br>(18) | 0.48±0.09<br>(17) | 0.50±0.07<br>(19) | 0.40±0.08<br>(19) | $H_3=8.19$<br>$P<0.05$ |
| Number of chicks fledged | 1.94±0.27<br>(18) | 1.29±0.24<br>(17) | 1.90±0.26<br>(19) | 1.42±0.29<br>(19) | $H_3=4.46$<br>N.S. |

[1] Laying dates - one-way ANOVA; all other data - Kruskal Wallis ANOVA

[2] For treatment 4, hatching success = proportion of b, c, and d-eggs hatched, since all a-eggs failed due to procedural problems.

[3] Treatments 1,2 and 3 only. Range tests show treatment 3 is significantly different from treatments 1 and 2.

[4] Range test indicates higher fledging success in treatment 1 compared with treatments 2 and 4.

### *Effects of increased egg production on breeding performance*

In order to investigate the effects of increased egg production on breeding performance, comparisons were made between gulls incubating and rearing clutches of three eggs, produced either with (treatment 2) or without (treatment 1) increased egg production costs, and also between gulls incubating and rearing clutches of four eggs, produced with (treatment 4) and without (treatment 3) increased laying costs (Table 4).

Table 4. Breeding performance, per pair, of gulls incubating and rearing clutches of three or four eggs, produced either with or without increased costs of egg production (mean ± S.E. (N), for definitions, see text).

| | Number of eggs hatched | Number of chicks fledged |
|---|---|---|
| Incubating and rearing three eggs | | |
| Unmanipulated laying costs | 2.40 ± 0.19<br>(15) | 2.07 ± 0.34<br>(14) |
| Increased laying costs | 2.23 ± 0.34<br>(13) | 1.54 ± 0.28<br>(11) |
| Incubating and rearing four eggs | | |
| Unmanipulated laying costs | 3.20 ± 0.26<br>(15) | 1.93 ± 0.32<br>(15) |
| Increased laying costs | (2.00 ± 0.25)*<br>(12) | 1.46 ± 0.43<br>(11) |

* No a-eggs hatched due to procedural problems

The first comparison suggested that experimentally increasing the cost of egg production tended to reduce the number of chicks hatched and fledged from clutches of three eggs, although the differences did not quite reach significance (number of eggs hatched $X^2_2=5.61$ $P=0.06$, number of chicks fledged $X^2_3=7.73$ $P=0.052$). The second comparison indicated that experimentally increasing laying costs had no effect on the number of eggs hatched or the productivity of clutches of four eggs (number of eggs hatched from b,c and d-eggs only, due to non-viability of a-eggs; $X^2_2=3.53$ $P>0.05$, number of chicks fledged $X^2_4=2.65$ $P>0.05$).

*Egg size*

The sizes of the three eggs laid following removal of the first egg, were examined in comparison with those of control clutches of three eggs, pooling data from both treatments in which females laid a normal clutch (1 and 3) and from those in which three further eggs were laid following egg removal (treatment 2 and 4; Table 5).

Table 5. Effect of egg removal on the size (mean ± S.E. (N)) of eggs subsequently laid, analysed by laying sequence. N.B. In treatments where the first egg was removed, eggs are referred to by their position in the laying sequence, excluding the first egg.

| Egg sequence | Control clutches | First egg removed |
|---|---|---|
| A-egg | 71.75 ± 0.97 (30) | 69.58 ± 1.34 (25) |
| B-egg | 70.27 ± 1.08 (30) | 65.89 ± 1.03 (25) |
| C-egg | 64.31 ± 1.07 (30) | 60.68 ± 1.18 (25) |

In addition to the expected effect of laying order (repeated measures ANOVA: Wilk's $F_{1,52}=145.85$ $P<0.001$), there were also significant treatment effects ($F_{1,53}=5.20$ $P<0.03$, interaction not significant). Gulls laying clutches of three eggs, following removal of their first egg, laid eggs which were significantly smaller than those produced by control females. This difference in egg size could potentially contribute to the poorer survival of chicks reared by gulls with experimentally increased laying costs suggested by the analysis above.

Whilst variation in egg size between clutches can have important implications for chick survival, variation in egg size within clutches may be of similar importance. The reduced size of the c-egg and hatching asynchrony are commonly viewed as brood reduction adaptations, which create a competitive hierarchy within the brood during the hatching period (Parsons 1975). The precise degree of hatching asynchrony, and the egg size differential within clutches, may be important in determining early chick survival. I therefore compared the size of b- and c-eggs, relative to the a-egg of the same clutch, between treatments. This indicated that the size differential between a- and c-eggs was similar for both treatments (c-egg volume/a-egg volume: control; 0.90 ± 0.01 (30), laying costs; 0.87 ± 0.01 (23) $t_{53}=1.61$ $P>0.05$), indicating that newly hatching c-chicks were at the same size disadvantage in both treatments. However, the same analysis of the relative size of b-chicks suggested that those in clutches laid following egg removal were at a greater size disadvantage than those in control clutches (b-egg volume/a-egg volume: control 0.98 ± 0.01 (30), laying costs; 0.94 ± 0.01 (23), $t_{53}=2.49$ $P<0.02$). There was no difference in the size of a-eggs between treatments ($t_{53}=1.35$ $P>0.05$).

### *Hatching spread and pattern of mortality*

There were no significant differences in the degree of hatching asynchrony of control clutches of three eggs compared with clutches of three produced following egg removal (2.21 ± 0.35 and 3.09 ± 0.58 days respectively, Mann Whitney U=54.5 $P>0.05$). The pattern of chick mortality is shown in Figure 2 for these two groups. For control broods there was no significant difference in the proportion of chicks dying within the first two weeks post-hatching, and the proportion dying subsequently ($X^2{}_1=0.039$ $P>0.05$), whereas for those with increased laying costs, a significantly higher proportion of chicks died within the first two weeks than subsequently ($X^2{}_1=4.369$ $P<0.05$).

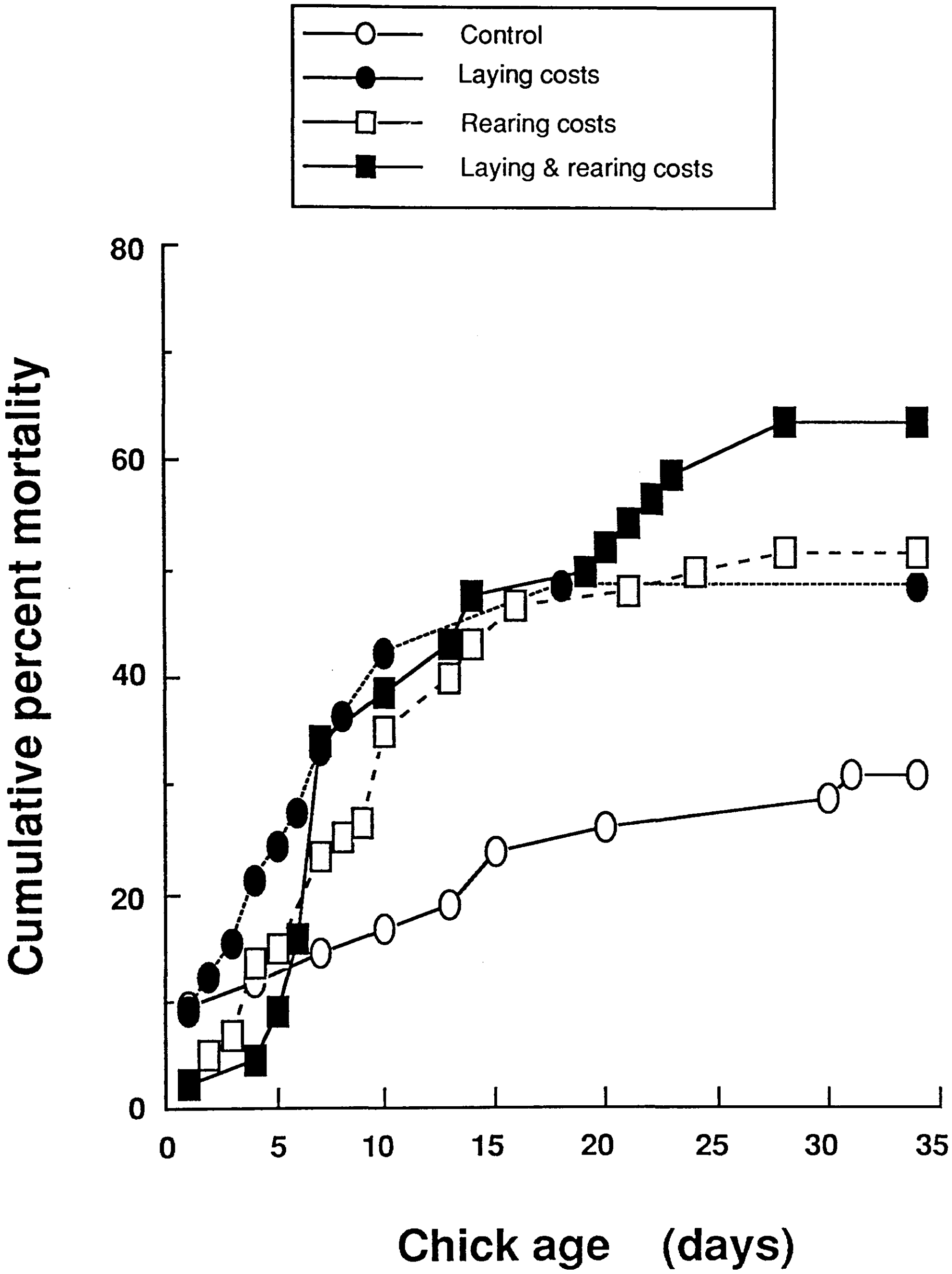
### *Size and weight of chicks at fledging*

There were no differences in either the fledging size or weight of chicks from broods of three, reared by parents with increased laying costs compared with controls (tarsus length: control; 59.44 ± 0.76mm (25), laying costs; 59.17 ± 0.94mm (15) $t_{18}=0.23$ $P>0.05$, weight: control; 679 ± 28g (25), laying costs; 640 ± 38g (15) $t_{18}=0.85$ $P>0.05$). (Due to non-independence of data from chicks of the same brood, the degrees of freedom have been reduced from (N-2) to (number of broods-2)).

### *Egg production and female body condition*

I examined whether the production of a replacement egg led to a decrease in body condition of females at the end of laying compared with control gulls laying a normal clutch. Pooling data from both treatments with (2 and 4), and both treatments without (1 and 3) increased laying costs, showed that there were no significant differences in the condition of females which had just completed

**Figure 2.** Cumulative percentage mortality of chicks in broods of three produced either with (closed circles) or without (open circles) laying costs, and broods of four produced either with (closed squares) or without (open squares) laying costs.


Control
Laying costs
Rearing costs
Laying & rearing costs
Cumulative percent mortality
80
60
40
20
0
0
5
10
15
20
25
30
35
Chick age (days)

enlarged clutches (ranging from two to four eggs) compared with controls laying unmanipulated clutches from one to three eggs (-0.308 ± 0.378 (14) and 0.308 ± 0.560 (14) respectively, $t_{26}$=0.91 P>0.05). There were also no differences in the condition of females which had just produced a normal clutch of three eggs (-0.388 ± 0.643 (7)) compared with those that had laid an additional egg to produce a total of four eggs 0.351 ± 0.529 (6) $t_{11}$=0.87 P>0.05).

I also examined the laying interval between the penultimate and last egg of enlarged and control clutches. Pooling data from all females in treatments with and without increased laying costs as above, showed that females whose first egg was removed, took on average 11 hours longer to lay their final egg, compared with females laying unmanipulated clutches (2.95 ± 0.16 days (40), and 2.49 ± 0.12 days (37) respectively, Mann Whitney U=547 P<0.04). I then compared the time taken to lay the final egg by females laying an unmanipulated clutch of three, compared with females which eventually produced four eggs after initial egg removal. Females laying four eggs took 16 hours longer to produce their final egg than females laying three eggs (3.04 ± 0.23 days (25) and 2.37 ± 0.10 days (30) respectively, Mann Whitney U=246.0 P<0.02).

### *Upper limits to clutch size: incubation capacity*

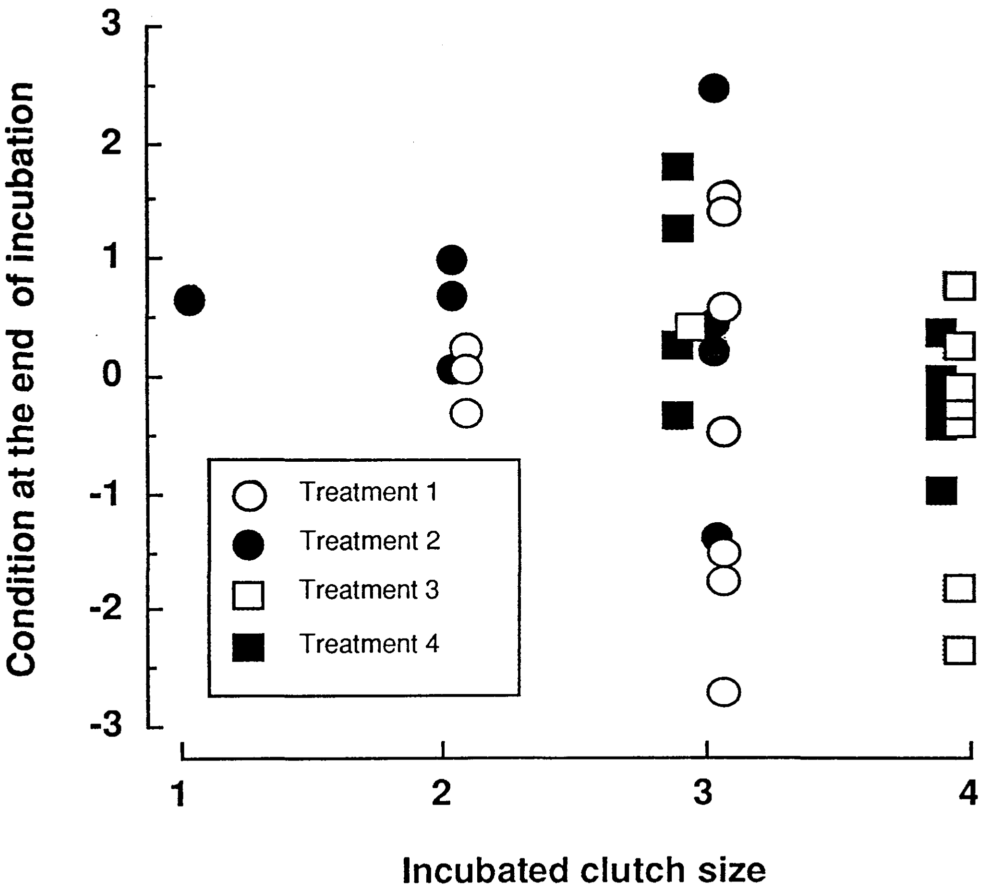
The potential costs associated with incubating an enlarged clutch were investigated, in isolation from the potential costs of increased egg production examined above, by comparing the breeding parameters of gulls which had laid a clutch of three eggs, and were incubating and rearing clutches of either three or four eggs.

There were no significant differences in the proportion of eggs hatched by pairs incubating control clutches of three eggs and those hatched by pairs with enlarged clutches of four eggs (hatching success 0.80 ± 0.06 (15) and 0.80 ± 0.07 (15) Mann Whitney $U=110.0$ $P>0.05$). This resulted in the experimentally increased clutches producing a larger number of hatched chicks (Table 4, $X^2_3=11.11$ $P<0.02$). Several authors have suggested that the hatching success of the last egg in experimentally enlarged clutches may be lower than that of the last egg in clutches of three, due to a decline in the amount of incubation by the parents after the first eggs have hatched (for example Reid 1987). The hatching success of last eggs in experimentally enlarged clutches of four (treatment 3) was not significantly different from that of last eggs in control clutches of three (treatment 1, failure rate: 25% of 15 d-eggs compared with 33% of 15 c-eggs, $X^2_1=0.172$ $P>0.05$).

### *Condition of males and females at the end of incubation*

The condition of males and females incubating enlarged and control clutches during the final week of incubation were examined. Condition was calculated for each sex separately, since there were differences in the regressions of total flight muscle protein on body size between sexes. Pooling data from all treatments, showed that among females there was generally a significant negative correlation between the number of eggs incubated and condition at the end of laying (Figure 3, Spearman's $r=-0.344$ $N=36$ $P<0.02$), although there was no relationship for males ($r=-0.018$ $N=26$ $P>0.05$). The effect of increased laying costs was then investigated, by comparing the condition of females which had either laid a normal clutch, or produced an additional egg, and were incubating clutches of the same size. There were sufficient data from females which were incubating clutches of three eggs and those incubating clutches of four eggs. These comparisons indicated that there were no detectable differences in condition at the end of incubation associated with increased laying costs (condition of females

**Figure 3.** Pectoral muscle protein condition (g.) of females at the end of incubation with the number of eggs incubated (see text for treatment definitions).


Condition at the end of incubation
3
2
1
0
-1
-2
-3
Treatment 1
Treatment 2
Treatment 3
Treatment 4
1
2
3
4
Incubated clutch size

incubating clutches of three eggs; control: -0.381 ± 0.637 (7), increased laying costs 0.463 ± 1.59 (4) Mann Whitney U=10.0 $P>0.05$, females incubating clutches of four eggs; control: -0.499 ± 1.05 (8), increased laying costs -0.225 ± 0.500 (5) U=19.0 $P>0.05$).

### *Upper limits to clutch size: chick rearing capacity*

As in the analysis of incubation capacity above, the following examination of chick rearing ability is mainly confined to pairs rearing control broods of three chicks (treatment 1) compared with those rearing broods of four chicks produced by adding an egg to a completed clutch (treatment 3). This allows the examination of the potential costs of chick rearing on breeding performance, in isolation from those associated with increased egg production.

#### *Duration of hatching and pattern of mortality*

The delay between the hatching of the first and last egg was calculated for control and enlarged (treatment 3) clutches. The hatching spread was on average 43 hours longer in clutches of four eggs than in clutches of three (mean hatching spread; control: 2.21 ± 0.35 days (14), enlarged: 4.00 ± 0.40 days (15), Mann Whitney U=41.0 $P<0.005$). Figure 2 shows the pattern of mortality of chicks from control broods of three (open circles), and enlarged broods of four (open squares). There were significant differences between these groups in the proportion of chicks dying within the first two weeks of life ($X^2_1=5.510$ $P<0.05$), but not subsequently ($X^2_1=0.00$ $P>0.05$).

#### *Productivity and fledging size and weight*

The productivity of control broods of three chicks and enlarged broods of four chicks were examined (Table 4). There were no differences in the number of chicks fledged per brood, despite significant differences in original brood size ($X^2_3$=4.50 P>0.05). Nor were there any significant differences in either the size (tarsus length) or weight of chicks fledging from initial broods of three chicks, or enlarged broods of four (tarsus length; control: 59.44 ± 0.76mm (25), enlarged: 58.96 ± 1.12mm (28), $t_{22}$ 0.35 P>0.05, weight: control; 679 ± 28g (25), enlarged 626 ± 34g (28), $t_{22}$=1.21 P>0.05, degrees of freedom adjusted as above).

## DISCUSSION

Clutch and brood manipulation experiments have frequently been used to investigate the ability of birds to rear a greater number of offspring than they would normally produce in a particular year. The results have often been interpreted in terms of the likely selection pressures which have operated in the evolution of the clutch size of the species. However, in the absence of some measurement of residual reproductive value of both the adults and offspring produced, such an approach is of limited value in understanding the ultimate factors which have shaped clutch size during the course of evolutionary history. However, this experimental method can provide useful information about the current pressures maintaining or limiting the number of eggs laid.

Brood enlargement experiments on a number of larid species have demonstrated that in some years, adults are able provide sufficient food for more chicks that they would normally produce (Harris & Plumb 1965, Ward 1973, Coulter 1973b Haymes & Morris 1977). This has been interpreted as evidence in support of the hypothesis that the current upper limit to clutch size in gulls may be set by incubation capacity, rather than chick rearing ability (Coulter 1987). However, the data presented here show that there were no differences in the hatching success of eggs in clutches of four eggs compared with control clutches of three. I interpret these results as evidence against the incubation capacity hypothesis; lesser black-backed gulls are physiologically capable of laying and incubating clutches of four eggs to hatching, which produce larger initial brood sizes than clutches of three.

The lack of a marked reduction in hatching success of four egg clutches is perhaps not surprising, since clutches of this size do occasionally occur, and one might expect behavioural or physiological compensation to increase the effectiveness of heat transfer to the eggs with increased clutch size (such as increased turning of the eggs, greater nest insulation, and increased vascularisation of the brood patches) in the absence of morphological plasticity.

There was no evidence to suggest that in the year of the study, broods of four were more productive than control broods of three. The enlarged broods showed significantly reduced fledging success, and did not produce more fledged chicks than controls, although there were no differences in the size (tarsus length) or weight of chicks which survived to fledging between treatments. This provides good evidence that, in some years at least, limits on chick rearing ability set the upper limit to clutch size at three eggs.

This finding is at variance with the results of many other brood enlargement studies on gulls. This discrepancy may be due to differences in food availability between studies, or a result of the rigorous attempt to maintain the normal pattern of laying and therefore hatching asynchrony in this study. Other brood enlargement manipulations appear to have been effected by merely adding an additional chick to the brood at, or immediately after "hatching" (for example Harris & Plumb 1965, Andersson 1976). The precise degree of hatching asynchrony can play an important role in determining gull chick survival, especially in the first few days of life (Reid 1987) and, acting in conjunction with the reduced size of the last-laid egg, is commonly believed to be a brood reduction adaption to maximise seasonal reproductive success in response to an unpredictable food supply (Parsons 1975, Hahn 1981, Reid 1987). Enlarged broods of four hatched over a considerably longer period than control broods of three, which may have led to a greater size and weight differential between chicks in enlarged broods, which could have survival consequences. The high levels of chick mortality during the first two weeks post-hatching which characterised enlarged broods of four chicks is consistent with this interpretation.

One important finding of this study, particularly in the context of brood manipulation experiments, is the suggestion that experimentally inducing gulls to lay an additional egg may result in decreased breeding success, although the data presented here do not quite reach significance. However, if this effect is real, it would be of considerable importance since it implies that costs associated with egg formation have implications for subsequent breeding performance and seasonal reproductive success. Many experimental studies have increased brood size and thereby demonstrated the existence of the costs of chick rearing, on both offspring and parental productivity in ensuing seasons. However, as emphasised by Partridge (1989), no experimental studies have attempted to manipulate all aspects of reproductive effort. In particular, few studies have attempted to manipulate the costs associated with egg formation, although there is evidence for their existence (Winkler 1985).

This result would have implications for brood enlargement studies which do not attempt to manipulate the costs associated with egg laying, since adults may be able to rear supernormal broods, if exempt from the associated costs of increased young production. However, this was not the case in the present study, since there was no difference in the productivity of enlarged broods produced either with or without increased laying costs.

The mechanism by which increased egg production could lead to a reduction in productivity is unclear. Although the role of body reserves may be important, females laying enlarged clutches of four eggs did not have significantly lower protein levels than controls. However, they took considerably longer to produce their final egg. This suggests that under normal circumstances protein requirements for egg formation are not met wholly from the diet, but body reserves are also required. It appears that females will not allow their body protein levels to drop below a certain threshold (chapter 6) and are therefore forced to acquire additional nutrients for production of replacement eggs from daily intake, which results in an increase in the time required to form the last egg of the clutch. There was little evidence to suggest that increased laying costs resulted in decreased body condition at the end of incubation. Whilst there was a general trend for females incubating larger clutches to be in poorer condition than those with fewer eggs, there were no differences in condition associated with experimentally manipulated laying costs. This implies that the suggested reduction in productivity of gulls induced to lay a fourth egg may not be mediated via impaired body condition.

It is unlikely the high levels of early mortality among broods of three produced with increased laying costs can be explained by variation in hatching asynchrony, as discussed above for enlarged broods. Although females laying a clutch of three eggs following egg removal took significantly longer to produce their final egg than controls, there were no significant differences in the hatching spread of the three eggs which were incubated.

A number of studies have demonstrated the importance of egg size in determining chick survival during the first weeks of life. The data presented here indicate that eggs laid following egg removal were significantly smaller than those of control clutches. They also showed that in addition to the typical size disadvantage of c-eggs, b-eggs of clutches laid following egg removal were also smaller than a-eggs of the same clutch, which could place newly hatching b-chicks at a greater competitive disadvantage at hatching. As in enlarged broods of four, most chick mortality in broods of three produced with increased laying costs occurred before chicks were 14 days old. This suggests that intra-brood competition, probably mediated via egg size rather than hatching asynchrony, may have been an important factor contributing to the high levels of chick mortality during the early stages of life.

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# CHAPTER 8

## GENERAL DISCUSSION

Advances in the understanding of the factors shaping the number and size of eggs laid by other groups of animals may be of relevance to the avian ecologist: investigations into the fitness consequences of egg size variation in Brook Trout *Salvelinus fontinalis* (Hutchings 1991), (in which parental effects are absent) have demonstrated a positive relationship between egg size and offspring survival, of the type shown here for lesser black-backed gulls (chapter 3). However, the relationship between egg size and survival in Brook Trout is modified by the availability of food to the hatchlings. Under a regime of low food availability, egg size is of considerable importance in determining offspring survival, since small-egg hatchlings die. The importance of egg size is greatly reduced when food supply is good, since the survival of small egg hatchlings is improved. It is likely that environmental variables such as food availability and ambient temperature may play an important role in modifying the relative importance of egg size as a determinant of chick survival in gulls. Optimal egg size may therefore vary between years, and also within years in a predictable fashion according to food availability, but may also be influenced by unpredictable variables, such as weather conditions during the first week of life.

In approaches to understanding the factors involved in shaping the number and size of eggs laid in a breeding attempt, it is commonly assumed that a trade off exists between offspring size and offspring number, of the type proposed by Smith & Fretwell (1974). Such a trade off must inevitably exist for species of fish and reptiles (for example Side-blotched Lizards *Uta stansburiana*, Sinervo & Licht 1991) which store all the eggs to be laid in a breeding attempt within the body prior to laying. However, it is not clear that this type of model is applicable to avian species which develop their eggs sequentially and lay each completed egg before ovulating the next follicle, although such an approach has been suggested (for example Martin 1987).

Such a trade off would exist if females were solely or heavily dependent on body reserves to form their clutch, with little contribution from the diet over the period of egg formation. Whilst some species of Arctic nesting geese form their clutches in this way, most other species are likely to rely heavily on dietary intake to provide a substantial proportion of the nutrients required for egg formation. The results presented in chapter 6 suggest that whilst the number of eggs laid by lesser black-backed gulls is related to the accumulation of body reserves, there is no detectable relationship between flight muscle protein condition and egg size. Although the provision of an egg protein supplement led to a 10% increase in egg size, it was not accompanied by an increase in flight muscle condition at the start of laying. This may be the result of a failure to detect the comparatively small differences in body condition which may accompany differences in egg size. Whilst differences in clutch size represent quantum changes in reproductive investment, accompanied by large variation in body reserves, differences relating to variation in egg size would be comparatively minor and possibly undetectable using the methods outlined here. Alternatively, body reserves may play little role in the control of egg size, which may be determined largely by dietary intake, during the days of egg formation. The difference between large and small eggs of the same sequence lies in the albumen content rather than the yolk. Unlike yolk proteins, which are concentrated from plasma lipoproteins by the ovarian follicle, the proteins required for albumen formation are synthesised *in situ* from free amino acids and other small molecules by the anterior oviducal gland cells (Romanoff & Romanoff 1949, Grau 1984). Most of the proteins that contribute to albumen formation are formed and accumulated during the intervals between successive eggs. It is not known to what extent birds are able to store the amino acids required for albumen proteins prior to the onset of ovulation, but it is possible that they are acquired from the diet during the days immediately preceding albumen secretion.

In order to investigate the factors influencing egg production in lesser black-backed gulls, an experimental approach was adopted throughout this study. Comparisons of breeding parameters of experimentally manipulated treatments with unmanipulated controls permitted the isolation of single independent variables (food abundance, food quality etc.) for examination. The experiments detailed in the preceding chapters provide evidence concerning the factors involved in the proximate determination of clutch size, the significance of egg size and the current selection pressures limiting clutch size to three eggs. It should be emphasised that the data presented here were collected in just three breeding seasons and are therefore unlikely to cover the extremes of fluctuations in environmental conditions, especially food supply. Despite this, there was marked variation in egg production between the three years. In particular, the average clutch sizes found in 1989 are among the lowest recorded for the species in Britain (chapter 4, Harris 1964, Brown 1967, Davies & Dunn 1976, Cramp 1983), whereas the clutch sizes recorded in the other seasons were more typical. This allowed an investigation of the effects of increased food supply/quality in contrasting years, when food was apparently either poor or good.

The results obtained from the experiments outlined in this study are therefore consistent with the following set of hypotheses:

1. The rate of accumulation of pectoral muscle protein reserves is the proximate determinant of the number of follicles ovulated, and hence, clutch size. The ultimate function is to adjust clutch size to local food supply, to produce the number of chicks which could reasonably be reared under the current conditions.

2. The upper limit to clutch size is fixed, almost invariably, at three eggs, since this represents the maximum brood size which can be reared, even in a good year. Brood sizes larger than three result in an increase in the degree of hatching asynchrony, leading to a greater competitive differential within broods and therefore higher levels of mortality early in post hatching development. The costs of producing a fourth egg may further limit brood size to three. Variation in food supply is therefore only likely to result in a decrease in clutch size downwards from three eggs.

3. Egg size may have fitness consequences for the chicks hatched, but a large proportion of the correlation between egg size and offspring survival in non-manipulative studies is a function of a parental effect, since parents which lay large eggs are also more successful in rearing young. The importance of egg size may also be modified by environmental conditions during the first few weeks of life. Harsh environmental conditions will ameliorate differential egg size related mortality.

4. Variation in egg size is related to variation in albumen, rather than yolk content. The synthesis of albumen proteins requires specific amino acids which are scarce in the diet. These amino acids are not stored in pectoral muscle

proteins prior to laying, but rather acquired from the diet during the period of albumen formation. The supply of these specific amino acids can therefore limit albumen production, and hence egg size.

A critical test of the hypothesis that limiting nutrients are required for albumen synthesis would be provided by a supplementary feeding experiment, similar to that described in chapter 5, in which experimental groups are provided with either a general protein supplement, or an egg-protein supplement during the prelaying period. Eggs would be collected and subjected to composition analysis. The suggestion that these nutrients are not stored in the pectoral muscles during the prelaying period, but rather derived from exogenous sources, could also be examined by providing an additional group with egg protein, over the period of egg formation only. If the specific nutrients are obtained from the diet during egg formation, the provision of egg protein, either for the duration of the prelaying period, or merely during egg formation, should result in increased albumen content compared with unfed controls. A general (fish) protein supplement should have no effect on egg composition.

The identity and function of the limiting nutrients required for egg formation (chapter 5) remain unclear, though circumstantial evidence suggests that the sulphated amino acids methionine and cysteine are possible candidates. Organic forms of the heavy metal mercury, such as methyl mercury, are known to bind selectively with the sulphyl groups of body proteins (Backstrom 1969), and the accumulation of methyl mercury into the albumen proteins of laying hens, with deleterious consequences on hatching success, may be the result of an unavoidable physiological requirement for sulphated amino acids for albumen synthesis.

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