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Reproductive decisions in the lesser black-backed gull *Larus fuscus* and their effects on reproductive success

by

Nicholas John Royle

Presented in canditure for the degree of Doctor of Philosophy

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DECLARATION

No part of this thesis has been previously submitted for a degree at this or any other University. All work is the author's own, except where indicated.

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ABSTRACT

The effect of several fundamental reproductive 'decisions' upon reproductive success were examined over a three year period at a large, inland gullery in the Pennines. Variations in reproductive parameters in relation to timing of breeding and reproductive success were compared among years. Determinants of the degree of hatching asynchrony were identified. Eggs from two years were taken for yolk lipid analysis, using gas chromatography. Variation in micronutrient content of eggs within clutches and between years was assessed in relation to egg size and yolk size, in order to examine resource allocation decisions of individuals. Timing of breeding of individuals was experimentally manipulated through the exchange of whole clutches of eggs between early and late laying birds, whilst controlling for variation in clutch size and egg-size, in order to assess whether the seasonal decrease in reproductive success was best explained by a decrease in food supply or differences in quality among parents. I experimentally manipulated the within-brood mass hierarchy of gulls, whilst controlling for variation in both chick quality and parental quality, in order to assess the effect of hatching asynchrony *per se* on chick growth and survival, and whether parents optimized the degree of hatching asynchrony with respect to the prevailing food supply. Brood size was experimentally reduced in order to assess the costs and benefits of the production of supernumary young. This was acheived by comparison of chick growth, feather development and chick survival of unmanipulated three-chick broods with broods where either the a-chick or the c-chick had been removed. I present a general discussion of the results within the context of life-history theory and a model for the evolution of hatching asynchrony in the lesser black-backed gull.

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CHAPTER 1

General Introduction

'All animals are equal, but some are more equal than others'

George Orwell (1945)

Several fundamental 'decisions' face a reproducing bird. Individuals must choose a mate (or mates) and select a suitable place to breed. Once these choices have been made individuals must then decide when to start laying eggs, how many eggs to lay (Daan *et al.* 1990), and how to apportion available resources among those eggs. These investment decisions are fundamental because timing of breeding (Daan *et al.* 1990), clutch size (Roff 1992) and egg-size (Bernardo 1996) can have a major impact upon both the number of offspring surviving to breed and the chances of further breeding for parents.

Trivers (1972, 1985) defined parental investment as '...any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring.' This thesis examines some aspects of the phenotypic expression of parental investment decisions related to egg-laying, such as within-clutch variation in egg-size and quality, the degree of hatching asynchrony, clutch size and timing of breeding and relates these to overall to reproductive success in the lesser black-backed gull *Larus fuscus*.

1.1. *Structure and content of avian eggs*

Avian eggs are remarkable because at the time of laying, all the nutrients required for development of the embryo (with the exception of oxygen) are packaged within the egg (Perrins 1994). Avian eggs exchange only gases (oxygen, carbon dioxide and water vapour), but not liquids or solids with their environment (Carey 1996). Female birds must accumulate or synthesize a wide variety of nutrients in order to provide the raw materials essential for egg formation (Carey 1996), which is energetically costly (Monaghan & Nager 1997). This discrete, almost closed, energetic system (Vleck & Hoyt 1991) has meant that avian eggs have been much used in the development of optimality theory with regard to parental investment (Bernardo 1996). It remains intriguing as to why birds are the only vertebrate class that is exclusively oviparous despite having a great diversity of species (Rahn 1991). The main components of eggs are described in detail below:

1.1.1. *Shell*

Avian eggs are characterised by hard, calcified shells (Perrins 1994). Eggshells provide a protective barrier between the developing embryo and the nest environment (Carey 1996). Shell thickness is a basic compromise between being strong enough to support the weight of the egg contents and the incubating adult, and being thin enough so that the embryo can break out of the shell at hatching (Board & Sparks 1991; Carey 1996). Gas exchange also occurs through the shell and porosity affects both the mechanical strength of the shell and the rate of water loss (Board & Sparks 1991). Eggshells are mainly (98%) composed of crystalline calcium carbonate ($CaCO_3$) interweaved with a protein-acid mucopolysaccaride complex organic matrix (Board & Sparks 1991). Most avian species also have a proteinatious cuticle covering the shell. Two membranes are bound to the shell separating it from the contents of the egg (albumen and yolk).

1.1.2. *Albumen*

Albumen is the largest proportion of egg content by weight, and consists primarily of water (88% in chicken eggs) and protein (11%), in addition to small amounts of carbohydrate, lipid and inorganic ions (*ca.* 1 % of weight; Carey 1996). Proteins in the albumen contribute significantly to the amino acid resources utilized by the embryo, but their main function is protection of the embryo from fungal and microbial attack through the binding of proteins to nutrients used by bacteria for growth (eg. riboflavin), so that these nutrients are then unavailable to the bacteria (White 1991).

1.1.3. *Yolk*

The vitelline membrane separates the albumen from the yolk (Carey 1996). Yolk of chicken eggs is mainly water (48%), lipid (33%) and protein (17%), with carbohydrates and ions comprising a minor fraction (Noble 1991). The lipid fraction is predominantly triacylglycerides (61 - 72%) and phospholipids (25 - 36%), with cholesterol esters, free cholesterol and free fatty acids comprising the remainder (Noble 1991; Carey 1996). Yolk proteins are involved in the transport of lipids, vitamins and ions from the maternal liver into the egg, where they are stored for later use during embryo development (Noble 1991). The proteins then provide amino acids upon degradation for synthesis of embryonic tissue (Murphy 1994). Most yolk proteins are derived from vitellogenins, which bind to calcium and iron, and are also the main source of phosphorous for the embryo (Carey 1996). Whilst the majority (*ca.* 80%) of the calcium required by the embryo for skeletal development and regulation of cellular processes is taken from the shell, the remaining fraction is derived from the yolk (Carey 1996).

1.2. *Egg and clutch synthesis*

Egg and clutch synthesis is proximately under hormonal control. The ovarian cycle is regulated by the hypothalamus through control of the release of follicle-stimulating hormone and lutenizing hormone from the pituitary gland (Carey 1996). The following brief description of egg formation is adapted from Carey (1996). Only lutenizing hormone itself is associated with the maturation of oocytes and their subsequent ovulation, although both follicle-stimulating hormone and lutenizing hormone are involved in the initial stimulation of growth of individual follicles in the ovary. As the follicles develop they secrete oestrogen, which stimulates such important reproductive processes as the synthesis of yolk proteins in the liver, the development of the oviduct and brood patch formation. Just before ovulation, the follicle stops secreting oestrogen and secretes progesterone. Subsequently there is a large increase in lutenizing hormone and ovulation begins. Once the follicle has ovulated it continues to secrete progesterone, which stimulates the secretion of albumen proteins and the formation of the shell in the shell gland, before the egg is subsequently laid.

Slow growth of follicles, in response to small increases in follicle-stimulating hormone and lutenizing hormone, starts well before the breeding season when the specific follicles that will make up the clutch are 'selected' in a size hierarchy. This means that follicle selection is determined well in advance of the time they will develop to ovulation during conditions which will be very different from those experienced during the forthcoming breeding season. During the slow winter growth, food availability may have an effect upon the number and quality of follicles selected

(Carey 1996). Current evidence indicates that rapid growth of follicles (the period of rapid yolk lipo-protein deposition prior to ovulation) is stimulated by a number of cues, including overall nutritional status of the female (Carey 1996). Timing of reproduction may also be affected by social factors in addition to nutritional state (Schultz 1991) and stimulated by changes in ambient temperature or photoperiod (Meijer *et al.* 1990).

1.3. *Female diet and micronutrient content of eggs*

During reproduction, female birds require nutrients both for maintenance of essential body components and for synthesis of eggs (Murphy 1994). Maternal diet, provided it meets a certain minimum standard for egg synthesis, has very little effect upon the amino acid composition of eggs, because dietary proteins are hydrolysed to free amino acids, which are precursors for species-specific maternal proteins that are eventually taken up in the egg. However, vitellogenin binds relatively non-specifically with respect to the lipids it transports to the oocyte (White 1991). Consequently the lipid composition of egg-yolk can vary in response to the composition of essential fatty acids in the maternal diet (Noble 1991; White 1991). It has also been suggested that vitellogenin synthesis itself is sensitive to the availability of essential amino acids, fatty acids and vitamins in the diet, which could explain why chickens on, for example, riboflavin-deficient diets cannot produce eggs (White 1991). It is not always apparent what the physiological implications of variation in egg micronutrient content are. What is clear is that, although female birds may have fairly good genetic control over the macronutrients deposited in their eggs (White 1991), deposits of essential micronutrients depend more specifically upon diet. Carey (1996) highlighted the deficiency of data available on micronutrient content among eggs of different wild bird species and raised a number of unresolved questions: to what extent (if at all) do differences in micronutrient content of eggs from different species occur, and how might this have evolved? In addition, do micronutrient concentrations vary among females of the same species, and among eggs within a clutch, and what are the effects of such variation upon embryo viability and chick survival? Furthermore, it is also not known with any certainty how egg-size reflects micronutrient content of eggs (Bernardo 1996).

1.4. *Macronutrient composition of eggs*

Macronutrient composition of eggs varies with the developmental mode of the species in question. In general, precocial species have proportionately larger amounts of yolk relative to other components (Carey 1996). Taking the developmental modes (altricial and precocial) separately, relative yolk content does not vary significantly when compared among species (Carey 1996). However, attempts to relate hatching precocity to egg composition within a taxonomic order (eg. Williams *et al.* 1982) have revealed no clear patterns. In addition, composition of eggs may vary within a species, between individuals in different populations or years (Arnold *et al.* 1991), within clutches (Parsons 1970) and with variation in egg size (Williams 1994). For example, in ring-billed gulls *Larus delawarensis* protein content of the albumen increased disproportionately with increasing egg size (Meathrel & Ryder 1987) whereas in most other species, protein content of albumen tends to scale proportionately to size (Carey 1996). This variation in egg composition with size, and among years and different populations, may be important when assessing fitness consequences of variation in egg size or the extent of parental investment. Although many studies have concluded that there is good evidence for a positive relationship between egg-size and nestling growth and survival in birds (Williams 1994), most of these studies have not taken into account other characters that may be phenotypically correlated with both egg-size and offspring fitness, such as female body size, age, experience (Carey 1996) or quality (Bolton 1991). Such factors, in addition to the variation in egg quality with food supply (varying among years and between different populations and different habitats), have been given little consideration, despite the common use of egg-size as an indication of parental investment.

1.5. *Egg-size*

Natural selection acts at several different life history levels and this causes trade-offs within the parent and between parents and offspring. Bernardo (1996) lists three main trade-offs that potentially affect egg-size:

1) The amount of resources that females can give to progeny is limited by the ability to gather those resources and the females' needs for assimilated energy, including maintenance.

2) When the resources available to females are limited, increased allocation of resources to one egg means that other eggs must be smaller or fewer eggs must be produced.

3) Increases in offspring fitness occur through further investment in individual eggs, but once eggs are large enough so that there is no likely increase in the survival probability of offspring to independence, parental fitness is enhanced by production of additional rather than larger eggs.

The effect of the enivironment further complicates the situation because the egg-size that a female produces and the relative fitness of the offspring from eggs of this size (compared to others in the population) both depend upon the environment in which they occur (Bernardo 1996).

1.6. *Clutch size*

Life history theory has identified several different trade-offs relating to clutch size and future reproductive success. Clutch size may be limited by a trade-off with parental survival, such that rearing a large clutch adversely affects survivorship or reduces future fecundity, leading to selection for reduced clutch size (Roff 1992; Stearns 1992). A large clutch size may also delay the next breeding attempt, which would be particularly deleterious for single brooded species, such as the lesser black-backed gull, with a strong association between timing of breeding and offspring fitness (Davis 1975; Godfray *et al.* 1991). The clutch size of an individual is also likely to be influenced by proximate factors such as physiological condition, which may act directly as a constraint on egg production, or indirectly through the effect of natural selection moulding the behaviour of the bird so that it produces the optimum clutch size for the current conditions (Godfray *et al.* 1991). Environmental conditions during growth may affect subsequent clutch size (Haywood & Perrins 1992) and clutch size may covary with other life history traits, including timing of breeding (Daan *et al.* 1990). Recent research has further highlighted the importance of energetic demands not just during the chick rearing period of parental care, but also during the laying and incubating of eggs, in determining clutch size (Monaghan & Nager 1997). Reproductive investment decisions are also shaped by mate choice; in particular the extent to which the partner invests in the current clutch or brood.

1.7. *Parent-parent relations*

Female produce larger gametes than males, so their parental investment in any single clutch or brood (even without consideration of parental care) is greater than that of males (Trivers 1972). This asymmetry in relative investment is important because it is predicted that females will be limited in their reproductive success by the amount of resources that they can invest in the breeding attempt, in contrast to males which can increase their number of matings to increase reproductive success (Harvey 1994). The lower potential reproductive rate of females affects the direction of mating competition such that females become a resource that males compete for, generating sexual selection (Parker & Simmons 1996). The dispersion of resources (females themselves and the resources needed for reproduction) and the ability of males to monopolise these resources affects mating systems (Harvey 1994). Lesser black-backed gulls are socially monogamous. Monogamy is unusual due to the asymmetry in gamete size between males and females, and although there are many mutual advantages to biparental care, there is potential for sexual conflict between parents over the relative contribution that each sex makes to rearing offspring (Mock *et al.* 1996). As a consequence of a lack of genetic relatedness between parents, there is a fine balance between co-operation and conflict, determined by factors such as the relative foraging abilities of each parent, availability of alternative sexual partners, social compatibility of the pair and the demands of the current brood (Mock *et al.* 1996). Empirical studies indicate that parents are often not working at maximal levels and can adjust their effort to the prevailing effort of their partners, in order to avoid potential future fitness costs (Mock *et al.* 1996). Although it is not altogether clear how social monogamy has evolved (Gowaty 1996) there are several benefits associated with monogamy and mate fidelity, including improved co-ordination of reproductive duties and prolonged biparental investment (Black 1996). These factors may contribute to changes in reproductive parameters such as clutch size or egg-size, especially in long-lived species (Pyle *et al.* 1991), and subsequently have a positive effect upon reproductive success (Mills *et al.* 1996).

1.8. *Parent-offspring relations*

Conflicts over clutch size are not just restricted to conflicts over reproductive investment between parents. Natural selection operating on genes expressed in offspring may have a different effect to natural selection operating on genes expressed in the parents (Godfray *et al.* 1991). Conflict over resource allocation arises because parents are equally related to all their young, so should favour an even division of resources, but each offspring values itself more highly than its siblings (on average siblings only share half their genes) and should be selected to acquire a disproportionate amount of parental investment, which is a greater share than parents are selected to provide (Kilner & Johnstone 1996). Theoretically offspring should prefer a small brood size to increase their own resource share, but parents should not (Clutton-Brock & Godfray 1991). In this context brood reduction would appear to be a good example of parent-offspring conflict. This assumes that brood reduction, as a result of sibling competition, has an adverse effect upon parental fitness, because parents would do better by raising all offspring. However, parents may benefit from a reduction in brood size so this conflict may disappear (Mock *et al.* 1996). Parents can increase their fitness by selectively reducing brood size when they cannot commit enough parental investment to rear the whole brood (due to resource limitations) or will not do so (due to sexual conflict or consideration of future reproduction; Clutton-Brock & Godfray 1991). In this situation, selection may favour variation in offspring size or quality so that parents can accelerate the process of preferential resource allocation to the largest chicks, thus maximizing gains from brood reduction (Haig 1990). Creating size hierarchies through staggering hatching intervals is a good way of achieving variation in offspring size.

1.8.1. *Sibling hierarchies*

Resource allocation decisions occur on two general levels: at the level of the parent and at the level of the offspring. Parents must decide how to allocate the resources they are prepared to invest in the current breeding attempt, and one way of maximizing the number of well nourished young reared to independence in a stochastic environment is to create a hierarchy of chick sizes by hatching the clutch asynchronously (Godfray 1995). In large gull species last hatched 'marginal' chicks are handicapped by their smaller size, and position in the hierarchy influences a chicks' survival chances and hence its reproductive value to parents (Parsons 1970). Identification of a 'runt' which will require greater future investment than other chicks in the brood may be an important component in minimizing the potential costs associated with brood reduction (Nilsson 1995).

1.9. *Sibling-sibling relations*

Chicks need to maximise their resource gains within the constraints imposed by the parentally determined hierarchy. Chicks may attempt to manipulate parents into allocating more resources than they would otherwise be willing to invest, through begging and solicitation (Kilner & Johnstone 1996). Parents may chose to ignore such demands but the costs of doing so could be severe if solicitation is an expression of real need, so parents are unlikely to evolve a complete indifference to begging (Clutton-Brock & Godfray 1991). Theoretical models indicate that the evolutionary stable strategy is a compromise between parental feeding and offspring begging rates (Parker & McNair 1979; Parker 1985). Despite the potential energetic costs associated with scramble competition for parental resources in gulls (Henderson 1975), allowing chicks to determine resource allocation through a size hierarchy may be adaptive if, by taking the resources the dominant chick increases its own survival probability by at least half that of the increase in survival the food item would confer on the next chick in the hierarchy (Parker *et al.* 1989). In reality, individual chicks are unlikely to have a complete monopoly over resource distribution and parents may differ in their food allocation decisions with respect to the sex of the parent and chick sizes (Stamps *et al.* 1985).

1.10. *Hypotheses for the evolution of hatching asynchrony*

Recent reviews list at least seventeen different hypotheses for the evolution of hatching asynchrony in birds that feed their offspring, not all of which are mutually exclusive (Magrath 1990; Stoleson & Beissinger 1995; Stenning 1996). Of these, only about half are potentially applicable to gulls. Although Dunlop (1910) was the first person to postulate the link between early incubation and hatching asynchrony, the study of hatching asynchrony did not receive significant further attention until David Lack's presentation of his brood reduction hypothesis (Lack 1947). The subject of hatching asynchrony was then little studied until the mid-1970s, since when it has attracted a considerable amount of interest (Magrath 1990). Recent reviews have tended to split the different hypotheses into one of three types. Hatching asynchrony may: 1) arise as a direct consequence of selection on the timing of events during the nesting cycle, or 2) may be an adaptive mechanism to promote reproductive success through reduction in brood size or increasing energetic efficiency of chick rearing, or 3) be a consequence of an environmental or physiological constraint.

1.10.1. *Timing of events in the nesting cycle*

Clark & Wilson (1981) developed the *nest failure hypothesis* to predict the optimal degree of asynchrony based on the probability of total nest failure at different phases of the breeding cycle (Stoleson & Beissinger 1995). This hypothesis suggests that asynchronous hatching decreases the amount of time that a nest contains only eggs and therefore reduces the amount of time before the first chick fledges. The model cannot, however, be tested experimentally and recent work has questioned some of the assumptions of the model (Stoleson & Beissinger 1995). The majority of its assumptions rely upon the effectiveness of size hierarchies in determining brood success (Magrath 1990).

1.10.2. *Adaptive hatching patterns*

The ***brood reduction hypothesis*** (Lack 1947) proposes that hatching asynchrony facilitates adaptive family planning in relation to food supply. In its modern incarnation (eg. Temme & Charnov 1987) it suggests that, in an unpredictable environment, brood size is adjusted to the ability of parents to supply food through the elimination of the nestlings that will require the greatest future investment. Although there have been many experimental tests of the hypothesis few studies have verified its predictions (Stoleson & Beissinger 1995). This is at least partly a consequence of the difficulties associated with experimental design and measurements of reproductive success (Magrath 1990). The primary predictions of Lack's hypothesis are that, when conditions are poor (but not when food availability is good) asynchronously hatched broods should produce more fledglings, fledglings of a higher mass or that nestling mortality will occur at a younger age than synchronous broods (Magrath 1990; Wiebe & Bortolotti 1995). It is important to demonstrate that the brood reduction mechanism makes hatching asynchronously more productive or efficient than synchronous hatching during conditions of resource limitation. In the only two studies to date where this has been demonstrated, using supplementary feeding in blackbirds *Turdus merula* (Magrath 1989) and American kestrels *Falco sparvarius* (Wiebe & Bortolotti 1995), these predictions were confirmed. Lack (1947) also suggested that, if food supply at laying is not a good indicator of food supply available during chick rearing then birds should lay an 'optimistic' clutch size (equal in size to the number of offspring that can be reared in good years) before opting for a reduction in family size if conditions are not that good. This production of supernumary young, or 'overproduction,' in some species of bird (Mock & Forbes 1995), has led to the development of the related *insurance hypothesis* for the evolution of hatching asynchrony (Stinson 1979). Insurance against hatching failure may be important in single-brooded species with small clutch sizes if there is a high chance of hatching failure or failure soon after hatching, as, for example, occurs in obligate brood-reducing species such as pelicans *Pelecanus spp.* (Evans 1996). Supernumary young, in addition to having an insurance function in poor conditions, may survive and provide extra reproductive value in years of good food supply (Mock & Parker 1986). The success of such a strategy depends upon the existence of an efficient brood reduction mechanism (Forbes 1993).

Parental manipulation of hatching intervals may also function to minimize the future costs of sibling competition (Mock 1994). The *sibling rivalry hypothesis* (Hamilton 1964; Hahn 1981) predicts that hatching asynchrony imposes a stable dominance hierarchy which results in avoidance of wasteful scamble competition and allows more efficient use of parental resources regardless of food supply. The rivalry reducing brood hierarchy is predicted to complement the brood reduction mechanism through increasing efficiency in resource share of chicks during years of food scarcity and so lead to an increase in breeding success (Hahn 1981). This hypothesis has received some empirical support (e.g. Mock & Ploger 1987; Wiebe & Bortolotti 1994a). The *peak load hypothesis* (Hussell 1972) suggests that hatching asynchrony maximizes parental efficiency by reducing the peak brood energy requirements, although there is little evidence to suggest that peak energy requirements are either particularly sharp or that the reduction in peak load as a result of hatching asynchrony is very large (Magrath 1990; Wiebe & Bortolotti 1994a). Many of these hypotheses for an adaptive function of hatching asynchrony are compatable and complementary.

1.10.3. *Energetic constraints*

It has also been suggested that hatching asynchrony is a by-product of energetic constraints during laying (Stoleson & Beissinger 1995). Greig-Smith (1985) suggested that poor food availability during egg laying may mean that birds have to spend more time foraging and therefore have less time for incubation, so that eggs hatch more synchronously than normal. Although this explanation is unlikely to be applicable to gulls because clutches tend to hatch more, not less, synchronously in years of good food supply (Pierotti & Bellrose 1986; Kilpi 1995), it does add support to the existing body of evidence that environmental conditions during laying can influence egg-size (Magrath 1990).

Hatching asynchrony may have evolved as a result of the hormonal mechanism involved in egg-laying and incubation (Mead & Morton 1985). It is argued that prolactin causes the termination of ovulation whilst also stimulating the initiation of incubation, so that incubation normally starts with the penultimate egg and is independent of clutch size. However, evidence indicates that there is much inter- and intraspecific variation in timing of incubation initiation, and if some of this variance is genetic then it is likely there will be a response to selection on patterns of incubation and the *hormone hypothesis* of Mead & Morton can be rejected (Magrath 1990). This conclusion does not undermine the possibility that hormonal mechanisms are important in the evolution of hatching patterns.

1.10.4. *Other hypotheses*

The more general *individual-optimization hypothesis* of Harper *et al.* (1994) provides a possible explanation for the observed widespread variations in incubation patterns between species. This hypothesis proposes that an individual adjusts its behaviour to suit the current environmental conditions, which suggests a strong element of phenotypic plasticity (Stenning 1996). In order for phenotypic plasticity to evolve there must be suitably reliable cues which can be used to predict likely future conditions and the phenotypic modification should be reversible (Lessells 1991). Wiebe & Bortolotti (1994b) have shown that American kestrels *Falco sparverius* provided with supplementary food during the pre-laying period are 'fooled' into 'thinking' that the food supply is better than it really is and although they lay larger eggs and hatch those eggs more synchronously than control broods, they have poorer reproductive success. Wiebe & Bortolotti (1994b) suggest that parents facultatively manipulate the degree of hatching asynchrony to food supply, and predict that the costs and benefits of the degree of hatching asynchrony during the nestling period should also be food-dependent (Wiebe & Bortolotti 1995). Although some studies (e.g. Stouffer & Power 1990) have suggested that hatching asynchrony is maladaptive because there is a cost of increased mortality of later-hatched chicks even in good years, theoretical studies have shown that, even without consideration of facultative manipulation of hatching spans, a general strategy of hatching asynchrony is favoured over synchronous hatching when good years are not very frequent, when the survival rate of last-hatched nestlings during good food years is high or when bad years are not that severe (Pijanowski 1992).

1.10.5. *Overview of hatching patterns*

Experimental studies manipulating food supply, such as those conducted by Wiebe & Bortolotti (1994a, 1994b, 1995) have illustrated the complementary nature of many of the hypotheses to explain hatching asynchrony in birds, whilst also highlighting the complex nature of the phenomenon. Variation in traits such as hatching asynchrony and incubation periods may ultimately be best explained by reference to common features of phylogenetic groups rather than particular attributes of a well-studied species, because hatching asynchrony may be an example of convergent evolution so that the trait serves many different functions (Stenning 1996; Ricklefs 1997).

1.11. *Characteristics of study species*

The study species was the lesser black-backed gull *Larus fuscus* of the subspecies *L. f. graellsii* (Figure 1.1). The lesser black-backed gull is one of three species of large gull that breeds in the British Isles, the other two species being the much larger great black-backed gull *Larus marinus*, and the closely related herring gull *Larus argentatus* (Lloyd *et al.* 1991). Although the lesser black-backed gull and the herring gull rarely interbreed in this country, and behave as distinct species (Tinbergen 1953), they are part of a chain of ten subspecies or forms of gull in the *fuscus-argentatus* group, which form a northern hemisphere longitudinal ring from the British Isles through Scandinavia, Russia, Siberia and the Bering Straits, to Alaska and Canada before coming full circle back to Britain (Maynard-Smith 1993). Whilst much of their general biology is similar there are notable differences between the two species in Britain. Lesser black-backed gulls are slightly smaller, with a lighter build and the adults have a dark ash-grey mantle and yellow legs, in contrast to that of the light grey mantle and pink legs of adult herring gulls (Grant 1986). In contrast to herring gulls, which are largely sedentary, most lesser black-backed gulls migrate

Figure 1.1 – *Adult lesser black-backed gulls*

Figure 1.2 – *Photograph showing 'normal' three-egg clutch*

south to overwinter off the coasts of Portugal, southwest Spain and northwest Africa, although more recently increasing numbers are overwintering in southern Britain (Lloyd *et al.* 1991). Lesser black-backed gulls and herring gulls often breed in mixed colonies, where the two species may nest in different areas, relating to vegetation type (Hosey & Goodridge 1980). Lesser black-backed gulls tend to establish territories later than herring gulls in mixed colonies, perhaps as a consequence of differences in overwintering habits (Hosey & Goodridge 1980), but the two species eat similar food stuffs (Mudge & Ferns 1982). Both species are omnivorous, opportunistic feeders (Cramp & Simmons 1983), although lesser black-backed gulls are more adept at taking fish (Mudge & Ferns 1982). Large gull species populations are often strongly dependent upon scavenging at refuse tips (Lloyd *et al.* 1991; Pons & Migot 1995), but the diet of both species is varied and includes invertebrates such as earthworms (*Lumbricidae*; O'Connell 1995), plant material and vertebrates of suitable size in addition to fish, marine crustaceans and rubbish from refuse tips (Cramp & Simmons 1983).

In addition to being colonial and omnivorous, the lesser black-backed gull is also generally long-lived and socially monogamous (Cramp & Simmons 1983). Monogamous pair-bonds usually persist for several years, although some extra-pair courting (mostly unsuccessful) does take place (MacRoberts 1973). Birds normally begin breeding at 4 years of age (range 3 - 6 years) and individuals may survive for as long as 26 years (Cramp & Simmons 1983). Modal clutch size is 3 eggs, with the third egg generally smaller than the first two (Figure 1.2). Both sexes incubate for *ca.* 27 days once the clutch has been completed (Cramp & Simmons 1983). Lesser black-backed gulls are single-brooded, laying only one clutch in any one season, although some individuals may re-lay if the first clutch is lost (Monaghan *et al.*

Figure 1.3 – *Asynchronously hatching brood of chicks (note pipped c-egg)*

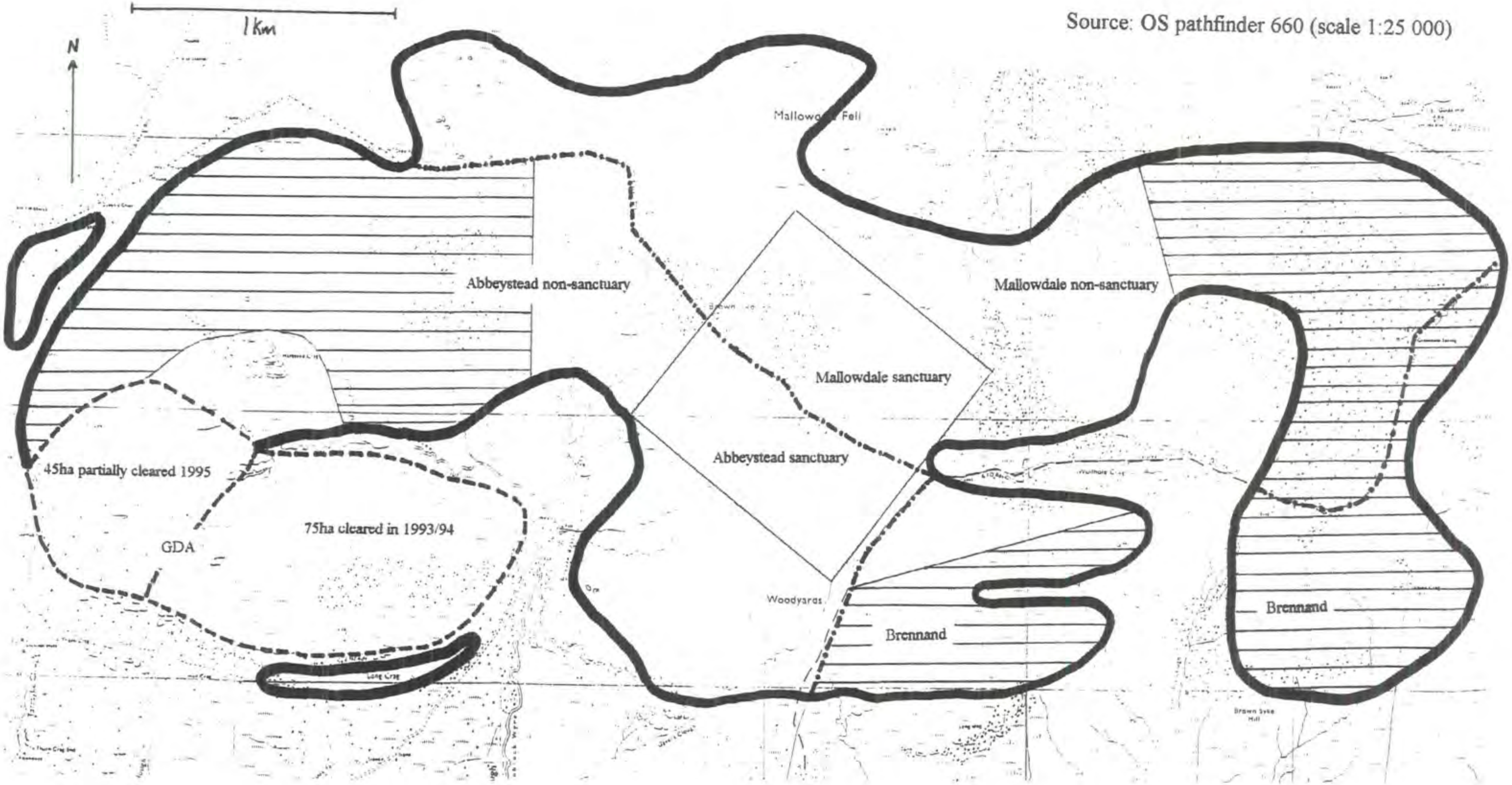
Figure 1.4 – *Photograph of territorial adults showing nesting habitat*

1995). Clutches hatch asynchronously (Bolton 1991; Figure 1.3) and the young, although covered in down and able to walk and thermoregulate soon after hatching (Dunn 1973), are fed and cared for by both parents and are thus termed semi-precocial. Chicks are fed small pieces of food by the parents during the first few days after hatching, but thereafter they indulge in scramble competition for larger pieces of regurgitated food (Henderson 1975). Partial brood losses are common, even in years of apparently good food supply. Chicks fledge after *ca.* 30 - 45 days and parental care may extend beyond this period. Adults go through a complete moult after breeding, prior to migration (Cramp & Simmons 1983).

1.12. *Study site*

The data presented in this thesis were primarily collected during a three year period from 1994 until 1996 inclusive, at the Tarnbrook Fell gullery near Abbeystead in the Forest of Bowland, Lancashire (SD614595). The gullery occupies *ca.* 6 km^2 of land owned by three private estates (Abbeystead, Mallowdale and Brennand), managed primarily for grouse *Lagopus lagopus scoticus*. The vegetation is dominated by heather *Calluna vulgaris*, interspersed with areas of cotton grass *Eriophorum augustifolium* and patches of exposed peat and rock (mainly millstone grit), at an elevation of between 450 and 515 metres (Figure 1.4). Rainfall is high (> 1500 mm per annum) and snow sometimes occurs at the site as late as mid-May. The colony consists mainly of lesser black-backed gulls (92 - 97% of the total) with smaller numbers of herring gulls, and is the largest inland colony of either species in the British Isles (Lloyd *et al.* 1991). Gulls were first recorded breeding at the site in 1938, and the population expanded rapidly in the years following the second world war (O'Connell 1995). Numbers peaked in the late 1970s at upwards of 48,000 birds. At this point culling, using baits laced with the narcotic alpha-chloralose, was

Figure 1.5 - *Map of the Tarnbrook Fell gullery showing the sanctuary, land cleared of breeding birds using disturbance techniques and cull areas* ▤



Source: OS pathfinder 660 (scale 1:25 000) introduced by the North-West Water Authority with the aim of reducing the suspected contamination of the local water supplies. Since the termination of the large scale culling operations in the mid-1980s numbers have fluctuated between 8,000 and 18,000 birds (Royle & Coulson 1995). More recently (from 1995 onwards) culling has been re-introduced as a management tool to supplement the disturbance methods employed at the colony since 1992 (see O'Connell 1995). The areas used for management are shown in Figure 1.5. A sanctuary area, where birds can breed without disturbance or management, was established in 1990 and covers 1.54 km^2 of moorland over two estates (Abbeystead and Mallowdale; Figure 1.6). All field data presented in this thesis were collected in the Abbeystead sanctuary, where the dominant vegetation type is cotton grass *Eriophorum augustifolium*, in short sheep-grazed swards growing on top of and amongst the peat 'hags'.

1.13. *Layout of thesis*

The following chapters describe both empirical and experimental studies of reproductive decisions in lesser black-backed gulls. An appreciation of the general breeding ecology of gulls at the colony was considered to be an important component of this work, and so *Chapter 2* describes annual patterns of variation in reproductive parameters at the colony in relation to reproductive success. *Chapter 3* assesses egg quality in relation to egg-size and laying order in terms of yolk lipid content of eggs. *Chapter 4* presents data from an experiment to manipulate timing of breeding, through exchange of clutches at different stages of incubation, and its subsequent effect on reproductive success independent of clutch size and egg-size variation. *Chapter 5* describes an experimental manipulation of the degree of hatching asynchrony, controlling for clutch size and egg-size, and its impact upon parental investment decisions and reproductive success. *Chapter 6* presents the results from an experiment to assess the effect of clutch size, and in particular the role of supernumary young, on reproductive decisions. *Chapter 7* summarises and discusses the results from the previous five chapters in the context of a general reproductive strategy for the lesser black-backed gull.

CHAPTER 2

Annual and seasonal variation in reproduction

2.1. SUMMARY

Various characteristics of reproduction (clutch size, egg-size, timing of breeding, hatching success, fledging success and chick growth) were compared over three years, during which time the population of breeding birds was declining, in relation to variation in reproductive success among years. In addition, a sample of clutches laid in 1996 was used to examine the factors involved in the determination of hatching patterns (after controlling for clutch size). Both clutch size and mean egg-size in three-egg clutches declined significantly through the breeding season, and there were no differences among years in these relationships. In all years last-laid (c-) eggs showed the strongest seasonal decline in size. C-eggs were significantly smaller than a- and b-eggs. The largest clutches (in terms of total volume) had the lowest intraclutch egg-size variation (IESV). IESV increased with hatch date. The degree of hatching asynchrony and the extent of the sibling size hierarchy were significantly related to IESV. Clutches with very little IESV hatched relatively synchronously. There were no indications that the smaller size of the c-egg shortened the time required for incubation or the hatching interval, so that c-egg size had no direct effect upon hatching asynchrony. There was, however, an indirect effect, because the onset of incubation (which determines the degree of hatching asynchrony) was related to IESV. Egg volume showed a strong correlation with chick mass at hatching, and less so (although still strongly) with tarsus length. Head-bill length was, in contrast, independent of egg volume. C-chicks were only slightly smaller in terms of skeletal size than a- and b-chicks, but were *ca.* 25% lighter than their earlier hatching siblings by the time of hatching mainly because immediate post-hatching growth is biased towards mass gain. Hatching success and fledging success (both in terms of the number of chicks fledged per egg laid and per egg hatched) were greater in 1996 than in 1994 or 1995, mainly due to a higher proportion of broods fledging two or three chicks in 1996. Timing of chick mortality was significantly later in 1994 than in 1995 or 1996. Chick growth rate was greater in 1996 than in 1995, which in

turn was greater than in 1994. These results are discussed with respect to sibling competition, brood reduction and parental reproductive decisions.

2.2. INTRODUCTION

For long-lived species such as the lesser black-backed gull, life-history theory predicts a trade-off between the amount of resources expended on the current reproductive event and adult survival and/or future fecundity (Roff 1992). A major component of the 'decision' an individual must make regarding the amount of effort to invest in any one reproductive event is the number of offspring produced in that event (Stearns 1992). In addition to the above trade-off, the number of offspring produced during the current reproductive episode may be limited by the likelihood of nest failure or current environmental conditions (Roff 1992). Plasticity of life-history traits that affect breeding success may therefore be important for many species (Lessells 1991).

Clutch size in gulls has been found to be related to the amount of protein reserves in the flight muscles of females (Houston *et al.* 1983; Bolton *et al.* 1993) and also to pre-breeding lipid reserves (Hario *et al.* 1991), whilst egg-size is more dependent on exogenous nutritional sources (Bolton *et al.* 1992). In gulls large egg-size has been shown to increase chick survival (Parsons 1970), notwithstanding the potentially confounding effects of parental quality (Bolton 1991), even though for most other bird species there is little unequivocal evidence that large egg-size *per se* has a positive effect on offspring survival (Williams 1994). In gulls egg-size, like clutch size, is related to food supply (Hiom *et al.* 1991; Oro *et al.* 1996) and to the ability of individuals to exploit that food supply (Coulson *et al.* 1982; Martin 1987; Spaans *et al.* 1987).

In many larids both clutch size and egg-size decrease seasonally (Parsons 1972; Davis 1975; Sydeman *et al.* 1991). Individuals laying larger clutches, with bigger eggs, earlier in the season have higher reproductive success and tend to be older (Davis 1975; Sydeman *et al.* 1991; Mills *et al.* 1996), more experienced (Pyle *et al.* 1991) birds and have greater coordination of reproductive duties with their mates (Burger 1986, Mills *et al.* 1996). All these factors tend to relate to foraging efficency (Greig *et al.* 1983) and the ability to exploit available food resources (Sydeman *et al.* 1991; Bukacińska *et al.* 1996). Evidence from food supplementation experiments suggests that females are resource limited during clutch formation (Salzer & Larkin 1990; Hiom *et al.* 1991; Bolton *et al.* 1993) so that differences in foraging efficiency may determine reproductive success, and clutch size therefore reflects likely differences in parental quality (Sydeman *et al.* 1991).

In addition to variation in clutch size and egg-size between individuals, for most larids with a modal clutch size of three eggs there is significant within-clutch egg-size variation, with the last laid (c-) egg being typically 8-10% smaller than the eggs immediately preceeding it in the laying sequence (Parsons 1972; Meathrel & Ryder 1987; Reid 1987a; Sydeman & Emslie 1992). The size of the c-egg is more sensitive to resource availability than those of a- or b- eggs, showing greater within season decline than a- or b- eggs (Parsons 1972) and a greater increase in size when supplemental food is provided in a year of poor food availability (Hiom *et al.* 1991). Where food availability is good and intraspecific competition for food is low, gulls lay large eggs with little or no intraclutch size variation, hatch chicks synchronously and have high breeding success (Pierotti & Bellrose 1986, Kilpi 1995). Within populations, low intraclutch egg-size variation and a high degree of hatching synchrony are associated with older individuals with more proficient foraging skills

(Sydeman & Emslie 1992), whilst relative size of the c-egg has also been shown to be related to the rate of courtship feeding by the male (Nisbet 1973; Salzer & Larkin 1990). Courtship feeding rates have been shown to be a good predictor of chick provisioning rates (Nisbet 1973; Niebuhr 1981), which in turn affect chick growth rates and survival (Martin 1987; Reid 1987b; Green & Krebs 1995).

The aim of this chapter is to examine variation among years in a range of parameters related to the production and incubation of eggs, in order to assess what effect these variables have on overall breeding success. In addition, because variation in sibling size hierarchies (and hatching asynchrony) has a strong effect on chick growth and mortality at this colony (*Chapter 5*), the current chapter also aims to establish the effects of variation in egg-size, the rate of laying and timing of clutch initiation on the degree of asynchrony at hatching.

2.3. METHODS

2.3.1. *Breeding success*

Two permanently marked 100m × 50m study plots in the undisturbed 'sanctuary' area of the colony were used to assess different aspects of breeding ecology in each of the three years from 1994 until 1996. Timing of nest-building and egg-laying were determined through regular marking and monitoring of nests (using a short length of bamboo cane marked with a nest number) every 1 - 2 days. In addition, in 1994 and 1996 all eggs were measured (length and breadth ± 0.1mm using dial calipers), marked with indelible ink according to nest number and laying sequence, and volume (V in cm^3) was calculated in each case using the following formula (from Bolton 1991):

$$V = (0.000476) \times (\text{length (mm)}) \times (\text{breadth (mm)})^2$$

Date of laying of the first egg and clutch size were established for each breeding pair. Upon hatching, chicks were individually marked with insulation tape 'bands' according to nest number and hatching order, and then subsequently given BTO rings once their legs had grown sufficiently to allow the ring to be retained (usually at *ca.* 10 - 15 days post-hatch). Chicks were assumed to have died if they could not be located on three or more consecutive visits (after Hamer *et al.* 1991), whilst chicks reaching 36 days post-hatching, when they were fully feathered, were considered to have fledged.

2.3.2. *Variation among three-egg clutches*

In addition to clutches in the two permanent study plots, in each of the three years a sample of three-egg clutches laid during the first two weeks of clutch initiation at the colony was measured and processed as above. Both sets of clutches were used to assess the relationship between mean egg-size and lay-date in different years, whilst controlling for clutch size. Each clutch was enclosed towards the end of incubation with chicken-wire mesh approximately 2 m^2 in area and 50 cm high to assist in locating chicks after hatching, and a wooden shelter was also provided. Adults incubated clutches and fed their chicks normally within these enclosures (as found in other studies e.g. Bolton 1991). Tarsus length and body mass of all chicks were measured every 3 - 6 days and fledging success and growth rates of surviving chicks were compared among years to assess possible effects on these chicks of variation in resource availability independent of effects of variation in clutch size or lay-date.

2.3.3. *Relationships between egg-size characteristics and hatching asynchrony*

In 1996 detailed data were collected on a total of 45 three-egg clutches in order to assess the relationship between within-clutch variation in egg size and hatching patterns. The variables measured or calculated for each clutch were:

1) Total clutch volume (cm^3)

2) Laying date - indicates the date upon which the first (a-) egg within a clutch was laid

3) Intraclutch egg-size variation (IESV) - size of the smallest egg in the clutch as a percentage of that of the largest

4) Laying interval - the time between the laying of the first (a-) egg and the last (c-) laid egg measured to the nearest hour

5) b-c egg-size variation - size of the c-egg as a percentage of that of the b-egg

6) b-c laying interval - the time in hours between the laying of the second (b-) and last (c-) laid egg

7) Egg incubation time - time in hours between laying of an egg and that egg hatching

8) Total incubation time - time in hours between laying of the first (a-) egg to the hatching of the last (c-) egg within a clutch

9) Hatch date - the date the a-chick hatched for each clutch

10) Hatch interval - the time in hours between the first (a-) chick and the last (c-) chick to hatch within a clutch

11) Chick biometrics - tarsus length (mm) and head-bill length (mm) were measured using dial calipers (± 0.1 mm), in addition to mass (g), using a Pesola spring balance (± 1 g). Every chick was measured twice: a) upon hatching, and b) upon brood completion when the last chick had hatched from the brood

12) Mass ratio - the ratio of the mass of the smallest chick in the brood (ie. the last hatched chick) to that of the largest, at brood completion. The mass ratio is a consequence of the differences in hatching size, hatching interval and post-hatching growth rate among chicks within a brood, and these factors determine the magnitude of the brood hierarchy.

2.3.4. *Statistical analysis*

The data for clutches laid in 1996 (investigating the link between IESV and hatching asynchrony) were analysed, except where indicated otherwise, using Pearson's product moment and partial correlation coefficients, after testing data for normality. In comparing among years, analysis of variance (ANOVA) was used to assess differences in egg-size and chick biometrics and G-tests were used to compare differences in fledging success. Analysis of covariance (ANCOVA) was used to assess growth rates of surviving chicks during the linear growth phase (3 - 24 days post-hatch; Furness & Monaghan 1987) using differences among years in body mass and tarsus length, with age as a covariate. Non-parametric tests were used where data were not normally distributed. All data were transformed where appropriate (Sokal & Rohlf 1995). All tests are two-tailed and means are presented ± 1 s.e. unless otherwise stated.

2.4. RESULTS

The number of breeding birds in the sanctuary and in the colony as a whole (as indicated by the annual census: see annual reports, eg. Royle & Coulson 1995) declined during the study by 23% and 37% respectively (Table 2.1). This was mainly due to the effects of management in the non-sanctuary areas of the colony.

Table 2.1 - *Total numbers of gulls breeding in the Abbeystead sanctuary and the colony as a whole, estimated from the annual census, for each year of the study*

Year	Sanctuary	Total in Colony
1994	5,308	18,254
1995 - pre-cull	4,812	18,250
1995 - post-cull	4,812	15,626
1996 - pre-cull	4,074	14,060
1996 - post-cull	4,074	11,458

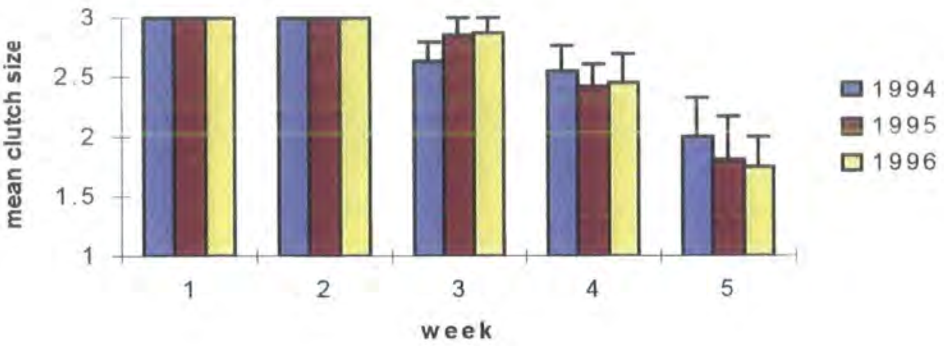
2.4.1. *Clutch Size*

There were no differences among years in the proportion of nests with clutches ($G_2 = 0.84$; n.s.; Table 2.2). High proportions of empty nests are a phenomenon apparently peculiar to the Tarnbrook Fell gullery among British gull colonies, and are discussed in some detail by O'Connell *et al.* (1997). Average clutch size and median laydate of clutches were similar in the three years (Table 2.2: clutch size; Kruskal-Wallis ANOVA; $\chi^2 = 1.89$, n = 128, n.s.; laydate; $\chi^2 = 3.65$, n = 128, n.s.). Clutch size declined with laydate (week 1 = 25 April to 1 May inclusive), with the relationship strongest in 1995 and 1996 (Spearman rank correlation; $r_{95} = -0.60$, n = 44, $P < 0.001$; $r_{96} = -0.54$, n = 66, $P < 0.001$) and least strong in 1994 ($r = -0.37$, n = 49, $P < 0.01$; Figure 2.1).

Table 2.2 - *Summary data for nests in the two permanent study plots*

	year		
	1994	1995	1996
number of nests	167	144	122
number of nests with clutches	49	44	40
% of nests with clutches	29%	31%	33%
number of eggs laid	131	114	105
average clutch size	2.67	2.59	2.63
median lay-date	13 May	12 May	14 May
number of eggs hatched	72	83	97
% of eggs hatched	55%	73%	92%
number of chicks fledged	46	44	71
% of eggs laid giving rise to fledged young	35%	39%	68%
% of eggs hatched giving rise to fledged young	64%	53%	73%
number of chicks fledged per clutch laid	0.94	1.10	1.78

Figure 2.1 - Mean clutch size (± 1 s.e.) for each week of laying in the colony



2.4.2. *Egg-Size*

Mean egg volume of three-egg clutches declined significantly with laydate (ANCOVA; $F_{1,195} = 12.90$, $P < 0.001$), but there were no differences among years in either the slope ($F_{2,195} = 0.58$, n.s.) or the elevation ($F_{2,195} = 1.69$, n.s.) of the relationship. Overall (all three years combined; n = 201 clutches) egg volume declined at a mean rate of 0.21 ± 0.06 cm³/day, which is equivalent to approximately 1.5 cm³/week. Analysis of eggs from each position in the laying sequence separately showed that c-egg volume had the strongest negative relationship with laydate ($F_{1,195} = 15.30$, $P < 0.001$) whilst a-eggs had only a barely significant negative relationship with laydate ($F_{1,195} = 4.12$, $P < 0.05$). Second laid (b-) eggs were intermediate in the strength of relationship ($F_{1,195} = 12.83$, $P < 0.001$). There were no differences among years in the relationship between egg volume and laydate (e.g. c-eggs; slope $F_{2,195} = 0.61$, n.s.; elevation $F_{2,195} = 2.09$, n.s.).

2.4.3. *Breeding Success and Chick Survival*

In the two semi-permanent study plots there were significant differences among years in the proportion of eggs that hatched ($G_2 = 44.74$, $P < 0.001$), with 1996 having the greatest proportion, followed by 1995, then 1994 (Table 2.2). Breeding success (number of chicks fledged per nest) was highest in 1996 in terms of both proportions of eggs laid ($G_2 = 28.84$, $P < 0.01$) and proportions of chicks hatched ($G_2 = 7.92$, $P < 0.05$), whereas 1994 and 1995 were similar to one another in these respects (Table 2.2). This pattern of breeding success was also similar (although slightly higher overall; Table 2.3) for the separate sample of three-egg clutches, with 1996 again the highest (chicks fledged/eggs hatched; $G_2 = 10.90$, $P < 0.01$). This resulted from the higher proportion of broods fledging two or three chicks in 1996 relative to the other two years (G-test on relative proportions of broods fledging either 0 & 1 or 2 & 3 chicks for 1996 compared with 1994 and 1995 combined; $G_1 = 3.86$, $P < 0.05$; Table 2.3).

Table 2.3 - *Number of chicks fledged from unmanipulated three-egg clutches in each year*

	number of chicks fledged per nest				
year	0	1	2	3	total
1994	3	4	5	2	14
1995	2	2	6	3	13
1996	2	1	5	12	20

2.4.4. *Timing of Chick Mortality*

Chick mortality occurred significantly later in 1994 (mean = 15.9 days post-hatching, n = 22, s.e = 2.0) than in either 1995 (mean = 9.3 days, n = 16, s.e = 1.9) or 1996 (6.9 days, n = 13, s.e = 1.6; Kruskal-Wallis ANOVA; $\chi^2 = 10.62$, n = 51, $P < 0.01$).

2.4.5. *Chick Growth*

There were significant differences among years in both tarsus growth and mass growth of chicks surviving to fledging age (Table 2.4). Tarsus length increased at a greater rate (ANCOVA; slope $F_{2,548} = 96.80$, $P < 0.001$) in 1996 than in 1995, which was in turn greater than in 1994 (as indicated by post-hoc parameter estimates). Mass growth changed across years in a similar way (slope $F_{2,548} = 98.31$, $P < 0.001$), with 1996 > 1995 > 1994 (as determined by pairwise post-hoc comparisons).

Table 2.4 - *Growth of chicks that survived to fledging age in each year of study*

	year		
	1994 (n = 20 chicks)	1995 (n = 23 chicks)	1996 (n = 47 chicks)
tarsus length (mm)			
at 10 days	30.03	33.61	36.46
at 20 days	37.88	43.42	49.87
growth rate/day (± 1 s.e.)	0.79 ± 0.03	0.98 ± 0.04	1.34 ± 0.03
mass (g)			
at 10 days	185.35	241.19	277.99
at 20 days	339.64	433.19	545.22
growth rate/day (± 1 s.e.)	15.43 ± 0.71	19.20 ± 0.77	26.72 ± 0.58

2.4.6. *Detailed data from 1996*

C-eggs were significantly smaller than both a-eggs (mean difference = 9.7%) and b-eggs (mean difference = 8.8%), but there was no significant difference in size between a-eggs and b-eggs ($F_{2,132} = 30.9$, $P < 0.001$; Tukey's range test; Table 2.5).

Table 2.5 - *Mean egg volume in 1996 clutches by order in which eggs were laid*

laying order	mean vol (cm^3)	s.e.	c.v.	n
a	73.58	0.62	5.7	45
b	72.83	0.66	6.1	45
c	66.45	0.81	8.2	45

c.v. = coefficient of variation (SD/mean × 100)

Total clutch volume declined as the season progressed (r_{Sp} = -0.31, n = 45, $P < 0.05$). The largest clutches (total clutch volume) had the lowest amount of intraclutch variation in egg-size (IESV; r_{Sp} = 0.34, n = 45, $P < 0.05$). Within-clutch egg-size variation also increased seasonally (IESV with hatch date; r_P = -0.31, n = 45, $P < 0.05$), with clutches of more uniform egg-size being laid earlier than clutches with a greater IESV. Hatching interval was significantly correlated with IESV (r_P = -0.31, n = 45, $P < 0.05$; ie. clutches where chicks hatched relatively synchronously tended to be clutches with little size difference among eggs). Mass ratio was similarly correlated with IESV (r_P = 0.46, n = 45, $P < 0.001$). However, there was no relationship between laying interval and IESV (r_P = 0.19, n = 45, n.s.) suggesting that producing a relatively small c-egg did not shorten the total time over which laying occurred. Indeed, the mean laying interval between b-eggs and c-eggs (2.29 ± 0.09 days) was significantly longer than the mean laying interval between a-eggs and b-eggs (1.84 ± 0.08 days; paired *t*-test, t_{44} = 3.25, $P < 0.01$). In addition, the hatching interval between the b-egg and the c-egg within each clutch was not related to the relative size difference between the b-egg and the c-egg (r_{Sp} = -0.16, n = 45, n.s.), showing that the relative size of the c-egg did not directly influence the degree of asynchrony at hatching.

Hatching intervals increased as the season progressed ($r_P = 0.33$, n = 45, $P < 0.05$) and the within-brood size hierarchy became more accentuated (mass ratio $r_P = -0.47$, n = 45, $P < 0.001$). Brood hatching intervals varied from 0 - 71 hours (mean = 27.96 ± 2.05 hours, n = 45), and this variation was mostly due to differences in the interval between the b-chick and the c-chick hatching (mean b-c hatch interval = 22.04 ± 2.00 hours, n = 45), whereas a- and b- chicks always hatched within a few hours of each other. Total incubation period varied from 674 - 792 hours (mean = 726.23 ± 3.21 hours, n = 45) and was not related to IESV ($r_{Sp} = 0.01$, n = 45, n.s.) or clutch volume ($r_{Sp} = -0.11$, n = 45, n.s.).

Eggs hatched in the order in which they were laid in all clutches except two (4.4%), where the b-egg hatched before the a-egg. In both of these cases the hatching interval was less than two hours between the two chicks and both chicks hatching from the second laid (b-) eggs were still slightly smaller than their siblings hatching from the a-egg. C-chicks (chicks hatching from c-eggs) hatched with significantly shorter tarsi (Table 2.6; $F_{2,129} = 20.3$, $P < 0.001$) than both a-chicks and b-chicks, which were of similar tarsus length at hatching (as indicated using Tukey's range test). C-chicks were also significantly lighter than both a- and b-chicks at hatching (Table 2.6; $F_{2,129} = 31.0$, $P < 0.001$; Tukey range test). However, head-bill length at hatching was significantly different only between a-chicks and c-chicks (Table 2.6; $F_{2,129} = 4.5$, $P < 0.01$; Tukey range test), with c-chicks less than 2.5% smaller in this measurement than a-chicks. By contrast c-chick mean tarsus length at hatching was 4.2% smaller than that of a-chicks, whilst hatching mass of c-chicks was 11.8% smaller than that of a-chicks; a difference similar to the difference in size between a- and c-eggs.

Table 2.6 - *1996 chick biometrics at hatching by hatching order*

tarsus length				
hatch order	mean length (mm)	s.e.	c.v.	n
a	22.61	0.12	3.4	44
b	22.57	0.12	3.6	43
c	21.65	0.12	3.8	45
head-bill length				
hatch order	mean length (mm)	s.e.	c.v.	n
a	46.79	0.24	3.4	44
b	46.51	0.20	2.9	43
c	45.89	0.21	3.1	45
mass				
hatch order	mean mass (g)	s.e.	c.v.	n
a	57.95	0.48	5.5	44
b	56.44	0.58	6.8	43
c	51.69	0.68	8.8	45

Egg volume and hatching mass were strongly correlated ($r_{132} = 0.85$, $P < 0.001$). This was only slightly affected by controlling for hatching sequence (partial $r_{129} = 0.80$, $P < 0.001$). The relationship between egg volume and tarsus length at hatching was less strong ($r_{132} = 0.65$, $P < 0.001$) but was also only slightly influenced by hatching sequence (partial $r_{129} = 0.55$, $P < 0.001$). Egg volume was a relatively poor, but nonetheless significant predictor of head-bill length at hatching ($r_{132} = 0.30$, $P < 0.01$).

At the point of brood completion, first hatched (a-) chicks had grown (in terms of tarsus length) by an average of 1.05mm since hatching (Table 2.7), an increase of 4.6% over mean hatch size at an average rate of 0.034mm/hr. Second hatched (b-) chicks had a similar immediate post-hatch tarsus growth rate to that of a-chicks, but were only 0.79mm (3.5%) larger because they hatched slightly later. Upon hatching c-chicks were thus a mean of 8.5% smaller than their first hatched sibling, and 7.3% smaller than their second hatched sibling, in terms of tarsus length. Differences among chicks in head-bill length were slightly less pronounced, with both a- and b-chicks growing at an average rate of 0.10mm/hr post-hatch, so that a-chicks were 5.8% larger, and b-chicks 4.9% larger at brood completion than they had been at hatching. At brood completion c-chicks were only, on average, 7.3% smaller than a-chicks and 5.9% smaller than b-chicks with respect to head-bill length (Table 2.7). However, for body mass the difference was much more pronounced. First hatched chicks put on an average of 10.7g in the time between hatching and brood completion, an increase of 18.4% from body mass at hatching. B-chicks put on an average of 7.7g, which was 13.7% of mean hatching mass. Consequently, at hatching c-chicks were severely disadvantaged in terms of the within-brood mass hierarchy, being on average almost 25% smaller than first hatched, and over 19% smaller than second hatched siblings (Table 2.7). Mass gain during the period between the first and the last chicks hatching in a brood was much more variable than for either tarsus length or head-bill length (Table 2.7). Variance in the mass of chicks at brood completion was significantly greater for a- and b-chicks compared to c-chicks (Levene's test for homogeneity of variance; $F_{2,132} = 6.50$, $P = 0.002$). However there were no differences in variance for either tarsus length (LV; $F_{2,132} = 1.33$, n.s.) or head-bill length (LV; $F_{2,132} = 2.94$, n.s.) among chicks of different hatching sequence at brood completion.

Table 2.7 - *1996 chick biometrics at brood completion by hatching order*

tarsus length				
hatch order	mean length (mm)	s.e.	c.v.	n
a	23.66	0.14	3.9	45
b	23.36	0.15	4.4	45
c	21.65	0.12	3.8	45
head-bill length				
hatch order	mean length (mm)	s.e.	c.v.	n
a	49.49	0.32	4.3	45
b	48.78	0.29	4.0	45
c	45.89	0.21	3.1	45
mass				
hatch order	mean mass (g)	s.e.	c.v.	n
a	68.62	1.55	15.2	45
b	64.18	1.38	14.4	45
c	51.69	0.68	8.8	45

2.5. DISCUSSION

In many species, including the *Laridae*, clutch sizes are larger in populations breeding in more favourable habitats or, within populations, in years of more favourable food supply (Martin 1987). At the Tarnbrook Fell colony, although there was a decrease in the density of conspecifics over the three years of study there were no changes in average clutch size or the overall timing of reproduction. However, the seasonal decline in clutch size commonly observed in the *Laridae* (Davis 1975; Pyle *et al.* 1991; Sydeman *et al.* 1991) did show slight annual variation. In 1994 the seasonal decline was slightly less pronounced than in the following two years although this may not have been a real effect because sample sizes of clutches in each week were not very large, and the pattern of decline was similar in each year (see Figure 2.1).

Several studies have indicated that clutch size in gulls is determined by the level of pre-breeding protein reserves laid down by the female. Houston *et al.* (1983) found a positive relationship between the amount of protein reserve in the flight muscles and the number of developing ova (potential clutch size), but no relationship between lipid reserves and the potential clutch size. This contrasts with the study by Hario *et al.* (1991), who found that females allocated stored fat to clutch formation, but not protein reserves. Provision of supplemental food to lesser black-backed gulls in a year of poor natural food availability significantly increased mean clutch size, but had no effect when natural food availability was 'normal' (Hiom *et al.* 1991). This suggests that food limitation *per se* may not limit clutch size to a normal upper level of three eggs (Hiom *et al.* 1991). Further supplementary feeding experiments by Bolton *et al.* (1993) indicated that egg-size was influenced by female size, except among those females that had received a supplement of food. This was interpreted as a consequence of competitive superiority of larger females which meant thay could gain access to a greater amount of nutrients, but the effect of supplementary feeding reduced this difference. When clutch size is at a normal level (*ca.* 2.7 eggs) supplementary food has no further effect on clutch size, probably due to smaller fluctuations in food availability or as a consequence of a life history strategy reflecting the trade off between current and future reproduction (Bolton *et al.* 1993). Clutch size in gulls varies with timing of breeding and both these parameters are also associated with increased breeding success.

In this study, as with most studies on gulls (eg. Parsons 1972, Sydeman *et al.* 1991), mean egg-size and clutch size decreased seasonally. There were no significant differences among years in the slope or elevation of this relationship but in all three years c-egg size showed the greatest rate of seasonal decline. Large egg-size has been shown to be beneficial for chick survival in gulls (Parsons 1970) even when controlling for variation in parental quality (Bolton 1991). The ability to produce large eggs was found to contribute significantly to the ability of pairs to raise chicks in an experimental study by Bolton (1991), and he concluded that this was a good indicator of parental quality. Large egg-size is partly a function of female size (Bolton *et al.* 1993), in addition to food availability and foraging ability (Bolton *et al.* 1992). A series of supplementary feeding experiments on lesser black-backed gulls by Bolton *et al.* (1992) revealed that it was probably specific nutrients rather than general food supply that limited egg production. Supplementary feeding of two different proteins resulted in an increase of *ca.* 10% over egg size of control clutches, but the difference between a- and c-egg size was maintained. Reid (1987a), however, found that provision of food supplements during the laying period resulted in a significant increase in the size of the c-egg, but not for either the a- or b-eggs, for a population of glaucous-winged gulls *Larus glaucescens*. Whilst this appears to contradict the data of Bolton *et al.* (1992) the glaucous-winged gulls on Protection Island did not appear to be food stressed (Reid 1987a). In Reid's study the first laid a- and b-eggs may have been, before the addition of extra provisions, at or very near a physiological maximum size, so that the extra available resources were allocated to the c-egg. Where environmental conditions are good and many birds can lay large eggs the advantages of hatching from a large egg may be reduced (Bernardo 1996) such that resources are better invested either in other offspring (either current or later broods) or later phases of reproduction, once egg-size has reached a certain size. If resources are scarce, egg-size (and in particular c-egg size) decreases (Spaans *et al.* 1987). This is because, for a long-lived species with the prospect of many other breeding events and a small clutch size, a reduction in egg-size is a less severe adjustment than a reduction in clutch size (Martin 1987; Bolton *et al.* 1992). When, however, egg-size drops below a threshold value, chick viability is drastically reduced and clutch size is truncated at two eggs (Parsons 1970, 1976).

At the Tarnbrook Fell gullery intraclutch egg-size variation (IESV), which in general reflects the relative size of the c-egg, increased seasonally in clutches of three eggs. Clutches with the least IESV also had the greatest mean egg-size. C-egg size has been found to be significantly related to courtship feeding performance of the male in common terns *Sterna hirundo* (Nisbet 1973), ospreys *Pandion halietus* (Green & Krebs 1995) and glaucous-winged gulls (Salzer & Larkin 1990), but was not assessed here. At the population level, low IESV, large mean egg size and high breeding success are associated with low levels of conspecific competition and an abundant food supply (Pierotti & Bellrose 1986; Kilpi 1995). Breeding success was predictable from the level of IESV, rather than mean egg-size (which may be confounded by body size) in a small population of herring gulls in the Baltics (Kilpi 1995). At Tarnbrook, IESV exhibited a seasonal trend, with earlier laying individuals having less variable egg-size. Earlier laying birds also had higher reproductive success than later laying pairs. IESV was related to both measures of the degree of hatching asynchrony (hatch interval & mass ratio), with clutches showing the least IESV hatching the most synchronously. This relationship between IESV and the degree of hatching asynchrony has also been recorded for western gulls (Pierotti & Bellrose 1986; Sydeman & Emslie 1992) and herring gulls (Kilpi 1995). Evidence for this relationship at the Tarnbrook Fell gullery does not support the suggestion that the small size of the c-egg is an adaptation to reduce the degree of asynchrony at hatching (eg. Parsons 1972; Bollinger 1994) because if this were generally so the opposite relationship between IESV and hatching asynchrony would prevail. In addition, evidence from the current study shows that production of a small c-egg did not shorten the time over which laying occurred (laying interval and IESV were not related). Nor did the hatching interval between the b- and c-eggs within a clutch relate to their relative size difference. Variation in hatching intervals was predominantly due to the difference in hatching time between b- and c-chicks. The total incubation time of a clutch was not related to either its total volume or its IESV. Timing of incubation onset can thus be confirmed as the primary determinant of the degree of hatching asynchrony. The relative size of eggs within a clutch had no significant effect upon hatching asynchrony directly, but it may have had an indirect effect because timing of incubation onset may have been adjusted to the IESV.

Initiation of incubation is proximately under hormonal control (Meijer *et al.* 1990). Parsons (1976) suggested that the tactile stimuli produced by the contact of the brood patch with the first laid a-egg induces the onset of incubation. This is associated with a decrease in levels of plasma-luteinizing hormone and steroidal hormones and a corresponding increase in the levels of prolactin (Stoleson & Beissinger 1995). It has been suggested that the hypothalamus monitors the level of amino acids and free fatty acids/glycerol circulating in the body, because a restriction in dietary protein has been found to cause a drop in luteinizing hormone in hens *Gallus domesticus* (Carey 1996). This indicates that a lack of nutrients acts at the level of the hypothalamus (Carey 1996). If this is generally the case then this could explain why gulls with relatively small c-eggs initiate incubation earlier (and consequently hatch broods more asynchronously) than gulls that can produce three large eggs of relatively uniform size. This suggests that the degree of hatching asynchrony is phenotypically plastic with respect to IESV (which is dependent upon food supply).

The size hierarchy at brood completion can have important implications for the division of parental resources and the timing of brood reduction (Godfray & Parker 1991; *Chapter 5*). Establishment of a size hierarchy (mass ratio) has two components, both indirectly related to food supply. The first of these is the size variation within a clutch (IESV), which has both a direct effect (c-chicks are smaller because they hatch from a smaller egg) and an indirect effect relating to the onset of incubation. The second component is the growth rate of a- and b-chicks in the period between hatching and brood completion. This latter component of the establishment of the within-brood size hierarchy represents another point in time (in addition to initiation of incubation) at which parents could re-assess environmental conditions and investment decisions. Mass ratio increased seasonally in the current study, such that the broods with the most accentuated size hierarchies occurred later in the season. Size hierarchies result in differences in competitive ability and this has been shown to dictate the distribution of food among siblings such that the lower in the hierarchy a chick is, the smaller its share of parental investment (Nilsson & Svensson 1996). The magnitude of the size hierarchy affects the timing of brood reduction and this may have important consequences for the amount of parental effort expended during a single breeding episode (see *Chapter 5*). Later laying pairs may be poorer quality individuals (see *Chapter 4*) and this may affect their ability to raise a full brood of three chicks if environmental conditions are unfavourable. It would therefore be expected that later laying individuals have a greater within brood size hierarchy than early laying birds in most years, except perhaps when food is superabundant.

In addition to hatching later than their siblings, c-chicks are also handicapped by hatching from a smaller egg. Egg volume is a good predictor of mass at hatching. C-chicks hatching mass was *ca.* 12% smaller than that of a-chicks in the current study, and by brood completion this difference had increased to 25%. Structural size differences were less pronounced, because tarsus length and head-bill length at hatching were less well predicted from egg volume than hatching mass. In comparison to mass there was much less variation in these parameters at both hatching and brood completion. Thus, despite hatching from a considerably smaller egg, c-chicks were only slightly disadvantaged in terms of skeletal size. In addition, post-hatch growth rates of both tarsus and head-bill length were apparently relatively invariable. This could be important because mass is easier to increase than structural size and c-chicks may have only a short amount of time to take advantage if an earlier hatched sibling ails (see *Chapter 6*). Conversely, if food is limiting and brood reduction occurs, c-chicks are likely to succome relatively quickly because they have fewer body reserves than a- and b-chicks.

There were significant annual differences in growth rates at the Tarnbrook Fell gullery. Variation in both food supply (Martin 1987; Sedinger *et al.* 1991; Lindholm *et al.* 1994) and aspects of parental care relating to increased foraging time (Pugesek 1995) and efficiency (Cooch *et al.* 1991; Hipfner 1997; Moreno *et al.* 1997) have been shown to affect growth rates, in addition to sibling competition (Werschul & Jackson 1979; Ricklefs 1982). Differences among years undoubtedly relate to changes in food availability as a consequence of changes in the level of intraspecific competition and/or annual variation in the food supply. For example, at the Tarnbrook Fell colony one of the major food sources for feeding chicks are earthworms *Lumbricus terresteris* gathered from fields during the cutting of grass for silage. The timing of silage cutting depends upon several factors determining the early season growth of the grass and the weather conditions around the proposed time of cutting. Weather conditions also affect earthworm availability. Growth rates and survival of chicks were highest in 1996 and lowest in 1994. Apart from variation in food availability, the degree of sibling competition may have also affected annual variation in growth rates. Overall mortality rates were lower in 1996 compared to the other two years and this resulted in a greater proportion of broods where all three chicks survived (1996 - 60%; 1995 - 23%; 1994 - 14%), and hence, a greater propensity for sibling competition. Size hierarchies determine resource distribution (Nilsson & Svensson 1996) and competition for food in larger broods may accelerate growth rates if adults increase provisioning rates in response to increased offspring solicitation (Ricklefs 1982) because in order to survive, the smallest chick must grow quickly so that it can increase its share of parental investment. A decrease in brood size (even temporarily) resulted in a decrease in sibling competition and fighting in cattle egrets *Bubulcus ibis*, even though parents responded by bringing less food

(Mock & Lamey 1991). If this a general pattern and brood size (and the resulting level of sibling competition) has a positive effect upon growth rate then this could contribute in explaining the among year variation in growth rates seen at Tarnbrook Fell. The lower growth rates in 1994 and 1995 may be a reflection of the greater environmental variation in these years and this illustrates the plasticity of growth rates (Cooch *et al.* 1991). Phenotypic plasticity of life-history traits may be vital to reproductive success for long-lived species in a stochastic environment, but this also makes the evolution of such life-history traits difficult to study (Cooch *et al.* 1991).

CHAPTER 3

Yolk lipid analysis: between year and within clutch comparisons

3.1. SUMMARY

Many micronutrients, such as specific fatty acids in yolk lipid, cannot be synthesised by the laying female and must originate from the diet. Analysis of micronutrient composition of eggs may elucidate the role of food availability in resource allocation decisions of wild birds. Yolk lipid analysis was carried out on gull eggs (whole clutches of three) collected in two separate years using thin-layer and gas-liquid chromatography in order to assess the extent (if any) of annual variation in macro-nutrient composition and within-clutch variation in both macro- and micro-nutrient composition of eggs. In addition, the commonly held assumption that egg size is a good indicator of egg resource content was evaluated. Although there were no significant differences between years in egg size or yolk weight among eggs of the same laying order, eggs collected in 1996 had significantly lower lipid content than those collected in 1997. In both years c-eggs were smaller, had smaller yolks and had significantly less absolute amounts of lipid compared with earlier laid (a- and b-) eggs. Eggs laid in 1996 had proportionately less yolk lipid than those laid in 1997, but in neither year were there any significant within-clutch differences in the relative amount of yolk lipid (g/10g). Nor did the ratio of yolk weight to egg weight vary with eggs of different laying sequence. Despite the differences in the total lipid content between eggs of different laying sequence there were no significant differences in the absolute amount of micronutrients deposited, except for cholesterol which was found in significantly greater amounts in a-eggs. Controlling for variation in the total amount of lipid, c-eggs were found to have a disproportionately high fraction of triglyceride, and a disproportionately low fraction of cholesterol ester compared to a- and b-eggs. Triglyceride and phospholipid content of eggs scaled proportionately with egg-size. Other minor components of the lipid fraction varied independently of egg-size. Egg-size, except within a clutch, was not a good indicator of resource content of the egg.

3.2. INTRODUCTION

Variation in propagule size has profound evolutionary and ecological significance because the amount of resources allocated to individual propagules affects the subsequent fitness of both mother and offspring (Bernardo 1996). Most theoretical and empirical studies of parental investment decisions assume that propagule size is a good predictor of the composition of resources packaged inside them (Clutton-Brock 1991; *Chapter 4*), but this is not necessarily the case (Bernardo 1996). Although not well studied in wild species of birds (Carey 1996) there is evidence to suggest that there is considerable variation in egg micronutrient composition (eg. specific lipid classes, fatty acids, amino acids) among individuals of domestic species (Noble 1991; Murphy 1994), related to diet during egg-laying (White 1991).

The main metabolic characteristic of developing avian embryos is the use of lipids within the yolk to provide energy through the β-oxidation of fatty acids (Speake *et al.* 1996). Lipids cover a broad range of complex substances that are insoluble in water but soluble in organic solvents (Noble 1991). Virtually all egg lipid is found in the yolk, and most of this is present as lipoprotein complexes (Noble 1991). Yolk proteins are involved in the transport of vitamins, ions and carbohydrates (in addition to lipids) from the mother to the egg during the period of rapid development of the yolk, where they are stored before use by the developing embryo (Carey 1996). Eggs of precocial species tend to have greater relative amounts of both yolk and lipid compared with eggs of altricial species (Williams *et al.* 1982; Blem 1990). Precocial young require a longer period of development in the egg and the extra lipid and protein are used to support the greater total metabolic costs of maintenance associated with a longer development time (Blem 1990), and the raw materials for the tissues required by precocial hatchlings, such as down, muscles and brain tissue (Carey 1996). The fractions of major lipid classes within the yolk also differ between altricial and precocial species (Vanheel *et al.* 1981; Noble 1991). The major lipid fraction of yolk is triacylglyceride, followed by phosphoglyceride (Noble 1991). In precocial species such as the domestic chicken *Gallus gallus domesticus* or the pheasant *Phasianus colchicus* triglycerides make up approximately 65 - 70 % of the total lipid fraction and phospholipids 20 - 27 % (Noble 1991; Speake *et al.* 1996). In altricial species, such as the domestic pigeon *Columba livia domestica* the proportion of triglycerides is lower (*ca.* 58 %) and the proportion of phospholipids (*ca.* 31 %) and cholesterol higher than those of precocial species (Vanheel *et al.* 1981). Triglycerides provide most of the energy (*ca.* 90 %) assimilated by the embryo during growth, for development and maintenance of embryonic tissue (Carey 1996). Phospholipids are structural lipids and are incorporated into cell membranes of the developing embryo, whilst cholesterol is primarily involved in the transport of lipoprotein complexes from the yolk to the liver of the embryo (Carey 1996).

Almost nothing is known about the egg micronutrient content of wild bird species (Bernardo 1996), nor how ecological and nutritional constraints have shaped the co-evolution of embryonic nutritional requirements and the ability of the laying female to provision the egg (Carey 1996). The primary aim of this study was to investigate the within-clutch variation in micronutrient composition of yolks in order to try to elucidate patterns of parental investment of resources among eggs of different laying sequence. In addition, annual variation in macronutrient composition of eggs and the validity of the use of egg size as an accurate indication of resource composition of eggs were also examined.

3.3. METHODS

3.3.1. *Fieldwork*

In both years of study (1996 & 1997) nests within an area (*ca.* 500m^2) of the Sanctuary were monitored at least twice daily during early May, the period of peak laying activity in the colony (*Chapter 2*). Nests were marked with a short length of bamboo cane upon clutch initiation (laying of the a-egg). The a-egg was then removed, marked with a nest number and the laying sequence, and replaced with a hard-boiled gull's egg to reduce the chance of clutch desertion. Nests so marked were then visited on a daily basis and the b- and c-eggs removed, sequentially, as laid. Eggs were measured (length and breadth ± 0.1 mm, for calculation of egg volume; see *Chapter 2*) and then refrigerated (at *ca.* 4° C) on the day they were collected. All complete (three-egg) clutches (n = 11 in 1996; n = 10 in 1997) collected were initiated within ± 3 days of each other. Upon completion of fieldwork the eggs were transported to the Scottish Agricultural College laboratories at Auchincruive, where they were kept refrigerated for *ca.* 36 hours prior to analysis.

3.3.2. *Laboratory analysis*

Gull eggs were carefully separated into their constituent parts (shell, albumen and yolk) and weighed before extraction of the yolk lipid. The yolk lipid extraction and separation of the different lipid classes was carried out by Dr. B. K. Speake and R. J. McCartney at the SAC, Auchincruive. A brief resumé of the laboratory protocol is given here; more detailed methods can be found in Speake *et al.* (1996).

Yolk samples were homogenised in chloroform/methanol (2:1, v/v), and the homogenates filtered. Non-lipid compounds were removed through the addition of potassium chloride solution, before extraction of the total lipid in the sample using chloroform. Thin-layer chromatography on silica gel, using a hexane/diethyl ether/formic acid (80:20:1, v/v/v) solvent, was then used to separate the lipid sample into its major fractions (triacylglycerol, phospholipid, free cholesterol, cholesterol ester and free fatty acid). The fatty acid profile of the major lipid fractions was determined, following methylation, using a packed column gas chromatograph. A suitable software package (EZ Chrom Data System; Scientific Software Inc., USA) was utilised to quantify the relative proportions (% w/w) of each fatty acid within the different lipid fractions, the amount of the original lipid fractions containing fatty acyls and the retention times. The retention times allowed the identity of the fatty acid methyl peaks to be verified using standard fatty acid methyl ester retention times. Cholesterol levels were determined using a kit. Absolute values of lipid fractions were calculated by multiplying the weight of total lipid in each sample by the proportion of the lipid fraction under consideration in that sample.

3.3.3. *Statistical analysis*

Data expressed as proportions were arcsine transformed prior to analysis or analysed using non-parametric techniques, but all other (absolute) data were found to conform to an approximately normal distribution when tested using Kolmogorov-Smirnov one-sample tests. These data were therefore not transformed before analysis. Correlation coefficients are Pearson's product-moment correlation coefficients, unless otherwise stated. Analysis of variance was used to compare means between years and among eggs of different laying sequence. Means are presented ± 1 standard error and all statistical tests are two-tailed.

3.4. RESULTS

Yolk lipid fractions (ie. triglyceride, phospholipid etc.) are presented only for eggs taken in 1997 because there was a problem with the analysis of these components in the yolk samples from 1996.

3.4.1. *Variation in egg size, yolk size and lipid content of yolks*

There were no significant differences between years in egg volume (two-way ANOVA; $F_{1,56} = 0.07$, n.s.), but in both years egg volume varied significantly with laying sequence ($F_{2,56} = 13.69$, $P < 0.001$), with a similar pattern (year and laying sequence interaction; $F_{2,56} = 0.93$, n.s.; Table 3.1).

Table 3.1 - *Between year comparison of the mean egg volume for three-egg clutches*

	year			
	1996		1997	
laying sequence	mean ± 1 s.e.	number	mean ± 1 s.e.	number
a-egg	73.07 ± 1.39	11	70.85 ± 1.98	9
b-egg	71.38 ± 1.26	11	73.02 ± 2.02	10
c-egg	63.97 ± 1.72	11	65.55 ± 1.42	10

Last-laid (c-) eggs were significantly smaller than a- or b-eggs (one-way ANOVA on egg weight for 1997 clutches; $F_{2,25} = 4.62$, $P < 0.05$; followed by range tests) in terms of both weight and volume, which were highly correlated ($r_{28} = 0.98$, $P < 0.001$). C-eggs also had significantly smaller yolks (one-way ANOVA; $F_{2,25}$ =

3.92, $P < 0.05$; range tests indicated that b-egg yolks were significantly larger than c-egg yolks), although there were no differences among eggs of different laying sequence in the proportion of egg weight that consisted of yolk (one-way ANOVA on arcsine transformed data; $F_{2,26} = 0.51$, n.s.; Table 3.2).

Table 3.2 - *Egg weights, yolk weights and proportionate yolk weights by laying sequence for eggs collected in 1997*

laying sequence	mean egg weight (g) ± 1 s.e.	mean yolk weight (g) ± 1 s.e.	yolk weight as a % of egg weight ± 1 s.e.
a-egg (n = 9)	78.68 ± 2.19	22.59 ± 0.71	28.70 ± 1.18
b-egg (n = 9)	79.75 ± 2.47	23.62 ± 0.86	29.55 ± 1.85
c-egg (n = 10)	71.85 ± 1.38	20.94 ± 0.49	29.19 ± 2.23

Yolks from eggs collected in 1997 had a significantly greater total amount of lipid than yolks from eggs collected in 1996 (two-way ANOVA; $F_{1,55} = 7.03$, $P < 0.01$), despite there being no significant difference between years in overall egg-size (see earlier) or yolk size ($F_{1,55} = 3.38$, n.s.). However, both yolk size and total lipid content of yolks were affected by laying sequence (Table 3.3). Yolks from a- and b-eggs were significantly larger than c-egg yolks ($F_{2,55} = 9.91$, $P < 0.001$), whilst a-egg yolks had the greatest total amount of lipid and c-egg yolks the least (effect of laying sequence; $F_{2,55} = 5.82$, $P < 0.01$; Table 3.3). The relationship between total lipid content and laying sequence was similar between the two years (interaction of year and laying sequence; $F_{2,55} = 0.38$, n.s.; Table 3.3), indicating that the lower total lipid levels in 1996 affected eggs of different laying sequence equally. In neither year were there any significant differences relating to laying sequence in the relative amount of lipid deposited in the yolk (g/10g: Kruskal-Wallis one-way ANOVA; 1996; χ^2 = 5.43, n = 33, n.s.; 1997; χ^2 = 3.83, n = 28, n.s.; Table 3.3). However, as indicated by the above data, eggs laid in 1997 had a significantly greater mean proportionate amount of lipid (g/10g) per yolk than that of eggs laid in 1996 (1996 mean = 2.96 ± 0.05, n = 33; 1997 mean = 3.09 ± 0.05, n = 28; Mann-Whitney; $Z = 3.39$, n = 61, $P <$ 0.001).

Table 3.3 - *Between year comparison of yolk weights and lipid content of yolks (absolute and relative values) according to laying sequence*

	mean yolk weight (g) ± 1 s.e.		mean total lipid content (g) ± 1 s.e.		mean relative lipid content (g/10g yolk) ± 1 s.e.	
laying sequence	1996	1997	1996	1997	1996	1997
a-egg	**22.36** ± 1.09	**22.59** ± 2.12	**6.84** ± 0.71	**7.23** ± 0.80	**3.07** ± 0.11	**3.20** ± 0.04
b-egg	**22.04** ± 1.49	**23.62** ± 2.59	**6.26** ± 0.84	**7.06** ± 1.11	**2.83** ± 0.08	**2.99** ± 0.10
c-egg	**19.97** ± 2.04	**20.94** ± 1.55	**5.97** ± 0.66	**6.44** ± 0.55	**2.99** ± 0.04	**3.09** ± 0.10

Note - sample sizes for 1996 are n = 11 for a-, b- and c-eggs; in 1997 n = 9 for a- and b-eggs but n = 10 for c-eggs

3.4.2. *Composition of yolk lipid*

Comparison of the yolk lipid fractions among eggs of different laying sequence revealed no significant differences in the absolute amount of the main constituent fatty acid group (triglycerides - TG; one-way ANOVA; $F_{2,25}$ = 1.24, n.s.; Table 4), nor the amount of phospholipid (PL; $F_{2,25} = 1.53$, n.s.) or free fatty acid (FFA; $F_{2,25} = 0.28$, n.s.), despite the difference in both yolk size and total lipid content of a- and b-eggs compared to c-eggs. There were, however, significantly greater amounts of cholesterol ester (CE) deposited in a-egg yolks than that in b- or c-egg yolks ($F_{2,25} = 13.00$, $P < 0.001$; followed by range tests). There was no effect of laying sequence upon free cholesterol (FC) content of eggs ($F_{2,25} = 2.81$, n.s.).

Table 3.4 - *Absolute values of yolk lipid fractions for eggs of different laying order (means ± 1 s.e. grams)*

laying seq.	TG	PL	FC	CE	FFA	number
a-egg	**4.72** ± 0.19	**1.87** ± 0.06	**0.41** ± 0.05	**0.16** ± 0.01	**0.07** ± 0.01	9
b-egg	**4.66** ± 0.23	**1.85** ± 0.15	**0.37** ± 0.04	**0.11** ± 0.01	**0.07** ± 0.01	9
c-egg	**4.35** ± 0.13	**1.66** ± 0.05	**0.28** ± 0.04	**0.08** ± 0.01	**0.07** ± 0.01	10

Comparison of the relative amounts of the different classes of lipid within the yolk showed that there were still significant differences in the composition of the lipid fraction of yolk among eggs of different laying order even after controlling for the effects of the total amount of lipid (Table 3.5). Levels of cholesterol ester (CE) remained significantly higher in first laid (a-) eggs than in either b- or c-eggs (one-way ANOVA on arcsine transformed data; $F_{2,27} = 9.00$, $P < 0.001$; followed by range tests), and c-egg yolks were found to have proportionately more triglyceride (TG) than a-eggs (as indicated by range tests; $F_{2,27} = 3.40$, $P < 0.05$). There were no differences in the relative proportions of phospholipid (PL; $F_{2,27} = 0.08$, n.s.), free fatty acid (FFA; $F_{2,27} = 0.01$, n.s.) or free cholesterol (FC; $F_{2,27} = 1.57$, n.s.; Table 3.5).

Table 3.5 - *Relative composition of yolk lipid mass (%; mean ± 1 s.e.) of eggs of different laying order*

laying seq.	TG	PL	FC	CE	FFA	number
a-egg	**65.24** ± 0.48	**25.91** ± 0.43	**5.62** ± 0.51	**2.20** ± 0.17	**1.03** ± 0.18	10
b-egg	**65.93** ± 0.85	**26.10** ± 0.66	**5.32** ± 0.40	**1.63** ± 0.20	**1.02** ± 0.07	10
c-egg	**67.52** ± 0.50	**25.85** ± 0.31	**4.41** ± 0.58	**1.23** ± 0.11	**1.01** ± 0.10	10

3.4.3. *Egg size as an indicator of resource content*

Changes in the proportions of egg constituents with egg size are not accurately measured by linear regression statistics because variation in average egg size and composition affect the slopes of such relationships. These problems may be overcome by using regression statistics based upon the logarithms of these variables (Ricklefs *et al.* 1982). Across laying sequence log-log regression of yolk weight upon egg weight indicated that yolk weight increased in direct proportion to egg weight (slope = 0.898 ± 0.134, $r^2 = 0.62$, $P < 0.0005$), because the confidence limits of the slope include 1.0 (0.622 - 1.174; Ricklefs *et al.* 1982). Similarly, total lipid content was found to be directly proportionate to egg weight (slope = 0.835 ± 0.216, 95% C.I's 0.391 - 1.279, $r^2 = 0.34$, $P < 0.001$). However, these relationships differed among eggs of different laying order. Both yolk weight (slope = 0.991 ± 0.180, 95% C.I's 0.565 - 1.417, $r^2 = 0.78$, $P < 0.001$), and total yolk lipid content (slope = 1.136 ± 0.214, 95% C.I's 0.643 - 1.629, $r^2 = 0.77$, $P < 0.001$) increased in direct proportion to egg weight for first-laid (a-) eggs. In contrast, both yolk weight (slope = 0.394 ± 0.392, $r^2 = 0.001$, $P = 0.35$) and total lipid content (slope = -0.379 ± 0.482, $r^2 = 0.04$, $P > 0.50$) were independent of egg weight for c-eggs. Consequently, egg weight was a good indicator of general resource content (yolk weight, total lipid mass) for a-eggs, but not for c-eggs. However, the across-laying-sequence log-log regression statistics indicated that within a clutch, egg-size provided a good indication of differences in yolk weight and total lipid mass.

Across-laying-sequence log-log regression of the different lipid fractions with egg weight showed that triglyceride (slope = 0.745 ± 0.221, 95% C.I's 0.291 - 1.199, $P < 0.01$) and phospholipid (slope = 0.940 ± 0.270, 95% C.I's 0.385 - 1.495, $P < 0.01$) increased in proportion to egg weight, whilst free cholesterol ($P > 0.05$), cholesterol ester ($P > 0.25$) and free fatty acid ($P > 0.90$) varied independently of egg weight. However, triglyceride (slope = 1.257 ± 0.240, $P < 0.001$) and phospholipid (slope = 0.885 ± 0.299, $P < 0.05$) scaled proportionately to egg weight for a-eggs but not for c-eggs (TG & PL $P > 0.50$). Variation in a-egg size between different females within any one year was thus a good indicator of the amount of the two major lipid fractions (TG & PL) packaged in the egg, but was not a good indicator of the minor lipid fractions present.

3.5. DISCUSSION

The yolk, as the major source of energy and nutrients utilized by the developing embryo, represents the largest energy investment in an egg by the laying female

(Astheimer & Grau 1990). In this study, in common with other studies of egg composition in Larids (Parsons 1971; Ricklefs *et al.* 1982; Meathrel & Ryder 1987), c-eggs were smaller and contained fewer nutrients than a- and b-eggs, suggesting that nutrient reserves of females were being depleted during egg formation (Meathrel & Ryder 1987). However, in contrast to previous work there were no differences relating to laying sequence in the proportion of yolk within eggs, so that the smaller size of the third egg was not just a result of reduced albumen deposition during egg formation. This may reflect species or population differences in egg composition.

Meathrel (1991), studying Silver gulls *Larus novaehollandiae*, found that rapid yolk deposition (RYD) occurred at a faster daily rate for a-eggs than for eggs laid later in the clutch. Although there were no differences in the total periods of RYD for different eggs within a clutch, and daily growth increments were independent of maternal control (and so reflected daily variation in female nutritional state), proportionately more of the available resources were allocated to a-eggs (Meathrel 1991). Whilst albumen synthesis is predominantly temporally separate from the period of RYD, in birds with clutches of more than one egg there is some overlap, and hence the potential for increased demand for resources, between yolk formation and albumen synthesis (Astheimer & Grau 1990). In addition, courtship feeding in gulls, which may be an important source of nutrients for egg formation, decreases sharply once the a-egg has been laid (Salzer & Larkin 1990). It is likely that the hierarchial difference in the allocation of resources among yolks undergoing rapid yolk development peaks once albumen synthesis has begun. A consequence of this is that the nutritional composition of the c-egg is likely to be more variable than that of the a-egg within a clutch because the yolk lipid composition of c-eggs will, at least partly, be dependent upon how much of the daily available resource ration is 'left over' after preferential allocation to yolks higher up in the hierarchy. In this study egg-size was found to be a good predictor of yolk size and total lipid content for a-eggs from different clutches and for eggs within a clutch, but not for c-eggs laid by different females, providing evidence in support of differential investment of maternal resources among eggs of different laying order.

Egg-size differences within a clutch provide a general indication of differences in investment of resources because smaller eggs have absolutely fewer nutrients. However, in contrast to a-eggs the amount of triglyceride and phospholipid allocated to c-eggs was not accurately predicted by egg-size. Furthermore, although the amount of cholesterol in egg yolks was significantly affected by laying sequence it varied independently of egg-size. Free fatty acid varied independently of both egg-size and laying sequence. In view of this, egg-size *per se* cannot be considered to be a good indicator of micronutrient composition. Neither is egg-size a good indicator of macronutrient composition of eggs, which varies both between species, and within species, between years as a likely consequence of differences in diet (Noble 1991).

Although it is not clear what consequences differences in yolk micronutrient composition have for chick survival, available evidence suggests that these differences arise through nutritional constraints on egg production. This is important as crucial investment decisions later in the reproductive cycle depend upon food availability. For example, although there were no significant differences in the total amount of phospholipid deposited among different eggs in the laying sequence, a-eggs had significantly more arachidonic acid (20:4, n-6; one-way ANOVA; $F_{2,25}$ = 5.26, $P < 0.01$) than b- or c-eggs. Arachidonic acid is an essential fatty acid that must originate from the diet (Carey 1996). There is also evidence to suggest that levels of essential vitamins and carotenoids decrease significantly with laying sequence (Surai, Speake & Royle; unpublished data). Differential investment of available resources among yolks undergoing RYD or during albumen synthesis, may affect the timing of incubation onset if the general nutritional status of females influences the release of hormones associated with incubation onset from the hypothalamus (*Chapter* 2). This would have important consequences for the development of sibling hierarchies and offspring survival (*Chapter 5*) if true.

In conclusion, whilst this study has posed more questions than it has given answers, it has shown that there may be significant differences in the nutrient content of eggs both between individuals of the same species and among eggs within a clutch. The use of egg-size as an indication of the magnitude of parental investment of resources should be treated with caution until it is known how variation in micronutrient composition within an egg affects offspring fitness.

CHAPTER 4

The effect of experimental manipulation of timing of breeding upon growth and survival of chicks

4.1. SUMMARY

In many species of bird reproductive success declines seasonally in conjunction with a decline in clutch size and egg-size. In the current study I exchanged whole clutches of eggs between early laying and late laying birds, whilst experimentally controlling for variation in both clutch size and egg size. Growth and survival of chicks in experimental groups were compared with that of unmanipulated control chicks in order to assess whether a decrease in food supply, or differences in quality between parents best explained seasonal variation in reproductive success of lesser black-backed gulls. There was no significant difference in fledging success among groups. Logistic regression analysis revealed that chick mass at hatching (except for chicks in delayed broods) and hatching date (which showed a strong interaction with hatching mass) were the most important factors affecting chick survival probability, but parental quality (foster lay-date) was also important. In delayed broods timing of chick mortality was significantly later than in either of the other treatments, and chick mass at hatching was not a good predictor of survival probability in this treatment group. Delayed brood chicks also had lower mass growth rates and exhibited lower asymptotic weights than chicks in advanced or control broods. Delayed pairs had lower breeding success while advanced pairs had higher breeding success than expected, providing support for the date hypothesis for timing of breeding. However, the effect of parental quality on chick survival was greatest when chicks hatched late or at low weights, which supported the parent quality hypothesis for timing of breeding.

4.2. INTRODUCTION

In gulls, as for most bird species nesting in temperate seasonal environments, both clutch size and breeding success are related to the timing of breeding, with earlier laying birds generally having larger clutches and higher reproductive success (eg. Parsons 1971; Davis 1975; Sydeman *et al.* 1991). Earlier fledging chicks also have a higher probability of future recruitment into the breeding population (Spear & Nur 1994). In many species birds lay particularly early in the season when feeding conditions are favourable (Martin 1987). Lack (1954) suggested that in general, breeding of birds is timed so that the period of peak nestling demand for food coincides with peak food availability; food supply thus determines the timing of breeding. Several authors have examined this experimentally, using food supplementation experiments (Svensson 1995). These have resulted in most cases in a shift to earlier breeding, suggesting that food supply does indeed affect timing of breeding. However this shift is usually relatively small indicating that other factors also act to constrain the timing of clutch initiation. These factors may include heredity, increased thermoregulatory costs of breeding earlier and predation pressure (Martin 1987).

In order to assess whether date of breeding and breeding success are causally linked, timing of breeding must be manipulated experimentally. This has been done for a number of species in recent studies. Verhulst & Tinbergen (1991) manipulated the timing of breeding in the great tit *Parus major* in two different forests by inducing birds to lay repeat clutches through removal of first clutches. Late breeding caused a decrease in clutch size, fledging success and nestling weight in a mixed wood forest but not the other (predominantly oak *Quercus robar*) forest, where female survival was subsequently reduced instead. However, for both forests recruitment of fledged young into the breeding population was much reduced and clutch size of the females in the following season was also adversely affected. Later hatched young appeared to have lower competitive abilities than earlier hatched young, with lower dominance in winter flocks, and therefore lower survival. Although the study provided evidence to support a causal relationship between timing and success of reproduction, the experimental design was flawed in that inducing repeat clutches through removal of first laid clutches was likely to have reduced their subsequent breeding performance, because production of eggs is energetically costly (Monaghan & Nager 1997). Nonetheless, a study on the same species by Barba *et al.* (1995) incorporating a different experimental design (as a result of sparrows *Passer domesticus* naturally delaying some pairs by nesting in the nest boxes in their territories), supported the general findings of Verhulst & Tinbergen (1991). Delayed pairs produced fewer fledglings, which were less likely to recruit into the breeding population. Additionally the degree of hatching asynchrony was higher among late layers and clutch size was reduced compared to control pairs. Barba *et al.* (1995) thus concluded that there was a cost to breeding late in the season and that there was a causal relationship between timing and success of breeding.

Nilsson (1994), working on blue tits *Parus caeruleus,* showed that there may also be a cost associated with breeding too early. Pairs of tits were manipulated into initiating clutch formation earlier through the provision of supplemental food that was then withdrawn once the female had started the clutch. Clutch size and egg-size were unaffected but both fledging success and subsequent female local survival were reduced in experimental broods, indicating a cost to reproducing too early. Nilsson (1994) suggested that food availability may not be the proximate factor determining timing of breeding but that it may need to reach a threshold level of nutritional reserves as an indicator of likely resource availability during later, more critical stages of the breeding cycle. The breeding phase that is the most energy demanding in relation to food availability will then be the bottle-neck that determines timing of breeding (Nilsson 1994). Selection pressure for breeding early means that females may start breeding before peak food availability. The exact timing of breeding would then depend upon the trade-offs among brood size, nestling condition, parental energy expenditure and the benefits of producing early young (Nilsson 1994).

Demonstrations of a causal relationship between timing and success of breeding are not restricted to the *Paridae*. Daan *et al.* (1990) found evidence of such a relationship in kestrels *Falco tinnunculus,* and it has also been demonstrated experimentally in coots *Fulica atra* (Brinkhof *et al.* 1993). All these studies indicate that individuals optimize timing of clutch initiation and the size of the clutch with respect to resource availability and nutritional status (Daan *et al.* 1990). Individual optimization may explain why clutch size tends to decline seasonally in many species (Daan *et al.* 1990). The earliest breeders in a population are often those with the greatest energy reserves due to the possession of superior foraging skills and/or competitiveness (Schultz 1991). In most cases early laying individuals tend to be older birds (eg. western gulls *Larus occidentalis;* Sydeman *et al.* 1991). In coots advancement of lay-date occurred as females matured (Perdeck & Cavé 1992), and this was due to increased experience rather than increased reproductive effort with age or differential survival of early laying versus later laying birds. Sydeman *et al.* (1991) came to the same conclusions for western gulls. Variation in coots' lay-date was predominantly attributed to seasonal variation in food supply (Perdeck & Cavé 1992) and this was taken as evidence to support Fisher's (1930) theory that as a trait's effect on fitness increases, genetic variance (and heritability) of this trait decreases. However, high heritability of lay-date has been found in other species (refs in Price *et al.* 1988), and so given that early breeding pairs tend to have higher reproductive success than birds that breed later in the season, it is not immediately clear why natural selection has not led to earlier breeding in birds. Price *et al.* (1988) modelled the evolution of lay-date under conditions of apparent directional selection, to show that there can be a correlation between a heritable trait (lay-date) and fitness whenever a non-heritable trait (nutritional state of the female) affects the expression of the character (lay-date) and fitness, at the same time but through different pathways. However, whether lay-date is heritable or not, timing of breeding is strongly related to female nutritional state (Price *et al.* 1988), and hence, food supply.

Food supplementation experiments with gulls show that, even in years of poor food availability, lay-date is not advanced by addition of extra food (Reid 1987a; Hiom *et al.* 1991; Bolton *et al.* 1992), although it is known that higher quality individuals lay earlier in the season than poorer quality birds (Sydeman *et al.* 1991). Instead the extra resources gained are put into production of more and/or larger eggs. This could be because gulls are colonial breeders and there may be costs associated with breeding too early (eg. predation). Alternatively, or additionally, the supplemental food may have failed to provide some limiting nutrient (Hiom *et al.* 1991).

In order to assess whether, in lesser black-backed gulls, date and success of reproduction are causally related, I exchanged clutches of eggs between early laying and later laying birds then subsequently monitored both chick growth and survival as an indicator of parental investment decisions. The experimental technique followed Brinkhof *et al.* (1993), except that I controlled for the potential covariates of both clutch size and egg size as part of the experimental design rather than post-hoc, as part of the statistical analysis. If a decrease in food supply causes the seasonal decline in reproductive success observed in gulls then pairs whose clutch hatching date has been advanced (by exchanging clutches) should have increased reproductive success, whilst for delayed pairs reproductive success should decrease. Similarly, if the seasonal variation in fledging success is due to differences between parents (eg. in their ability to exploit the available food supply) then the original laying and/or hatching date of the (foster) parents will have a greater effect on chick survival probability than the actual lay and/or hatch date (Brouwer *et al.* 1995). If timing of breeding is individually optimized then any manipulation should result in a decrease in fledging success in comparison to that expected from the seasonal pattern.

4.3. METHODS

A total of approximately 100 nests in the 'sanctuary' area of the colony was located and marked with a short length of bamboo cane near the nest during nest-building in April 1995. Upon the initiation of egg-laying at these nests, all eggs were measured (length and breadth ± 0.1mm using dial callipers) on the day they were laid, and marked according to nest number and laying sequence. From these nests, 33 clutches of three eggs laid during the first two weeks in May with similar total clutch volumes (one way ANOVA; $F_{2,29} = 0.09$, n.s.) were selected and assigned to one of three groups:

1) experimental 'Advanced' clutches (median laydate 11 May)

2) experimental 'Delayed' clutches (median laydate 3 May)

3) unmanipulated 'Control' clutches (median laydate 8 May)

4.3.1. *Experimental procedure*

Upon clutch completion (laying of the c-egg) in treatment 1, whole clutches were swapped with those of birds in treatment 2 through pairing the most closely sized clutches together (paired t-test on total clutch volume; $t_9 = 0$, n.s.; paired t-test on arcsine transformed data for IESV (see *Chapter 2*); $t_9 = 0.98$, n.s.) from birds laying at least a week earlier in the breeding season (mean difference in laying date = 8.8 ± 0.3 days). Thus treatment 1 parents had their timing of breeding advanced whilst treatment 2 parents had their breeding attempt delayed, by over a week. A consequence of this cross-fostering of clutches was that delayed (treatment 2) pairs were engaged in incubation of eggs for almost twice as long as advanced (treatment 1) pairs (mean total incubation time; delayed pairs = 38.7 ± 0.4 days; advanced pairs = 21.9 ± 0.6 days), with a mean difference of 16.8 ± 0.9 days between the two treatment groups. Clutches were briefly removed in control (treatment 3) nests to control for the effect of handling eggs, before being returned, and incubated normally by the parents.

4.3.2. *Data collection*

Once manipulations were completed, every nest was surrounded with a chicken-wire enclosure (approx. 2 m^2 in area, and 50 cm high) to assist in finding chicks after hatching. A wooden shelter was also provided for the chicks in each enclosure. Chicks were individually marked according to hatching sequence and were weighed (± 1g on a Pesola balance) and measured (tarsus length ± 0.1mm using dial calipers), at 3 - 6 day intervals, from hatching until 36 days post-hatching. It was assumed that chicks reaching 36 days of age (when they were fully feathered and almost capable of flight) fledged successfully.

4.3.3. *Statistical analysis*

Growth rates of chicks were compared among treatments using analysis of covariance of body mass or tarsus length with treatment during the linear phase of growth (3 - 24 days post hatching; Furness & Monaghan 1987), with age as a covariate. Chick survival was modelled using stepwise logistic regression (Hosmer & Lemeshow 1989) because survival probabilities were binary (chicks either survived or they did not). Suitability of each independent variable for inclusion in the model was assessed using the Likelihood Ratio Statistic (LRS) at each step. LRS is analagous to the residual sum of squares in linear regression (Hosmer & Lemeshow 1989). Independent variables selected ($P < 0.05$) for initial inclusion in the analysis were:

1) lay-date - date of clutch initiation of the original parents of clutch (pre-manipulation)

2) foster lay-date - date of clutch initiation of the foster parents of clutch (post-manipulation)

3) hatch-date - date of hatch of each individual chick

4) treatment group - as a categorical variable

5) hatch weight - weight (grams) of individual chick at hatching

6) hatch tarsus - tarsus length (mm) of individual chick at hatching

In addition, an index of condition was calculated for each chick by regressing weight on tarsus length and expressing the residuals as a proportion of the predicted value in each case. This was used to compare the body condition of surviving chicks among treatment groups and also allowed a comparative assessment of within brood variation in body condition and mean brood body condition during nestling development. Data were transformed where appropriate (following Sokal & Rohlf 1995) and means are presented ± 1 s.e. unless otherwise stated.

4.4. RESULTS

4.4.1. *Chick biometrics at hatching*

There were no differences among treatments (post-manipulation) in chick weight at hatching (three-way ANOVA; $F_{2,87} = 0.58$, n.s.), but both hatching sequence ($F_{2,87} = 18.76$, $P < 0.001$; first hatched chicks larger than last hatched chicks) and survival to fledging ($F_{1,87} = 10.78$, $P < 0.01$) were significantly related to hatching mass. However, the interaction between treatment and status was significant ($F_{2,87} = 7.96$, $P < 0.001$) indicating that the relationship between hatch weight and survival was different among treatments (Table 4.1). Delayed pairs hatched chicks that were significantly smaller in terms of tarsus length than the other two treatments ($F_{2,87} = 5.31$, $P < 0.01$) despite there being no significant difference in pre-manipulation clutch volume among the three treatments (see **Methods**). Tarsus length was also significantly related to both hatching sequence ($F_{2,87} = 7.75$, $P < 0.001$) and status ($F_{1,87} = 5.47$, $P < 0.05$). However, in contrast to hatch weight, tarsus length did not significantly interact with any of the above variables.

Table 4.1 - *Chick size (tarsus length) and mass at hatching by treatment group and survival to fledging*

treatment group	tarsus length (mm) (mean ± 1 s.e.)	mass (g) (mean ± 1 s.e.)	number
advanced: died	21.91 ± 0.22	53.60 ± 1.23	15
advanced: survived	22.71 ± 0.21	56.22 ± 0.94	18
control: died	21.24 ± 0.24	50.36 ± 1.03	14
control: survived	22.27 ± 0.22	58.72 ± 0.89	18
delayed: died	21.58 ± 0.26	53.17 ± 0.99	12
delayed: survived	21.68 ± 0.20	54.92 ± 1.54	13

4.4.2. *Fledging success*

The number of chicks fledged was modelled both as the proportion of fledged chicks per egg laid and as the proportion of fledged chicks per hatched egg.

4.4.2.1. Seasonal trend of unmanipulated broods

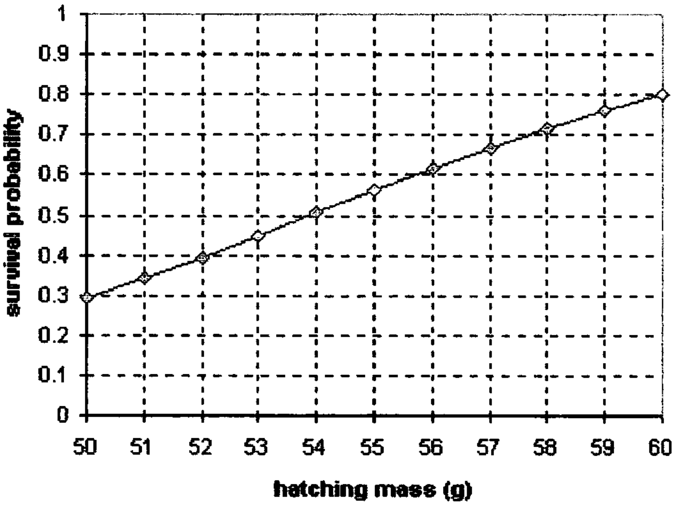
Only hatch weight and hatch date contributed significantly to the explained variation in fledging success in unmanipulated Control broods. Hatch weight was selected on the first step ($LRS_{1,30} = 13.09$, $P < 0.001$), and hatch-date significantly improved the fit of the regression line ($LRS_{1,30} = 3.84$, $P < 0.05$).

4.4.2.2. Inclusion of experimental groups

In a multivariate model for all treatment groups (with treatment group as a catagorical variable, and also including foster lay-date and all possible interaction terms) hatch weight was found to be the most important variable affecting the chick survival probability ($LRS_{1,89} = 20.06$, $P < 0.001$; Fig. 4.1). Hatch weight showed a strong interaction with both hatch-date ($LRS_{1,89} = 13.07$, $P < 0.001$) and lay-date

($LRS_{1,98}$ = 11.98, P < 0.001), so in order to assess the effect of the experimental manipulation of timing of breeding upon fledging success, hatch weight was incorporated in subsequent models in terms of its interactions with other variables.

Figure 4.1 - *Variation in probability of surviving to fledging with hatching mass*



A logistic regression was then developed incorporating lay-date, hatch-date and treatment group, and all the possible two way interactions between these variables. Hatch-date was selected on the first step of the model ($LRS_{1,89} = 9.83$, $P < 0.01$), while lay-date significantly improved the fit of the regression and was selected on the second step ($LRS_{1,89} = 6.69$, $P < 0.01$). There was no effect of treatment upon fledging success. This implies that the probability of fledging was strongly dependent on hatch date of chicks. When lay-date was replaced in the model by foster lay-date to investigate the effect of parental quality upon the probability of surviving to fledging, only the interaction term foster lay-date × hatch-date was selected, because this significantly improved the fit of the regression ($LRS_{1,89} = 7.21$, $P < 0.01$). This suggests that the quality of the foster parents also had an effect on the survival probability of chicks. Introduction of the interaction terms foster lay-date × hatch weight and hatch-date × hatch weight indicated that foster lay-date had a stronger association with hatching weight than with hatch-date, because it was selected on the first step of the revised regression analysis ($LRS_{1,88} = 15.10$, $P < 0.0001$), whilst foster lay-date x hatch-date improved the fit further (2nd step; $LRS_{1,88} = 7.04$, $P < 0.01$).

The change in incubation regime when clutches were swapped may have affected the hatching weight of chicks because advanced clutches were incubated for longer than delayed clutches, although the difference was not significant (Mann-Whitney, $Z = 1.41$, $n = 22$, n.s.). However, a greater number of eggs failed to hatch in the delayed broods (8 out of 33 cf. 1/33 in control and 0/33 in advanced broods) although this was not related to either laying order (Mann-Whitney for clutches where eggs failed to hatch, $Z = 0.58$, $n = 18$, n.s.) or egg-size (unhatched mean egg-size = 70.49 cm^3, s.e. ± 2.96, n = 8; hatched mean = 69.64 cm^3, s.e. ± 0.82, n = 25).

The regression analyses illustrate that mass at hatching and hatching date were the most important variables affecting chick survival probability but that parental quality was also important. In order to illustrate the relative effects of hatching weight, hatching date and parental quality upon chick survival it was necessary to calculate survival probabilities using the following expression (Norusis 1993):

$$P = 1 / 1 + e^{-z}$$

where z = constant - (foster lay-date x hatch weight) + (foster lay-date x hatch-date) and P = the probability of surviving to fledging age

Figure 4.2 - *Variation in probability of survival of a 55 gram chick with hatch date (1 = 1 January) and treatment group (parental quality)*

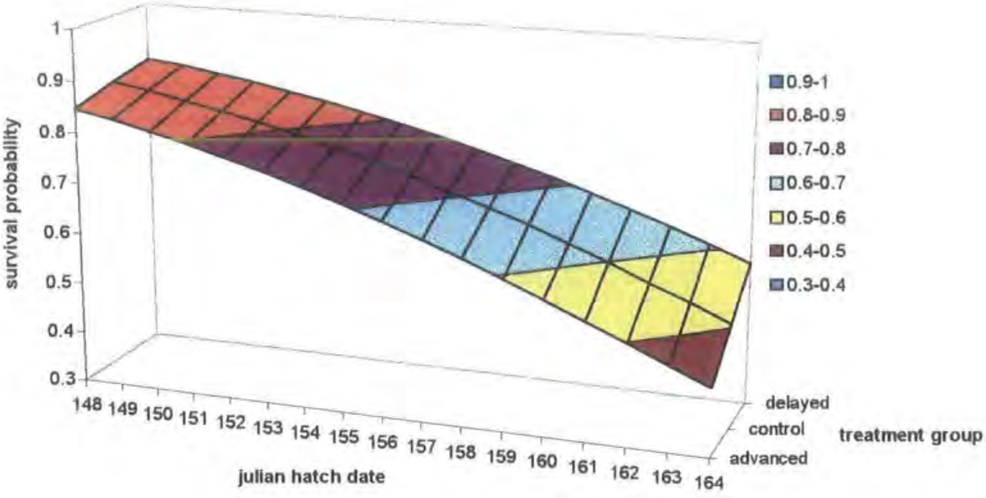
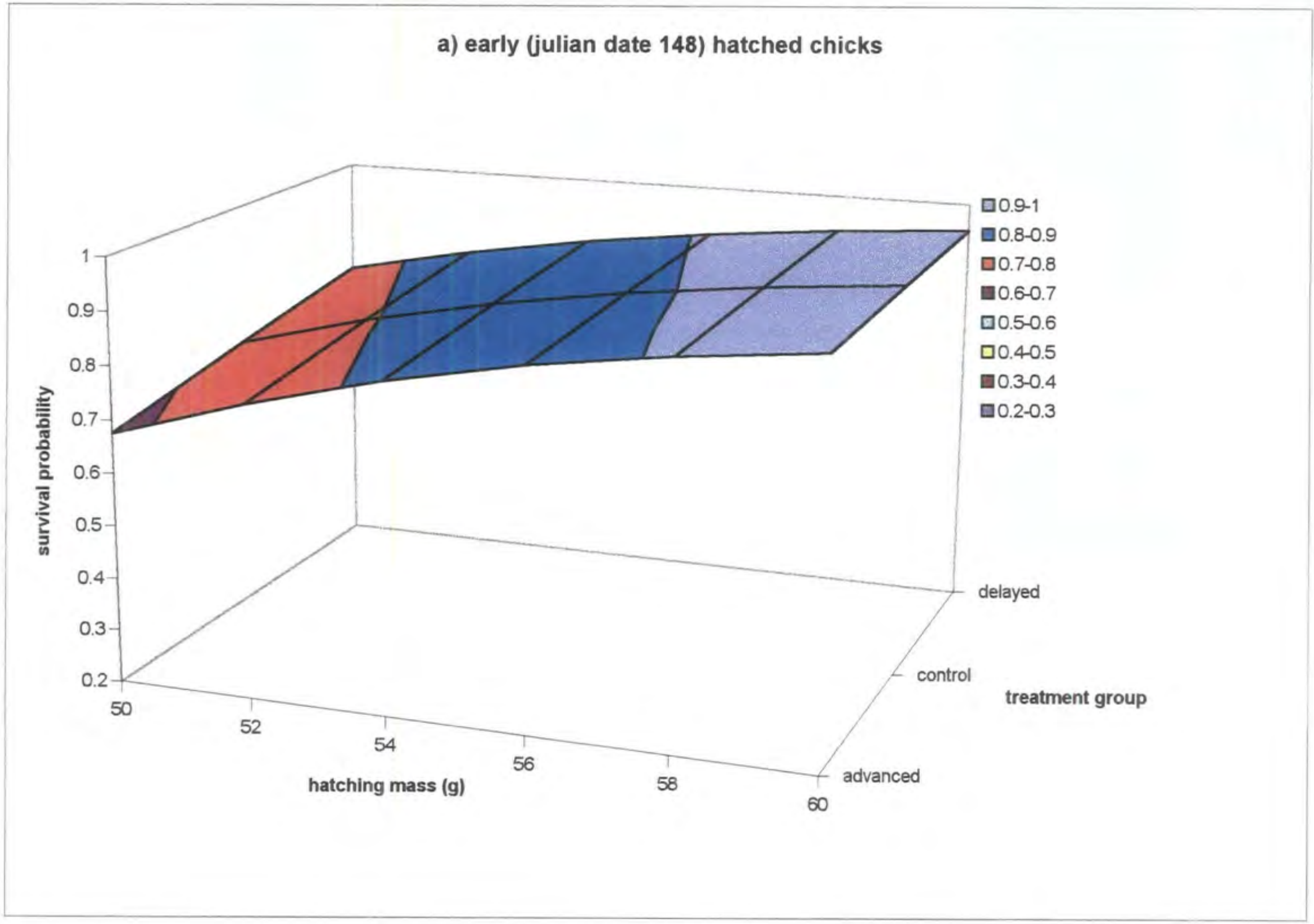


Figure 4.2 illustrates the probability of survival of a chick of average hatch weight (55g) with a 27 day incubation, for a range of hatch dates, under the three different treatments (eg. for a chick hatching on day 156, delayed group parents have an original lay-date of day 120 and the chicks survival probability is 0.78, whilst advanced and control parents have lay-dates of 138 and 129 and chick survival probabilities of 0.68 and 0.73 respectively). The curves clearly show that for any particular hatch date, whilst statistically controlling for chick hatch weight and incubation period, survival probability varies with foster lay-date (treatment group), and thus parent quality. Parental quality has a greater impact on survival probability at later hatch dates than when chicks hatch early in the season.

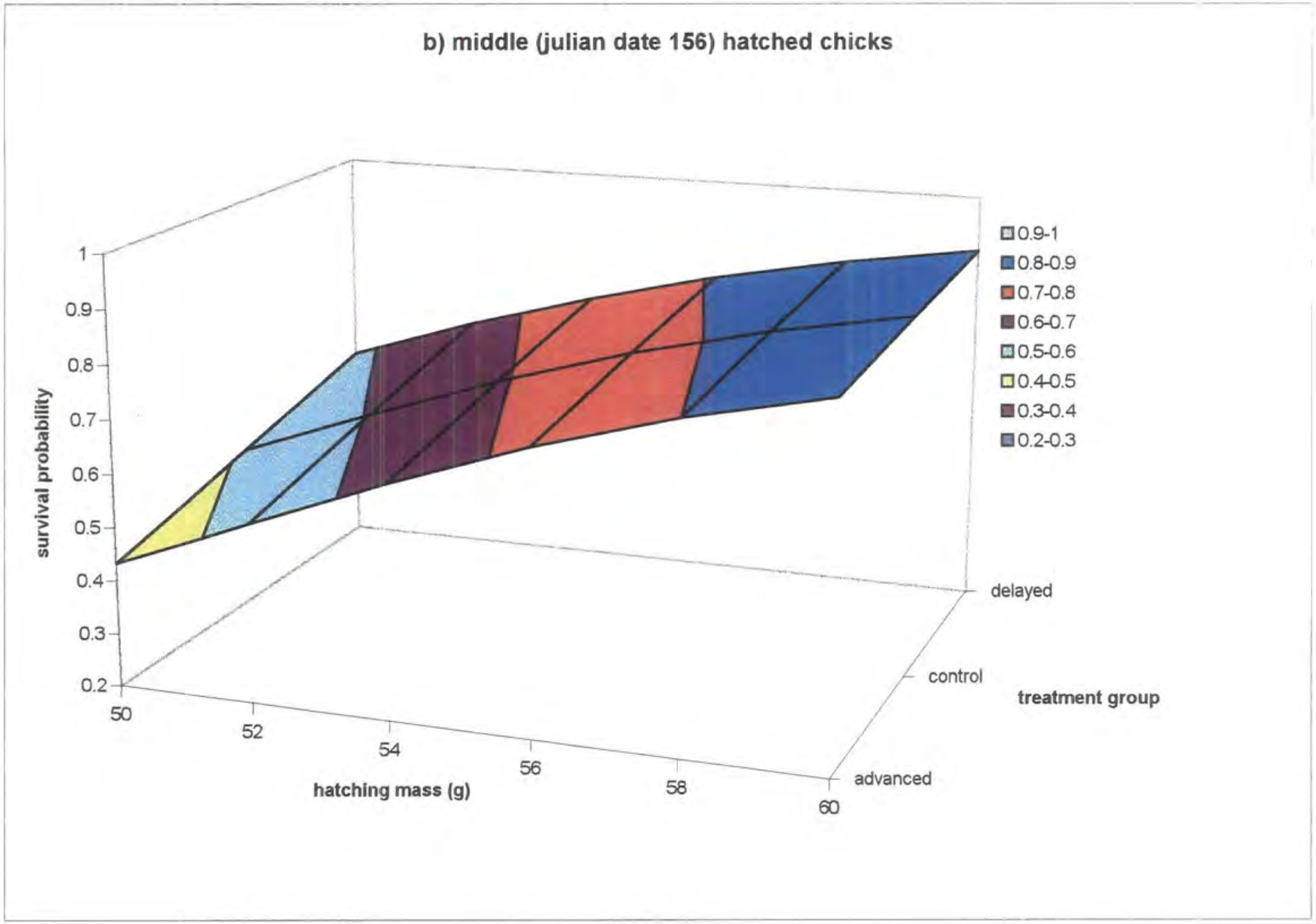
This can also be seen in Figure 4.3, which shows how survival probability varies for chicks hatching a) early, b) during the middle of the season, and c) late in the season with hatch weight and treatment (parental quality). For early hatched (julian date 148) large chicks (60g) parental quality (foster lay-date) has very little effect upon survival probability, but parental quality becomes more important for

Figure 4.3 - *Variation in survival probability with hatching mass and treatment*



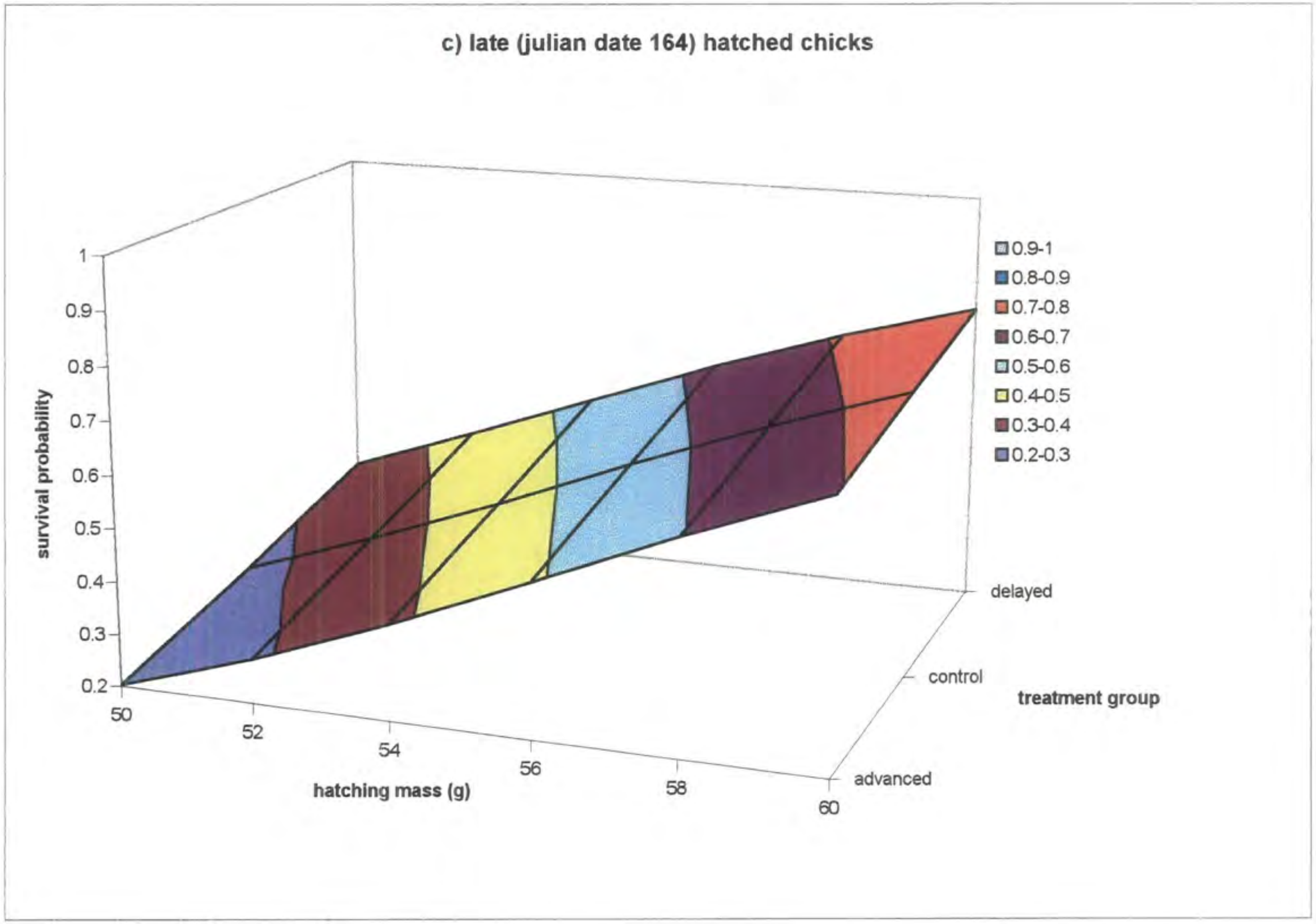
a) early (julian date 148) hatched chicks

Figure 4.3 - *Variation in survival probability with hatching mass and treatment*



b) middle (julian date 156) hatched chicks

Figure 4.3 - *Variation in survival probability with hatching mass and treatment*

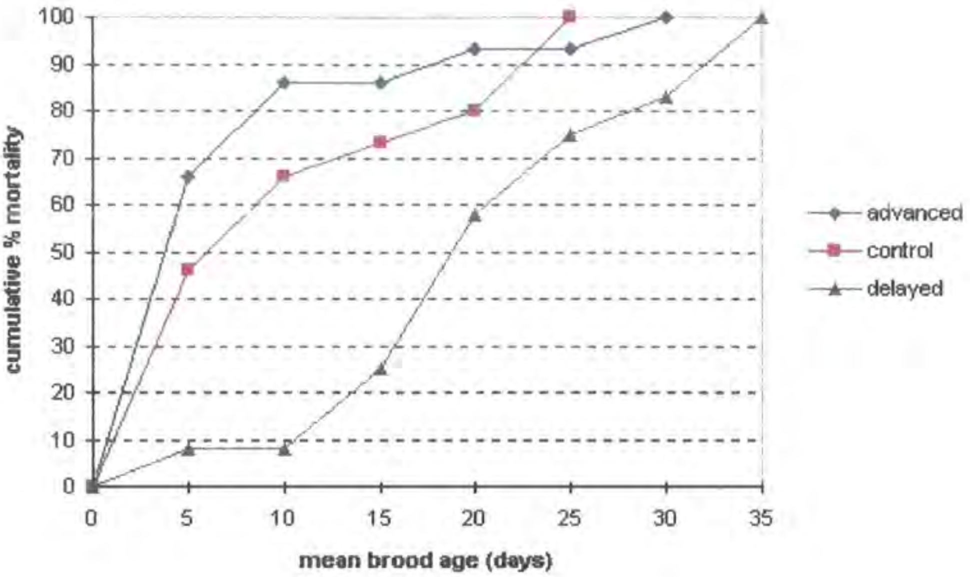


c) late (julian date 164) hatched chicks small early hatched chicks (50g) even though survival probability does not fall greatly compared to large chicks. For middle (julian date 156) and late (164) hatched chicks both hatch weight and parental quality have increasingly greater effects upon survival probabilities than for early hatched chicks.

4.4.2.3. Timing of chick mortality

Mortality of chicks that hatched in delayed broods was significantly later than in either advanced or control broods (Kruskal-Wallis $\chi^2 = 13.15$, $n = 42$, $P < 0.001$), with a median age of death over three times later than for the other two treatment groups (Figure 4.4).

Figure 4.4 - *Chick mortality curves for the three treatment groups*



4.4.2.4. Chick growth

Growth characteristics of surviving chicks showed some significant differences among treatment groups (Table 4.2). Tarsus growth rates were similar for the three treatments (slope $F_{2,305} = 0.18$, n.s.), and the difference in elevation among the three treatment groups was not quite significant ($F_{2,305} = 2.53$, $P = 0.08$). Weight growth rate was significantly different among the three treatment groups (slope $F_{2,305} = 6.86$, $P < 0.001$); delayed broods had lower growth rates than advanced broods (parameter estimate, $P < 0.001$). Control broods had similar weight growth characteristics to those of advanced broods (parameter estimates for both slope and elevation; n.s. Table 4.2).

Table 4.2 - *Growth rates of chicks surviving to fledging age for each treatment group*

	treatment		
	delayed (n = 13)	advanced (n = 18)	control (n = 18)
tarsus length (mm)			
at 10 days	34.48	32.21	32.84
at 20 days	45.58	43.41	44.34
growth rate/day (± 1 s.e.)	1.11 ± 0.04	1.12 ± 0.05	1.15 ± 0.05
mass (g)			
at 10 days	237.62	223.68	225.68
at 20 days	420.92	459.98	448.72
growth rate/day (± 1 s.e.)	18.33 ± 1.11	23.63 ± 0.99	22.32 ± 0.96
asymptotic mass (g) (± 1 s.e.)	489.23 ± 31.71	625.56 ± 29.74	582.78 ± 30.80

As a consequence of these differences, chicks in delayed broods were in poorer condition than advanced or control brood chicks by midway through the nestling period (condition at 12 days of age, Kruskal-Wallis $\chi^2 = 8.33$, n = 64, $P < 0.05$), and overall mean brood condition was also significantly lower (Kruskal-Wallis $\chi^2 = 7.02$, n = 27, $P < 0.05$). Delayed pairs also raised chicks with significantly lower asymptotic weights than either of the other two treatments (Table 4.2; one-way ANOVA $F_{2,46} = 4.76$, $P < 0.01$; tukey range test).

4.5. DISCUSSION

The results of this experiment indicate that both food availability and parental quality significantly affect the seasonal variation in fledging success in the lesser black-backed gull. Delayed pairs had reduced breeding success whilst breeding success of advanced pairs was enhanced (suggesting food supply was declining). Hatching mass was the single most important variable affecting the survival probability of chicks, but this also varied with hatching date. There were no significant differences among treatments in either the proportion of fledged chicks per egg laid or the proportion of fledged chicks per egg hatched. However, as Figures 4.2 & 4.3 clearly show for any given hatching date and/or hatch weight, survival probability of chicks increased as a function of parental quality (foster lay-date). The effect of parental quality on chick survival prospects was greatest when chicks hatched late and/or hatched at low weights. These results are in accordance with those of Brouwer *et al.* (1995), who performed a similar manipulation of timing of breeding (but without consideration of chick growth rates) in herring gulls *Larus argentatus*.

In the current study chicks raised by delayed pairs showed significantly lower rates of mass gain throughout development and were consequently in poorer condition than chicks in the other two treatment groups. Chicks in delayed broods subsequently either fledged at lower weights (and in poorer condition), and/or they required a longer fledging period than chicks in advanced or control broods. Both of these possibilities are likely to have had adverse consequences on post-fledging survival and recruitment into the breeding population. In gulls hatching date is the most important factor affecting survival and recruitment (Nisbet & Drury 1972; Parsons *et al.* 1976; Spear & Nur 1994). This may be due to a direct effect (eg. early fledging young may gain valuable foraging experience and dominance over later fledged young; Spear & Nur 1994) and/or an indirect effect (parental quality). Brood size also affects survival and recruitment probability (Nisbet & Drury 1972; Spear & Nur 1994) with chicks from larger broods being more likely to recruit, although this relationship may change with hatch date (Parsons *et al.* 1976), suggesting that parental quality is an important component of the relationship between hatch date and post-fledging survival (Spear & Nur 1994). Consequently, although fledging success was similar among treatment groups in this study, for chicks in delayed broods post-fledging survival was likely to have been reduced relative to the other treatment groups (particularly if hatch date *per se* had a greater effect upon post-fledging survival than parental quality). This may have offset any benefits gained through the indirect effects of higher parental quality (eg. better parental care) compensating for the less favourable environmental conditions apparently encountered by delayed pairs (Brouwer *et al.* 1995).

Chicks fostered by delayed pairs were significantly smaller (tarsus length) than those hatching in advanced and control broods in the current study, despite there being no difference in egg-size among treatments. This raises the possibility that the eggs laid by advanced pairs (and subsequently fostered by delayed birds) were either of poorer quality (eg. different yolk lipid composition or amino acid composition; *Chapter 3*, Murphy 1994) and/or that the change in incubation regime, as a result of the exchange of clutches between pairs at different stages of incubation, affected embryo development. The latter suggestion is more plausible because delayed clutches had a disproportionately large number of eggs that failed to hatch. Attentiveness of parents (in terms of the percentage time that eggs are covered) increases steadily until 10 - 12 days after incubation initiation from *ca.* 65% up to *ca.* 98%, at which point the curve reaches a plateau (Drent 1970). Clutches from treatment 1 (advanced) birds were swapped at the point of clutch completion (c-egg laid) with clutches laid by treatment 2 pairs (laid approximately 9 days earlier), so that eggs fostered by delayed pairs may have been incubated with greater levels of attentiveness than would have been normal for a newly completed clutch. This may have affected embryo temperature (and therefore viability of that embryo; Drent 1970) and/or rates of evaporative water loss, which is known to influence chick size at hatching (Ar 1991).

Another possible effect of the experimental manipulation is through increased (delayed treatment) or reduced (advanced treatment) costs of incubation to parents. If incubation entailed significant energetic costs to breeding pairs then it might be expected that any manipulation that artificially increased or decreased the length of this phase of reproduction may have affected reproductive success. In gulls incubation is not thought to be energetically costly (Norstrom *et al.* 1986), indeed birds may increase weight and replenish energy reserves during this time (Hario *et al.* 1991). However, recent experimental studies involving manipulation of incubation effort through addition of an extra egg in common terns *Sterna hirundo* (Heaney & Monaghan 1996) and by extension of the incubation period in barnacle geese *Branta leucopsis* (Tombre & Erikstad 1996) found that artificially increased incubation demands reduced chick growth rates and female body condition respectively. It is therefore possible that the reduced growth rates of the delayed group chicks may be explained by increased costs of prolonged incubation incurred by adults in this group. If this was the case then the observed patterns of fledging success could be due to the effect of increased incubation costs on the trade-off between the incubation and chick rearing phases of reproduction, rather than a causal relationship with timing of breeding. There are two reasons why this is unlikely to be the case. Firstly, in contrast to barnacle geese, gulls are biparental incubators (Cramp & Simmons 1983) and both sexes have an equitable share of incubation duties (Drent 1970; Burger 1980), which would leave each parent plenty of time to forage (Brinkhof *et al.* 1993) and would explain why both sexes tend to replenish energy reserves during incubation rather than loose condition (Hario *et al.* 1991). Secondly, as Brouwer *et al.* (1995) argue, if the duration of incubation had altered the nutritional condition of pairs subjected to experimental manipulation then it would be expected that advanced pairs should have had greater fledging success and delayed pairs lower fledging success than control birds. In this study, as for Brouwer *et al.* (1995), this was not the case, because there were no differences in fledging success among treatments.

There were, however, significant differences among treatment groups in the patterns and condition of chicks during growth. Chicks in the delayed group had lower growth rates which may have been a result of a seasonal decrease in food availability (Brinkhof & Cavé 1997). The later timing of mortality in delayed broods was probably related to the smaller initial brood size (due to a lower proportion of eggs hatching in this group compared to advanced and control clutches) and presumed lower levels of competition among siblings.

In gulls, earlier breeding individuals tend to be older (Davis 1975; Sydeman *et al.* 1991) and more experienced (Pyle *et al.* 1991) than later breeding birds and may be more efficient at foraging (Greig *et al.* 1983; Marchetti & Price 1989). However, timing of breeding also influenced reproductive performance independently of parental age in the western gull *Larus occidentalis* (Sydeman *et al.* 1991). This supports the findings of the current experiment, that aspects of both parental quality and some seasonally varying environmental factor influence breeding success. Brouwer *et al.* (1995) suggested that this environmental factor might be increased levels of conspecific predation as the season progressed due to a shortage of food. Synchronization of breeding, with an emphasis on early breeding, may be important as a defence against such predators (Svensson 1995). However, at the Tarnbrook Fell gullery nests were enclosed with a chicken-wire surround for experiments, and all but a few bodies of chicks that died were recovered. The indications were that most chicks had died because their body condition had reached a critically low point. Thus, increased conspecific predation can be discounted as a likely reason for the seasonal decline in fledging success at the Tarnbrook Fell colony. The results of this experiment suggest that there are distinct advantages to breeding early. On the other hand, if timing of breeding was individually optimized, advanced pairs should have had poorer, not better, fledging success after manipulation, and this suggests that there are also constraints on early breeding.

Schultz (1991) suggested that the earliest breeding birds are those with the greatest somatic energy reserves due to superior foraging skills or competitiveness. If, in general, birds in temperate climates breed as early as possible, but the timing of breeding is constrained by having sufficient energy to reproduce, then poorer quality individuals' energy requirements will only be met later in the breeding season as food availability increases. Schultz (1991) suggested that the models of Fisher (1930) and Price *et al.* (1988), which assume a linear relationship between nutritional state and timing of breeding may not be accurate. Instead he proposed that birds have a 'saturation' level of somatic reserves. Below this point breeding is delayed, but above this limit timing of breeding is not related to energy reserves but to some other proximate cue such as photoperiod. Empirical evidence suggests that this model is valid, but that the relationship between timing of breeding and energy reserves may not always be evident if, for example, food availability is particularly high (Schultz 1991). Although food supply may therefore be the most important factor limiting early breeding it is not necessarily the ultimate factor that determines timing of reproduction. For single-brooded species such as gulls the decision of when to start breeding should be based on reliable cues to the level of food availability, and birds with high costs and low benefits associated with breeding too early should wait until conditions are optimal for breeding (Svensson 1995). If early hatched, and fledged, young exhibit greater post-fledging survival and recruitment (Nisbet & Drury 1972; Spear & Nur 1994) independent of the effects of parental quality, then earliness relative to other breeding conspecifics rather than absolute earliness will be adaptive (Svensson 1995). Reproductive value (RV) of offspring would then decrease during the course of the breeding season (Nilsson 1994). Parents in the advanced treatment group could take advantage of the higher RV of their offspring by investing more effort in raising the brood. However, because these pairs originally started breeding later in all likelihood because they were not as proficient at foraging as early laying birds (and therefore took longer to reach a threshold of somatic reserves), fledging success was not quite as good as expected from the seasonal trend. This argument works in reverse to explain the fledging success but lower growth rates of delayed treatment broods.

In addition to the factors discussed above, timing of breeding may also be constrained by heredity (Martin 1987). This is a consequence of the expectation that character traits that have fitness benefits are heritable (Hakkarainen *et al.* 1996). However, although several studies have revealed moderate heritability estimates for reproductive traits such as laydate (Price *et al.* 1988) this may be largely a result of a common environment shared between parents and offspring where natal dispersal is restricted (Hakkarainen *et al.* 1996). In Tengmalm's owls *Aegolius funereus* natal dispersal is wide-ranging and food supply is highly stochastic. Heritability estimates for laydate and other reproductive and morphological parameters were low and this was mostly attributed to non-additive genetic variance (Hakkarainen *et al.* 1996). This could promote adaptability to a stochastic environment through the production of a wide variety of different phenotypes so that there should always be some individual offspring suited to the current environmental conditions (Hakkarainen *et al.* 1996). Phenotypic plasticity of reproductive traits such as timing of breeding is thus likely to be highly beneficial when environmental conditions vary between years and so can be considered to be a trait under natural selection (Hakkarainen *et al.* 1996).

If this is generally so then it might be expected that if individuals vary in their foraging ability (eg. Greig *et al.* 1983), although food supply may be an important constraint on very early breeding (Nilsson 1994) the ultimate determination of the timing of breeding may be limited by the most energy demanding phase of reproduction, which is chick rearing (Nilsson 1994; Nager *et al.* 1997). Food supplementation experiments indicate that net energy balance does not limit the timing of breeding (eg. Bolton *et al.* 1992; Nager *et al.* 1997) and this suggests that there is good support for a threshold function of body reserves (Schultz 1991) whereby food supply acts as a proximate cue to resource availability later in the season, rather than a restriction on egg formation (Nager *et al.* 1997). Experiments on blue tits *Parus caeruleus* by Svensson & Nilsson (1995) showed that pairs in poor quality territories responded to supplemental food by advancing date of laying to a greater extent than pairs in good quality territories, providing support for the threshold hypothesis. In addition, a comparative analysis of the effect of supplemental food on laydate from a range of studies revealed that young birds (which are usually poorer foragers; Marchetti & Price 1989) tended to advance timing of breeding to a greater extent than older birds (Nager *et al.* 1997).

There is thus evidence that parents maximise their fitness by basing their decision of when to initiate breeding, once they have reached a threshold level of somatic reserves, upon the anticipated food supply during the most energy demanding phase of reproduction (Daan *et al.* 1990). The relationship between timing of breeding and reproductive success in this experiment on lesser black-backed gulls is most probably a consequence of these factors. The effect of parental quality varies with timing of breeding because birds that can reach a threshold level of breeding reserves earlier in the season are either better foragers or are more efficient at coordinating breeding activities. They are therefore more likely to be able to provide higher levels of parental care than later breeding birds, and so have higher reproductive success. Moreover, if there is a causal relationship between hatching (and fledging) date and probability of recruitment, then the benefits of early breeding and the differences in parental quality between early and later breeding birds are compounded due to a decrease with date in the reproductive value of those offspring that do fledge (Daan *et al.* 1990).

CHAPTER 5

*Hatching asynchrony and sibling size hierarchies in gulls: effects on parental investment decisions, brood reduction and reproductive success**

* submitted as a paper to the Journal of Avian Biology, coauthored by K. C. Hamer, and reproduced here, apart from stylistic alterations, in the form in which it was submitted

5.1. SUMMARY

Hatching asynchrony is an almost ubiquitous phenomenon amongst birds which feed their young during the nestling period. Whilst there are many theories that attempt to explain hatching asynchrony few theories are unequivocally supported by empirical data. This can be attributed at least in part to the complex nature of hatching asynchrony, making it difficult to distinguish the effects of hatching patterns on reproductive success, from those of other life history traits such as clutch size, egg size and timing of laying. For this reason, in order to investigate the adaptive significance of hatching asynchrony it is necessary to control experimentally for as many potentially confounding variables as possible, although few studies have done so. We manipulated the within-brood mass hierarchy of lesser black-backed gulls *Larus fuscus* and controlled for chick quality by swapping last hatched (c-) chicks in experimental broods with a- or b- chicks from residual broods to create either synchronous broods (all 3 chicks in the brood approximately the same mass) or asynchronous broods (same within-brood difference in mass between largest and smallest chicks as that of unmanipulated broods), whilst also controlling for variation in characteristics of parental quality (clutch size, egg size and timing of laying). This was done in order to assess *i*) the effect of hatching asynchrony *per se* on chick growth and survival, and *ii*) whether the degree of hatching asynchrony was optimized with respect to the prevailing food supply. Subsequent chick growth and survival for each brood were monitored in comparison with naturally asynchronous, unmanipulated broods. There were no differences among treatments in fledging success

or growth characteristics of surviving chicks. However, chick mortality occurred significantly later in synchronous broods and this was a consequence of the smaller within-brood hierarchical differences in size and body condition of these chicks. Parents could not apparently regulate resource allocation in synchronous broods, and invested a greater amount of parental investment in chicks that later died. These results are discussed within the context of life history strategies.

5.2. INTRODUCTION

Birds are unusual among animals in that the embryo develops externally to the mother's body and development is generally initiated by transfer of heat from the parent. Hatching intervals of offspring can therefore be influenced by the parent through the timing of initiation of incubation (Stoleson & Beissinger 1995). Avian eggs are usually laid at the rate of one per day or two days, and if incubation is delayed until the final egg in the clutch has been laid, then hatching occurs synchronously. Conversely, initiation of incubation before the last egg has been laid results in asynchronous hatching. This often results in the establishment of a size hierarchy among nestlings at brood completion (Bryant 1978) which may contribute directly to the premature death of smaller, later-hatched chicks (Magrath 1989). This apparent waste of parental effort was first explained in adaptive terms by Lack (1947), who suggested that bird species with an unpredictable food supply at the time of initiation of clutch formation should lay an optimistic clutch size. Then, if food supply is poor during the nestling period, brood size can be reduced accordingly, with this brood reduction facilitated by asynchronous hatching. Implicit in this hypothesis is the assumption that hatching asynchrony leads to the establishment of a hierarchy of chick sizes, and that chicks highest in the hierarchy gain the greatest share of available resources. The brood reduction hypothesis has subsequently been modified to suggest that hatching asynchrony facilitates efficient brood reduction through swift elimination of the chick(s) that will require the greatest future parental investment (Temme & Charnov 1987).

The brood reduction hypothesis has two main predictions: that food supply at the time of laying should be a poor predictor of food availability during the nestling period, and that when food supply during chick rearing is poor, asynchronously hatched broods should produce more fledglings, fledglings of higher quality, or both, than synchronous broods. Whilst some studies have found partial support for the hypothesis (Husby 1986; Gibbons 1987; Magrath 1989), most studies have produced inconclusive results in the field (Mock & Forbes 1994). This is possibly a consequence of the difficulty in establishing the underlying causes and subsequent effects of such a complex phenomenon as hatching asynchrony, within the restrictions of working in the field (Stoleson & Beissinger 1995). For this reason it is necessary to control experimentally for as many potential covariates as possible, *a priori*.

A more recent hypothesis, the individual-optimization hypothesis (Harper *et al.* 1994; Wiebe & Bortolotti 1994), focuses on variation in the degree of hatching asynchrony and suggests that there are potential costs associated with having an 'inappropriate' degree of hatching asynchrony in relation to food supply. Parents therefore adjust the degree of hatching asynchrony to current environmental conditions in order to maximise breeding success. This hypothesis predicts that broods manipulated to have a greater or lesser degree of asynchrony compared to unmanipulated broods should have poorer reproductive success.

In addition to hatching asynchrony, most multiparous birds show variation in egg-size (and chick quality) within a brood, and this is particularly marked in those species of gulls (Laridae) with a modal clutch size of three eggs (Meathrel & Ryder 1987; Reid 1987; Sydeman & Emslie 1992; Kilpi 1995). In these species the last-laid egg (termed the c-egg) is typically *ca.* 10% smaller than the first and second-laid eggs (termed a- and b-eggs respectively), and produces a smaller chick which has a lower probability of survival to fledging (Parsons 1970, 1976).

In lesser black-backed gulls *Larus fuscus* a- and b-eggs are similar in size and usually hatch within a few hours of each other, whilst c- eggs are, on average 10% smaller than first laid eggs (Cramp & Simmons 1983; *Chapter 2*). The smaller c-egg also hatches considerably later (usually *ca.* 7-15 hours after the b-egg), so that the greatest difference in the size hierarchy occurs between the last-hatched chick (the c-chick) and the chick immediately preceeding it (the b-chick). In addition, c- chicks have a body mass on average *ca.* 12% smaller at hatching than that of a- chicks, but skeletal size (tarsus length) is only *ca.* 4% less (*Chapter 2*). Thus, chicks hatching from last laid eggs have a different body composition ('quality') to that of a- and b- chicks. It is therefore important to control for possible confounding effects of variation in the size and laying order of eggs from which chicks were hatched, in order to assess the effects of hatching asynchrony and sibling size hierarchies *per se* on chick growth and survival (Magrath 1990; Stoleson & Beissinger 1995). Recent work on within-clutch variation in testosterone concentrations of eggs for canaries *Serinus canaria* (Schwabl 1993, 1996) and siblicidal cattle egrets *Bubulcus ibis* (Schwabl *et al.* 1997) highlighted the fact that size may not be the only difference in terms of within-clutch allocation of parental resources, further emphasising the need to control for laying order of eggs in experimental studies of hatching asynchrony.

In addition to variation in chick quality, our experimental design also controlled for potential differences in adult quality through the control of clutch size, total clutch volume (egg-size) and timing of laying. In gull species, all three of these variables are interrelated and reflect individual quality (Sydeman *et al.* 1991). Clutch size is correlated with female nutritional status (Houston *et al.* 1983; Meathrel 1991; Bolton *et al.* 1993) whilst the ability to produce large eggs relates both to courtship feeding rates (Salzer & Larkin 1990) and the ability of parents to provision chicks (Bolton 1991). Early breeding individuals have higher reproductive success and lay both larger and a greater number of eggs than later breeding birds. These individuals tend to be older birds (Davis 1975; Pyle *et al.* 1991; Sydeman *et al.* 1991). Holding these variables constant, and thereby minimizing unexplained variance, effectively controls for both seasonal variation in food availability and parental quality (the ability of individuals to exploit that food supply). This paper examines the adaptive significance of hatching asynchrony in lesser black-backed gulls *Larus fuscus*, whilst controlling for potential differences in both chick and adult quality, through manipulation of the within-brood size hierarchy.

5.3. METHODS

The study was conducted at a colony of *ca.* 9,000 lesser black-backed gulls at Tarnbrook Fell, North Lancashire (grid ref. 614595). A total of 120 nests was located during nest-building in April 1995, and marked with a short length of bamboo cane placed near the nest. Every nest was then checked once daily until clutch completion. All eggs were measured (length and breadth ± 0.1mm using dial calipers) on the day they were laid, and marked with indelible ink according to nest number and laying sequence. Egg volumes (V in cm^3) were calculated from the following equation, after Bolton (1991):

$$V = (4.76 \times 10^{-3}) \times (\text{length (mm)}) \times (\text{breadth (mm)})^2$$

5.3.1. *Experimental manipulation*

From the 120 nests marked before egg-laying, we selected 39 nests with three-egg clutches laid within ± 3 days of the modal laying date and with similar total clutch volumes (within 5% of the overall mean). Towards the end of incubation, each of these nests was surrounded with a chicken-wire enclosure approximately 2 m^2 in area and 50 cm high, to assist with finding chicks after hatching. A wooden shelter was also provided for the chicks in each enclosure. Adults incubated clutches and fed their chicks normally within these enclosures, as was found in previous studies (e.g. Bolton 1991).

Before hatching, each of these 39 nests was assigned at random to one of three groups, with 13 nests in each group:

(1) Asynchronous with the youngest chick hatching from a small egg (unmanipulated controls)

(2) Asynchronous with all three chicks hatching from eggs of similar size

(3) Synchronous with all three chicks hatching from eggs of similar size.

In groups 2 and 3, the chick from the c-egg (termed the c-chick) was replaced with a chick from an a-egg or a b-egg from a residual brood. In group 2, the foster chick was approximately the same size as the c-chick that it replaced; in group 3, the foster chick was the same size as the two other chicks in the brood that it joined. As a result of this, the body mass ratio within each brood (body mass of the smallest chick divided by body mass of the largest) immediately following manipulation was significantly higher in group 3 (0.91, s.e. ± 0.01), than in group 2 (0.73, s.e. ± 0.02) or group 1 (0.79, s.e. ± 0.02; arcsine transformed data followed by Tukey's range test; one-way ANOVA; $F_{2,36} = 27.6$, $P < 0.001$). There was no significant difference among the three groups in hatching date (Kruskal-Wallis one-way ANOVA; $\chi^2_2 = 0.29$, n.s.) or in premanipulation clutch volume (one-way ANOVA; $F_{2,35} = 0.97$, n.s.).

All chicks from these nests were individually marked and were weighed (± 1g using a Pesola balance) and measured (tarsus length ± 0.1mm using dial calipers) at 3 - 6 day intervals from hatching until 36 days post-hatching. Chicks not seen on consecutive visits were presumed to have died, and chicks reaching 36 days post-hatching, when they were almost capable of flight, were assumed to fledge successfully. Growth rates were compared amongst treatments using analysis of covariance of body mass or tarsus length upon age during the linear phase of growth (3 - 24 days post-hatching; Furness & Monaghan 1987) with treatment as a cofactor. Use of feeding rates, as a measure of parental effort, are not accurate for gulls due to the low numbers of feeds per day (Henderson 1975) and the variability of both the size and composition of the feeds, so chick growth rates were used as an indirect measure of parental effort. This assumes, after experimentally controlling for variation in food supply, parental quality and initial brood size, that there were no differences among treatment groups in the food conversion rate, so that any differences in growth rates would be due to the amount of and/or rate of food delivered (Martin 1987). In addition to growth rate, an index of body condition was calculated for each chick by regessing weight on tarsus length and expressing the residuals as a proportion of the predicted value in each case. The mean of these values was calculated for each chick as an index of condition during development, and this was then used used to compare within-brood differences in body condition among treatment groups.

5.4. RESULTS

5.4.1. *Fledging success and timing of chick mortality*

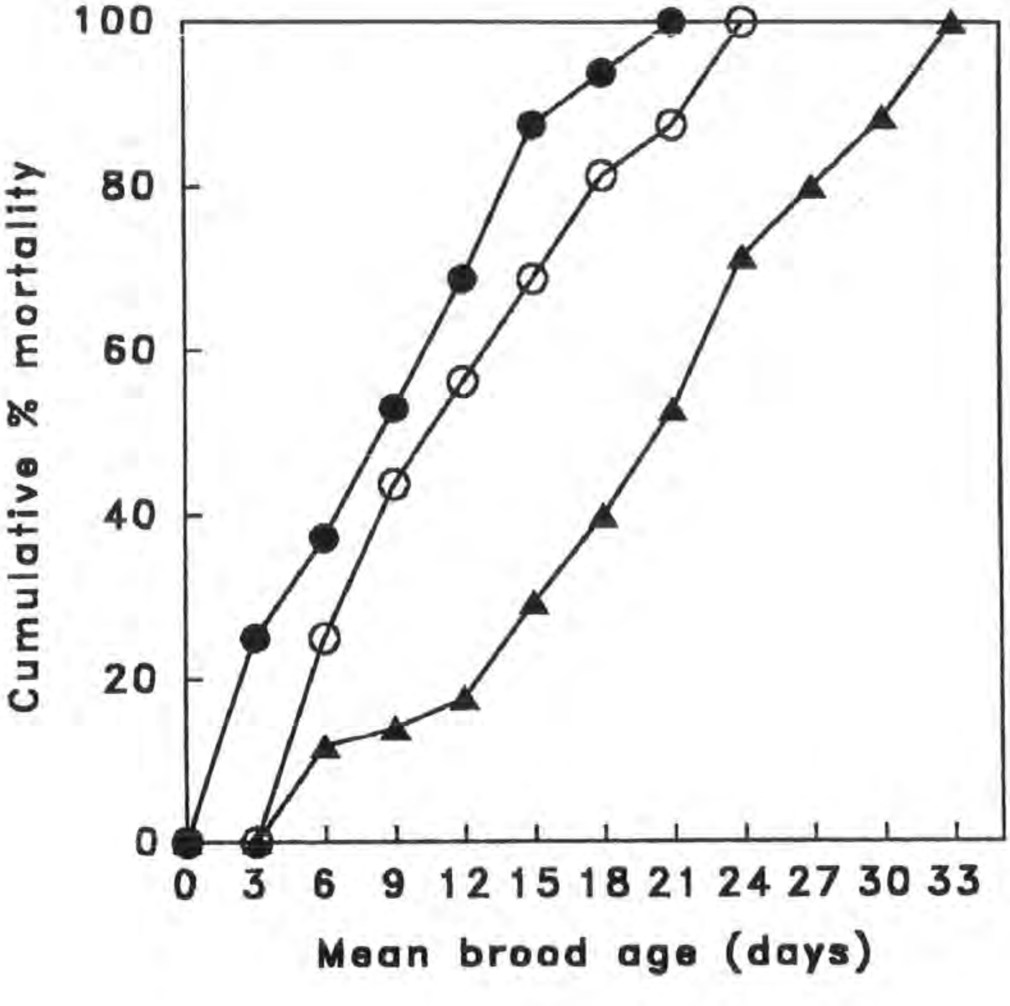
There was no difference among the three treatment groups in either the proportion of chicks per brood that survived to fledging or the total number of chicks fledged per treatment group (Table 5.1; G-test on pooled data (numbers of broods fledging 0 & 1 or 2 & 3 chicks); $G_2 = 0.72$, n.s.).

Table 5.1 - *Number of lesser black-backed gull chicks fledged per brood in each treatment group.*

	number of chicks fledged per brood				
treatment	0	1	2	3	total
1) unmanip.	2	2	6	3	23
2) async.	2	3	4	4	23
3) sync.	1	5	4	3	22

However, the mean age at which chicks died in synchronous broods (mean = 20.4 days, n = 17, s.e. ± 2.1) was considerably later than in the two treatments with asynchronous broods (group 1, mean = 9.3 days, n = 16, s.e. ± 1.9; group 2, mean = 12.3 days, n = 16, s.e. ± 1.8; $F_{2,46} = 6.47$, $P < 0.01$). In groups 1 and 2, mortality was relatively constant until 22 - 25 days post-hatching, by which time 90% of total pre-fledging mortality had occurred (Figure 5.1). By contrast, mortality in synchronous broods was low until 12-15 days post-hatching then increased rapidly, with over half the total mortality occurring between this period and 25 days post-hatching.

Figure 5.1 - *Cumulative percentage mortality over time of 1) unmanipulated asynchronous broods (filled circles); 2) experimental asynchronous broods (open circles); 3) experimental synchronous broods (triangles)*



5.4.2. *Effect of initial position in hierarchy on chick mortality*

For all three treatment groups, in broods where partial brood reduction occurred (ie. broods where 1 or 2 chicks died), chicks that died before fledging were significantly lower in their initial brood size hierarchy than those chicks that survived to fledging (Group 1, $Z = 2.98$, $n = 24$, $P < 0.01$; Group 2, $Z = 2.10$, $n = 21$, $P < 0.05$; Group 3, $Z = 2.31$, $n = 27$, $P < 0.05$; all Mann-Whitney tests) and the pattern of chick mortality within-broods was similar among treatments ($G_4 = 0.48$, n.s; Table 5.2).

Table 5.2 - *Relative mortality of a-, b- and c-chicks in each treatment group.*

treatment	a-chicks (%)	b-chicks (%)	c-chicks (%)	total mortality
1) unmanip.	3 (19%)	4 (25%)	9 (56%)	16 (100%)
2) async.	4 (25%)	4 (25%)	8 (50%)	16 (100%)
3) sync.	4 (24%)	5 (29%)	8 (47%)	17 (100%)

5.4.3. *Chick growth*

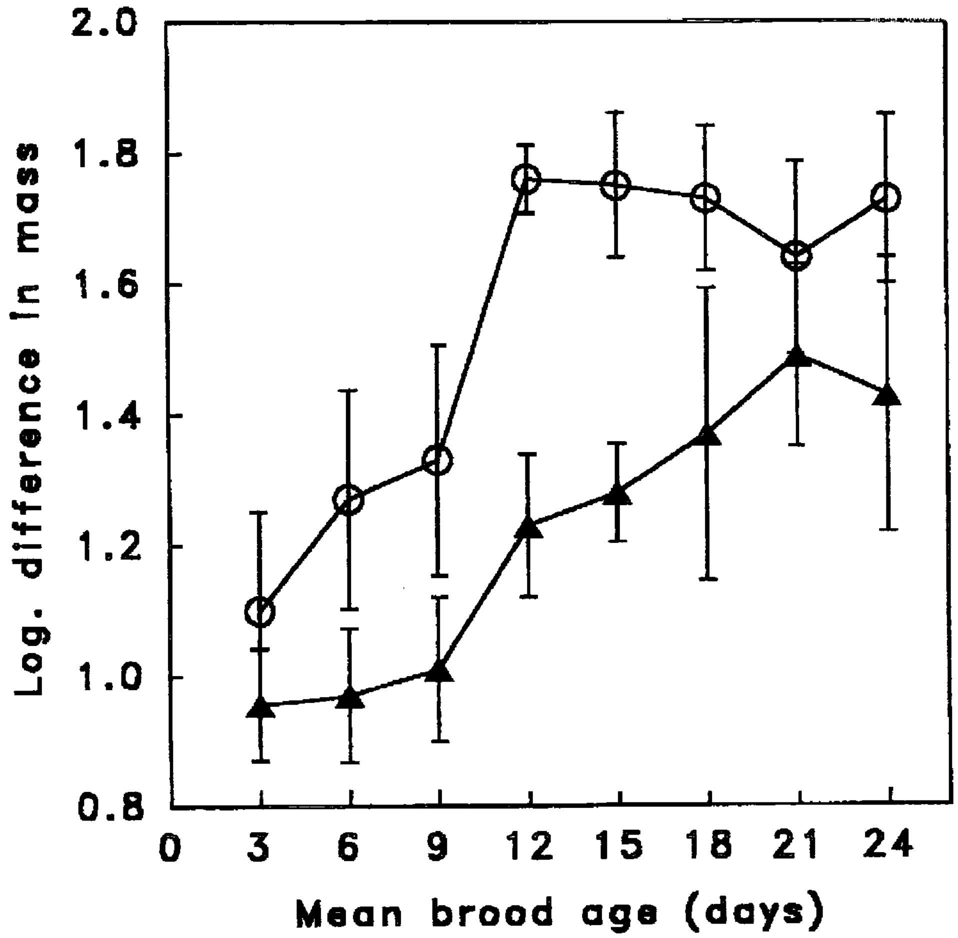
There was no difference among groups in the mean growth rate of surviving chicks in each brood (Table 5.3), either in terms of body mass ($F_{2,466} = 0.20$, n.s.) or in terms of tarsus length ($F_{2,466} = 1.26$, n.s.). In addition, there was no difference amongst groups in the asymptotic weights of surviving chicks (one-way ANOVA, log-transformed data $F_{2,65} = 0.44$, n.s; Table 5.3).

Table 5.3 - *Growth of lesser black-backed gull chicks that survived to fledging age in each treatment group.*

	treatment		
	unmanipulated (n = 23 chicks)	asynchronous (n = 23 chicks)	synchronous (n = 22 chicks)
tarsus length (mm)			
at 10 days	33.61	34.40	33.76
at 20 days	43.42	44.36	43.07
growth rate/day (± 1 s.e.)	0.98 ± 0.04	1.00 ± 0.03	0.93 ± 0.03
mass (g)			
at 10 days	241.19	257.30	247.17
at 20 days	433.19	452.00	435.71
growth rate/day (± 1 s.e.)	19.20 ± 0.77	19.47 ± 0.69	18.85 ± 0.70
asymptotic mass (± 1 s.e.)	577.83 ± 23.37	609.35 ± 25.55	584.77 ± 24.21

However in the two treatments controlling for chick quality (treatments 2 & 3), asynchronous broods exhibited greater within-brood variation in condition of chicks than did synchronous broods (Mann-Whitney, $Z = 2.19$, $n = 26$, $P < 0.05$). Moreover, the difference in mass between the smallest chick and the next smallest in the hierarchy (where the greatest difference in fitness occurs; Parker *et al.* 1989) was consistently greater in asynchronous than in synchronous broods (Figure 5.2, ANCOVA using log-transformed data; elevation $F_{1,156} = 5.22$, $P = 0.02$), although there was no difference in the rate of change in size difference over time (slope $F_{1,156} = 0.01$, n.s.).

Figure 5.2 - *Temporal change in within-brood difference in log_{10} (body mass + 1) between the smallest chick and the next smallest in the hierarchy for a) experimental asynchronous (circles) and b) experimental synchronous (triangles) treatment groups. Expressed as means ± 1 s.e.*



5.5. DISCUSSION

This study provides the first experimental evidence for a species with marked within-clutch variation in egg and chick size that brood reduction is facilitated primarily through hatching asynchrony and not by within-brood variation in chick quality (see also Magrath 1992). There was no difference among groups in either the total number of chicks fledged or the pattern of fledging (ie. proportions of broods fledging 0 & 1 or 2 & 3 chicks), showing that the initial brood mass hierarchy did not affect fledging success of treatments. Nor was there evidence to suggest that post-fledging survival prospects were likely to be different for chicks fledging from the three treatment groups, as there were no differences among treatments in mass or age at fledging and our experimental design controlled for variation in hatching date, which is the most important variable affecting post-fledging survival in gulls (Nisbet & Drury 1972; Spear & Nur 1994). However, chick mortality occurred significantly earlier in asynchronous broods compared to synchronous broods, irrespective of the degree of within-brood variation in chick quality, in terms of weight or size at hatching. This was related to the greater magnitude of within-brood differences in size (and presumably competitive abilities; Godfray & Parker 1991) in asynchronous compared to synchronous broods (Figure 2). There was also greater within-brood variation in growth characteristics and condition among chicks in asynchronous broods. As a result, chicks lowest in the initial hierarchy experienced higher levels of mortality and were usually first to die in those broods where brood reduction occurred. In the synchronous group within-brood differences were much less pronounced, but chicks initially lowest in the size hierarchy nonetheless had equally high levels of mortality as chicks lowest in the hierarchy in asynchronous broods (Table 2). In addition, chicks that survived to fledging in synchronous broods were those chicks that were slightly larger than their nestmates early in development, suggesting that for individual chicks, the early establishment of even slight differences in size among synchronous broods affected the probability of survival to fledging. This confirms the view that although asynchronous hatching is not necessary for the establishment of a social hierarchy, it leads to the immediate establishment of a size hierarchy, which facilitates early brood reduction (Temme & Charnov 1987).

The later timing of chick mortality in synchronous broods imposes a potential cost to parents in terms of wasted parental investment (Gibbons 1987) and the burden of extra energy costs (Wiebe & Bortolotti 1994) associated with feeding chicks for longer before they died. This may be costly with respect to the trade-off between current and future reproductive success (Mock & Forbes 1994). Parents whose broods were manipulated to hatch more synchronously than 'expected' could not apparently adjust to this increased degree of brood synchrony (Mock & Ploger 1987), as there was no differences among treatments in growth rates of surviving chicks, but within-brood differences in condition were significantly lower, and chicks that died did so later, in synchronous broods. This strongly suggests that the original (pre-manipulation) degree of hatching asynchrony was 'individually-optimized' (Harper *et al.* 1994) to the prevailing resource availability. The reason why parents could not readjust their behaviour to the manipulated degree of synchrony, and avoid the potential extra investment costs, is related to the way in which parents feed their chicks.

Gull chicks are fed a short distance from the nest during the first few days after hatching (Graves *et al.* 1984) and first hatched, larger chicks are more mobile and behaviourally better developed, and therefore receive more food. Subsequently, larger pieces of partially digested food are regurgitated and chicks indulge in scramble competition for resources, resulting in the larger, more aggressive chicks taking most of the food (Henderson 1975). Where size (and therefore competitive) hierarchies are pronounced (asynchronous broods) and resources are relatively scarce, fitness differences between chicks are most exaggerated. The greatest difference in fitness occurs between the last two members of the hierarchy as a consequence of the reduced access to resources of the smallest chick in scramble competition (Parker *et al.* 1989). Hence if resources are limiting, the effect of the hierarchy is to compound the initial competitive differences between chicks until brood reduction occurs. If the initial hierarchy is reduced (synchrony), chicks are on more equal terms in scramble competition for resources and consequently brood reduction is delayed. By allowing offspring to control resource allocation once the degree of hatching asynchrony has been set, parents may ensure both that the maximum number of high quality chicks survive (Godfray 1995) and the chicks that die are eliminated as early as possible.

In general, synchrony is likely to be favoured only when food supply is superabundant over a sustained period (eg. Pierotti & Bellrose 1986), and the potential benefits of synchrony (eg. earlier fledging, which may result in dominance over later fledged young, Spear & Nur 1994), are not countered by negative fitness costs of wasted parental investment as described above. Long-lived birds such as gulls may pay an especially steep fitness penalty for hatching chicks synchronously in years of poor food supply (Mock & Forbes 1994), so that a conservative, bet-hedging strategy of hatching clutches asynchronously is better in the long run in a stochastic environment

(Pijanowski 1992). However, this means that in years of good food supply there may be a cost associated with hatching asynchrony (unwanted brood reduction), which may explain why several studies (reviewed by Magrath 1990, Stoleson & Beissinger 1995 and Stenning 1996), having found experimentally synchronous broods to fledge more young, have concluded that hatching asynchrony is maladaptive.

CHAPTER 6

Brood size and the production of supernumary young

6.1. SUMMARY

Brood size was experimentally reduced in order to assess the potential costs and benefits of producing supernumary young (overproduction) in the lesser black-backed gull. Two different 'brood reduction' groups were created. In the first group the last-hatched 'marginal' chick was removed (R_c). There was then no difference in size between the remaining two chicks. In the second group the first hatched chick was removed in order to assess whether the remaining 'marginal' chick could fullfil an insurance function, enabling it to grow and survive sufficiently well to replace a 'core' brood member. In this group (R_a) there was a distinct size hierarchy between the two remaining chicks, which has been shown to affect resource distribution under conditions of resource limitation. Structural growth, mass gain, rates of feather development and survival of chicks in the experimental broods were compared with those of chicks in unmanipulated (three chick) control broods. Control group broods fledged a higher total number of chicks than the two chick broods in the experimental groups. However, there were no differences among groups in the proportion of chicks fledged as a function of the post-manipulation total. During this study marginal chicks were mostly raised in addition to older siblings, which suggested that overproduction may function as a resource tracking mechanism. However, marginal chicks also provided an insurance reproductive value in 27% of cases where c-chicks survived to fledging age. Tarsus (and, to a lesser extent, weight) growth rates of chicks in R_a broods were greater than those of chicks in R_c broods so that, despite being initially smaller than chicks in R_c broods, R_a chicks were of similar size by fledging. Control brood chicks were initially of similar size to R_a chicks, but had slightly slower growth rates. Chicks lowest in the hierarchy generally obtain a smaller, more variable share of parental resources, and so for these chicks, decisions concerning which aspects of growth to allocate limited resources to may have important effects on survival prospects. Timing of feather development was negatively affected by increasing brood size, although this was only evident for

marginal chicks. For parents, overproduction and differential investment in progeny, combined with an efficient brood reduction mechanism could be a good way of producing the maximum number of high quality young in a stochastic environment.

6.2. INTRODUCTION

David Lack (1947) was the first researcher to postulate an adaptive explanation for the variations in clutch size seen both between species and within populations of certain species, in terms of individual selection (Godfray *et al.* 1991). He suggested that birds tend to lay the size of clutch that fledges the most offspring. This is now known as the Lack clutch and his idea has been more recently expressed such that if nestling mortality increases as the clutch size increases, and no other effects are important, the optimal clutch size is the one that produces the most fledglings (Stearns 1992). However, this notion does not take into account the effects of an increase in clutch size on parental survival or residual reproductive value (ie. future reproductive output; Roff 1992). Trade-offs between current and future reproduction, in addition to the possible effects of parent-offspring conflict, or temporal variation in environmental conditions, often result in the optimal clutch size being smaller than the Lack clutch (Roff 1992; Stearns 1992). Conversely, an increase in clutch size above that producing the most fledglings ('overproduction': Mock & Forbes 1995) can also occur, due to mechanisms evolved in response to fluctuating or unpredictable resource levels.

Overproduction is characterised by species in which the average number of young reared to fledging is commonly less than the number of eggs laid (Mock & Forbes 1995; Stoleson & Beissinger 1995). Parents encounter several problems that may be resolved by overproduction of young: critical resources may vary unpredictably, some offspring may die suddenly (eg. accident or predation) or during development some offspring may prove to be intrinsically flawed (Mock & Forbes 1995). These potential problems are largely unpredictable, which is an added complication best resolved through the production of extra offspring before a subsequent downwards modification of brood size, if necessary (Forbes 1991; Mock & Forbes 1995). In this context, it is useful to distinguish a 'base' or 'core' brood (the number of young normally reared to fledging) plus additional 'marginal' or 'surplus' brood members (overproduction: Forbes 1991; Mock & Forbes 1995).

Overproduction of offspring may have several functions:

Resource Tracking - Also known as *Bet-hedging* (Kozlowski & Stearns 1989), this hypothesis assumes that the optimal brood size varies unpredictably among breeding attempts. Overproduction of offspring occurs and brood size is then flexibly adjusted accordingly (Stearns 1992). The extent of overproduction depends upon the costs of producing and supporting 'marginal' offspring before brood reduction occurs (Mock & Forbes 1995). In order for overproduction to evolve, the unit costs of producing marginal offspring that die before independence must be low relative to the cost of producing an independent offspring (Kozlowski & Stearns 1989). If unit costs are relatively high then selection will favour a conservative clutch size (Mock & Forbes 1995). In addition, a high degree of environmental variation, especially the frequency of occurrence of bad years with respect to critical resources, is also important in selecting for a conservative clutch size because the potential cost of producing too large a clutch in bad years is greater than the cost of producing a small clutch during good years (Roff 1992).

As well as resource tracking there are hypotheses to explain the function of marginal offspring that emphasise offspring quality. The *Selective Abortion* hypothesis (Stearns 1992), otherwise known as *Progeny-choice* (Temme & Charnov 1987) suggests that where there is an identifiable difference in fitness between offspring, parents should overproduce and then eliminate those with the lowest fitness expectations. This hypothesis makes several assumptions: 1) that zygotes are cheap to produce, 2) that there is continued parental investment in progeny after fertilization, 3) that progeny vary in fitness, 4) that this variation in fitness can be identified early in development, and 5) that parents kill or allow to die progeny with low fitness expectations in relation to other progeny (Kozlowski & Stearns 1989). The *Insurance* hypothesis suggests that females should lay a 'pessimistic' clutch (ie. overproduce) so that, if a 'core' chick dies prematurely parents have a backup in the form of a 'marginal' chick (Forbes 1991; Stoleson & Beissinger 1995). Without such backup parents may have to invest in low-quality offspring (Mock & Forbes 1995).

The overall effectiveness of overproduction depends on the existence of an efficient brood reduction mechanism, because failure to remove any 'redundant' offspring would result in wasted parental investment (Evans 1996). An efficient brood reduction mechanism allows parents to avoid the cost of redundant offspring in years of low critical resource availability by eliminating such chicks, whilst gains may be maximized if marginal offspring can be reared when conditions are favourable (Roff 1992). Survival of supernumary young represents two different kinds of reproductive value to parents: extra reproductive value, when last hatched chicks survive in addition to older siblings, and insurance reproductive value obtained when at least one older sibling dies first (Mock & Parker 1986). For overproduction to be favoured, replacement of failed core brood members by marginal offspring must occur at least occasionally, although not necessarily in all breeding attempts.

However, overproduction is increasingly likely to be favoured as post-fledging survivorship of offspring increases (Forbes 1991).

The present study was conducted on the lesser black-backed gull. In common with most large gulls lesser black-backed gulls have a modal clutch size of three eggs. This is not very flexible because gulls have only three brood patches (Lack 1947) and do not appear to be able to incubate more than three eggs effectively (Reid 1987a). Moreover, even when egg production costs (see Monaghan *et al.* 1995) are not taken into account, parents artificially rearing more than three chicks have lower subsequent survival (Reid 1987b). The small maximum clutch size of lesser black-backed gulls does not allow clutch size to be finely tuned, and so adjustments in breeding are initiated on egg size (Martin 1987; Bolton *et al.* 1992). Intraclutch egg-size variation (IESV) shows much greater variation than clutch size among females (see *Chapter 2*), and the degree of hatching asynchrony is related to IESV. Most clutches contain three eggs and the last-laid (or c-egg) in three-egg clutches is significantly smaller than the similarly sized a-and b-eggs, and hatches a smaller, lighter chick. In addition, c-chicks hatch considerably later than a-and b-chicks so that the greatest difference in the size hierarchy within the brood occurs between the b- and c-chicks rather than the a- and b-chicks (*Chapter 2*) and parents normally rear only two chicks to fledging age (Cramp & Simmons 1983), further emphasising the apparently 'marginal' status of c-chicks.

In order to investigate the potential costs and benefits of overproduction in lesser black-backed gulls, three-chick broods were experimentally manipulated to produce broods of two chicks, and the number and size of the resultant fledglings were compared. Broods of three chicks should produce more fledglings on average than broods where a chick has been removed (Stoleson & Beissinger 1995). To assess the ability of 'marginal' c-chicks to successfully act as backup offspring by effectively replacing a 'core' brood member, some of the experimental two-chick broods were created through removal of the a-chick whilst the remaining two-chick broods were created by removal of the c-chick. Chick growth, feather development and survival were compared amongst all three groups, because theoretical and empirical studies (Parker *et al.* 1989; Nilsson & Svensson 1996) indicate that growth and fitness differences are affected by the degree of competitve asymmetry within a brood.

6.3. METHODS

6.3.1. *Data collection*

Nests in the study area were marked with a length of bamboo cane during April 1996. Individual nests were numbered and checked for eggs at least once daily so that accurate lay-dates could be obtained and egg laying order determined. All eggs were measured and marked and the volume calculated (see earlier chapters for details). A total of 58 three-egg clutches was selected and towards the end of incubation, each clutch was enclosed with chicken wire and provided with a wooden shelter.

At hatching chicks were weighed (± 1g using a Pesola balance) and two measurements of skeletal size were taken; tarsus length and head-bill length (both ± 0.1mm using dial calipers). Chicks were subsequently banded (marked with nest number and hatching sequence) with coloured insulation tape round the tarsus, then broods were allocated to one of three groups:

1) Unmanipulated (control)

2) Experimental 1 (R_a) - a-chicks removed

3) Experimental 2 (R_c) - c-chicks removed.

A proportion of the broods (n = 20) was left unmanipulated (controls), whilst the two experimental groups were created through removal of chicks at hatching to create two-chick broods. Broods were manipulated only when all chicks from a clutch had hatched (brood completion). In the first experimental group (known as R_a, n = 20), first hatched (a-) chicks were removed. Thus brood size was reduced to two chicks, and the second hatched chick (originally the b-chick) elevated to the top position in the size hierarchy. In the second experimental group (R_c, n = 18) last hatched (c-) chicks were removed, with the result that the second hatched (b-) chicks were now the smallest chicks in the reduced (two-chick) brood hierarchy.

Chicks in all groups were weighed and measured every 3 days until the youngest chick in the brood was 21 days of age, when surviving chicks had their insulation tape removed and replaced with an individually numbered BTO ring. Chick biometrics were taken again at 24 days, then every 6 days until the brood (age of youngest chick) was 36 days of age when the chicks were sufficiently feathered to fly.

6.3.2. *Feather growth*

In addition to the biometric measurements taken on each visit to a brood, an index of feather development, using terminology from Grant (1991) was used to assess the timing of initiation and completion of feather growth in chicks from the three groups:

Initiation of feather growth - feathers initially begin to develop on the scapulars. The start of feather growth is defined as the point at which there are feathers developing on the scapulars, but before there is any feather development on the greater and median coverts of the wing.

Completion of feather growth – there is no down evident by this stage, except on the nape of the neck. Feathers begin to grow on the crown. Chicks were almost able to fly by this stage and were very mobile and often difficult to find. For this reason feather growth was considered to have been completed (Figure 6.1).

Figure 6.1 – *Gull chick at final stage of feather development*

6.3.3. *Statistical analysis*

Analysis of covariance (ANCOVA) was used to assess chick growth. Unless otherwise indicated, only chicks surviving to fledging age were used in analysis of growth. An index of condition was also calculated for each chick (see earlier chapters) in order to compare within brood differences in condition during growth among treatments, for broods where all chicks survived to fledging age. In addition, one-way ANOVA and t-tests were also used. Data were transformed where appropriate, and all means are presented ± 1 s.e, unless otherwise stated. Data not conforming to the assumptions underlying the use of parametric statistical tests were analysed using the appropriate non-parametric formulae (Sokal & Rohlf 1995). All statistical tests are two-tailed.

6.4. RESULTS

6.4.1. *Biometric comparisons*

Prior to manipulation, there was no difference among the three groups in clutch volume (one-way ANOVA; $F_{2,55} = 1.18$, n.s), intraclutch egg-size variation ($F_{2,55} = 2.01$, n.s), date of clutch initiation ($F_{2,56} = 0.49$, n.s) or hatch date ($F_{2,56} = 0.42$, n.s). Within the unmanipulated control group, post-hoc range tests indicated that last-hatched (c-) chicks hatched from significantly smaller eggs (one-way ANOVA; $F_{2,63} = 14.51$, $P < 0.001$), with a shorter tarsus ($F_{2,62} = 12.33$, $P < 0.001$) and a lower weight ($F_{2,62} = 15.66$, $P < 0.001$) than both the first-hatched (a-) and second-hatched (b-) chicks (see *Chapter 2*). However there was no significant effect of hatching sequence on head-bill length (one-way ANOVA; $F_{2,62} = 2.98$, n.s). There was no difference among groups in measurements of b-chicks at hatching (tarsus $F_{2,52}$ =

0.31, n.s; head-bill $F_{2,52}$ = 0.42, n.s; weight $F_{2,52}$ = 0.45 n.s). Nor were there biometric differences at hatching between control and R_a c-chicks (t-test; tarsus t_{38} = 0.68, n.s; head-bill t_{38} = -1.13, n.s; weight t_{38} = 1.74, n.s). When data from all chicks in the experimental groups were pooled (R_a = b-and c-chicks, R_c = a-and b-chicks) there were significant differences between the two groups in terms of hatching tarsus length (t_{65} = -2.58, $P < 0.01$) and weight (t_{65} = -3.71, $P < 0.001$) due to the presence of the smaller c-chicks in the R_a group. However there was no difference in head-bill length (t_{65} = -0.51, n.s). Post-manipulation mass ratios (mass of smallest chick in the brood divided by that of the largest, a measure of the initial intrabrood size hierarchy; *Chapter 2*) were significantly different in the two-chick brood groups, with R_a broods having a greater initial difference in size between the two chicks (R_a mean mass ratio = 0.832 ± 0.021) compared to R_c broods (R_c mean mass ratio = 0.933 ± 0.017; t_{36} = 4.02, $P < 0.001$), as expected. Consequently, there was no significant difference in size between chicks in R_c broods (paired t-test; t_{17} = 1.07, n.s), but the older chick in R_a broods was significantly larger than its younger sibling (paired t-test; t_{19} = 6.33, $P < 0.001$).

6.4.2. *Chick survival*

The unmanipulated control group fledged a greater number of young per brood than either the R_a or the R_c groups (G-test; G_2 = 7.80, $P < 0.05$). However there were no differences among treatments in the number of chicks fledged as a proportion of the initial number of chicks post-manipulation in each group (G-test; G_2 = 2.46, n.s; Tables 6.1 & 6.2).

Table 6.1 - *Fledging success of gulls in the three treatment groups*

treatment	number of chicks fledged per egg laid	number of chicks fledged per brood	mean number of chicks fledged per brood ± 1 SD	coefficient of variation (%)*
control	47/60 (78%)	47/60 (78%)	2.35 ± 0.99	42
R_a	36/60 (60%)	36/40 (90%)	1.80 ± 0.52	29
R_c	30/54 (55%)	30/36 (83%)	1.67 ± 0.59	35

* coefficient of variation = (SD/mean) × 100

Variation in the number of chicks fledged per brood was greatest in three-chick broods (control) and least in the two-chick broods with the greatest difference in initial size hierarchy ($R_{a;}$; Levene's test for homogeneity of variances; $F_{2,55} = 4.99$, $P < 0.01$), as indicated by the coefficient of variation (Table 6.1).

Table 6.2 - *Chick survival per brood*

	number of chicks per brood				
treatment	0	1	2	3	total broods
control	2 (10%)	1 (5%)	5 (25%)	12 (60%)	20
R_a	1 (5%)	2 (10%)	17 (85%)	n/a	20
R_c	1 (6%)	4 (22%)	13 (72%)	n/a	18

Overall mortality was low and there was no relationship between chick mortality and initial position in the hierarchy either for unmanipulated or experimental broods (Table 6.3).

Table 6.3 - *Chick mortality in relation to position in the size hierarchy following manipulation, in the three treatments*

	position in hierarchy			
treatment	1	2	3	total mortality
control	4	4	5	13
R_a	2	2	n/a	4
R_c	2	4	n/a	6

6.4.3. *Chick growth*

For unmanipulated broods an analysis of covariance for chicks that survived to fledging revealed no effect of hatching sequence on the slope of growth for head-bill length, tarsus length or weight, and the elevation of the lines differed at the 6% level but not at the 5% level for both head-bill length ($F_{2,280} = 2.83$, $P = 0.06$) and tarsus length ($F_{2,280} = 2.90$, $P = 0.06$). When chicks that died before fledging were included in the analysis, hatching sequence had a significant effect on elevation for head-bill length ($F_{2,298} = 4.00$, $P < 0.05$) and tarsus length ($F_{2,298} = 3.91$, $P < 0.05$). This indicated that chicks highest in the size hierarchy maintained their larger size, and that chicks that died tended to be those that were smallest skeletally. When all surviving chicks were included and growth was compared among the three treatments, tarsus growth rate was significantly greater in R_a broods ($F_{2,754} = 2.80$, P

< 0.05) than in the other two treatments, whilst the elevation of the growth in head-bill length was significantly higher for R_c chicks ($F_{2,754} = 3.51$, $P < 0.05$) because there were no c-chicks in this group (Table 6.4). For the two experimental treatments, although chicks in R_c broods were initially larger than those in R_a broods in terms of both tarsus length and weight (see preceeding section), chicks in R_a broods showed significantly faster growth rates than chicks in R_c broods for tarsus length ($F_{1,470} = 7.68$, $P < 0.01$) and weight ($F_{1,470} = 4.00$, $P < 0.05$) so that differences between groups in size and weight were minimal by fledging.

Table 6.4 - *Growth rates of chicks surviving to fledging age for each treatment group*

	treatment		
	control (n = 47)	R_a (n = 36)	R_c (n = 30)
tarsus length (mm)			
at 10 days	36.46	36.87	38.28
at 20 days	49.87	51.02	51.40
growth rate/day (± 1 s.e.)	1.34 ± 0.03	1.42 ± 0.03	1.31 ± 0.03
head-bill (mm)			
at 10 days	67.84	67.80	69.45
at 20 days	86.02	86.10	87.30
growth rate/day (± 1 s.e.)	1.82 ± 0.04	1.83 ± 0.04	1.79 ± 0.03
body mass (g)			
at 10 days	277.99	281.77	295.15
at 20 days	545.22	558.77	557.38
growth rate/day (± 1 s.e.)	26.72 ± 0.58	27.70 ± 0.64	26.22 ± 0.61

The two treatments with c-chicks (controls & R_a) had greater within-brood variation in body condition than the only group where the c-chick was removed as part of the experimental treatment (R_c; Kruskal-Wallis; $\chi^2 = 6.93$, 2df, $P < 0.05$; followed by non-parametric range tests).

6.4.4. *Reproductive value of c-chicks*

The reproductive value of last-hatched chicks is related to their chances of survival, and can be split into two components: extra reproductive value (RV_e) and insurance reproductive value (RV_i). Total reproductive value of last hatched chicks can be calculated as the sum of:

$$RV_e = q \cdot P_e$$

&

$$RV_i = (1 - q) \cdot P_i$$

where q is the proportion of broods in which the last hatched chick was not the first chick to die, P_e is the fraction of q where the last hatched chick survives, and P_i is the fraction of c-chicks that survive in the other 1 - q broods (Mock & Parker 1986). The total reproductive value (RV_{total}) of last hatched chicks in unmanipulated broods was 0.75. Only 27% (RV_i = 0.20) of this total was insurance reproductive value. Of the six broods where older siblings died first, the last hatched chick survived on four occasions. Conversely, c-chicks provided extra reproductive value in 73% (RV_e = 0.55) of the broods where last hatched chicks survived.

6.4.5. *Feather growth*

In order to compare the effect of brood size on feather growth, experimental broods were compared with the two chicks in unmanipulated control broods that were in the same position in the initial pre-manipulation size hierarchy. For example chicks in R_a broods were compared with control b-and c-chicks, whilst R_c chicks were compared with control a-and b-chicks.

There was no significant difference in the age, tarsus length, head-bill length or weight at which feather growth started between chicks in R_a broods and their corresponding control chicks. Nor were there significant differences in the size or weight at which feather growth was completed. However, R_a chicks completed feather growth at a significantly earlier age ($t_{28.8} = 2.50$, $P < 0.05$), and exhibited less variance in this age (Levenes' $F_{1,38} = 6.24$, $P < 0.05$) than control chicks (Table 6.5). By contrast, although there was less variance associated with the age at which feather growth started for chicks in R_c broods compared to controls ($F_{1,44} = 4.83$, $P < 0.05$), there were no differences in size or weight when feather growth started. Nor were there any differences in the size, weight or age of R_c chicks compared to controls in terms of completion of feather growth (Table 6.5).

Table 6.5 - *Comparison of variables taken on completion of feather growth for the different treatments (means ± 1 s.e.)*

	treatment group			
variable	R_a (n = 22)	control-a (n = 18)	R_c (n = 23)	control-c (n = 20)
age (days)	24.77 ± 0.28	26.11 ± 0.46	24.52 ± 0.43	25.55 ± 0.34
tarsus (mm)	54.91 ± 0.73	53.94 ± 0.79	52.99 ± 0.65	53.82 ± 0.68
head-bill (mm)	93.34 ± 1.04	94.03 ± 1.23	91.29 ± 1.07	93.78 ± 0.99
weight (g)	693.0 ± 25.6	698.0 ± 22.5	625.2 ± 28.5	669.0 ± 19.1

6.5. DISCUSSION

The results of this experiment show that during the year of study unmanipulated three-chick broods produced more chicks to fledging than did artificially reduced two-chick broods, but variance in the number of fledglings produced was higher. The low mortality and high growth rates of chicks indicated that the year of study was not poor in terms of critical resource availability (see *Chapter 2*). The benefit of marginal chicks primarily as insurance is more likely to apply during years of poor food supply, where they are only likely to survive if a core brood member dies. Nonetheless, calculation of the reproductive value of last hatched chicks in unmanipulated broods indicated that c-chicks provided insurance reproductive value in 27% of broods in which they survived to fledge. The high total reproductive value of last hatched chicks, and the random variation in deaths of offspring with respect to hatching sequence meant that during the year of this study (1996), most marginal chicks (73%) that survived did so in addition to their older siblings, providing extra reproductive value to parents (Mock & Parker 1986). In earlier years (see *Chapter 2*), when chick growth rates and survival were significantly lower in unmanipulated three chick broods, last hatched chicks had lower survival rates (and, hence lower total reproductive value) than their siblings and, when they did survive, provided insurance reproductive value as often as extra reproductive value. Despite the higher growth rates and survival of chicks during 1996 compared to those of chicks hatching in 1994 and 1995, the proportion of broods in which a marginal chick was not the first chick to die was greater in 1996 (0.30) than in the earlier years (1994 = 0.21; 1995 = 0.15) indicating that offspring failure, like food supply, may vary unpredictably among years.

As predicted by Parker *et al.* (1989) growth rate differences within each brood were greatest in the two-chick broods where the initial competitive asymmetry was largest (R_a group). Conversely, there were no differences in growth between chicks in R_c broods, where there were no significant initial differences in age and size. This suggests that available resources were not limiting in two-chick broods so that resources were shared evenly between chicks, and hence their growth rates were similar. In this situation the a-chick would gain little from denying the b-chick access to such resources (Parker *et al.* 1989). However, in R_a broods the initial hierarchical difference in size between the two chicks meant the smaller chick had to grow at a faster overall rate (in terms of tarsus size and, to a lesser extent, weight) in order to 'catch-up' with its larger sibling. This provides evidence of marginal chicks' ability to slot into the 'survivor space' created by the unexpected loss of an older sibling, given the right environmental conditions for survival (Mock & Forbes 1995). Chicks in R_a broods had faster tarsus and weight growth rates than chicks in the other treatment groups. These data suggest that a size hierarchy in a brood of two chicks promotes greater competition for resources between chicks, leading to higher growth rates through increased parental provisioning. If food supply varies in a stochastic manner and there is a possibility of further brood reduction, the smallest chicks in the hierarchy have a lower reproductive value than larger chicks and is consequently less likely to be fed if resources become limiting. Chicks of equal size in a brood of two will have equal reproductive value to parents and are therefore equally likely to survive.

In general the difference in growth patterns between unmanipulated chicks and chicks in experimental broods was relatively small, which suggests that chicks from both brood sizes were growing at or close to a maximal rate. Assuming this is so, then parents with two-chick broods are likely to have utilized fewer resources in rearing their chicks than those with three-chick broods, as was found for artificially reduced broods of egrets by Mock & Lamey (1991). This may have important implications for lifetime reproductive success in long-lived birds, and suggests that in the conditions prevalent during the study there was little or no benefit gained, in terms of growth and survival of the remaining two brood members, from brood reduction (simulated by the experimental removal of one chick from the brood).

Chicks in R_a broods completed feather growth at a significantly earlier age than control chicks, indicating a negative effect of increased brood size on feather growth of chicks. However there were no differences in the timing of completion of feather growth between chicks in R_c broods and their corresponding control brood chicks, which suggests that the effect of brood size was significant only for later hatched chicks in unmanipulated control broods. Availability of parental resources may not have been as high for these chicks in unmanipulated broods, leading to a slower feather development. For chicks lowest in the size hierarchy, tarsus size may be important when chicks compete in scramble competition for parental resources (Nilsson & Svensson 1996). The lower in the hierarchy a chick is, the smaller its share of parental resources. Given this lower and more variable resource intake, the decision of how and where to allocate scarce resources relative to the growth and development of different body components is crucial (Nilsson & Svensson 1996). Gebhardt-Henrich & van Noordwijk (1994), studying great tits *Parus major*, found that growth of nestlings was significantly influenced by both brood size and variations in resource availability, and these factors had a greater relative effect (compared with genetic variation) upon growth during poor environmental conditions. However, even when resources are not overtly limiting (as during this experiment) growth of marginal chicks may be primarily affected by the size and growth rate of their siblings (Werschel & Jackson 1979; Nilsson & Svensson 1996).

Avian species that produce supernumary young require an efficient brood reduction mechanism so that parents do not incur the costs of raising too many offspring when resource availability is poor (Evans 1996). One way of ensuring that birds raise as many high quality chicks as possible in a stochastic environment is to create a hierarchy of chick size through altering the degree of hatching asynchrony (*Chapter 2*). A last-hatched marginal chick is handicapped by hatching from a smaller egg and its position in the hierarchy (determined mainly by the degree of hatching asynchrony) influences its chances of survival, and hence its reproductive value to parents (see *Chapter 5*; Mock & Parker 1986). Where resource availability is stochastic, parents may benefit from unequal investment in brood members, if this enhances the efficiency of brood reduction (Forbes 1993).

All three of the hypotheses for the production of supernumary young discussed here could explain overproduction in the lesser black-backed gull. However, the hypotheses are not mutually exclusive (Mock & Forbes 1995). Theoretical studies identify three key elements in the evolution of overproduction: 1) variation in offspring failure, 2) the nature of environmental variation, and 3) the costs of producing marginal offspring that die before fledging (Kozlowski & Stearns 1989; Forbes 1991). Lesser black-backed gulls produce a preponderance of three-egg clutches, even in years of poor food supply (*Chapter 2*). It is thus highly probable that the unit costs of producing marginal offspring are low relative to the rate of offspring failure and the costs associated with rearing offspring to independence in lesser black-backed gulls. Years of poor critical resource availability may be relatively rare or it may be that the cost of overproducing in a year of poor resource availability is reduced or avoided through the efficient elimination of redundant offspring (Forbes 1991). This study has shown that marginal chicks can provide extra reproductive value to parents when conditions are good for breeding (*resource tracking*). When conditions are poor or the rate of offspring failure is high, marginal chicks can also survive and grow well enough to replace failed older siblings (*insurance*). Moreover, the previous chapter has shown that, where resource availability is not sufficient to rear the whole brood, unequal investment in chicks within a brood enhances the efficiency of brood reduction at the expense of the last hatched, marginal chick (*progeny choice*). In contrast to the single-brooded lesser black-backed gull, silver gulls *Larus novaehollandiae,* breeding in Western Australia, where resource availability is neither seasonal nor limiting, lay several clutches in a season (Wooller & Dunlop 1979). Here, modal clutch size is reduced to two eggs presumably because investment per individual clutch is lower when there are several opportunities to breed within a season.

CHAPTER 7

General Discussion

7.1. *Clutch formation, egg resource allocation and the timing of breeding*

Evidence from the experiment presented in *Chapter 4* and related published work (e.g. Schultz 1991; Nager *et al.* 1997) suggests that, in addition to parental quality, food supply and reproductive value of offspring decrease seasonally for lesser black-backed gulls. Earlier breeding individuals are likely to be more proficient foragers, and may be older, more experienced birds. Although individuals may have to reach a threshold level of somatic reserves in order to breed (Schultz 1991), the actual 'decision' of when to start breeding is probably based upon the anticipated food availability during the most demanding phase of reproduction (*Chapter 4*). Data from *Chapter 4* showed that timing of breeding affects reproductive success independently of both clutch size and egg-size. However, clutch size and egg-size also decline seasonally in relation to food availability and with variation in parental quality (*Chapter 2*; Sydeman *et al.* 1991). The energetic requirements for producing a semi-precocial chick may put a premium on egg-size for lesser black-backed gulls. This could result in a trade-off between egg-size and clutch size, such that this trade-off, in addition to only having three brood patches, acts as a constraint on the natural upper limit of clutch size to three eggs.

Chicks hatching from a large egg have a greater body mass than chicks hatching from small eggs, which can have a positive effect on chick survival (*Chapter 4*; Bolton 1991). Allocation of parental resources is apparently biased towards first laid eggs in lesser black-backed gulls when resources are limiting, so that last-laid eggs are often significantly smaller than a- and b-eggs (*Chapters 2 & 3*). Where resource availability and/or total parental investment of resources is high, investment will be equalized across eggs within a clutch (*Chapters 2 & 3*; Sydeman & Emslie 1992; Kilpi 1995). This is because when chick survival probability is high, there may be no further benefits from an increase in the size of a- or b-eggs, so parental fitness will be enhanced by investing more resources in other eggs and/or later phases of reproduction (Bernardo 1996). Moreover, body size effects mean that there is a physiological maximum egg-size that an individual can lay. Conversely, where environmental variation in resources is highly stochastic, differential investment of maternal resources to eggs within a clutch may promote identification of the offspring with the highest fitness expectations (see also Anderson *et al.* 1997; Schwabl *et al.* 1997). This could be important if brood size has to be trimmed during later stages of breeding.

7.2. *Hatching asynchrony and brood size*

Is hatching asynchrony adaptive? To answer this fundamental question requires answers to two related questions: 1) can parents control hatching patterns, and if so, how?, and 2) what are the benefits of hatching asynchrony, and do they outweigh the costs? Evidence presented in *Chapter 5* suggests that lesser black-backed gull parents optimize the degree of hatching asynchrony with respect to current environmental conditions. More specifically the experiment showed that when all other things were equal, a reduction in the size hierarchy (synchrony) from that 'set' by parents subsequently resulted in wasted parental investment in chicks that died before fledging. This illustrates the potential cost of having the 'wrong' degree of hatching asynchrony for the prevalent conditions, and suggests that there are distinct advantages to phenotypic plasticity of the degree of hatching asynchrony.

In *Chapter 2* I presented evidence in support of intraclutch egg size variation (IESV) as a proximate cue to environmental conditions, because the degree of IESV is dependent upon food supply (Kilpi 1995). Moreover, comparative evidence that initiation of incubation, which is under hormonal control regulated by the hypothalamus, may be influenced by the nutritional status of the female (Carey 1996) provides further support for phenotypic plasticity of hatching asynchrony in gulls. A change in phenotype dependent upon environmental conditions can provide increased environmental tolerance. This is because where environments are heterogeneous with respect to the availability of critical resources, it is unlikely that any single phenotype will confer high fitness in all situations (Via *et al.* 1995). Phenotypic plasticity of hatching spans in response to local changes in food supply have been empirically demonstrated for American kestrels *Falco sparvarius* by Wiebe & Bortolotti (1994a, b, 1995). In this population of lesser black-backed gulls both IESV and hatching spans increased seasonally (*Chapter 2*) as likely resource availability declined (*Chapter 4*), indicating a possible plastic response to food supply. However, there were no significant differences among years in seasonal patterns of clutch size or egg size, despite significant differences among years in the growth and survival of offspring (*Chapter 2*). If this reflects the stochastic nature of seasonal variation in resource availability, then it would pay individuals to be conservative with respect to clutch size and the degree of hatching asynchrony. Hatching too synchronously for the current conditions may have costs associated with wasted parental investment (see above), or increased risk of losing the whole brood. Conversely, the main cost of hatching a clutch asynchronously when food supply is good may be an increased risk of unwanted brood reduction, as a consequence of the largest chicks in a brood taking an excessive amount of available food. In years of good food supply there may also be greater sibling rivalry and competition for resources in asynchronous broods compared to synchronous broods, due to the size difference among chicks (*Chapter 6*). In contrast, during years where resources are limiting, synchrony of hatching is more costly in terms of both sibling rivalry (Mock & Ploger 1987) and efficiency of brood reduction (*Chapter 5*) as a result of the lack of size differences among chicks within broods. In the simplest terms then the costs and benefits of a strategy of synchronous hatching relative to hatching asynchrony will depend upon the frequency and magnitude of years of good and poor food supply. Pijanowski (1992) modelled the costs and benefits of asynchrony and synchrony and showed that asynchrony would be favoured as a general strategy when good years are not very frequent, when the survival rate of last-hatched chicks during good years is high, or when years of poor food supply are not that poor. In reality food availability is likely to vary more continuously than this; but even so asynchronous hatching of chicks has been shown to be more efficient in tracking available resources than hatching synchrony (Temme & Charnov 1987).

The degree of hatching asynchrony may be dependent on several factors in addition to food supply. For example, females may adjust the degree of hatching asynchrony to the males' willingness to invest directly in reproduction (eg. courtship feeding: Niebuhr 1981; Green & Kerbs 1995), chicks may communicate with each other during hatching to increase the synchrony with which they hatch (Schwagmeyer *et al.* 1991), or parental age may be important. Survival probability of old individuals is lower than that of young birds, so that the current reproductive attempt makes up a greater proportion of residual reproductive success for old relative to that of young birds (Trivers 1985). Older individuals may be more willing to invest in the current reproductive attempt and hatch clutches more synchronously (e.g. Sydeman & Emslie 1992). However, in general synchrony is likely to be favoured only when food supply is superabundant or predictable (Pierotti & Bellrose 1986), so that the benefits of synchrony in a year of good food supply (reduced sibling rivalry and reduced dominance over later fledged young) are not countered by negative fitness costs such as wasted parental investment. Long-lived birds such as gulls may pay an especially steep fitness penalty for hatching chicks synchronously in years of poor or highly stochastic food supply (Mock & Forbes 1994), so that a conservative bet-hedging strategy of hatching clutches asynchronously, but fine-tuning to current conditions, is better over the long run.

Similarly, although the upper limit on clutch size in the lesser black-backed gull may be constrained by morphology (the number of brood patches) and the trade-off between egg-size and clutch size, within the narrow range of natural clutch sizes (1 - 3 eggs) the decision of how many eggs to lay is related to food availability (*Chapter 6*; Martin 1987). Unless food availability is so poor that the cost of producing a third 'marginal' offspring is very high, parents should always produce a clutch of three and then adjust the brood size downwards if necessary. This requires an efficient brood reduction mechanism to be effective. Production of a size hierarchy among chicks facilitates early brood reduction (*Chapter 5*). The effect of differential investment of available resources to eggs within a clutch and the subsequent timing of onset of incubation affect the nature of the brood hierarchy and thus the timing of brood reduction (*Chapter 5*). Offspring solicitation for parental resources may represent an honest signal of true need and may also be the product of sibling competition over parental resources (Mock *et al.* 1996). Parents failing to respond to honest signals of condition could lose offspring unnecessarily (Kilner & Johnstone 1997). However, this also leaves parents open to manipulation by offspring into feeding them more food, or distribution of available resources among chicks in such a way as to lower parental fitness. Avian parental workloads are flexible, and particularly for individuals in long-lived species, an increase in current parental effort may have an adverse effect on future reproductive success (Reid 1987b; Roff 1992; Daan *et al.* 1996). Current brood size may therefore have important implictions for lifetime reproductive success, and parents should try to avoid the costs of an over-large brood size (in relation to the amount of resources parents are willing to invest) and the concurrent effects of parent-offspring conflict over limited parental resources.

The effect of sibling rivalry upon parental investment decisions can be seen by comparison of species such as gulls, which have a modal clutch size of three, with that of species where modal clutch size is only one. For example, *Procellariiforms* successfully shunt all experimentally induced reproductive costs to their offspring (Mauck & Grubb 1995), because investment decisions over resource allocation are made with respect to only one chick and parents regulate food delivery at a less than maximal, flexible rate (Johnsen *et al.* 1994; Cook & Hamer 1997) so that parental effort is adjusted to the body condition of the adult and the reproductive value of the offspring (Erikstad *et al.* 1997). In contrast, gulls may have difficulty in regulating the shares of parental investment when chicks within a brood do not differ significantly in reproductive value, but the total parental investment allocated is insufficient to raise all chicks within the brood (ie. artificially induced synchrony; see *Chapter 5*). This illustrates the potential costs of sibling rivalry when parental workrates are flexible but less than maximal (Mock *et al.* 1996). A reduction in brood size may therefore reduce the degree of parent-offspring conflict over resource allocation (Godfray & Parker 1991), reduce sibling rivalry (Mock & Lamey 1991) and maximize reproductive success, within the boundaries set by the amount of parental investment committed to the current reproductive attempt.

Brood reduction should occur as soon as possible when demand for parental resources exceeds (anticipated) supply, but if environmental resource availability and offspring failure rates vary stochastically then parents need to wait as long as possible in order to assess the level of risk before reducing brood size (Forbes 1993). Parents can only effectively do this if they have fairly good control over resource allocation decisions and, hence, fitness of individual offspring. Establishment of sibling hierarchies facilitates the identification of the reproductive value of offspring through differential investment of available resources, and this can maximize reproductive success when tracking environmental resources. Parents have two points at which to assess current environmental conditions during the establishment of a sibling size hierarchy: 1) at the onset of incubation, and 2) during the period of nestling provisioning before all chicks have hatched. Several authors have noted that feeding rates of chicks are often very low immediately after hatching, but before brood completion (e.g. Parsons 1971; Graves *et al.* 1984). There are also wide variations in growth rates of chicks among broods during this period (*Chapter 2*). It seems likely that parents are fine-tuning the brood size hierarchy in response to the latest environmental information available. This would be advantageous if food supply varies stochastically.

Variation in the phenotypic characteristics of individuals, and in particular maternal phenotypic characters, are likely to play an important role in reproductive decisions and consequently reproductive success. For example, age, breeding experience, pair-bond duration, body size and extent of nutrient reserves may all affect timing of breeding, clutch size, egg size and hatching asynchrony. In western gulls *Larus occidentalis* nesting on the Farallon Islands in California, clutch size was found to be related to both parental age and timing of breeding with the seasonal decline greatest for younger birds (Sydeman *et al.* 1991). Clutch size also declined seasonally independent of age effects and was related to food availability (Sydeman *et al.* 1991). The relative effects of age and experience on breeding parameters have been assessed by Pyle *et al.* (1991). The increase in breeding success associated with was primarily a function of increased foraging skills with age and the acquisition of nesting skills through an increase in breeding experience (Pyle *et al.* 1991). This is a general trend in studies of gulls, with most empirical evidence suggesting that successful breeding pairs of gulls tend to be those that are more efficient at foraging and co-ordinating reproductive activities (Morris 1987; Mills *et al.* 1996). There were no data on age, experience or size of individuals breeding at Tarnbrook, but seasonal trends in reproductive parameters and success were similar to other studies as above. Ideally the data presented in this thesis would have been augmented by details of parental phenotypes, because there are likely to be multivariate correlations between maternal characters and reproductive parameters, such as egg-size, which may affect both parent and offspring fitness (Bernardo 1996). Due to time and manpower restrictions I could not gather the required data, so experimental field manipulations were introduced in order to attempt to control for as many of the potentially confounding variables as possible.

7.3. *A general life-history model of reproductive decisions in the lesser black-backed gull*

There follows a general account, based on evidence presented in this thesis, suggesting a possible 'plasticity of life-history traits' hypothesis for hatching asynchrony in large gull species:

In order to begin breeding individuals must first reach a threshold of nutrient reserves, once environmental cues (e.g. photoperiod) have stimulated hormonal changes associated with reproduction. This threshold may vary between individuals depending upon foraging ability and other factors such as age or breeding experience. Proximate determination of clutch size is linked to the extent of overwinter follicle growth and nutritional status. Once this threshold has been reached the actual timing of breeding will be determined by the energetic bottle-neck of the most costly phase of reproduction, i.e. chick rearing.

The size of eggs within a clutch is related to the quality and quantity of food taken in during the period of rapid yolk development. Courtship feeding may be an important component of female nutrition during this stage. Males may have a direct effect upon the nutritional status of the female, whilst females can use courtship feeding as an indication of the male's willingness to invest in reproduction. Resource allocation is biased towards eggs highest in the follicular hierarchy, with the result that the resource content of last-laid eggs is more variable among females than that of the first-laid eggs. This differential investment of within-clutch resources means that c-eggs are significantly smaller than a- or b-eggs when maternal resources are limiting. When resource availability is very good the benefits of hatching from a large egg are reduced and parental fitness is enhanced by equalizing investment of resources across eggs within a clutch. Onset of incubation is determined by the nutritional condition of females and is related to the degree of differential investment in eggs within a clutch. In general the IESV is a fair indicator of the extent of within-clutch differential investment in eggs. Where resource availability is poor and nutritional condition of females is low, there is greater within-clutch variation in parental investment of resources and clutches are hatched asynchronously. When resource availability is not limiting parental investment of resources will be similar for all eggs within a clutch and parents hatch clutches synchronously. If resources vary unpredictably parents can re-adjust the level of investment differential among offspring through varying the feeding rate of a- and b-chicks during the short period (up to 2 - 3 days) before the c-chick hatches, to manipulate the size hierarchy of the brood.

The extent of the size hierarchy among chicks within a brood determines the likelihood and timing of brood reduction and allows efficient tracking of resources. Production of three chicks is beneficial when rates of chick loss and food supply vary unpredictably. When conditions are generally poor the third hatched 'marginal' chick provides mainly insurance cover against premature loss of an older sibling and can be eliminated relatively quickly if it becomes 'redundant'. When conditions are generally good parents can benefit from the extra reproductive value provided by survival of the c-chick, to maximize reproductive success. In a stochastic environment it is better in the long term to hatch clutches with at least a degree of hatching asynchrony, because the costs of being too synchronous are greater than those of being too asynchronous if feeding conditions deteriorate. However, parents should hatch their clutch synchronously whenever costs can be minimized. This explains the general prevalence of hatching asynchrony among lesser black-backed gulls. However, specific characteristics relating to size, age, breeding experience or foraging ability will alter the costs and benefits of the degree of hatching asynchrony for individuals in any given year.

7.4. *General conclusions*

The lesser black-backed gull is a good species for the study of reproductive decisions because of the plasticity of many of its life-history traits. However, this plasticity also makes the evolution of such traits difficult to study (Cooch *et al.* 1991). It is apparent that many reproductive decisions are based upon food availability, and this thesis could be criticised for not attempting to assess environmental variation in food supply. Unfortunately one of the most important factors that contributes to the plasticity of life-history traits, the varied diet, also makes an accurate direct assessment of variation in food availability almost impossible. It is clear too that it is not just quantity of food that may be important but quality (*Chapter 3*; Selman & Houston 1996). Future studies should attempt to address these problems.

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