# SIBLING RIVALRY IN GULLS:

## EFFECTS OF ASYNCHRONOUS HATCHING AND EGG-CHARACTERISTICS ON OFFSPRING GROWTH, SURVIVAL AND BEHAVIOUR.

## A STUDY ON LESSER BLACK-BACKED GULLS (*LARUS FUSCUS*).

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When you collect a lot of data and start to analyse them all you first find is confusion and chaos. After a while, however, what you'll see is beauty.......

Bernd Heinrich.

## SUMMARY

In many bird species, eggs within a clutch differ in their size and content composition (egg characteristics). Additionally, in asynchronously hatching broods, chicks also differ in age and relative size due to staggered hatching. The last-hatched chick within a brood generally has the shortest incubation duration, is socially subordinate to its older siblings and has the lowest fledging success. Both hatching rank and egg characteristics have been shown to correlate with the duration of incubation, chick growth, chick survival and chick behaviour. Many studies have investigated the significance of those differences and whether it is the hatching rank or egg characteristics which causes the differences between siblings, however, these two factors are often confounded in natural broods. The last-hatching offspring often also hatches from an egg different from its older siblings. Therefore it is hard to distinguish between the two alternative explanations. Recent studies suggest that mainly hatching asynchrony affects offspring success. Chicks of last-laid eggs lag behind in growth and experience and are therefore disadvantaged in competitive situations. The egg characteristics of the last-laid egg, though, might actually bear some advantages for its chick to overcome the disadvantage given by the last hatching rank. In several species, last-laid eggs were found to contain a higher level of testosterone, which has been suggested to increase the embryonic and neonatal growth of the chick as well as the aggressive behaviour in order to enable the last-hatched chick to catch up with its siblings. However, other studies have shown that a high testosterone level might also decrease the chick' success by weakening the immune system, delaying hatching and decreasing growth rates of the last-hatched chick. These results show that it still remains unclear whether the hatching rank or the egg characteristics affect incubation duration, chick growth, chick survival and chick behaviour, i.e. fledging success. Lesser black-backed gulls (*Larus fuscus*) generally lay a clutch of three eggs with the last egg being smaller and showing differences in egg composition (e.g. nutrients, hormones) compared with the other two eggs of the same clutch. Chicks hatching from last-laid eggs have been shown to differ in terms of size, growth, immune response, condition and survival compared to their siblings. I manipulated the hatching order relative to the laying order of lesser black-backed gull broods with clutches of three eggs in such a way that chicks of first-laid eggs were the last chick to hatch within a brood, and chicks of last-laid eggs were the first chick to hatch within a brood. This experimental design separated the effects of hatching rank from the effects of egg characteristic and therefore enabled me to investigate whether it is the hatching rank or the egg characteristic which affects incubation duration, chick growth, chick survival and chick behaviour. Emphasis was laid on chicks of first-laid eggs (A-chicks) and of last-laid eggs (C-chicks). When hatching last, C-chicks had a shorter incubation duration than A-chicks due to a shorter hatching duration. When hatching first, A- and C-chicks did not differ in hatching duration. These results indicate that it is the characteristics of C-eggs which enables these offspring to respond to the presence of older chicks in the nest by hatching more quickly. C-chicks had a poorer condition at hatching than A-chicks independent of hatching rank, thus, the differences in condition between A- and C-chicks was due to the same differences in characteristics of A- and C-eggs. Nevertheless, there were no differences in growth, fledging success and cause of mortality between A-and C-chicks. C-chicks begged more intense than A-chicks, independent of hatching rank, and within the last hatching rank, only C-chicks were able to monopolise food and to address begging behaviour towards their nestmates. Within control nests, C-chicks were fed as frequent as A-chicks, and when hatching last C-chicks were fed even more frequently than A-chicks. When hatching first, however, A-chicks were fed more often than C-chicks. These results suggest that C-chicks are more adapted to the last hatching rank within a brood.

Many studies explain the third chick disadvantage as a mechanism to facilitate brood reduction. In the case of scarce resource abundance, parental birds could adjust the brood size to the low resource abundance by expelling the chicks in which they have invested the least and which has the lowest survival probability. Alternatively, last-laid eggs might be an insurance strategy. In the case that one or all of the first chicks die very soon after hatching. The lack of a distinct third chick disadvantage in this study contradicts the brood reduction hypothesis. The predictions of the insurance hypothesis can not be confirmed either since last-hatched chicks did not show a significant insurance value within a brood, i.e. they did not survive significantly more often when either one or both of the earlier hatched chicks died than when both of the older nestmates survived. The results of this study give evidence of a co-evolution of hatching asynchrony and differences in egg characteristics.

# CONTENTS

# CHAPTER I.

# GENERAL INTRODUCTION ON SIBLING RIVALRY

## *BEGGING BEHAVIOURS*

In many species across the animal kingdom parents have more than one offspring at a time (Storch and Welsch, 1997). If the young are dependent on their parents for food, protection from predators and/or keeping warm, siblings might compete over the parental resources, especially if these are scarce (reviewed in Mock and Parker, 1997). Each offspring might try to gain the largest share of these resources and to get more than the parents are able or willing to give (Trivers, 1974; Godfray, 1995a). Therefore, parental care is often accompanied by vigorous begging behaviours of the young. There are many examples. Domestic piglets (*Sus scrofa*) call when they are hungry (Weary and Fraser, 1995). Poison-arrow frog (*Dendrobates pumilio*) tadpoles perform swimming movements to stimulate their mother to lay trophic eggs for the young to eat (Weygoldt, 1980). Within birds there is a large variety of strategies chicks employ to obtain resources from their parents. Chicks use begging calls and begging movements (e.g. gapping that shows a colourful pattern inside the bill to the parent) to stimulate parents to feed the young (Pough et al., 1996). In some avian species chicks position themselves at a superior place within a nest to increase their chance to obtain food (Smith and Montgomerie, 1991; Price et al., 1996). Chicks of the herring gull (*Larus argentatus*) and the lesser black-backed gull (*Larus fuscus*) peck the parents' mandibles to make them regurgitate a half-digested food bolus (Tinbergen, 1953).

Hungrier offspring beg more and begging increases parental care (e.g. Henderson, 1974; Price et al., 1996). It also has been suggested that offspring negotiate with their siblings about the distribution of resources among them in order to avoid high energy expenditures and the risk of injuries of competitive fights (Forbes, 1991; Roulin et al., 2000).

In several bird species it has been found that chicks start to vocalise when they are still in the egg (Goethe, 1955; Tinbergen, 1953, Evans, 1988). Little squeaking sounds can be heard when the chicks have pecked a hole through the shell but have not yet hatched completely. At this stage the communication between parents and their offspring, as well as between individual offspring, has started, with both parents and chicks responding to each other's sounds (Goethe, 1955; Tinbergen, 1953, Evans, 1992).

After hatching, signalling both vocally and non-vocally among nestlings is a matter of begging and competition. Signals given by the individual chicks are thought to reflect their condition (Cotton et al., 1999), needs (Weary and Fraser, 1995; Price et al., 1996; Iacovides and Evans, 1998; Leonard and Horn, 1998, 2001) and/or competitive abilities, e.g. their size (Neuchterlein, 1981; Leonard and Horn, 2001). The latter might define their position in the brood's hierarchy. Many authors suggest that solicitation in offspring is a signal of need, since chicks with higher needs (e.g. which are hungrier) beg more (e.g. Smith & Montgomerie. 1991; Price & Ydenberg, 1995; Price et al., 1996). On the other hand, larger chicks may be able to out-compete smaller siblings by begging more and/or more efficiently (Ryden & Bengtsson, 1980; Fujioka, 1985; Greig-Smith, 1985). In this case the chick's signals would not correctly reflect its needs. Another possibility is that begging might signal the chick's condition rather than its needs to the parents, as Cotton et al. (1999) suggested. The condition (i.e. long-term need) of chicks may not therefore be related to a certain short-term need e.g. hunger (Price et al., 1996). Some behaviours might also express a chick's rank in the social hierarchy and not relating to the needs or condition of the young (Price and Ydenberg, 1995). Access to parental resources might be defined by signals specific to the chick's position within the brood. In this case parents actually allocate food among their young according to their rank (Mock & Ploger, 1987; Price and Ydenberg, 1995). For example, older nestmates might be able to monopolise positions towards the front of the nest, where they have a higher probability of being fed (Cotton et al., 1999; Kilner, 1995).

Various papers attempted to explain which of the cues parents use to decide their allocation of resource among the young (Impekoven, 1971; McArthur, 1982; Leonard and Horn, 1998; Evans, 1992) but remained inconclusive. However, chick begging behavioiur is also more complex than initially thought. Ryden and Bengtsson (1980) discussed begging behaviour only as a mechanism of communication between parent and offspring. Since chicks respond to their nestmates' begging either by increasing or by reducing their own solicitation it is clear that offspring signals are important for the between-chick relationship as well (Godfray, 1995; Leonard & Horn, 1998; Roulin et al., 2000).

It has been widely discussed whether offspring signals are honest and if parents can actually rely on them (MacNair and Parker, 1979; Godfray, 1995; Kilner and Johnstone, 1997; Leonard and Horn, 1998; Lotem, 1998). When parents respond to the chicks' begging by increasing their effort in parental care it seems very likely that cheating and exaggerated signalling might evolve in order to obtain the highest possible share of parental resources. Trivers (1974) pointed out that the young are often selected to try to gain more resources than the parents are selected to distribute. This parent-offspring conflict about parental resources is an important issue in animal behaviour (reviewed in Mock & Parker, 1997). Trivers (1974) stressed the idea that parent-offspring conflict has caused the evolution of solicitation in order to compete over, and to obtain extra resources. On the other hand, many authors argue that signalling, and especially vocal begging, bears costs to the individual chick through energy expenditure, and to its nestmates and parents in terms of increased predation risk (for a review see Kilner and Johnstone, 1997).

Therefore, with this cost in mind, begging should be honest in order to prevent a decrease of fitness. If vocal begging indeed bears predation costs for all nestmates, large nestlings are expected to beg less in order to quiet their smaller siblings (Zahavi,1977). Another cost of begging might be fight for food. Forbes (1991) suggested that a less-hungry nestling signals to its nestmates that it will not contest the next prey item in order to avoid aggression. This shows again that chicks may actually respond to each other's signals and needs. Roulin et al. (2000) described the solicitation of barn owl (*Tyta alba*) chicks as an actual negotiation about resources when parents are absent. However, the influence of a chick's solicitation on its sibling's behaviour is not consistent across the species that have been tested to date (Leonard and Horn, 1998).

In gulls, a certain number of characteristic calls and postures of both parents and chicks have been described that are used by the birds for communication (Tinbergen, 1953; Goethe, 1955). Impekoven (1971) found different calls for different needs in the black-headed gull, the herring gull, and others. A list presenting some of the offspring signals found in gulls is shown later in this thesis (Chapter IV).

### *SIBLING AGGRESSION*

Competition between siblings over parental resources can become quite aggressive and may even lead to the death of the weakest young. Sibling rivalry has been described in insects, birds and mammals (reviewed in Mock and Parker, 1997). Domestic piglets compete vigorously with their siblings for access to the sow's teats (Hartsock and Graves, 1976; Fraser and Thompson, 1991). Within the first few hours of life, a "teat order" is established with most piglets returning repeatedly to a particular teat which they defend against their siblings, and if there are more piglets than teats, the losers in this early competition are likely to die due to starvation (Fraser, 1990). In the polyembryonic wasp

*Copidosoma floidanum* (Hymenoptera: Encyrtidae), whose eggs proliferates clonally during the larval development, female precocious larvae kill their brothers during the larval stage in order to secure more resources for themselves and their clonal sisters (Grbic et al., 1992). Also young of spotted hyaena *Crocuta crocuta* (Frank et al., 1991; Smale et al., 1999), osprey *Pandion haliaetus* (Forbes, 1991) and cattle egret *Bubulcus ibis* (Fujioka, 1985; Ploger and Mock, 1986) have been described to show aggression against their siblings or even to practice siblicide.

### *BIRDS AS A SPECIES TO STUDY SIBLING RIVALRY*

Sibling rivalry has been investigated mostly in birds (reviewed in Mock and Parker, 1997) which are very suitable for this investigation due to various characteristics. Parents are able to alter the resource allocation to individual offspring from egg production onwards (reviewed in Mock & Parker, 1997; Royle and Hamer, 1998; Nager et al., 2000a). Furthermore, the embryonic development of the chicks is initiated through the body heat of the adults during incubation. Parents can alter incubation and therefore the development of their offspring by choosing the onset of incubation (Ricklefs and Smeraski, 1983; Stoleson and Beissinger, 1995). If females start to incubate before the last egg has been laid, the chicks hatch asynchronously which leads to an age-size hierarchy among siblings (e.g. Meyburg, 1973; Stinson, 1979; Hahn, 1981). Differences between the ranks of this hierarchy might be increased through differences in both egg size and egg composition (egg characteristics) across the laying order (Parsons, 1975; Hario and Rudbäck, 1996, 1999). Furthermore, feeding can easily be controlled in such a way that food can be monopolised by a strong chick or be selectively delivered to a chick favoured by the parent (Ryden and Bengtsson, 1980; Drummond et al., 1986; Price and Ydenberg, 1995; Krebs and Margrath, 2000). Studies on the evolutionary significance of the within-brood size-age hierarchy and of the differences in size, condition, growth, survival and/or behaviour between offspring of the same brood in birds have often not distinguished between the possible causations of these differences.

*EGG-CHARACTERISTICS*

In many bird species female parents allocate their resources asymmetrically among the eggs (reviewed in Mock & Parker, 1997). Eggs have been shown to differ in their size, mass, and composition in relation to laying order (Slagsvold et al., 1984; Schwabl, 1996a; Royle and Hamer, 1998; Nager et al., 2000a). These differences may affect the survival of chicks (Parsons, 1970) and chick behaviour (Schwabl, 1996a).

Many gull species usually lay a clutch of three eggs per nest (Cramp, 1985). These three eggs show a decline in size with increasing laying sequence (Parsons, 1975; Royle and Hamer, 1998; Royle et al., 1999; Nager et al., 2000a). While the first and second eggs are of similar size, the third egg is significantly smaller (Harris, 1963), lighter (Parsons, 1972) and the chick that hatches from this egg has a relatively lower body mass and smaller size than its siblings (Royle & Hamer, 1998; Nager et al., 2000a). Parsons (1970) and Bolton (1991) suggested that the survival of the last chick increases with increasing egg size.

Eggs within a gull nest also differ in their composition. The most striking differences are often found in the last-laid egg, which contains more testosterone (Royle et al., 2001; Eising et al.. 2001), less antioxidants (vitamine E and carotenoids) (Royle et al., 1999), and has less lipid (Royle et al., 1999; Blount et al., 2002) than earlier-laid eggs.

A significantly lower concentration of vitamin E and carotenoids in last-laid lesser black-backed gull eggs (Royle et al., 1999) is thought to decrease the embryonic and neonatal growth and development of the chicks. Antioxidants might reduce the susceptibility to pathogens and increase the ability of the embryo to withstand the oxidative stress at hatching. These advantages of a high antioxidant concentration are not available to the last-hatched chick.

Furthermore, Hario and Rudbäck (1996, 1999) found a higher susceptibility to diseases in last-hatched lesser black-backed gull chicks. Dead C-chicks had significantly more frequent signs of degeneration and inflammation of severalimportant internal organs than their siblings. This might have caused the higher mortality among third-egg chicks in that study. Similarly, Blount et al., (2002) and Nager et al. (ms) found a weaker immune response in lesser black-backed gull chicks hatched from the last egg. Both the observed low antioxidant reserves and the higher content of testosterone could weaken the immune system of the chick hatching from the last-laid egg (Folstad and Karter, 1992; Owens and Wilson, K., 1999; Blount et al., 2002).

Differences in both egg size and egg composition between eggs within a clutch might also lead to different abilities, needs and conditions of the chicks (Bolton, 1991; Schwabl, 1996a), which in return might affect the chick's behaviour.

### *ASYNCHRONOUS HATCHING*

Additionally to within-clutch differences in egg quality, in many avian species, parents start to incubate before clutch completion so that the chicks hatch asynchronously (Clark and Wilson, 1981). Asynchronous hatching leads to size and age differences among the offspring, especially between the first and the last hatched chick (reviewed in Clark and Wilson 1981). Both size and age differences among offspring lead to a social hierarchy within broods with its ranks defining the success of the offspring (Fujioka, 1985; Maynard-Smith and Parker, 1976; Hahn, 1981; Griffiths, 1992; Heg and van der Velde, 2001). Mortality among last-hatched chicks is usually higher than in earlier hatched chicks. Hahn (1981), Griffiths (1992) and Heg and Van der Velde (2001) all found a decrease in survival with increasing hatching order. Furthermore, last-hatched chicks are usually the first to die in the nest (Parsons, 1975). This so-called "Last Chick Disadvantage" has been discussed in a wide range of studies (reviewed in Stoleson and Beissinger, 1995) and might be a result of both within-clutch differences in egg characteristics and within-brood differences in hierarchy ranks, which both have been shown to decrease the success of last-hatched chicks compared to its siblings.

Overall, asynchronous broods have been shown to fledge more young than do synchronous broods (reviewed in Stoleson and Beissinger, 1995). These findings support the idea that asynchrony is an advantageous adaptation. However, other studies have found that the success of asynchronous broods varied between years and even that fledging success of synchronous broods was equal or greater than that of asynchronous broods (reviewed in Stoleson and Beissinger, 1995). Hence, the evidence that hatching asynchrony is advantageous for offspring survival after hatching is controversial.

The most commonly hypothesis of the significance of asynchronous hatching is Lack's (1947, 1954) brood reduction hypothesis. It describes asynchrony as a mechanism by which brood size can be adjusted to the food availability during the nestling period. In the case of scarce food resources, asynchronous hatching would enable parents to allocate food to the chicks that have the highest survival probability. Parents may even systematically expel the chick(s) in which they have invested the least or that will require the most future investment in order to reduce brood size (Hahn, 1981; Hebert and Barclay, 1986). Another hypothesis suggests that asynchrony shifts the peak of the chicks' food requirements, thus gives the parents a greater chance to feed and raise all of their young (Peak Load Hypothesis, Hussel, 1972). The last-laid egg might also be an insurance strategy of the parents for the case that one or all of the first chicks die very soon after hatching. With an "extra" egg the parental birds would still be able to raise at least one chick. However, if the first chicks survive, not many resources are given to the last-hatched chick (Insurance Hypothesis, Stinson, 1979).

Many authors propose that siblings aggression is not required for brood reduction, since rank differences among offspring are sufficient to enforce the starvation of the smallest chick (Lack, 1954, 1968; Howe, 1978; Werschkul, 1979; Ryden & Bengtsson, 1980; Hahn, 1981; Mock, 1984a; Inoue, 1985). Hamilton (1964) described asynchronous hatching as a mechanism by which sibling rivalry is reduced. In this case, age and size differences between the chicks give rise to a dominance hierarchy, with the hierarchy's ranks rather than competitive fights defining the access to resources. Forbes' (1991) work on ospreys (*Pandion haliaetus*) supported this hypothesis, suggesting that aggression in asynchronous broods is diminished because the senior siblings' dominant status is not threatened. However, Fujioka (1985) showed in his work on cattle egrets (*Bubulcus ibis*), that with increasing brood size the success rate of begging decreased, and more actual fights occurred among siblings. Hence, aggressive competition arose when resources were low. Additionally, Parker et al. (1989) demonstrated that the largest discrepancy exists between the last and the penultimate chick, and it is between these two chicks where most of aggressive behaviour and competitive fights occur.

First-hatched nestlings are bigger, stronger and more experienced since their development is more advanced. For example, they have better motor skills and a higher developed nervous system and therefore better competitive abilities. Hence, the last chick's chance to succeed over its older siblings is relatively low in most cases, with the older offspring out-competing the youngest chick (Maynard-Smith and Parker, 1976; Hahn, 1981; Fujioka, 1985; Ploger and Mock, 1986; Heg and van der Velde, 2001). Last-hatching chicks might also have a lower chance to escape from predators (Hillström et al., 2000).

Since in most nests only the older chicks survive, the question arose as to why birds produce low-quality last-eggs and hatch them asynchronously. Why do they not delay incubation until clutch completion so that, at least, the last chick is not confronted with a rank disadvantage? Relatively recent studies suggested that the last egg might not be just a poor quality egg but that it might actually also bear some advantages for the last chick to reduce the disadvantages caused by hatching asynchrony. A number of studies have found a significant increase of higher testosterone level with increasing laying order (e.g. Schwabl, 1996b; Lipar and Ketterson, 2000; Sockman and Schwabl, 2000; Eising et al., 2001; Royle et al., 2001). The higher testosterone content of last-laid eggs might have a number of advantages. It might lead to a faster growth of the chick, which is thought to be an advantageous adaptation for the youngest nestling to catch up with its siblings who are initially bigger and stronger (Schwabl, 1996b; Eising et al., 2001). The hormone also has an effect on the aggressive behaviour (e.g.Schwabl, 1996b). Females might provide the last egg with a high amount of testosterone to increase the aggressiveness and thus the competitive ability of the young in order to increase its ability to compete with its nestmates. However, Ellis et al. (2001) did not find a correlation between egg laying rank and androgen level in egg yolks within neither synchronous nor asynchronous broods of house wrens (*Troglodytes aedon*). However, the last hatched nestling was still able to overcome any age- and size-related disadvantages of hatching asynchrony (Ellis et al., 2001). Thus, these results on within-clutch patterns of androgen deposition into eggs did not support Schwabl's (1996b) hypothesis of improved breeding success.

The increasing androgen level with increasing laying order might also be an evolutionary adaptation in order to increase the embryonic growth and thus to decrease the incubation duration of the last-hatching chicks. As a consequence asynchrony and therefore the size-age differences between the chicks is decreased. Parsons (1972) showed in his study on herring gulls *(Larus argentatus)* that embryos of within-clutch last-laid eggs, which supposedly have higher androgen levels than earlier- laid eggs, reduced the interval in development between B- and C-eggs from 36 hours during the first 4 days of incubation to 18-24 hours at hatching, while the interval in embryonic development between A- and B-eggs remained on average 12 hours throughout the whole incubation period. These different results show that it is still unclear what effects differences in egg characteristics have on the incubation (i.e.embryonic development). In support of this suggestion, Eising et al. (2001) found that black-headed gull (*Larus ridibundus*) eggs with a high amount of androgens had a significantly shorter incubation duration than eggs with a lower androgen concentration. However, other studies did not support this hypothesis and even found the opposite effect, i.e. that testosterone delays hatching and reduces nestling growth (Sockman and Schwabl, 2000; Ellis et al., 2001).

Another factor that might explain hatching asynchrony is that the egg viability might decrease if eggs have not been incubated from laying onwards (Veiga and Vinuela, 1993). If parental birds wait until the last egg is laid the survival probability of the first egg might be decreased (Veiga and Vinuela, 1993). In this case hatching asynchrony would be essential to avoid a high mortality of the first-laid eggs.

### *SEXUAL DIMORPHISM*

Stamps (1990) and Drummond et al. (1991) discussed the role of sexual size dimorphism in sibling competition. In bird species in which competition over parental resources is affected by chick size. sexual size dimorphism could result in sex-biased mortality and biased fledging sex ratio. The larger sex might have an advantage over the smaller sex because of its greater size enabling it to out-compete its siblings in competition over parental resources (Teather, 1992; review: Mock & Parker, 1997; Oddie, 2000).

Competition between male and female chicks due to size differences in dimorphic species might not be immediately recognisable when the chicks hatch asynchronously since the effect of age differences among the siblings on competitive ability might overshadow any sex effects (Bortolotti, 1986). Accordingly, in lesser black-backed gulls, Bradbury and Griffiths (1999) showed that the sex-biased mortality was only found in asynchronous broods, but not in artificially synchronised broods.

Alternatively, the larger sex could be more vulnerable to starvation due to greater food needs (Clutton-Brock and Iason, 1986; Griffiths, 1992). In lesser black-backed gulls where the male is of larger size, Griffiths (1992) showed a male-biased nestling mortality in unmanipulated nests. Nager et al. (2000b) demonstrated in the same species a strongly reduced pre-fledging survival of male chicks in all-male broods when the parent's poor condition was experimentally reduced, whereas survival of female chicks was not affected by the condition of their parents or the sex of their siblings. Male chicks hatched from poor quality eggs survived less well than female chicks hatched from poor quality eggs (Nager et al., 1999). Hence, in lesser black-backed gulls, male offspring are more vulnerable to poor resource supply than female offspring both as an embryo and as a chick.

### *EXPERIMENTAL APROACH OF MY THESIS*

The various aspects of sibling rivalry discussed above have been the subjects of a wide range of studies and have provided vary and contradictory result. However, few studies so far have looked at the significance of differences in egg-characteristics on sibling competition.

For this thesis, I chose the lesser black-backed gull (*Larus fuscus*) as the experimental species to investigate the influence of egg-characteristics (size and content) on sibling rivalry. The lesser black-backed gull has many advantages as a study species. There is a large amount of background information which gives a good picture about egg composition, the third chick disadvantage and sex differences in survival in this species.. Furthermore, breeding colonies of the lesser black-backed gull give good opportunities of both manipulations and observations of broods.

This gull belongs to the order *Charadriiformes* and the family *Laridae*. It is a common breeding species along the coasts from North-West Europe to North-Middle-Siberia and is rather rarely seen inland (Bezzel, 1985). Five subspecies are recognised within Middle-*Europe (L.f. graellsii, L.f. intermedius, L.f. fuscus, L.f.heuglini, L.f. taimyrensis*) of which the *Larus fusucs graellsii* breeds at the coasts of Great Britain

(Bezzel, 1985). These gulls reach the mature stage at the age of 3-4 years and have seasonal monogamous pair bonds. The breeding season begins in early spring (end of March/beginning of April) with egg laying starting at the end of April (Bezzel, 1985). Nests are generally built on the ground often protected by vegetation patches and of litter from the sea washed onto the beach (Bezzel, 1985). Female *L. fuscus* normally lay a clutch of three eggs (Harris, 1963; Cramp, 1983; Monaghan et al., 1998) and only one clutch per year. However, in the case of egg predation or nest failure, more eggs or even an entirely new clutch can be laid (Houston et al., 1983). Eggs hatch asynchronously and show differences in egg characteristics as described above. Chicks hatched from last-laid eggs generally show the lowest fledging success within a brood (Griffiths, 1992; Hario and Rudbäck, 1996, 1999). Fledging production in *L. fuscus* is recorded as between 0.75 and 1.5 fledglings per brood (Bezzel, 1985).

Fieldwork was conducted in a large mixed breeding colony of herring gulls (*Larus argentatus*) and lesser black-backed gulls (*Larus fuscus*) at the South Walney Nature Reserve on Walney Island, Cumbria (NW England), where the gulls have been guarded and protected during the breeding seasons since 1951 (Dean, 1990). The reserve's habitat is characterised by saltmarshes, shingle beaches, a few mature grey dunes and a wide area of flat grass dunes (Dean, 1990). The gull colony is situated on this relatively flat and sandy ground, which offers reasonably good conditions for observations (Brown, 1967; Tinbergen and Falcus, 1970). My study was carried out within five different experimental plots, which were all relatively close (50-300m) to each other near the centre of the colony.

The aim of my project was to separate the effects of hatching rank and egg characteristics on chick performance.

First-laid eggs are called A-eggs, second-laid eggs B-eggs and third-laid eggs C-eggs. Similarly, the chicks are called A-, B-, and C-chick referring to the egg they hatched from. Additionally, eggs being the first to hatch within a nest are called F-eggs independent of initial laying rank. Similarly, eggs to hatch second are called S-eggs and eggs to hatch last are called L-eggs. Accordingly, chicks hatching from F-, S-, and L-eggs are called F-, S-, and L-chicks, respectively. In natural broods of three chicks, A-chicks are usually the F-chicks and C-chicks the L-chicks.

I exchanged A-and C-eggs between nests, such that a C-egg took the F-rank and an A-egg took the L-rank (figure 1).

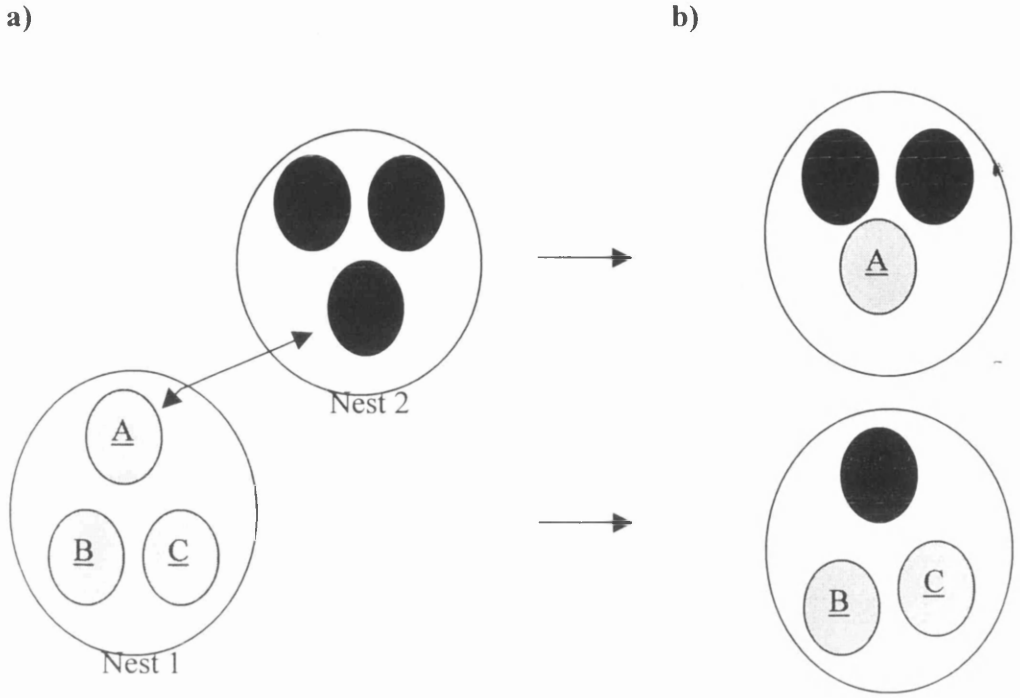

**Figure 1. Experimental nests (a) before and (b) after the exchange of A- and C-eggs between nests in order to reverse hatching order relative to laying order.**

This led to a reversed hatching sequence in relation to the laying sequence and thus to a separation of initial egg-quality effects and effects of within-clutch hatching rank differences. During my work, I concentrated on the questions whether the poor success of C-chicks in gull broods is due to the size and/or composition of the egg or due to the C-chicks' low hatching rank compared to A- and B-chicks. I looked at differences in embryonic development (incubation duration) and nestling development (chick growth and survival) in relation to egg characteristics and hatching rank. Since both factors might affect the chick's competitive ability I also studied whether the chicks show distinctive behaviours relative to either their hatching rank or their egg characteristics (figure 2).

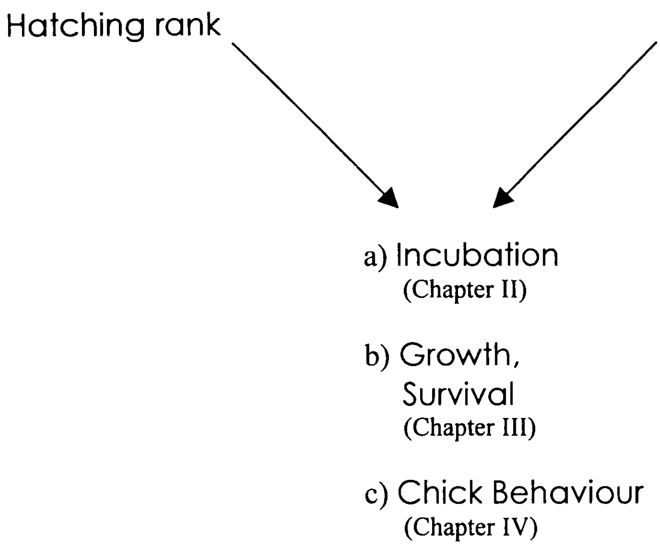

**Figure 2. Aim of my study: to separate and determine the effect of hatching rank from the effect of egg characteristics on (a) incubation duration, (b) chick growth and survival, and (c) chick behaviour.**

In **Chapter II**, I recorded the incubation duration (period from the day an egg has been laid until the chick emerged from the shell (hatching)) and the interval between the stage when a chick had pecked a small hole through the egg shell (pipping) and hatching. Cross-fostering of eggs took place either the day they have been laid (*experiment A*) or at the stage of pipping (*experiment B*). In *experiment A*, the day an A-egg was laid it was exchanged with a C-egg of another nest and which was laid at the same day. In *experiment B*, A-eggs at the pipping stage from one nest were exchanged with pipped C-eggs of another nest. In the control group of both *experiment A* and *B*, either an A-egg was exchanged with another A-egg or a C-egg with another C-egg. I recorded incubation duration and pipping-hatching intervals of each egg in either the F- or the L-rank, i.e. first hatched C-chicks, normal A-chicks, last-hatched A-chicks and normal C-chicks. In this chapter, I tested whether egg quality influences the duration of incubation and of the hatching process in lesser black-backed gulls. I separated experimentally the effects of egg quality from the effects of hatching rank by cross-fostering eggs between nests in such a way that chicks from last-laid eggs were expected to hatch first and chicks from first-laid eggs were expected to hatch last within a brood. I predicted that chicks of last-laid eggs have a shorter incubation period than first-laid eggs when corrected for hatching rank.

In the experimental work of the next two chapters, I cross-fostered eggs in the same way as described for *experiment B* of chapter II. Both the growth and the survival of the chicks from hatching onwards until the age of 28 days at which I considered them as successfully fledged are described and analysed in **Chapter III.** The aim of this chapter was to investigate the effects of egg quality and hatching rank on chick growth rate, mortality and fledging success in the lesser black-backed gull. If differences in the composition of the last-laid egg are adaptive, I predict that when hatching last, chicks from first-laid eggs are less successful compared to chicks from last-laid eggs. In contrast, I expect that when hatching first, chicks from first-laid eggs are more successful than chicks from last-laid eggs. Moreover, if the observed within-clutch differences in egg quality are adaptive, I expect that experimental alteration of these differences should result in a poorer breeding success of manipulated broods compared to control broods.

Both hatching asynchrony and egg characteristics influence the chick's size, condition, need and success and therefore might affect its behaviour (figure 2). In **Chapter IV**, I recorded the frequency and duration of behaviours shown during begging, feeding and aggression towards siblings between. I investigated whether chick behaviour is affected by the characteristics of the egg it hatched from or the chick's hatching rank within the brood. Furthermore, I compared the behaviour between male and female chicks.

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CHAPTER II.

# DETERMINANTS OF INCUBATION DURATION IN THE LESSER BLACK-BACKED GULL (*LARUS FUSCUS*): RELATIVE CONTRIBUTIONS OF EGG CHARACTERISTICS AND HATCHING RANK

## ABSTRACT

Incubation duration has been shown to be dependent on parental quality, size and composition of eggs and laying order. However, in many studies the effects of egg characteristics and laying order are confounded. In Larids, broods hatch asynchronously and this could be due to differences between eggs across the laying order or due to incubation starting before laying is completed. In this study on lesser black-backed gulls (*Larus fiscus*), I examined (a) whether independent of variation in incubation start, egg characteristics influence the duration of incubation and the hatching process and (b) whether the social environment in the nest affects incubation duration. I experimentally manipulated the hatching order relative to the laying order within broods of three chicks. Through exchanging eggs between nests chicks from first-laid eggs became the last to hatch, and chicks from last-laid eggs became the first to hatch. This experimental design separated effects of egg characteristics from effects of hatching rank on the incubation duration. When hatching last, chicks from last-laid eggs (C-chicks) had a shorter incubation duration than chicks from first-laid eggs (A-chicks). This difference was mainly due to a shorter hatching duration (interval between the stage at which a chick had pecked a small whole through the shell until the chick had completely emerged from the egg shell) in last-hatched C-chicks compared to last-hatched A-chicks. There was no difference in hatching duration in first-hatched chicks between A- and C-chicks. This suggests that only C-chicks had the ability to accelerate hatching when its nestmates had already hatched. It might be that a shortened incubation duration of the last-laid egg could decrease asynchrony and its disadvantageous effects on the survival of the chick from that egg.

## INTRODUCTION

Incubation duration of eggs varies either within avian species or even within the same clutch (e.g. Hötker, 1998; Strausberger, 1998). However, the proximate factors affecting this variation is not yet understood.

The duration of embryonic growth and development of avian offspring may depend on parental care (i.e. incubation behaviour) (Stoleson and Beissinger, 1995) as well as on size and composition of the eggs (Parsons, 1972; Bolton, 1991; Bollinger, 1994; Eising et al., 2001).

During incubation parents transfer heat to the eggs, and it is this heat transfer which initiates and maintains chick development (Stoleson and Beissinger, 1995). Through the timing of the incubation onset parents may influence the length of incubation (Ricklefs and Smeraski, 1983) and the hatching intervals of their young (Stoleson and Beissinger, 1995; Wiebe et al., 1998).

When incubation starts before the last egg is laid chicks within a brood hatch asynchronously. This is known to occur in a wide range of avian species (reviewed in Clark and Wilson, 1981; Stoleson and Beissinger, 1995). The incubation duration of eggs within asynchronous clutches has been shown to differ across the laying order with the last egg having the shortest incubation duration (e.g. Parsons, 1972; Vinuela, 1997). Initiation of incubation before the last egg is laid is commonly believed to be responsible for differences in incubation length across the laying sequence. However, there might also be egg effects on incubation duration independent of variation in incubation start (Parsons, 1972). Asynchronous hatching leads to a size-age hierarchy within broods with the last-hatched chick being disadvantaged in competitive situations due to its smaller size, younger age and delayed development compared with its siblings (Maynard-Smith and Parker, 1976; Fujioka, 1985; Hahn, 1981; Heg and Van der Velde, 2001). This size-age disadvantage often leads to a higher mortality in last-hatching chicks (reviewed in Mock and Parker, 1997). Differences in the duration of incubation could be adaptive in order to decrease asynchrony and its disadvantageous effects for last-hatching chicks (Parsons, 1972).

In several bird species, eggs of the same clutch have been shown to differ in their size and composition (Slagsvold et al., 1984; Williams, 1994). It has been suggested that the poor success of last-hatching chicks is mainly due to asynchronous hatching (Parsons, 1970, 1975, Hahn, 1981, Royle and Hamer, 1998). However, last-laid eggs have often also been suggested to be of poorer quality, which in addition to hatching later than its siblings might reduce the expectations of survival of that chick (Parsons, 1975; Hario and Rudbäck, 1996, 1999). In contrast, more recent studies have pointed out that last-laid eggs may actually bear some advantages for their chicks to deal with their particular situation (Schwabl, 1996; Eising et al, 2001; Lipar and Ketterson, 2000). Both egg size as well as egg composition (egg characteristics) are believed to influence incubation duration (Parsons, 1972; Ricklefs and Smeraski, 1983; Martin and Arnold, 1991; Bollinger, 1994; Sockman and Schwabl, 2000; Eising et al., 2001). Smaller eggs may take less time to hatch (Lack 1968; Parsons, 1972; Martin and Arnold, 1991; Arnold, 1993; Strausberger, 1998), which might be due to a lower energy requirement for embryonic development compared to large eggs (Parsons, 1972). Egg composition, independent of egg size, which also might also affect the duration of incubation. Eising et al. (2001) found that black-headed gull (*Larus ridibundus*) eggs with experimentally increased amounts of androgens had a significantly shorter incubation duration than eggs with a lower androgen concentration. However, Sockman and Schwabl (2000) showed that androgen injections in American kestrel (*Falco sparverius*) delayed hatching.

The incubation duration can also be affected by the offspring themselves. Persson and Andersson (1999) showed that pheasant (*Phasianus colchicus*) and mallard (*Anas platyrhynchos*) embryos were able to alter their timing of hatching so as to hatch synchronously and therefore to avoid asynchronous hatching. Hence, last-hatching embryos might accelerate the hatching process in response to signals from hatching siblings. It is usually the last-hatched chick that responses to signals from nestmates. However, whether this ability to respond to siblings present in the nest depends on differences between eggs has not been looked at yet.

Lesser black-backed gulls (*Larus fuscus*) usually lay a clutch of three eggs with the last egg being smaller, of lower mass and having a different egg composition (Harris, 1963; Parsons, 1972; Royle et al., 1999; Royle et al., 2001). Chicks within a brood hatch asynchronously with the last-laid egg hatching last and that chick suffers higher nestling mortality than their siblings (Royle and Hamer, 1998).

In this study, I tested whether egg quality influences the duration of incubation and of the hatching process in lesser black-backed gulls. I separated experimentally the effects of egg quality from the effects of hatching rank by cross-fostering eggs between nests in such a way that chicks from last-laid eggs were expected to hatch first and chicks from first-laid eggs were expected to hatch last within a brood. I predicted that chicks of last-laid eggs have a shorter incubation period than first-laid eggs when corrected for hatching rank.

## MATERIAL & METHODS

The experimental work was conducted in a large gull colony on Walney Island, Cumbria (NW England) in 2001. In this colony, about 24,000 pairs of lesser black-backed gulls breed every year. This colony is situated on relatively flat and sandy ground and offers reasonably good conditions for experimental manipulations (Nager et al., 2001).

Five different areas, all located near to each other (50-300m apart) within the centre of the colony, were searched daily for new nests, which were individually marked with numbered stones and randomly assigned to an experimental treatment. Eggs were weighed to the nearest 0.1g with an OHAUS balance on the day they were laid and were individually marked with a non-toxic, water-resistant marker according to their position in the laying sequence. The first-laid egg was called A, the second-laid egg B and the third-laid egg C. Similarly, chicks hatching from A-, B- and C-eggs are called A-chick, B-chick, and C-chick, respectively.

I carried out two experiments. *Experiment A* was conducted in one of the five experimental plots, while *experiment B* was conducted in the other four plots, which did not differ in pre-treatment egg mass of A-eggs and C-eggs (oneway ANOVA, A-eggs: $F_{3,120} = 0.44$, $p = 0.726$; C-eggs: $F_{3,119} = 0.66$, $p = 0.578$) and were therefore combined for the experimental manipulations and statistical analysis.

It has been shown that parental birds differ in their qualities. Poor quality parents lay their eggs relatively late in the breeding season (Brouwer et al., 1995), lay smaller clutches (Monaghan et al., 1998), and/or lay relatively smaller eggs (Parsons, 1972; Bolton, 1991) than the high quality pairs. In order to reduce the variation in quality of birds used in the experiments, I only included nests with three eggs that were laid in the early part of the laying season (i.e. before May 16th), and with eggs of average mass in order to insure equal conditions for the chicks. Fresh egg mass was compared to the average egg mass for each laying rank recorded in previous years at that colony and only eggs within ± 9g of these averages were included in the experiment. A total of 170 lesser black-backed gull nests, each with a clutch of three eggs of average size were marked.

*Experiment A*:

At egg laying, I created experimental clutches of three eggs where the expected hatching order was changed in relation to the laying order. The day the C-egg from one nest was laid it was exchanged with the A-egg from another nest that started to lay at the same day.

*Experiment B*:

Shortly before hatching, I created experimental clutches of three eggs where the expected hatching order was changed in relation to the laying order. The C-egg from one nest was exchanged with the A-egg of another clutch where both eggs were pipping.

Through the manipulation of the expected hatching order relative to the laying order, in both experiment A and experiment B, chicks from last-laid eggs became more advanced in the hatching process relative to their new nestmates whereas chicks from last-laid eggs became delayed compared to their nestmates. In control nests, eggs of the same laying rank (either two A-eggs or two C-eggs) were exchanged between nests in order to disturb these nests at a similar level than experimental nests.

Egg-exchange led to the following two experimental groups and those were compared to a control group. All nests were randomly assigned to one of the three treatment groups.

*Experiment A*:

1. Control group ABC (n = 16).

   Nests with a clutch of three eggs, which hatch at their initial hatching rank.

2. Treatment group ABA (n = 10).
   Nests with a clutch of three eggs where eggs hatched in the order they were laid.
3. Treatment group CBC (n = 20).
   Nests with a clutch of three eggs of which the first-hatched chick is a C-chick.

*Experiment B:*

1. Control group ABC (n = 49).
   Nests with a clutch of three eggs, which hatched according to their initial hatching rank.
2. Treatment group ABA (n = 40).
   Nests with a clutch of three eggs of which the last-hatched chick is an A-chick.
3. Treatment group CBC (n = 35).
   Nests with a clutch of three eggs of which the first-hatched chick is a C-chick.

At pipping, non-toxic acrylic paint was applied with a fine grass stem onto the egg tooth of each chick (one colour for each rank) in order to assign which chick hatched from which egg once the chicks had hatched.

During the hatching period, nests were visited twice a day in order to monitor and record the time of pipping (when the eggs were cross-fostered) and the time of hatching. Hatching was defined as complete emergence from the shell and was recorded to the nearest 24 hours. Incubation duration (the time from the day an egg was laid until hatching) and the interval between pipping and hatching (p-h-interval) were calculated for each chick. Furthermore, within all nests the laying interval (the day an egg was laid until the day the following egg within the nest was laid) between eggs and the hatching interval (the day a chick hatched until the day the next chick within the nest hatched) between chicks were determined.

In *experiment A*, I compared laying dates (the date at which the eggs were laid across the laying season), laying intervals, egg mass and incubation duration both between control ABC nests and treatment group ABA nests, and between control ABC nests and treatment group CBC nests. Thus, eggs of different quality but at the same within-brood rank were compared (AB**C** – AB**A**; **A**BC – **C**BC). For the statistical analysis I used oneway ANOVA and paired t-test.

In *experiment B*, I compared laying dates, laying intervals, incubation duration, the interval between piping and hatching, and hatching intervals between treatment groups as well as within treatment groups. Thus, I compared

(a) eggs of both different quality and different within-brood ranks (**ABC**) using oneway ANOVA, Pearson correlation and paired t-test,

(b) eggs of the same quality but with different within-brood ranks (**ABA**, **CBC** using oneway ANOVA and paired t-test,

(c) eggs of different quality but at the same within-brood rank (AB**C** – AB**A**; **A**BC – **C**BC) using oneway ANOVA and paired t-test.

Chicks hatched first within a nest are called F-chicks independent of initial laying rank. Similarly, chicks hatched second are called S-chicks and chicks hatched last L-chicks. The hatching ranks across the hatching order within a brood are called 1 for F-chicks, 2 for S-chicks and 3 for L-chicks.

## RESULTS

Figure 1 shows the mean mass of A-, B- and C-eggs in control ABC nests. There was a significant difference in egg mass between eggs of different laying rank (figure 1).

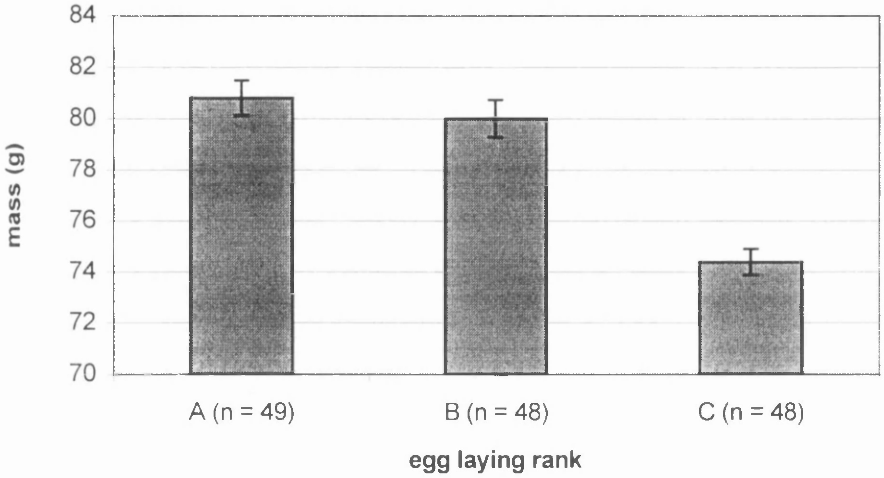


**Figure 1. Pre-treatment A-, B- and C-egg mass (mean ± s.e.) of control ABC nests. There were significant differences in egg mass between the three laying ranks (ANOVA, $F_{2,142} = 6.86$, $p = 0.001$). The significant differences of mean values are between A and C and between B and C (post-hoc Tukey test).**

Within control ABC nests, the incubation duration of the three eggs differed significantly across the laying sequence (figure 2). The duration of incubation decreased with increasing laying order with A-eggs (28.0 ± 0.16 days, n = 39) being incubated on average more than 2 days longer than C-eggs (25.8 ± 0.08 days, n = 39).

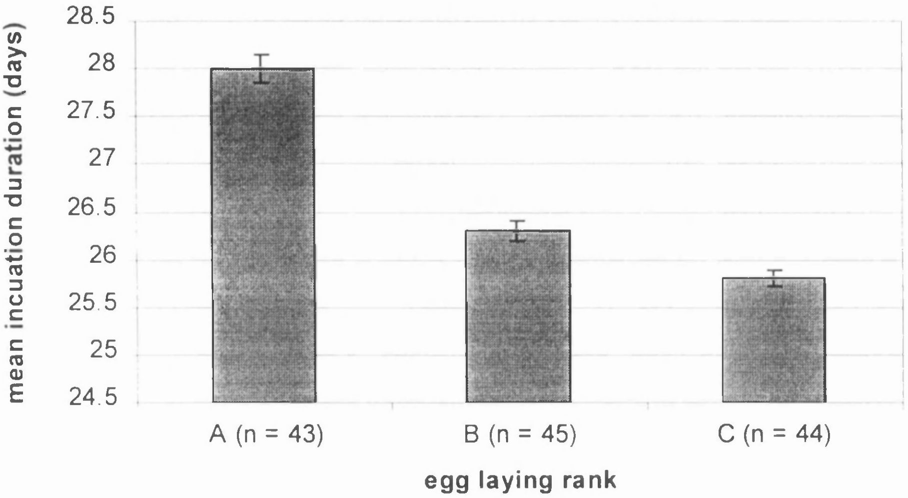
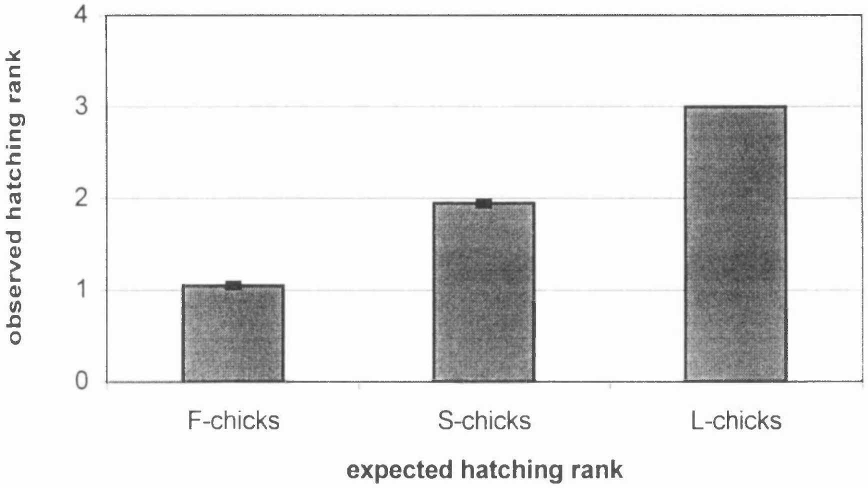


**Figure 2. Incubation duration (mean ± s.e.) of A-, B- and C-eggs in control ABC nests. There were significant differences in incubation duration between eggs of different laying rank (ANOVA, $F_{2,129} = 100.71$, $p < 0.001$). A-eggs (n = 39) had a significantly longer incubation duration than C-eggs of the same nest (n = 39) (paired t-test, $t_{38} = 13.36$, $p < 0.001$).**

There was no correlation between egg mass and incubation duration in any of the three laying ranks within control ABC nests (Pearson correlation, A-egg: $r = 0.24$, $p = 0.121$, $n = 43$; B-egg: $r = 0.13$, $p = 0.387$, $n = 44$; C-egg: $r = 0.19$, $p = 0.226$, $n = 43$).

Within both *experiment A* and *experiment B*, the exchange of eggs between nests successfully led to the expected change of the hatching order in relation to the laying order. The actually observed rank (1, 2, or 3) at which F-, S- and L-chicks hatched relative to the expected hatching rank due to manipulation is shown in figure 3. The same proportion of clutches hatched in the excepted order in all 3 treatment groups (experiment A: $\chi^2 = 4.17$, df = 2; $p = 0.125$; experiment B: $\chi^2 = 2.19$, df = 2, $p = 0.334$). Therefore the three treatment groups were combined for figure 3.

a)



b)

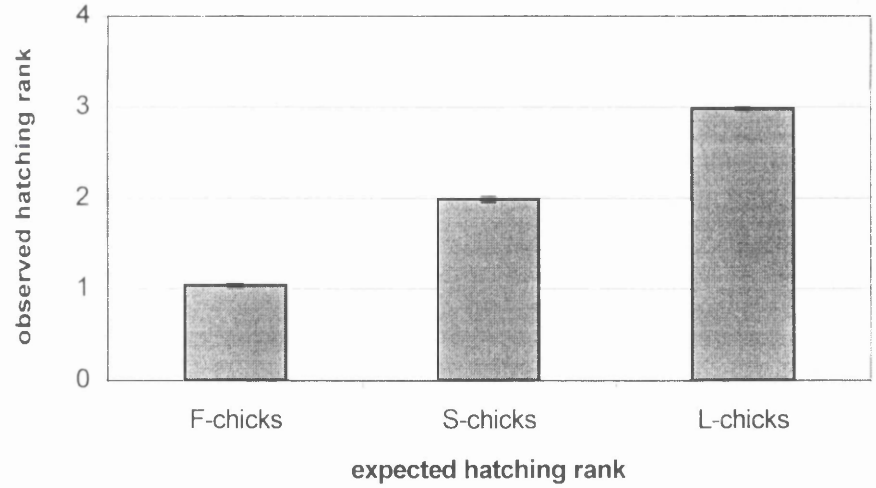


Figure 3. Actually observed rank (mean ± s.e) at which chicks of F-, S- and L-eggs hatched relative to the expected hatching rank (1,2,3) within all three treatment groups combined in (a) *Experiment A* and (b) *Experiment B*.

*Experiment A:*

There were no significant differences in laying date and the second laying interval (second to the last-laid egg) between control group ABC and treatment group ABA (laying date: $F_{1,24} = 0.48$, $p = 0.495$; laying interval: $F_{1,24} = 0.34$, $p = 0.466$). Last eggs were laid 2.1 ± 0.20 days after the second egg.

L-eggs in control group ABC and treatment group ABA did not differ in their egg mass despite their different initial laying ranks (A-egg: 77.6 ± 1.34g, n = 10; C-egg: 78.6 ± 1.82g, n = 16; ANOVA, $F_{1,24} = 0.15$, $p = 0.701$).

The incubation duration of L-eggs did not differ between control group ABC and treatment group ABA despite the different quality of the eggs (table 1).

**Table 1. Mean incubation duration of last-hatched eggs in control ABC nests and treatment group ABA nests.**

| treatment group | L-eggs |
|---|---|
| ABC | 26.2 ± 0.16 days (n = 16) |
| ABA | 26.4 ± 0.22 days (n = 10) |

There were no significant differences in L-egg incubation duration between the two treatment groups (ANOVA, $F_{1,24} = 0.62$, $p = 0.440$)

The mean laying date of the first egg differed significantly between control group ABC (42.4 ± 1.04 days, n = 16) and treatment group CBC (46.1 ± 1.10 days, n = 20; ANOVA, $F_{1,34} = 5.84$, $p = 0.021$).

The laying interval between the first and second egg did not differ between control ABC nests (1.9 ± 0.13 days, n = 16) and treatment group CBC nests (2.1 ± 0.07 days, n = 20; ANOVA, $F_{1,34} = 2.76$, $p = 0.106$).

F-eggs of control group ABC and experimental group CBC differed significantly in their mass (A-eggs: 84.5 ± 1.23g, n = 16; C-eggs: 74.8 ± 1.20g, n = 20; ANOVA, $F_{1,34} = 31.17$, $p < 0.001$). F-eggs in CBC nests (initial C-eggs) were on average 9.7g lighter than F-eggs in control nests (initial A-eggs).

The mean incubation duration of F-eggs did not differ between nests of the control ABC group and of treatment group CBC (table 2).

**Table 2. Mean incubation duration of first-hatched eggs in control ABC nests and treatment group CBC nests.**

| treatment group | F-eggs |
|---|---|
| ABC | 28.8 ± 0.32 days (n = 16) |
| CBC | 28.8 ± 0.23 days (n = 20) |

The mean incubation duration of F-chicks did not differ between the two treatment groups (ANOVA, $F_{1,34} = 0.03$, $p = 0.871$).

*Experiment B:*

Laying dates and laying intervals between eggs within nests of the three experimental groups are shown in table 3. There were no significant differences between experimental groups both in laying dates and in laying intervals between either A- and B-egg or B-egg and C-egg.

**Table 3. Mean laying dates and laying intervals per experimental group.**

| treatment group | laying date (± s.e.) | laying interval A-B (days) (± s.e.) | laying interval B-C (days) (± s.e.) |
|---|---|---|---|
| ABC (n = 49) | 41.1 ± 0.70 | 2.1 ± 0.04 | 2.2 ± 0.05 |
| ABA (n = 40) | 39.8 ± 0.92 | 2.1 ± 0.04 | 2.2 ± 0.07 |
| CBC (n = 35) | 40.8 ± 0.79 | 2.1 ± 0.04 | 2.1 ± 0.05 |

No significant difference between groups both in laying dates (ANOVA, $F_{2,121} = 0.77$, $p = 0.466$) and in laying intervals between either A- and B-egg or B- and C-egg (ANOVA, $F_{2,121} = 0.03$, $p=0.967$ and $F_{2,121} = 0.26$, $p=0.773$, respectively).

Furthermore, the pre-treatment mass of A-eggs and of C-eggs was not significantly different between the three experimental groups (ANOVA, A-eggs: $F_{2,121} = 0.79$, $p = 0.457$; C-eggs: $F_{2,121} = 0.15$, $p = 0.859$).

Among F-chicks, p-h-interval did not differ between A- and C-eggs. However, among L-chicks the p-h-interval was significantly longer in A-eggs (1.3 ± 0.11 days, n = 36) than in C-eggs (1.0 ± 0.07 days, n = 71) (table 4).

**Table 4. Mean interval between pipping and hatching (p-h interval) of first-hatched chicks and last-hatched chicks per treatment group.**

| | mean pipping-hatching interval (days ± s.e.) | |
|---|---|---|
| treatment group | F-chicks (n) | L-chicks (n) |
| ABC | $1.2^{a}$ ± 0.13 (43) | $1.1^{ab}$ ± 0.10 (43) |
| ABA | $1.0^{a}$ ± 0.11 (28) | $1.3^{a}$ ± 0.11 (36) |
| CBC | $1.2^{a}$ ± 0.10 (35) | $0.9^{b}$ ± 0.12 (28) |

There were no significant differences in the p-h-interval of F-chicks between groups (ANOVA, $F_{2,103} = 0.56$, $p = 0.573$). However, there were significant differences in the p-h-interval of L-chicks between groups ($F_{2,104} = 4.02$, $p = 0.021$). Mean values followed by different letters differ significantly (post-hoc Tukey test). Numbers in brackets are sample sizes.

Within nests of the treatment group ABA, A-chicks did not differ in their p-h-interval when they hatched first or last (Paired t-test, $t_{26} = 1.19$, $p = 0.247$). C-chicks within treatment group CBC, however, showed significant difference in p-h-intervals between hatching ranks. When a C-chick was the first chick to hatch the p-h- interval was significantly longer than when it hatched last (figure 4).

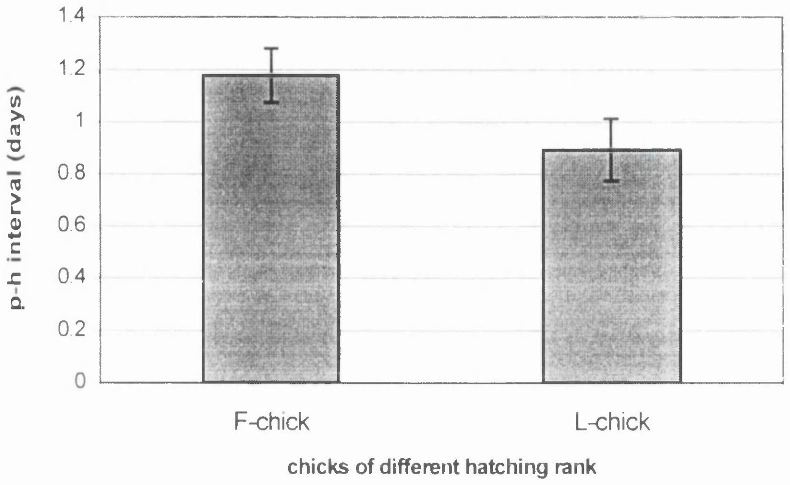


**Figure 4. Pipping-hatching interval (mean ± s.e.) of C-chicks of CBC nests at different hatching rank. F-chicks (n = 28) had a significantly longer p-h interval than L-chicks (n = 28) (paired t-test, $t_{27} = -2.12$, p=0.043).**

The incubation duration of F-chicks differed significantly between the three treatment groups (table 5.a). First-hatched A-chicks (28.3 ± 0.16 days, n = 73) had significantly longer incubation duration than first-hatched C-chicks (27.9 ± 0.14 days, n = 35) (table 5.a). Similarly, among L-chicks the incubation duration was significantly shorter in C-eggs (25.6 ± 0.10, n = 73) than in A-eggs (26.1 ± 0.11, n = 38) (table 5.b). Furthermore, the incubation duration of last-hatched C-eggs differed significantly between control ABC nests and treatment CBC nests (table 5.b).

**Table 5.a. Mean incubation duration of (a) first-hatched eggs (F-chicks) and (b) last-hatched eggs (L-chicks).**

a)

| | treatment group | | |
|---|---|---|---|
| | ABC (n) | ABA (n) | CBC (n) |
| mean (days) | 28.0 $^a$ (43) | 28.5 $^b$ (30) | 27.9 $^a$ (35) |
| ± s.e. | 0.15 | 0.16 | 0.14 |

The incubation duration of F-chicks differ between the treatment groups (ANOVA, $F_{2,105} = 4.31$, $p = 0.016$). Mean values followed by different letters differ significantly (post hoc Tukey test). Numbers in brackets are sample sizes.

b)

| | treatment group | | |
|---|---|---|---|
| | ABC (n) | ABA (n) | CBC (n) |
| mean (days) | 25.8 $^a$ (44) | 26.1 $^b$ (38) | 25.4 $^c$ (29) |
| ± s.e. | 0.08 | 0.11 | 0.12 |

Significant difference between the three treatment groups (ANOVA, $F_{2,108} = 8.42$, $p < 0.001$). Mean values followed by different letters differ significantly (post hoc Tukey test). Numbers in brackets are sample sizes.

The hatching interval between S- and L-chicks (S-L hatching interval) was significantly longer than the hatching intervals between F- and S-chicks (F-S hatching interval) within both control nests and ABA nests. However, within CBC nests there were no significant differences between S-L and F-S hatching intervals although there was a tendency in the same direction (table 6).

**Table 6. Comparison of hatching interval between first- and second-hatched chicks (F-S) and between second- and last-hatched chicks (S-L) using paired t-test.**

| treatment group | F-S hatching interval (± s.e.) (n) | S-L hatching interval (± s.e.) (n) | paired t-test | p |
|---|---|---|---|---|
| ABC | 0.47 ± 0.12 (36) | 1.56 ± 0.11 (36) | $t_{35} = 6.94$ | < 0.001 |
| ABA | 0.20 ± 0.18 (25) | 1.44 ± 0.20 (25) | $t_{24} = 3.67$ | 0.001 |
| CBC | 0.78 ± 0.15 (23) | 1.13 ± 0.16 (23) | $t_{22} = 1.28$ | 0.213 |

There was no statistically significant difference in the hatching interval between F- and L-chicks between the three treatment groups (Figure 5).

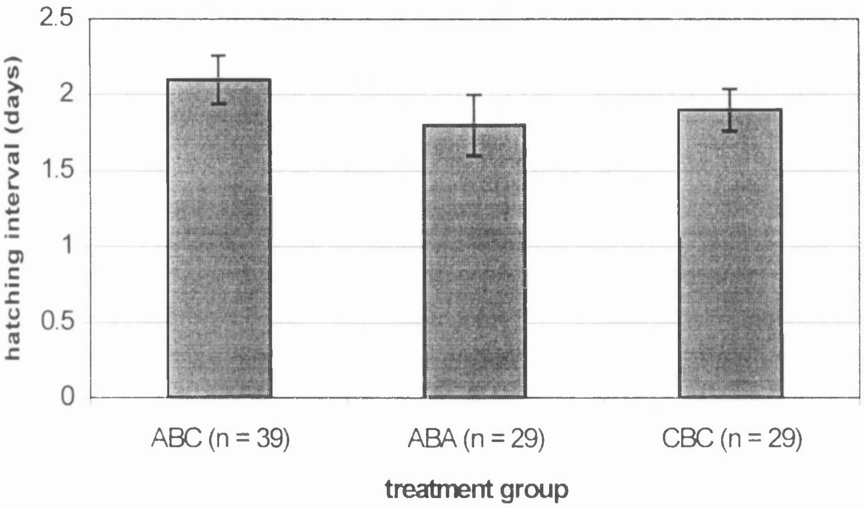


**Figure 5. Hatching interval (mean ± s.e.) between first- and last-hatched eggs per treatment. There was no significant difference in the hatching interval between treatment groups (ANOVA, $F_{2,94}$ = 1.00, p = 0.373).**

# DISCUSSION

The results of my study show that in lesser black-backed gulls (*Larus fuscus*), incubation duration differed between A-eggs and C-eggs, when hatching last. The same extent of differences between A- and C-eggs were also found in the pipping-hatching interval (hatching duration). The results therefore confirm my prediction that chicks of last-laid eggs have a shorter incubation duration than chicks of first-laid eggs, and suggest that these differences are mainly due to differences in the time it takes them to hatch.

In both *experiment A* and *experiment B*, cross-fostering of eggs successfully led to a change of the hatching order in relation to the laying order as I had expected (figure 3). Eggs that were set up to hatch first generally hatched also first in the nest and eggs that were set up to hatch last were generally the last to hatch. I had considered exchanging eggs not only of the same age, but also of a similar size. An A-egg as small as a C-egg, however, might have been of very poor quality (due to the poor quality of the parental birds, or other environmental factors), and so might have been the chick that would have hatched from this egg. C-chicks hatching from poor quality eggs might not have the same competitive ability/behaviour as other first-hatched chicks and thus the outcome of the experiments might have become confounded. Similarly, C-eggs of the size of an A-egg might give rise to chicks of above average quality. Accordingly, I decided to work only with eggs that were of about the average size for their laying sequence. Unfortunately, in *experiment A*, last-hatching A-eggs and last-hatching C-eggs were inadvertently not of significantly different mass since the ranges of mass of which I chose to collect the eggs turned out to be too wide. These results have too be kept in mind while discussing my data since it might have had an effect on my interpretation of the results. Nevertheless, first-hatched A-and C-chicks of *experiment A* as well as both first- and last-hatched A and C-chicks of *experiment B* were as expected of significantly different mass.

Within control nests the incubation duration of A-eggs (28.0 ± 0.16 days) was significantly longer than the incubation duration of C-eggs (25.8 ± 0.08 days). The initiation of incubation is thought to start when the second egg is laid (Bollinger, 1994). Thus, the incubation of A-eggs is delayed. Since B-eggs are generally laid 2 days after the A-egg (see table 3), full incubation starts 2 days after the A-egg has been laid, and therefore the incubation duration of A-eggs is about 26 days and not so much longer as C-egg incubation duration, after all. However, Parsons (1972) argues that incubation starts before the second egg is laid. If this is the case, the incubation duration of A-eggs might indeed be longer than of C-eggs.

There was no significant correlation between egg size and incubation duration within neither of the three laying ranks (A, B, and C) of control ABC nests. These results are contradictory to Parsons' (1972) study on herring gulls (*Larus argentatus*), which showed a significantly longer incubation duration in large A-eggs compared to small A-eggs. However, for this study only clutches with eggs of average size according to their laying rank were chosen for the experiments. The range of egg mass within a laying rank of the experimental nests combined was therefore smaller compared to the range of egg mass of the entire colony. Thus, the correlation between egg mass and incubation duration might not have been significant due to the very small differences in egg mass within a laying rank. However, Vinuela (1997) in his study on black kites (*Milvus migrans*) also did not find a correlation between egg size and incubation duration.

The results of *experiment A*, in which eggs were swapped between nests the day they were laid and therefore addressed differences in incubation duration between eggs of different laying rank, show that both in F-chicks and in L-chicks the duration of incubation did not differ significantly between A- and C-eggs. Nevertheless, within the last hatching rank, the incubation duration of A-eggs took on average 0.2 days longer compared to the incubation duration of C-eggs (table 1). Although not significant, this delay in *experiment A* is similar to the delay in the p-h interval of L-eggs found in *experiment B* (table 4). The p-h interval of last-hatched A-chicks was on average 0.3 days longer than the p-h interval of last-hatched C-chicks from *experiment B*. This difference in p-h interval between A-and C-chicks hatching last was statistically significant. In *experiment A*, the very small sample size of both control group ABC (n = 16) and treatment group ABA (n = 10) might be responsible for the difference of 0.2 days in the incubation duration of 26 ± 0.33 days being not significant. If the sample sizes would have been similar as in *experiment B*, this difference might well have been significant and could then be explained with the difference in p-h intervals of last-hatched A- and C-chicks.

In *experiment B*, C-eggs had a significantly shorter incubation duration than A-eggs both when hatching last and when hatching first within their nest. When hatching last, the different incubation duration might have been caused by a shorter interval between pipping and hatching in C-eggs than in A-eggs. From table 4 we could argue both that when hatching last A-eggs take unusually long to hatch or that C-eggs could speed up hatching. but not A-eggs. However, the analysis of p-h intervals of last- and first-hatched eggs within both treatment group ABA and CBC with paired t-test supports the latter interpretation. Thus, C-chicks were able to accelerate the hatching process at the end of incubation once they had fully developed. This ability was not found in A-chicks and might therefore be explained by egg-quality (size and composition) differences. Accordingly, Parsons (1972) showed in his study on herring gulls *(Larus argentatus*) that within natural broods embryos of C-eggs reduced the interval in development between B- and C-eggs from 32 hours during the first 4 days of incubation to 18-24 hours at hatching. The interval in embryonic development between A- and B-eggs remained on average 12 hours throughout the whole incubation period. In Parsons' work it remained unclear when

C-chicks catch up with B-chicks. This study suggests that they can catch up through hatching faster. In contrast, first-hatched C-chicks did not have a shorter p-h interval than first-hatched A-chicks but still showed a significantly shorter incubation duration. However, in *experiment B*, cross-fostering took place right at the end of incubation. The experimental design therefore did not allow to determine the exact incubation duration and thus cannot explain the shorter incubation duration in first-hatched C-chicks compared to first-hatched A-chicks. *Experiment A*, though, found no significant difference in the duration of incubation between first-hatched A- and C-chicks. I therefore conclude that only C-eggs hatching last can accelerate their p-h interval and that therefore this ability was due to differences between A-and C-eggs. It still remains unclear if this ability is strictly given through a small egg size or is affected by other properties of the eggs as well.

Parsons (1972) suggested that the reduction of the interval between B-and C-eggs between laying and hatching was due to the smaller size of C-eggs. Accordingly, Bollinger (1994) found a positive correlation between incubation duration and egg size in common terns (*Sterna hirundo*). The hormone level of the egg yolk might also have an effect on incubation duration. Royle et al. (2000) found in lesser black-backed gulls a higher testosterone level in C-eggs in comparison with the levels in A- and B-eggs. Yolk testosterone concentration has also been shown to increase with the position of an egg in the laying sequence in the lesser black-backed gull colony of this study (Verboven, pers. comm.). Elevated testosterone levels are thought to increase the rate of development of the chicks (Schwabl, 1996). Accordingly, Eising et al.'s (2001) work on black-headed gulls (*Larus ridibundus*) showed that A-eggs with higher androgen concentration than normal A-eggs had a significantly shorter incubation time. In that study it was not determined whether elevated androgen levels caused faster embryo development or shorter hatching duration. Lipar and Ketterson (2000) found a positive correlation between testosterone concentration and the mass of the hatching muscle *musculus complexus* in the red-winged blackbird (*Agelaius phoeniceus*). The muscle enables the chicks to break through the shell using dorsal and lateral head movements (Gross, 1985). Both testosterone concentration and *musculus complexus* mass increased with laying order. A bigger hatching muscle could translate into a decreased hatching duration and therefore might explain the faster p-h-interval in lesser black-backed gull chicks shown in this study. The higher testosterone level in C-eggs might not decrease the duration of the embryonic growth but enhance the growth of certain features of the chicks which in return helps the chick to decrease the hatching interval and therefore asynchrony. However, Sockman and Schwabl (2000) showed in their work on American kestrel (*Falco sparverius*) an opposing effect of testosterone, which delayed hatching when injected into A-eggs, and therefore the effects of androgens on embryonic development and hatching process might differ between species.

The fact that in F-eggs, C-chicks did not have a shorter p-h interval (i.e. the hatching duration) than A-chicks indicates that the hatching duration was also dependent on hatching rank. Persson and Andersson (1999) showed that mallard and pheasant embryos in experimental broods either decreased or increased the incubation duration depending on their hatching rank in order to hatch synchronously. Clues for their position within the hatching order were given through vocalisation of the siblings while they were still in the egg. In this work, cross-fostering of individual eggs did not only lead to a different hatching rank for these chicks within their broods but also changed the environment they hatched into. Vocalisation in gull chicks starts before the chicks have actually hatched (Tinbergen, 1953; Goethe, 1955). Communication between parents and their offspring, as well as between individual offspring, starts at the pipping stage with both parents and chicks responding to each other's sounds (Tinbergen, 1953; Goethe, 1955). Hence, a few days before C-chicks reach the pipping stage, their siblings start to vocally communicate with the parents. Furthermore, once A- and B-chicks have hatched parents start to feed the young and there is more activity within the nest. Thus, C-chicks hatch into a relatively "noisy" environment. This "noise" might give the last-hatching chicks clues about their hatching rank (Vinuela, 1997). Experimental C-chicks that hatched first within the nest did not experience the sounds and action of older siblings and their parents when they were the first chick to hatch and therefore did not have any indications to accelerate hatching. An increased development of C-chicks is thought to be an evolutionary adaptation for the C-chick to decrease the negative effects of asynchrony on last hatchlings' development and survival (Parsons, 1972; Schwabl, 1996). The decrease of the p-h-interval of last-hatching C-chicks might not only be an evolutionary adaptation to decrease asynchrony but also to increase the survival probability of C-chicks at hatching. Once A- and B-chicks have hatched incubation of the last egg might lack its constancy since the parental birds have to feed and care for their chicks, thus are not able to stay on the nest all the time. Hatching is a critical stage of development where the lack of constant heat supply by the incubating parent may cause death (Lee et al., 1993) or oxidative stress to the chick (Royle et al., 1999).

C-chicks had a shorter hatching duration than A-chicks, therefore, the manipulation of the hatching order relative to the laying order might have affected the synchrony within broods. Since asynchrony decreases the survival of the last chick (Maynard-Smith and Parker, 1976; Hebert and Barclay, 1985; Fujioka, 1985; Hahn, 1981; Heg and Van der Velde, 2001), a change in asynchrony might affect the survival probability of either the last-hatching chicks or the first-hatching chicks as well. However, the hatching interval between F- and L-chicks did not differ significantly between the three treatment groups (figure 5). Thus, cross-fostering of eggs in such a way that A-eggs are made to hatch last did not increase asynchrony in those experimental nests.

My work showed that, in C-eggs, having an advantageously high testosterone level does not necessarily lead to the chick hatching faster once it has pipped. The chick needs to hatch after its siblings in order to make use of its advantage. This result indicates the co-evolution of asynchrony and egg-quality and therefore gives evidence for an evolutionary advanced egg quality of last-laid eggs in lesser black-backed gulls.

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CHAPTER III.

# EFFECTS OF EGG CHARACTERISTICS AND ASYNCHRONY ON CHICK DEVELOPMENT AND SURVIVAL IN LESSER BLACK-BACKED GULLS (*LARUS FUSCUS*).

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## ABSTRACT

Asynchronous hatching leads to a within-brood size and age hierarchy with the last-hatching chick being disadvantaged in competitive situations due to its smaller size, younger age and delayed development compared to its nestmates. Additional within-brood differences in egg quality may also affect the success of the last-hatched chick. I examined the adaptive significance of the order in which different egg types hatched in lesser black-backed gull (*Larus fuscus*) broods. Through cross fostering of eggs, chicks from first-laid eggs (A-chicks) were made to hatch last and chicks from last-laid eggs (C-chicks) were made to hatch first within a clutch of three eggs. Growth rates, fledging success and mass and condition at fledging, did not differ between A- and C-chicks both within and between different hatching ranks. Therefore, unlike other studies no significant third chick disadvantage could be found. The result did not support predictions of the brood reduction hypothesis but support the theory of the C-chick being an insurance in the case A- and B-chicks die at an early stage.

## INTRODUCTION

In many bird species, parents allocate resources asymmetrically among their offspring both before and after hatching (reviewed in Mock and Parker, 1997).

In many species, eggs within a clutch show differences in their size and/or composition in relation to laying order (Slagsvold et al., 1984; Williams, 1994). These differences are reflected in the chicks: the last-hatched chick is generally smaller (Furness, 1983; Hario and Rudbäck, 1999; Nager et al., 2000a), of poorer condition (Bolton, 1991) and shows a lower survival rate than its siblings (e.g. Hahn, 1981; Griffiths, 1992; Heg and Van der Velde, 2001). These differences are enhanced through asynchronous hatching. Initiation of incubation before the entire clutch has been laid leads to a staggered hatching order. The last hatching chick is smaller and younger than its nestmates (Parsons, 1972) and therefore disadvantaged since its siblings have an advanced growth and are more experienced, both enabling these chicks to gain more resources (food, warmth) from their parents (Maynard-Smith and Parker, 1976; Hahn, 1981; Fujioka, 1985; Heg and Van der Velde, 2001) and a better chance to escape from predators (Hillström et al., 2000) than their youngest and smallest nestmate. However, the relative contributions of differences in egg size and asynchronous hatching to the poorer survival of the last-hatched chick still remain unclear.

This disadvantage has often been interpreted as an adaptation that enables parents to adjust their brood size to food availability during the nestling period; they will by allocate scarce resources only to the chicks with the highest survival probability (Brood Reduction Hypothesis, Lack, 1954). Alternatively, the last-laid egg might be an insurance strategy for the case that one or all of the first chicks die very soon after hatching. With an "extra" egg the parental birds would still be able to raise at least one chick. However, if the first chicks survive, not many resources are given to the last-hatched chick (Insurance Hypothesis, Stinson, 1979).

Recent findings on differential allocation of hormones, especially androgens, in relation to laying order have brought renewed interest to sibling rivalry. A higher level of testosterone in last-laid eggs might be an evolutionary adaptation to increase the last chicks' development and competitive behaviour in order to reduce the disadvantageous effects of hatching asynchrony (Schwabl, 1993; Lipar and Ketterson, 2000). Hence, last-laid eggs might not be just poor-quality eggs but also provide some specific advantages for last-hatching chicks.

Most gull species of the genus *Larus* lay a clutch of three eggs, which differ in their size, mass and composition (egg characteristics) across the laying sequence. The last-laid egg is smaller, lighter and has a different composition of egg components such as nutrients, hormones and others (Harris, 1963; Parsons, 1972; Royle et al., 1999, 2001; Nager et al, 2000a; Blount et al., 2002). The chick hatching from the last egg hatches later (chapter II) and has been shown to be smaller and of poorer condition (Bolton, 1991). Accordingly, last-hatched chicks generally have the lowest fledging success (Parsons, 1975; Royle and Hamer, 1998).

Various studies on the evolutionary significance of this third chick disadvantage in gulls, have shown that chicks from first- and second-laid eggs usually still fare better than the last chick even when the latter has been made to hatch synchronously and is of equal mass to its nestmates (Parsons, 1970, 1975, Hahn, 1981; Royle and Hamer, 1998). These results point out that the poor fitness of younger chicks was primarily caused by within-brood variation in egg or chick quality and not by hatching order. However, these studies compared between eggs of equal quality but at different hatching ranks rather than between eggs of different quality within ranks, thus mainly asynchrony has been addressed. Furthermore, Parsons (1974) showed that herring gull (*Larus argentatus*) chicks from last-laid eggs that were made to hatch first within a brood suffered less mortality than at their initial hatching rank. Similarly, chicks from first-laid eggs made to hatch last had a lower survival than normal first-hatching chicks. These results indicated that chick survival is not dependent on the hatching rank, but that egg quality may also affect chick survival. For example, a significantly higher testosterone level was found in the last-laid egg in comparison with the hormone levels in the first two eggs (Eising et al., 2001; Royle et al., 2001). This might lead to a faster growth of the chick, which is thought to be an advantageous adaptation for the youngest nestling to catch up with its siblings who are initially bigger and stronger (Schwabl, 1996; Lipar and Ketterson, 2000; Eising et al., 2001). Females might also provide the last egg with a high amount of testosterone to increase the aggressiveness of the young in order to enhance its ability to compete with its nestmates over parental resources (Schwabl, 1996), which could increase their growth as well.

There might be both disadvantages (size, age) and advantages (e.g. testosterone) given to last-hatching chicks. However, the effects of hatching rank and egg characteristics are difficult to separate in natural broods.

The aim of my study was to investigate the effects of egg quality and hatching rank on chick growth rate, mortality and fledging success in the lesser black-backed gull (*Larus fuscus*). Through the manipulation of the hatching rank of individual chicks I experimentally separated the effects of hatching rank from the effects of egg-quality on development and survival. Emphasis was put on the chicks of first- and last-laid eggs.

If differences in the composition of the last-laid egg are adaptive, I predict that when hatching last, chicks from first-laid eggs are less successful compared to chicks from last-laid eggs. In contrast, I expect that when hatching first, chicks from first-laid eggs are more successful than chicks from last-laid eggs. Moreover, if the observed within-clutch differences in egg quality are adaptive, I expect that experimental alteration of these differences should result in a poorer breeding success of manipulated broods compared to control broods.

## MATERIAL AND METHOD

The experimental work was conducted in the large gull colony on Walney Island, Cumbria (NW England) in 2001. In this colony, about 24,000 pairs of lesser black-backed gulls (*Larus fuscus*) breed every year. This colony is situated on relatively flat and sandy ground and offers reasonably good conditions for observations (Brown, 1967; Tinbergen and Falcus, 1970; Nager et al., 2001).

During nest building, three separate plots within the central area of the colony were searched daily for new experimental nests and were marked individually with numbered stones. The plots were all close to each other and near the centre of the colony. The study plots did not differ in laying date (ANOVA, $F_{2,101} = 0.47$, $p = 0.627$), egg mass (A-egg: $F_{2,101} = 0.57$, $p = 0.566$; C-egg: $F_{2,100} = 0.84$, $p = 0.435$) and hatching date ($F_{2,87} = 0.43$, $p = 0.653$). Therefore, I combined the data from the three study plots.

Every nest was checked daily in order to determine egg laying dates and laying sequence until the clutches were complete. Birds differ in their qualities; poor quality parents lay their eggs relatively late in the breeding season (Brouwer et al., 1995), lay smaller clutches (Monaghan et al., 1998), and/or lay smaller eggs (Parsons, 1972; Bolton, 1991) than the average breeding pairs. In order to reduce the variation in quality of birds used in the experiments, I only included nests with three eggs that were laid in the early part of the laying season (i.e. before May 16th ), and with eggs of average mass in order to ensure equal conditions for the chicks. Fresh egg mass compared to the average egg mass for each laying rank recorded in previous years at that colony and only eggs within ± 9g of these averages were included in the experiment. A total of 104 nests with these standardised conditions were found and experimentally manipulated for this study.

Each egg was weighed to the nearest 0.1 g with an OHAUS balance within 24 hours after laying and marked individually with a waterproof non-toxic marker according to its rank in the laying order. First-laid eggs were called A-eggs, second-laid eggs B-eggs and third-laid eggs C-eggs. Similarly, the chicks are called A-, B-, and C-chick referring to the egg they hatched from.

During the hatching period, the nests were visited twice a day to control the eggs for pipping stages (when chicks have pecked a small whole through the shell). At that stage, I applied non-toxic acrylic paint with a fine grass stem onto the egg tooth of each chick (one colour for each rank) in order to assign which chick hatched from which egg once the chicks had hatched.

At pipping, cross fostering of eggs took place. The day the C-egg from one nest was in the pipping stage, it was exchanged with the A-egg from another nest where the chick started to peck a hole through the shell at the same day. Thus, after the cross-fostering initially low-quality chicks (C-chicks) had an advantage of a high rank, while chicks with an advantage of a high initial quality (A-chicks) were the last-hatching ones. I did not match eggs to be exchanged by their size, because an A-egg as small as a C-egg might have been of poor quality (due to the poor quality of the parental birds, or other environmental factors (Bolton, 1991), and so might have been the chick that would have hatched from this egg. A poor-quality A-chick wouldn't have had the same competitive ability/behaviour as other first-hatched chicks and could have confounded the outcome of the experiment. The same could have happened with C-eggs with a size of an A-egg. Accordingly, I decided to work only with eggs that were of about the average size according to their laying sequence.

Cross-fostering led to three experimental groups. Nests were randomly assign to one of these treatment groups.

1.) Control group A B C (n = 39).

Nests with a clutch of three eggs where eggs hatched in the order they were laid.

2.) Treatment group A B A (n = 38).

Nests with a clutch of three eggs where both the last-hatched (L) and the first-hatched (F) chick have hatched from A-eggs.

3.) Treatment group C B C (n = 27).

Nests with a clutch of three eggs where both the first-hatched (F) and the last-hatched (L) chick have hatched from C-eggs.

The day the chicks hatched, they were marked individually with little colour dots on each wing and on the throat using live stock dye (Stay-on Stock Marker Spray, Trilanco, Lancashire). In addition each chick was marked with numbered leg rings. These rings were made out of 10cm-long pieces of white isolation tape, which were folded in the middle so that the two sticky sides got to lay on top of each other and were then put around one of the chicks' leg and stapled at the ends. I wrote the nest number onto these rings in order to assign which chick belonged to which nest. For the colour marking I used two different coloured dyes, blue and red, to spray two chicks of each nest, independent of rank or age. Thus, each nest had a red-marked and a blue-marked chick and one without any colour. These markings were important for the behavioural observations in order to distinguish between the three chicks of a brood from the distance.

At hatching, chicks were weighed to the nearest 0.1g with an OHAUS balance, their tarsus and head-bill (back of the head to the tip of the bill) were measured to the nearest 0.1mm using a dial calliper, and the lengths of wing (maximum wing chord) was taken to the nearest 1.0 mm using a wing ruler. From the time the chicks had reached a mass of 100g onward they were weighed to the nearest 1.0g using a spring balance, and after they had reached a mass of 300g the mass was taken to the nearest 25g using another spring balance. The 5 measurements were first taken when hatchlings were found and their plumage was dry, and from then onwards every 4 days until ca. day 28.

From the age of about three weeks onwards it became very difficult to catch and/or find the chicks to take the measurements. Chicks either ran away and chasing them would have been too great a disturbance for the colony, or the chicks were hiding in the vegetation and were not found. Thus, data on older chicks are less complete. However, observation of older colour-marked birds supplemented the information on chick survival. I first looked at survival and growth of the chicks until 21days of age. Chicks that survived until day 28 were considered as fledglings, since they are close to fledging (36-40 days, Cramp and Simmons, 1983; Bolton, 1991) and the mortality after 28 days is very low (Nager et al., 2000b). The number of fledglings and mortality before fledging was determined through observations of the nests and dead chicks found during nest checking. Nests where it was impossible to determine if chicks were missing, dead or actually fledged (since the vegetation was too high for observations of the nests) were grouped as "unknown" cases. Dead chicks found either on or near the nest were considered to have died from starvation, whereas chicks that have neither been found during nest checking nor seen during observations have probably been predated by other gulls, foxes or stoats and were therefore recorded as dead (missing). In the case a chick was missing I continued looking for it on a daily bases during the following 8 days in order to be sure of its disappearance. Therefore, we distinguished two causes of death: (i) starvation, and (ii) predation. Chicks were recorded to have died or to have been predated at either the day they were found dead or the day they were last seen alive. Chicks that died from botulism and unknown cases were excluded from the analysis of mortality. Both botulism and unknown cases were relatively rare and occurred equally often in F-chicks of treatment groups ABC and CBC, in L-chicks of treatment groups ABC and CBC, and in both F- and L-chicks within control nests (Chi-square test, F-chicks: $\chi^2 = 0.78$; L-chicks: $\chi^2 = 0.0$;

control nests: $\chi^2 = 0.0$; all $p > 0.250$). As a result, the fledging success determined in this study is a minimal estimate.

I determined the growth rate of mass, wing length, tarsus length and head-bill length between the days 5 and 21 (time of linear growth) of individual chicks by calculating the instantaneous growth rates $R = (\log W_2 - \log W_1)/(t_2 - t_1)$ where W = mass or length according to measurement and t = time. Only chicks that survived until the age of 28 days were included in this calculation of growth rate since I was only interested in successful chicks in order to investigate whether A-chick fledglings and C-chick fledglings differed in their growth pattern. I took measurements from day 28 as the fledging mass/size.

Body size of hatchlings and of fledglings was calculated from wing, tarsus and head-bill lengths using PCA. The first component of this analysis describes 60% (hatchlings) and 66% (fledglings) of the variance of the three measurements representing size. Residuals from the regression analysis of mass on size were taken as a measure of. I took the residuals as a proportion of the predicted values to present the condition of the chicks at hatching and at fledging (arcsin transformed for statistical analysis). Growth rates and fledging size, mass and condition has been compared between treatment groups using ANCOVA with brood size at fledging as the covariate. Sample sizes of the analysis of size, condition and mass of the chicks at hatching differ in some cases since I determined the hatching size of more chicks than I took the mass of hatchlings.

To compare the growth rates of A- and C-chicks of control nests, I first looked for any differences between the growth rates of A- and B-chicks within control nests using oneway ANOVA. If the growth rates of these chicks did not differ significantly I took the mean value of the growth rates of A- and B-chicks and compared this with the growth rate of control C-chicks using oneway ANOVA and adjusted the p-values following to the Bonferroni transformation.

Finally, the productivity per brood was recorded as the number of fledglings per brood and was compared between experimental group.

The data on condition, growth and survival were analysed in relation to hatching rank (F- and L-chicks) and quality of eggs they hatched from (A-and C-eggs):

*1) Comparisons of A- and C-chicks in control ABC nests*

I compared A- and C-chicks of ABC control nests in order to compare chicks that differ in both hatching rank and quality of the egg they hatched from using oneway ANOVA, paired t-test and chi-square test.

*2) Comparison of chicks hatching from the same egg quality, but differ in hatching rank*

I compared F-and L-chicks within both treatment group ABA and CBC in order to compare chicks which hatched from eggs of similar quality but differ in hatching rank using paired t-test, oneway ANOVA and chi-square test.

*3) Comparison of chicks of the same hatching rank hatching from different egg qualities.*

I compared L-chicks of treatment groups ABC and ABA, as well as F-chicks of treatment groups ABC and CBC, in order to compare chicks within hatching ranks and which differed only in the quality of the egg they hatched from using ANCOVA, oneway ANOVA, chi-square test, paired t-test, Fisher's exact test, and Kruskal-Wallis test.

## RESULTS

### *1) Comparisons of A- and C-chicks in control ABC nests*

Within control nests, F-chicks and L-chicks differed significantly in hatching mass and hatching condition (table 1). However, size did not differ between these chicks (paired t-test, $t_{30} = 0.66$, $p = 0.512$).

**Table 1. Mean hatching mass and hatching condition of A-chicks and C-chicks within control nests.**

| Chick | mass (g) ± s.e. | condition ± s.e. |
|---|---|---|
| A (n = 30) | 56.3 ± 0.62 | 0.021 ± 0.064 |
| C (n = 30) | 52.2 ± 0.53 | -0.019 ± 0.065 |

Significant differences between A- and C-chicks in hatching mass and hatching condition. (paired t-test, mass: $t_{29} = 5.38$, $p < 0.001$, condition: $t_{29} = 2.55$, $p = 0.016$).

Control A- and B-chicks did not differ in any of the four growth measurements (mass: $F_{1,12} = 1.98$, $p = 0.185$, tarsus: $F_{1,12} = 1.65$, $p = 0.223$, wing: $F_{1,12} = 2.895$, $p = 0.115$, head-bill: $F_{1.12} = 2.40$, $p = 0.147$). C-chicks tended to have a higher tarsus growth rate than their older siblings, whereas growth rates of mass, wing, and head-bill length did not differ between C-chicks and A/B-chicks (figure).

Mass, body size and condition of chicks at fledging were analysed in the same way as the growth rate. No differences were found in fledging mass, fledging size or fledging condition between A- and B-chicks (ANOVA, mass: $F_{1,10} = 0.47$, $p = 0.509$; size: $F_{1,10} = 0.19$, $p = 0.676$; condition: $F_{1,16} = 1.65$, $p = 0.227$). C-chick fledglings did not differ significantly in mass. size or condition from the mean of A- and B-chicks (ANOVA, mass: $F_{1,15} = 0.14$, $p = 0.710$: size: $F_{1,15} = 1.17$, $p = 0.297$; condition:$F_{1,15} = 0.53$, $p = 0.239$).

A-chicks (n = 31) and C-chicks (n = 34) within control nests did not differ in their fledging success or the causes of their death (fledging success: chi-square test, $\chi^2 = 0.12$, df = 1,

$p > 0.50$; cause of death: fisher's exact, df = 1, $p = 0.645$, 1-tailed). 16 (51.6%) A-chicks and 16 (47.1%) C-chicks survived until fledging. Moreover, the age at which chicks died did not differ between A- and C-chicks (figure 2). However, within control nests, A-chicks died before the C-chick more often (in 80% of all A-chick deaths) than C-chicks died before the A-chick (in 44.4% of all C-chick deaths) (chi-square test, $\chi^2 = 4.31$, df = 1, $p < 0.05$).

Cross-fostering of pipped eggs successfully changed the hatching order in the experimental groups (Chapter II). Hatching success within experimental nests was very high for all ranks (78-100%) indicating that egg swapping did not affect survival at hatching. Furthermore, laying dates and hatching dates did not differ between experimental groups (table 2).

**Table 2. The mean date at which eggs where laid and the mean date when chicks hatched in control ABC nests, experimental ABA nests and experimental CBC nests.**

| treatment groups | laying date (mean ± s.e.) | hatching date (mean ± s.e.) |
|---|---|---|
| ABC | 41.4 ± 0.81 (n = 39) | 69.1 ± 0.84 (n = 34) |
| ABA | 39.9 ± 0.95 (n = 38) | 68.1 ± 1.23 (n = 29) |
| CBC | 41.6 ± 0.91 (n = 27) | 69.4 ± 0.90 (n = 27) |

Laying dates and hatching dates did not differ between experimental groups (ANOVA, laying date: $F_{2,101} = 1.07$, $p = 0.347$. hatching date: $F_{2,82} = 0.45$, $p = 0.637$).

### 2) *Comparison of chicks hatching from eggs of the same characteristics, but differ in hatching rank*

In treatment ABA nests, A-chicks that hatched first did not differ from A-chicks that hatched last in terms of hatching mass, hatching size and hatching condition (paired t-test, mass: $t_{21} = 0.03$, $p = 0.980$, condition: $t_{20} = -0.09$, $p = 0.952$, size: $t_{21} = 1.31$, $p = 0.205$).

Fledging success and proportion of different causes of mortality did not differ between F-and L-chicks (fledging success: chi-square-test, $\chi^2 = 0.16$, df = 1, $p > 0.50$; causes of death: Fisher's exact, df = 1, 1-tailed, $p = 0.376$). A-chicks that hatched last tended to die at a younger age than A-chicks that hatched first (figure 3a), although, this difference was not statistically significant. In 46.2% (n = 13) of all F-chick deaths F-chicks died before the L-chick within their broods, whereas L-chicks predeceased F-chicks within the same brood in 22.2% (n = 18) of all L-chick deaths. However, this difference was not statistically significant (chi-squared test, $\chi^2 = 1.96$, df = 1, $p > 0.25$).

F-chicks and L-chicks within treatment group CBC did not differ significantly in hatching mass, hatching size and hatching condition (paired t-test, mass: $t_{19} = 0.83$, $p = 0.416$, size: $t_{18} = 0.11$, $p = 0.910$, condition: $t_{18} = -1.01$, $p = 0.325$).

C-chicks of different hatching ranks did not differ in fledging success or in the causes of death (fledging success: chi-square test, $\chi^2 = 0.03$, df = 1, $p > 0.75$; cause of death: Fisher's exact, df = 1, $p = 0.674$). Furthermore, the age at which chicks died did not differ significantly between F- and L-chicks (figure 3b). C-chicks that hatched first died before the last-hatched C-chick in 40% (n = 15) of all first-hatched C-chick death, whereas last-hatched C-chicks died before the first-hatched C-chick in 33.3% (n = 15) of all last-hatched C-chick deaths. This difference was not significantly ($\chi^2 = 0.14$, df = 1, $p > 0.50$).

### *3) Comparison of chicks of the same hatching rank hatching from egg of different characteristics.*

While L-chicks of the control group ABC and treatment group ABA had a similar hatching size (ANOVA, $F_{1,65} = 2.25$, $p = 0.138$), hatching mass and hatching condition of these chicks differed significantly between the two groups (table 3).

**Table 3. Mean hatching mass and hatching condition of L-chicks from control nests (ABC) and experimental nests (ABA).**

| Treatment | mass (g) $\pm$ s.e. | condition (%) $\pm$ s.e. |
|---|---|---|
| ABC (n = 30) | 52.7 $\pm$ 0.54 | -0.032 $\pm$ 0.012 |
| ABA (n = 27) | 57.2 $\pm$ 1.09 | 0.038 $\pm$ 0.016 |

Significant differences between treatments in L-chick mass and condition (ANOVA, mass: $F_{1,65} = 14.621$, $p < 0.001$, condition: $F_{1,52} = 12.32$, $p = 0.001$).

Using ANCOVA I compared growth rates, fledging mass, fledging size and fledging condition of L-chicks in nests of treatment groups ABC and ABA, with brood size at hatching as a covariate. There was no effect of the interaction egg characteristics x brood size on L-chick tarsus, wing or head-bill growth rates in ($p > 0.66$). Furthermore, growth rates of wing and head-bill did not differ between chicks hatching from eggs of different characteristics (wing: $F_{1,18} = 2.12$, $p = 0.163$, head-bill: $F_{1,18} = 0.05$, $p = 0.822$) or between broods of different sizes (wing: $F_{1,18} = 1.37$, $p = 0.258$, head-bill: $F_{1,18} = 0.39$, $p = 0.542$). Tarsus growth rate did not differ between broods of different sizes ($F_{1,18} = 0.85$, $p = 0.368$), but the tarsus of L-chicks that hatched from C-eggs tended to grow faster than the tarsus of L-chicks that hatched from A-eggs although the difference was not statistically significant ($F_{1,18} = 3.67$, $p = 0.072$). Similarly, there was no significant effect of brood size and egg characteristics on body mass growth (interaction: $F_{1,16} = 3.15$ , $p = 0.095$; egg characteristics: $F_{1,17} = 0.10$, $p = 0.761$; brood size: $F_{1,17} = 0.003$, $p = 0.956$).

There were no differences in mass, size and condition at fledging between L-chicks hatched from different egg characteristics (ANCOVA, mass: $F_{1,17} = 0.18$, $p = 0.679$, size: $F_{1,18} = 1.98$, $p = 0.177$, condition: $F_{1,17} = 0.26$, $p = 0.616$) or L-chicks raised in broods of different size (mass: $F_{1,17} = 0.11$, $p = 0.740$, size: $F_{1,18} = 1.10$, $p = 0.310$, condition: $F_{1,18} = 0.24$, $p = 0.628$). Moreover, the effects of egg characteristics and brood size at hatching did not interact in any of these three aspects ($p > 390$).

Table 4 compares fledging success and causes of mortality between L-chicks of control ABC and treatment group ABA nests. In control nests, 47.1% of L-chicks fledged, whereas in ABA nests only 37.9% of L-chicks fledged. This difference was, however, not statistically significant (table 4). There were also no significant differences in the causes of mortality between treatments (table 4), although, L-chicks that hatched from A-eggs tended to be found dead less likely but disappeared more often.

**Table 4. Nestling mortality and cause of mortality of L-chicks in ABC and ABA nests given as the absolute number of observed cases and as a percentage of total number of chicks per treatment.**

| | Mortality | | | |
|---|---|---|---|---|
| Treatment | found dead | missing | total | Fledged |
| ABC (n = 34) | 6 (17.6%) | 12 (35.3%) | 18 (52.9%) | 16 (47.1%) |
| ABA (n = 29) | 1 (3.4%) | 17 (58.6%) | 18 (62.0%) | 11 (38.0%) |

There were no significant differences between L-chicks of ABC nests and of ABA nests in Fledging success (chi-square test, $\chi^2 = 0.51$, df = 1, $p > 0.05$) and in the frequency of causes of death (Fisher's exact, one-tailed, $p = 0.044$).

L-chicks in ABA nests died at an earlier age than L-chicks in ABC nests, although the difference was not statistically significant (figure 4a).

F-chicks of control ABC nests and treatment CBC nests differed significantly in mass and condition at hatching (table 5), whereas hatching size was not different between these groups (ANOVA, $F_{1,64} = 1.77$, $p = 0.189$).

**Table 5. Mean hatching mass and hatching condition of F-chicks from control nests (ABC) and experimental nests (ABA).**

| Treatment | mass (g) ± s.e. | condition (%) ± s.e. |
|---|---|---|
| ABC | 56.4 ± 0.60 (n = 32) | 0.038 ± 0.012 (n = 32) |
| CBC | 52.5 ± 1.02 (n = 23) | -0.055 ± 0.015 (n = 22) |

Significant differences between treatments in F-chick hatching mass and condition (ANOVA, mass: $F_{1,53} = 11.97$, $p = 0.001$, condition: $F_{1,52} = 25.39$, $p = 0.001$).

There was no effect of the interaction between treatment and brood size on any of the growth rates of mass, tarsus, wing and head bill of chicks that reached the fledging age of 28 days ($p > 0.139$). Growth rates of mass, tarsus, wing and head-bill in F-chicks were also not affected by brood sizes or eggs characteristics (effect of brood size on mass: $F_{1,20} = 0.68$, $p = 0.421$, tarsus: $F_{1,20} = 2.73$, $p = 0.114$, wing: $F_{1,20} = 2.78$, $p = 0.111$, head-bill: $F_{1,20} = 2.56$, $p = 0.125$; effect of egg characteristics on mass: $F_{1,20} = 0.001$, $p = 0.982$, tarsus: $F_{1,20} = 0.52$, $p = 0.479$, wing: $F_{1,20} = 0.93$, $p = 0.346$, head-bill: $F_{1,20} = 0.04$, $p = 0.843$).

At fledging, F-chicks of ABC and CBC nests with either 1, 2 or 3 fledglings did not differ in mass (egg characteristics: $F_{1,15} = 0.15$, $p = 0.704$, brood size: $F_{1,15} = 0.05$, $p = 0.827$), size ($F_{1,15} = 0.18$, $p = 0.680$; $F_{1,15} = 0.50$, $p = 0.489$), or condition ($F_{1,15} = 1.63$, $p = 0.223$; $F_{1,15} = 1.10$, $p = 0.312$).

F-chicks of the control group died at an earlier stage of development than F-chicks of treatment group CBC, although the mean age at which chicks died did not differ significantly between treatment groups (figure 4b).

Fledging success and causes of mortality of F-chicks did not differ significantly between treatment groups (table 6).

**Table 6. Nestling mortality and cause of mortality of F-chicks in ABC and CBC nests given as the absolute number of observed cases and as a percentage of total number of chicks per treatment.**

| | Mortality | | | |
|---|---|---|---|---|
| treatment | found dead | missing | total | fledged |
| ABC (n = 31) | 5 (16.1%) | 10 (32.3%) | 15 (54.3%) | 16 (51.6%) |
| CBC (n = 24) | 3 (12.5%) | 12 (50%) | 15 (66.6%) | 9 (37.5%) |

There were no significant differences between F-chicks of ABC nests and of CBC nests both in nestling mortality (Chi-square test, $\chi^2 = 1.08$, $p > 0.05$) and in frequency causes of death (Fisher's exact: one-tailed, $p = 0.341$).

The proportion of eggs which produced a fledgling per clutch of three eggs did not differ significantly between treatment groups (figure 5).

In over 50 % of treatment CBC nests none of the chicks fledged, whereas in only 35% of control ABC nests and only 34% of treatment ABA nests produced no fledgling at all (Chi-square test, $\chi^2 = 1.01$, df = 2, $p = 0.603$). Only 20.5%, 18.4%, and 14.8% of ABC, ABA and CBC nests, respectively, fledged three young (Chi-square test, $\chi^2 = 0.35$, df = 2, $p = 0.539$)

Within both control ABC nests and treatment ABA nests, L-chicks did not have a different mortality rate when their older siblings either died or survived (insurance value) (Fisher's exact, ABC: df = 1, one-tailed, $p = 0.099$; ABA: df = 1, one-tailed, $p = 0.044$).

Furthermore, last-hatched A-and C-chicks did not differ in their insurance value (Fisher's exact, df = 1, one-tailed, $p = 0.323$).

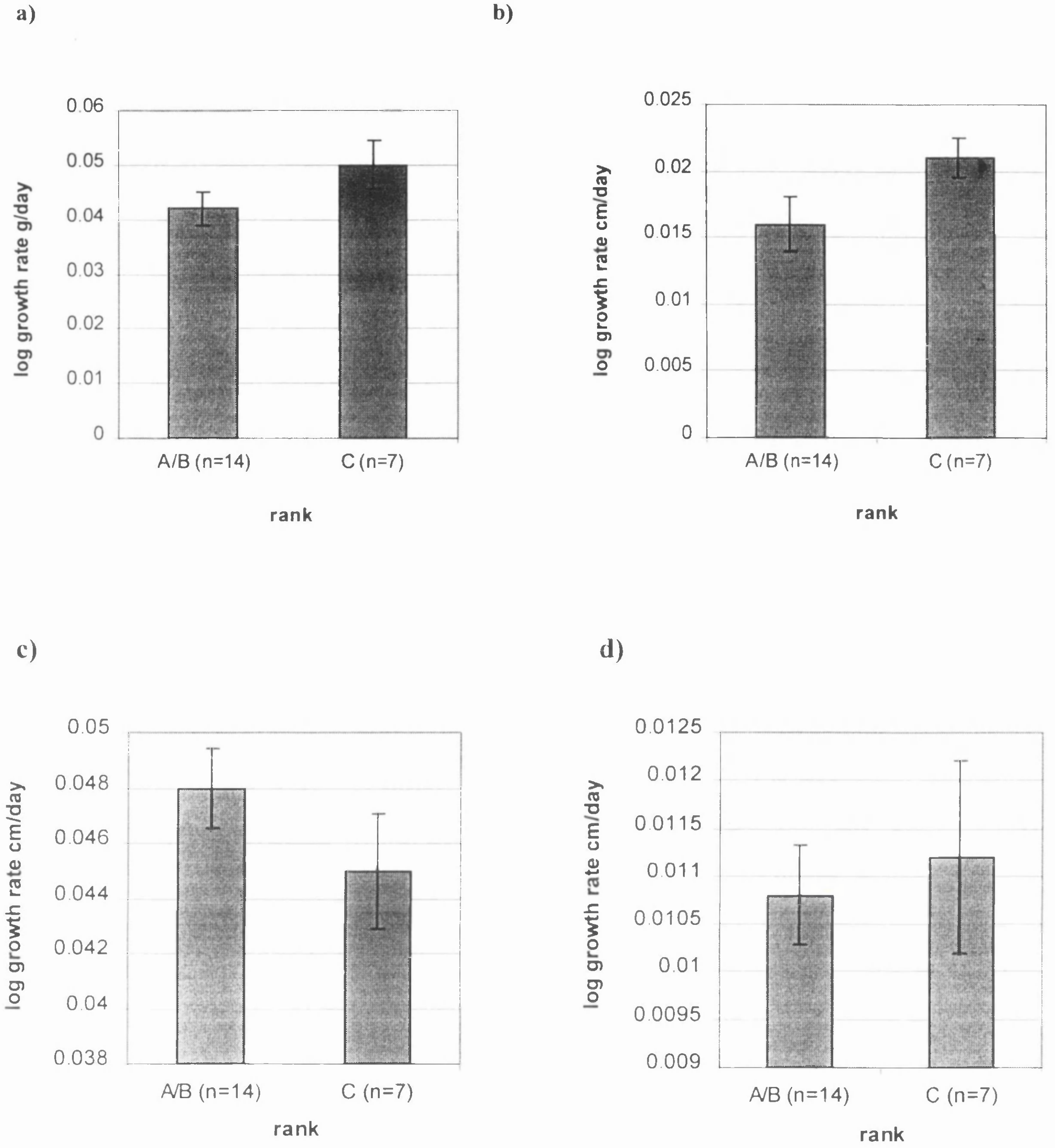


Figure 1. Comparison of growth rates (mean ± s.e.) between C-chicks and their older siblings. Growth rates were calculated between the ages of 5 and 21 days of chicks that successfully fledged (see methods). There were no statistically significant differences in growth rates between siblings for (a) mass (ANOVA, $F_{1,19} = 1.81$, $p = 0.195$), (b) tarsus length ($F_{1,19} = 2.57$, $p = 0.063$), (c) wing length ($F_{1,19} = 1.33$, $p = 0.262$), and (d) head-bill length ($F_{1,19} = 0.15$, $p = 0.704$). Due to multiple tests only $p < 0.05/4 = 0.0125$ should be considered to be statistically significant (Bonferroni transformation).

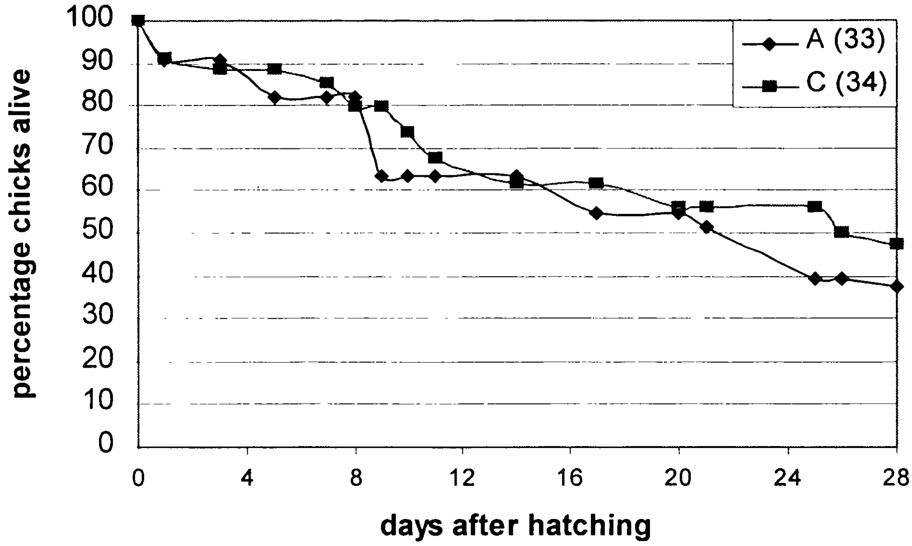
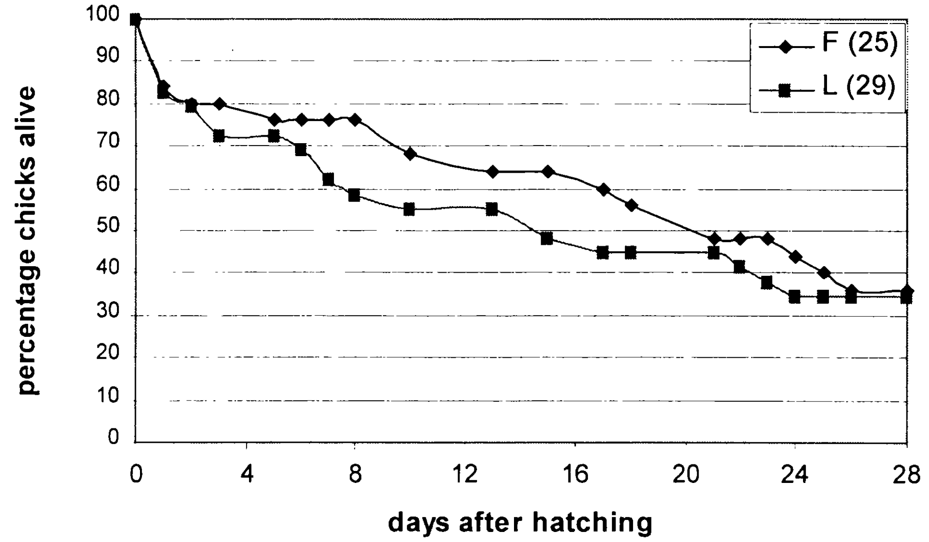


Figure 2. Cumulative mortality over time of A-chicks and C-chicks within control nests. There was no difference in the age at which they died or were last seen alive between A- and C-chicks (ANOVA, $F_{1,62} = 0.04$, $p = 0.835$).

**a)**



**b)**

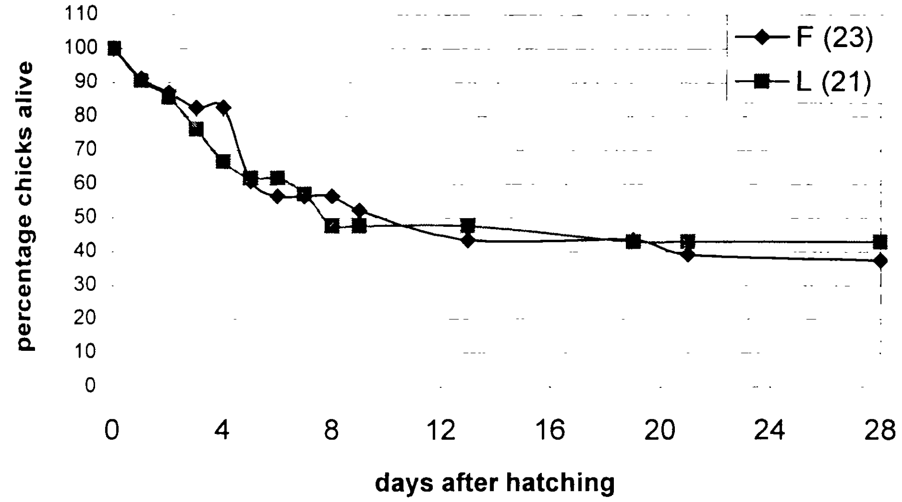
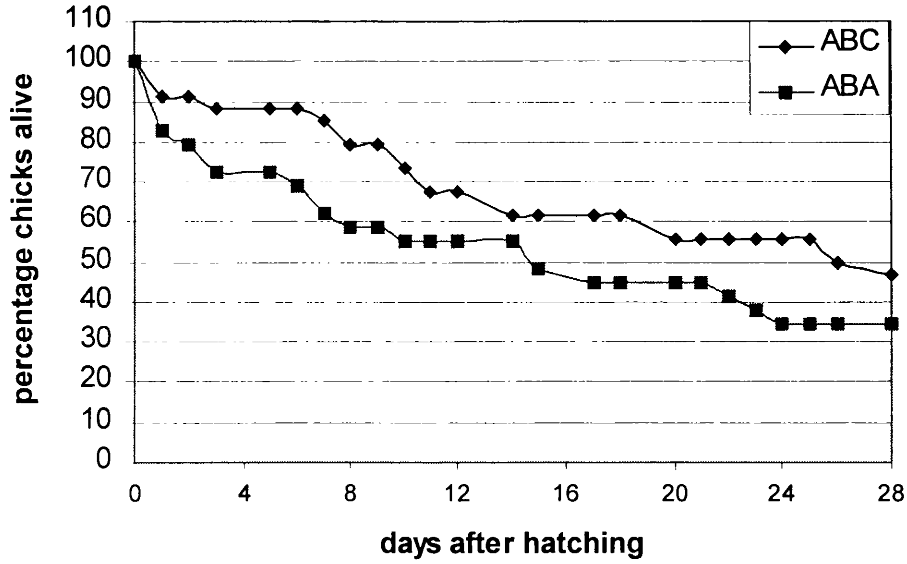


Figure 3. Cumulative mortality over time of F and L-chicks within (a) ABA nests and (b) CBC nests. There was no difference between F- and L-chicks in the age at which they died in both (a) ABA nests (ANOVA, $F_{1,44} = 0.45$, $p = 0.506$) and (b) CBC nests ($F_{1,38} = 0.03$, $p = 0.879$).

a)



b)

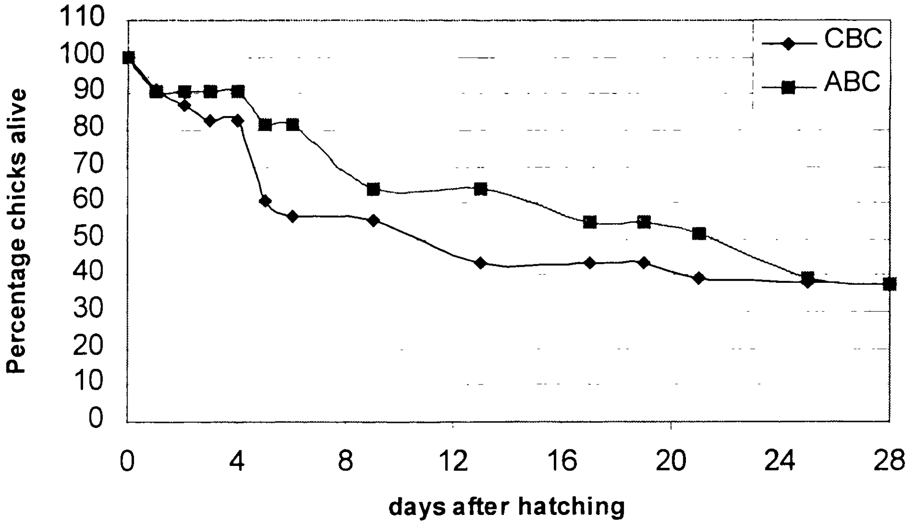


Figure 4. Cumulative mortality over time of (a) L-chicks in ABC (n = 33) and ABA (n = 25) nests and of (b) F-chicks in ABC (n = 31) and CBC (n = 20) nests. There were no significant differences in the age at which they died or were last seen alive between (a) L-chicks chicks in ABC and ABA nests (ANOVA, $F_{1,56} = 0.97$, p = 0.329) and between (b) F-chicks in ABC and CBC nests ($F_{1,49} = 0.14$, p = 0.715).

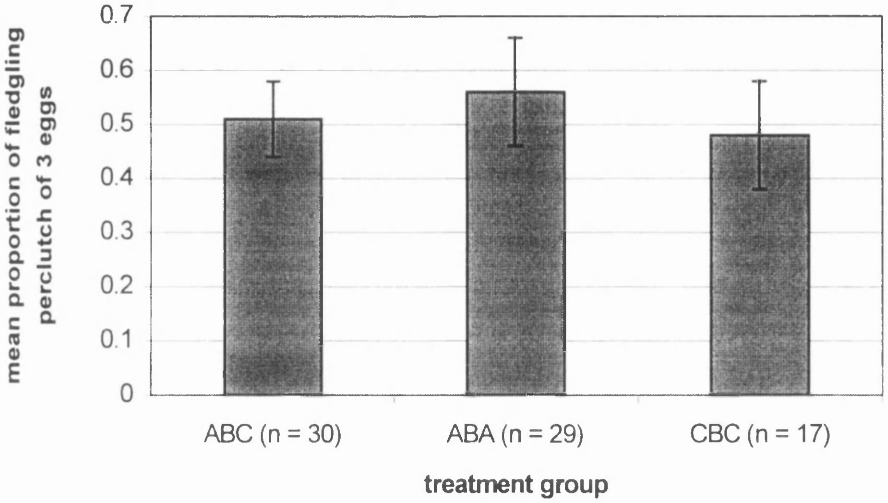


Figure 5. Proportion (mean $\pm$ s.e) of fledglings per clutch of 3 eggs in relation to treatment group. There were no significant differences between the mean of the three treatment groups (Kruskal-Wallis test, $\chi^2 = 0.488$, df = 2, p = 0.784).

# DISCUSSION

The results of this study show that there was no difference in growth or survival in relation to egg quality and hatching rank and therefore does not support my hypothesis that first-hatched chicks are more successful when they hatched from a first-laid eggs than from a last-laid egg, or that chicks from last-laid eggs are more successful than chicks from first-laid eggs when hatching last. Additionally, unmanipulated control broods did not on average fledge more young than manipulated broods. Thus, the hypothesis of an advantage of the naturally occurring within-brood allocation of resources compared to manipulated broods cannot be confirmed.

### *1) Comparison of A-and C-chicks in control ABC nests*

Within non-manipulated nests, chicks that hatched from A-eggs were in a better condition and had a higher mass at hatching than chicks hatched from C-eggs. These differences in hatchlings from A-and C-eggs are consistent with observations of many studies (Parsons, 1975; Hebert and Barclay, 1986; Bolton, 1991; Hillström et al., 2000). Age and size asymmetry among hatchlings is thought to help parents to reduce brood size in the case it is necessary, e.g. when food resources are low (Lack, 1954). Accordingly, Royle and Hamer (1998) showed that in lesser black-backed gulls mortality occurred significantly later in synchronous broods than in asynchronous broods as a consequence of smaller within-brood hierarchical differences and chick mortality was constant throughout the nestling period. In my study, mortality within control nests was also relatively constant fledging. Nestling survival and causes of mortality did not differ between A-and C-chicks. C-chicks did also not die at a younger age than A-chicks. In fact, A-chicks predeceased C-chicks significantly more often than vice versa. Other studies on lesser back-backed gulls found that hatchlings from C-eggs have a weaker immune system than their older siblings

(Blount et al., 2002) and C-chicks often die not due to starvation but due to infections (Hario and Rudbäck, 1996, 1999). Their weaker immune system might be related to the high testosterone content in C-eggs. For example, a negative relationship between high levels of testosterone and low immunocompetence has been found in male superb fairy-wrens (*Malurus cyaneus*) (Peters, 2000). Furthermore, C-eggs have a lower antioxidant content (vitamin E and carotenoid) than A- and B-eggs (Royle et al., 1999; Blount et al., 2002). The low antioxidant content of C-eggs might increase the oxidative stress at hatching and therefore the mortality both at and shortly after hatching. However, these two disadvantages might have had little effect on chick survival during my study, whereas other causes of mortality (e.g. predation) that are independent of egg characteristics or laying order mainly determined nestling survival.

C-chicks lag behind A-chicks in terms of development and experience and therefore are disadvantaged in competitive situations within their nest (Maynard-Smith and Parker, 1976; Fujioka, 1985; Hahn, 1981; Heg and Van der Velde, 2001). Royle et al. (1999) suggested that C-chicks might have an increased development due to the high testosterone content in the egg. However, in this study, chick as well as fledgling size, mass and condition did not differ between A- and C-chicks. Only the growth rate of the tarsus length tended to be higher in C-chicks than in A- and B-chicks. These results are consistent with other works on lesser black-backed gulls (Bolton 1991, Royle and Hamer 1998) where growth characteristics and fledging mass did not vary with laying order. Thus, hatching rank and egg quality did not affect the rate of development and the final size and condition of the chicks. Similarly, in common terns (*Sterna hirundo*) and arctic terns (*Sterna paradisaea*), Robinson and Hamer (2000) found that hatching position within brood had no effect on the rate of development or final fledging mass or size of the chicks. Nevertheless, the differences in condition and mass at hatching between chicks hatched from C-eggs and chicks hatched from A-eggs were no longer present at fledging, thus C-chicks must have caught up with their older siblings. Hebert and Barclay (1985) suggested in herring gulls C-chicks may already catch up with their older siblings during the first 5 days after hatching. Hence, this study did not find a marked C-chick disadvantage in terms of growth and fledging success in unmanipulated lesser black-backed gull broods despite the low condition and the low mass at hatching.

*2) Comparison of chicks hatching from eggs of the same characteristics, but differ in hatching rank*

Within both experimental groups (ABA and CBC), there were no differences in mass, size and condition between F- and L-chicks both at hatching and at fledging. Mortality rates were also not different between A-chicks at different hatching ranks within ABA nests and between C-chicks at different hatching ranks within CBC nests. Moreover, the mean age at which chicks died did not differ between F- and L-chicks in both treatment groups. Therefore, development, final fledgling characteristics and fledging rates were not affected by hatching ranks.

Parsons (1975) found in his work on herring gulls that A-chicks made to hatch last suffered a higher mortality rate than first-hatching A-chicks and concluded that it is hatching rank rather than egg characteristics causing the lower survival of the third chick in gulls. Within ABA nests of this study, F-chicks tended to die before the L-chick more often than L-chicks died before the F-chick. Within treatment group CBC, there was no difference in the timing of mortality between F- and L-chicks. Hatching at a higher rank within the brood did therefore not improve the C-chick's survival. I did not find an explanation for the different results of chick mortality in respect to hatching rank and egg characteristics shown in Parsons' (1975) and my study. This therefore remains an open question.

L-chicks in ABC nests had a lower mass and were in poorer condition at hatching than L-chicks in ABA nests. Similarly, F-chicks in ABC and CBC nests differed in mass and condition at hatching. These results are comparable to those of the within-treatment comparisons. Mass and condition at hatching seem to be determined only by egg characteristics. Chicks hatching from last-laid eggs have been shown to be able to speed up hatching (Chapter II). Persson and Andersson (1999) found in their work on mallard ducks and pheasants that chicks of these species could either decrease or increase the length of incubation in order to hatch synchronously within a brood. Both mallard and pheasant chicks, which decreased the incubation length, had shorter wings and tarsi and mallard chicks even a lower mass at the age of 10 days post-hatching (Perssons and Nilsson, 199). A shorter hatching duration in last-hatching C-chicks observed in this study might therefore be at the expense of hatching condition.

Last-hatching A-chicks and C-chicks had similar growth rates of tarsus, mass, wing and head-bill lengths, as well as similar fledging rates and final fledging mass and condition. Nestling survival and timing and causes of mortality were not different between L-chicks in ABA nests and L-chicks in ABC nests. Growth, survival fledgling mass and fledgling condition did not differ between F-chicks of treatment groups ABC and CBC. Therefore, within both the last hatching rank and the first hatching rank, egg-quality did not seem to affect the growth and survival of chicks. Similarly, Royle and Hamer (1998) found no significant differences between asynchronous ABC nests and asynchronous ABA nests in either the proportion of chicks that fledged per brood, the total number of chicks fledged or the mean age at which chicks died. Moreover, mean growth rate (mass and tarsus length) and fledging mass per brood did not differ between the two groups. However, they did not distinguish between individual hatching ranks.

In this study, within both control ABC broods and treatment groups ABA and CBC, C-chicks survived as well as A-chicks despite poorer condition at hatching. This result arouses the question what might be if C-chicks are in equally good condition at hatching compared to A-chicks. Would C-chicks then have a much stronger competitive ability, would out-compete its older siblings and even have a higher fledging rate than its nestmates?

Lack's (1954) brood reduction hypothesis predicts an early occurrence of mortality since in the case of food shortage parental birds should adjust the brood size right after hatching to ensure the survival of at least some chicks. Accordingly, many studies on gulls have found an early nestling mortality (Parsons 1970, 1975; Hebert and Barclay, 1986; Bolton, 1991; Hillström et al., 2000) with C-chicks suffering from highest mortality rates (Parsons, 1975; Hario and Rudbäck, 1999) and are therefore in agreement with one prediction of the brood reduction hypothesis. The lack of a distinct third chick disadvantage in terms of growth and survival, as well as the constant mortality throughout the nestling period found in this study, however, contradicts the prediction of the brood reduction hypothesis.

Nevertheless, within control nests A-chicks predeceased C-chicks more often than the other way around. Stinson (1979) suggested that the C-egg might be an insurance strategy of the parents in case the A- or B-chick dies very soon after hatching. With the "extra" C-egg the parental birds would still be able to raise two chicks. However, if A- and B-chicks survive, not many resources are given to the last-hatched chick. Stinson's insurance hypothesis Stinson's (1979) could also not be confirmed since last-hatching chicks did not show a significant insurance value, i.e. they did not survive significantly more often when either one or both of the earlier hatched chicks died than when both of the older nestmates survived. The equal fledging success of A-and C-chicks both between and within different hatching ranks observed in this study does therefore not support any predictions of the evolutionary adaptation of asynchronous hatching.

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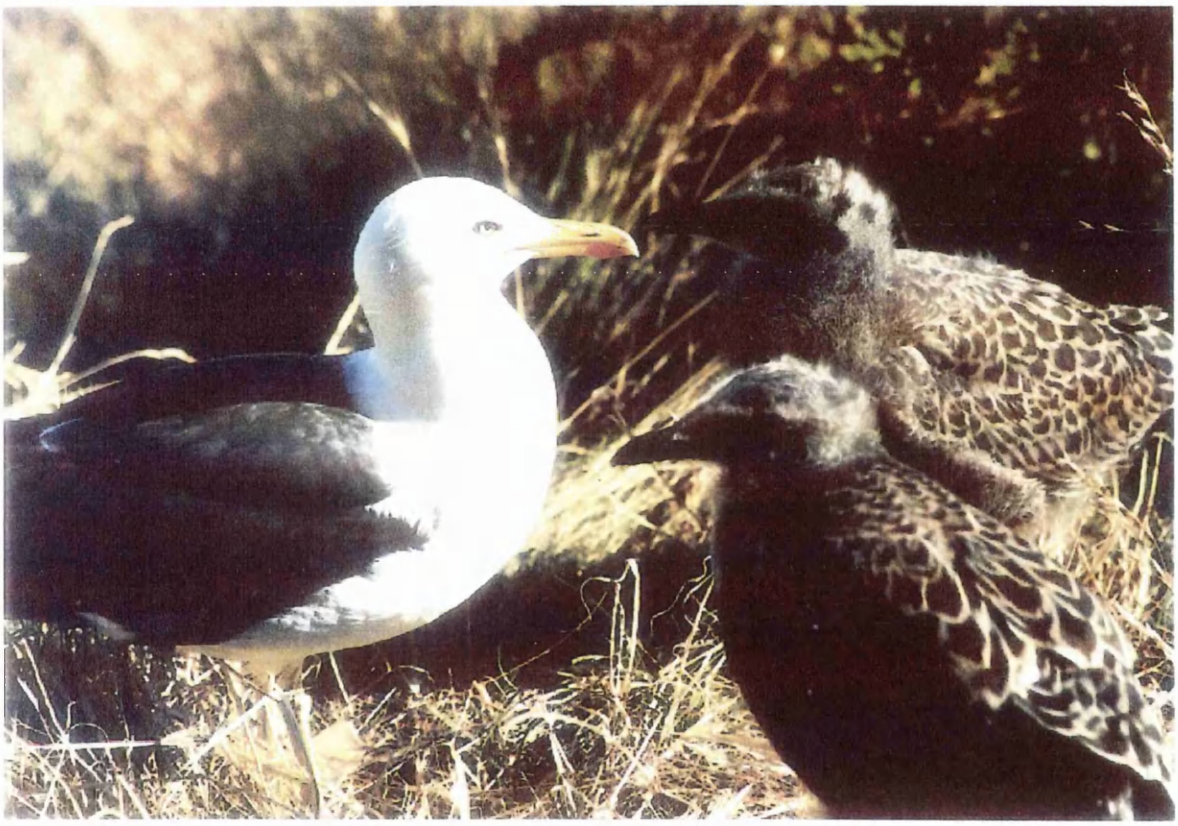
CHAPTER IV.

# VARIATION IN CHICK BEHAVIOUR IN LESSER BLACK-BACKED GULLS (*LARUS FUSCUS*): RELATIVE CONTRIBUTIONS OF EGG CHARACTERISTICS, SEX AND HATCHING RANK



## ABSTRACT

In asynchronously hatching birds, differences in begging behaviour might be due to the hatching rank or to differences in egg characteristics (egg size and/or composition) across the hatching order which both affect the needs and the condition of chicks. In natural broods, hatching rank and egg characteristics are not mutually exclusive. It is therefore difficult to determine whether hatching rank or egg characteristics influence the behaviour of the chick. In order to distinguish between these two possibilities I manipulated the hatching order relative to the laying order of lesser black-backed gull (*Larus fuscus*) broods. Using cross fostering of eggs, chicks from first-laid eggs were made to hatch last and chicks from last-laid eggs were made to hatch first within a brood. C-chicks spent more time begging than A-chicks corrected for hatching. These results therefore indicate that both egg characteristics and hatching rank influence the chick's begging behaviour. Male and female chicks did not differ in begging behaviour or feeding frequency. However, only last-hatched female chicks were able to monopolise food or beg food from nestmates, but last-hatched male chicks were unable to monopolise food or beg food from nestmates. Control A- and C-chicks were fed equally often by their parents. However, last-hatched C-chicks were fed more frequently than last-hatched A-chicks, whereas first-hatched A-chicks were fed more often than first-hatched C-chicks. Furthermore, last-hatched A-chicks did not monopolise food, whereas first- and last-hatched C-chicks as well as first-hatched A-chicks did so. Overall parents fed chicks from control broods more often than chicks from broods where hatching order was manipulated. Therefore, the observed natural within-brood differences seemed to be the most advantageous pattern and A-chicks were less able to cope with the last-hatching position within a brood.

## INTRODUCTION

In birds, parental care (food, warmth, protection) for dependent young is often solicited by the offspring through specialised behaviours such as vocalisation and/or certain body postures (Tinbergen, 1953; Goethe, 1955; Kilner and Johnstone, 1997). Such behaviours have been described to be used by the offspring to communicate with their parents (Evans, 1984; Cotton et al., 1999) and their siblings (Roulin et al., 2000), i.e. to out-compete nestmates and to gain a larger share of the parental resources than the parents would give them (Trivers, 1974; Godfray, 1995a,b). Observational studies in asynchronously hatching broods have shown that begging strategies differ between offspring within the same nest. Late-hatched chicks generally beg more than their older and larger siblings (Ryden and Bengtsson, 1980; Drummond et al., 1986; Price and Ydenberg, 1995; Cotton et al., 1999). Furthermore, it has been shown that an increase in solicitation behaviour leads to an increase in parental care such as incubation (Evans, 1984) or provision of food and warmth to dependent young (Smith and Montgomerie, 1991; Leonard and Horn, 1998). However, large chicks often receive more food than their younger and smaller siblings despite their less frequent begging (Ryden and Bengtsson, 1980; Drummond et al., 1986; Price and Ydenberg, 1995). It is not clear if the biased feeding of the parents towards older and bigger offspring is due to parents actually choosing large chicks over small ones or due to competition between chicks. The former is predicted by the brood reduction hypothesis (Lack, 1954). It describes asynchronous hatching as a mechanism by which brood size can be adjusted to food availability during chick rearing. When food is scarce and parents cannot raise all young they will allocate most food to the oldest and/or most dominant chicks that then have the highest survival probability. However, the factors causing differences in behaviour of chicks within the brood remain unclear.

Differences in begging behaviour among offspring of the same brood could be caused by differences in offspring's need (Impekoven, 1971; Henderson, 1975; Leonard and Horn, 2001), condition (Price et al., 1996), size (Leonard and Horn, 2001) and/or hatching rank within the nest (Mock and Ploger, 1987; Price and Ydenberg, 1995; Cotton et al., 1999). These four aspects might differ between chicks of the same brood due to asynchronous hatching, which leads to a size-age hierarchy among siblings (Maynard-Smith and Parker, 1976; Fujioka, 1985; Hahn, 1981; Heg and Van der Velde, 2001). Within asynchronously hatching broods, last-hatched chicks, as the youngest and relatively smallest chick within the nest, lag behind in growth, development and experience. Thus they are disadvantaged in competitive situations (Maynard-Smith and Parker, 1976; Fujioka, 1985; Hahn, 1981; Heg and Van der Velde, 2001). Alternatively, females of many bird species allocate their resources asymmetrically between the eggs (reviewed in Mock and Parker, 1997), which may also affect the phenotype of offspring. Within-clutch differences of eggs have been described for their size, mass and composition. Last-laid eggs are often smaller (Slagsvold et al., 1984) and may differ in their composition from earlier laid eggs (Parsons, 1972; Schwabl, 1993; Royle et al., 1999; Eising et al., 2001; Royle et al., 2001; Blount et al., 2002). These differences in egg size and egg composition (egg characteristics) may affect the survival and behaviour of the last chick. An initially smaller size of chicks from last-laid eggs lowers the chicks' survival probably through its lower competitive ability (Parsons, 1975; Fujioka, 1985; Ploger and Mock, 1986; Bolton, 1991; Royle and Hamer, 1998; Hillström et al., 2000). Sometimes last-laid eggs also have lower contents of antioxidants (vitamin E and carotenoids) and immunoglobulins which could negatively affect the survival of chicks hatching from those eggs (Royle et al., 1999; Blount et al., 2002). In contrast, in several species last-laid eggs have an elevated androgen which might increase the embryonic and neonatal growth rate, as well as the aggressive behaviour (i.e.

competitive ability) of last-hatched chicks (e.g. Schwabl, 1996; Royle et al.,2001; Lipar and Ketterson, 2000; Eising et al, 2001). These effects of a high androgen content are described as an evolutionary adaptation for the youngest chick to catch up with its older, more experienced and larger siblings (Schwabl, 1996). It is not yet fully understood whether observed differences in offspring behaviour among chicks of the same brood depend on hatching rank and/or egg characteristics.

Another factor that may affect variation in chick behaviour within a brood could be the offspring's gender. In bird species in which competition over parental resources is affected by chick size, sexual size dimorphism could result in differences in chick behaviour and mortality between the sexes (Drummond et al., 1991). The sexes may also differ in their energy requirements and/or their competitive ability independent of size (Clutton-Brock and Iason, 1986; Teather, 1992; Mock and Parker, 1997; Oddie, 2000). However, the differences in behaviour between male and female chicks have been little studied so far.

In this study, I manipulated the hatching order relative to the laying order of lesser black-backed gull (*Larus fuscus*) broods in order to separate the effects of hatching rank from the effects of egg characteristics on chick behaviour. Lesser blacked-backed gulls lay a modal clutch of 3 eggs, which hatches asynchronously and the eggs differ in size, mass, and composition (Harris, 1963; Parsons, 1972; Royle and Hamer, 1998; Royle et al., 1999; Nager et al., 2000a; Royle et al., 2001; Blount et al., 2002). Offspring size, mass, condition and survival rate vary across the laying sequence (Parsons, 1975; Griffiths, 1992; Hario and Rudbäck, 1996, 1999; Royle and Hamer, 1998; Nager et al., 2000a), whereas offspring size, mass and survival rates also vary between the sexes (Griffiths, 1992; Nager et al., 1999; Nager et al., 2000b).

In this study, I investigated whether chick behaviour is affected by the characteristics of the egg it hatched from or the chick's hatching rank within the brood. In order to separate the effects of egg characteristics from effects of hatching order on chick behaviour, I experimentally altered the chicks hatching order. Through exchanging eggs between nests, chicks from first-laid eggs were made to hatch last and chicks from last-laid eggs were made to hatch first within a brood. Furthermore, I compared the behaviour between male and female chicks.

## MATERIAL AND METHODS

Fieldwork for this study was conducted in 2001 in a large gull colony on Walney Island, Cumbria (NW-England) where about 24.000 lesser black-backed gull (*Larus fuscus*) pairs breed each year on flat sandy grass dunes. The study area has been shown to be well suited for behavioural observations (Brown, 1967; Tinbergen and Falcus, 1970) and experimental manipulations of gull broods (Nager et al., 1999, 2000a,b).

Three separate plots, ca. 50-300m apart from each other and situated within the centre of the breeding colony, were searched daily for new nests. The latter were mapped and marked individually with numbered stones. During egg laying, nests were controlled daily. New eggs were weighed to the nearest 0.1g with an OHAUS balance and individually marked with a non-toxic, water-resistant marker according to their position in the laying sequence as A, B or C, respectively, on the day they were laid. Similarly, chicks are called according to the egg they have hatched from as A-, B- or C-chick.

In order to control for effects of egg mass and laying dates on chick development (Parsons, 1972, 1975; Bollinger, 1994) only nests with clutches of three eggs, which have been laid before May 16th and with eggs of average mass (within ± 9g of the mean their laying rank comparing to egg masses recorded in previous years) at that colony were selected for the experiment. Furthermore, selected nests were situated in a range of 4-100m to one of three observation hides (one per experimental plot) which were used for behavioural observations of the chicks. In cases where small patches of vegetation reduced the visibility of nests in such a way that observations were not possible, the vegetation was cut at one side of the nest that was facing the hide. However, within all plots there were patches off high vegetation, which occasionally made continuous recordings of behaviour impossible since chicks and/or parental birds walked behind and/or into these vegetation patches. A total of 28 lesser black-backed gull nests, each with a clutch of three eggs of average size and all suitable for observations were selected. These 28 observation territories were evenly distributed within the area of the three experimental plots and did not spatially overlap, so individuals from different nests were not confounded. My behavioural observations of the experimental chicks during the nesting period showed that chicks did generally not walk further away from their nest than approx. 4m (except during threatening situations, e.g. when I walked through the colony in order to measure chicks). In the case they came too close to a neighbouring territory, they were chased away by the neighbours. Therefore, individual chicks were not mistaken for chicks from another nest.

At the time of hatching, nests were visited twice per day in order to monitor and record the time of pipping (when the chicks have pecked a small hole through the shell) and the time of hatching (when chicks have completely emerged from the egg shell). At pipping, I applied acrylic paint onto the egg tooth of each chick (green for A-chicks, white for B-chicks, yellow for C-chicks) in order to determine the hatching rank of each chick once they have hatched. All chicks were successfully identified from which egg they hatched. Within 12 hours after hatching, chicks were weighed to the nearest 0.1g with an OHAUS balance, tarsus length and head-bill length (back of the head to the tip of the bill) were measured to the nearest 0.1mm using callipers and wing length (maximum wing chord) was measured to the nearest 0.5mm using a wing ruler. Furthermore, hatchlings were marked individually with live stock dye (Stay-on Stock Marker Spray, Trilanco, Lancashire) by applying little dots on each wing and on the throat, and with numbered leg rings. These rings were made out of 10cm-long pieces of white isolation tape, which were folded in the middle so that the two sticky sides got to lay on top of each other and were then put around one of the chicks' leg and stapled at the ends. I wrote the nest number onto these rings in order to recognise which chick belonged to which nest. The colour markings were important during the behavioural observations in order to distinguish between the chicks from the distance. I used two different coloured dyes, blue and red, to spray two chicks of each nest, independent of rank or age. Thus, each nest had a red-marked and a blue-marked chick and one without any colour.

At the age of 1-20 days, a small droplet of blood was taken from each chick under Home Office Licence using a fine needle to pierce the leg vein. Blood samples were stored in BLB buffer in the freezer for ca. 3 months until they were processed in the laboratory. The sexes of the chicks were determined based on a W chromosome repeat (Griffiths et al., 1996).

To separate the effects of egg characteristics from the effects of hatching rank on chick behaviour I created experimental clutches of three eggs where the hatching order was changed in relation to the laying order. At the time of pipping, the C-egg from one nest was exchanged with the A-egg of another clutch, which was at a comparable stage of development to the C-egg. Thus, initially last-laid eggs then hatched relatively early compared to its nestmates, while A-eggs that should have hatched first became the last-hatched ones. I did not match eggs to be exchanged by their size, because an A-egg as small as a C-egg might have been of poor quality (due to the poor quality of the parental birds. or other environmental factors (Bolton, 1991; Monhagan et al., 1998), and so might have been the chick that would have hatched from this egg. A poor-quality A-chick wouldn't have had the same competitive ability/behaviour as other first-hatched chicks and thus the outcome of the experiments would be confounded. The opposite could have happened with C-eggs of the size of an A-egg. Accordingly, I decided to work only with eggs that are of about the average size according to their laying sequence (see above).

Nests were randomly assign to one of the three treatment groups. The egg-exchange led to two experimental groups and these were compared to an unmanipulated control group.

1.) Control group ABC (n = 11)
Nests with a clutch of three eggs where eggs hatched in the order they were laid

2.) Experimental group ABA (n = 11)
Nests with a clutch of three eggs where the last-hatched chick is an A-chick

3.) Experimental group CBC (n = 6)
Nests with a clutch of three eggs where the first-hatched chick is a C-chick

Between 3-28 days after hatching (depending on the visibility of the nest site and the chicks) each brood was observed on average every three days. However, due to weather conditions (strong wind and rain, when no observations were made) the interval between single observations sometimes varied between 1-5 days. Each nest was observed for 0.5-2 hours and only one nest was observed at a time. During each observation I recorded frequency and duration of the chick and parental behaviour involved in begging and feeding, described below and listed in table 1.

*Offspring begging behaviour and parental feeding behaviour*

On the arrival at the territory of a parent, the offspring (either all three or only one-two chicks) approached this parent in a submissive posture (head and upper body bowed towards the ground to be parallel with the ground) either by walking or by running. The approach was often accompanied by vigorous bobbing (up- and down movement of the upper body part) and/or calling. The parental bird usually ignored the chick begging behaviour and turned away from the chicks to stare into the other direction. The chicks then stayed very close to their parent either with stretched necks or in the submissive posture and attempt to peck or even grasp the parent's mandibles. The parent often walked away from its offspring in order to avoid the harassment. In this case, the chicks might follow the adult bird similarly to their first approach. Chick begging behaviours sometmes stimulated the parent to feed its young either as an immediate response or after 1-30 minutes begging duration. The parent regurgitated a food bolus and kept it either in its bill so that the offspring could feed directly out of the parent's beak, or the parent dropped the food onto the ground and the young had to pick it up themselves. The ability of chicks to monopolise food delivered by the parent and offspring begging at siblings is described as aggressive behaviour in this work. Pecking and grasping of parental mandibles was classified as type b begging, whereas the other chick begging behaviours were classified as type a begging.

**Table 1. Definitions of behaviours recorded and analysed for this study.**

| Behaviour | | Defenition |
|---|---|---|
| Begging attempt: | a) successful | Duration the chick begged for food until it obtained food |
| | b) unsuccessful | Duration the chick begged for food and did not obtain food |
| Begging frequency | a) type a | Number of begging attempts (successful and unsuccessful combined), which showed behaviours classified as type a only (see text), during the total time the nest was observed |
| | b) type b | Number of begging attempts (successful and unsuccessful combined), which showed behaviours classified as both type a and type b (see text), during the total time the nest was observed |
| Proportion Begging | | Proportion of observation time when one or both parents were present that a chick spent begging (type a and type b combined) |
| Feeding frequency | | Number of feedings a chick received per hour |
| Monopolisation frequency | | Percentage of chicks that were able to monopolise a food bolus regurgitated by one of the parents |
| Sibling begging frequency | | Percentage of chicks that begged food from siblings. This begging from siblings did never result in feeding. |

Begging was recorded as unsuccessful (not obtained any food) when either the parental bird left the nest site or the begging chick gave up and went into a vegetation patch or sat down at the nest site.

Offspring behaviour might change with increasing age (Tinbergen, 1953; Fujioka, 1985); number of siblings could also influence begging and feeding behaviour of both parents and offspring. Therefore, I first compared the mean brood ages when observations were done and the mean number of chicks per brood between treatments.

I compared the collected data between A-and C-chicks within the control group (**ABC**), between first-hatched chicks (F-chicks) of control and CBC nests (**ABC** – **CBC**) and between last-hatched chicks (L-chicks) of control and ABA nests (AB**C** – AB**A**) using Fischer's except test for presence-absence data and Mann Whitney U test for duration and proportions. I also compared brood averages of the behaviours between the three treatment groups using oneway ANOVA, and between male and female L-chicks using both logistic regressions and ANCOVA with egg type as the covariant. Finally, I compared feeding frequency between A- and C-chicks of all treatment groups combined using two-way ANOVA with egg type and hatching rank as independent variables. Differences were recorded as significant at a level of $p \leq 0.05$.

## RESULTS

### *1) Comparison between broods of control group ABC, treatment group ABA and treatment group CBC*

There were no differences between treatment groups in the mean age at which individual broods were observed (ANOVA, $F_{2,25} = 0.15$, $p = 0.866$) or the mean number of chicks per brood ($F_{2,25} = 0.26$, $p = 0.777$).

Differences between the treatment groups were found in the attendance of parents at the nest site (figure 1). In treatment group ABA parents spend significantly less time at the nest than in the CBC group. Parents of control broods stayed for an intermediate proportion of the total observation time at the nest compared to ABA and CBC nests.

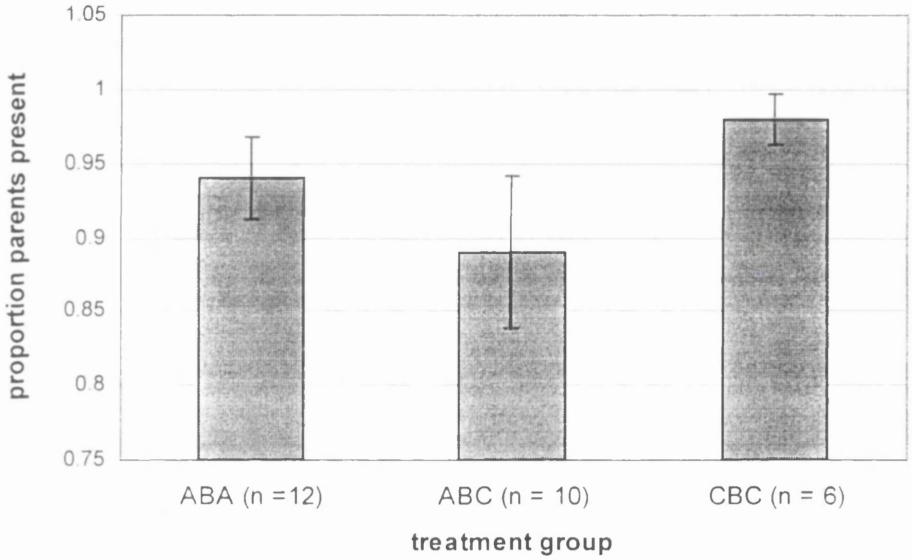


**Figure 1. Mean proportion of time that parents were present at the nest per treatment. The error bars show the standard error of the mean. There were no significant differences between treatment groups (ANOVA, $F_{2,25} = 1.15$, $p = 0.333$).**

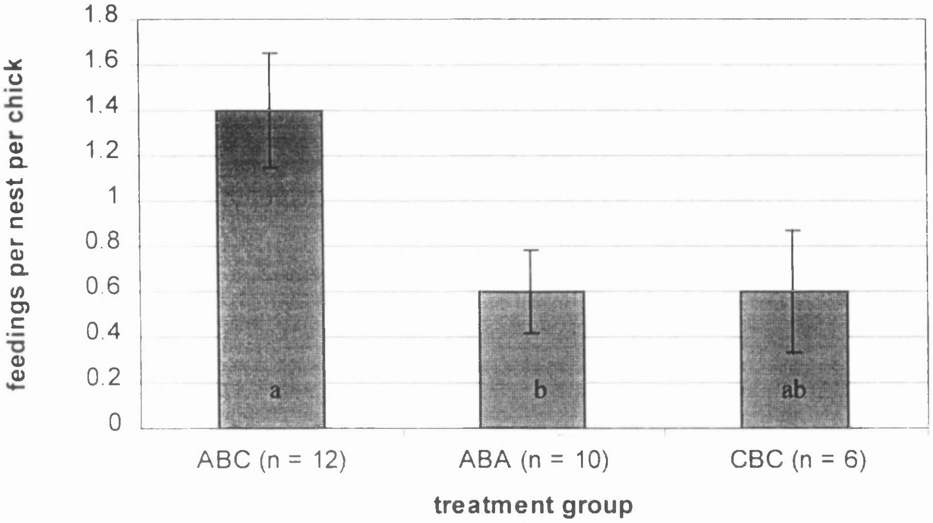


**Figure 2. Mean feeding frequency per chick per hour compared between three treatment groups. There was a significant difference between treamtent groups (ANOVA, $F_{2,25} = 3.91$, $p = 0.033$). Bars labelled by different letters differ significantly (post-hoc Tukey test).**

*2) Comparisons of A- and C-chicks in control ABC nests*

Within the control group, no significant differences were found between A- chicks and C-chicks in the duration of both successful and unsuccessful begging attempts (table 2). Furthermore, the begging frequency both type a and type b was not significantly different between the two chicks (table 2). There was also no difference between A-and C-chicks in proportion begging (table 2). The frequency of food monopolisation by one chick and the sibling begging frequency did not differ between control A- and C-chicks, either (table 2).

**Table 2. Differences in behaviour (mean ± s.e or percentage) between A-and C-chicks within control nests. Numbers in brackets are sample sizes.**

| Behaviour | A-chick (n) | C-chick (n) | Test |
|---|---|---|---|
| Begging attempt: | | | |
| a) successful | 1.53 ± 0.39 min (11) | 2.3 ± 0.45 min (9) | Mann Whitney,<br>U = 30.50, p = 0.149 |
| b) unsuccessful | 5.64 ± 0.88 min (11) | 8.16 ± 2.10 min (10) | Mann Whitney,<br>U = 42.00, p = 0.360 |
| Begging frequency | | | |
| a) type a | 0.2 ± 0.033 (11) | 0.18 ± 0.028 (11) | Mann Whitney,<br>U = 41.00, p = 0.200 |
| b) type b | 0.03 ± 0.009 (11) | 0.03 ± 0.008 (11) | Mann Whitney,<br>U = 58.00, p = 0.869 |
| Proportion Begging | 0.2 ± 0.03 (11) | 0.2 ± 0.04 (10) | Mann Whitney,<br>U = 59.00, p = 0.922 |
| Feeding frequency | 1.5 ± 0.30 (11) | 1.5 ± 0.30 (11) | Mann Whitney,<br>U = 59.50, p = 0.948 |
| Monopolisation frequency | 27.3% (11) | 27.3%(11) | Fisher's Exact,<br>p = 1.00 (2-tailed) |
| Sibling begging frequency | 0% (11) | 27.3%(11) | Fisher's Exact,<br>p = 0.107 (2-tailed) |

*3) Comparison of L-chicks between control ABC nests and treatment ABA nests*

L-chicks of ABC and ABA nests did not differ in the duration of both successful and unsuccessful begging attempts (table 3). However, L-chicks of ABA nests had a lower begging frequency compared to L-chicks of ABC nests, although the difference was statistically significant only for begging of type a (table 3). Proportion begging of L-chicks differed between the control group and treatment group ABA, although, this difference was not statistically significant (table 3). Furthermore, L-chicks did not differ significantly between treatments in sibling begging frequency and food monopolisation frequency (table 3). Nevertheless, control L-chicks had a significantly higher feeding than ABA L-chicks (table 3, figure 2).

**Table 3. Differences in behaviour (mean ± s.e or percentage) between L-chicks of control group ABC and L-chicks of treatment group ABA. Numbers in brackets are sample sizes.**

| Behaviour | ABC (n) | ABA (n) | Test |
|---|---|---|---|
| Begging attempt: | | | |
| a) successful | 2.30 ± 0.45 min (9) | 1.32 ± 0.61 min (4) | Mann Whitney,<br>U = 10.50, p = 0.247 |
| b) unsuccessful | 8.16 ± 2.10 min (10) | 4.10 ± 0.56 min (9) | Mann Whitney,<br>U = 26.00, p = 0.119 |
| Begging frequency | | | |
| a) type a | 0.18 ± 0.028 (11) | 0.10 ± 0.027 (9) | Mann Whitney,<br>U = 24.50, p = 0.057 |
| b) type b | 0.03 ± 0.008 (11) | 0.01 ± 0.003 (10) | Mann Whitney,<br>U = 25.00, p = 0.030 |
| Proportion Begging | 0.2 ± 0.04 (10) | 0.1 ± 0.06 (10) | Mann Whitney,<br>U = 29.00, p = 0.067 |
| Feeding frequency | 1.5 ± 0.30 (11) | 0.4 ± 0.17 (10) | Mann Whitney,<br>U = 19.00, p = 0.010 |
| Monopolisation frequency | 27.3% (11) | 0% (10) | Fisher's Exact,<br>p = 0.124 (2-tailed) |
| Sibling begging frequency | 27.3% (11) | 40% (10) | Fisher's Exact,<br>p = 0.438 (2-tailed) |

### *4) Comparison of F-chicks between control ABC nests and treatment CBC nests*

Begging frequency of both types a and b as well as proportion begging did not differ significantly between F-chicks of control ABC and treatment CBC nests (table 4). Duration of unsuccessful begging attempts in F-chicks did not differ between the two treatments, whereas successful begging attempts lasted longer in F-chicks of CBC nests than in F-chicks of control nests (table 4), although, this difference was not statistically significant. There was a significant difference in the feeding frequency between control F-chicks and CBC F-chicks (table 4, figure 2) with control F-chicks being fed more often than CBC F-chicks. Monopolisation and sibling begging frequencies did not differ between the two treatment groups (table 4).

**Table 4. Differences in behaviour (mean ± s.e or percentage) between F-chicks of control group ABC and F-chicks of treatment group CBC. Numbers in brackets are sample sizes.**

| Behaviour | ABC (n) | CBC (n) | Test |
|---|---|---|---|
| Begging attempt: | | | |
| a) successful | 1.53 ± 0.39 min (11) | 8.25 ± 5.63 min (3) | Mann Whitney, U = 4.00, p = 0.052 |
| b) unsuccessful | 5.64 ± 0.88 min (11) | 4.21 ± 1.15 min (6) | Mann Whitney, U = 25.00, p = 0.421 |
| Begging frequency | | | |
| a) type a | 0.2 ± 0.033 (11) | 0.22 ± 0.061 (6) | Mann Whitney, U = 26.00, p = 0.482 |
| b) type b | 0.03 ± 0.009 (11) | 0.02 ± 0.007 (6) | Mann Whitney, U = 31.50, p = 0.879 |
| Proportion Begging | 0.2 ± 0.03 (11) | 0.2 ± 0.10 (6) | Mann Whitney, U = 33.00, p = 1.000 |
| Feeding frequency | 1.5 ± 0.30 (11) | 0.8 ± 0.31 (6) | Mann Whitney, U = 69.00, p = 0.047 |
| Monopolisation frequency | 27.3% (11) | 0% (6) | Fisher's Exact, p = 0.243 (2-tailed) |
| Sibling begging frequency | 0% (11) | 0% (6) | Fisher's Exact, p = 1.00 (2-tailed) |

### 5) Comparison of feeding frequency between A-and C-chicks of all treatment groups combined

There was no significant interaction egg type x hatching rank in feeding frequency type a (figure 2). However, F-and L-chicks differed significantly in their feeding frequency type a when corrected for egg type (figure 2).

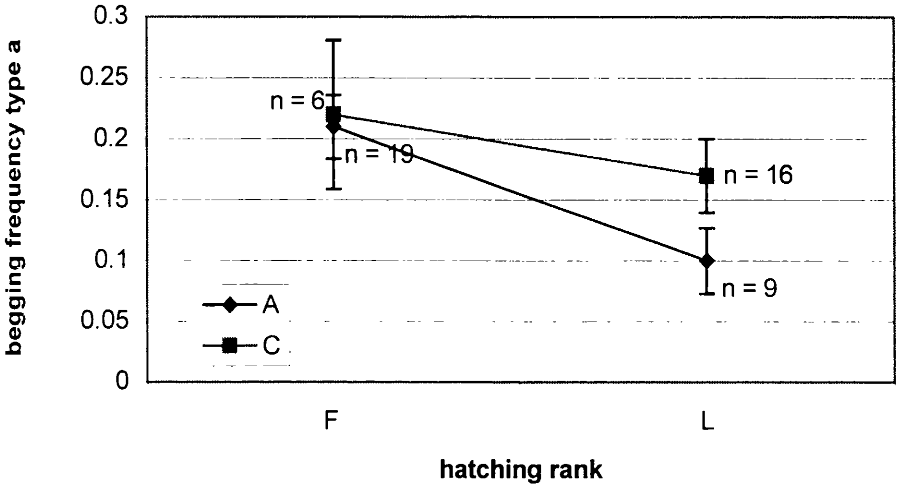


**Figure 2. Begging frequency type a (± s.e.) of A-and C-chicks within both the F- and the L-hatching rank. There was no significant interaction egg type x hatching rank (twoway ANOVA, $F_{1,46} = 0.45$, $p = 0.507$). However, there was a significant difference in begging frequency type a between A-chicks and C-chicks ($F_{1,47} = 5.34$, $p = 0.025$).**

Figure 3 shows the feeding frequency of A- and C-chicks within the F-hatching rank and the L-hatching rank. There was a significant interaction egg type (A and C) x hatching rank (F and L) (figure 3). A-chicks were fed more frequently when hatching first than when hatching last, whereas C-chicks were fed more often when hatching last than when hatching first.

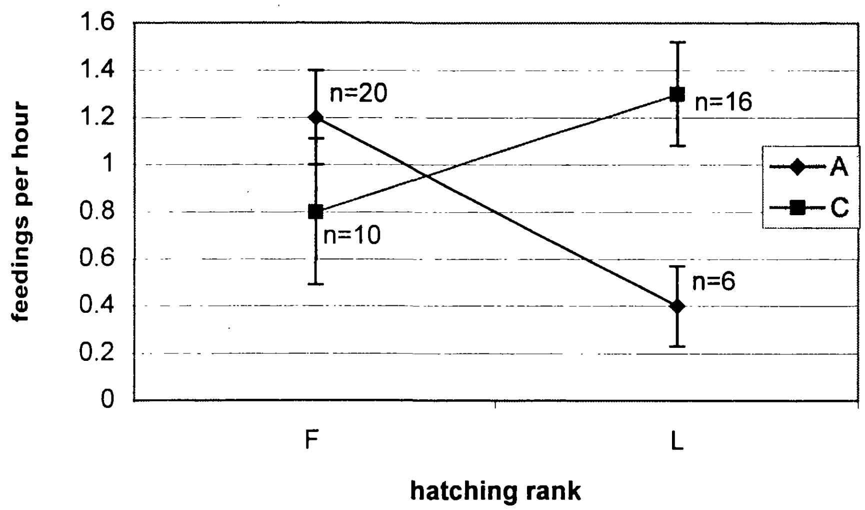


**Figure 3. Feeding frequency (+ s.e.) of A-and C-chicks within both the F- and the L-hatching rank. There was a significant interaction egg type (A and C) x hatching rank (F and L) (Two-way ANOVA, $F_{1,48} = 6.59$, p = 0.013).**

*6) Comparison of female and male chicks of all treatment groups combined in respect to hatching rank*

The number of male and female chicks per treatment group and hatching rank are listed in table 5. The number of chicks of unknown gender did not differ between treatment groups.

**Table. 5. Number of male and female chicks per treatment group and hatching rank.**

| | | gender | | |
|---|---|---|---|---|
| treatment group | hatching rank | male | female | unknown |
| ABC | F | 5 | 4 | 2 |
| | L | 3 | 4 | 4 |
| ABA | F | 2 | 4 | 3 |
| | L | 3 | 3 | 4 |
| CBC | F | 3 | 0 | 3 |
| | L | 0 | 2 | 3 |

There were no differences in chick number of unknown gender between treatment groups ($\chi^2 = 0.31$, df = 2, p = 0.857) and there was no difference in offspring sex ratio between treatment groups ($\chi^2 = 0.51$, df = 2, p > 0.05) or between hatching rank ($\chi^2 = 0.83$, df = 2, p > 0.05).

There was no interaction sex x egg type in the duration of begging attempts (both successful and unsuccessful), begging frequency (both type and type b), proportion begging and feeding frequency (table 6). I therefore combined the chick behaviour of A- and C-chicks in table 6 to show differences in chick behaviour of female and male L-chick. Male and female L-chicks did not differ in either of the five begging behaviours mentioned above when corrected for egg type (table 6).

**Table 6. Differences in behaviour (mean + s.e. or percentage) between female L-chicks and male L-chicks of all treatment groups combined. Corrected for egg type, i.e. is in the model.**

| Behaviour | Male (n) | Female (n) | Test |
|---|---|---|---|
| Begging attempt:<br>a) successful | 1.72 ± 0.55 min (4) | 5.74 ± 2.90 min (7) | ANCOVA, sex:<br>$F_{1,9}$ = 0.41, p = 0.538 |
| b) unsuccessful | 3.86 ± 0.57 min (4) | 6.62 ± 2.45 min (9) | ANCOVA, sex:<br>$F_{1,11}$ = 0.35, p = 0.565 |
| Begging frequency<br>a) type a | 0.12 ± 0.045 (6) | 0.14 ± 0.027 (9) | ANCOVA, sex:<br>$F_{1,13}$ = 0.02, p = 0.892 |
| b) type b | 0.02 ± 0.014 (6) | 0.02 ± 0.007 (9) | ANCOVA, sex:<br>$F_{1,13}$ = 0.85, p = 0.376 |
| Proportion Begging | 0.11 ± 0.02 (6) | 0.21 ± 0.067 (9) | ANCOVA, arcsin transformed data, sex:<br>$F_{1,13}$ = 1.24, p = 0.287 |
| Feeding frequency | 1.3 ± 0.46 (6) | 1.1 ± 0.27 (9) | ANCOVA, sex:<br>$F_{1,13}$ = 1.00, p = 0.336 |
| Monopolisation frequency | 0% (6) | 22.2% (9) | Logistic regression, sex x egg type: ($\chi^2$ = 0.01 df = 1, p = 0.943; sex: ($\chi^2$ = 3.01, df = 1, p = 0.083 |
| Sibling begging frequency | 33.3% (6) | 44.4% (9) | Logistic regression, sex x egg type: ($\chi^2$ = 9.60 df = 1, p = 0.002 |

All interactions sex x egg type that are not shown in this table were non-significant (p > 0.282).

There was a significant interaction sex x egg type in sibling begging frequency (table 6, figure 4). In A-chicks, less female L-chicks begged at siblings then male L-chicks, whereas in C-chicks, 50% of female L-chicks begged at siblings, but no male L-chick showed this behaviour.

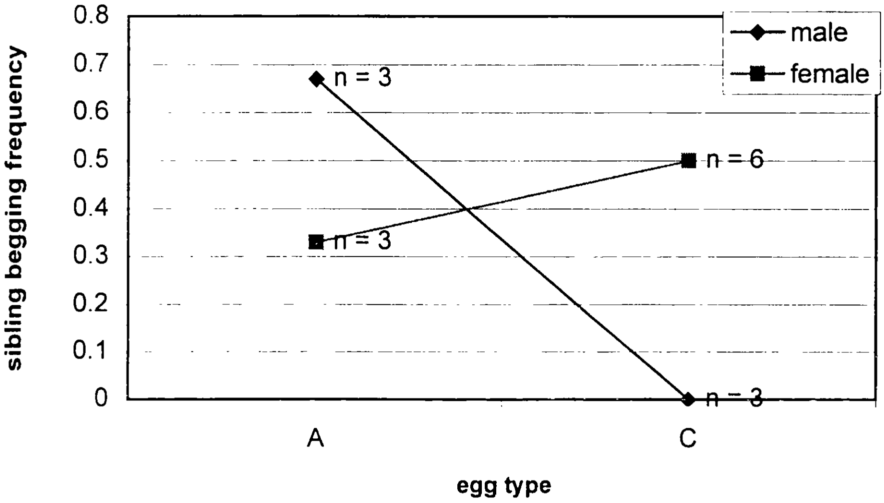


**Figure 4. Proportion of L-chicks, which begged at siblings analysed by sex and egg type. There was a significant sex x egg type interaction in sibling begging (logistic regression, $\chi^2 = 9.60$, df = 1, p = 0.002).**

Male and female L-chicks differed in their ability to monopolise food (table 6), although, this difference was not statistically significant. Only female L-chicks were able to monopolise food, but no male L-chicks (table 6, figure 5).

*6) Comparison of female and male chicks of all treatment groups combined in respect to egg type.*

When F- and L-chicks combined, male and female chicks differed significantly in their ability to monopolise food in respect to egg type (figure 5). About 10% of female and male

A-chicks monopolised food, whereas over 30% of female C-chicks, but no male C-chicks monopolised food.

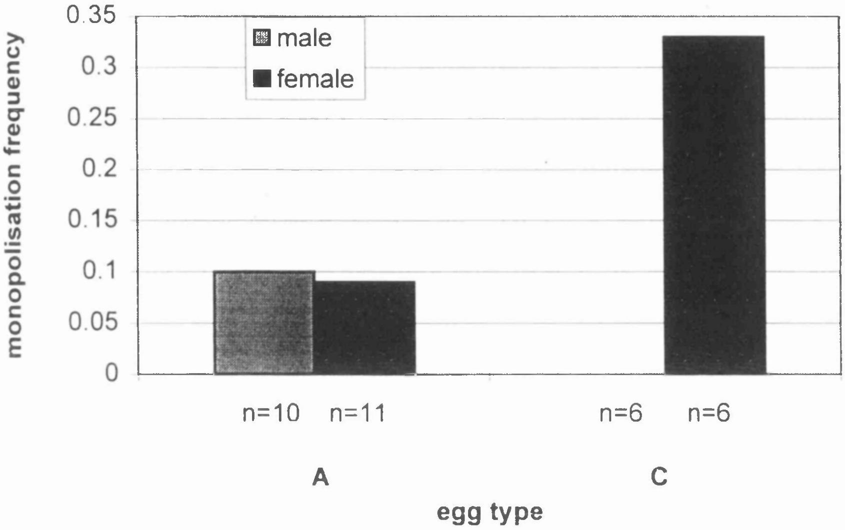


**Figure 5. Proportion of chicks, which were able to monopolise food at least once analysed by sex and egg type. There was a significant effect of the interaction between offspring sex and egg type on monopolisation frequency between male and female chicks (logistic regression, $\chi^2 = 5.47$, df = 1, p = 0.019).**

## DISCUSSION

The results of this study showed that there was an affect of egg type, hatching rank and/or offspring sex on chick behaviour. These three factors either affected chick behaviour independently or there was an interaction between two of them in the behaviour of the offspring.

The three treatment groups (ABC, ABA group and CBC) did not differ in the mean age at which individual broods were observed and in the mean number of chicks per brood. Therefore, possible changes in chick behaviour with age and brood size should not affect the results of the comparison since I covered the same age period and brood sizes in all groups.

The behaviours observed in begging and feeding by both the parents and the chicks (see page 14) were consistent with the behaviours described in the studies of Tinbergen and Falcus (1970), Tinbergen (1953), Goethe (1955) and Henderson (1975).

### *Effects of characteristics of the egg the chick hatched from and of the chicks's hatching rank within a brood on begging behaviour*

In control nests, A-and C-chicks did not differ in their begging behaviour and aggression towards siblings (food monopolisation, sibling begging). In experimental nests, L-chicks that hatched from C-eggs begged more frequently and tended to spend more time begging than L-chicks hatched from A-eggs. F-chicks hatched from C-eggs also tended to persist longer on begging until they obtained food from their parents compared to F-chicks hatched from A-eggs. These results suggest that differences in begging behaviour are at least partly due to differences in egg characteristics independent of the hatching order. The question is why did chicks that hatched from C-eggs spend more time begging and begged more frequently than chicks that hatched from A-eggs?

Henderson (1975) showed in his food-deprivation experiment on glaucous-winged gulls (*Larus glaucescens*) that the intensity of begging behaviour increased with increasing hunger level. Similarly, the begging behaviour off yellow-headed blackbird (*Xanthocephalus xanthocephalus*) chicks correlated with hunger level (Price and Ydenberg, 1995; Price et al., 1996). In this study, however, control C-chicks were fed as often as control A-chicks and even more often than last-hatched A-chicks. Thus a higher hunger level in C-chicks is rather unlikely to explain the observed differences in begging behaviour.

Some studies have found an increase in feeding with increasing begging behaviour (Smith and Montgomerie, 1991; Leonard and Horn, 1998). This work is partly consistent with these findings since last-hatched C-chicks both begged more intense and in return were fed more than last-hatched A-chicks. The higher feeding frequency in last-hatched C-chicks compared to last-hatched A-chicks suggests that C-chicks are more adapted to the last hatching rank within a brood. However, within the F-rank, A-chicks were fed more often despite the lower begging intensity than in C-chicks.

Parents might also feed according to criteria other than begging signals such as offspring size or position of the chicks within the nest (Smith and Montgomerie, 1991). This would leave the begging signals as the only option for smaller chicks to compete with their siblings and this could explain the higher begging frequency and longer begging attempts in the smallest chick (Ryden and Bengtsson, 1980; Drummond et al., 1986; Price and Ydenberg, 1995; Cotton et al., 1999). In this case, begging would reflect the siblings' rank in the brood hierarchy. since only last-hatched chicks would show a more intense begging behaviour. Such begging strategies have been shown by several studies (Leonard and Cohen, 1999; Mock and Ploger, 1987; Price et al., 1995; Cotton et al. 1999). For example, in European Starlings (*Sturnus vulgaris*) Cotton et al. (1999) found that in experimentally asynchronous broods the youngest chick spent more time begging than its oldest sibling, whereas, no differences were found in synchronously hatched broods. Therefore, differences in begging were explained by hatching rank rather than egg characteristics. The data on lesser black-backed gulls of this study, however, show that differences in begging behaviour occurred between F- and L-chicks as well as, within the L-rank, between A- and C-chicks. Thus, chick behaviour was affected by both egg type and hatching rank.

### *Aggressive behaviour between nestmates*

Within control nests, A- and C-chicks were equally able to monopolise food. Begging from siblings, however, did only occur in C-chicks.

Both first-hatched A-chicks and first-hatched C-chicks were able to monopolise food but never begged from their siblings. Last-hatched A-chicks never monopolised food boluses, but begged from their siblings, whereas last-hatched C-chicks showed both behaviours, food monopolisation and sibling begging. These results show that only last-hatched chicks directed begging behaviour towards their older siblings, and that within the L-rank, only C-chicks were able to monopolise food. These results contradict the findings of Fujioka (1985) and of Ploger and Mock (1986), which found that in cattle egrets (*Bubulcus ibis*) first-hatched A-chicks were able to monopolise food more often than their younger siblings. However, in cattle egrets, the hatching intervals between chicks of the same brood (Ploger and Mock, 1986) are larger than in lesser black-backed gulls. In this study, lesser black-backed gull C-chicks hatched 1.56 ± 0.11 days after the B-chick (Chapter II), whereas cattle egret C-chicks hatched 2.1 ± 0.087 days after their siblings (Ploger and Mock, 1986). Therefore, due to a higher level of asynchrony in cattle egrets, last-hatched C-chicks were even smaller relative to their nestmates than in lesser black-backed gulls. The smaller size difference between offspring may be an explanation for the absence of a difference in the success of food monopolisation between A- and C-chicks in lesser black-backed gull nests.

### *Possible mechanism of egg characteristics effect on chick behaviour*

Eggs differ in many aspects across the laying sequence such as androgen level, size, nutrients and conditions of their hatchlings.

Price et al. (1996) showed for yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) that chicks in poor condition begged more than chicks in good condition independent of hunger level. Furthermore, they found when they exchanged young between nests where offspring differed in size, chicks altered their begging intensity relative to the size of their new nestmates. Price et al. (1996) therefore hypothesised that yellow-headed blackbird chicks were able to assess their relative size and to express this size through their begging behaviour. Chapter III of my thesis showed that hatchling size differences within broods disappeared soon after hatching. Therefore, size differences among nesmates are rather unlikely to explain the differences in begging intensity found between A- and C-chicks of this study. However, C-chicks that hatched both first and last within a brood had a significantly lower condition at hatching than first- and last-hatching A-chicks (chapter 2). Therefore, the observed higher begging frequency and longer begging attempts in C-chicks compared to A-chicks could be explained by differences in hatching condition between these chicks.

Royle et al.(2001) found a higher testosterone level in lesser black-backed gull C-eggs in comparison with the hormone levels of A- and B-eggs. Schwabl (1996), Lipar and Ketterson (2000) and Eising et al. (2001) suggested that this increased androgen level might increase the C-chick's aggression and therefore its competitive ability. This study confirms this suggestion, since C-chicks showed more intense begging behaviour than A-chicks independent of hatching rank and when hatching last only C-chicks were able to monopolise food. Lipar and Ketterson (2000) found in red-winged blackbirds (*Agelaius phoeniceus*) an increasing mass of the hatching muscle *musculus complexus* with increasing laying order. This increase is thought to be due to the increasing testosterone concentration in later-laid eggs (Lipar and Ketterson, 2000). The hatching muscle may enable chicks to hatch more quickly, but also to extend their necks more, which is advantageous for effective begging behaviour (Ashmore et al., 1973). A stronger *musculus complexus* could therefore explain the C-chicks' ability to beg for longer and at a higher frequency.

### *Comparison of chick behaviour between sexes*

There were no differences in begging behaviour between male and female L-chicks. Within the last hatching rank, offspring sex therefore is not a factor that influences within-brood differences in begging behaviour in lesser black-backed gulls. This is contradictory to the study of Price et al. (1996) who found in yellow-headed blackbirds that males begged more than females, independent of hunger level. Similarly, Teather (1992) showed that in red winged blackbirds (*Agelaius phoeniceus*), male chicks begged more often than female chicks and were offered more food by the parents. This difference in feeding frequency between the sexes might also be dependent on hatching order, i.e. male and female chicks may differ compared both within and between hatching ranks. In lesser black-backed gulls of this study, both sexes were fed equally often, however, I only compared male and female chicks within the L-rank, thus hatching order was not addressed in this analysis.

The aggressive behaviour of chicks differed between the sexes. Independent of hatching rank, food monopolisation as well as begging from siblings did only occur in female C-chicks but not in male C-chicks. In A-chicks, food monopolisation and sibling begging were observed in equal frequencies in both sexes. These results show that these two behaviours presenting sibling aggression are dependent on both sex and egg characteristics. Although male lesser black-backed gull chicks are skeletally bigger, they are more likely to die under poor rearing conditions than females (Griffiths, 1992; Nager et al., 1999, 2000b). However, how female chicks are able to survive better under poor conditions still remains unclear. It has been suggested that this might be due to higher resource requirement of the bigger sex (e.g. Nager et al., 1999). Alternatively, Teather (1992) suggested that when competition between nestlings becomes more intense, and/or when nestlings are subjected to stressful conditions, female offspring may be able to compete more successfully. This hypothesis can be confirmed with my observations that female C-chicks could monopolise food and begged from their siblings but male chicks did not, thus female C-chicks appeared more aggressive. This work therefore showed that the characteristics of C-eggs increased especially the female C-chicks success within its particular disadvantageous rank.

Unfortunately, due to no data on first-hatching female C-chicks, I could not directly compare the behaviour of male and female chicks of different egg types among first-hatched chicks only.

### *Parental behaviour*

The mean feeding frequency per chick per hour was highest in broods of control group ABC. The attendance of at least one parent at the nest site, however, was more frequent in CBC nests than in ABC nests, but less frequent in ABA nests than in ABC nests. These results show that the order in which the different egg types (A, B, C) hatch within a brood also affects the behaviour of the parents. In unmanipulated broods, parents fed both A- and

C-chicks more frequently than in experimental broods. This study also showed that in terms of chick behaviour A-and C-chicks from control nests were more similar than in manipulated nests. The differences in egg characteristics across the laying sequence in natural lesser black-backed gull broods may therefore be evolutionary adaptive.

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# CHAPTER V.

## GENERAL DISCUSSION ON SIBLING RIVALRY

The aim of this study was to separate and determine the effect of hatching rank from the effect of egg characteristics on incubation duration, chick growth and survival, and chick behaviour. My results show that either hatching rank or egg characteristics affected these aspects of offspring performance, and sometimes hatching rank and egg characteristics acted simultaneously. Fledging success of both F- and L- chicks as well as the mean productivity of broods did not differ between treatment groups. The lack of a distinct third chick disadvantage contradicts the results of many other studies on Larus species. This does not support predictions of Lack's brood reduction hypothesis.

The results of **Chapter II** showed that C-chicks that hatched last in a brood had a shorter incubation duration due to a shorter pipping-hatching interval than A-chicks hatching last, i.e. they could emerge from the egg more quickly once they were fully developed. However, this phenomenon was only observed in last-hatched C-chicks, whereas first-hatched C-chicks had the same pipping-hatching interval than first-hatched A-chicks. These results demonstrate that both egg characteristic and hatching rank influenced incubation duration. Only C-chicks possessed the ability to hatch more quickly but they only did so when hatching last.

Chapter II showed a co-evolution of effects of hatching asynchrony and effects of egg characteristics on incubation duration. The characteristics of C-eggs allow them to catch-up a bit with their older siblings and might increase the C-chick's chance to fledge successfully.

The results of **Chapter III** showed a significantly lower hatching condition in C-chicks in both the F-rank and the L-rank within a brood compared to A-chicks. The low hatching condition could therefore be explained by the characteristics of the egg. Nevertheless, growth, survival and fledging condition did not differ between A- and C-chicks at neither of the two hatching ranks. The low hatching condition of C-chicks therefore had no detectable effect on the development of the chicks.

The results from this study suggest that the within-nest asymmetries found in lesser black-backed gulls are an evolutionary adaptation in order to increase the C-chick's success within its disadvantageous last hatching rank.

Both Chapter II and Chapter IV found an effect of hatching rank on the success of C-chicks. Chapters II and IV also found an effect of egg characteristics on the chick's behaviour. These results propose a co-evolution of hatching asynchrony and differences in egg characteristics within a brood.

Persson and Andersson (1999) found in their work on mallards and pheasants that offspring of these species could either decrease or increase the length of incubation in order to hatch synchronously within a brood. In both mallards and pheasants the length of incubation was positively correlated with tarsus and wing size at hatching, and in mallard chicks the length of incubation was also correlated with the growth rates of mass, tarsus and wing length (Perssons, 2000). Thus, mallard and pheasant chicks, which decreased the incubation length, had shorter wings and tarsi and mallard chicks even a lower mass at the age of 10 days post-hatching (Perssons, 2000). In this study, last-hatched C-chicks decreased their hatching duration and thus the length of incubation. The decreased hatching duration could be an explanation for the low hatching mass and poor hatching condition of lesser black-backed gull C-chicks. However, hatchlings from C-eggs that hatched first and did not accelerate the hatching process were also in poorer condition. I also did not find an effect of advanced hatching on the C-chicks growth rate or chick size.

The results of chapter **Chapter IV** demonstrated a strong effect of egg characteristics on chick behaviour. C-chicks begged for longer and often had a higher begging frequency than A-chicks irrespective of hatching rank. Furthermore, first-hatched C-chicks had a similar feeding frequency than first-hatched A-chicks and were fed even more frequently than last-hatched A-chicks. These results are consistent with the hypothesis that the high testosterone content in C-eggs increases the chicks' aggressiveness and competitive ability.

In C-chicks, only females begged from their siblings and monopolised food. Female lesser black-backed gull chicks despite their smaller size seemed to be better adapted to the last hatching rank than male chicks.

Chicks from control nests were fed both more frequently than chicks from experimental broods of the other two treatment groups. Chapter IV therefore suggests that the observed differences in egg characteristics across the laying sequence within broods of lesser black-backed gulls might represent an evolutionary adaptation.

The question is why C-chicks differ from their siblings. Last-hatching lesser black-backed gull C-chicks of this study were able to decrease the hatching duration, i.e. to emerge from the egg shell faster than A-chicks. Lipar and Ketterson (2000) found a positively correlation between testosterone concentration and the mass of the hatching muscle *musculus complexus* in the red-winged blackbird (*Agelaius phoeniceus*). The muscle enables the chicks to break the shell through dorsal and lateral head movements (Gross, 1985). Both testosterone concentration and *complexus* mass increased with laying order in the red-winged blackbird (Lipar, 2001) but data from lesser black-backed gulls are missing. A bigger hatching muscle of C-chicks in lesser black-backed gulls, however, may be an explanation for the shorter hatching duration in these chicks. Effects of androgen on *musculus complexus* may also explain the results of Chapter IV. C-chicks showed a more intense begging behaviour than A-chicks. The *musculus complexus* is also responsible for the extension of the neck during begging (Ashmore et al., 1973). A larger *musculus complexus* is suggested to allow nestlings to lift the head more rapidly and/or to maintain a begging posture for longer (Lipar, 2001). The higher begging intensity of lesser black-backed gull C-chicks may therefore also be explained by an increased mass of the hatching muscle. Both a shorter hatching duration and a higher begging intensity might increase the C-chicks success within its brood since both may decrease the effects caused through asynchronous hatching, i.e. differences in both size/age and competitive ability among siblings. Within control nests, C-chicks were fed at a similar frequency as A-chicks and were even fed more frequently than last-hatched A-chicks. Thus, chicks hatched from C-eggs did indeed have a high success in terms of competition over food resources.

At hatching, C-chicks had a lower mass and a lower condition than A-chicks independent of hatching rank. However, no difference in mass and condition was found between A- and C-chicks at fledging. C-chicks must therefore have caught up with its nestmates in terms of growth and development. I did not find a higher growth rate, neither of mass nor of body size, in C-chicks compared to A-chicks. However, I only compared growth rates between 5-21 days of age. Hebert and Barclay (1985) showed that within herring gull broods A- and B-chicks grew faster than C-chicks during the first 5 days after hatching. Even though these results are contradictory to this study, it shows that the differences in growth rates may only occur during the early nesting period (see also Nager et al., 2000b), which then could explain similar condition and mass of fledglings coming from A- and C-eggs. The higher feeding frequency observed in last-hatched C-chicks in chapter IV could explain how these chicks might have caught up with their older siblings as observed in chapter III.

It has also been observed that the differences in chick behaviour are at least partly due to characteristics of nestmates. Oddie (2000) found in cross-fostering experiments of great tit (*Parus major*) broods, that, not surprisingly, small nestlings suffered from higher mortality when mixed with larger and older nestlings from another brood. Price et al. (1996) also found that when offspring of different size were exchanged between nests, yellow-headed blackbird (*Xanthocephalus xanthocephalus*) chicks altered their begging intensity relative to the size of their nestmates. However, focal chicks of this study always had the same set of nestmates (table 1). Therefore, the effect of nestmates on chick performance should have been equal among broods, which were compared in this study.

**Table 1. Egg types of nestmates of focal chicks compared between control and experimental nests.**

| | first-hatched chicks | | | last-hatched chicks | | |
|---|---|---|---|---|---|---|
| treatment | focal chick | | nestmates | nestmates | | focal chick |
| control nests | A | -- | **BC** | **AB** | -- | C |
| experimental nests | C | -- | **BC** | **AB** | -- | A |

However, in both control nests and experimental nests, chicks that hatched from eggs that were swapped between nests did grow up with non-siblings. Non-siblings might change the overall behaviour within a brood. Several studies suggest that individuals may show behavioural traits that confer a net cost to themselves but therefore increase the fitness of their kin (Mock and Parker, 1997). If nestmates are not related to each other, these behavioural traits might change, e.g. the individual offspring might become more selfish and therefore might affect the fitness of the whole brood. However, control broods in this study consisted like experimental broods of non-kin nestlings. Thus, an effect of non-kin siblings on behaviour should not be responsible for differences I found between treatment groups.

In lesser black-backed gulls, where the male is of the larger size, male offspring are more vulnerable to poor resource supply than female offspring, both as an embryo and as a chick (Griffiths, 1992; Nager et al., 1999, 2000a). A sex-biased nesting mortality rate has also been shown in several other studies (reviewed in Stoleson and Beissinger, 1995). Griffiths (1992) and Nager et al. (2000a) suggested that the low survival of male offspring might be due to a higher metabolic rate and energy requirement, i.e. a higher demand for parental resources, which may lead to starvation of male offspring during periods where food is not very abundant. Accordingly, Stolesson and Beissinger (1995) proposed that sex-biased mortality is a nonadaptive consequence of difference in susceptibility to starvation between the sexes. However, Teather (1992) suggested that in red-winged blackbirds, when competition between nestlings becomes more intense, and/or when nestlings are subjected to stressful conditions. females, which are smaller and generally less successful in this species. may be able to compete more successfully. The results on lesser black-backed gull chick behaviour in this study showed that female chicks were more aggressive and more successful in terms of food monopolisation than male chicks when being the last-hatching chick within a brood. i.e. in a disadvantageous environment, and therefore confirm this hypothesis. It would be of great interest to investigate differences in incubation duration, pipping-hatching interval, growth and fledging success between male and female lesser black-backed gull chicks of this study in order to determine whether female C-chicks are more successful in other aspects of the nesting stage as well. Griffiths (1992) showed that in lesser black-backed gulls male offspring grow faster than female offspring. However, it is generally very little known about differences in the growth process of male and female nestlings.

Furthermore, the lack of a distinct third-chick-disadvantage in all three chapters contradicts the predictions of the brood reduction hypothesis (Lack, 1954). The predictions of Stinson's (1979) insurance hypothesis could also not be confirmed since last-hatching chicks did not show a significant insurance value, i.e. they did not survive significantly more often when either one or both of the earlier hatched chicks died than when both of the older nestmates survived. Several studies on lesser black-backed gulls have found a lower fledging rate in C-chicks compared to chick A and chick B and therefore provided support for brood reduction within this species (Griffiths, 1992; Hario and Rudbäck, 1996, 1999). The differences between the results of those studies and the results of my study may be due to differences in food availability and predation frequency between both the study sites and between the years the studies were conducted. In this study, control broods produced 1.5 ± 0.2 fledglings, similar to the breeding success of the lesser black-backed gull described by Bezzel (0.75-1.5 fledglings per brood). Heg and Van der Velde (2001) described in their study on oystercatchers (*Haematopus ostralegus*) an interaction of the brood reduction hypothesis and the insurance hypothesis. In years with high food abundance and low predation rates, brood reduction may operate and enhance the survival of the early hatchlings while reducing the survival of the last chick. In years of poor food abundance and high predation rates, the last chick increases the parents' possibility to rear at least one chick. Mortality recorded in lesser black-backed gull chicks in this study was mainly due to chick predation: a lot of chicks went missing, while I also observed adult gulls predating neighbouring offspring and found several remains of chicks that had been predated by foxes. The observed high mortality confirm the prediction of Heg and Van der Velde (2001) that in years of high predation parents do not facilitate brood reduction. However, this study did not confirm the predictions of the insurance hypothesis, either. Predation occurred at random and did not relate to the within-brood hatching order. Thus, the high predation rate may have randomly affected my results of chick performance and could therefore explain the observed equal fledging success of A-and C-chicks which does not support either of the two hypotheses on the significance of asynchrony.

Several studies that investigated the significance of hatching asynchrony found that broods which hatched asynchronously differed in their productivity compared to synchronously hatched broods (reviewed in Stoleson and Beissinger, 1995). Several studies showed that asynchronous broods had a higher fledging rate than synchronous broods, suggesting an evolutionary adaptation of hatching asynchrony in order to increase the breeding success of individual pairs (reviewed in Stoleson and Beissinger, 1995). Nevertheless, hatching asynchrony has also been shown to decrease the survival of last-hatched chicks and therefore seems maladapted for the individual offspring's survival (Maynard-Smith and Parker, 1976; Hebert and Barclay, 1985; Fujioka, 1985; Hahn, 1981; Heg and Van der Velde, 2001). In addition, fledging success of experimentally synchronised nests was frequently equal to or even greater than the fledging success of asynchronous broods (reviewed in Stoleson and Beissinger, 1995). Asynchronous hatching is obviously still not fully understood, and evidence for its advantages for offspring survival is contradictory. Nevertheless, there is an effect of the level of synchrony on the fledging success of individual offspring and/or the entire brood. In this study, a longer hatching duration in last-hatched A-chicks did not result in an increased asynchrony. Hence, the experimental manipulation of the hatching order did not affect the level of asynchrony, which was similar in the three treatment groups and therefore could not skew the results of any of the three chapters towards one of the experimental groups. Furthermore, the results of chapter II and chapter IV showed that the C-chicks' advantage during both incubation (shorter hatching duration) and during competition over food resources (high feeding frequency) was stronger when the C-chicks hatched last. The effect of the hatching rank on C-chick success, but not A-chicks, therefore supports the hypothesis that C-eggs have special characteristics that allow C-chicks to deal with the disadvantageous of hatching asynchrony.

Chapter IV showed that the within-brood asymmetries played a role not just in chick behaviour but also in parental behaviour, as well. The proportional attendance of parents at the nest site was higher in CBC broods but lower in ABA broods compared to control ABC broods. Furthermore, the feeding frequency was highest for both F- and L-chicks in control nests than in experimental nests. Since the level of hatching asynchrony was not different among the three treatment groups (Chapter II), the described differences in parental performance are likely to be explained by differences in the within-brood asymmetries. Differences in parental behaviour may in return affect offspring behaviour as well as offspring growth, development and survival (Dewey and Kennedy, 2001). The role of parental performance on chick survival and behaviour is very difficult to separate from other factors that affect offspring such as nestmates, egg characteristics and hatching asynchrony (Clutton-Brock, 1991). Normally studies on asynchrony focus on advantages and disadvantages to the chicks. Nevertheless, few studies looked at the cost and benefits to the parents. This study does not distinguish between effects of quality and behaviour of parents and effects of egg characteristics on the success of the offspring. Nevertheless, the results of my work indicate that this matter is even more complicated since egg characteristics might also affect the behaviour of parents.

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