Sibling Rivalry in Black-legged Kittiwakes

(*Rissa tridactyla*)

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Candidate's declaration

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

Adriana Vallarino Moncada

January 2008

To my father, Roberto Vallarino (1955-2002)

for his rebel word against the establishment

“El que agandalla no batalla”

Mexican popular saying about people getting what they want without caring for others.

ABSTRACT

Chicks of several species compete with their siblings for parental provisioning of resources and care. This competition is mainly manifested by begging or food hoarding and in only few species, as in the black-legged kittiwake (*Rissa tridactyla*) direct aggression between the offspring is present.. It has been proposed that the degree of asymmetry between the members of a brood influences the severity and outcome of this conflict. Several inequalities between the offspring have been identified (e.g. age, size, egg quality). First- hatched chicks (A) are older, larger and hatch from eggs of different quality and size than second- hatched chicks (B). These inequalities provide different advantages to the chicks within a brood, but their influence in sibling rivalry has not been widely tested. In the present thesis the components of A and B eggs were analysed, comparisons of behaviour, survival and growth of chicks in two different years were made and egg size and quality were experimentally manipulated in order to test their importance in the sibling rivarly outcome of the black-legged kittiwake.

Chapter II describes composition analyses of A- and B- eggs within a clutch. It was found that mothers allocated more carotenoids and less testosterone to A- eggs, while corticosterone, lipid and protein content did not differ, although A- eggs were 4% larger than B- eggs. In **chapter III** comparisons of behaviour, growth and survival between two environmentally different years were made. 2004 was a year with poor quality food, whilst 2005 was a year with good food quality. In 2004, the majority of second-hatched chicks died before reaching 10 days of age and all were dead before 15 days; in 2005, more than 80% of second-hatched chicks fledged. The same behaviour patterns were performed by A and B chicks in the two years, but their pattern of change with age differed. In 2004, aggression increased with age while begging and feeding decreased in A and B chicks; these behaviours did not change with age in 2005. In **chapter IV** the influence of egg size on sibling rivalry was experimentally tested by eliminating age and egg quality differences within a brood and manipulating only the egg size differences. Comparisons of survival, growth and behaviour were made between experimental broods and control broods in which all the natural asymmetries were present. Differences in egg size determined which chick became dominant, but these differences did not change the brood behaviour. Furthermore, experimental broods showed very different behaviour from control broods and the frequency of aggression was different between experimental broods formed by A- or B- eggs. In order to test if eggs are adapted for their hatching position, in **chapter V** an experimental manipulation of the brood composition was carried out. Eggs were swapped between clutches in such a way that they hatched in a different

position from the one they would have had if left undisturbed. The natural asymmetries in age and egg size were maintained. Broods with two first-laid eggs were less aggressive than control broods and than broods with two second-laid eggs. If A- and B- chicks are provisioned in a manner usited for their hatching position, their stress response should differ when they were artificially made to hatch on the same position and exposed to a stressor. This was tested on **chapter VI** and it was found that second hatched chicks from A- and B eggs did not differ on their stress response when facing a handling-stress protocol. In each year, natural broods fledged in higher proportion than experimental ones, which indicates that asymmetries within a brood are adaptive. It seems that eliminating within-brood asymmetries is costly for the parents and perhaps these differences are optimal for maintaining a efficiency for the parents in terms of the amount of investment and the number of fledged chicks. Thus apparently, the main asymmetry influencing sibling rivalry is the difference in age of the offspring caused by hatching asynchronously.

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Chapter I

General Introduction

Sibling rivalry was defined by Mock and Parker (1997) as "any features of animals or plants that have the effect of promoting individual survival and/or reproduction at the expense of siblings". The cost to the disadvantaged siblings is not always an immediate death, but can take the from of diminished growth and survival (Mock & Parker, 1998). Obligate or facultative siblicide can be present as a consequence of this rivalry. Sibling rivalry is widespread in mammals (e.g. pigs, hyenas, seals, lynxes; reviewed in: (reviewed in: Fraser et al., 1995; Golla et al., 1999; Trillmich & Wolf, 2008), birds, (reviewed in: Mock & Parker, 1998) and parasitoids (Pexton & Mayhew, 2002). However, sibling rivalry has been mainly studied in the avian taxa, mainly due to the number of species showing some type of competition between sibling and to the fact that birds can more readily be observed in natural conditions and/or captivity compared with mammals.

Chicks from several species compete with their siblings for resources and parental care (reviewed in Mock & Parker, 1998; Drummond, 2001a). Chicks can contend through begging or agonism to obtain food; brooding and protection from thermal stress, predation and infanticide from other adults (reviewed in Mock & Parker, 1998; Drummond, 2001a). Begging competition is present in the majority of the avian species' chicks (Drummond, 2004). Usually the first hatched chick is more developed thus performs begging in a more efficient way and in a better position within the nest which will provide it with more food from the parents (Glassey et al., 2002). This confers first-hatched chicks with an advantage towards their siblings and increases their chances of surviving (Forbes et al., 1997). On the other hand, species that compete with their siblings with agonism need to perform aggressive acts (pecking, biting and pushing) in order to gain access to resources (Mock & Ploger, 1987; Mock & Parker, 1998). This aggression can be constant and open or can be present in a low frequency only attacking the necessary to establish a dominance hierarchy depending on the species (Mock & Ploger, 1987; Mock & Parker, 1998; Drummond, 2001a).

In species of birds where siblicide occurs, the agonistic relation between these siblings varies from terrible aggression to a ritualised dominance – subordination relationship (Drummond 2001a). Species that show an uncontrollable aggression between siblings (usually two or three per brood) and always perform siblicide are known as obligate brood reducers. Chicks of species that show aggression but do not always kill their siblings are called facultative brood reducers. Facultative reduction seems to be modulated by the amount of food present during the rearing period. If food is sufficient for raising the whole brood all the hatched chicks will survive but siblicide will occur if food is not enough (Braun & Hunt, 1984; Mock & Ploger, 1987; Forbes, 1991; Drummond, 2001b).

A conflict between offspring and parents could arise because differences between the optimum distribution of parental resources for the parent and the offspring normally exist (Drummond et al., 1986; Forbes, 1993; Godfray, 1995; Rodriguez-Girones, 1996; Ricklefs, 2002). Access to food brought by parents depends on the size-related competitive abilities of the young (Lipar & Ketterson, 2000). Chicks within a brood will usually hatch asynchronously from eggs of different size and quality (Williams, 1994; Simmons, 1997; Vinuela, 2000). Asynchronous hatching will provoke first hatched chicks to start growing earlier and gain developmental advantages towards their siblings (Slagsvold & Lifjeld, 1989; Amundsen & Slagsvold, 1996). These within-brood asymmetries regulate the competition for food because an age/size hierarchy is usually established and nestlings learn their social positions without having to compete each time to gain access to resources (Osorno & Drummond, 1995). In facultative brood reducer species dominance-subordinate hierarchies establishes and once they are formed, the competition for food decrease (Drummond, 2001a). Moreover, each chick is able to monitor the level of solicitation displayed by its brood mate (Godfray, 1995). Thus chicks are simultaneously receivers and signallers, which have the potential of also modulate their competition for food (Godfray, 1995).

In species that show offspring agonism parents might monitor aggression levels as an indicator of brood needs (Godfray, 1995). Therefore within-brood aggression plays two roles: one signalling and another one determining food allocation in the brood (Rodriguez-Girones, 1996). If food amount affects aggressiveness, then parental food allocation will exert a direct influence on nestling agonism (Rodriguez-Girones, 1996). However, it is uncertain to what extent food allocation is controlled by parents rather than by chicks (Drummond 2001a). Evidence exists showing that provisioning behaviour depend on signals from all the brood members and the interpretation of these signals can vary between and within populations (Hinde & Kilner, 2007). Parents should use the information concerning the nutritional state of their offspring to allocate food in a manner that maximizes their own fitness (Ricklefs, 2002).

Apparently parents do not directly interfere with the chicks' decision of performing siblicide. The amount of aggression seems to be regulated by the availability of food during the rearing period, but a maternal influence could be present since the egg stage. Females of several species allocate eggs within the same clutch with different amounts of nutrients and hormones according to the laying order (reviewed in Royle et al., 2001; Groothuis et al., 2005; Eising et al., 2006). This manipulation has the potential to influence chicks' behaviour and survival depending on the environmental conditions present (Groothuis et al., 2005; Eising et al., 2006). It should also favour mother interests if chicks can not do anything to protect themselves against this manipulation at the egg stage, although this is unlikely (reviewed in Muller et al., 2007). Chicks should have developed tools in order to cope with the maternal influence at the egg stage if this manipulation goes against their interests (Muller et al., 2007). As mentioned before, it is unlikely that a conflict between mothers and offspring is absent, therefore, some response from the chicks should be present to be able to defend themselves against maternal manipulations (Winkler, 1993; Muller et al., 2007).

Parents lay the maximum number of eggs they can rear during a good year very early on the season, before they can assess the quality of that present year (Lack, 1947). There are various hypotheses to explain why parents overproduce and allow their offspring to show sibling rivalry without interfering:

- Egg Insurance: Dorward (1962) proposed that parents lay more eggs than they can always rear because extra eggs work as replacement eggs, in case some of them are lost during the incubation period. Extra eggs should occur where hatch failure is not trivial and second eggs are inexpensive (Forbes, 1990).

- Chick Insurance: Depending on the asymmetries between the chicks of a brood (e.g. age and size), it is the time it will take senior chicks to eliminate the junior chick. If conditions are bad, seniors can get rid of their senior siblings soon after hatching but if conditions are good, the extra chick will increase parents' fitness (Mock & Parker, 1986).
- Additional egg hypothesis: Females lay a second egg when offspring from both eggs sometimes fledge (Tershy et al., 2000).
- Ice box hypothesis: An extra sibling is produced on the nest in order to feed the stronger chicks of a brood if the amount of food provided is insufficient (Ingram, 1959).

In the present thesis I studied the factors influencing the outcome of sibling rivalry in a species that show facultative brood reduction, the black-legged Kittiwake (*Rissa tridactyla*). Kittiwakes lay from one to three eggs which hatch with a difference of 1.5 to 2 days. Siblicide in this species is regulated by the food amount available (Braun & Hunt, 1984), but it is not clear how factors like egg size and egg quality influence chicks' behaviour and physiology and the outcome of the conflict between the siblings. Usually the first hatched chick within a brood becomes dominant over its second hatched sibling, which will be subordinate. The term dominance was defined by Schjeldereup-Ebbe (1922) as follows: "Dominance is an attribute of the pattern of repeated, agonistic interactions between two individuals, characterized by a consistent outcome in favour of the same dyad member and a default yielding response of its opponent rather than escalation. The status of the consistent winner is dominant and that of the loser subordinate. Dominance status refers to dyads while dominance rank, high or low, refers to the position in a hierarchy and, thus, depends on group composition". Dominance is a relative measure and not an absolute property of individuals. Later on, this concept included the priority of access to resources that dominance confers to the individuals.

Fieldwork was carried out on the Isle of May on the East Coast of Scotland, Firth of Forth (fig.1.1) during 2004 and 2005. Number of kittiwakes on this colony have decline since the early 90's and it was aggravated by the presence of an industrial fishery during 8 years. When the fishery was closed there was an increase in the population, but numbers went down again soon after this imminent increase (Frederiksen et al., 2004). Understanding better the factors by which kittiwakes decide or not to perform siblicide could help to provide more tools to prevent kittiwakes from disappearing from the North Sea. If other factors apart from the amount of food availability exert a big influence on kittiwake's sibling rivalry, they could potentially be manipulated in order to enhance their survival chances.

Figure 1.1 Map of the Isle of May in the Firth of Forth, Scotland. The kittiwake population from this island is spread out in several sub-colonies. Sub-colonies studied in the present work are marked with a black triangle ▲.

In **chapter II** whole clutches of kittiwakes were collected soon after being laid. Lipids, protein, water, carotenoids, testosterone and corticosterone from these clutches were measured. It was compared if first and second laid eggs had different amounts of nutrients, carotenoids and hormones. These analyses were done to establish if kittiwake mothers manipulate egg components according to the laying order and to know if she favours eggs in a certain position (Schwabl et al., 1997). Differential egg allocation could affect chicks' behaviour and influence the outcome of the conflict between siblings (Schwabl et al., 1997; Groothuis & Schwabl, 2002; Groothuis et al., 2005).

In **Chapter III** I studied behaviour and survival of broods in two consecutive but different years in terms of survival and food quality. Broods used as controls for the experiments carried out on each year were studied and compared between years. Comparisons were made in order to determine the influence of environment on within-brood chicks' competition. It is known that food amount available plays an important role in determining the presence and outcome of sibling rivalry (Braun & Hunt, 1984). Having behavioural recordings as well as growth and survival rates from two consecutive years that turned to be very different from each other was lucky. It is, perhaps, a unique opportunity to assess how food availability or quality during the rearing period is able to influence chicks survival and sibling rivalry.

In **chapter IV** the influence of egg size in sibling rivalry of kittiwakes was studied. The importance of egg size on the conflict between offspring has seldom been studied. It is not well established if the egg size differences within a clutch are a consequence of mothers' decision or if it is a consequence of laying consecutive eggs (Bowden et al., 2004). Egg size could be an important factor on sibling rivalry because a positive relationship exists between egg size and the size of newly hatchlings (Deeming & Birchard, 2007). Hatching from a larger egg is advantageous because it provides more nutrients and a larger size at hatching, which should confer an advantage if one has to compete for resources with another hatchling (Mock et al., 1990). The experimental designed used to test the importance of size is shown in Figure 1.2.

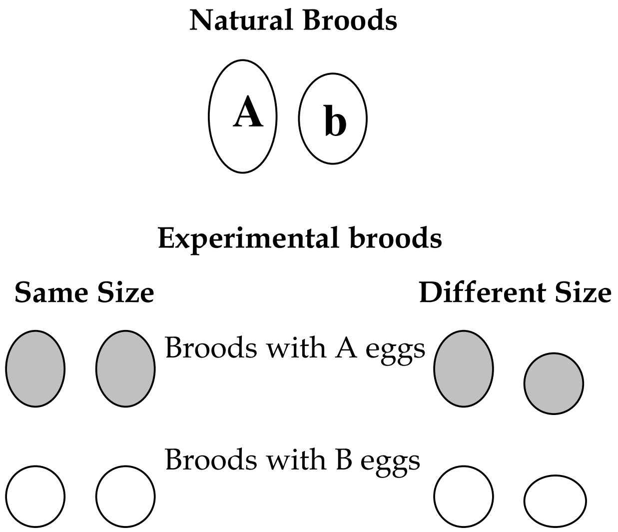

Fig 1.2 Experimental manipulation of kittiwake broods in 2004. Age, laying date and egg quality were matched between egg pairs. Only size was similar or different between the eggs. Broods with A eggs are gray coloured and broods with B eggs are white coloured.

If mothers are making within-brood different quality eggs according to the laying order, it is expected that they favour each of their chicks depending on their specific hatching position. For this reason, in **chapter V** experimental manipulations were carried out to alter the natural brood composition and compare the behaviour of altered broods with that of naturals. Only one member of the clutch was altered and all the natural within-brood asymmetries were maintained (fig. 1.3).

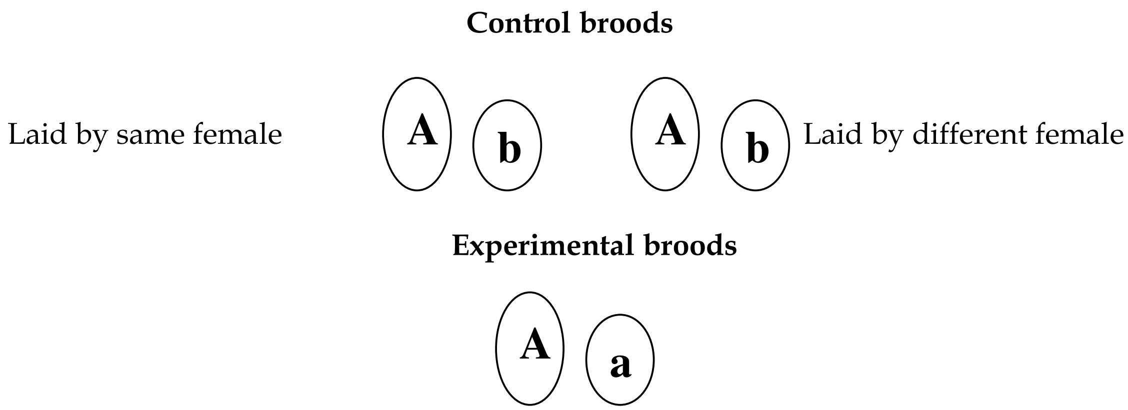

Fig 1.3 Experimental manipulation of broods in 2005. Two control groups were observed: one with eggs laid by the same female and another one with eggs laid by different females. Controls were compared with one experimental group in which the second egg was originally laid on the first position. The natural asymmetries in egg and size were maintained in the three groups.

If mothers provide and advantage to her chicks by differentially egg allocating various components, chicks hatched on a different position than the one they were meant to be should behave and respond different to stressors than chicks hatched on their natural position (**chapter V and VI**).

To conclude, in chapter **VII** I discussed the within-brood asymmetries' influence on the outcome of sibling rivalry as well as some new insights the results from this thesis offer. I examined what it needs to be done to clarify or increase the understanding of the sibling rivalry not only in kittiwakes but in other brood reducer species.

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Chapter II

Opposite Within-Brood Patterns in Hormone and Nutrient Concentrations in Kittiwake Eggs

Introduction

In many avian species two or more eggs are laid in the same clutch and asymmetries between them are present. Chicks within a brood hatch asynchronously according to their laying order. The first functional explanation for hatching asynchrony was proposed by Lack (1947). He stated that parents lay as many eggs as they can raise under optimal rearing conditions and the asynchronous hatching can effectively down-regulate the size of a brood if the amount of food is less than optimal.

Parents have the option to produce more eggs than the number of chicks they could fledge even if conditions are not good at a time where it is uncertain how the present reproductive season will be in terms of food availability. By doing this they promote the brood reduction if food is not enough but have the chance of produce a higher number of chicks if the food available is sufficient without diminishing their fitness (brood reduction strategy, O'Connor, 1978). This strategy works mainly due to the hatching asynchrony within a brood which cause asymmetries in age and size (reviewed in Stenning 1996). Hatching asynchrony is present because parents often start to incubate before the clutch is complete thus embryos start their development at different times (Stoleson & Beissinger, 1995).

There are other less studied factors differing within a clutch: egg size and egg components. Egg size variation within a clutch is regulated by physiological limitations during egg formation like the resources available during the laying period (Ylimaunu & Jarvinen, 1987) or the females' condition (Houston et al., 1983). It has been proposed that species that adopt a brood reduction strategy (O'connor, 1978) lay a smaller last egg than species that adopt a brood survival strategy (Clark & Wilson, 1981). Contrary to the brood reduction strategy proposed by O'connor, the brood survival strategy proposes that parents attempt to decrease the competitive differences between nest mates. Parents are able to diminish the differences between their offspring by reducing the hatching asynchrony, making eggs of similar sizes (Slagsvold et al., 1984) and allocating egg components in similar amounts. The adoption of these different strategies not only varies between species but within species (e.g. Muller et al., 2004). Adults are capable of modifying the strategy they use depending on the environmental conditions of each season or to fast changes that take place within a season (Tobler et al., 2007a).

As previously mentioned, mothers influence offspring phenotype by manipulating the amount of resources (e.g. carotenoids, hormones, antibodies) they allocate to their eggs (e.g. Eising et al., 2001; Royle et al., 2001; Grindstaff et al., 2005). The differential manipulation of the amount of these egg components can have profound effects on growth, development, behaviour and fitness of the offspring (reviewed in Nager, 2006). It has been suggested that these manipulations are reproductive strategies where mothers invest according to ecological factors or partner attractiveness that may indicate rearing capabilities or potential provisioning on the present reproductive attempt (Winkler, 1993; Muller et al., 2007). The egg components mothers have the potential to manipulate and have received more attention are hormones (e.g. androgens and corticosterone) and carotenoids (due to the unclear evidence of their antioxidant properties (Costantini & Moller, 2008).

Maternal hormones transferred into the egg seem to play an important role in causing asymmetries between the siblings (Winkler, 1993; Muller et al., 2007). Elevated levels of yolk androgens enhance the development of the embryo’s hatching muscle thus accelerating the hatching time (Lipar et al., 1999; Lipar & Ketterson, 2000) and thereby having the potential of decreasing hatching asynchrony (Eising et al., 2001; Gorman & Williams, 2005; Gil et al., 2007). The deposition of androgens can indirectly increase aggression by decreasing the hatching asynchrony (Ketterson et al., 1992) and altering the begging behaviour (Schwabl et al., 2002; Eising & Groothuis, 2003) or directly increase aggression by exerting an amount-dependant effect on this behaviour (Groothuis & Ros, 2005). Androgens can also have detrimental effects on the newly-hatched chicks by increasing oxidative stress and metabolic rate, and suppressing the immune system (Sockman & Schwabl, 2000; Royle et al., 2001; Gasparini et al., 2007; Tobler et al., 2007b).

In some species deposition of yolk androgens increases over the laying sequence, in others decrease, and in others do not differ (reviewed in Groothuis et al., 2005). By allocating more androgens to the later-laid eggs of a clutch, the mother could be compensating for the difference in age and size of the last hatched chick (brood survival strategy, Schwabl, 1993; Eising & Groothuis, 2003; Muller et al., 2004; Navara et al., 2005). Mothers could also favour older siblings to out-compete younger ones when food is not sufficient to rear the whole brood by decreasing the level of androgens in later-laid eggs (brood reduction strategy, Schwabl et al., 1997; Tobler et al., 2007). In cattle egrets (*Bubulcus ibis*), a species where nestlings show strong sibling rivalry and siblicide, androgen yolk levels were higher in the first-laid eggs (in clutches of three) and decreased with the laying sequence (Schwabl et al. 1997). Schwabl et al. (1997) proposed the hormonal parent favouritism (PHF) hypothesis. They stated that mothers allocate egg hormones within a clutch in a way that favours the more advantaged chick (the first-hatched) in order to eliminate its sibling faster. The amount of androgens mothers allocate to an egg can vary depending on the environmental conditions of the breeding season. Gasparini et al. (2007) showed that kittiwake mothers in bad condition allocated more androgens to their eggs probably because they were not able to provide enough resources and this increase could in turn favour their chicks on the short term developing phase.

Stressed mothers can lay eggs with high levels of corticosterone (Hayward & Wingfield, 2001; Saino et al., 2005). Although exposure to maternal glucocorticosteroids can have detrimental and long-lasting effects on development, growth and learning (Schwabl, 1999), and immune functions (Rubolini et al., 2006). Corticosterone may alter offspring phenotype in order to maximize fitness under suboptimal conditions by increasing provisioning rate and fat reserves and producing an intense adrenocortical response to acute stress (Hayward & Wingfield, 2004). Experimentally elevating glucocorticoids in the albumen of domestic chicks increased embryonic mortality, developmental instability of the skeleton, impaired embryonic development, and reduced hatchling weight and growth (reviewed in Rubolini et al., 2005). High circulating levels of corticosterone in chicks might increase begging behaviour (Kitaysky et al., 2001), impair learning abilities (Kitaysky et al., 2003), determine social status (Nunez de la Mora et al., 1996), increase submissiveness in an indirect way and influence the behaviour of siblings that are not necessarily stressed (Drummond et al., 2003). But the relationship between egg corticosterone and chick's corticosterone titres has been studied only in a few number of species (Hayward & Wingfield, 2004; Rubolini et al., 2005).

Other egg components that can sometimes vary depending on the laying order are carotenoids (Royle et al., 2001; Blount et al., 2002; Torok et al., 2007; Berthouly et al., 2007). They are powerful antioxidants and immunostimulants that trap free radicals released during normal physiological processes (Young & Lowe, 2001 but see Constantini & Moller, 2008) and provide the yolk with its characteristic bright yellow colour (e.g. Blount et al. 2000). Carotenoids in the eggs are higher if their consumption by the mother is high (Blount et al., 2002; Blount et al., 2004; McGraw et al., 2005). Chicks with high levels of carotenoids are less likely to contract infectious diseases and can cope better with stressful events than chicks with lower levels (Rock, 1997). Females could increase the levels of carotenoids they put into their eggs to provide protection to the offspring and enhance their fitness (Royle et al., 2001; McGraw et al., 2005).

Yolk hormones (androgens and corticosterone) and carotenoids have opposing effects on the immune system; while carotenoids boost the immune response, elevated androgen and corticosterone levels affect it detrimentally (Saino et al., 2003; Muller et al., 2005; Berthouly et al., 2007). In the lesser black-backed gull (*Larus fuscus*), carotenoids decreased over the laying sequence whereas testosterone increased (Royle et al. 2001). In species with the mentioned within clutch androgens allocation, high levels of these hormones will favour a chick hatched in the last position. Chicks will be more competitive and capable of getting more food thanks to the androgens effect in increasing begging and favouring growth (Schwabl, 1993; Schwabl, 1996; Eising et al., 2001; Eising & Groothuis, 2003). If the food availability is not good, first-hatched chicks will cope better than second-hatched chicks with this lack thanks to the antioxidant protection of the extra carotenoids (e.g. enhancing the immune system, Royle et al., 2001; Blount et al., 2002a; Saino et al., 2003). On the other hand, second- hatched will have an opportunity to survive due to the increased competitive abilities provided by the maternal androgens (reviewed in Muller et al., 2007). These two opposing maternal effects may allow parents to regulate the relative costs and benefits of having an extra chick in good or poor years (Royle et al., 2001; Groothuis et al., 2006).

By manipulating the egg size and composition mothers will be primarily affecting the chick size (Slagsvold et al., 1984; Vinuela, 1997; Budden & Beissinger, 2005). Larger eggs in general are considered to have a higher absolute nutrient content (e.g. lipids and proteins) than smaller eggs (Howe, 1976; Ricklefs, 1977; Williams, 1994). Depending on the species larger eggs will have more albumen or more yolk (Williams, 1994). Larger eggs with relatively higher albumen or yolk mass have more water or lipids (respectively) than smaller eggs (Williams, 1994). Mothers could allocate these components differently according to the laying order and the type of sibling competition present in each species.

Egg composition within broods of facultative reducer species have seldom been studied (e.g. cattle egret, Schwabl et. al., 1997). It is not known if in other siblicidal species mothers favour the competition of the first-laid egg by allocating more androgens or if it regulates the conflict by assigning other hormones or nutrients to second laid eggs.

The aim of this study was to test in the black-legged kittiwake (*Rissa tridactyla),* a species where chicks show facultative siblicide how mothers allocate different egg nutrients and hormones according to the laying order. It was tested whether the mother decreases the amount of androgens with the laying sequence as predicted by the parental favouritism hypothesis (Schwabl et al., 1997) or if she adopts a different strategy like favouring the last laid egg or not allocating egg components in different manner to her eggs. Levels of lipids, protein, carotenoids, testosterone and corticosterone were measured in fresh eggs to investigate the within brood composition of kittiwake eggs without the confounding influence of embryo age.

High levels of testosterone could be exerting an influence in two directions: one to compensate for poor egg quality (Eising et al., 2001) and the other to counteract for the disadvantage of hatching asynchronously. I predict that kittiwake mothers will allocate more testosterone to last laid eggs in order to compensate for these two factors contrary to what Schwabl et al., (1997) proposed in the parental favouritism hypothesis. Corticosterone concentrations will be higher in second-laid eggs due to the potential benefits it provides by enhancing the provisioning rate and maximising energy reserves through metabolic changes. Besides, second hatched chicks could benefit from higher egg-corticosterone levels if it influences their phenotype in order to cope better with stressful situations (Hayward & Wingfield, 2004). Another prediction is that carotenoid, protein and lipid contents will be higher in first-laid eggs in order to give them an extra tool to cope with poor environmental conditions and enhance their immune system.

Methods

This work was carried on the Isle of May, Firth of Forth, Scotland (fig. 1.1) in May 2004, under a Scottish National Heritage (SNH) license for kittiwake egg collection.

Kittiwakes are cliff-nesting birds that breed in several sub-colonies around the island. The majority of females lay two eggs (80-90%) with an average interval of 2 days. Eggs in this colony are laid very synchronously over a period of around 14 days with a peak 7 days after the first egg was laid. In order to examine the composition of first- and second-laid eggs, eleven two-egg clutches were collected from three different sub-colonies where accessible nests were present. Nests were checked every day to establish the exact day each egg was laid. First-laid eggs (hereafter called A-eggs) were taken from the nest on the day they were laid and replaced with a dummy chicken egg (previously boiled and painted) equal in size and colour to the original egg in order that the females kept laying in a normal way and did not desert the nest. On the following days, checks were made to detect the laying date of the second-laid egg (hereafter called B-egg), which was also taken on the day it was laid and replaced with another dummy egg. Two days after this, another visit was carried out to verify that no third egg was laid (never happened) and the dummy eggs were removed to allow the parents to relay a second clutch. The eggs were collected from the top of the cliffs using a long pole with a net attached at one end. On the day each egg was laid, it was then individually marked, and length (l) and breadth (b) measured to the nearest 0.1 mm with a calliper to calculate the volume with the formula $V=0.4866(b^2)l$ (Coulson, 1963).

Eggs were then wrapped in cling film and newspaper and frozen at -20 °C until laboratory analyses were carried out back at Glasgow University (see below).

Characteristics of the collected broods

During 2004 and 2005 I carried out some experiments involving egg swapping. All the clutches used for these experiments were also measured and the volume was calculated although eggs were collected only in 2004. The sample of collected eggs was taken from three different sub-colonies that were not included in the experiments. In the past, the sub-colonies from which eggs were collected experienced a high predation rate and almost none of the chicks fledged (F. Daunt, pers. obs.). A total of 118 clutches in 2004 and 256 in 2005 were measured.

Egg composition analysis

Yolk, albumen and egg lipids content of 11 whole clutches were measured. Eggs were thawed and separated into shell, albumen and yolk. Each part was dried at 60 °C and then weighed. Lipids were extracted only from the yolk using a Soxhlet extraction with petroleum ether as a solvent (Dobush et al., 1985). The lean lipid-free part of yolk was then dried and weighed to give the protein content of the yolk (lean dry yolk mass). Adding dry albumen (assumed to be only protein) mass to the lean dry yolk mass gave an estimate of the total protein content of the egg. By subtracting the mass of the dry lean yolk mass from the dry yolk mass before lipid extraction gave the lipid content of the egg (Nager et al. 2000). The yolk coloration was measured using the Roche colour fan (Vuilleum, 1969) on a scale from 1 to 15 (15 dark orange, 1 light pale yellow). It has been shown that the yolk colour relates to yolk carotenoid levels in a Laridae species (Verboven et al., 2005). In the results' section I will talk about carotenoid contens estimated using the yolk colour rank.

Hormone assays

Hormones were analysed from the yolk with radioimmunoassays for testosterone and corticosterone from the 11 complete clutches. Yolk testosterone and corticosterone were measured following the protocol of Verboven et al. 2005 and Robertson et al.

(pers.comm), respectively. Homogenized yolk was mixed with an equal amount of water (1:1 w/w) to avoid the variation in concentrations of hormones in different layers of the yolk (Lipar et al. 1999). Then a sub-sample of known weight from this homogenized mixture was taken for the hormone assays. To measure testosterone 6000 cpm titriated testoterone were added to each sample to calculate the extraction recovery. Two ml of methanol were added to the samples, these were vortexed for 40 minutes and then centrifuged at -8 °C for 10 minutes. 300 µl of the supernatant was transferred to a new vial and diluted with 2700 µl of water. The samples were purified using pre-conditioned isolute C18 columns (International Sorben Technology, UK). After running the sample through the column, the column was washed with 3 ml of water. Testosterone was eluted with 3 ml of methanol diluted at a 70% concentration.

To measure corticosterone, 100 µl of tritiated corticosterone ([1,2,6,7-^{3}H] Corticosterone, TRK406, Amersham Biosciences, UK) was added to each sample, to allow assessment of recovery efficiency. Triplicates of the tritiated corticosterone (100 µl) were added to plastic assay tubes for a measure of maximum recovery efficiency and were compared to samples taken after the two main stages of extraction (the methanol stage and the column stage). The tubes were then vortexed briefly and placed at 4˚C. After 24 hours of incubation, samples were mixed with 2.5 ml of 100% methanol (MeOH) (HPLC Grade Methanol, Rathburn Chemicals, Walkerburn, UK) and the tubes vortexed for one hour, before being centrifuged for 10 minutes at 4000 rpm. The resultant supernatant was poured off into new 12x75 tubes. From each sample, 500 µl of supernatant was transferred to a scintillation vial, scintillant (Ecoscint A, National Diagnostics, Hull, UK) added and counted on a Packard Tri-Carb Liquid Scintillation Counter (PerkinElmer Life And Analytical Sciences, Connecticut, USA) to obtain the MeOH recovery estimate (a measure of how much hormone it can be recovered alter an extraction with MeOH for further calculations). Another 1500 µl of each sample was mixed with 13500 µl of diluted water before being passed through a C18 column (200 mg, 3 ml C18-220-0020-B, Isolute, International Sorbent Technology, UK). After passing through the column, 1500 µl of each post-column sample was added to new 12x75 glass tube and dried down on a heat block (60°C) under a stream of air using a sample concentrator (Teche, Cambridge, UK). Dried down samples were then resuspended with 330 µl of assay buffer (Phosphate Buffered Saline with 0.25%), and triplicates of 100 µl transferred to plastic tubes ready for assay.

For measuring both testosterone and corticosterone, samples were run in a double antibody radioimmunoassay along with a standard curve of known amounts of the hormone (20 ng/ml – 0.038 ng/ml). Extraction efficiency (estimated from the recovery of titriated testosterone) for the yolk samples averaged 81.5 ± 2.6% for testosterone and 82.3 ± 1.9% for corticosterone. The intra-assay variation was 11.5% for testosterone and 9.2% for corticosterone. All the samples were assayed in one assay for each hormone.

Statistical analysis

Generalized Linear Mixed Models (GLIMMIX) were carried out to analyse 1) egg volume with egg order as fixed factor and laying date as covariate with nest and sub-colony as random factors. And 2) egg components (mass in grams of: dry and wet yolk, dry shell, dry albumen, total protein and lipid content; colour rank as a measure of carotenoids; picograms of testosterone per gram of wet yolk and nanograms of corticosterone per gram of wet yolk) with egg order as fixed factor, laying date and egg volume as covariates and nest and sub-colony as random factors. The analyses were performed with SAS statistical package v.9 (SAS Institute, Inc.). This package uses the Satterthwaite method to derive degrees of freedom (Gaylor & Hopper, 1969). Because testosterone and corticosterone levels were not normally distributed, one was added to each data point and then log transformed in order to normalize the data. To test the influence of egg order, egg volume and laying date on testosterone and corticosterone levels, multivariate models with backward stepwise elimination of non-significant effects were carried out using SPSS Inc. v.14. All the factors and two way interactions were tested in each model.

Results

From all the clutches measured in both years of the field work (118 in 2004 and 256 in 2005), A- eggs were on average 3.7% bigger than B- eggs independently of the year and the laying date (laying order: $F_{1,251}=53.94$, $p<0.0001$; year: $F_{1,250}=0.39$, $p=0.53$; laying date: $F_{1,498}$=0.71, p=0.40; mean volume of A-eggs: 44.91±0.54 cm^3; and B-eggs: 43.23±0.49 cm^3, fig. 2.1). When comparing volume between A- and B- eggs from the 11 collected clutches, there was an effect of the interaction between laying order and laying date on egg volume (laying order: $F_{1,10}$=9.68, p=0.011; laying date: $F_{1,21}$=3.73, p=0.067; laying date*laying order: $F_{1,11}$=10.69, p=0.008). Post hoc analyses showed that only A eggs were larger if they were laid later in the season while no differences in size was found in B-eggs according to the laying date (A-egg volume: laying date: $F_{1,10}$=12.48, p=0.0054; B-egg volume: laying date: $F_{1,11}$=0.13, p=0.72; fig. 2.2).

Apart from volume, dry shell mass and carotenoids differed between A and B eggs. Levels of these components were higher in A- than in B-eggs independently of their volume and laying date (table 2.1 and table 2.2). Dry and wet yolk, dry albumen, lipid and protein content did not differ between A- and B-eggs (table 2.1 and table 2.2) but were higher in larger eggs independently of the laying order and laying date (table 2.1, fig.2.3). Although the mass of lipids increased with laying date independently of egg volume (table 2.1, fig. 2.4).

Testosterone levels in the yolk were higher in B- eggs than in A-eggs from the same brood and these levels were not influenced by the egg volume or laying date (table 2.1 and table 2.2, fig. 2.5).

Corticosterone concentration did not differ between A- and B-eggs (table 2.1). Testosterone and corticosterone concentrations were not influenced by the nutritional egg composition and they were not related with each other (table 2.3). A marginal effect of carotenoid levels were found on testosterone levels: chicks with higher levels of testosterone had lower levels of carotenoids (table 2.3, fig. 2.6).

Discussion

As predicted, kittiwake mothers did not allocate higher levels of testosterone to A- eggs but they assigned more testosterone to B eggs. No differences in corticosterone concentrations between A- and B- eggs were present. Of the components expected to be higher in A- eggs, only carotenoids was in fact hgher and no differences occurred in protein and lipid content, although A- eggs were larger than Bs.

Opposite to Schwabl et. al. (1997) findings on cattle egret, kittiwake mothers did not allocate higher levels of testosterone to first laid eggs (parental favouritism hypothesis) but they assigned more testosterone to last laid eggs. Both species, cattle egrets and kittiwakes are facultative brood reducers thus a similar androgen allocation pattern was expected. Perhaps mothers manipulate the levels of this hormone in order to favour the chick that more needs it. By doing this kittiwake mothers could favour themselves more in terms of fitness than cattle egret mothers. Schmaltz et al. (2007), found that testosterone levels in the eggs of smooth-billed ani (*Crotophaga ani*) did not reflect the females' hormone levels at the time of laying. This supports the idea that females can allocate hormones to manipulate chick performance according to specific environmental circumstances thus the amount of hormones allocated do not necessarily reflect their circulating levels of the hormone. Verboven et. al., 2003 found that lesser black-backed gull (*Larus fuscus)* mothers in good condition allocated less androgens to their eggs despite the fact that they had high circulating levels of androgens after laying. The pattern of testosterone levels within the clutch in kittiwakes is similar to the pattern found in lesser black-backed and black-headed gulls (*Larus ridibundus*) in which this hormone levels in yolk also increased with laying order (Royle et al., 2001; Eising et al., 2001; Verboven et al., 2003). Testosterone could compensate the last egg for hatching last due to hatching asynchrony of the broods (Royle et al., 2001; Eising et al., 2001).

Eggs that had higher levels of testosterone showed a tendency of having lower carotenoid levels, which indicates that yolk testosterone may compensate for other differences in egg composition (Groothuis & Schwabl, 2002). Perhaps mothers in bad condition allocated more testosterone to her eggs in order to compensate for the lack of nutrients (e.g. carotenoids), but this is unclear due to the fact that no other of the measured egg components that could indicate egg quality (e.g. lipids and proteins) influenced testosterone levels.

A- eggs had higher concentration of carotenoids than B-eggs. This pattern of allocation could increase the senior chicks' survival and decrease junior chicks' chance of surviving when conditions are not favourable. When carotenoid availability was experimentally increased in zebra finches, females produced eggs with higher levels of this component and the experimental broods fledged more chicks than control broods

(Blount et al. 2003). Perhaps last-laid eggs will be more prone to suffer from diseases, grow slower and show a retarded development (Hario & Rudback, 1999; McGraw et al., 2005; Groothuis et al., 2005a; Rubolini et al., 2006).

Another way in which carotenoid levels could benefit senior chicks is by influencing chicks gape colour as it happens in the barn swallows (*Hirundo rustica*) (Saino et al., 2000). Swallow parents preferentially fed chicks with redder gapes, which is positively correlated with the amount of carotenoids in the egg (Saino et al., 2000). It is uncertain if this also happens in kittiwakes but it could be a mechanism used by the mother to favour senior chicks when food is scarce. If carotenoids affect the gape colour and gape colour affects begging success then kittiwake junior chicks will receive less food than its senior sibling. This avoids spending resources on a chick that has low chances of survival. In good years where there is sufficient food for all chicks, gapes colour would not differ and parents will feed both chicks equally.

Corticosterone levels did not differ between A- and B- kittiwake eggs. In Japanese quail, herring (*Larus argentatus*) and lesser black-backed gulls no differences of corticosterone between eggs within a clutch were found either (Hayward & Wingfield, 2004 and Nager, pers. comm.). This could be indicating that perhaps corticosterone in the egg yolk does not influence individual characteristics of senior and junior chicks and does not affect offspring differently. Corticosterone levels could be a reflection of the mothers stress levels and thus affect the whole clutch but not provoking differences within the clutch. There is not much information about corticosterone egg yolk levels within clutches of species with brood reduction.

It has been shown that eggs laid late in the season are poor quality eggs and if parents are in poor condition offspring from these parents will survive less (Blount et al., 2003). In the present study, kittiwake eggs laid later had higher lipid contents, which could indicate that parents' from these clutches were of good quality or perhaps the resources available at the laying time were abundant. Bad quality parents or parents in poor condition are not able to allocate high amounts of lipids if they do not have the resources to do so (Muck & Nager, 2006).

Females might lay eggs with a similar within-brood pattern allocation of carotenoid and hormone levels in good and poor years and the influence of these antagonistic effects in the survival and behaviour of kittiwakes will depend on the amount of food present in a given year. It will be easier for kittiwake females to allocate egg components in the same way year after year because at the time of egg laying feeding conditions later in the season may be difficult to predict. If this is the case, the pattern of testosterone, corticosterone and carotenoids allocation found in kittiwakes' eggs in the present study will work differently in years of good and poor food availability. If there is sufficient food to feed two chicks, the junior chick will be able to compete successfully with the older and bigger sibling thanks to the benefits of the extra maternal testosterone. On the other hand, if food availability is low B- chicks will be weaker than senior chicks for the reasons discussed above: directly due to the lack of food and indirectly because of the increase in oxidative stress. Oxidative stress is higher in hungrier chicks because of the increased physiological stress hungry produces and because they lack the nutrients (vitamins and carotenoids) obtained from the food to overcome the normally occurring production of reactive oxygen radicals that cause oxidative stress (Prakash et al., 1998; Finkel & Holbrook, 2000). Moreover, in a poor year chicks will be more susceptible to diseases due to their poor condition and the immunosupressory effect of testosterone and the lack of carotenoids (Royle et al., 2001; Muller et al., 2005; Martin-Vivaldi et al., 2006; Berthouly et al., 2007).

By egg-allocating these components with the described pattern according to the laying sequence, females will facilitate brood reduction in poor years and promote the survival of B- chicks in good years by giving them extra tools to compensate for size and age differences. There is no data indicating that the species within-brood pattern of different components allocation changes according to the year conditions, which will support the idea of mothers allocating similar amounts of nutrients independently of the environmental conditions. The relative amount of these components in A- and B- eggs may make the B- egg an insurance (brood reduction strategy) when food is scarce and may promote their survival when the food availability allows it. In the next chapters I will try to link the pattern of nutrients' and hormones' egg deposition with the outcome of sibling rivalry in black-legged kittiwake chicks.

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Tables

Table 2.1. The influence of laying order, volume and lay date and all the two way interactions on the amount of egg yolk, albumen, shell, lipids, lean mass, carotenoids, testosterone and corticosterone of black legged kittiwakes was tested using a mixed model with sub-colony and nest as random factors. Non-significant interactions were removed from the model using the stepwise regression method. Significant results or tendencies are marked with bold characters.

	Egg Order			**Volume**			**Lay Date**			**Zone**		**nest**	
COMPONENT	*df*	*F*	*p*	*df*	*f*	*p*	*df*	*f*	*p*	Z	P	Z	*p*
Wet yolk	1, 9	0.97	0.35	**1,19**	**7.06**	**0.01**	1,20	0.14	0.71	0.57	0.57	1.49	0.13
Dry yolk	1,10	0.01	0.99	**1,19**	**7.83**	**0.01**	1,16	1.32	0.27	0.49	0.62	1.49	0.13
Dry albumen	1,8	0.01	0.99	**1,13**	**13.17**	**0.003**	1,13	0.20	0.66	0.15	0.87	1.09	0.28
Dry shell	**1,11**	**8.93**	**0.01**	**1,18**	**3.59**	**0.07**	1,13	0.23	0.64	0.56	0.57	0.13	0.89
Lipids	1,10	0.01	0.97	**1,20**	**10.50**	**0.004**	**1,13**	**4.67**	**0.05**	0.91	0.36	1.98	0.04
Lean mass	1,11	0.66	0.43	**1,18**	**7.62**	**0.01**	1,18	0.67	0.42	0.65	0.51	1.48	0.13
Carotenoids	**1,11**	**67.55**	**0.001**	1,11	0.32	0.58	1,19	0.003	0.86	0.71	0.48	0.30	0.76
Testosterone	**1,10**	**4.68**	**0.04**	1,19	0.81	0.38	1,21	1.51	0.23	1.22	0.22	2.16	0.03
Corticosterone	1,11	0.32	0.58	1,16	0.83	0.37	1,21	0.20	0.66	0.70	0.48	1.18	0.23

Table 2.2. Mean values and standard error of the amount of egg components (yolk, shell, albumen, lipids, lean mass, carotenoids, testosterone and corticosterone) measured in A- and B- eggs as well as the mean difference and standard error between them.

	A-eggs		**B-eggs**		**Differences (A-B) within a clutch**	
COMPONENT	*X*	*SE*	*X*	*SE*	*X*	*SE*
Dry yolk (g)	5.53	0.10	5.62	0.13	0.09	0.03
Wet yola (g)	12.79	0.17	13.05	0.26	0.25	0.08
Dry shell (g)	2.87	0.03	3.53	0.19	0.66	0.16
Dry albumen (g)	3.57	0.08	3.62	0.08	0.05	0.00
Lipids(g)	1.67	0.07	1.75	0.08	0.08	0.01
Lean mass(g)	2.38	0.04	2.38	0.05	0.01	0.01
Carotenoids (rank colour)	11.92	0.12	13.79	0.25	1.88	0.13
Testosterone (pg/g of yolk)	48.36	3.97	38.72	3.48	-9.65	-0.49
Corticosterone (ng/mg yolk)	5.53	0.10	5.62	0.13	0.09	0.03

Table 2.3. The influence of the amount of yolk, albumen, lean mass, shell, lipids and carotenoids from black-legged kittiwake chicks in testosterone and corticosterone levels was tested using a univariate mixed model with sub-colony and nest as random factors. Significant results or tendencies are marked with bold characters. NA=Non Aplicable.

	Testosterone			**Corticosterone**			**Random Factors**			
COMPONENT	*df*	*F*	*p*	*df*	*F*	*p*	*Testosterone*			
Wet yolk	1,21	0.08	0.77	1,11	0.56	0.47	Zone		Nest	
Dry yolk	**1,15**	**4.41**	**0.053**	1,9	0.05	0.82	*Z*	*p*	*Z*	*p*
Dry albumen	1,16	1.55	0.23	1,17	1.85	0.19	0.87	0.38	2.22	0.023
Lean mass	1,18	1.48	0.24	1,19	0.21	0.65	*Corticosterone*			
Dry shell	1,21	0.001	0.97	1,11	0.13	0.72	Zone		Nest	
Lipids	1,17	0.38	0.55	**1,20**	**3.33**	**0.083**	*Z*	*p*	*Z*	*p*
Carotenoids	**1,11**	**4.34**	**0.06**	1,16	0.04	0.85	2.03	0.04	1.40	0.16
Corticosterone	1,11	1.82	0.21	NA	NA	NA				
Testosterone	NA	NA	NA	1,11	0.26	0.62				

Figures

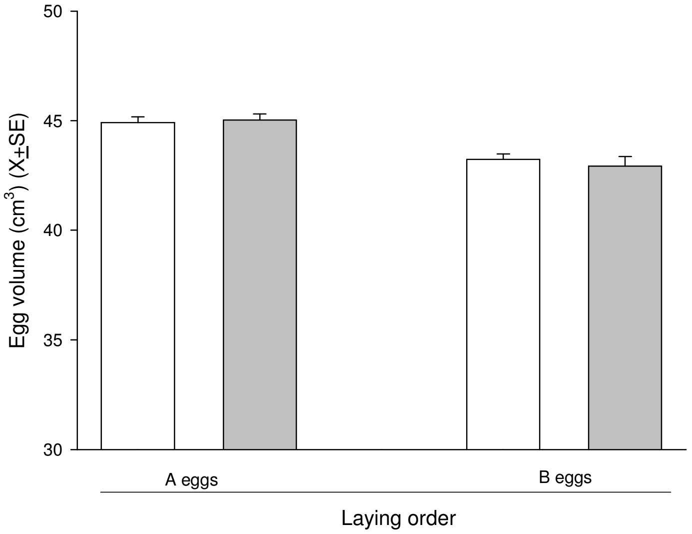

Figure 2.1. Mean (± SE) egg volume of A- and B- eggs in 2004 ○ and 2005 ●. A- eggs were larger than Bs in both years and no differences in size were present between A- and B- eggs in different years.

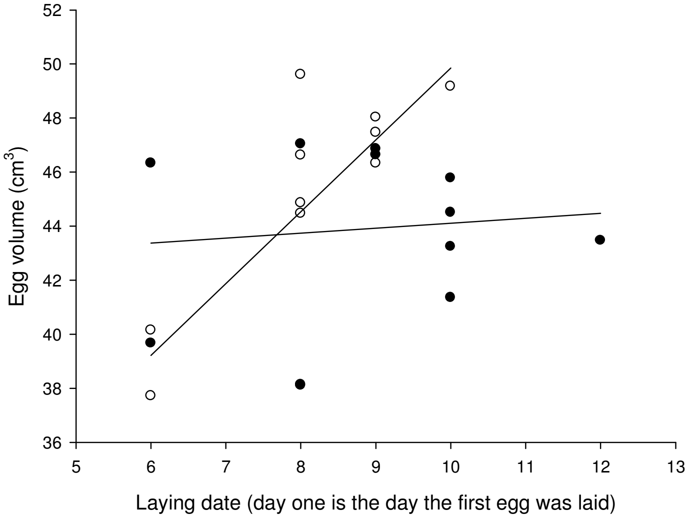

Figure 2.2. Egg volume of A- ○and B- ●eggs according with the laying date. Egg volume increased with laying date in A-eggs while it did not change in B-eggs.

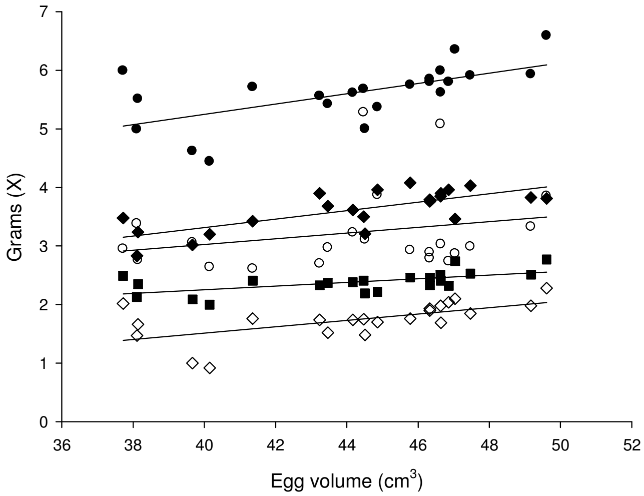

Figure 2.3. Mean dry yolk ●, dry shell ○, dry albumen ◆, lipids ◇and lean mass ■of kittiwake eggs. These components were higher in lager eggs independently of the laying order.

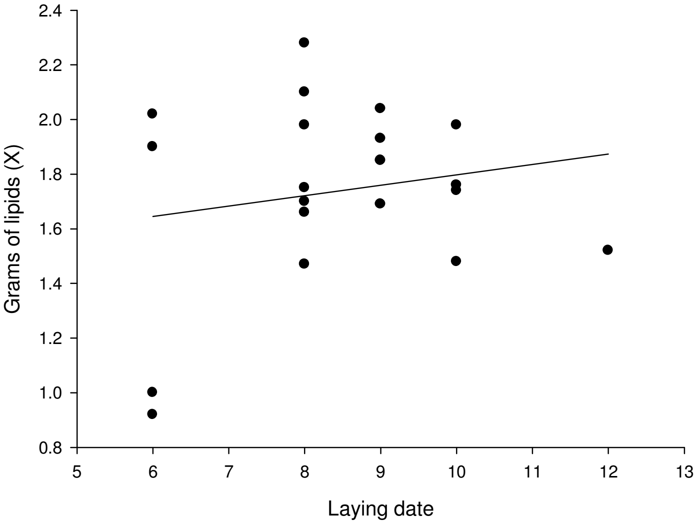

Figure 2.4. Average lipid mass on kittiwake eggs. Lipids' mass increased as the laying date progressed independently of laying order.

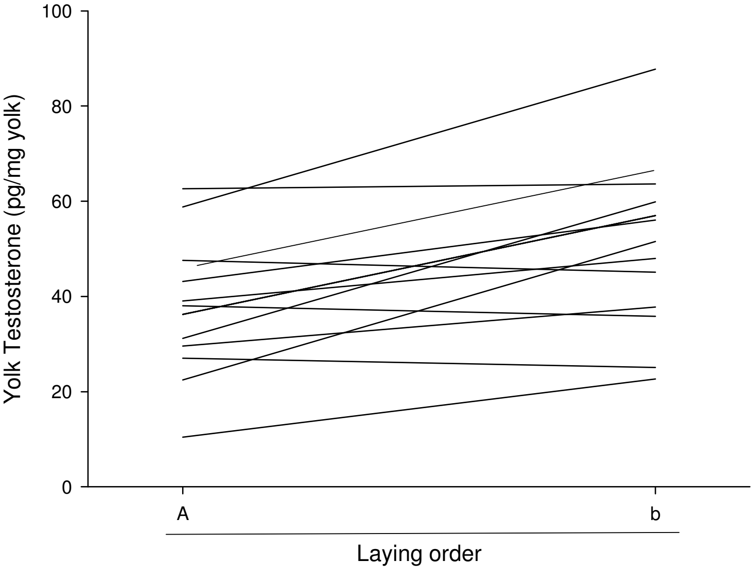

Figure 2.5. Mean yolk testosterone levels of A- and B-eggs in 13 two-egg clutches. The line indicates the difference between A- and B- eggs within a clutch. In the overall sample of eggs measured in two years, B-eggs were 3.7% smaller than A-eggs. On the egg sub-sample used for the composition analyses this pattern was found in 9 out of 13 clutches.

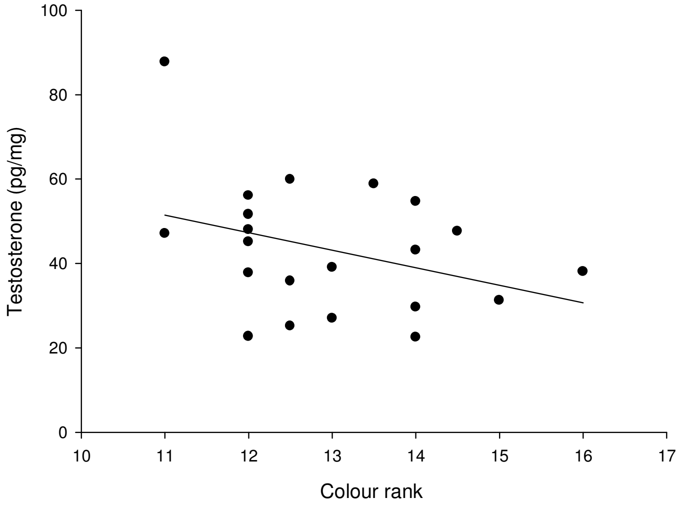

Figure 2.6. Testosterone levels in relation with carotenoid levels. Levels of testosterone were marginally related to carotenoid levels independently of the laying order and the laying date. Eggs with higher levels of testosterone had lower amounts of carotenoids.

Chapter III

Are Differences in Yearly Breeding Success of Kittiwakes Related to Differences in Offspring Behaviour?

Introduction

The concept of dominance was widely used after the 1920's when researches discovered that groups of birds, mammals and other animals organized themselves according to an agonistic hierarchy. Counting the pecks that each hen (*Gallus gallus*) directed towards the others in a group Schjelderup-Ebbe (1932) was capable of building a linear sequence from the dominant individual who attacked the most to the subordinate individual who never attacked and only received aggression (cited from Archer 1988). This study provided the origin for the concept of dominance, which in subsequent research included not only aggressive interactions, but more importantly the priority of access to resources that this dominance should confer. Having priority of access to resources without having to engage each time in a fight is clearly advantageous for the dominant individual. For the subordinate it is more beneficial to distinguish the dominant's aggressive behaviour and submit because it is less costly than getting involved in a fight with little chances of winning (Archer, 1988).

In dominance-subordinate relationships, it has also been questioned if the submission of the subordinate individual, rather than the aggression of the dominant determines the stability of a relationship because submissive signals have the potential to stop aggression (reviewed in Archer 1988). It is important to highlight that the term dominance is a description of a series of fights that have been won or lost. It is simplistic to assume that dominance is always fixed because the direction of the dominance between two individuals can change depending on each situation (reviewed in Huntingford, 1987; Archer, 1988). Both individuals in a dyad have a specific role between itself and the opponent that not necessarily reflects its role towards other individuals from the group (Pagel & Dawkins, 1997).

For an establishment of a dominance-subordinance relationship individuals need to fight to each other or know who the winner of a fight is (Dugatkin & Earley, 2004). In the traditional formation of linear hierarchies in hens and chickens, an individual recognition is necessary and therefore, these hierarchies depend on dyadic knowledge (Chase et al., 2002). Usually the dyad with a bigger difference between the participants will be the one that has to fight less because a weaker rival will submit faster or choose not to fight at all, whilst a pair of individuals that are very similar in size and other traits will have to fight for longer for a winner to emerge (Chase et al., 2002).

Hierarchies' formation does not only occur in adults but happen in chicks as well. Chicks of several species form clear hierarchies from early ages onwards. Nestling birds can compete for parental provisioning through begging or aggression. Competition through begging is more common in birds and there are empirical (Smiseth et al., 2003; Mathevon & Charrier, 2004; Budden & Wright, 2005) and theoretical (Royle et al., 2002; Johnstone, 2004) studies of competitive interactions between nestling birds.

The mechanisms for the formation of hierarchies through aggression have been also mathematically modelled using three learning processes: trained losers and winners, individual recognition and the evaluation of the site where the aggression takes place. These processes can be applied alone or in combination to explain the formation of hierarchies between individuals. Few models on wild animals, and fewer on birds, particularly on species with open nestling aggression have been proposed (e.g. Forbes, 1993; Forbes & Lamey, 1996; Rodriguez-Girones, 1996; Rodriguez-Girones et al., 1998; Rodriguez-Girones, 1999; Rodriguez-Girones et al., 2001). Most of the time models have been made using captive and domesticated animals with non-realistic assumptions, which makes it hard to experimentally demonstrate them (reviewed in Mock & Forbes 1992).

Aggressive sibling rivalry is present in only a few species of altricial birds and it serves two purposes: obtain resources and a dominance status (Drummond, 2001a). It is usually the oldest nestling that dominates the younger members of the brood. As a result of this aggression a hierarchy is formed and it does not need an individual recognition, it is usually created through a process by which chicks learn to be winners or losers (Drummond & Osorno, 1992).

Some factors that can influence the presence and occurrence of sibling aggression are food availability, the method of parental food delivery and the size of the prey (Mock & Parker, 1998). When food was directly given to cattle egret (*Bubulcus ibis*) chicks, their aggression was higher and it increased as the prey size decreased (Mock et al., 1987). But Drummond (2001b) pointed out that Mock et. al. interpretations may not be completely correct because their prey size experiment did not take food amount into account. Moreover, aggression was also influenced by developmental factors with chicks becoming more aggressive with age independently of the feeding method that were not taken into account either. Brood size and differential growth caused by age differences or offspring sex can also influence sibling aggression (Mock & Parker, 1998). If one of the sexes grows faster at an age where the hierarchy is not yet well established, this sex will have an advantage over the other sex.

In species where siblicide occurs, little is known about how the hierarchies are formed and whether they change over time. It has been shown in the black guillemot (*Cepphus grille*) and the blue-footed booby (*Sula nebouxii*) that a stable dominant-subordinate relationship is established during the first two weeks of life; chicks from these two species showed a peak of aggression in the second week after hatching which decreased thereafter once the dominance was formed and remained at low frequencies until fledging (Drummond et al., 1986; Cook et al., 2000; Valderrabano-Ibarra et al., 2007). In the laughing kookaburra (*Dacelo novaeguineae)* aggressiveness within the brood was also influenced by the brood sex composition (Nathan, 2001). In this species, broods with a first-hatched male and a last-hatched female showed more aggression in broods of two and three chicks (Nathan, 2001). In these three species, aggression was influenced by hatching asynchrony and chicks' body and mass size differences, supporting the idea that these asymmetries might cause fitness benefits to the older sibling by promoting aggression (Drummond et al., 1986; Cook et al., 2000; Nathan, 2001).

The aims of this study were (1) to compare behavioural interactions among siblings between years with different productivity, and (2) to test whether hierarchy formation is differentially influenced by different environmental conditions. To test these assumptions Black-legged kittiwakes (*Rissa tridactyla*) were used. Kittiwakes only eliminate their sibling when food is not enough (facultative brood reducers; Braun & Hunt, 1984; Irons, 1992). Juniors show agonism soon after the second chick is hatched and thereafter maintaining a dominant-subordinate relationship that lasts until they fledge or one of them dies depending on food availability (Braun & Hunt, 1984; Irons, 1992). Usually the first-hatched chick becomes dominant due to the size and/or age advantage from hatching first and as a result it gains better access to food and develops faster than its younger nest mate (Braun & Hunt, 1984). Aggression in this species is usually followed by a submission or retreat from the attacked chick (usually the junior) and this behaviour presumably stops escalation of aggression on most occasions.

Adult kittiwakes are sexually dimorphic (Helfenstein et al., 2004) with males being on average 10% larger than females. Therefore, it is expected that males hatched on a first position with a female sibling will be more aggressive than females on a first position independently of the sibling’s sex.

Kittiwake females from the studied population usually lay two eggs with two days of difference and senior chicks hatch from a larger egg than that of juniors ca 1.5 days before their junior sibling (**chapter II).** It is an ideal population to accomplish the proposed aims. In order to compare hierarchies’ formation under different circumstances and to evaluate how it will influence breeding success, behavioural observations of chicks were carried out in two consecutive years in which breeding success was different (Harris, 2004; Harris, 2005; Wanless et al., 2007).

Methods

The black-legged kittiwake colony from the Isle of May, Firth of Forth, Scotland (fig. 1.1) was used for carrying out this work. Between 4000 and 7000 kittiwake pairs distributed in several sub-colonies nest each year on the Isle of May. During 2004 and 2005 the study nests were selected in sub-colonies where nests were accessible to manipulate eggs and chicks. Six different sub-colonies dispersed all over the island were used (fig. 1.1). During laying, nests were visited daily and first- and second-laid eggs were identified on the day of laying and marked. When the clutch was completed the length (l) and breadth (b) of each egg was measured using callipers to the nearest 0.1mm and the volume was calculated using the formula: $V=0.4866(b^2)l$ (Coulson, 1963). These broods served as control broods of cross fostering experiments in 2004 and 2005 (**chapter IV and V**). In 2004 the complete clutch was moved to another nest within the first two weeks after being laid and in 2005 there were two control sub-groups: one in which again the whole brood was moved to another nest and a second group in which a first- and a second-laid egg from two different clutches were moved into a foster nest maintaining the normal within-clutch differences in egg size (first-laid eggs are on average 4% larger than second-laid eggs, **chapter II**) and laying date (2 days).

Statistical analyses were made to compare the behaviour of the two control groups in 2005 (1) biological siblings (n=12) and (2) non-siblings raised by foster parents (n=10). No differences were found in any of the observed behaviours: activity ($F_{1,31}$=1.02, p=0.32), begging ($F_{1,42}$=2.73, p=0.11), feeding ($F_{1,32}$=0.02, p=0.87), aggression ($F_{1,28}$=1.52, p=0.22) and submission ($F_{1,23}$=1.94, p=0.17). Because of this, both groups were pooled and treated as one control group for 2005 in all subsequent analyses.

Hatching date, sex, growth and survival.

Kittiwakes usually lay the two eggs of their clutch (modal clutch size in this population, Harris & Wanless, 1990) within 2 days. To establish the exact hatching date daily nests checks were made and each chick was randomly marked with sheep dye of one of two different colours on head, neck and rump as they hatched to be able to distinguish between first- (seniors) and last- hatched (juniors) chicks. When new chicks were found on the nest a blood sample of less than 10µl was taken from the medial metatarsal vein (under a UK Home Office license) to determine their sex using molecular techniques (Griffiths et al., 1996). Measures of tarsus, bill and ulna were taken with callipers to the nearest 0.1mm and chicks were weighed with a spring balance to the nearest gram on the same day in which the blood sample was collected and measured thereafter every 4 to 5 days until they were 20 days old. After this age kittiwake chicks become very sensitive and can jump out from their nests if they are disturbed. It was not possible to always do these procedures on the day of hatching due to the weather conditions (rain and gales) but they were always carried out within 24 hours of hatching. In 2004 chicks hatched between the 18th of June and the 2nd of July and in 2005 they hatched between the 27th of June and the 9th of July. Instantaneous growth rate was used in the analysis by calculating the slope of the regression line of log transformed weight (in g) on age (in days) for chicks that survived until at least 9 days.

Because every nest was checked at least once a day, the exact date a chick died or disappeared from a nest was recorded. Missing chicks could have been predated or expelled from the nest by their siblings but the exact cause could usually not be identified. Chicks were considered to have fledged if they survived until 40 days of age (Cullen, 1957) and the successful fledging was confirmed by checking the nest and its surroundings because fledged kittiwakes often return to their nests to receive food from their parents (Cullen, 1957).

Chick's regurgitates

The amount of food each chick received from their parents was unknown. The only information regarding this was their feeding frequency but it might not be related with the amount of food chicks got. Sometimes while chicks were manipulated, they regurgitated part or all of the food they were digesting. These regurgitates were collected and analysed as explained in Lewis et al. (2001). Their biomass was calculated and the proportion of several prey distinguished to give an estimate of the size of individual feeds (table 3.1). It is unknown if the collected regurgitates were all or only part of the stomach content of the chick because the time since the last feed was unknown. Regurgitates from unknown adults' from all over the island were also collected. Overall, regurgitates collected from adults in 2004 were 22.5% lighter than in 2005 (Harris, 2004; Harris, 2005; table 3.1). There were not enough regurgitates from the broods where the behavioural recording was made in order to run a proper statistical analysis to compare feed size of senior and junior chicks.

Behavioural recording.

Behavioural observations were made every other day during three hours. Three behavioural shifts of three hours each were made each day (from 6 to 9 am; from 10:30

to 13:30 and from 15:30 to 18:30). On each shift a maximum of five broods located close together were observed at the same time at a distance of between 3 and 7 meters. Each brood was observed during a total of 12 hours on average (ranging from 3 to 18 hours) and a total of 35 broods were observed in both years (13 in 2004 and 22 in 2005). The watches of the brood started one day after the second chick hatched. On the first observation the senior was on average 1.43 ± 0.75 days old (n = 35). The observations were done by two observers each year and they were unaware of the treatment of the brood they were observing. Observations were a combination of behaviour sampling and behaviour scanning (Martin & Bateson, 1994). Behaviour sampling was used to record the absolute frequency of each chick's begs, feeds and aggressive acts towards the nest mate and whether the recipient of each aggressive act responded by adopting or sustaining a submissive posture (behaviours described below). Every minute and a half each brood was scanned to record whether chicks were active (awake with the head up on the substrate and visible) or not. The behavioural method described here was based on Drummond et al. (2003) and the kittiwakes' behaviours were taken from Cullen (1957) who makes a detailed description of each behaviour: 1) Begging: rhythmical oscillation of the head with bill pointed upward or pecking the adult's bill; this behaviour can be accompanied by a call but sometimes is undistinguishable due to the colony noise. 2) Feeding: bills from the chick and the adult hooked together and food pass is evident from the chicks' throat distinctive distension. 3) Aggression: pecks and bites are performed from one of the chicks directly to the other when both are awake. 4) Submission: the attacked chick respond with a semi- or complete-stereotyped posture with the bill down and the face away. Whole broods were observed until the junior chick was 20 days of age because growth measurements also stopped at this age. Observations also stopped when one of the chicks of the brood died. Behavioural measurements based on the behaviours described above were calculated for each three-hour observation shift:

A bout is an event of the same behaviour (begging, feeding, aggression) shown in successive behavioural scans. The following behaviours were recorded:

- Activity = minutes per hour that a chick spent awake with the head visible.

- Begging frequency = number of begging bouts per chick per hour of observation.
- Successful begging bouts = proportion of begging bouts that were followed by a feeding act.
- Feeding frequency = number of feeding bouts per chick per hour of observation.
- Shared feeding bouts = feeding bouts in which both chicks received food.
- Aggression frequency = number of aggressive bouts per chick per hour of observation.
- Aggression while feeding = proportion of aggressive bouts present during a feeding bout.
- Submission ratio = proportion of aggressive attacks responded with a submissive act.

Statistical Analysis

Analyses of egg size, sex ratio and behaviour were carried out using the data of 13 broods in 2004 and 22 broods in 2005. After the age of 10 days the number of observed broods decreased to 6 in 2004 and to 17 in 2005 due to chick mortality.

To determine differences between the size of first-and second-laid eggs and between different years repeated measures ANOVAs were carried out. To test whether offspring sex depended on hatching order and year, a logistic regression testing the probability of being a female in relation with hatching order and year was performed. To establish whether body condition of the chicks influenced their behaviour, ANCOVAS were used with the mean of each chicks' behaviour (feeding, begging, aggression) recorded when chicks were from 4 to 10 days old as the dependent variable, year as factor and body condition (residuals of the regression between each chicks' weight and its wing length at 7 days) as covariate. If the year*condition interaction was significant, correlations were made between behaviour and condition in each year separately. If no significant interaction was present a correlation with the pooled data for both years was done. For these analyses SPSS Inc. v 14.0 was used.

Generalized Linear Mixed Models (GLIMMIX) with different types of error distribution depending on the error distribution of the behavioural data (activity: normal distribution; begging, feeding and aggression: poisson distribution; aggression,

aggression while feeding, begging success and submission: binomial distribution) were used. These models were chosen because the data was clustered in groups (nest), each nest contained two factors (both chicks) and they were observed several times at different ages and day times. Therefore, chick and nests were used as random factors. The macro GLIMMIX in SAS statistical package version 9 (SAS Institute, inc.) was utilized to run these analyses with the Satterthwaite method to derive degrees of freedom (Gaylor & Hopper, 1969). In the models, the following factors and all the two way interactions were tested: year, age, sex, social rank, hatching date and hatching asynchrony. When the interactions were not significant, they were removed from the model using the stepwise regression method and only significant interactions were reported. Preliminary tests showed that sub-colony, observer and time of day when chicks were observed had no effect on the behaviours ($p>0.2$) and therefore these factors were not included in the models. When differences were present between years, between seniors and juniors or between males and females mean and standard error are reported. If a change with age was found, the slope of the regression is reported.

Results

During 2004 and 2005 kittiwakes on the Isle of May laid their first egg later in the season compared to the long-term average. The mean food load mass of adults during chick-rearing was very similar in the two years, but the percentage of sandeels in the diet was higher in 2005 than in 2004 (Wanless et al., 2007; table 3.2). Sandeels are the main component of kittiwake's diet (reviewed in Wanless and Harris, 1992). The higher percentage of this fish found in chicks' regurgitates in 2005 indicate that sandeels (*Ammodytes marinus*) were more abundant in 2005 than in 2004 (table 3.1). The main components of the studied chicks' diet were sandeel (0 and 1+ group), clupeids and gadids (data from the regurgitates of the observed broods in both years; table 3.1). Breeding success was higher in 2005 than in 2004. Compared to the long-term average in number of chicks fledged per nest, 2004 was below the lower 95% colony average and 2005 was above the upper 95% colony average.

Egg size and chicks' sex

The difference in size between first-(A) and second- laid (B) eggs was similar in both years, with A-eggs being larger than B-eggs (2004: A-egg=44.2±0.44 mm^3; B-egg: X=42.9±0.46 mm^3; 2005: A-egg =46.9±1.01 mm^3; B- egg =44.7±1.06 $mm^{3)}$. Egg size did not differ between years and laying date did not influence egg size (Repeated measures ANOVA, laying order: $F_{1,33}$=7.93, p=0.002; hatching asynchrony: $F_{1,31}$=1.63, p=0.27; year: $F_{1,32}$=2.72, p=0.19; laying order*year: $F_{1,30}$<0.01, p=0.97;).

There was no difference in offspring sex ratio between 2004 and 2005 or between hatching order (n=13 and 22 broods, respectively; logistic regression on probability of being a female, social rank: Wald=0.19, p=0.83; year: Wald=0.26, p=0.61; order*year: Wald=0.46, p=0.37).

Behavioural observations

Seniors were more active than juniors at any age (social rank: $F_{1,97}$=9.73, p=0.002; seniors: X=20.9±1.2 minutes of activity/h; juniors: X=16.3 ±1.1 minutes of activity/hr). Chicks increased their activity with age independently of social rank and there were no differences in activity between male and female chicks and between years (age: $F_{1,160}$=119.60, p<0.0001; year: $F_{1,50.1}$=0.10, p=0.75; sex: $F_{1,133}$=1.24, p=0.26; fig.3.1).

Begging and feeding behaviours did not differ between senior and junior chicks and between males and females but all begging and feeding behaviours were influenced by an interaction between age and year (table 3.3). In 2005 chicks increased their begging with age (p=0.001, X=0.63 ±0.08 begging bouts/h, b=0.3), but feeding did not change with age (p=0.96, X=0.1±0.05 feeding bouts/h, b=0.05) while in 2004 older chicks decreased their begging (p<0.001, X=0.2±0.04 begging bouts/h, b=-0.21) and feeding frequency (p<0.001, X=0.33 ±0.02 feeding bouts/h, b=-0.16) compared to younger chicks (figure.3.2 and figure 3.3, respectively). In 2004 the proportion of begging acts that were followed by food provisioning (successful begging bouts) decreased with age, while in 2005 the proportion did not change (analysed for each year separately: 2004: age: $F_{1,51}$=10.22, p=0.002, X=0.31, ±0.03 successful begging bouts, b= -0.15; 2005: $F_{1,111}$=0.20, p=0.99, X=0.02, ±0.05 successful begging bouts, b =0.04; table

3.3, fig. 3.4). An interaction between age and year was present when the proportion of shared feedings was analysed (table 3.3). When each year was analysed separately it was found that in 2004 chicks decreased the proportion of shared food with age (p=0.002, X=0.46 ±0.03 shared feeding bouts, b=-0.17) while in 2005 chicks did not change this proportion with age (p=0.99, X=0.09 ±0.01 shared feeding bouts, b=-0.012).

While the provisioning behaviour was not different between senior and junior chicks, three interactions were present when aggression was analysed: age*rank, age*year and rank*year (table 3.4, fig.3.5). In 2005 senior chicks did not change their aggression frequency with age ($F_{1,24}$=0.26, p=0.61, X=0.19±0.07 aggressive bouts/h, b=0.003) while junior chicks decreased their aggression with age ($F_{1,28}$=4.24, p=0.049, X=0.27±0.1 aggressive bouts/h, b=-0.19). In 2004 senior chicks increased their aggression with age ($F_{1,25}$=8.51, p=0.0073, X=0.58±0.08 aggressive bouts/h, b=0.28) while. junior chicks did not change their aggression with age ($F_{1,14}$=2.02, p=0.17, X=0.11±0.02 aggressive bouts/h, b=0.006) The frequency of aggression was not influenced by the chick's sex (table 3.4). Chicks that received aggression can respond either with submission, retaliation or not respond at all. Junior chicks were more submissive than senior chicks (table 3.4) and responded to 80% of aggressive acts with submission, 3% with retaliation and did not respond at all to the remaining 17% of the attacks. Senior chicks responded to an aggression with submission 30% of the times, 47% of the times with retaliation and 23% of the times did not respond at all. This pattern was consistent between years. An interaction between sex and age in the proportion of aggression responded with submission was found (table 3.4). When age was tested for each sex separately only a tendency was found indicating that males decreased their submissiveness with age ($F_{1,15}$=3.26, p=0.091, X=0.29 submissive acts in response to an aggression, b=0.17) while females did not change their submissiveness with age ($F_{1,20}$=0.05, p=0.83, X=0.14 submissive acts in response to an aggression, b=0.095). This analysis was carried out in 9 males and 6 females (the only survivors who showed submissiveness after 10 days of age). For this reason the power of the tests is very low and the results should be taken with caution.

Growth rates and survival

The instantaneous growth rate did not differ between senior and junior chicks and between males and females; although the growth rate was higher in 2005 than in 2004 (social rank: $F_{1,49}$=0.44, p=0.51; year: $F_{1,37}$=40.66, p<0.0001; sex: $F_{1,54}$=0.17, p=0.68; random effect: nest: Z=1.2, p=0.22; fig.3.6).

Survival rates were lower in 2004 than in 2005 in senior and in junior chicks (year: $F_{1,34}$=17.16, p=0.0002; social rank: $F_{1,33.5}$=12.53, p=0.001; year*rank: $F_{1,33.5}$=3.55, p=0.068; random factor: nest: Z=1.61, p=0.1; fig.3.7). Sex and hatching date did not influence the survival rate of the chicks (sex: $F_{1,58}$=1.01, p=0.31; hatching date: $F_{1,30}$=2.55, p=0.17). In both years, senior chicks had a higher survival rate than junior chicks (social rank 2004: $F_{1,12.5}$=5.61, p=0.034; random factor: nest: Z=1.02, p=0.31; social rank 2005: $F_{1,21}$=3.5, p=0.075; random factor: nest: Z=2.43, p=0.015; fig.3.7). In 2004, 75% of the junior chicks were dead before 20 days of age and only one fledged (it was expelled from its nest by its senior sibling, established itself in a neighbouring nest with younger chicks and became dominant by expelling the chicks in the adopted nest). In the same year, 50% of senior chicks were dead before 30 days old and the other 50% fledged. In 2005, 75% of the junior chicks were alive after 20 days of age and 65% of them fledged. 87% of senior chicks in 2005 were alive after 30 days old and all of them fledged.

Behavioural correlations

ANCOVAS to test the influence of body condition on begging, feeding and aggression were carried out. There was a marginally significant interaction between year and condition on begging frequency (year: $F_{1,55}$=4.04, p=0.049; condition: $F_{1,55}$=4.31, p=0.043; year*condition: $F_{1,55}$=3.22,. p=0.078; post hoc analyses explained below), and a significant year*condition interaction on feeding frequency (year: $F_{1,56}$=3.29, p=0.075; condition: $F_{1,56}$=1.78, p=0.18; year*condition: $F_{1,55}$=4.45, p=0.012; post hoc analyses explained below). Year and body condition had no effect on aggression (year: $F_{1,56}$=0.64, p=0.42; condition: $F_{1,56}$=0.76, p=0.38; year*condition: $F_{1,55}$=0.022, p=0.88).

The influence of chicks' aggressiveness on the age they died was also tested and a significant interaction between year and the age of death was found (year: $F_{1,66}$=5.18, p=0.026; age of death: $F_{1,66}$=8.14, p=0.006; year*age of death $F_{1,66}$=8.14, p=0.006).

Due to the strong interaction effects I made separate Spearman correlations of complete broods between condition and begging, between condition and feeding and between age of death and aggression for each year. A positive correlation was present between begging frequency and condition in 2004 (r=0.63, p=0.036, n=21) but not in 2005 (r=-0.07, p=0.67, n=38); a negative tendency correlation between feeding and condition was present in 2004 (r=-0.42, p=0.058, n=21) but not in 2005 (r=-0.10, p=0.55, n=38) and a negative correlation was found between aggression frequency and age of death in 2004 (r=-0.24, p=0.021, n=21) and no correlation was present in 2005 (r=-0.007, p=0.91, n=38). A correlation between aggression and condition with pooled years was done because no interaction between year and condition was found. A negative correlation between aggression and condition was found when both years were tested together (r=-0.34, p=0.008, n=59). The mean aggressive bouts each chick performed and the age at which they died (or fledged) were negatively correlated in 2004 (r=-0.75, p=0.012, n=13) but no correlation was found in 2005 (r=-0.13, p=0.40, n=22).

Discussion

Comparison of the behaviour of siblings in 2004 and 2005 showed different patterns of begging, feeding and agonistic behaviours. Older senior and junior chicks in 2004 showed a lower begging frequency, begging success and feeding frequency and higher aggression frequency than younger senior and junior chicks and than senior and junior chicks of the same age in 2005. Activity levels did not differ between years but senior chicks spent more time active than juniors at any age. No differences in egg size and sex ratio between the years were present. Moreover, a dominance-subordinate relationship was formed in both years, but the aggression peak and the frequency of aggression at different ages differed between years. It was expected that, due to sexual dimorphism among adult kittiwakes, A-males were more aggressive than A-females, but the frequency of aggression did not differ between sexes.

There is some evidence indicating that 2004 was a poor year in terms of breeding success compared with 2005. Although adults' regurgitates' size did not differ between 2004 and 2005, an important characteristic of 2004 was the low energy content of the fish present in the regurgitates of several birds from the island (Wanless et al., 2005) . 0

group sandeels were of a very small size and of low lipid contents compared with sandeels in previous years (Wanless et al., 2005). These fish characteristics will directly affect nestlings because this prey item is the main food provided by their parents (Lewis et al., 2001).

The low energetic contents of food in 2004 were correlated with a low fledging rate and poor condition of the chicks that managed to fledge on that year. The yearly differences between 2004 and 2005 and the population data of at least 7 years for some parameters like the date of the first appearance of 0 group-sandeels up to 23 years data for date of first egg laid, clearly shows that 2004 was on the bottom 5% and 2005 was on the top 5% for breeding success. This is important to notice because it gives the appropriate basis for making behavioural comparisons between 2004 and 2005 and try to understand why the breeding success was so different in two consecutive years and how chicks adapted their behaviour to cope with environmental constraints.

In both years senior chicks always became dominant and juniors acquired the subordinate role. A linear dominance hierarchy was formed in both years during the first week of life as a consequence of agonistic encounters. It seems likely that this hierarchy was not created through a winning and looser training process (Drummond & Osorno, 1992) because when juniors had the chance to become dominant towards unknown chicks they did, meaning that they measure the strength of their rivals or recognize some trait(s) from them (Chase, 1982; Chase et al., 1994; Chase et al., 2002; Dugatkin & Earley, 2004) and not only learnt to behave like losers. However, junior chicks only retaliated to their older sibling 3% of the times they were attacked and never inverted the dominance roles.

Patterns of aggression were different between years. In 2004 seniors' aggression increased with age and reached a peak after 10 days old while juniors did not change the frequency of aggressive acts. In 2005 senior chicks' aggression frequency was constant through the whole breeding season and did not change with age while junior chicks decreased it. A high aggression level at a young age is what is expected from a species in which chicks have to establish a dominance-subordinate relationship. Once established, aggression levels should decrease if there is adequate food availability (Drummond, 2006; Valderrabano-Ibarra et al., 2007). In 2005 aggression remained constant through age and in 2004 aggression increased with age probably because food supply was inadequate to cover the needs of both chicks of the brood. The negative correlation found between chicks' aggression and their condition supports this idea: senior chicks in poor condition were more aggressive perhaps they were hungrier. Moreover, a negative correlation was found between aggression levels and age of death in 2004. This indicates that independently of the social rank more aggressive chicks that presumably had higher needs died earlier in the season. It seems that their increased aggression did not provide them with more food and they died earlier than less aggressive chicks that were perhaps in better condition.

It has been proposed that siblicide could be present if killing the sibling augments the food intake of the siblicide performer (Forbes, 1993; Rodriguez-Girones, 1996) but there is little empirical evidence of this (e.g. Mock et al., 1987; Mock & Lamey, 1991; Ploger, 1997; Drummond, 2001a). These studies seem to show that food intake did not increase in the chick that performs siblicide. Brown pelican (*Pelecanus occidentalis*) A-chicks in broods of three that performed siblicide did not increase their food intake after eliminating the C-chick from the brood, but B-chicks did. Moreover, aggression did not decrease after committing siblicide, which is not expected from the theoretical models. In the cattle egret it was found that after removal of the C-chick (simulating siblicide) chicks got the same amount of food than before the chick removal (Mock & Lamey, 1991; Mock et al., 1987). More aggressive kittiwake senior chicks in 2004 seem to not have benefited from reducing the brood because they died earlier compared with less aggressive seniors.

Although in 2004 the mortality of junior chicks (46%) when they were young was higher than young junior's mortality in 2005 at the same age (30%) it seems that it was a consequence of starvation more than a consequence of aggression because aggression rates when they were young did not differ between the years. The aggression rates did not differ between years but it is unknown if the intensity of the aggression was. Perhaps the intensity was not different between years but chicks in 2004 were weaker than in 2005 and aggression received in 2004 with the same frequency and intensity could have been fatal or exert them a bigger damage. Even though the size of the food loads each chick received remains unknown it seems from the chicks' regurgitates data that in general they were smaller in 2004 than in 2005. Although these data should be taken with care because it is unknown how long after eating chicks regurgitated.

But why did chicks not increase their begging rate with age in 2004 if indeed they were starving but instead decreased their begging frequency? Hungrier chicks should beg more than less hungry chicks (Kilner, 1997). If the energy contents and perhaps the amount of food received in 2004 was lower than in 2005 it is expected that in 2004 chicks would have begged more and their begging rates increased with age because as chicks grow they need more food (Godfray, 1991; Godfray, 1995). It could be that in 2004 junior chicks were prevented from begging by their dominant nest mates, but that does not explain why senior chicks were not begging more than junior chicks and instead decreased their begging frequency as well. Something was stopping chicks from increasing their begging effort. Perhaps in 2004 chicks stopped begging to save energy. In 2004 the proportion of successful begging bouts of older chicks was very low compared with begging success of older chicks in 2005. This may indicate that in fact in 2004 begging was not supplying more resources. More over, it seems from the positive correlation between begging rates and chicks’ condition present in 2004 that only chicks in good condition were able to beg. Blue-footed booby chicks that were artificially food-deprived and had a low condition did not beg as much as food-deprived chicks with good condition although parents provided more food (but less frequently) to chicks in poor condition (Villasenor & Drummond, 2007). The high mortality rate of kittiwakes indicates that in 2004 parents were not able to increase neither the food amount nor the quality of the food they provided to their chicks. All the evidence points out that begging could not be increased due to the low chicks’ condition and that parents were not able to provide more or better quality food.

Bize et al., (2006) found that in years with low food availability parents of the Alpine Swift (*Apus melba*) and the European Starling (*Sturnus vulgaris*) favoured the chick that was more likely to survive while in years with enough food they favoured the disadvantaged chick. In kittiwakes this favouritism was not observed, in both years senior and junior chicks received food at a similar frequencies, although the quantity of food might have differed. In 2005 senior and junior chicks increased their begging rates as they grew older but neither the feeding rate nor the proportion of successful begging bouts increased. I propose two explanations to explain these behavioural results in 2005: 1) parents increased the amount of food they gave in each feeding bout without increasing the frequency or/and 2) parents had a threshold above of which they do not feed more despite increased begging of the chicks to prevent cheating and avoid a decrease in fitness (Price & Ydenberg, 1995; Kilner, 1997; Smiseth et al., 2003; Royle, 2002; Glassey et al., 2002).

Even with the low amount of nutrients in 2004 junior chicks did not increase their aggressiveness. Junior chicks in poor condition and likely to die did not act like desperado chicks (Drummond et al., 2003). Perhaps junior chicks were assessing the strength of the senior nest mate. If the senior chick was ill or weak then the junior chick might have a chance of reversing the dominance (its only chance of surviving), but this situation never occurred in the studied nests as all junior chicks died before their senior nest mate. Junior chicks might have failed in reversing the dominance relationship because they were disadvantaged (in size and age) compared to their senior nest mate. When junior subordinate chicks had the chance to become dominants after being expelled from their nests and luckily got into another one inhabited by younger chicks they were able to do so in both years (in five occasions, pers. obs.). Perhaps junior chicks learn to be submissive in their natal nests in order to increase their chances of survival if the submission posture serves to stop aggression from their senior siblings (Matsumura & Hayden, 2006). However, when juniors had the opportunity they were capable to act as dominant chicks showing high levels of aggression and expelling chicks from their nests. It seems that chicks identified their opponent competitive abilities (size, aggressiveness, condition, etc.) thus acting aggressively without previously have been in contact with it.

In 2004 senior chicks did not kill their junior nest mates as soon they hatched, but the majority of them did it when juniors were older than 7 days. Perhaps senior chicks could not eliminate their nest mate earlier because they did not have the strength and the maturation to do so (Drummond et al., 2003). Apart from the physical strength seniors might need junior chicks to be weak (poor condition) before they can eliminate them from the nest. Senior chicks could have detected the lack of food at a certain age or a threshold on its own condition indicating that was time to commit siblicide in order to survive. Senior blue-footed booby chicks that were between 20 to 25% below their optimal weight became more aggressive and committed siblicide in higher proportion than chicks on their optimal weight (Drummond et al., 1986).

The differences in behaviour, growth and survival of kittiwake chicks in two consecutive, but very different years showed that environmental constraints can influence the flexible behaviour of kittiwake chicks. In a year where breeding success was poor, presumably due to poor food availability(2004), junior kittiwake chicks died in 100% of the study nests in contrast to less than 20% of junior chicks dead in a year where the food quality was better (2005).

In this chapter it was shown how flexible and sensitive kittiwake chicks behaviour is in responding to environmental challenges. Moreover, it seems that chicks learn how to behave on their social position and are capable of changing and adapting their behaviour if the social and environmental conditions change.

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Tables

Table 3.1. Proportion of different fish prey found in chicks regurgitates in 2004 and 2005. In both years the chicks mainly regurgitated 0 group sandeels, but in 2004 the remaining fish prey were equally divided between 1+ group sandeels, clupeids and gadids, while in 2005 clupeids were the second most important fish prey. Data collected from chicks in control groups in 2004 (n = 8) and 2005 (n = 11). Statistical comparisons between regurgitates of senior and junior chicks from the study nests could not be made do to the small number of regurgitates collected for chicks with known identity (four seniors and four juniors in 2004 and 2 juniors and 9 unknown chicks in 2005).

YEAR	**0 group sandeel**	**1+ group sandeel**	**Clupeid**	**Gadids**	**Average weight**
2004	73.3%	8.8%	8.8%	7.8%	6.8 g
2005	84.7%	0.5%	13.8%	0.8%	8.8g

Table 3.2. Comparisons of several aspects of the breeding biology of kittiwakes on the Isle of May between 2004 and 2005 and the data from 23 years. Data from kittiwake adults taken from Wanless et al., 2007.

	2004		**2005**		**means 1981-2003**		
	n	*mean*	*n*	*mean*	*n*	*mean*	*95% CI*
First egg date	*	22 May	*	30 May	23	08 May	6 - 12 May
Breeding success (chicks fledged per nest)	476	0.29	675	0.85	19	0.59	0.39 - 0.79
Adult mass (g)	362	365	420	373	15	374	366 - 382
First appearance of 0-group	*	23 May	*	30 May	7	22 May	16-28 May
Mean food load mass during chick-rearing	114	12.16	116	12.22	17	17.18	14.89-19.46
% sandeels in diet by mass (diet samples)	120	79	116	92	17	83	76 - 89

Table 3.3. The influence of age, rank, year, sex and hatching date and all two way interactions between these factors on the frequency of begging, feeding and the proportion of shared feeding bouts and successful begging was tested using a mixed model with nest and chick as random factors. Significant results are marked with bold characters. Non significant interactions were dropped from the model using the stepwise regression method. NA=Non applicable.

	Begging			*Feeding*			*Shared Feeding Bouts*			*Successful Begging*		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Age	**1,191**	**8.85**	**0.003**	**1,168**	**19.04**	**<0.001**	**1,144**	**6.51**	**0.001**	**1,126**	**7.32**	**0.008**
Rank	1,94	1.10	0.29	1,104	0.41	0.52	NA	NA	NA	1,105	0.15	0.69
Year	**1,41**	**6.72**	**0.013**	1,119	2.15	0.14	**1,88**	**3.89**	**0.051**	**1.124**	**8.93**	**0.003**
Sex	1,150	1.07	0.302	1,148	<0.01	0.80	NA	NA	NA	1,146	0.04	0.83
Hatching date	1,50	0.66	0.42	1,41	<0.01	0.94	1,27	0.77	0.39	1,41	0.96	0.33
Age*Year	**1,160**	**3.85**	**0.051**	**1,168**	**19.03**	**<0.001**	**1,144**	**10.35**	**0.001**	**1,126**	**18.36**	**<0.001**
Random Factors	Z	p		Z	p		Z	p		Z	p	
Nest	0.64	0.52		1.9	0.056		1.64	0.10		1.92	0.052	
Chick	1.77	0.077		1.78	0.074		3.33	0.009		0.97	0.33	

Table 3.4. The influence of age, rank, year, sex and hatching date and all two way interactions between these factors on the frequency of aggression and the proportion of submissive acts responded with submission was tested using a mixed model with nest and chick as random factors. Significant results are marked with bold characters. Non significant interactions were dropped from the model using the stepwise regression method.

	Aggression			*Submission*		
	df	*F*	*p*	*df*	*F*	*p*
Age	1,187	0.19	0.66	**1,33**	**8.56**	**0.006**
Rank	1,169	3.82	0.05	**1,32**	**22.4**	**<0.001**
Year	1,79	0.45	0.50	1,36	1.76	0.19
Sex	1,135	0.04	0.84	1,33	3.75	0.061
Hatching date	1,42	1.35	0.25	1,54	1.19	0.28
Age*Year	**1,190**	**12.37**	**0.005**	1,22	0.16	0.69
Age*Rank	**1,155**	**12.93**	**<0.001**	1,32	1.71	0.2
Age*Sex	1,178	0.14	0.71	1,41	8.81	**0.006**
Rank*Year	**1,110**	**18.54**	**<0.0001**	1,44	0.38	0.54
Random Factors	Z	p				
Nest	0.9	0.3		1.69	0.09	
Chick	3.3	0.0008		0.44	0.65	

Figures

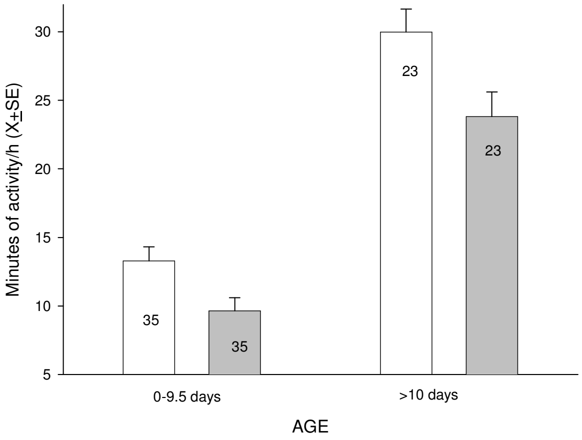

Figure 3.1. Mean (±SE) minutes per hour spent active by senior ○and junior ● in relation to chicks age in 2004 and 2005 (pooled data). Senior chicks were more active than juniors and activity increased with age in seniors and juniors. Mean values for activity of chicks below and above 10 days old is shown for presentation purpose only. Age was used as a continuous variable in the analysis (from this figure and thereafter). Sample sizes (number of broods observed) are indicated inside each bar. The number of broods observed are on each bar, fewer broods were observed at ages > 10 days due to chick mortality.

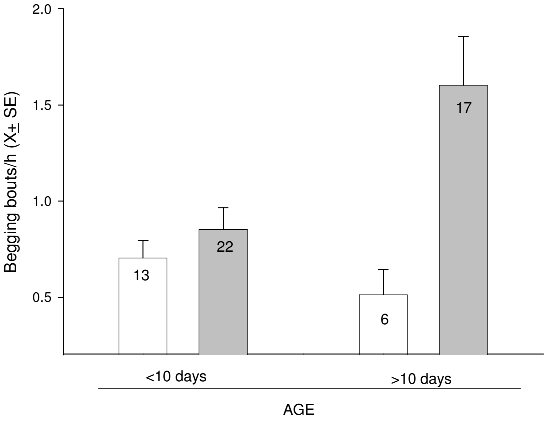

Figure 3.2. Mean (±SE) begging frequency per hour during 2004 ○ and 2005 ●in relation to chicks age. Frequency of begging decreased with age in 2004 and increased with age in 2005. Sample sizes (number of broods observed) are indicated inside each bar.

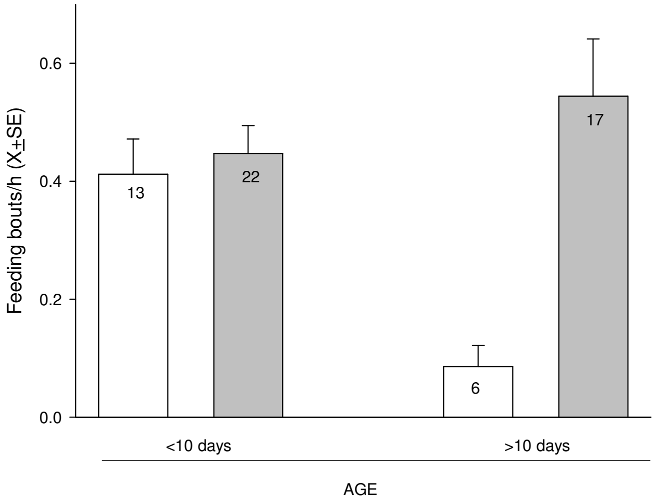

Figure 3.3. Mean (±SE) feeding frequency during 2004 ○ and 2005 ● in relation to chicks age. The frequency of feeding was not different between 2004 and 2005 when chicks were young. In 2004 the feeding frequency decreased with age while in 2005 it did not change. Sample sizes (number of broods observed) are indicated inside each bar.

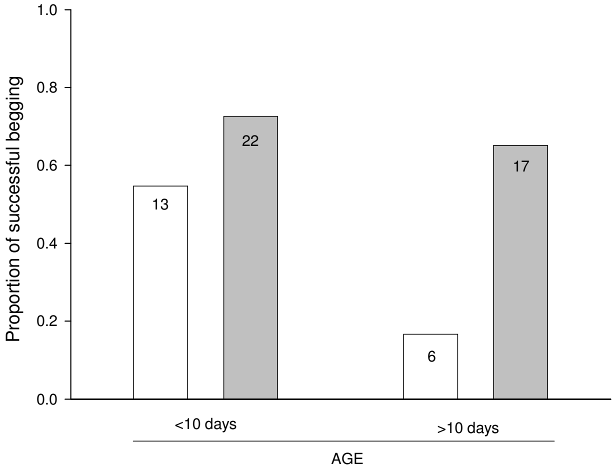

Figure 3.4. Proportion of successful begging in 2004 ○ and 2005 ● in relation to chicks age. Chicks in 2005 begged more successfully than in 2004. Begging success decreased with age in 2004 and did not change with age in 2005. Sample sizes (number of broods observed) are indicated inside each bar.

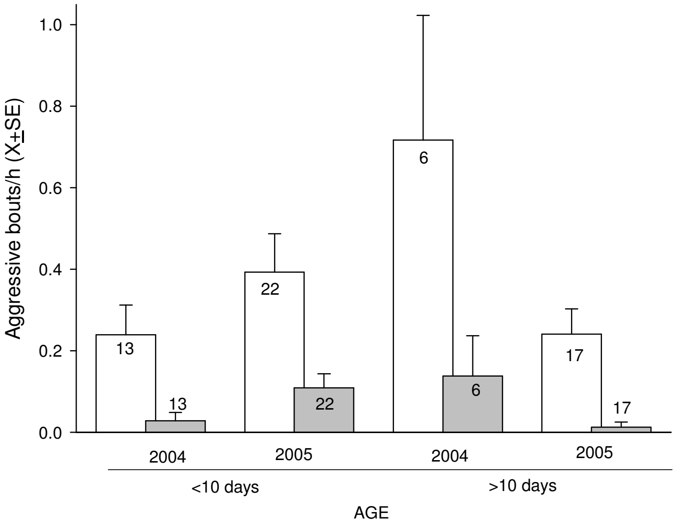

Figure 3.5. Mean (±SE) aggressive bouts per hour during 2004 and 2005 in senior ○and junior ●chicks in relation to their age. Aggression frequency was higher in seniors than in junior chicks, but the difference depended on chicks age and year. In 2004 aggression increased with age while in 2005 it decreased with age. Sample sizes (number of brood observed) are indicated inside each bar.

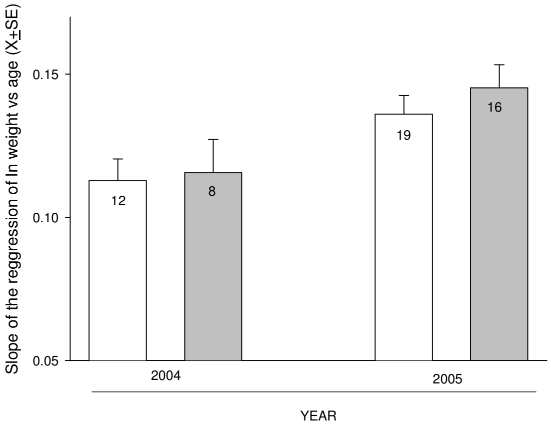

Figure 3.6. Mean (±SE) instantaneous growth rate of senior ○and junior ●chicks in 2004 and 2005. Growth was not different between senior and junior chicks but it was different between 2004 and 2005. Chicks grew faster in 2005 than in 2004 independently of hatching order.

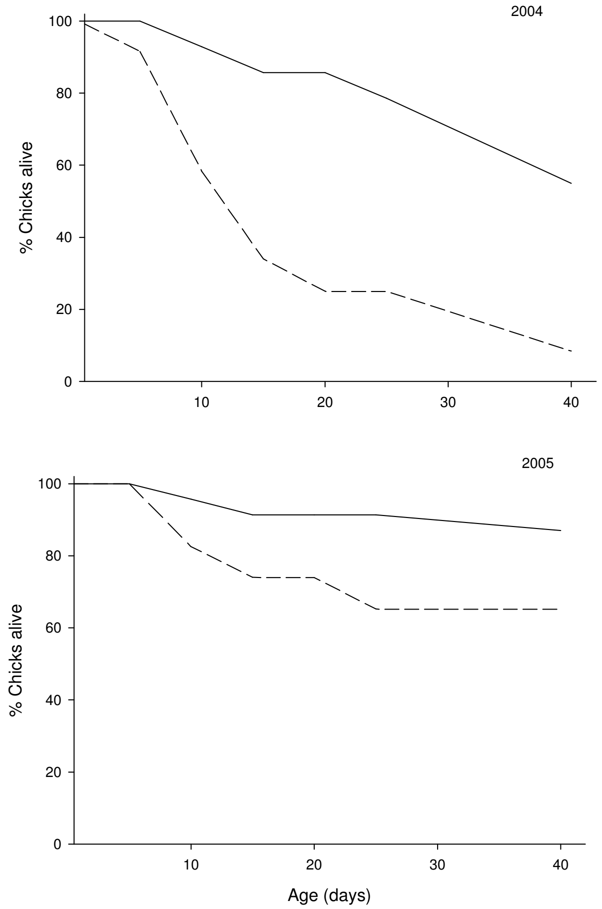

Figure 3.7. Cumulative survival curves for senior and junior chicks in 2004 (top) and 2005 (bottom). Senior chicks (−) survived better than junior chicks (--) in both years, although the difference tends to be more pronounced in 2004 (see text for analyses). Chick survival up to fledging was higher in 2005 than in 2004.

Chapter IV

The Influence of Egg Size in the Sibling Rivalry of Black-legged Kittiwakes

Introduction

Brood reduction is a common cause of chick loss (Lack, 1947; O'connor, 1978; Stinson, 1979; Mock & Parker, 1998). Lack (1947) suggested that birds produced broods with the maximum number of chicks they can successfully fledge during years with good food availability. Any asynchrony within the brood such as asynchronous hatching will create a hierarchy. This hierarchy will favour some chicks over others making it more likely for the favoured chicks to survive when the food conditions are not good (Lack, 1947). Reduction of the brood in asynchronously hatching birds occurs in two ways: when younger chicks starve as older chicks satisfy their nutritional needs first, or when siblicide occurs through aggressive actions from the older chick that inhibit feeding of the younger siblings, kill them or eject them from the nest (Cash & Evans, 1986; Mock & Parker, 1998; Ploger & Medeiros, 2004).

Parents can affect the outcome of brood reduction by manipulating hatching asynchrony (Magrath, 1989; Stoleson & Beissinger, 1995), egg size (Howe, 1976; Zach, 1982; Stokland & Amundsen, 1988; Williams, 1994) and egg composition including hormones and carotenoids (Schwabl, 1993; Schwabl et al., 1997; Blount et al., 2000; Groothuis et al., 2005a). It is not well understood if these potential asymmetries are present to specifically favor one of the chicks depending on the environmental conditions and their influence on sibling rivalry. Studies analyzing the importance of the asymmetries within a brood on sibling rivalry usually confound differences in chicks’ size due to egg size, and as a consequence of hatching asynchrony.

The best studied parental manipulation of the brood influencing sibling rivalry is hatching asynchrony. It has been a topic of discussion for more than 60 years with around 19 hypotheses attempting to explain it (reviewed in Vinuela, 2000). Hatching asynchrony has usually used to explain the influence of size differences between nest mates on sibling rivalry. The degree of asynchrony will determine size and maturation differences between the chicks (Edwards & Collopy, 1983; Mock & Ploger, 1987; Wiebe, 1995; Stenning, 1996; Beissinger & Stoleson, 1997). Experimentally reducing or removing the hatching asynchrony can increase the fledging rate of a brood in a specific season by increasing parental effort in food provisioning as long as the resources are abundant (Fujioka, 1985b; Stenning, 1996; Beissinger & Stoleson, 1997).

Another factor that may influence sibling rivalry is the pattern of maternal allocation of egg components within a brood in relation to laying order. The main egg components studied so far are hormones (principally androgens and corticosterone) (Schwabl, 1993; Groothuis & Schwabl, 2002) and carotenoids (Royle et al., 2001; Fenoglio et al., 2003; Torok et al., 2007) that are differentially allocated according to the laying order. It has been shown that maternal deposition of these components into the egg can influence chick's behaviour and survival. For example, in cattle egrets (*Bubulcus ibis*), a facultative brood reducing species, first-laid eggs had higher levels of testosterone than later-laid eggs which have the potential of promote aggressiveness towards junior siblings and facilitate brood reduction (Schwabl et al., 1997). High levels of androgens have various effects depending on the laying position and the species (e.g. diminished immune response, increased developmental rate; reviewed in Muller et al., 2005). On the other hand, egg carotenoids enhance the immune function (Royle et al., 2001) and corticosterone in eggs diminish the developmental rate and decreased the immune function (Rubolini et al., 2005). Patterns of hormones' allocation differ between species and their effects vary as well (reviewed in Groothuis et al., 2007).

The important factors in the survival of last-hatched chicks in species with brood reduction are: 1) the size difference between siblings and 2) the junior vulnerability provoked by hatching on a second place from a different quality egg, and not necessarily the absolute size of the junior (Fujioka, 1985a; Machmer & Ydenberg, 1998; Royle & Hamer, 1998). Females could control the egg size or they just run out of resources after laying a first egg (Bowden et al., 2004). Size of the chicks will be positively correlated with the size of the egg from which it hatches (Ricklefs et al., 1978;

Grant, 1991; Deeming & Birchard, 2007). Chicks from later-laid eggs are usually smaller and in several species are more likely to die earlier than larger and older chicks of the same brood (Howe, 1976; Bolton, 1991; Mock & Parker, 1998).

In order to test the influence of egg size differences on the agonistic behaviour and survival of the facultative brood reducer black-legged kittiwake (*Rissa tridactyla*) experimental broods with no hatching asynchrony and differences in egg composition were created. Eggs of the same laying position, laid on the same day and controlling for size differences between the eggs (matching egg size or simulating natural egg size differences) were placed into the same nest. By doing this, other factors influencing sibling rivalry (age difference, egg type, hatching asynchrony) were avoided and it was possible to compare agonism and parental provisioning between chicks hatched from eggs of similar or different size.

I predict that (1) in broods with differences in egg size chicks hatched from the larger egg will become dominant and will require less aggression to establish a stable dominance-subordinate relationship compared with broods with no differences in egg size (Chase et al., 2002). (2) Dominance in both types of experimental broods (equal and different egg size) will be expressed with a higher rate of sibling aggression and a higher feeding frequency from the parents compared to control broods (Drummond et al., 1986; Drummond, 2006). (3) The survival proportion of control broods will be higher than the survival proportion of experimental broods if asymmetries within a brood serve parents to optimize resources and allocate in a differential manner without compromising their future fitness (Fujioka, 1985a; Fujioka, 1985b).

Methods

Kittiwakes are colonial birds with a modal clutch size of two eggs. Chicks show facultative brood reduction (chicks perform siblicide only when the food is insufficient to successfully fledge two chicks). They show a hatching asynchrony of ca. 2 days with the first-laid egg (A- eggs) being 4% bigger and having higher levels of carotenoids and lower levels of corticosterone than the second-laid egg (B-eggs) (**chapter II**). First-hatched chicks (A- chicks) usually become dominants and may eliminate their siblings when food is scarce (Braun & Hunt, 1984). Because of these characteristics, kittiwakes are a good species to do experiments that could explain the influence of egg size on the outcome of sibling rivalry.

In 2004, the black-legged kittiwake colony on the Isle of May, Firth of Forth, Scotland (fig.1.1) where between 4000 and 7000 pairs breed was used for this experiment. Eggs of the same clutch were laid and hatched typically two days apart (n=87). A dominance-subordinate relationship was established soon after hatching and maintained throughout the brooding period or until one of the chicks died (usually the younger one).

One hundred and twenty accessible nests distributed in 6 sub-colonies were used for this work. Nests were inspected daily to record the date when each egg of a clutch was laid and then marked on the day of laying in order to identify A- and B- eggs. Egg length (l) and width (b) was measured using calipers to the nearest 0.1mm and volume was calculated with the formula $V=0.4866(b^2)l$ (Coulson, 1963).

Experimental broods were created with two eggs laid on the same day from the same laying order (A or B eggs within a brood) to control for egg quality and age by swapping eggs between nests. In order to control for egg size differences, experimental clutches made of two A- or two B-eggs were each divided into two treatment groups. Four experimental groups were thus created: two groups made of two A- or two B-eggs where both eggs were of similar volume (AA and BB, respectively) laid on the same day and their volume differing less than $3\text{cm}^{3.}$ The other two groups were made of two A- or two B-eggs laid on the same day and the volume between them differing more than 5cm^3 (Aa and Bb, respectively).

It was tested if the size asymmetries between the equal- or different- size groups were similar by using a repeated measures ANOVA with egg volume as the within - subjects factor, treatment of the eggs as the between- subjects factor and the egg size differences between the eggs of the brood as covariate (repeated measures ANOVA: treatment: $F_{1,31}=0.017$, $p=0.89$; egg size differences: $F_{1,31}=3.14$, $p=0.086$; treatment*egg size difference: $F_{1,31}=4.53$, $p=0.041$). These results indicate that the egg size differences between the eggs of a brood differed between groups. The differences between eggs of

AA-broods were 1.7%; between Aa 14%; between BB 1.3% and between Bb 12%. A group of control broods was also created by cross-fostering whole clutches laid by the same female. This group conserved the natural asymmetries in size, age and quality within a clutch.

Hatching date, growth and survival.

Kittiwakes incubate in average 28 days (Cullen, 1957). The cross fostering to create the experimental broods was made within ten days of the eggs being laid. Daily checks of the broods were made from 25 days after clutch completion onwards in order to know the exact hatching date of each chick. Eighty experimental clutches were created, but some of the eggs failed to hatch or the chicks of a nest did not hatch on the same day. Only broods in which chicks hatched within 24 hours were used for the statistical analyses. On the day each chick hatched it was randomly marked on head, neck and rump with red or blue sheep dye for individual identification. Chicks were weighed with a spring balance to the nearest gram every 4 to 5 days if weather conditions permitted it (since kittiwakes nest on cliffs, it was not possible to access the nest when it was raining or during periods of strong wind) until they were 20 days old. After this age kittiwake chicks become very sensitive to human disturbance and can jump off their nests. Instantaneous growth rate was used in the analysis by calculating the slope of the regression line of log transformed weight (g) on age (days) over the linear growth period (7 to 16 days, Lance & Roby, 1998) using only chicks that survived until the age of 9 days. The exact date that a chick died or disappeared from its nest (predated, pushed off the nest by its sibling or dead inside the nest) was known from daily visual nest checks. If a chick survived until 40 days of age it was considered to have fledged (Cullen, 1957). A confirmation of this was made by checking the nest and its surroundings to try to spot fledglings because it is common that fledged kittiwakes go back to their nests to be fed by their parents for some days or even weeks after fledging (Cullen, 1957).

Behavioural recording.

Behavioural observations of broods started when juniors were one day old (age = 0 is day of hatching) and followed up until chicks were 20 days old or one of the chicks died. A total of 38 broods were observed: AA=10, Aa=10, BB=7 Bb=11 plus 13 control broods. Behavioural observations of the available nests were made every other day during three hour shifts. The observation day was divided into three shifts: morning (6:00 – 9:00), afternoon (10:30-13:30), and evening (15:30-18:30) and on each observation day nests were randomly allocated to one of the three shifts. On each shift a maximum of five nests were observed at the same time. On average each nest was observed for a total of 12 hours, ranging from 3 to 18h. Observers did not know the identity of the experimental nests they were observing and they were seated at a distance of 3-7m from the observed nests. A combination of behavioural and scan sampling observations (explained in Martin & Bateson, 1994) was used to record chicks' behaviour. In the behavioural sampling the absolute frequency of each chick's begs (oscillating the head rhythmical with bill pointed upward, or pecking the adult's bill); feeds (chick's bill inside of the adult's bill or direct observation of food passing from the adult to the chick) and aggressive acts (pecks and bites); and whether each aggressive act elicited a submissive posture (bill down, face away or both) from the receiver was registered. Behavioural scanning was carried out every 1.5 minutes to record if the chicks were active or not. The criteria to record these behaviours were modified from the protocol used by (Drummond et al., 2003) for recording the behaviour of blue footed- *(Sula nebouxii)* and brown-booby (*Sula leucogaster)* chicks and adjusted from behavioural descriptions of black-legged kittiwake chicks by Cullen (1957). The only behaviour that was never observed in this colony was the wing display by the subordinate chick showing its black band to a dominant chick, presumably to indicate its subordinate status. For a detailed description of how each behaviour was defined and its frequency calculated refer to **chapter III**.

Statistical Analysis

Generalized Linear Mixed Models (GLIMMIX) were carried out to compare the behaviour between experimental broods. Nest and chick were used as random factors in every model because the data was clustered in nests with each nest containing two factors (both chicks) and were observed several times on different days and day times. Different types of error distribution were utilized depending on the behaviour analysed: activity: normal distribution; begging, feeding and aggression: poisson distribution; submission and successful begging: binomial distribution. The same type of analyses (GLIMMIX) were also used for growth and survival using a normal and a binomial distribution respectively. SAS statistical package (SAS Institute, inc.) was used to run the analyses, this package uses the Satterthwaite method to derive degrees of freedom (Gaylor & Hopper, 1969). Before running the final model for behaviour, growth and survival, the following factors were tested using univariate models to see whether they were influencing chick's behaviour: sub-colony, observer, and time of the day of the observation. They were never significant ($p>0.26$). In each model the chick's age, difference in egg size, egg type (A- or B-egg) and all possible two way interactions were tested. When the interactions were not significant, they were removed from the model using the stepwise regression method.

The result section is split into two sections: 1) Comparison of experimental broods taking dominance into account. In these analyses it was tested if the behaviour of dominants and subordinates differed in the different experimental groups. 2) Behaviour of experimental and control groups were compared. This comparison is complicated because control broods differ from experimental broods (longer hatching intervals, two different egg types of different size) in different ways than experimental broods differ among each other. Comparisons between experimental and control broods were done through simplifying models (Crawley, 1993) by comparing the deviances between full and simplified models. The full model had 5 groups. In a first step I tested whether simplifying the model by dropping egg type and/or egg size differences would not affect the comparison as judged by the change in deviance between the full and the simplified model. In the behaviours where in the previous analysis no effect of egg size difference and egg type was present (feeding, begging, and submission) the 4 experimental groups were pooled and compared to control broods. In order to compare activity (influenced by the egg size difference within a brood) two groups of experimental broods (with- and without-egg size difference) were compared to control broods. To compare aggression (differing in A- and B-chicks) two groups of experimental broods (A or B) were compared against control broods. The change in deviance between full and simplified models is approximately chi square distributed and its significance evaluated accordingly.

To test how levels of aggression correlated with the feeding rate of a chick and its survival, Spearman correlations were carried out in the 4 experimental and the control broods. Throughout the text I report mean values ± 1 standard error.

Results

Dominance assessment

It was established if a dominant-subordinate relationship was created and which of the chicks became dominant in every brood by examining aggression This dominance assessment was carried out using a logistic regression with the aggression rate as the dependent variable (logistic regression, egg size difference: wald=0.83; p=0.031; egg type: wald=0.059, p=0.71). It turned out that 70% (14 out of 21) of chicks hatched from smaller eggs became dominant in the broods where eggs differed on size irrespective of egg type. In broods where chicks hatched from eggs of similar size it was not possible to identify a characteristic trait deciding which of both chicks become dominant (logistic regression, egg size difference: wald=0.032, p=0.84, egg type: wald= 0.031, p=0.85).

Behaviour, growth and survival

Dominants vs. subordinates chicks

Because a dominant-subordinate relationship was found in all of the nests independently of their experimental treatment, behavioral analyses from this section always took into account the rank of each chick within a brood and the egg size differences as well as the type of egg each brood hatched from (A- or B-eggs).

Behaviour

Broods where chicks hatched from eggs with different sizes were more active than broods with no egg size differences independently of the egg type (table 4.1). Activity increased with age in dominants and subordinates, independently of egg size differences and egg type (fig.4.1, table 4.1). Dominant chicks begged and fed with higher frequency than subordinate ones independently of their egg type and the egg size difference (table 4.2). Begging success was higher in dominant chicks irrespective of their egg type and the egg size difference (table 4.2).

When aggression was analysed two interactions were found (table 4.3) and post-hoc analyses from each interaction were carried out: 1) age*rank: the frequency of aggression of dominant chicks did not change with age (age: $F_{1,82}=3.20$, $p=0.077$; fig.4.2) while subordinate chicks decreased their frequency of aggression as they got older (age: $F_{1,75}=10.44$, $p=0.002$; fig.4.2). 2) egg type*rank: dominant chicks that hatched from B-eggs were more aggressive than dominant chicks that hatched from A-eggs ($F_{1,28}=36.84$, $p<0.0001$; fig.4.3) while subordinate chicks from different egg types did not differ in their frequency of aggression ($F_{1,39}=1.39$, $p=0.24$; fig.4.3). Subordinate chicks responded to attacks with a submissive act more frequently than dominants did when they were attacked and submissiveness increased with age (table 4.3, fig.4.4). Aggression was seldom answered by retaliation from the attacked chick. There were no differences in the submission between experimental groups (table 4.3).

Growth rates and survival.

Dominant chicks gained weight faster than subordinate chicks independently of the egg size difference or type of egg they hatched from (rank: $F_{1,20}=6.23$, $p=0.007$; egg size difference: $F_{1,25}=2.36$, $p=0.14$; egg type: $F_{1,25}=0.52$, $p=0.47$; random factors: nest: $Z=0.04$, $p=0.97$, chick: $Z=2.04$, $p=0.04$; fig. 4.5). Dominant B chicks had higher fledging success than subordinates and than dominant As (rank: $F_{1,25}=36.56$ $p<0.0001$; egg type: $F_{1,25}=0.67$, $p=0.43$; rank*egg type: $F_{1,25}=7.03$, $p=0.018$; random factors: nest: $Z=0.33$, $p=0.73$, chick: $Z=4.83$, $p<0.0001$, fig. 4.6). The age at which chicks died did not vary with egg type, egg size difference or aggressiveness of their nest mate (rank: $F_{1,28}<0.01$, $p=0.96$; egg type: $F_{1,21}=0.17$, $p=0.68$; egg size difference: $F_{1,21}=0.93$, $p=0.34$;

aggressiveness: $F_{1,19}<0.01$, p=0.94; random factors: nest: Z=0.06, p=0.94, chick: Z= 3.53, p=0.0004).

Comparisons with control broods.

In order to compare the behaviour of experimental broods with that of control broods, simplified models were used (see methods). No effects of egg size difference and egg type were found on begging, and feeding frequencies and submissiveness. Hence for these behaviours the 4 experimental groups were pooled and compared with control broods. Activity differed between broods where eggs were matched in size and where the two eggs differed in size, thus only egg types within the egg size difference treatments were pooled and compared to control broods. Aggression differed between broods with different egg type therefore broods of the same egg type were pooled and compared with control broods. χ^2-tests comparing the models always indicated that pooling was justified (begging: $\chi^2=0.13$, p=0.94; feeding: $\chi^2=2.55$, p=0.28; submission: $\chi^2=0.43$, p=0.81; activity: $\chi^2=0.93$, p=0.42; aggression: $\chi^2=0.79$, p=0.67). Because of these results, the groups were pooled as previously described in order to compare behavioural traits of experimental and control groups.

Broods with B-chicks were more active than broods with A-chicks and than control broods independently of egg size differences and rank (egg type: $F_{2,41}=46.13$, p<0.0001; age: $F_{1,164}=0.51$, p=0.47; size: $F_{1,45}=0.25$, p=0.61; rank: $F_{1,302}=0.18$, p=0.67; random factors: nest: Z=0.61, p=0.54; chick: Z=1.94; p=0.052; fig.4.7). Control broods begged at lower frequencies than experimental broods independently of the age and rank (group: $F_{1,34}=4.26$, p=0.041; age: $F_{1,121}=1.01$, p=0.32; rank: $F_{1,318}=1.40$, p=0.24; random factors: nest: Z=0.37, p=0.70; chick: Z=0.28; p=0.78). An interaction between age and group was present when analysing successful begging and feeding (successful begging: group: $F_{1,175}=0.51$, p=0.47; age: $F_{1,180}=0.26$, p=0.61; group*age: $F_{1,180}=3.73$, p=0.05; random factors: nest: Z=0.05, p=0.96; chick: Z=0.36; p=0.71; feeding: group: $F_{1,135}=2.09$, p=0.15; age: $F_{1,94}=12.65$, p=0.006; group*age: $F_{1,152}=4.15$, p=0.04; random factors: nest: Z=0.23, p=0.82; chick: Z=1.55; p=0.12). Post hoc tests indicate that in control broods begging success decreased with chicks' age and feeding frequency increased with chick's age while in experimental broods these behaviours did not change with age (successful begging: control broods: age: $F_{1,63}=4.29$, p=0.023; experimental broods: age: $F_{1,107}=0.92$, p=0.37; feeding frequency: control broods: age: $F_{1,31}=3.17$, p=0.015; experimental broods: age: $F_{1,127}=13.71$, p=0.0004; fig.4.8 and fig.4.9 respectively). Aggressiveness was lower in A-chicks' broods than in B-chicks' broods and than in control broods and it was not influenced by chicks' age or rank (egg type: $F_{2,93}=3.87$, p=0.024; age: $F_{1,91}=2.22$, p=0.11; rank: $F_{1,195}=1.00$, p=0.38; random factors: nest: Z=0.63, p=0.52; chick: Z=4.29; p<0.0001; fig.4.10). Submissiveness was higher in control than in experimental broods (group: $F_{1,13}=16.43$, p=0.0014; random factors: nest: Z=1.33, p=0.18; chick: Z=0.39; p=0.69; fig.4.11).

Growth and survival

Chicks from control broods did not grow differently from chicks in experimental broods ($F_{1,43}=2.32$, p=0.14; fig.4.12) but chicks from control broods were more likely to fledge than chicks in experimental broods ($F_{2,92}=35.5$, p<0.0001; fig.4.13).

Relationships between aggression, feeding rate and survival.

Correlations were made to test whether there was a relationship between aggression and feeding rate and between these behaviours and the survival of dominant and subordinate chicks. Neither dominant nor subordinate chicks showed a correlation between aggressiveness and feeding rate in any of the groups (p>0.12). No correlation was present between dominant's feeding and aggression frequency and the survival rate of subordinates (p>0.37).

Discussion

I predicted that (1) in experimental broods with differences in egg size, chicks hatched from larger eggs would be domina tand that aggression would be higher in broods hatched from similar size eggs. However, the opposite happened: chicks hatched from smaller size eggs became dominant in higher proportion than chicks hatched from larger eggs. Moreover, chicks did not differentially compete (through begging or aggression) or differentially obtained food according to their egg size differences. It was also predicted and corroborated that (2) sibling aggression would be higher in experimental than in control broods and (3) that control chicks were more likely to fledge than experimentals.

The only behavioural difference found between equal and different egg size groups was that broods hatched from different egg size were more active than broods hatched from similar size eggs. No differences in aggression, submission, begging and feeding rates were found between these two brood types. It is uncertain why broods from eggs of different size were more active than broods from eggs of similar size. It could be advantageous to spend more time active in order to compete for food using the extra active time being aggressive or begging for longer time but differences on these behaviours were not found.

In several species it has been shown that chicks hatched from larger eggs grow better and have a higher chance of survive than chicks hatched from smaller eggs (Williams, 1994; Ferrari, 2006). However, within clutches egg size often correlates with laying order and egg size is confounded with the size asymmetries that are a consequence of hatching asynchrony (Stokland & Amundsen, 1988). In the present study no differences in provisioning behaviour or growth rates were found between clutches hatched from eggs of different or similar sizes when other naturally occurring differences between nest mates were controlled (hatching asynchrony and egg type). These results suggest that kittiwakes hatched from smaller eggs did not have impairments for growing at similar rates as chicks hatched from larger ones.

A dominant-subordinate hierarchy was always formed in the experimental broods independently of the egg size difference as it happens in natural asynchronous broods. In natural conditions the older and larger chick is usually the dominant (Braun & Hunt, 1984). However, in the experimental broods where the two eggs differed in size but were matched for age and egg quality the opposite happened: 70% of the chicks hatched from the smaller egg became dominant and in broods where both chicks hatched from eggs of similar size none of the tested traits (age difference, relative egg size or egg type) determined which chick become dominant. These findings could be indicating that when no other differences are present within the brood, the difference on egg size is the one directing the pattern of dominance and not the egg type.

Why did chicks hatched from smaller eggs become dominants in higher proportion than chicks hatched from larger eggs in broods with different egg size?

Perhaps smaller eggs developed faster and hatched before its larger sibling of the same age. It is possible that chicks hatched only a few hours before their nest mates have a greater advantage and this small difference in age is sufficient to become the dominant chick (Wiebe & Bortolotti, 1994). The daily nest checks made to record the hatching date of chicks was not fine enough to provide this data on the kittiwakes of the present study. Embryos from last laid eggs that are usually smaller than embryos from A- eggs can accelerate their hatching in order to compensate for being smaller and younger by using vibration and acoustic signals from their older nest mates (Persson & Andersson, 1999; Muck & Nager, 2006). In the lesser black-backed gull (*Larus fuscus*) last-laid eggs accelerated hatching only when they were the last to hatch, when they were manipulated to hatch in the first position they did not accelerate hatching and A- eggs never accelerated hatching in any position (Muck & Nager, 2006). In the present experiment kittiwake chicks hatched from a smaller egg did not have the stimulus from an older nest mate to accelerate their hatching because both hatched on the same day thus is unlikely that they accelerated their hatching due to the sibling stimulus.

Behavioural asymmetries between dominants and subordinates of different experimental groups

Several differences were present between dominant and subordinate chicks independently of their egg type and egg size differences. Dominants begged and fed in higher frequencies than subordinates and subordinates showed a submissive posture after being attacked more frequently than dominants. Since the dominance assessment was made using only the aggression frequency these results confirm that this trait indicates with accuracy the dominant individual. Dominant chicks from A- and B-broods differed on their aggression frequency. Dominant chicks from B-broods were more aggressive than dominant chicks from A-broods independently of the egg size difference. Perhaps the differential maternal influence on A- and B- eggs provoked this difference in aggression. On **chapter II** it was found that B- eggs had higher levels of testosterone than A-eggs and the former had lower carotenoid levels than the latter.

Having high levels of testosterone in the yolk could be advantageous because it can increase aggressiveness (Schwabl, 1993; Groothuis & Ros, 2005) and early competitive abilities (Groothuis et al., 2006) by promoting readiness to beg and obtain food (Schwabl, 1996; Quillfeldt et al., 2006; but see Groothuis & Ros, 2005). Mothers could favour B chicks for hatching on a disadvantageous position by allocating more testosterone therefore promoting aggressiveness in case they have the chance to reverse the dominance. Thus it is not surprising that dominant B-chicks showed an elevated aggressive frequency compared to dominant A-chicks, although dominants from A- and B- broods did not differ on their begging and feeding behaviour.

Behavioural asymmetries between control and experimental broods.

In the present study, dominant chicks from experimental broods did not behave differently from dominant chicks from control broods and it is remarkable that kittiwakes formed a stable dominance hierarchy with a nest mate matched in age, size and quality in the same way as chicks did in control broods. When experimental broods were compared with control broods, one of the main differences in behaviour was that in control broods begging and feeding frequencies were lower, but at the same time begging success was higher than in experimental broods. This result could be showing that the efficiency (less effort for higher productivity) of control broods is better than that of experimental broods. Control chicks did not have to beg more in order to obtain more food, while it seems that experimental chicks did. Moreover, experimental broods with chicks hatched from A-eggs showed less aggression than broods with B-eggs and than control broods. This might indicate that the natural differences between nest mates do not exert an effect on the aggression rate but perhaps the egg composition of the clutch does. A-chicks in natural conditions are often the dominant chicks and therefore the most aggressive ones. However, it seems that when they are with a matched opponent they do not need to be as aggressive as when they compete towards a chick hatched from a B-egg. Regardless of being as aggressive as B-broods, control broods were more submissive than experimental broods independently of the egg quality or size differences. Perhaps this difference in submissiveness between control and Bb-broods determined the increase in the fledging proportion of control broods.

Submission could have provoked a decrease on the intensity of aggression (not recorded) without necessarily decreasing the frequency of aggression (Deag & Scott, 1999; Matsumura & Hayden, 2006).

As mentioned before, the proportion of fledged chicks was higher in control than in experimental broods and the survival rate was higher in dominant than in subordinate chicks from the experimental and control groups. Apparently the egg size difference within a brood it is not important on the survival rate of chicks and the way a dominance-subordinate relationship establishes. It seems that the natural asymmetries between A- and B- eggs are convenient for the survival of the dominant (usually the senior) chick. Experimentally eliminating the asymmetries in age and quality between nest mates resulted in a decrease in fledging success despite of the fact that experimental broods begged and fed in higher proportion than natural broods. Perhaps parents increased the feeding frequency due to the high levels of begging but they were not able to provide more food (i.e. they provided smaller feds).

Fujioka (1985b) found in cattle egret chicks that experimentally synchronous broods had higher survival than natural asynchronous broods and presumably parents fed them with more food because it was a year with abundant resources. The environmental conditions of this kittiwake colony were not good: food was not abundant and the energy content was low (Wanless et al., 2005), thus the fledging success of the whole colony was low (Harris, 2004). The absence of asymmetries between siblings within a brood could be energetically costly for the parents when the food is scarce. Both chicks need high amounts of energy at the same time. If there is too little food, chicks are more likely to die because they have to compete with a more equally matched opponent. This competition is likely to be more costly than competing with a younger and smaller chick (Gibbons, 1987; Bollinger, 1994; Vinuela, 2000). Perhaps experimental chicks fledged in lower rates because it was expensive to compete towards a matched opponent. The extra food they could receive from the increased feeding frequency would have been used into this competition thus not exerting a benefit for increasing their survival.

If differences in size within a clutch are not important in the outcome of sibling rivalry and do not influence chick's behaviour and survival, it could be that mothers do not decide the egg size but it is a consequence of a diminish of endogenous resources necessary for egg formation through the laying sequence (Bernardo, 1996). Kittiwake females could compensate for this asymmetry in egg size by allocating more testosterone into second-laid eggs. If the decrease in egg size according to the laying order is a consequence of sequentially laying eggs (Bernardo, 1996) and can not be controlled by the females it will make sense to compensate the second-laid egg (Stokland & Amundsen, 1988). If the first-laid egg dies, second-laid eggs can work as an insurance even with the disadvantages of hatching in a second position (Anderson, 1990). Both factors, the decrease in size and the increase of androgens could be counteracting for each other (Schwabl et al., 1997; Budden & Beissinger, 2005).

The results from this study are suggesting that the difference in eggs size within a brood *per se* do not influence the broods' behaviour or their fledging success. Natural within-brood asymmetries seem to promote a better efficiency than when no asymmetries are present without a detrimental effect on the parents' fitness. Perhaps parents favour the presence of these inequalities within a brood in order to increase their inclusive fitness, although several experiments are needed to find out which are the more efficient asymmetries within a brood in terms of parental investment.

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Tables

Table 4.1. The influence of age, rank, egg size and egg type on the minutes that chicks spent active was tested using a mixed model with nest and chick as random factors. Significant results are marked with bold characters. Non significant interactions were dropped from the model using a stepwise regression method. All interactions were P>0.27.

	Activity		
Behaviour	*Df*	*F*	*p*
Age	**1,32**	**41.5**	**<0.0001**
Rank	**1,40**	**4.0**	**0.05**
Size	**1,27**	**9.1**	**0.005**
Egg Type	1,18	1.6	0.22
Random Factors	*Z*	*p*	
Nest	2.47	0.01	
Chick	9.18	<0.0001	

Table 4.2. The influence of age, rank, egg size and egg type and all the two way interactions between these factors on begging and feeding frequency was tested using a mixed model with nest and chick as random factors. Significant results are marked with bold characters. Non significant interactions were dropped from the model using a stepwise regression method. All interactions were P > 0.17.

	Begging			***Successful Begging***			***Feeding***		
Behaviour	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Age	1,133	0.14	0.71	1,194	1.18	0.27	1,178	0.08	0.78
Rank	**1,43**	**4.52**	**0.003**	**1,68**	**8.14**	**0.0057**	**1,20**	**5.36**	**0.03**
Size	1,18	0.48	0.49	1,23	0.03	0.86	1,23	1.03	0.32
Egg type	1,15	0.51	0.48	1,21	1.39	0.25	1,23	0.45	0.50
Random Factors	*Z*	*p*		*Z*	*p*		*Z*	*p*	
Nest	0.58	0.56		1.73	0.08		1.4	0.16	
Chick	2.17	0.029		2.32	0.02		2.34	0.019	

Table 4.3. The influence of age, rank, egg size and egg type and all the two way interactions between these factors on aggression frequency and the proportion of this aggression responded with a submissive act was tested using a mixed model with nest and chick as random factors. Significant results or tendencies are marked with bold characters. Non significant interactions were dropped from the model using a stepwise regression method.

	Aggression			***Submission***		
Behaviour	*df*	*F*	*p*	*df*	*F*	*p*
Age	1,67	10.72	0.001	**1,33**	**4.7**	**0.04**
Rank	1,58	7.43	0.008	**1,34**	**18.7**	**0.0001**
Size	1,19	1.74	0.20	1,17	1.9	0.39
Egg Type	1,79	8.46	0.004	1,16	2.1	0.16
Age*Rank	**1,57**	**14.69**	**0.0003**	1,25.5	0.1	0.73
Egg type*Rank	**1,58**	**7.01**	**0.01**	1,26.5	0.08	0.78
Random Factors	*Z*	*p*		*Z*	*p*	
Nest	1.22	0.22		1.43	0.15	
Chick	6.89	0.0001		0.31	0.75	

Figures

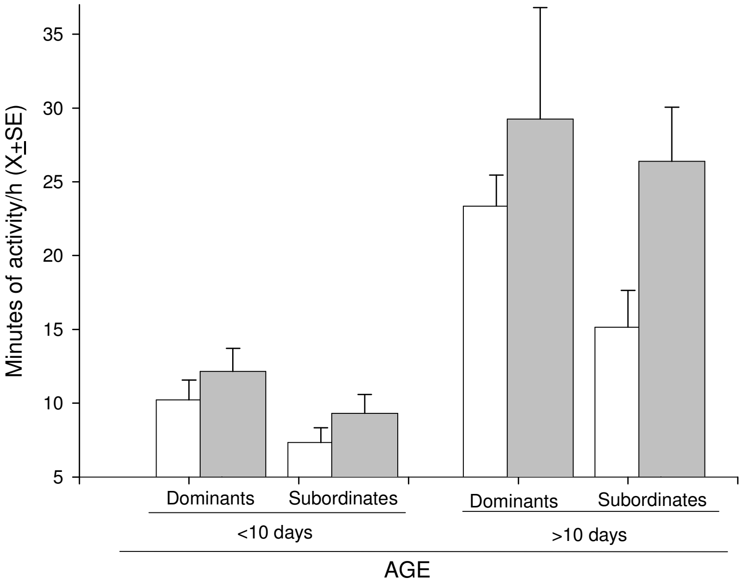

Figure 4.1. Mean (+SE) activity in dominant and subordinate chicks from equal- ○ and different size- ● eggs within a brood in relation to age. Chicks increased its activity with age and dominants were more active than subordinates independently of their experimental group.

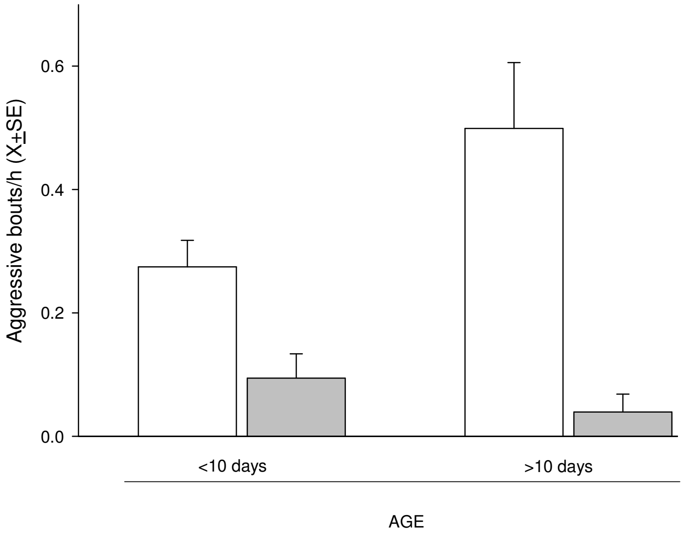

Figure 4.2. Mean (±SE) aggressive bouts in dominant ○ and subordinate ● chicks in relation to age. Dominant chicks increased its aggression with age while subordinates decreased it independently of their experimental group.

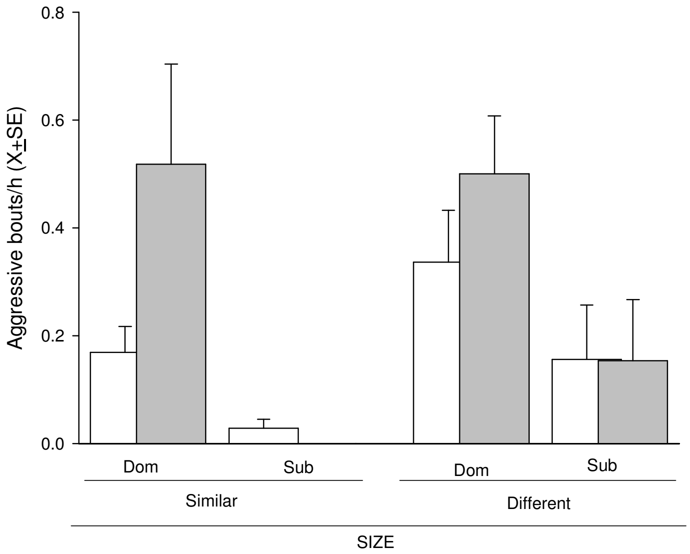

Figure 4.3. Mean (±SE) aggression of dominant chicks that hatched from A- ○ and B-eggs ● in broods that were matched for egg size or the two eggs differed in size. Dominant chicks from B-eggs were more aggressive than dominant chicks from A-eggs and than subordinate chicks. Subordinate chicks from B-eggs in broods where egg sizes were matched never showed aggression. Subordinate chicks from A- and B-eggs showed aggression at similar frequencies.

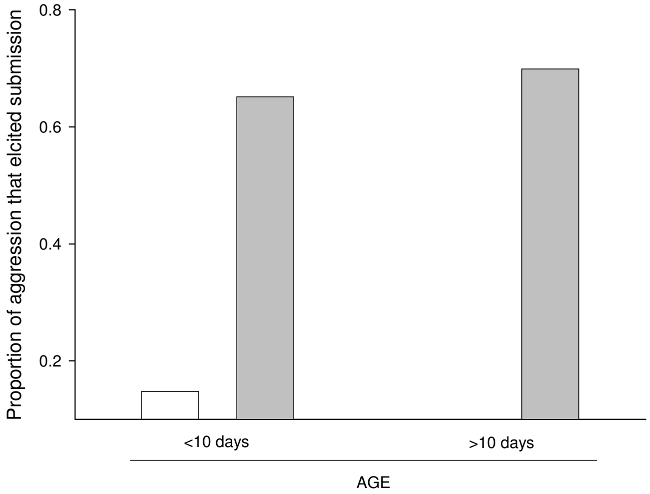

Figure 4.4. Proportion of submissive acts in response to aggression in dominant ○ and subordinate ● chicks in relation to age. Subordinate chicks were more likely to show

submissive behaviour after being attacked than dominant chicks and they increased their submissiveness with age while dominant chicks never showed submission when they were older than 10 days.

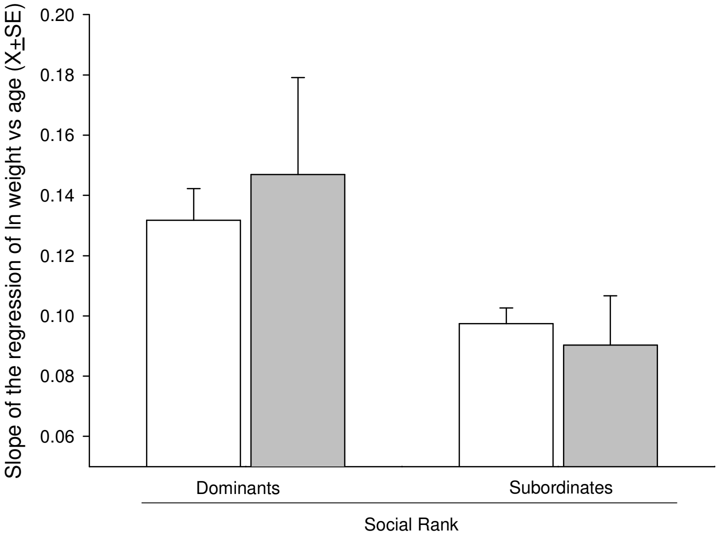

Figure 4.5. Mean (±SE) growth rates of dominant and subordinate chicks in the equal ○ egg size group and the different ● egg size group. Dominant chicks grew faster than subordinate chicks independently of their treatment group.

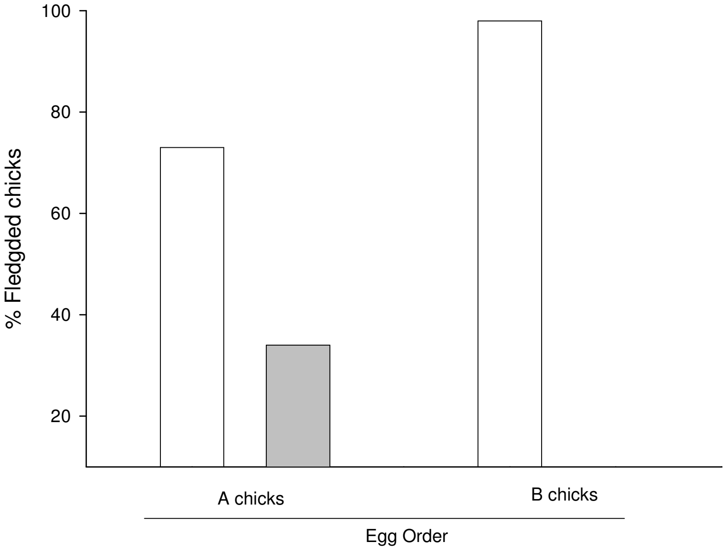

Figure 4.6. Proportion of fledged chicks in relation to their egg type and rank. Dominant chicks ○ fledged at a higher proportion than subordinates ● independently of their treatment group. In broods of B-eggs only dominant chicks fledged compared with broods of A-eggs where almost 40% of subordinates did.

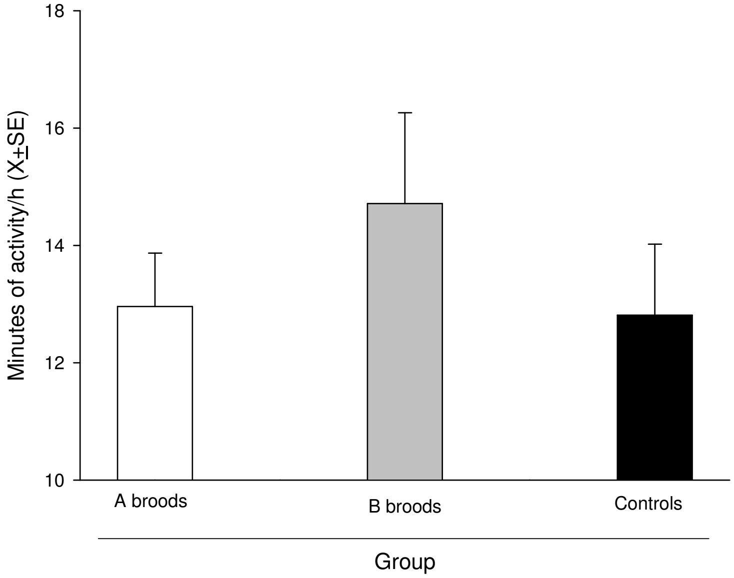

Figure 4.7. Mean (±SE) activity of control, A- and B- broods. Chicks from B-broods were more active than chicks from A-broods and control broods independently of the egg size differences and rank.

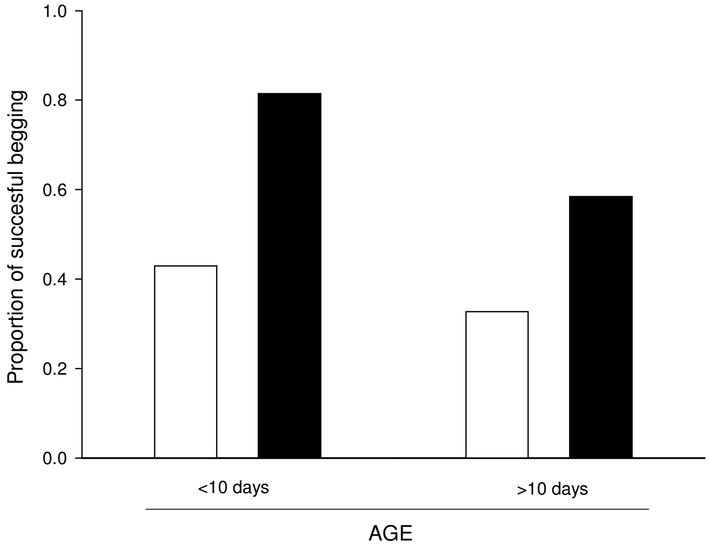

Figure 4.8. Comparison of the proportion of successful begging of experimental ○ and control ● broods in relation to age. Control broods begged more successfully than experimental broods. Successful begging decreased with age in control broods and did not change with age in experimental broods.

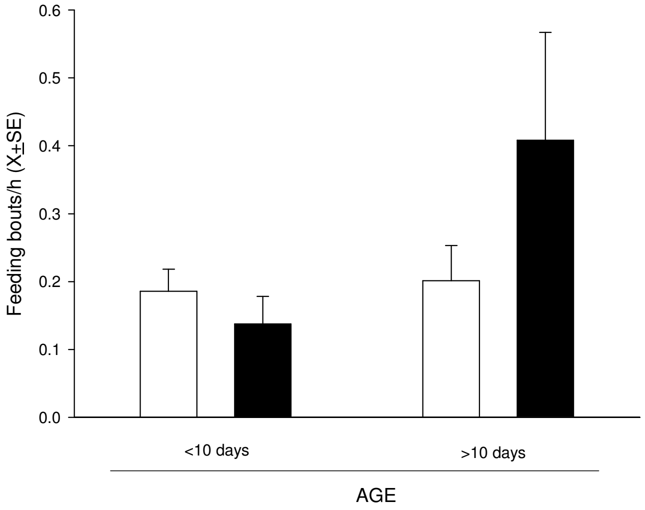

Figure 4.9. Mean (±SE) feeding frequency of experimental ○ and control ●broods in relation to age. Control broods received food more frequently than experimental broods. Feeding frequency increased with age in control broods and did not change with age in experimental broods.

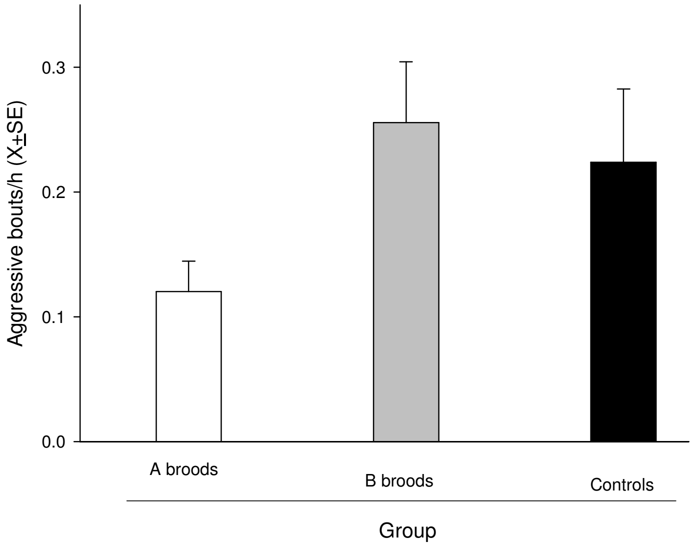

Figure 4.10. Mean (±SE) aggression frequency of control, A- and B- broods. Chicks in A-broods broods were less aggressive than chicks in B-broods and than controls independently of their egg size differences and rank.

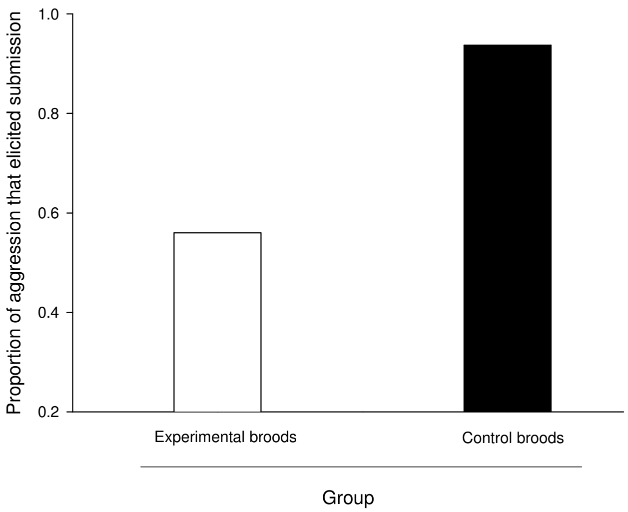

Figure 4.11. Comparison of submissive acts in response to aggression between experimental and control broods. Control broods showed submission more frequently after being attacked than experimental broods.

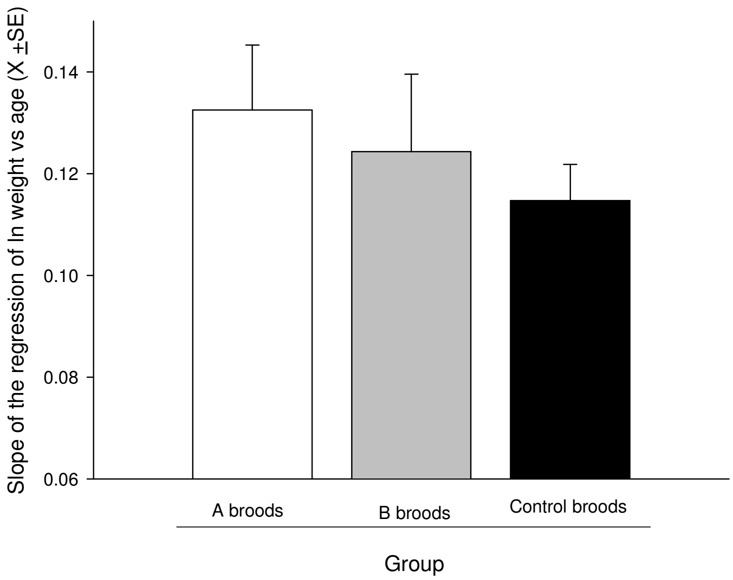

Figure 4.12. Mean (±SE) growth rate of control, A- and B- broods. Growth was similar between experimental and control broods independently of the size difference between the chicks

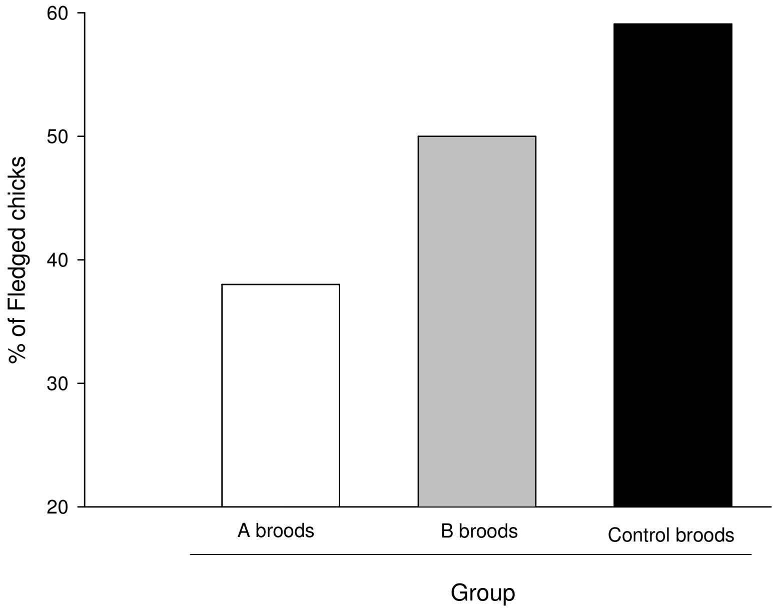

Figure 4.13. Comparison of the proportion of fledged chicks between control, A- and B- broods. The proportion of fledged chicks was higher in control broods than in any other group. B- chicks fledged in higher proportion than A- chicks.

Chapter V

Does hatching in a different position alter sibling competition?

Introduction

Young of several species compete with their siblings for resources. through begging, food hoarding or direct aggression (Archer, 1988; Mock & Parker, 1998b; Mock & Parker, 1998a; Drummond, 2006). In avian species with large clutches, the competition for food can be done through enhanced begging thus chicks will receive more food if the signal is honest (Kacelnik et al., 1995; Price et al., 1996). Chicks can also affect the amount of food they obtain by choosing a favourable place in the nest where it is more likely to be fed by adults (Kacelnik et al., 1995; Kolliker et al., 1998; Drummond, 2006). In species with smaller broods often a dominant-subordinate relationship is formed through aggressive encounters and siblicide might be present (Drummond et al., 1986; Mock et al., 1990; Mock & Forbes, 1992; Drummond, 2004).

The dominance-subordinate relationship is formed due to inequalities between siblings. These are inequalities in age, size and quality. The main factor provoking inequalities between the offspring of a brood is hatching asynchrony. Lack (1947) proposed that hatching asynchrony is a mechanism that parents use to facilitate brood reduction when the food availability is unpredictable and they can not raise the whole brood. Apart from leading to differences in age, hatching asynchrony also causes differences in size because the first-hatched chick starts to grow before the rest of the brood. These within-brood differences are present because parents start to incubate before the clutch is complete, resulting in asynchronous hatching (Lack, 1947; Howe, 1976; Slagsvold, 1986; Forbes, 1993; Mock, 2004). Within clutch asymmetries provoked initially by the parents will adjust the probability of brood reduction and the length of time extra chicks could function as insurance offspring (insurance hypothesis, Dorward, 1962; Anderson, 1990; Hardy, 1992; Evans, 1996; Forbes & Mock, 2000).

The influence of the differences in age and size are the main factors studied in the occurrence of sibling rivalry (Bolton, 1991; Drummond & Osorno, 1992; Williams, 1994; Royle & Hamer, 1998; Mock & Parker, 1998a). Recently it has been shown that not only size and age differ between chicks in a brood but the composition of the eggs they hatch from. Mothers can differentially allocate several egg components (e.g. lipids, water, carotenoids, antibodies, hormones) in relation to the laying order and sex (reviewed in Groothuis & Schwabl, 2002; Groothuis et al., 2005b; Eising et al., 2006). Differences in egg composition can influence offspring phenotype (Williams, 1994; Groothuis et al., 2005b; Eising et al., 2006) and in turn affect the outcome of sibling rivalry (Schwabl, 1993; Williams, 1994; Blount et al., 2002; Groothuis & Schwabl, 2002; Hargitai et al., 2006; Pihlaja et al., 2006).

In a large number of species, including the black-legged kittiwake (*Rissa tridactyla*), androgen deposition increases with laying order (**chapter II**, reviewed in Groothuis et al., 2005b). In one facultative siblicidal species, the cattle egret (*Bubulcus ibis)* androgens were lower in the third-laid egg (Schwabl et al., 1997). High levels of androgens *in ovo* can enhance chick aggressiveness (Groothuis et al., 2005a), promote begging behaviour (Schwabl, 1996; Eising & Groothuis, 2003; Groothuis & Ros, 2005; Quillfeldt et al., 2006) reduce hatching asynchrony (Lipar & Ketterson, 2000) suppress the immune response (Muller et al., 2005; Groothuis et al., 2005a) and increase the metabolic rate (Tobler et al., 2007).

In species where mothers assign more androgens to last-laid eggs females could be providing them with extra tools to deal with an older and more competitive sibling. Carotenoid egg levels can also vary with the laying order (e.g. Royle et al., 2001). Carotenoids enhance the chick's immune system by protecting it against oxidative stress (Royle et al., 2001). Carotenoids decreased with laying order in lesser black backed gulls (*Larus fuscus*) (Royle et al., 2001; Blount et al., 2003) and black-legged kittiwakes (**chapter II**) in the opposite direction of the androgen pattern found in these species. This could indicate that it is beneficial for the last-hatched chick to invest resources in enhancing competitive abilities rather than in immune functions. Energy invested in increasing the chances to survive or even out compete an older sibling could be more useful than utilizing it to mount an immune response if disease is not common (Muller et al., 2005).

Schwabl et al., (1997) proposed the parental favouritism hypothesis and Tarlow et al. (2001) the developmental advantage hypothesis in which they stated that in siblicidal species mothers allocate steroids to favour the first-hatched chick within a brood in order to facilitate siblicide. Schwabl et al., (1997) found in the facultative brood reducer cattle egret that yolk androgen levels decreased with laying order thus mothers were favouring first hatched chicks to commit siblicide. The opposite pattern was found in kittiwakes, another facultative brood reducer species. Kittiwake mothers seem not to be directly favouring first laid eggs but allocated androgens to compensate second laid eggs for hatching asynchronously (**chapter II**).

It has been proposed that mothers allocate different amount of androgens to male and female embryos (Petrie et al., 2001) but this findings should be taken carefully because androgen levels could change with age and do not reflect maternal assignment levels (Eising et al., 2001; Royle et al., 2001; Verboven et al., 2003; Pilz et al., 2005). The rate of androgen utilization could differ between male and female embryos and the maternal social position will affect androgen deposition on the eggs (reviewed in Muller et al., 2002). It is unknown if differential maternal deposition of androgens according to the embryo's sex could influence males and females differently in terms of aggressiveness or competitive abilities independently of the laying order. In some species with sexual dimorphism, the bigger sex is more aggressive when hatched on a first position. For example, in the laughing kookaburra (*Dacelo novaeguineae*) where females are larger than males, broods with females hatched on the first position aggression was higher than in the rest of the broods (Nathan, 2001), but as far as I know hormonal levels of their eggs have not been studied.

To my knowledge, no one has experimentally manipulated the egg order within a brood to study the importance of within-clutch differences in egg composition on the conflict between siblings. In order to test it an experimental manipulation of black-legged kittiwake broods was made in the present study. Kittiwake females lay a modal clutch of two eggs and show asynchronous hatching (Cullen, 1957). Mothers differentially allocate androgens and carotenoids to eggs in relation with laying order in opposite directions (increased androgens and decreased carotenoids' concentrations over the laying sequence (**chapter II**)). Kittiwake siblings establish a dominance-subordinate relationship in which the first-hatched chick is the dominant and can perform siblicide if food availability or food quality is low (Braun & Hunt, 1984).

Two experimental groups were created by manipulating the brood composition by swapping eggs between nests. The second-laid egg (B-egg) of one clutch was swapped with the first-laid egg of another clutch that was laid on the same day. This created one treatment group where the first-laid egg (A-egg) hatched in the second position and competed against an older chick that also hatched from a first-laid egg (Aa-broods). In the second treatment group created by egg swapping as well, a B- egg hatched first competed with a younger chick hatched from a B- egg (Bb-broods). Natural differences in age and size within the brood were maintained. Behaviour and survival of the two experimental groups were compared with control broods where egg and hatching order were not manipulated.

I predict that chicks hatched in the same order as they were laid (Ab broods) show less competition in terms of begging and aggression than chicks from experimental broods and thus Ab broods will be more likely to fledge. I also predict that second hatched chicks from A- eggs will show lower levels of aggression than chicks from B- eggs hatched on a second position. Kittiwakes are sexually dimorphic with males being larger than females (Helfenstein et al., 2004). Because of that I predict that broods with a second-hatched male will show an increased aggression compared with broods with a second-hatched female.

If mothers favour each of their eggs to be more successful when hatching in the order they were laid, survival should be higher in control than in experimental broods. With this work I expect to distinguish the maternal influence on sibling rivalry through differential allocation of egg components within her clutch.

Methods

Black-legged kittiwakes breeding on the Isle of May, Firth of Forth, Scotland (fig.1.1), were used for this study from May to August of 2005. Kittiwakes in this population lay on average two eggs that hatch with ca 1.5 days of difference and the A- egg is 4% larger than the B-egg. The laying date of each egg from 150 broods was recorded by checking the nests daily. On the day they were laid, each egg was marked and once the clutch was completed egg length (l) and breadth (b) were measured to the closest 0.1mm with callipers to calculate volume with the formula $V=0.4866(b^{2)}l$ (Coulson, 1963). Six sub-areas of the island where nests were accessible were used (fig. 1.1).

Hatching date, sex, growth and survival.

From the expected hatching date onwards (day the last egg of a clutch was laid + 25 days) daily checks of the nests were made to know the exact day chicks hatched. On the day chicks hatched they were marked on head, neck and rump with different coloured non-toxic sheep dye to identify them from the distance. Chicks were called senior and junior according to their hatching order. Each chick was weighed with a spring balance to the nearest gram and a blood sample of less than 10μl was taken (under a UK Home Office license) from the medial metatarsal vein to determine the sex using molecular techniques (Griffiths et al., 1996). These procedures were made whenever possible on the hatching day but it was not always achievable due to weather conditions (rain and gales) and they were carried out within 24 hours after hatching.

Chicks were weighed every 4 to 5 days thereafter until the senior chick was 20 days old. After this age kittiwake chicks become very sensitive to human disturbance and can jump off their nests in response to disturbance. The instantaneous growth rate was calculated as the slope of the regression of log-transformed weight on age during kittiwake linear growing phase which ranges from 7 to 16 days old (Lance & Roby, 1998). Only chicks that reached the age of 9 days were included on the growth analyses. Nests were monitored daily until chicks were 40 days old, age at which kittiwakes fledge (Cullen, 1957). Fledging was then confirmed by checking the nest and its surroundings to try to find the fledged chicks because it is common for kittiwakes to return to its nest to be fed by their parents for sometime after leaving the nest (Cullen, 1957).

The date when a chick died or disappeared was recorded although the cause of death could not always be identified (direct sibling aggression, predation or starvation). In a few cases (n=3 out of 67; two from control and one from the Bb group) chicks left their natal nest as consequence of sibling aggression and established themselves in a new brood by eliminating the original chicks living in that nest. For the purpose of the analyses they were included as dead because they lost the sibling competition in their natal nest. When analyses were carried out including the chicks that presumably died by predation it did not make a statistical difference compared with the analysis that were not including them ($p>0.1$).

Experimental design

Two experimental groups were created: the first group was made up from two A- eggs conserving the natural laying asynchrony and egg size differences (Aa-group). The second group was formed by two B- eggs again with the natural laying asynchrony and egg size differences (Bb-group). Two control groups to compare with the experimental broods were created (Ab-group). The first control group was created by cross-fostering entire clutches between nests to control for any parental effects. The second control group was made from two unrelated eggs (an A- and a B- egg) with the natural differences in laying interval and egg size. This second group was created to control for any differences that could arise for having two unrelated chicks sharing the same nest in comparison with a brood composed by two siblings hatched from eggs laid by the same female (the possibility of extra pair copulations can not be discarded although they are very uncommon in kittiwakes, Helfenstein et al., 2004b). The behaviour and survival was not different between the two types of control broods ($p>0.1$) and therefore were pooled and used as one control group.

Differences in egg volumes between experimental (Aa and Bb) and control broods (Ab) were tested with a repeated measures ANOVA. The egg to hatch first was larger than the egg to hatch second, but there was no difference in egg size between the treatment groups (repeated ANOVA: treatment: $F_{2,106}= 1.79$, $p=0.13$; hatching order: $F_{1,107}= 26.44$, $p=0.001$; treatment*hatching order: $F_{2,105}= 0.065$, $p=0.94$; n=98 clutches). The mean hatching span was not different between experimental and control groups (Kruskall-Wallis test: $X^2 =0.8$; df=2; $p=0.67$). The mean laying date of A- eggs was not different between experimental and control broods (Kruskall-Wallis test: $X^2 =2.8$; df=2; $p=0.24$).

Behavioural recording.

Behavioural observations of each brood were carried out every other day during 3 hours shifts distributed as follows: from 6am to 9am; from 10:30 to 13:30 and from 15:30 to 18:30 by two different observers. Observers did not know the treatment of the brood they were observing. On each shift a maximum of five nests close to each other were observed at the same time. Each brood was observed on average 12 hours (ranging from 3 to 18 h). Due to the synchrony of kittiwakes hatching within the colony, and the lack of field assistants, sub-samples from all the experimental and control groups were observed. Twenty two control broods (Ab-group), 22 Aa broods and 23 Bb broods were observed. Behavioural recording of the broods started when hatching was complete and the senior chick was on average 1.5 ± 0.7 days old (n=67). Watches were done at a distance of 3-7 meters by using a combination of behaviour sampling and scan sampling (Martin & Bateson, 1994). The absolute frequency of each chick's begging, feeding and aggression and whether the recipient of each aggressive act responded by adopting or sustaining a submissive posture was recorded (see **chapter III** for a detailed description of each behaviour and how frequencies were calculated). Additionally, every minute and a half a scan sampling was done to record whether each chick was inactive (head resting on any substrate or invisible under the parent) or visible and clearly awake (Drummond et al., 2003) to measure how many minutes per hour each chick was active. Broods were observed until junior chicks were 20 days of age or until one of the chicks died.

Statistical Analysis.

Because the two chicks from the same nest can not be considered independent and each chick was observed repeatedly, Generalized Linear Mixed Models (GLIMMIX) with the appropriate error distribution for each behaviour were used. For activity and growth normal distribution; for begging, feeding and aggression poisson; and for successful begging, submission and survival binomial error distribution. SAS statistical package version 9.0 (SAS Institute, inc.) was used. Chick and nest were used as random factors. This package uses the Satterthwaite method to derive degrees of freedom (Gaylor & Hopper, 1969).

With separate univariate analyses it was first tested whether observer, sub-colony, time of the day, hatching interval and hatching date had an effect on the chicks' behaviour. No significant effect was present ($p>0.15$) in any of the studied behaviours except on begging and feeding when time of the day was tested. Begging and feeding were more frequent during the afternoon but it was a biased result because only when chicks were older observations at this time of the day were done. An interaction between age and time was present and graphs showed that this was because when chicks were younger almost no observations were made at this time of the day. Therefore, I removed time from the model and did not include it in the feeding or begging results. Multivariate analyses were used on the following variables: experimental treatment, hatching order (senior or junior), age, sex and all the possible two way interactions. Interactions were tested and removed from the model when non significant using a stepwise approach. Since I do not have a 4x4 experimental design, the analyses were split in two: 1) comparisons between Aa-broods and control broods and 2) comparisons between Bb-broods and control broods. When a significant interaction was present in the model post hoc tests were done and reported alongside the mentioned result. Statistically significant differences were considered when $p<0.05$ and a tendency was considered when the p value ranged from 0.051 to 0.089. Non-significant differences were considered when $p>0.089$. When significant differences were present I reported mean ± standard error of the groups compared.

Rather than comparing juniors vs juniors and seniors vs seniors from different experimental groups, analyses using the brood as a whole were made. The behaviour of one of the chicks depends on the behaviour of the other and on the parents' behaviour as well. Therefore it would not be biologically significant to test the behaviour of only one individual from the brood as the chicks are not isolated units; a brood is a whole and can not be arbitrarily divided.

Results

Independently of the experimental group, the results of the behavioral recordings showed that chicks in all broods established a dominance-subordinate relationship in which seniors and juniors became dominants and subordinates respectively. Senior chicks were more aggressive than juniors ($p<0.0001$) and juniors were submissive more often after being attacked than seniors ($p<0.001$). Moreover, seniors tended to receive food more frequently than juniors ($p<0.072$), which is another indicator of a dominance status within a hierarchy (full models and results reported below).

In order to test for differences on the sex ratio of the experimental groups a chi square test was made using a table with the 4 brood sex ratios (MM, FF, MF, FM) along one axis and the three treatment groups along the other axis (n=67, df=6). Sex ratios did not differ between control and any of the experimental groups at hatching ($X^2=9.17$, df=6, p=0.2).

Comparisons between Ab and Aa broods

Behaviour

Activity increased with age in both groups and was higher in senior chicks (seniors: X=24.1±1; juniors: 19.6±1 minutes per hour; table 5.1, fig. 5.1). Feeding frequency was affected by a marginally significant age*treatment interaction (table 5.2) but post hoc analyses did not find a change in feeding with age in any of the two groups (Aa-broods: $F_{1,37}=0.71$, p=0.40; control-broods: $F_{1,31}=0.001$, p=0.96; fig.5.2). Begging frequency increased with age independently of the experimental group and the hatching order (less than 10 days: X=0.89±0.06 begging bouts/h; more than 10 days: X=1.47±0.1 begging bouts/h; table 5.2, fig.5.3). Junior chicks tended to receive food less frequently irrespective of their experimental treatment (juniors: X=0.45±0.04 feeding bouts/h; seniors: X=0.38±0.03 feeding bouts/h; table 5.2, fig.5.2) possibly because junior chicks begged less successfully than senior chicks (proportion of begging bouts followed by feeding in seniors: X=0.44; proportion of begging bouts followed by feeding in juniors: X=0.35; table 5.2, fig.5.4). Aggressiveness did not differ between control and experimental broods nor between males and females (table 5.3, fig.5.5). Senior chicks were more aggressive than juniors (seniors: X=0.28±0.04 aggressive bouts/h; juniors: X=0.06±0.02 aggressive bouts/h) and both decreased their aggressiveness with age (less than 10 days: X=0.22±0.04 aggressive bouts/h; more than 10 days: X=0.22±0.04 aggressive bouts/h; table 5.3, fig.5.5,). A sex*hatching order interaction was present when submissiveness was analysed. Senior females in Aa-broods never had the chance of being submissive because they were never attacked. In control broods, senior males showed a tendency of showing a submissive posture more often after being attacked (X=0.5±0.05) than senior females (X=0.15±0.03; $F_{1,6}$=3.83, p=0.098) and no differences were found between junior males and females independently of their treatment group ($F_{1,10}$=0.04, p=0.84; fig.5.6).

Growth and survival

No differences in growth rates (determined by the slope of the growth curve) were present between control and experimental broods but senior chicks grew better than junior chicks independently of treatment or sex (table 5.4, fig.5.7). Senior chicks gained 3% more weight daily than junior chicks. The probability of survival was not different between treatments, hatching order or sex (table 5.4, fig.5.8).

Comparisons between Ab and Bb broods

Behaviour

Activity of seniors and juniors increased with age and senior chicks were more active than juniors at any age (seniors: X=23.15±0.97 minutes/h; juniors: X=18.7±0.95 minutes/h; table 5.1, fig.5.1). An age*experimental treatment interaction was present when begging frequency was analyzed (table 5.2, fig.5.3). Post hoc analyses showed that when chicks were less than 10 days experimental broods begged less (X=0.4±0.02 begging bouts/h) than control broods (X=0.7±0.02 begging bouts/h) but after 10 days their begging rate was similar (younger than 10 days: $F_{1,23}$ =6.09, p=0.015; more than 10 days: $F_{1,41}$ =0.3, p=0.58; experimental broods: X=1.62±0.17 begging bouts/h; control broods: X=1.42±0.15 begging bouts/h; table 5.2, fig.5.3). Experimental broods begged less successfully (X=0.28 begging bouts responded with feeding) than control broods at any age (X=0.58 begging bouts responded with feeding; table 5.2, fig.5.4). As a consequence of this, chicks in experimental broods got food less often (X=0.30±0.04 feeding bouts/h) than in control-broods (X=0.44±0.03 feeding bouts/h; table 5.2, fig.5.2).

There was an interaction between treatment group and hatching order on frequency of aggression (table 5.3). Experimental senior chicks were more aggressive than control senior chicks (treatment group seniors: $F_{1,39}$ =12.86, p=0.001; experimental seniors: X=0.54±0.12 aggressive bouts/h; control seniors: X=0.32±0.06 aggressive bouts/h; fig.5.5) and junior chicks from both treatments did not differ in their aggression ($F_{1,43}$ =0.39, p=0.53; table 5.3, fig.5.4). Males and females did not differ on their aggression frequency (table 5.3). Moreover, experimental senior chicks tended to be more submissive than control seniors (proportion of aggressive acts received responded with submission in control senior chicks: X=0.79; proportion of aggressive acts received responded with submission in experimental senior chicks: X=0.92; table 5.3). In the experimental group, senior males were more submissive than senior females (proportion of aggressive acts received responded with submission in experimental senior males: X=0.66; proportion of aggressive acts received responded with submission in experimental senior females: X=0.33) and junior females were more submissive than junior males (proportion of aggressive acts received responded with submission in experimental junior males: X=0.83; proportion of aggressive acts received responded with submission in experimental junior females: X=0.97; table 5.3, fig.5.7).

Growth and survival

Growth rate did not differ between control and experimental broods but senior chicks tended to grow faster than junior chicks (table 5.4, fig.5.7). The survival rate was not different between experimental treatments or between senior and junior chicks and was not influenced by their sex (table 5.4, fig.5.8).

Discussion

The first prediction of control broods showing less aggression than experimental ones, was only corroborated when compared to Bb broods. Aa broods did not differ in aggression from the controls. However, as predicted, B junior chicks were more aggressive than A juniors. Higher aggression rates were predicted in junior male chicks than in junior females, but no differences in aggression between males or females were found, with within broods or between groups. However, submission was more frequent in senior males than in senior females in Aa broods, while junior females were more submissive than junior males in Bb broods and no differences occurred in controls.

When the brood composition of a facultative brood reducing species was modified by altering the position in which one of the eggs hatched, several behaviours of the chicks and parents were altered. Because egg composition varies systematically within clutches and affects offspring performance (e.g. Groothuis et al. 2005b) one of the brood members was of different quality compared to the chick of the same hatching order in control broods as a result of the experimental manipulation. Regardless of the treatment group, senior chicks always became dominant. They received food more frequently and were more aggressive and less submissive than juniors independently of the brood composition.

The dominance-subordinate relationships formed in different ways in the different treatment groups. Control and Aa-broods formed a similar dominance-subordinate relationship (no interactions between hierarchy and treatment present) and this relationship showed a similar development (no interactions between experimental treatment and age found in any of the studied behaviours). Contrary to this, Bb-broods showed a different dominance-subordinate relationship compared to controls. Senior chicks that hatched from B- eggs were more aggressive than control senior chicks (hatched from A-eggs). Furthermore, chicks in Bb-broods showed a differential development of the begging behaviour because they begged more than chicks in control broods as they got older. These differences in the dominance-subordinate relationship and in the behavioural development shows that indeed the egg quality from which each chick hatched influences chicks behaviour and sibling rivalry.

In the first place, older chicks in Bb-broods begged more than chicks in Aa- and control broods but there was no clear differences in begging frequency between control and Aa- broods. However, parents of Bb-broods seemed to respond to the increased begging with a decreased feeding rate. As chicks in Bb-broods had the lowest begging success, parents may have responded less efficiently to their begging signal if begging is considered a honest signal of need (reviewed in Kilner, 1997) although it is uncertain how much food these broods got. Since experimental Bb-broods did not grow slower than controls it is unlikely that they were receiving less food, but differences in metabolic rate and other physiological traits between experimental and control broods could not be discarded. Senior chicks grew faster than junior chicks in control- and Aa-broods and a tendency in the same direction was present in Bb-broods. This growth pattern could indicate two things: 1) that the amount of food provided to each group was not different, but the way it was divided between the members of the brood was or 2) that energy was allocated differently between growth and other behaviours such as aggression in the experimental Bb-broods.

Tarlow et al. (2001) found out in the Nazca booby (*Sula granti*), an obligate siblicidal species, that dominant chicks in broods of two gained mass faster than singletons and this extra mass facilitated siblicide. They concluded that differences in mass between the siblings influenced the outcome of sibling competition rather than the absolute difference in size (Tarlow et al., 2001). This was not observed in kittiwakes: senior chicks from second-laid eggs begged more but parents did not respond to their begging and they even got food less frequently than control senior chicks. It could be that mothers design chicks to begg in a way that optimizes parents feeding method, amount and frequency (Hinde & Kilner, 2007). Perhaps in obligate siblicidal chicks parents favour the A-chick in order to win the competition while in facultative siblicidal species the amount of food determines if only one chick survives.

The food amount hypothesis states that the aggression of a chick towards its sibling is inverse to the amount of food the chick gets and that aggression is mediated by hunger (Mock et al., 1987). Sibling aggression in kittiwakes is thought to be mediated by the amount of food parents provide to their siblings (Braun & Hunt, 1984; Irons, 1992). In a poor year (2004) on the Isle of May 100% of B- chicks were victims of sibling competition before they reached 15 days of age (**chapter III**). Higher levels of aggression in Bb-broods are consistent with them receiving food less frequently and perhaps in lower amounts.

Possibly the diminished quantity of food did not translate into reduced growth because physiological differences between chicks from A- and B- eggs make the latter to grow, survive and fledge more efficiently on sub-optimal provisioning conditions. When second hatched Nazca booby chicks had the opportunity to fight towards their sibling, a downregulation of steroids took place and they were able to do so (Tarlow et al., 2001). Perhaps some kind of hormone downregulation takes place in B-chicks in order to efficiently cope with inclement situations.

The increased aggression of Bb-broods could have been favoured by similar growth rates of both chicks within the brood (independently of egg size differences). Similarities in size could provoke increased aggression because seniors are at a higher risk of loosing their dominant position by a sibling which is of similar size and quality (hatched from a similar egg type). Aggression of non-experienced blue-footed booby (*Sula nebouxii*) chicks depended on its relative size (Drummond & Osorno, 1992). In the present study junior chicks from Bb-broods did not try to reverse the hierarchy despite having a similar size as their sibling. When the nest is shared with an aggressive sibling it could be expensive to show aggression and chicks may be less likely to try to do so even when there are no differences in size and competition (Drummond et al., 2003; Matsumura & Hayden, 2006). It seems from the marginal significance found in submissiveness that junior chicks in Bb-broods showed submission at a higher proportion than control junior chicks, possibly indicating that they needed to be more submissive to stop the aggression from their senior nest mate. The higher aggression frequency observed in B-senior chicks would not necessarily translate into higher submission rates by their sibling since the proportion of aggression responded with submission was used in the analyses and not only the total submission.

The influence of differences in egg hormone content between A- and B- eggs on chick's aggression can not be discarded (Groothuis et al., 2006; Eising et al., 2006). In **chapter II** it was shown that B- eggs of kittiwakes had higher levels of testosterone than A- eggs. The maternal hormone *per se* is unlikely to persist until 10 days after hatching, but its organisational effect could be reflected in chick's behaviour and physiology (Schwabl, 1996; Saino et al., 2001). For example, yolk hormones could influence the amount of hormone produced by the chick; alter the number of hormone receptors in the individual or influence brain differentiation that in turn will affect not only behaviour but the function of the endocrine system (Schwabl et al., 1997; Hayward & Wingfield, 2004; Groothuis et al., 2006; Carere & Balthazart, 2007).

Although some behavioural differences between control and experimental broods were found (mainly in Bb-broods) they did not translate into differences in growth and survival between treatment groups. Moreover, high aggression rates of senior B-chicks did not secure them more food. High fledging success show that 2005 was a good breeding season for kittiwakes (Harris, 2004; Harris, 2005). Because of these factors, the egg swapping and the experimentally manipulation of the chicks' hatching position could have not exerted a detrimental effect on the growth and survival rate of experimental broods compared with controls. If conditions are good, brooding and feeding a manipulated brood may not have been detrimental for the parents or the chicks as it is expected to be when breeding conditions are poor (e.g. low food availability Fujioka, 1985).

The results from this work are novel because there are no works in which the brood composition of one species is manipulated and the behaviour of the chicks recorded and related to their growth and survival. It seems that parents may produce broods that will show specific behaviours in such a way to optimize the resources they invest in the current reproductive attempt by allocating different amounts of nutrients and hormones to the eggs (Groothuis et al., 2005a). When the expected behaviour of the brood is not present parents may change their own behaviour to compensate for the differences in order to not increase their effort (Hinde & Kilner, 2007).

If chicks within a brood would not differ in their abilities to survive and cope with specific social circumstances and instead were equal, their survival probability could decrease and at the same time the cost for the parents to raise chicks increase. This may have been the case in 2004, a year with low food amount and poor energy content of the prey and low fledging success (Wanless et al., 2005). In that year where experimental broods had much reduced within-brood competitive asymmetries they also had a lower fledging success and poorer growth than control broods (**chapter IV**). This suggests that specific differential maternal allocation of resources to eggs could benefit the brood depending on the amount and quality of food available during chick rearing.

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Tables

Table 5.1. The influence of age, hatching order, sex and experimental treatment and all the two way interactions on the minutes that chicks spent active was tested using a mixed model with nest and chick as random factors. Results of the model comparing control- and Aa-broods are shown on the upper line. On the lower one results comparing control- and Bb-broods are displayed. Non significant interactions were dropped from the model using the stepwise regression method. Significant results or tendencies are marked with bold characters.

	Activity		
Factors	*Df*	*F*	*p*
Age	**1,398**	**212.6**	**<0.001**
	1,394	**45.6**	**<0.0001**
Hatching order	**1,46**	**5.1**	**0.028**
	1,38	**7.07**	**0.011**
Sex	1,82.7	0.05	0.81
	1,51.7	0.01	0.93
Experimental treatment	1,41.3	0.01	0.91
	1,43	0.06	0.80
Random Factors	***Z***	***p***	
Nest	2.34	0.019	
	1.72	0.085	
Chick	0.49	0.62	
	0.27	0.79	

Table 5.2. The influence of age, hatching order, sex and experimental treatment on begging, successful begging and feeding frequencies and all the two way interactions was tested using a mixed model with nest and chick as random factors. Results of the model comparing control and Aa-broods are shown on the upper line. On the lower one results comparing control- and Bb- broods are displayed. Non significant interactions were dropped from the model using the stepwise regression method. Significant results or tendencies are marked with bold characters.

	Begging			***Successful Begging***			***Feeding***		
Factors	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Age	**1,358**	**31.77**	**<0.0001**	1,314	2.56	0.11	**1,398**	**4.85**	**0.03**
	1,393	**48.69**	**<0.001**	1,303	0.31	0.57	1,384	1.30	0.25
Hatching order	1,37	0.57	0. 45	**1,23**	**7.84**	**0.01**	**1,37**	**3.41**	**0.07**
	1,29	0.03	0.87	1,35	0.04	0.83	**1,19**	**3.98**	**0.07**
Sex	1,69	<0.01	0.85	1,47	0.68	0.41	1,96	0.08	0.78
	1,55	0.76	0.38	1,46	<0.01	0.78	1,26	0.13	0.72
Experimental treatment	1,33	0.68	0.41	1,29	1.65	0.20	1,33	1.12	0.29
	1,23	**3.96**	**0.05**	**1,58**	**15.45**	**0.002**	**1,36**	**7.46**	**0.009**
Age*experimental treatment	1,366	0.45	0.50	1,311	0.47	0.49	**1,402**	**3.47**	**0.06**
	1,394	**6.67**	**0.01**	1,312	0.33	0.56	1,383	0.35	0.55
Random Factors	**Z**	***p***		**Z**	***p***		**Z**	**P**	
Nest	1.17	0.24		1.98	0.04		2.59	0.42	
	1.69	0.09		0.42	0.67		2.28	0.022	
Chick	1.02	0.30		2.17	0.03		5.74	<0.0001	
	0.44	0.65		7.52	<0.0001		5.56	<0.0001	

Table 5.3. The influence of age, hatching order, sex and experimental treatment and all the two way interactions on aggression and submission frequencies was tested using a mixed model with nest and chick as random factors. Results of the model comparing control- and Aa-broods are shown on the upper line. On the lower one results comparing control- and Bb-broods are displayed. Non significant interactions were dropped from the model using the stepwise regression method. Significant results or tendencies are marked with bold characters.

	Aggression			***Submission***		
Factors	*df*	*F*	*p*	*Df*	*F*	*P*
Age	**1,369**	**4.84**	**0.02**	1,26	0.32	0.57
	1,388	0.81	0.36	1,14	1.14	0.30
Hatching order	**1,51**	**22.19**	**<0.0001**	**1,14**	**23.48**	**0.0002**
	1,25	2.68	0.11	**1,59**	**18.43**	**0.0001**
Sex	1,72	0.93	0.33	**1,15**	**9.18**	**0.008**
	1,73	0.80	0.37	**1,60**	**12.00**	**0.001**
Experimental treatment	1,34	2.02	0.16	1,4	0.86	0.40
	1,59	2.16	0.14	**1,17**	**3.20**	**0.09**
Experimental treatment*hatching order	1,44	0.60	0.44	1,45	0.001	1.00
	1,26	**38.50**	**<0.0001**	1,7	0.20	0.67
Sex*hatching order	1,71	0.12	0.72	**1,20**	**9.61**	**0.007**
	1,49	0.32	0.57	**1,61**	**14.54**	**0.0003**
Random Factors	***Z***	***P***		***Z***	**P**	
Nest	0.62	0.53		0.35	0.72	
Chick	1.21	0.22		0.01	0.99	

Table 5.4. The influence of hatching order, experimental treatment and sex and all the two way interactions on growth and survival was tested using a mixed model with nest and chick as random factors. Results of the model comparing control- and Aa-broods are shown on the upper line. On the lower one results comparing control- and Bb-broods are displayed. Non significant interactions were dropped from the model using the stepwise regression method. Significant results or tendencies are marked with bold characters.

	Growth			***Survival***		
Factor	*df*	*F*	*p*	*df*	*F*	*p*
Hatching order	**1,31**	**8.75**	**0.0059**	1,71	0.04	0.83
	1,45	**3.64**	**0.06**	1,39	0.03	0.85
Experimental	1,20	2.96	0.101	1,52	0.01	0.97
treatment	1,38	1.8	0.18	1,39	0.56	0.46
Sex	1,42	0.46	0.62	1,71	0.01	0.99
	1,70	0.41	0.52	1,65	0.05	0.83
Random Factors	***Z***	***p***		***Z***	***p***	
Nest	0.05	0.95		0.6	0.07	
	2.1	0.03		0.8	0.42	
Chick	1.52	0.12		0.24	0.81	
	5.59	0.01		1.22	0.22	

Figures

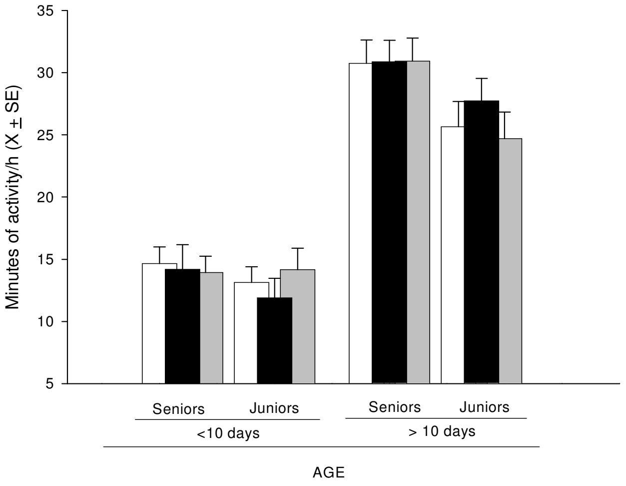

Figure 5.1. Mean (±SE) activity (minutes per hour of observation) in senior and junior chicks from control ○ , A- ● and B- ◍ broods at different ages. Activity increased with age in all three groups. Senior chicks were always more active than junior chicks and activity did not differ between the treatment groups.

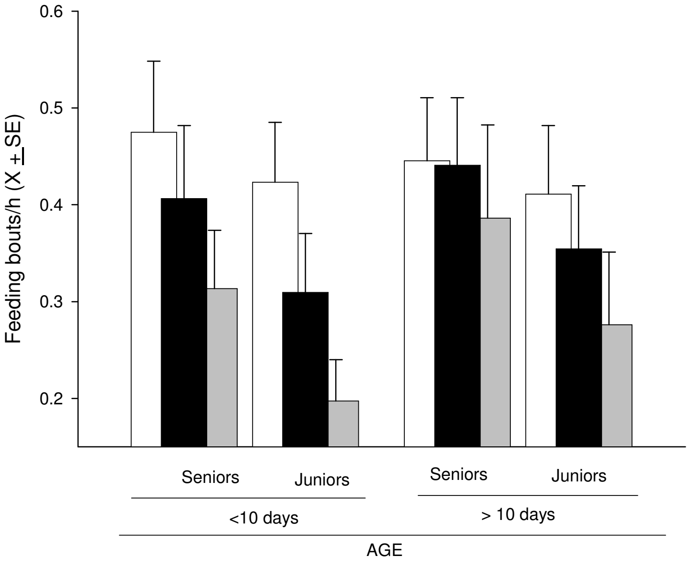

Figure 5.2. Mean (±SE) feeding frequency of senior and junior chicks from the three treatment groups at different ages. Senior chicks showed a tendency of eating more frequently than junior chicks in all the groups. Bb-broods ◍ ate less frequently than control broods ○. Feeding frequency increased in Aa-broods ● with age, decreased in controls and did not differ in Bb-broods.

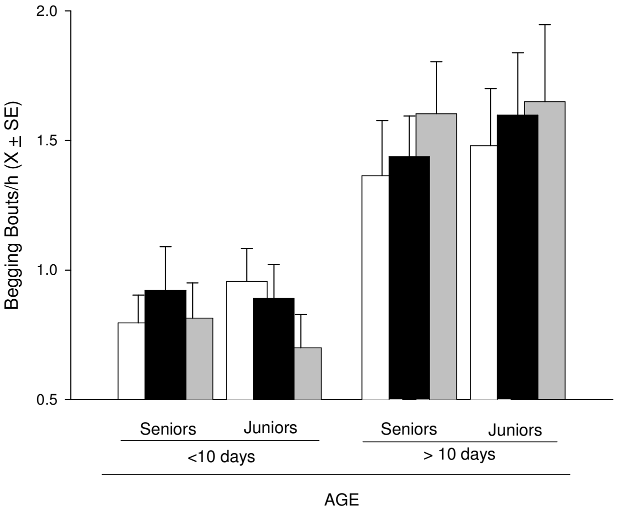

Figure 5.3. Mean (±SE) begging frequency of senior and junior chicks from the three treatment groups at different ages. Begging bouts were higher in Bb-broods ◍ than in control ○ and Aa-broods ● when they were older. Begging frequency increased with age in junior and senior chicks in all three treatment groups.

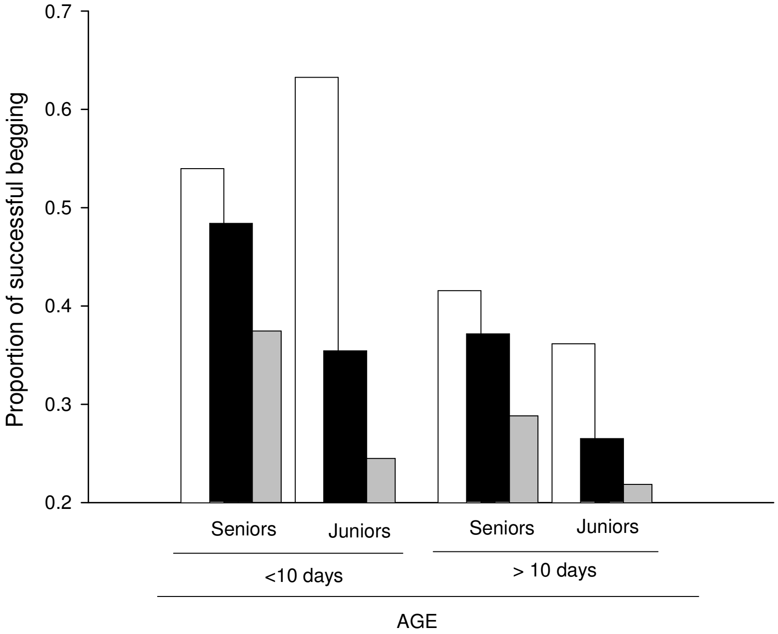

Figure 5.4. Proportion of successful begging bouts of senior and junior chicks from the three treatment groups at different ages. Senior chicks in Aa-broods ● begged more successfully than in control-broods ○. Control-broods begged more successfully than Bb-broods ◍ but their begging success did not differ from Aa-broods. Begging success did not change with age in any of the treatment groups.

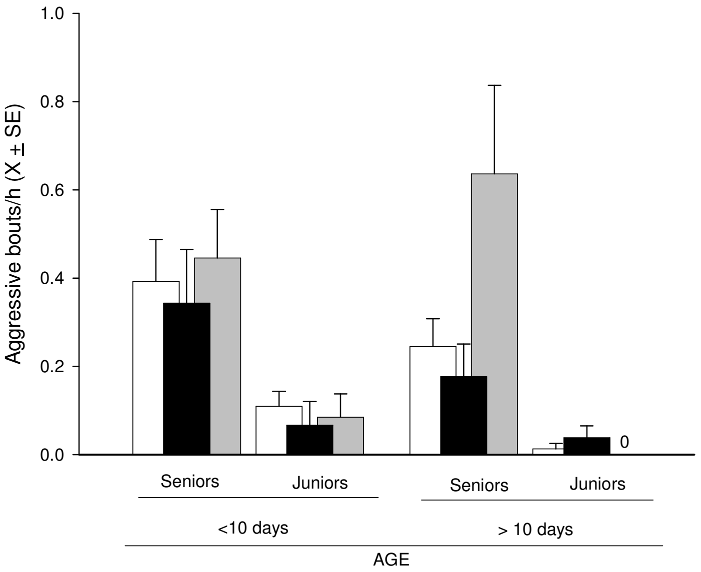

Figure 5.5. Mean (± SE) aggression frequency (bouts per hour) in senior and junior chicks from the three treatment groups at different ages. Senior chicks were more aggressive than junior chicks in the three treatment groups at all ages. Senior chicks decreased their aggression with age but junior chicks did not. Seniors from Bb-broods ◍ were more aggressive than control- ○ and Aa-broods ● regardless of their age.

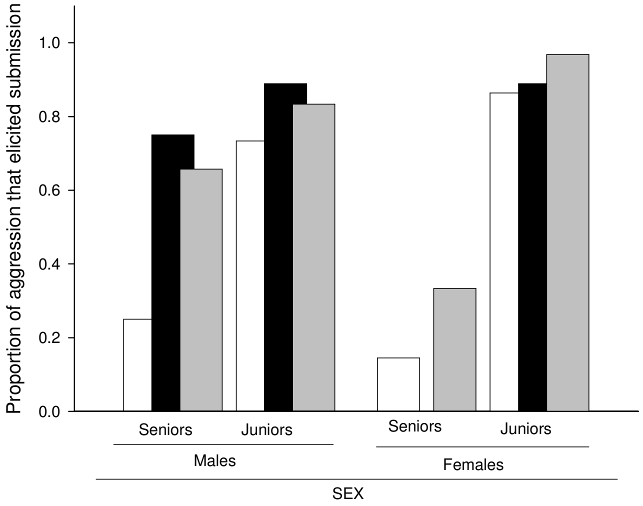

Figure 5.6. Submissiveness (proportion of received aggressive bouts responded with a submissive posture) of senior and junior males and females in the three treatment groups at different ages. Males and females from Bb-broods ◍ did not differ from control broods. Males from Aa-broods ● were more submissive than control ○ males when they were attacked. Senior females were less submissive than senior males and no differences were found between junior males and females independently of their treatment group. Females of Aa-broods were never attacked and therefore unable to show submission.

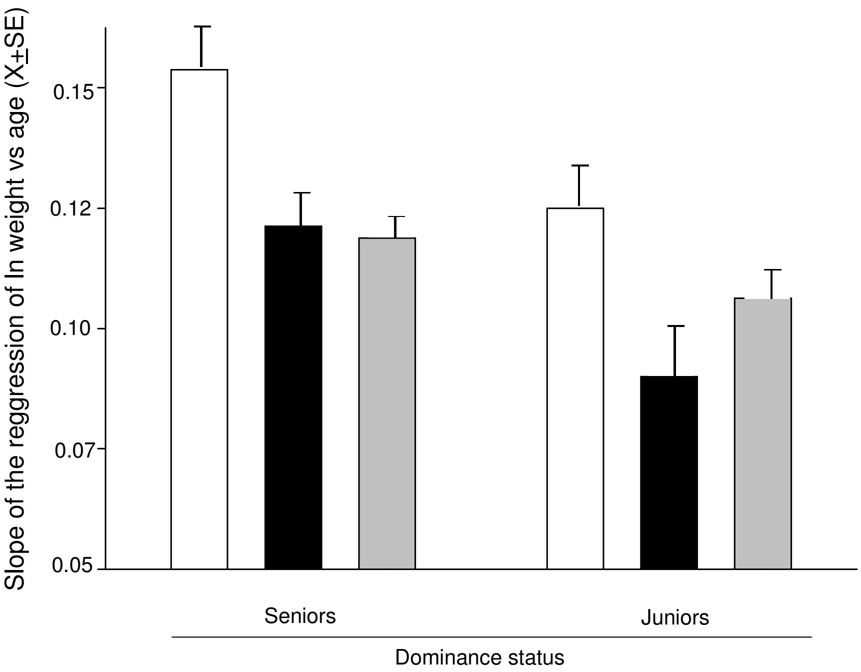

Figure 5.7. Mean (± SE) growth rate of senior and junior chicks from the three treatment groups. Senior chicks grew faster than juniors in control ○ and Aa-broods ● and the same tendency was present in Bb-broods ◍. Senior and junior chicks in Bb-broods grew at very similar rates compared with jseniors and juniors from any other group.

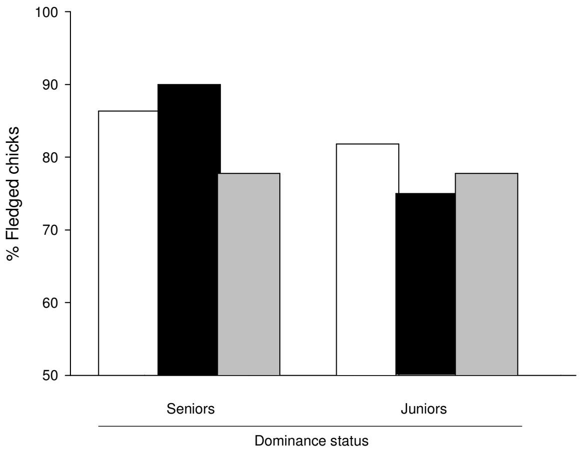

Figure 5.8. Percentage of fledged senior and junior chicks from control-○, Aa- ● and Bb-◍ broods. The proportion of fledged chicks did not differ between experimental and control broods and did not differ between senior and junior chicks.

Chapter VI

Stress Response and Testosterone Levels of Junior Black-legged Kittiwake Chicks Hatched From Different Quality Eggs

Introduction

Organisms from all taxa incur costs to maintain a physiological equilibrium (reviewed in Creel, 2001). Individuals need energy to complete their life cycles, to cope with changes in their environment and to adapt to new situations. In order to respond to these changes, physiological responses take place: the amount of released hormones change, affecting metabolism and the immune function amongst other physiological processes (reviewed in Sapolsky et al. 2000). A widely used term to define all these changes is stress. The word stress is commonly used with negative implications but the physiological changes that take place in response to diverse stimuli are beneficial to the organism in the short term (reviewed in Wingfield & Kitaysky, 2002).

Causes of stress are diverse and can be a consequence of environmental (e.g. inclement weather, lack of food, predation risk) or social constraints (e.g. dominance establishment, competition for food, e.g. Romero et. al., 2000). The physiological response to these situations is mainly the release of adrenocortical hormones, neurotransmitters and cytokines that act to help the organism to cope with the harmful event by moving energy from fat deposits, investing energy from secondary functions into increased metabolism and stop non-vital functions such as reproduction (Sapolsky, 1992). When these critical periods are sustained for long periods of time, they damage the organism and can provoke severe health detriment and diseases (Wingfield et al., 1992; Sapolsky, 1992).

Nestling chicks of several species need to compete with their siblings to obtain resources provided by their parents. Nestlings can compete for these resources through direct aggression and if necessary kill their siblings (reviewed in Mock & Parker, 1998).

Chicks in such situations show a high adrenocortical activity because they are constrained to a nest without being able to escape from the social stressors they are exposed to (e.g. food competition and aggression, Nunez de la Mora et. al. 1996). Therefore, the level of stress will depend on the degree of competition they have to deal with. Usually last-hatched chicks in species that show direct sibling competition are constantly attacked, receive less food and have to be submissive in order to survive (reviewed in Mock & Parker, 1998). For example, Eurasian kestrel (*Falco tinnunculus*) chicks that had an older female sibling were more stressed than chicks with an older male sibling because in this species females are larger and require more food, hence males with an older female sibling experienced a stronger competitive situation (Blanco et al., 2003).

In species that show sibling rivalry, parents can influence sibling competition in several ways (Slagsvold & Amundsen, 1992). Mothers can produce eggs of different size, start to incubate before the clutch has been completed resulting in hatching asynchrony (Stoleson & Beissinger, 1995) and allocate egg components (nutrients and hormones) in different amounts to each egg (Groothuis et al., 2005). If these differences favour the quick formation of a stable dominance-subordinate relationship, parents might save resources. The whole brood will need less parental effort and dominant chicks will show aggression only when necessary in species that do not show obligate siblicide.

Tarlow et. al. (2001) proposed the developmental advantage hypothesis. They found that parents favour the obligate siblicidal first-hatched Nazca booby (*Sula granti*) chicks through faster mass gain, but not faster increase in size to out compete their younger and smaller sibling (Tarlow et. al., 2001). Mothers can also favour chicks by manipulating egg hormones. In the cattle egret (*Bubulcus ibis)* mothers allocate more testosterone to the first-laid egg than to last-laid eggs (Schwabl et al., 1997). Testosterone can increase embryo developmental rate (Lipar & Ketterson, 2000; Tobler et al., 2007), muscle strength (Lipar, 2001), postnatal growth rate (Schwabl, 1996), provisioning behaviour (Quillfeldt et al., 2006; Goodship & Buchanan, 2007) and promote aggressiveness (Wingfield, 1994). However, the role of testosterone in the development of a dominance hierarchy among chicks has not been well established (Ramos-Fernandez et al., 2000; Tarlow et al., 2001; Ferree et al., 2004).

Another hormone that can also influence offspring phenotype and behaviour is corticosterone. It has been proposed that high levels of corticosterone exert a facilitative effect on individuals to be more submissive (Louch & Higginbo.M, 1967; Leshner & Politch, 1979; Leshner et al., 1980). On the other hand dominant chicks down regulate their corticosterone levels in order to increase their body mass and have a competitive advantage over their junior sibling (Tarlow et al., 2001). Contrary to this kittiwake (*Rissa tridactyla*) chicks with experimentally elevated levels of corticosterone in aggression trials showed more aggressive acts compared to chicks with non-elevated levels of this hormone (Kitaysky et al., 2003). These differences in the relationship between corticosterone and behaviour may depend on social experience (Creel, 2001). Junior blue-footed boobies (*Sula nebouxii*) that had the experience of being losers and subordinates showed a two-fold increase in baseline corticosterone levels compared with their dominant sibling (Nunez de la Mora et al., 1996). Black-legged kittiwake chicks in the previous study were naïve and exposed to a novel stimulus of having experimentally elevated levels of corticosterone (Kitaysky et al., 2003).

The amount of corticosterone allocated to eggs is influenced by the mother's circulating levels of this hormone (Hayward & Wingfield, 2001; Hayward & Wingfield, 2004). High corticosterone levels in eggs of Japanese quail (*Coturnix japonica*) were correlated with a slow growth rate of chicks and an enhanced adrenocortical activity towards acute stressors (Hayward & Wingfield, 2004). It also increased the hatching success and decreased the cellular immune response in yellow-legged gull chicks (*Larus michahellis*) (Rubolini et al., 2005). Differential allocation of corticosterone to eggs in relation to laying order has not yet been reported in any avian species, but its effects on chicks physiology and behaviour could play an important role in sibling rivalry.

In this study I tested the hypothesis that within-clutch differences in egg composition affect the establishment of the dominance hierarchy between siblings and the capacity of the last-hatched, subordinate chick to cope with the stress of sibling competition. I carried out this study on black-legged kittiwakes that typically lay two eggs and clearly differ in their egg composition **(chapter II)**. First-laid eggs (A-) are larger, have lower testosterone and higher carotenoid levels than second-laid eggs (B-)(**chapter II**). First-hatched chicks (A-) become dominants and constantly attack and food restrain second-hatched chicks

(B-), (Braun & Hunt, 1984; **chapter III**) which are ca 1.5 days younger than A chicks. For testing the stated hypothesis I changed the type of eggs from which junior chicks hatched by swapping eggs of known laying order between nests. This allowed me to compare junior chicks that hatched from two different egg types (A- and B- eggs) when competing in a standardised situation against an older, dominant chick. Because maternal allocation of nutrients and hormones to the egg may influence the establishment of the dominance hierarchy (first 10 days after hatching (**chapter III**)) I took behavioural and physiological measurements from control broods and broods where their composition was manipulated during that period.

I predict that control junior chicks (hatched from a B- egg) will be better able to cope with the social stress of being subordinate by showing a decreased stress response (release corticosterone slower and recover to basal levels also slower) than experimental junior chicks (hatched from A- eggs). Moreover, control junior chicks experienced higher testosterone levels in the egg than experimental junior chicks (**chapter II**), wich could influence testosterone production in the chick (Birkhead et al., 2000). I predict that higher testosterone yolk levels will make control junior chicks more resistant to attacks and will promote aggression in case they have the chance to reverse the dominance-subordinate relationship. Moreover, adult kittiwake males are 10% larger than females (Helfenstein et al., 2004) and hence the sexes may respond differently to social stress and I therefore included offspring sex in my analyses. These results will improve our understanding of the influence of differential resource allocation to eggs depending on laying order in sibling competition and in the stress response of the nestlings.

Methods

Black-legged kittiwakes on the Isle of May, Firth of Forth, Scotland (fig. 1.1) from the 2005 breeding season were used for this work. In this population, kittiwakes typically lay two eggs with 2 days of difference and chicks hatch asynchronously (ca 1.5 days). During the laying period, 150 active nests were visited daily and eggs individually marked on the day they were laid in order to know their laying order. Once the clutch was complete, the breadth (b) and length (l) of each egg was measured and egg volume was calculated with the formula $V=0.4866b^2l$ (Coulson, 1963).

From the expected hatching date onwards (date the first egg was laid + 25 days) nests were visited daily and checked for new hatchlings. Eggs hatched at least 24 hours apart and therefore it was possible to identify the egg from which each chick hatched. On the hatching day, or if weather did not permit it soon after hatching (within a day), hatchlings were weighed and a blood sample of less than 10µl was taken under a Home Office UK license in order to sex the chicks using molecular techniques (Griffiths et al., 1996). Hatchlings were individually marked using two different colours of sheep dye sprayed on head, neck and rump. The dye was reapplied at every nest visit to maintain a good marking for the behavioural observations. After hatching, nests were visited every five days (weather permitting) and chicks were weighed to the nearest gram using a spring balance. Wing and tarsus length were also measured to the nearest 0.1 mm. Growth measurements were stopped when nestlings were 20 days old because it was no longer possible to handle the nestlings without disturbing them and provoking them to jump out from their nest. Nests were checked daily until day 40 to record fledging success (Cullen, 1957).

Experimental design

In order to compare the stress response of junior chicks that hatched from either A-or B-eggs, experimental clutches were created by exchanging the b-egg of some nests with an a-egg from other nests that were laid on the same day. In some control broods, both eggs were swapped between nests, whereas in other control broods both eggs were exchanged together between nests. This latter control group was created to test whether any form of kin recognition may affect the results. Control groups did not differ on their behaviour ($p>0.15$; **chapter V**). Eggs were swapped within 10 after being laid. Care was taken that after eggs were exchanged the egg to hatch first was on average 4% larger than the egg to hatch last. This created two groups where a control junior chick hatched from a B-egg or an experimental junior chick hatched from a A-egg in both cases competing with an older nest mate hatched from a A-egg. The within-brood egg size difference and hatching span did not differ between experimental and control groups (for a detailed experimental design see **chapter V)**. In all control and experimental nests offspring were raised by foster parents.

*Blood collection***.**

Blood was taken only from junior chicks because it was logistically impossible to sample both chicks within three minutes of approach (in order to obtain corticosterone basal levels). Chicks between 6 and 8 days of age were sampled because at this age a dominance-subordinate hierarchy is being formed between the siblings (**chapter III**). On the sampling day control and experimental junior chicks were exposed to a handling-restraint stress protocol, which requires blood being collected repeatedly (Wingfield et al., 1992). The protocol was carried out as follows: a first blood sample was taken in less than 3 minutes after the initial approach (mean interval between first approach of the nest and taking the blood sample did not differ between control and experimental groups; control group: 2.01 ± 0.26 min (n = 11); experimental group: 2.15 ± 0.21 min (n = 17);timing of 2 samples missing; independent sample t-test: t_{26}=0.65, p=0.51) in order to measure corticosterone basal levels. To measure an acute stress response, the chick was kept inside a fabric bag in the interior of a shaded bucket without any disturbances. A second blood sample was taken 10 minutes after first approaching the nest when corticosterone levels peak and a third sample after another 20 minutes to measure how fast chicks recovered from the acute stressor (Kitaysky et al., 1999). Corticosterone basal, acute and recovery levels were taken from 19 experimental junior chicks (hatched from A-eggs) and 12 control junior chicks (hatched from B-eggs).

Blood samples were taken under licence of the UK Home Office by puncturing the brachial or tarsal veins with a 25GI needle, directly collecting the blood into a heparinised syringe and then transferred to a 1.5ml heparinised Eppendorf tube. The total amount of blood taken from each chick was less than 750µl (which is less than 10% of the body blood volume of a 7-day old kittiwake chick weighing on average 123 g (own data), Morton et. al., 193). Blood was kept inside a thermos bag with iced gel until it was brought back to the field lab (always within 2 h of blood sampling). In the lab, blood was centrifuged at 6,000 rpm for 4 minutes in order to separate blood cells and plasma. Plasma was collected with a 1 ml plastic Pasteur pipette, transferred into a new vial and stored at -20^{o}C until further analyses back in Glasgow University.

Hormone analysis.

Hormone levels were determined using radioimmunoassays (Wingfield & Farner, 1975; Wingfield et al., 1992). Corticosterone was measured from plasma in triplicates after an ether extraction from 30μl aliquotes. Anticorticosterone antiserum (code B3-163, Esoterix Inc Endocrinology, San Diego, CA, USA) and [1,2,6,7-3H]-corticosterone label (Amersham, Little Chelfont, U.K.) were used. Triplicates of the same blood sample and samples from the two treatment groups were randomly allocated between two assays. Assays were run with 50% binding at 1.02 pg/tube and the extraction efficiency was between 70 and 90%. The intra-assay variability was 3% and the inter-assay variability was 12%. The detection limit was 0.078ng/ml.

The integrated stress response (ISR) is a measure of how much corticosterone was released during the whole period of handling-restraint (30 minutes). It was calculated by measuring the area under the curve (AUC) of corticosterone levels vs. time using ImageJ v.1.47 with graphs produced in SigmaPlot v.10.0. The AUC is a measure of how much corticosterone is secreted by an individual over a period of time (Cockrem & Silverin, 2002) and it gives an integrated measure of the rate of corticosterone release and clearance (Breuner et al., 1999). Overall, having a lower integrated corticosterone response implies that chicks released less corticosterone after an acute stress and/or recovered to basal levels faster.

Since testosterone levels should not change after an acute stressor because it is not produced in the adrenal cortex (stimulated when the individual is exposed to a stressor, Adkins-Regan, 2005), the remaining blood of the three samples of each individual were pooled and analysed as one sample per chick in the same assay. Testosterone was measured from plasma duplicates after a chloroform extraction from 50μl aliquotes. Antitestosterone antiserum (code 8680-6004, Biogenesis, Poole, U.K.) and [I_{125}]-testosterone labelled (code 07-189126, Basingstoke, U.K.) were used for the assay. Intra-assay variation was 8%. The 50% binding was at 0.22pg/tube for 50μl plasma and the detection limit was 0.005ng/ml.

Behavioural observations

Observations of provisioning and agonistic behaviour were carried out during 3 hour observation periods every third day starting when the junior chick was 1 day old and until it was 9 days old for an average of 9 hours of observation per nest. The behavioural data of each chick for the first 9 days of life were averaged because no behavioural changes were present during this period (**chapter III**). These means were used in the subsequent analyses. Behavioural observations were carried out on 7 control broods (junior chick hatched from a B-egg) and 15 experimental broods (junior chick hatched from a A-egg). Activity, parental feeding, chick begging, aggressive acts towards the nest mate, aggressive acts received from the nest mate and the proportion of submission in response to an aggressive act received (see **chapter III** for a detailed descriptions of how each behaviour was measured) plus the proportion of feeding bouts with priority access to food (being the only one to receive food or being fed first) and the proportion of feeding bouts with no priority access to food were compared between control and experimental broods. In order to obtain a dominance index for each chick a principal component analysis (PCA) using the frequency of aggressive acts, aggression received, submission ratio and priority access to food was done and the first principal components obtained were used as a measure of the dominance status.

Statistical analysis.

Statistical tests were carried out in SPSS 14 (SPSS Inc., Chicago, IL, U.S.A.). Corticosterone and testosterone levels were not normally distributed and therefore one was added to each data point and then log transformed. Repeated measure ANOVAs were used to test for differences in corticosterone levels between treatment groups at 0, 10 and 30 minutes. Generalized Linear Models (GLM) were used to test the influence of egg type, sex, and chick condition (residuals of the regression of weight on wing length at 7 days of age) on corticosterone levels. In these analyses 19 experimental and 11 control broods were used because in one of the control broods sex was missing and in another one it was not possible to calculate chick's condition. Since it is almost impossible to disentangle between the cause and effect between high levels of hormones promoting a specific behaviour or a behaviour influencing hormone levels, Spearman correlations between each behaviour and the ISR were done.

GLMs were also used to test the influence of egg type, sex, and chick condition on testosterone (using the same sample sizes). Correlations between testosterone levels and behaviour were done, as well as correlations between testosterone and the three measurements of corticosterone. Three different correlations between testosterone and each of the three corticosterone levels as covariates along with sex, condition and egg type were performed. Sub-colony, hatching date and identity of observer were tested in preliminary univariate tests and as they were not significant ($p > 0.1$) were not considered in the final analysis. Mean ± SE is reported when differences between groups are present.

Results

Behaviour

Two factors were obtained from the PCA; the first principal component (PC-1) explains 46% of the variance and the second principal component (PC-2) explains an additional 21% of the variation (table 6.1). From the variables used to construct the PCA, the two with most weight on PC-1 were submissiveness and aggressions received, while none of the other variables explained PC-2 for more than 60% (table 6.1). The PC-1 explained the submissive behaviour and therefore will be considered as a subordination index. Higher values of the PC1 are for more subordinate chicks. The PC-1 and PC-2 differed between control and experimental junior chicks and these differences varied according to the sex of the chick: (PC-1: treatment*sex: $F_{1,25}=22.2$, $p<0.0001$; treatment: $F_{1,25}=12.6$, $p=0.002$; sex: $F_{1,25}=31.3$, $p<0.0001$; PC-2: treatment*sex: $F_{1,25}=9.52$, $p=0.005$; treatment: $F_{1,25}=5.37$, $p=0.03$; sex: $F_{1,25}=7.05$, $p=0.014$). Control females had a higher subordination index than experimental females and than control and experimental males (fig.6.1).

Corticosterone

There was no difference in the ISR between whole cross-fostered control broods (consisting of full siblings) and control broods created with eggs laid by two different females (repeated measures ANOVA with base levels, acute response and recovery levels as within-subject factor, between groups: $F_{1,10}$=0.26, p=0.61). For this reason, all the broods with junior chicks hatched from B-eggs were pooled together into one control group.

Kittiwake chicks showed a clear stress response after being exposed to a handling restraint stress protocol (repeated measures ANOVA, within subject factor: $F_{1,28}$=29.8, p<0.0001; fig.6.2); corticosterone levels differed between basal and acute levels (post-hoc comparisons: $F_{1,28}$=43.7, p<0.0001) and between the acute response and the recovery levels (post-hoc comparisons: $F_{1,28}$=7.2, p=0.007). There were no differences in the ISR between experimental and control junior chicks, sexes and chick's condition or age (table 6.2). Corticosterone basal levels did not differ between control and experimental junior chicks and was low in both groups (treatment: $F_{1,24}$=0.47, p=0.49; controls: X=2.8 ± 0.11 ng/ml; experimentals: X=3.5 ± 0.07 ng/ml) and were not influenced by condition, age, sex, or the nest mate's sex, (sex: $F_{1,23}$=1.57, p=0.22; sibling's sex: $F_{1,22}$=0.11, p=0.74; condition: $F_{1,26}$=0.73, p=0.40; age: $F_{1,25}$=0.65, p=0.42). The time taken between approaching the nest and getting the first sample was not correlated with basal, acute or recovery corticosterone levels (Pearson correlation: basal: r_{26}=0.061, p=0.75, n=28; acute: r_{26}=0.13, p=0.48, n=28; recovery: r=-0.056, p=0.77), which indicates that the first sample reflects corticosterone baseline levels.

When each behaviour was correlated with the ISR separately, a negative correlation between this response and begging success was present only in control junior chicks (r_5=-0.92, p=0.008; fig.6.3) but no other correlations were significant (table 6.3). There was a negative correlation between the subordination index and the ISR in experimental junior chicks (r_{13}=-0.52, p=0.017) while no correlation was present in control junior chicks (r_5=0.07, p=0.88; fig. 6.4). No correlation was found between the subordination index and chick's condition (r_{22}=-0.071, p=0.72).

Testosterone

No difference in testosterone levels between control and experimental junior chicks was present but males had higher levels of testosterone than females (treatment group: $F_{1,24}$=1.46, p=0.24; sex: $F_{1,27}$=5.57, p=0.026; condition: $F_{1,26}$=2.35, p=0.13; age: $F_{1,25}$=2.59, p=0.12; fig.6.5). Testosterone levels were not related to any of the three corticosterone measurements in control junior chicks (basal levels: r_7= 0.59, p=0.072; acute response: r_7= 0.27, p=0.44; recovery levels: r_7=-0.35, p=0.29). Levels of this hormone were negatively correlated with the acute corticosterone response in experimental junior chicks (acute response: r_{17}=-0.49, p=0.026) and not with basal (r_{17}=0.05, p=0.83) and recovery levels (r_{17}=-0.18, p=0.43). The ISR was correlated with testosterone levels in experimental junior chicks (r_{17}=-0.44, p=0.048) but not in control junior chicks (r_7= 0.03, p=0.93; fig.6. 6).

No correlations were found between testosterone levels and any of the behaviours analysed (p>0.2; table 6.3) nor between the subordination index and testosterone (r= -0.008, p=0.97).

Discussion

It was predicted that the stress response of junior B- and A- chicks would differ, with a stonger the response in A-chicks.However, no differences in the overall stress response between A- and B- junior chicks was found. I also predicted that the stress response would be different between males and females, but again no differences were found. Higher testosterone levels in B-junior chicks compared to As was alos predicted, but no differences on testosterone were found.

Control and experimental junior chicks differed in some aspects of their physiology and behaviour when they were exposed to similar environmental and experimental stressors. Although no differences in growth and survival were present between the two groups (**chapter V**), in the present chapter it was found that a calculated subordination index differed between treatment and experimental groups at the age when dominance hierarchies were established (**chapter III**). Because 2005 was a good year in terms of food availability and breeding success (Harris, 2005), mothers could have followed a brood survival strategy by increasing juniors' chances of survival (reviewed in Hillstrom, 1999). As a consequence of such a strategy the differences between control and experimental junior chicks could have been less pronounced than during a poor year.

Corticosterone levels and the integrated stress response

Junior kittiwakes as young as 6 days old were capable of mounting a normal stress response after being captured and restrained. American kestrels (*Falco sparverius*) and

Magellanic penguins (*Spheniscus magellanicus*) chicks exposed to a handling-restraint protocol at different ages showed a stress response at all ages, but their response increased with age and was only fully developed when reaching independence (Love et al., 2003; Walker et al., 2005). The hypothalamic-pituitary-adrenal (HPA) axis in semi-altricial birds takes longer to maturate than in precocial species in which the stress response is fully developed just days after hatching (Sims & Holberton, 2000). Sims & Holberton (2000) proposed that a delayed maturation of the HPA axis is beneficial for semi-altricial chicks because they cannot avoid stressful situations such as food and sibling competition when they depend on their parents to obtain food and cannot leave the nest. But corticosterone might have a functional role when altricial and semi-altricial chicks are chronically stressed (Walker et al., 2005). Junior kittiwakes of 7 days old should be suffering high levels of stress because they are physically competing with an older sibling for food and establishing a dominance hierarchy that lasts until they fledge or die (Braun & Hunt, 1984; **chapter III**). Thus it is not surprising that chicks of this age showed a stress response similar to adult kittiwakes during the early breeding period (Kitaysky et al., 1999). Kitaysky et al. (2001) predicted that the HPA axis of seabird chicks will be fully developed at early ages but to my knowledge it has not been tested before, thus the present work is a confirmation of their hypothesis.

No differences in the stress response between control and experimental chicks were found. 2005 was a particular good year for breeding kittiwakes on the Isle of May, food was abundant and the proportion of broods with two fledglings was high (Harris, 2005; **chapter III**). Therefore, junior chicks might not have been as stressed as they would have been when food is scarce. For example, in 2004, a considerably poorer year when 100% of B- chicks died or were expelled from their nests by the senior chick, junior chicks might have been exposed to greater stress in the nest. If the environment is not stressful (e.g. 2005), phenotypic differences due to differences in egg composition may be less obvious because they are not needed. Maternal manipulations in-ovo are specifically made to favour chicks in a challenging environment (Schwabl et al., 1997; Salvante & Williams, 2000; Saino et al., 2005). Otherwise, these manipulations should not exert a difference in behaviour and survival when compared with chicks that were not maternally favoured to cope with stressful environments.

The ISR indicates the chicks' exposure to corticosterone released in an acute way and the clearance rate of this hormone in the recovery period (Breuner et al., 1999). The two components of the ISR (acute and recovery levels) are not independent of each other thus the ISR gives an idea of how efficient an individual is coping with an acute stressful situation. A negative correlation between successful begging and the ISR was found only in control chicks and no correlation was present between begging bouts or feeding frequency and the ISR. This result seems to contrast with the findings of Kitaysky et al. (2001) and Kitaysky et al. (2003). They found that kittiwake chicks with experimentally elevated corticosterone levels increased their provisioning behaviour by increasing their begging rate and aggressiveness. Possibly acute corticosterone elevations do not exert the same effect as more sustained elevations as it was the case in both experiments with kittiwakes, although overall, chicks with a higher ISR were exposed to higher levels of corticosterone for a longer time than chicks with a lower ISR.

The subordination index obtained from the PCA was higher in control junior females than in experimental junior females and junior males from both groups. Kittiwake females are 10% smaller than males when they are adults (Helfenstein et al., 2004) thus coping with the stressful situation of being junior and smaller is crucial. Perhaps females have to be more submissive than males in order to survive probably because they are smaller. Possibly some behavioural changes that were not recorded like an increase in the intensity of begging or aggression took place and could explain why females showed a higher subordination index than males. On the other hand, experimental chicks showed a negative correlation between the submissive factor and the ISR which perhaps indicate that more submissive experimental chicks mounted a less enhanced stress response but control chicks presented a "normal" stress response independently of their submissiveness.

Testosterone

B- kittiwake eggs had higher levels of testosterone than A eggs (**chapter II**), but testosterone levels of 6-8 day-old kittiwake junior chicks were not different between control and experimental groups. High levels of testosterone in the egg could have organizational effects on the chicks and not necessarily exert a direct influence on their testosterone plasma levels (Birkhead et al., 2000). I predicted that higher yolk testosterone levels in control broods were going to promote aggressiveness in this group compared with the experimental broods but no differences in aggressiveness were found (**chapter V**). The subordination index varied between groups but only differed in control female chicks. Interestingly, females had lower levels of testosterone than males independently of their treatment group (discussed below). It could be that yolk testosterone levels exert some organizational effect which allows females to be more submissive than males. It has been shown that sex hormones have organizational effects on the hypothalamic-pituitary axes (McCormick et al., 1998). These influence could in turn affect the way individuals respond to different social stimulus and could vary depending on the individual's sex (Panzica et al., 1998; Love et al., 2005). If indeed yolk testosterone has a behavioural organizational effect, same environmental changes and stressful stimuli will affect male and female chicks from control in a different way than male and female chicks in experimental broods (Love et al., 2005). Maternal manipulations at the yolk level differentially influenced the way chicks respond to similar stimulus in similar social situations.

The challenge hypothesis states that individuals that face a challenge situation (e.g. aggression) will increase their testosterone production in order to cope with this new situation, but will return to previous levels soon after the challenge stops (Wingfield et al., 1990). A conclusive relationship between testosterone and aggressiveness was found by Ferree et al., 2004 in the siblicidal Nazca booby. They found an increase in testosterone levels when birds were sampled during aggressive encounters, but not after or before these encounters. When this hypothesis was tested in the facultative siblicidal blue footed booby chicks (*Sula nebouxii)* testosterone levels were very low and did not vary in chicks with different aggression levels (Ramos-Fernandez et al., 2000). Perhaps they were not sampled after an aggressive encounter and their basal levels do not need to be high in order to respond to an aggressive attack (attack or being attacked) (Ferree et al., 2004). Chicks should be able to regulate their testosterone production to avoid detrimental consequences of having elevated circulating levels of this hormone (e.g. immunosupression and metabolic costs, Wingfield et. al. 2001). Nazca boobies are able to do so in an efficient way, returngin to basal levels soon after the end of an aggressive encounter attack (Ferree et al., 2004) . If kittiwakes' physiology is similar to the Nazca boobies, we could expect no differences in testosterone levels between the experimental and control broods, because blood was not taken after aggressive encounters. If junior A- and B- chicks differed in their testosterone production after being challenged, one should expect to find different testosterone levels soon after being attacked only.

Overall, control junior chicks did not cope better than experimental juniors with an acute stressor. In general their stress response was similar although the way this response correlated with provisioning behaviour and testosterone levels differed between experimental and control chicks. It is unknown why they differed or the mechanism behind the variation. The only feasible explanation for the differences between the groups is a differential allocation of egg components by their mothers. These groups only differed on the quality of the egg they hatched from. Yolk hormones should exert organisational effects that shape the way chicks cope with stressful situations and the way the whole neuroendocrine system reacts to similar stimuli. Perhaps differences were not clear due to the good conditions in the study year but even so, chicks from both groups showed physiological differences that were not translated into survival or growth disparities. However, the influence of this physiological variation on adults' phenotype can not be discarded and future studies are needed in order to test how it might be affected.

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Tables

Table 6.1. Factors used in the principal components analysis (PCA). The weight each variable had in the calculation of the two principal components derived from this analysis is reported. Significant results are those higher than 0.70 and are marked with bold characters.

	Component	
Behaviour	*1*	*2*
Aggression_given	-0.462	0.547
Aggression_received	**0.870**	0.429
Submisiveness	**0.900**	0.353
Feeding priority_secured	-0.481	0.642
Feeding priority_failed	0.540	-.242
% Variance explained	46.06	21.58

Table 6.2. The influence of egg type, sex, condition, age and all the two way interactions in the ISR was tested using a GLM. Non-significant interactions were dropped from the model using the stepwise regression method.

Factor	*Df*	*F*	*p*
Egg type	1,25	0.017	0.89
Sex	1,27	0.44	0.51
Condition	1,26	0.062	0.81
Age	1,28	0.81	0.37

Table 6.3. Spearman rank correlations between different behaviours with the ISR and testosterone levels. In the first line results from control chicks are shown. In the second one, results from experimental chicks are reported. Significant results are marked with bold characters.

	ISR		**Testosterone**	
Behaviour	*r*	*p*	*r*	*p*
Begging Frequency	-0.5	0.24	0.37	0.36
	-0.04	0.88	-0.23	0.40
Begging success	**-0.93**	**0.008**	0.38	0.40
	0.15	0.58	-0.21	0.49
Feeding Frequency	-0.31	0.45	0.57	0.24
	0.35	0.19	-0.03	0.90
Aggression	0.64	0.35	-0.26	0.46
	-0.04	0.87	0.10	0.69
Submission	0.37	0.53	-0.13	0.75
	-0.15	0.61	-0.17	0.23

Figures

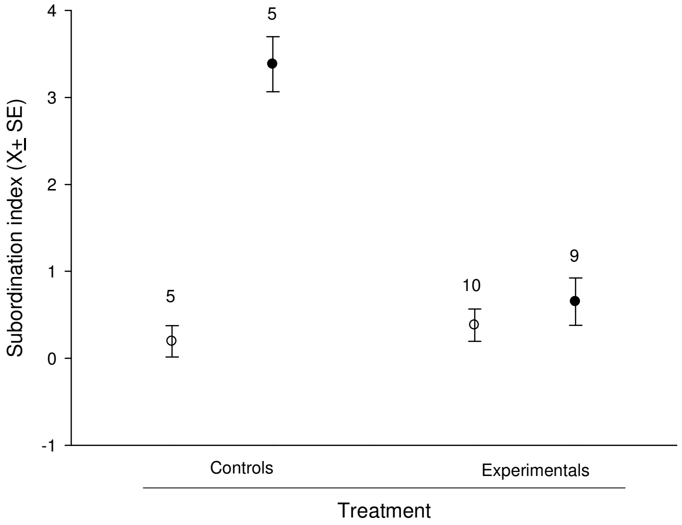

Figure 6.1. Mean (±SE) subordination index in males and females. Females● in the control group had a higher subordination index than experimental females and than males ○ in both groups. Sample sizes are reported above each bar.

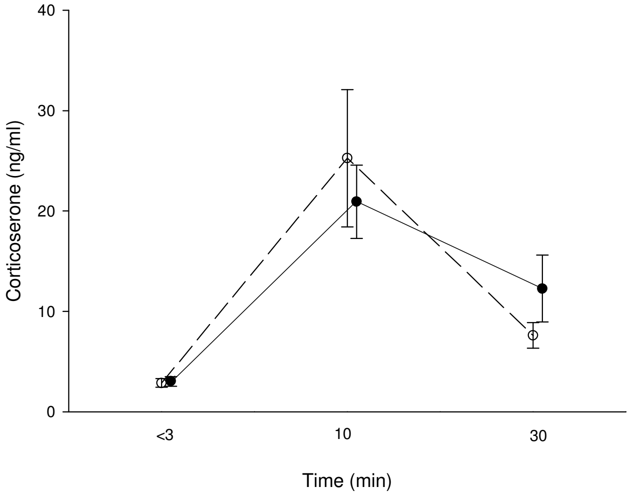

Figure 6.2. Mean (±SE) baseline (3 min), acute (10 min) and recovery (30 min) corticosterone levels of control ● (n=11) and experimental chicks ○ (n=19) at an age between 6 and 8 days in a handling restraint protocol.

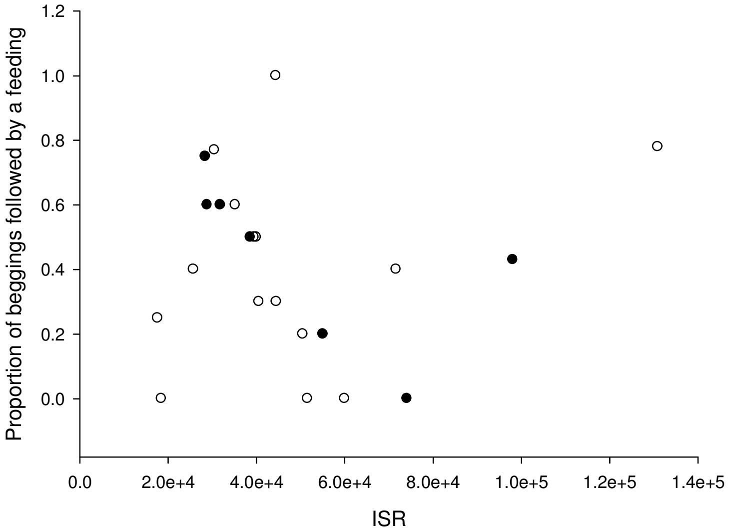

Figure 6.3. Correlation between begging success and the ISR. A negative correlation between begging success and the ISR was present in control chicks ● (n=7) while no correlation was found in experimental individuals ○ (n=15).

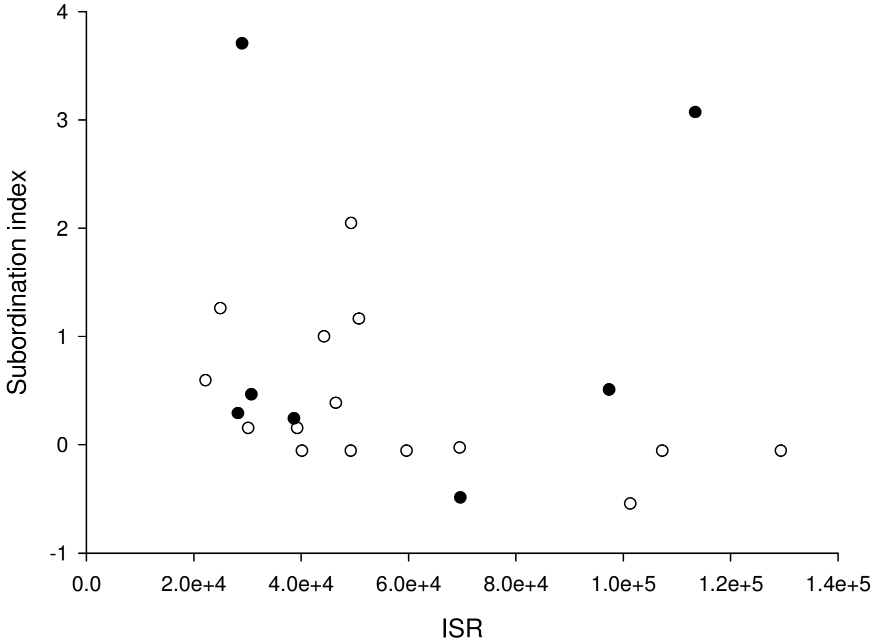

Figure 6.4. Correlation between the subordination index and the ISR. Experimental chicks ○ (n=15) showed a negative correlation between the subordination index (PC-1) and the ISR while control chicks ● (n=7) did not show a pattern.

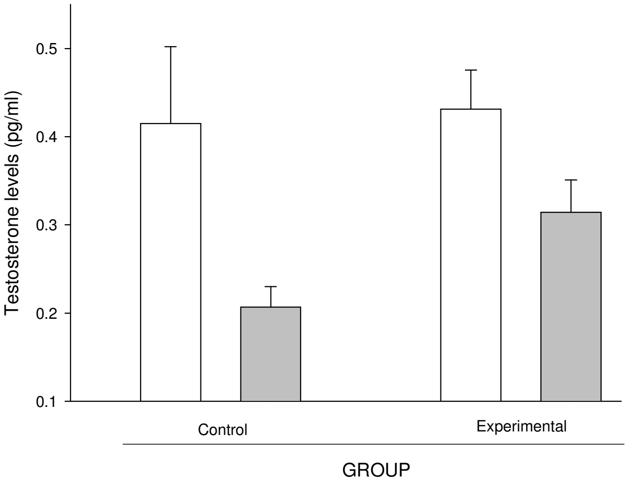

Figure 6.5. Mean (± SE) testosterone plasma levels in control and experimental male ○and female ●chicks. Testosterone levels were higher in males than in females independently of their experimental group and did not differ between different treatment groups.

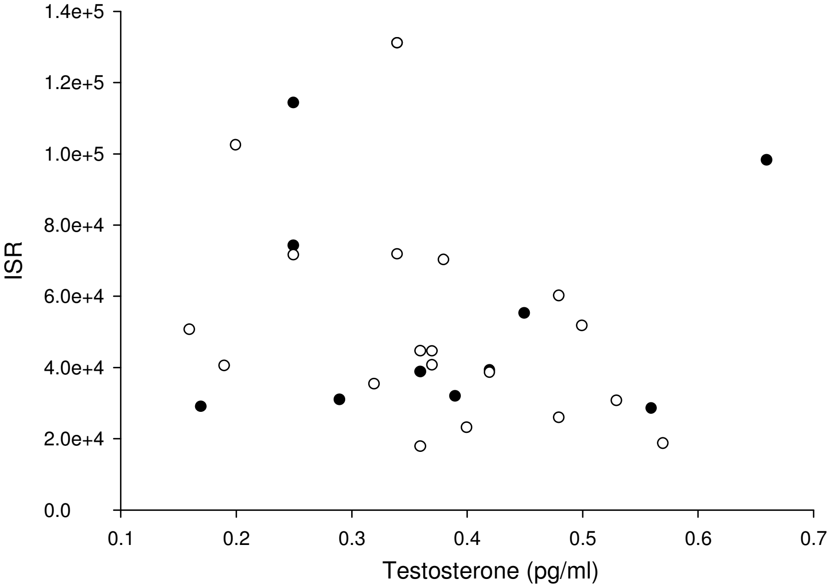

Figure 6.6. Correlation between testosterone plasma levels and the ISR. Testosterone levels were negatively correlated with the integrated stress response (ISR) in experimental chicks ○ (n=19) while no relation was present in controls ● (n= 9).

Chapter VII

General Discussion

Sibling rivalry by direct aggression is not common in animals. The findings from this work in one of the few avian species that performs it, the black-legged kittiwake, give new insights of the factors influencing the outcome of this conflict.

Chapter I outlines the various hypotheses that have been proposed to explain why parents sometimes lay more eggs than they can rear. Throughout the present thesis some of those hypotheses were directly or indirectly tested. In the following pages I will summarize some of the hypotheses, and compare them with the findings of the experiments made with kittiwake eggs in the present work.

Insurance Hypotheses

Dorward (1962) proposed the egg insurance hypothesis, stating that mothers of siblicidal species should lay more than one egg per clutch if the eggs or chicks from these clutches die sometimes before siblicide takes place and these chicks have chances of surviving and fledging. Laying a second egg as a replacement, should be less costly than not having the replacement at all (Trivers, 1974). When parents over produce the number of eggs in siblicidal species, they also influence asymmetries between the offspring in order to facilitate the brood reduction if necessary (Mock & Parker, 1986). Mock & Parker (1986) proposed the "insurance reproductive value", in which they state that parents will magnify the differences between offspring in order to respond to an unpredictable brood size and not to unpredictable resources after hatching.

In **chapter III** it was found that second hatched chicks (B-) from natural broods in a year with poor food quality did not survive. No B- chicks from the studied broods survived on their natal nest, compared with the high survival rate of B chicks the following year, in which the food quality was better. None of the B-eggs from the natural broods studied served as a replacement egg in either of the studied years. None of the A- eggs or chicks ever died before the B- eggs or chicks during; Bs never had the opportunity to be a replacement for the A- egg/chicks. The "additional-egg hypothesis"

(Tershy et al., 2000) states that "mothers can be selected to lay a second egg when offspring from both eggs sometimes fledge". High quality parents will benefit the most from laying a second egg, especially in years with high food availability (Tershy et al., 2000). In the control kittiwake broods we found that the extra egg translated in an extra chick only when food conditions were good. It is unlikely that only good quality parents benefited from laying an extra egg since more than 80% of the studied broods during this good quality year were able to fledge an extra chick (**chapter III**).

The ice box hypothesis predicts that extra siblings could be produced in order to feed stronger chicks when food is insufficient. This was not observed in any of the kittiwake chicks studied (experimental or control broods). Usually the B- chick disappeared from the nest as a consequence of direct aggression or predation but only in a few cases the dead body remained inside the nest. On these few occasions the surviving chick never eat their sibling; the dead body remained in the nest for long periods of time until it became part of the nest (pers. obs.).

It is important to emphasize that without the parental influences on the asymmetries between offspring, producing an extra chick in order to become insurance will be extremely costly for the parents (Trivers, 1974). The differences in egg size, age and egg components created or permitted by the parents to facilitate the occurrence of siblicide or sibling rivalry. Closely matched opponents will spend more resources in establishing a dominance-subordinate relationship than more different individuals, although these differences should not be either too big or otherwise the disadvantaged chick would not have any chance to survive against a much bigger or old opponent (Drummond et al., 1986).

In the present thesis, some of the between-offspring asymmetries, such as egg size, age and egg components, were experimentally manipulated in order to test their importance in the outcome of sibling rivalry. Theory predicts that hatching from a larger egg is advantageous because it provides more nutrients that enhance growth and facilitates survival (e.g. enhancing immune system; Slagsvold et.al., 1984; Williams, 1994; but see Maddox &Weatherhead, 2008). Chicks from larger eggs are larger than chicks from smaller eggs (Deeming & Birchard, 2007). Being larger confers an advantage during aggressive encounters, because usually larger and stronger chicks win these competitions (Mock & Parker, 1998). The findings of **chapter V** do not support this prediction. Our findings showed a tendency in the opposite direction, with chicks hatched from smaller eggs becaming dominant in higher proportion than chicks hatched from larger eggs when age and egg quality were equally matched. The effects of egg size and asynchrony are hard to separate. The effect of one of these two factors could be mediated by the other one (e.g. hatching asynchrony depending on egg size differences). Hatching asynchrony could be more or less detrimental depending on the egg size differences within a brood, or according to the egg size of a specific chick in a brood (Maddox & Weatherhead, 2008).

Overall, the findings of this thesis seem to indicate that the most influential within-brood asymmetry is hatching asynchrony. Perhaps a few hours of difference between the hatching of the siblings could determine which of them become dominant. Apparently hatching some hours before the sibling is enough to confer this sibling a very big advantage towards its sibling and mark the difference between being dominant or subordinate. At the end, this subtle difference in hatching time will produce the difference between living and surviving depending on the environmental conditions of each year.

Parents do not directly intervene in the physical aggressive encounters between their offspring but it seems that mothers can influence the occurrence and outcome of these encounters by manipulating the amount of nutrients and hormones she allocates to her eggs. The influence of these components on the chicks' competition will depend on the environmental conditions present during the rearing period. Mothers accomplish this by assigning components to her eggs that favour chicks on a disadvantaged position (juniors) in years with enough food but increases their vulnerability and facilitate siblicide on years where the food availability is low.

It is still unknown that if apart from allocating different amount of nutrients to first and second laid eggs, kittiwake mothers assign components to her eggs differently according to the males' quality or depending to the existence and frequency of extra pair couplations (although infrequently observed in black-legged kittiwakes, Helfenstein et al., 2004). Females could be making eggs in a way in which the conflict between her offspring favours her interests. Perhaps by putting more testosterone into their eggs mothers can make that a good quality father invest more on the brood (e.g. Quillfeldt et al., 2006). If the gape's colour influences the feeding frequency (Bize et al., 2006) mothers could also manipulate carotenoid levels to get more resources from their partners. If siblings of a brood are not fathered by the same male it could be advantageous to fabricate eggs that produce chicks that are less ready to show aggression or that are more prone to submit depending on their within-brood position thus maximising both chicks survival.

Another important broadly unstudied factor related to egg composition is the variability of asymmetries between within-clutch egg components. It is well known how egg nutrients and hormones can be allocated depending on the laying order but it is not known if the degree of differences is modified depending on the conditions of a specific breeding season. The maternal influence on the conflict between her siblings is much bigger than just creating asymmetries in age and size through hatching asynchrony, at the end she has the tools to decide who decides.

Natural inequalities between the eggs of a clutch should increase parents' fitness (Gibbons, 1987; Slagsvold & Lifjeld, 1989; Wiebe, 1995; Royle & Hamer, 1998). For parents it might be advantageous to have different age chicks' within a brood because chicks would not be on the peak of their growth and demanding big amounts of food at the same time. Senior chicks also benefit themselves from the asymmetries present between them and their junior siblings: if food is enough both survive, if it is not, only they have the opportunity do so (Braun & Hunt, 1984). Junior chicks only have chances of surviving when food is enough, when their senior sibling dies or when they are expelled and luckily can get into another nest inhabited by smaller chicks. It was observed that junior chicks have the tools and the behavioural plasticity to attack, overcome and out-compete a weaker opponent if they have the chance. Moreover, experimental broods with two B-chicks were more aggressive than broods with two A-chicks independently of their egg size when no age differences were present. If mothers favour second laid eggs by facilitating their competitive abilities through differential egg allocation this benefit could manifest when chicks have the chance of competing towards a very similar opponent.

If almost no extra-pair couplations occur in kittiwakes (Helfenstein et al., 2004) why kittiwake adults accept unknown chicks into their nests if this is obvious detrimental for their fitness? Adopting an unknown chick will undoubtedly damage their fitness. Parents have to provide food to the new chick, which probably will decrease the amount they provide to their own chicks. Moreover, the adopted chick has the potential of killing the natal chicks which of course is a big damage to parents' interests. Unless kittiwakes of an Island sub-colony are all genetically related, adopting a chick expelled from its nest seems as *contra*-nature.

From the experiments carried out on this thesis, a finding that I would like to remark is that manipulations of the brood at the egg stage not only provoked behavioural changes on the manipulated chick but also that from the sibling and the parents even when only one member of the brood was altered (**chapter V**). The manipulation of one of the members of a brood at the egg stage was enough to provoke behavioural changes in the rest of the family (parents and offspring).

Finally, I would like to discuss about how to relate the results from this work to the decreased kittiwake population on the Isle of May in particular and the North Sea in general. A better understanding of the sibling conflict of kittiwake chicks could give more tools to deeply understand the decline of the kittiwake populations in the North Sea. Kittiwake numbers have dramatically decreased since the early 90's in the North Sea and specifically in the Isle of May (Frederiksen et al., 2004). The establishment of a fishery near by was blamed for the decline on kittiwake feeding pray. Soon after its closure some years latter, the population seemed to recover but after a couple of years the population started a decrease that was more pronounced each year (Wanless et al., 2007). Braun & Hunt (1984) proposed that the amount of food was directly influencing offspring conflict in kittiwakes and the results from this thesis seem to confirm their proposal. Chicks fledging rates were very different depending on the food availability of each year (**chapter III**).

Perhaps it is humanly impossible to do anything to influence the sibling rivalry on a population of between 4,000 and 7.000 breeding pairs. It will be absurd to think that any manipulation of the broods of the island is feasible and could provoke a change on kittiwake numbers. However, understanding the factors influencing sibling rivalry could help to detect changes on the pattern of egg production and kittiwakes' adoption of different strategies in order to maximize their fitness and cope with conditions that can be extremely severe. For example, the recent observed switch in feeding items from parents to their chicks. Normally parents feed their chicks mainly with sandeels (*Ammodytes marinus*) (Lewis et al., 2001) but in recent years, an alarmingly increased in Sneak Pipefish (*Enteleurus aequoreus*) as a feeding item has been recorded (Harris et al., 2007). It could be that in a near future kittiwake mothers will stop laying two eggs because it will save them from investing resources on a chick that never serves as insurance and that has null possibilities of surviving if the actual trend on food availability continues.

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