Consequences of Competitive Asymmetry in Broods of the Black Guillemot *Cepphus grylle*

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Presented in candidature for the degree of Doctor of Philosophy, to the Faculty of
Science, University of Glasgow. December 1999.

I dedicate this thesis to the memory of my father, Richard Cook, whose enthusiasm for the natural world set me in this direction.

ACKNOWLEDGEMENTS

I gratefully acknowledge the help I have received from a great many people during the course of this project. Firstly I would like to express my sincerest gratitude to all the inhabitants of Papa Westray, for making my stay on their island such a memorable experience. A few of the islanders, however, deserve a particularly special mention. The field work would not have been possible without the Rendall family, Neil, Jocelyn and John, to whom I am extremely grateful for allowing us to work on their land, and for all their wonderful hospitality. My sincerest thanks go also to the Rendalls of Daybreak and Backaskaill, the McNabs, the Millers, all the workers in the Co-op, and the Hewitson family. The latter family rarely failed to come out to get a ball kicked at them and thus deserve my very special thanks. Most of all, though, I would like to thank Jim Davidson, who not only supplied us with our boat and logistical support, but most importantly because he was such a very good friend to us as well. I also have fond memories of Stuart Groat, who kindly supplied us with fresh crabs and cheerful chat; and Tommy, for his friendly, if pungent and incomprehensible visits in his tractor. The island will sadly miss these two characters.

I would also like to thank all the undergrads: Duncan Falconer, David Hughes, Chris Roger, Fiona McPhie, Linda Wilson and Dan Gates, who not only provided invaluable assistance in the field but were also a source of continual amusement. I particularly thank David Hughes for going beyond the call of duty on more than one occasion.

Most of all I thank Tom Sawyer and Rob Field. Not only did they provide invaluable assistance in all aspects of fieldwork, but they also provided incredibly amusing company and damned good curries. I learned a great deal from these two and my time on Orkney would not have been the same without them!

Back in Glasgow I would like to thank Richard Griffiths, Bob Dawson and Kate Orr for all their invaluable experience in helping me get through the trials and tribulations of determining chick sex. I also thank Martin Burns for all his advice and help in setting up the CCTV system and other aspects of the electronic side of the project. Other members of the department have also been very helpful. Ruedi Nager in particular has always been extremely glad to lend a willing hand with statistics and his enthusiasm for ecology is nothing less than infectious. I must also thank all my friends and colleagues in the department for making the place the friendly and stimulating environment it is today. In particular I mention Francis Daunt Dave Stevens, Alexandra Hay and landlady of the Millennium, Kate Greaves. Most of all, however, I would like to thank Pat Monaghan, who has been nothing less than a perfect supervisor. I consider myself to have been extremely fortunate to have worked under her supervision and have gained a great deal from her considerable experience.

Above all, I thank Deacem Hamilton for being such an incredible distraction.

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ABSTRACT

I examined natural variation in the reproductive strategy of the black guillemot, with particular emphasis on the consequences for nestlings. Data were collected from a population of c. 65 breeding pairs on the Holm of Papa Westray, northern Scotland.

Temporal differences in reproductive strategy are likely to reveal the reproductive constraints most pertinent to a species, exhibit how reproductive costs are manifest, and highlight the potential trade-offs selected to maximise reproductive success in the face of such costs. Constraints appeared to operate both at the egg production and chick rearing stages, with annual and seasonal effects influencing egg size, chick growth and survival. However, females did not alter the allocation of resources between eggs, nor the degree of hatching asynchrony, suggesting that females maintained the level of competitive asymmetry within the brood, despite these constraints.

The relationship between egg size and breeding success was investigated. Chick hatching size and quality were positively related to egg size, but I found no effect of egg size on chick growth or survival. Within clutches, first-laid eggs (a-eggs) were larger than second-laid (b-eggs), but egg-size disparity decreased with decreasing a-egg size. Thus females producing large eggs (i.e. potentially higher quality females) appeared to be striving for within-clutch egg size disparity, inferring that such a difference might be adaptive in the context of sibling competitive asymmetry. Paradoxically, however, egg-size disparity exhibited no relationship with hatching asynchrony. Egg size was a significant predictor of hatching success in the b-egg, suggesting that egg viability decreases once a certain minimum size was attained.

I also examined breeding success in relation to natural variation in hatching asynchrony. At all levels of hatching asynchrony, a-chicks attacked their siblings more frequently than vice versa. Consequently, a-chicks in asynchronous broods grew faster, reached higher asymptotic weights and were more likely to survive to fledging than b-chicks. No such differences were evident between siblings in synchronous broods, despite a-chick aggressive dominance in these broods. Overall, broods hatching with a two-day hatching interval achieved the highest breeding success.

I investigated food amount and competitive asymmetry as potential proximate cues for sibling aggression. Parental provisioning rates were experimentally manipulated in broods comprising a range of hatching intervals over a twelve-hour period. Aggression became evident only after parental provisioning rates were experimentally reduced. When parental provisioning resumed, adults did not increase their feeding rate to compensate for the induced food deficit

and the result of sibling rivalry was a change in the allocation of parental deliveries from one of equality to one in favor of the dominant chick. Food deprived chicks from synchronous broods were more aggressive than those from asynchronous broods, suggesting that one benefit of hatching asynchrony in the black guillemot is to establish an efficient competitive hierarchy among siblings which minimizes the need for costly aggressive interactions. Nonetheless, dominance was always established by the chick hatching from the first-laid egg, suggesting that factors in addition to size disparity are important in establishing competitive hierarchies. These results provide the first evidence that short-term food shortage per se acts as an initial trigger for aggression, yet also reveal that the aggressive response is complicated by factors associated with hatching and laying order.

Before the functional significance of hatching asynchrony within any avian species can be resolved, it is first necessary to determine the proximate mechanisms controlling the pattern of hatching. To date, most studies have tacitly assumed that hatching patterns are effected predominantly by parental incubation behaviour. I compared incubation periods of male and female black guillemot (*Cepphus grylle*) embryos to ascertain whether development rates are a function of embryo sex and, if so, the effects of clutch sex-composition on hatching pattern. Chick sex was determined using a molecular DNA technique based on the CHD gene. Laying date and egg mass had no significant effect on incubation period, but eggs containing male embryos developed significantly faster than those containing females. The onset of incubation in relation to clutch completion is variable in black guillemots. Thus, in mixed-sexed clutches where the first-laid embryo is male, hatching asynchrony was attained regardless of the incubation regime employed. These results clearly show that mechanisms in addition to incubation behavior are important in establishing avian hatching patterns.

I also demonstrated that pre-laying maternal allocation varied according to progeny sex and brood sex composition. Furthermore, survival probability was found to depend not only on the sex of the individual and its position in the laying/hatching sequence, but also on the sex of its sibling. Females appeared to respond to this gender related mortality by dramatically skewing the frequency of brood sex compositions in favour of those less prone to mortality.

CHAPTER 1

GENERAL INTRODUCTION

Reproductive tactics frequently vary among individuals of the same population, yet the causes and consequences of such intraspecific variation and how they relate to reproductive success are poorly understood. An important focus in behavioural and evolutionary ecology is investigating the functional significance and population consequences of this intraspecific variation (e.g. Stearns 1989, Williams 1992, Sutherland 1996). Although it has been demonstrated that age and experience are important factors influencing reproductive decisions (for reviews see Ryder 1980, Partridge 1989, Seather 1990), it is likely that individual differences in 'quality' also play a major role in the reproductive strategy adopted. In other words, individuals may differ permanently in their capacity to rear young (e.g. Coulson and Porter 1985), and in the costs they incur in doing so, due for example to differences in breeding strategy. Parents, thus, are likely to adopt a strategy of progeny investment to match their capabilities. Coupled with this is the increased recognition that many avian parents do not necessarily aim to sustain a full brood size all the way to fledging (Mock and Forbes 1995) and thus do not invest in all members of a brood equally, despite their equal coefficient of relatedness. A high degree of intraspecific variation in reproductive strategy is therefore likely to be associated with a corresponding array of fitness effects for the offspring.

In this thesis, I aim to examine the considerable variation in the breeding strategy of the black guillemot *Cepphus grylle*, and how this variation becomes manifest in terms of consequences for the offspring. In short, I will examine: 1) the extent of this variation in the study population, the likely environmental constraints influencing the reproductive 'decision' and the ensuing trade-offs selected to maximise reproductive success; 2) the consequences of both the egg production strategy and the degree of hatching asynchrony for nestlings; 3) the proximate cues promoting sibling aggression, 4) the role of offspring gender in effecting sibling hierarchies, and 5) the facultative parental manipulation of sex allocation.

In the remaining sections of the general introduction I provide an outline of why nidicolous species frequently produce more offspring than they are capable of rearing, and the theory underlying the advantages of inducing competitive asymmetry among siblings. I also summarise some of the means by which parents impose competitive handicaps on their young and the conclusions drawn from studies investigating the consequences of differential parental investment. Finally, I provide a brief introduction to the breeding biology of the black guillemot, and its suitability as a study species.

Initial overproduction in nidicolous young

Nidicolous avian neonates, i.e. those that are reared within a confined nest-space and parentally provisioned until independence, develop extremely rapidly, generally transforming from egg to independence within a matter of weeks. As a consequence, nestlings of such species require vast quantities of parentally delivered food, which is often in critically short supply. The ensuing resource bottleneck generates intense sibling rivalries, which in turn may potentially affect both the direct and indirect components of inclusive fitness. Resource distribution decisions during such competitions may be made through either one or more of the following behaviours; parental favouritism, sibling scramble competitions or sibling aggressive exclusion, depending on phylogeny and a number of ecological precursors (e.g. suitable weaponry and monopolisability of prey; Mock and Parker 1997).

Initially, aggressive interactions among siblings were regarded as clear manifestations of Trivers' (1974) concept of parent-offspring conflict (POC) (O'Connor 1978), the contention being that dominant siblings are selected to sequester a greater proportion of parental investment than the parent is selected to give. What O'Connor failed to consider, however, is that above a certain parental threshold, sibling aggression (or even siblicide) might serve the fitness interests of parents in addition to those of the dominant sibling. If O'Connor's idea is correct, and sibling competition is indeed a true case of POC, then one might expect parental behavioural strategies to have evolved to counter the effects of such aggression. Parental intervention of the dominant sibling's efforts to monopolise resources might include preferentially feeding smaller nestlings or physically interrupting aggressive sibling interactions. Yet, in by far the majority of siblicidal species examined so far, the anticipated documentation of parental interference has failed to materialise. For example, it has been explicitly asserted for the following siblicidal species that parents make no attempt to prevent sibling aggression: Ardeids (Mock 1987), Kittiwakes *Rissa tridactyla* (Braun and Hunt 1983), boobies *Sula neboxii, S. dactylatra* (Drummond et al. 1986, Drummond 1987, 1989, Anderson 1990), pelicans *Pelicanus erythrorynchus, P. occidentalis* (Cash and Evans 1986, Pinson and Drummond 1993) and eagles (Edwards and Collopy 1983). There are, however, a number of anecdotal reports claiming that South Polar skuas, *Catharacta mccormickii*, parents either settle on to chicks (Spelberg 1971), give spurious alarm calls (Young 1968), or, as is the case in a number of other species, separate young within the territory (Skuas and owls: Ingram 1959; and Cranes: Harvey et al. 1969). Further evidence to discredit O'Connor's contention that sibling conflict is detrimental to parental fitness is based on parental provisioning strategies. Both correlational and experimental studies have demonstrated that parents routinely withhold resources from their offspring (Fujioka 1985, Mock 1985, Mock and Ploger 1987), even though starvation forms the basis for most brood reduction systems and additional provisioning would abate sibling rivalries. Parental behaviour of this kind is therefore highly consistent with the idea that parents may be restricted to a set level of expenditure on the current brood.

Thus, in considering the functional significance of why parents often adopt strategies that require a necessary secondary downward adjustment of family size, the focus is increasingly shifting towards the long-term fitness consequence of the parents. One of the most influential developments in life history theory, the 'Demographic Theory of Optimum Reproduction' (Williams 1966), suggests that costs incurred at one stage of the life cycle must be repaid elsewhere. Hence, if critical resources are limited, any increase in current reproductive effort is likely to limit the investment available for somatic investment (e.g. growth, homeostasis, predator avoidance and immune function), thereby reducing future fecundity. Thus, a trade-off ensues between current reproductive output and residual reproductive value, which in turn is optimised by natural selection. Life-history models have demonstrated that only a very small improvement in parental survival probability is required to compensate for offspring mortality (Charnov 1982, Mock and Forbes 1994). Thus, in long-lived species, high investment in current offspring is rarely favoured because a small reduction in adult survival will reduce the number of subsequent breeding attempts (Curio 1988).

Therefore, in the interests of reserving residual reproductive value for investing in future offspring, it appears that parents do not necessarily intend rearing all members of a current brood to independence. This is particularly striking in obligate brood-reducing species, such as various raptor species in which two-eggs are laid but only a single chick is fledged (e.g. Gargett 1967, 1970, Brown et al. 1977), and even more so in species that practice infanticide (Urrutia and Drummond 1990, St. Clair et al. 1995). However, this still leaves us with the glaring questions as to why parents routinely overproduce in the first place, and what benefits accrue to both parent and senior sibling by sacrificing such a large proportion of inclusive fitness? Indeed, at first sight, this overproduction seems a maladaptive waste of essential parental resources, yet parents stand to gain a number of potential benefits from such behaviour (Kozlowski and Stearns 1989, Mock and Forbes 1995). Firstly, by initially over producing, parents can profit if environmental conditions prove to be better for rearing a brood than originally expected. (This, incidentally, provides the basis behind Lack's (1947, 1954) brood reduction hypothesis to account for the phenomenon of hatching synchrony.) Secondly, certain members of the brood may stand in as an insurance/backup if certain core members prove to be flawed or die (otherwise known as the 'replacement offspring hypothesis', Mock and Forbes 1995). Finally, extra progeny may assist core brood members. This help is usually considered within the context of avian cannibalism, although in other taxa forms of help such as those exhibited in eusocial insects are important. Thus, as a consequence of environmental stochasticity, the potential parental benefits of laying an optimistic clutch may outweigh the costs of initial over-investment. These benefits may be enhanced by features that facilitate brood reduction at a later date, as and when required. Assuming that a brood consists of a core set of offspring (i.e. the number of offspring expected to fledge), plus a number of marginal offspring, parents are likely to gain fitness benefits by maintaining the marginal offspring in a state that does not threaten the core. This perspective accounts for many levels of parental favouritism, and demonstrates that parents do not value all offspring equally. Indeed, it appears that fatal sibling competition may often enhance parental fitness (Mock and Forbes 1992, 1994, Drummond 1993, Forbes 1993, Godfray 1995).

Strategies that impose handicaps on certain brood members take many forms, ranging from frequently studied phenomena such as asynchronous hatching (Lack 1954, Ricklefs 1965), egg size (Schifferli 1973, Howe 1978, Ricklefs et al. 1978, O'Connor 1979, Birkhead and Nettleship 1982) and brood sex ratio adjustment (Trivers and Willard 1973, Howe 1976, Fiala 1981), to less persued topics such as preferential feeding of certain offspring (Boersma and Stokes 1995), differential allocation of maternally derived steroids within the clutch (Schwabl 1993, 1996, Schwabl et al. 1997), and egg quality (Gill et al. 1999, Lipar et al. 1999, Monaghan (in press). However, by far the most prevalent handicap employed by brood reducing species appears to be hatching asynchrony. By incubating prior to the completion of laying, parents give the first produced embryos a head-start that leads to hatching asynchrony and a consequent size hierarchy among brood-mates. Because it appears that parents can control the hatching span, its effects on their fitness (as measured by the growth and fledging rates of offspring) have been used to evaluate whether the observed levels of hatching asynchrony are beneficial. This has been pursued primarily through experimental swapping of chicks between nests, designed to reduce or eliminate size hierarchies. The major conclusion derived from a large number of experimental studies on hatching asynchrony is that asynchronous broods are no more successful than synchronous broods (Amundsen and Slagsvold 1991, Stoleson and Beissinger 1995, Mock and Parker 1998), even when post-fledging mortality is accounted for (Lessells and Avery 1989, Magrath 1989, Harper et al. 1992). However, a few studies in which the swap protocol was accompanied by sampling of parental effort uncovered a complicating artefact. For some reason parents supply considerably more food to artificially synchronised broods than to controls. This not only shows that such parents routinely withhold available food from their families, but it also seriously complicates the interpretation of studies in which parental effort was not monitored because accelerated food deliveries are likely to inflate nestling growth and survival. The few studies where hatching interval has been evaluated in terms of its impact on parental efficiency have shown that parents fare better with asynchronous hatching (e.g. Mock and Ploger 1987, Osorno and Drummond 1992, Machmer and Ydenberg 1999).

How birds manipulate breeding effort in relation to environmental and individual 'quality' is poorly understood. The investment strategy employed might have been a result of non-adaptive constraints or, alternatively, selected either to decrease the reproductive costs of the parent or to increase nestling survival probability. Either way, regardless of whether the resulting asymmetry is the cause or effect of the parental strategy, the consequence is often an unfair advantage to certain members of the brood and the reproductive values of brood members are no longer equal. As Mock and Parker (1998) eloquently state, "the nursery can simultaneously be a haven from malevolent extrinsic forces such as predators or abiotic threats, and a trap where victims may be treated as expendable, possessing only ephemeral or conditional value to more powerful family members".

THE BLACK GUILLEMOT

Reproduction is costly in the black guillemot

Although the relationship between egg size and body weight in the black guillemot is comparable to that of other auk species, it is unique among Atlantic alcidae in that the common clutch size is two eggs rather than one. Consequently, overall clutch investment is approximately double that of other species. Although it has been suggested that the short travel time of the inshore foraging black guillemot means that food is relatively easily attained (Birkhead and Harris 1985), recent evidence suggests that on average black guillemots travel equally as far to forage sites as more oceanic feeding species (Sawyer 1999). Indeed, production of a second egg evidently requires considerable effort in some birds, with young birds tending to lay only a single, relatively small egg (Asbirk 1979, Ewins 1989). The interval between laying the first and second egg is on average 3 days, but can be up to 10 days (Asbirk 1979, Peterson 1981, Ewins 1989). During this interval the birds obtain important nutrients to put into the second egg (as is evident from an observed correlation between the duration of the inter-laying interval and the subsequent size of the b-egg relative to the a-egg; the longer the interval, the larger the b-egg tends to be (Ewins 1986)).

Unlike the majority of avian species, which incubate more or less continuously until hatching, black guillemots are unusual among birds in that even older, experienced pairs laying two-egg clutches vary the onset of incubation in relation to clutch initiation. Furthermore, although both parents share the costs of incubation, many pairs take extended breaks throughout the incubation period (Petersen 1981). Indeed, black guillemot eggs are incubated on average for only 84% of the time (Cramp 1985), and are extremely resistant to chilling ((up to 15d of continual neglect; Bergman 1971, quoted in Harris and Birkhead 1985). These adaptations are extreme among birds, matched only by certain procellariform species (Boersma 1982) and it is likely that they are a response to the potential inability of the parents to sustain continuous incubation.

In order to raise two chicks black guillemots clearly need to make more foraging trips than alcids raising only a single chick. They achieve this by feeding close inshore and (in comparison with ledge-nesting species) by leaving the chick unattended, thereby freeing both parents for simultaneous provisioning. The rate of food delivery to chicks is highest between 2-3 weeks of age and by this stage, broods of one chick receive more feeds per chick than do broods of two; up to 10% of chicks starve to death at this age, and by three weeks chicks in broods of two are growing at a slower rate, and have a lower peak rate, than those in broods of one (Cairns 1981, Ewins 1986).

Variation in breeding strategy

The black guillemot Cepphus grylle is unusual among birds in that it exhibits extraordinarily large differences in reproductive strategy, particularly in the pattern of progeny development. For example, not only is it unique among Atlantic Alcidae in that 80% of breeding females produce two eggs rather than one (Asbirk 1979, Ewins 1989), but individual pairs differ considerably in the time taken to produce the same clutch size, to initiate incubation, and to hatch and fledge their offspring (Petersen 1981, Ewins 1986). Moreover, variability in incubation pattern results in marked differences in hatching patterns, ranging from synchronously hatched broods to those with a hatching span of ten days. Such variation in the extent to which the first egg is incubated also occurs in the congeneric pigeon guillemot *Cepphus columba* (Drent 1965). The parents thus determine the extent to which the chicks hatch synchronously or asynchronously. The result of staggered hatching is a size and age hierarchy within the brood, where older and larger nestlings may potentially outcompete their younger siblings for parental provisions. In addition to hatching asynchrony, nestling size hierarchies may also be affected by intra-clutch variation in egg size. Depending on how these two variables interact, the potential exists for considerable variation in competitive asymmetry among siblings.

This variation in reproductive strategy therefore provides an excellent opportunity to examine the consequences for the chicks in terms of the level of sibling conflict and begging behaviour, the distribution of food within the brood, growth parameters, and survival rates until fledging. The majority of research on hatching patterns has focused on the adaptive significance of hatching asynchrony with respect to the parent. By contrast, this project aims to focus attention on the consequences for the chicks at different degrees of competitive asymmetry.

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CHAPTER 2

GENERAL METHODS

1. STUDY SITE

Data presented in this thesis were collected from the Holm of Papa Westray, Orkney, Scotland (59° 22'N, 2°53'W) from the beginning of May through August, 1994 to 1997. The Holm is approximately 0.8 x 0.3 km, relatively low-lying with the black guillemot colony situated among the low-level cliffs and boulder beaches of the NE shore. The island is uninhabited by humans and used largely for low-intensity sheep farming and tourism. Mammalian predators are absent from the island, although the occasional Eurasian otter *Lutra lutra* has been reported taking eggs and nestlings (personal observation). Other potential predators and kleptoparasites on the island include c. 100 pairs of lesser black-backed gulls *Larus fuscus*, 60 pairs of herring gull *Larus argentatus* and 35 pairs of great black-backed gulls *Larus marinus*. In addition, great skuas *Catharacta skua* and arctic skuas *Stercorarius parasiticus*, from nearby Papa Westray, are frequently seen patrolling the black guillemot colony during the breeding season. Black guillemots on the Holm of Papa Westray have been monitored, at various intensities, for the past two decades; Glasgow University has collected breeding data annually from 1992 to 1997, and chicks have been ringed by the RSPB since 1982. Although the population during the 1990's has remained relatively stable at approximately 65-70 breeding pairs, this represents a declined since the 1980's when the Holm supported approximately 100 breeding pairs (Gray 1987).

The Holm lies approximately 1km to the east of Papa Westray and access to the island during the study was thus by boat. The boat used was an 18ft dory powered by a Yamaha 30hp outboard, and carried spare fuel, flares, an anchor, a marine VHS, tools, and sufficient life jackets for all crew. The island was visited daily, weather permitting. A base camp was set up on the island using a wooden shed securely staked and lashed to the ground, in which all electronic equipment and weather sensitive equipment was stored and behavioural monitoring took place.

This PhD thesis predominantly uses data collected by the author during the 1996 and 1997 breeding seasons. However, in places, it will also incorporate data collected in 1994 and 1995 by Paul Walton. Unless otherwise stated, the methods described here were employed during the field seasons of both 1996 and 1997, which follow closely those employed in 1994 and 1995.

2. THE PRE-BREEDING PERIOD

Adult black guillemots breed in loose aggregations, nesting cryptically within boulder caves on rocky shores, among cracks in cliffs, and occasionally in disused rabbit burrows. The largest concentration of nests were situated at the north end of the island, which contained the largest area of boulder beach. During the pre-breeding season (early May), occupied nest sites from previous years were relocated and potential new nest sites were sought by a series of morning hide watches. All nest sites were numbered with unobtrusive white paint (i.e. similar to guano prevalent on the island) and, where necessary, boulder cave nests were slightly modified at an appropriate point to allow easy access to the chicks. We ensured that the entrance to each of these man-made access points was suitably covered with a stone slab to prevent predation and that the internal structure of the nest cavity was not altered. In order to limit disturbance to the adults during egg laying and incubation, we also installed and set-up the camera system in all suitable nests at this stage (see section 5 of this chapter).

3. EGG LAYING

As in many species of bird, excessive disturbance of black guillemots, particularly during incubation, can lead to reduced breeding success. All nest visits during incubation and early post-hatching therefore occurred during the period of low adult attendance, i.e. between 12.00 and 17.00h BST (Ewins 1989). Most adults would vacate the nest chamber when disturbed, returning to the nest within minutes of our departure from the area. If an adult remained at the nest, the nest was left unexamined. Particular care was taken not to disturb a sitting adult during the first few days of incubation or if recently hatched chicks were being brooded. All visits to nests were as brief as possible, nest checking in any particular area of the colony taking no longer than 20 minutes.

Nests were checked daily for the presence of an egg so that the laying date of each egg and laying interval for each clutch was obtained. On the day of laying eggs were measured (length [L] and breadth [B]) to the nearest 0.1mm using Vernier callipers and weighed to the nearest 0.1g using a 200g electronic balance. Egg volume (V) was determined from the equation: $V=0.51LB^2$, in which V=egg volume (mm^3), B=breadth or maximal diameter (mm), and L=length (mm) (Hoyt 1979). Each egg was marked according laying sequence using a permanent marker pen ('a' or 'b' for the first and second laid eggs, respectfully); if the laying order was not known, eggs were randomly marked '1' or '2'.

On completion of the clutch, nests were undisturbed until a few days before estimated a-egg hatching date (eggs are incubated for c. 28 days; Ewins, 1986), whereupon daily nest checking resumed to determine hatching dates, hatching interval and incubation period. In those nests where I considered clutches might hatch synchronously, I undertook more frequent checks to ensure that the egg origin of each chick was ascertained. (Since the first signs of hatching can be seen at least two days before the chick emerges from the shell [Preston 1968, from Harris and Birkhead 1985], we were able to approximate the level of hatching asynchrony prior to hatching.) The first signs of hatching can be seen at least two days before the chick emerges from the shell and the chick is not usually dry until a day after hatching. I was therefore able to estimate hatching times of these chicks to within 24 hours using this method, even if hatching was missed by a day or two.

All eggs hatched according to laying order and a note was made of the egg from which each chick hatched. Within each brood, siblings were referred to as either a- or b-chick. To facilitate sibling identification, the a-chick in each brood was regularly marked on the head with a small streak of correcting fluid (Tipp-Ex®). This mark looked similar to faecal spots that regularly appeared on nestlings. Since the egg origin of each chick was established, we therefore knew the laying order, sex and development time for each egg laid.

4. CHICK GROWTH AND SURVIVAL

Where possible, chicks were weighed on hatching (using a 200g electronic balance) unless brooded or still too wet to remove from the nest. A note was also made of the egg origin of each chick. Chicks in all study nests were checked approximately every three days (depending on weather conditions) when chick mass and a number of other biometric measurements were recorded. I also attempted to measure chick mass at day 12 post-hatching, when possible. Each measuring period began at approximately 10.30 hours, the period when adult provisioning is lowest (Paul Walton, pers. com.). Body mass was measured to the nearest 1g using a 200g electronic balance or 500g spring balance, depending on chick mass. Right and left wing lengths (maximum flattened chord, from the carpal joint to the tip of the longest primary feather) were measured to the nearest 1mm using a stopped rule, and right and left tarsometatarsal length (tarsus) and head plus bill length were measured with slide callipers to an accuracy of 0.1mm. To minimise the risk of adult desertion, chicks were not removed if an adult was present in the nest. Where possible, nests containing chicks approaching 25d were checked and weighed daily until fledging to determine maximum weight, weight at fledging and fledging date. Obtaining this data often proved difficult due to time constraints on nest checking during the peak fledging period.

Moreover, a large number of chicks 'disappeared' in the week prior to fledging, usually as a result of great black-backed gull predation. 'Fledging' is defined here to mean surviving until at least 30 days post-hatching. That is, after the maximum age at which asymptotic mass is reached and thereby beyond the period of maximum food requirements. Mortality was categorised as follows: 'predation' (where predation was either observed directly or evidence such as blood or gull pellets were observed in close proximity to the nest); starvation (nestling obviously malnourished); or unknown (where chick mortality could not be established). During 1994 and 1995, Paul Walton recorded that b-chicks believed to have starved frequently displayed injuries to the head and neck suggesting that siblicide is prevalent in some nests. However, the cause of mortality, whether it was directly a result of aggression or starvation, could not be inferred.

Numerous nests were discovered after laying or hatching had taken place, and thus complete breeding data is not available for all nests.

5. CHICK BEHAVIOUR

Since direct observations of behaviour inside the nest cavity were not possible, we developed a system of miniature CCD charge-couple cameras with infra-red LED illuminators linked to CCTV and Sony-walkman® video recorders. These cameras provided an infra-red light source and were therefore capable of monitoring chick behavioural interactions and parental provisioning within the dark confines of the nest cavity. Suitable nest sites (i.e. those that were in range of the CCTV system and safely accessible) were located and the camera system installed before egg laying commenced. Using a simplified version of this system, pilot data from 1995 showed that nestling behaviour and adult provisioning could be quantified.

Sibling behavioural data were recorded from a maximum of six nests consecutively using the CCTV micro camera system: four cameras were linked to monitors in a hut 150m from the colony where direct observations were made, and two were linked to Sony video walkman recorders at the nest site for analysis at a later date. Data was recorded continuously for either a three-hour or twelve-hour period (more details on the monitoring protocol are given in the relevant chapters).

Specified behavioural events and states were recorded directly on to data sheets using the focal-animal sampling technique and instantaneous sampling technique (Altmann, 1977). Aggression was quantified in terms of the number of attacks (violent pecks, jabs or grasps) that each chick directed at its sibling, each individual attack being recorded as a discrete event. Agonistic behaviour was recorded in terms of both the attack intensity of the aggressor and its sibling's response.

Preliminary video recordings in 1995 enabled attack behaviour by the aggressor to be categorised into low intensity and high intensity aggression (hereafter called LI and HI aggression, respectively) before fieldwork commenced. LI aggression was employed by either sibling and entailed the aggressor facing its sibling and inflicting insubstantial, non-injurous pecks about the bill.

HI aggression was employed exclusively by the larger sibling and usually involved several bouts of violent pecking and tousling of the smaller chick, usually about the nape of the neck, face or back of the head. The recipient chick tended to assume a submissive posture and rarely attempted to retaliate or evade its attacking sibling. Submission from HI attacks was characterised by crouching as low as possible, any attempt to raise the head often being met with further bouts of aggression. B-chicks frequently died in unmanipulated nests where parental provisioning was poor, although the cause of death, whether directly through aggression or indirectly through starvation, could not be established. The response of the attacked chick from both levels of aggression was graded into three categories: 'evasive' if it attempted to avoid the aggressor; 'submissive' if it showed no active response to the aggressor; and 'defensive' if it retaliated. The intensity of the defensive retaliation of the attacked chick was recorded using the same two categories as that of attacking behaviour. A fight bout usually consisted of a series of blows between siblings: two blows were considered to be part of the same bout if they occurred within 10 seconds of each other. A nestling was considered to have won a bout if its sibling was evasive, submissive or attacked/defended at a lower intensity. State behaviours recorded include: being brooded, resting, preening, standing, walking and exercising wing muscles.

Adult black guillemots transport whole prey to the nest crosswise in their bills, and on entering the nest chamber food allocation is determined largely by scramble competition; the sibling that first reaches the provisioning parent generally receives the prey item. Thus, only one sibling is fed during each parental delivery. A record was made of the total number of parental deliveries to each nest and the recipient chick of each feed. In addition to data on sibling behavioural interactions, details of parental provisioning and food procurement success of each chick were collected during each observation period. The following information was obtained on each feeding occasion: (1) the recipient chick of the parental delivery, (2) the inter-sibling distance (cm) at the time of the parental delivery, (3) the relative position of siblings to the burrow entrance immediately prior to the parental delivery, (4) the foraging behaviour of each chick in terms of securing a parental delivery (behaviour was recorded as either: i) no movement, ii) walks, or iii) runs towards delivering parent), (5) the length and species of the prey item (length calculated in relation to the length of the adult's bill) (6) whether competition occurred between siblings over parental deliveries and the outcome of such interactions, and (7) whether chicks were temporarily satiated due to the presence in the crop of previous feeds.

The parents behaved quite naturally in response to the presence of the cameras, feeding nestlings on average once per hour, which corresponded to the adult provisioning rate in camera free nests (obtained from hide watches; see later). However, when video recorders were left too close to a nest, a number of parents appeared visibly stressed and data from these nests were thus omitted from calculations.

6. ADULT PROVISIONING

Monitoring only growth and survival of nestlings is insufficient for assessing the consequences of asymmetry because nestlings in asynchronous broods may appear to develop as well as those of synchronous broods if the parents are working harder to provide for the former brood type. It was therefore necessary to measure both chick growth and food supply rate.

Adult provisioning data was obtained by a series of hide watches on approximately 30 nests. Two 3-hour watches took place each day, on six successive days out of seven, throughout the duration of the chick rearing period. On each watch day approximately 14 nests were observed; one group of seven nests during the morning and another during the afternoon. On the following day the order in which the two sites was observed was reversed. Every two days a different group of 14 birds were observed.

Adult provisioning rates are a reasonable reflection of the work rate or energy expended by the parents, but are not an accurate measure of the biomass or energy provided to the brood, i.e. some parents might deliver a greater number of smaller, less nutritious prey. It was therefore necessary to determine the length and species of fish each parent returned to the nest with, thus providing an approximation of the energy supply each nestling and each brood received.

Adult black guillemots transport prey to the nest crosswise in their bills, thus it was possible to record the species and estimate the length of the fish carried by each adult. Fish lengths were estimated using the length of the adult bill as a guide (adult bill length is approximately 30-35mm).

7. IDENTIFICATION OF SEX

As in mammals, avian sex is determined chromosomally, but in birds it is the female rather than the male that is the heterogametic sex (females WZ, males ZZ). Chick sex was ascertained in 1996 and 1997 using a molecular DNA technique based on the presence or absence of the highly conserved W-linked (female specific) CHD1 gene (chromodomain-helicase-DNA-binding protein W-linked) using genomic DNA isolated from blood samples (live chicks) or muscle samples (dead chicks; after Griffiths et al. 1996).

Blood extraction

Blood samples for molecular sexing were obtained under U.K Home Office licence from 5-20d old nestlings by means of tarsal veinipuncture using a sterile hypodermic needle. Approximately 50ul of blood was transferred via capillary tube to a 1.5 millilitre (ml) “eppindorph” tube containing an equal volume of EDTA buffer (“Bill’s lethal brew”: 2% sodium dodecyl sulphate (SDS), 50mM EDTA, 50mM Tris (pH8)). Such buffers are required for the preservation of blood samples because nucleases naturally present in the blood break down DNA into fragment lengths inappropriate for analysis (Suetin et al. 1991). This catabolic activity is considerably reduced by EDTA, a major constituent of these buffers (nucleases are divalent cation dependent enzymes). Blood samples were kept cool in the field, placed in a refrigerator within 5 hours of collection and stored for up to 15 months prior to sexing. No chick mortality occurred within three days of blood sampling.

A number of chicks died prior to blood sampling. In such incidences, the corpse was frozen and the muscle tissue used subsequently as a source of DNA. The protocol for the extraction of muscle DNA followed that of the blood samples but with slight modifications: muscle tissue required at least 48h in proteinase K for complete digestion.

DNA extraction

The extraction of DNA from black guillemot blood samples was based on that of Wetton (1990), with slight modifications.

Sample Preparation

1. Approximately 10 microlitres (µl) of each blood sample was placed into a 1.5 millilitre (ml) eppendorf microcentrifuge tube and resuspended in 200µl SET buffer (100mM NaCl, 1mM EDTA, 100mM Tris Cl {pH8}). Dilution of the blood in this way prevents DNA overload. To aid transference of blood solutions that had become viscous during storage it was necessary to use cut pipette tips. Such tips also minimise any damage to DNA caused by its repeated movement through narrow-bored tubes.

2. For degradation of potentially DNA-damaging enzymes and lysis of cell membranes, 8µl of 10mgml⁻1 proteinase K stock solution (stored in –20°C freezer) and 20µl of 10% SDS (sodium dodecylsulphate) detergent were added, respectively. The solutions were mixed on a rotary mixer and incubated for 12-hours in a rotary incubator at 55°C.

Extraction of DNA for Polymerase Chain Reaction (PCR)

1. An equal volume (approx. 200µl) of 0.6mM buffered phenol solution was added to the blood sample and mixed by shaking for 20 minutes.

Note: if purchased buffered phenol is unavailable it may be prepared as follows. Phenol crystals are dissolved in an equal volume of 1M Tris.HCl pH8.0. A few crystals of 8-hydroxyquinoline are added to assist in preventing the rapid oxidation of phenol, and also act as a colour change indicator (yellow through brown to pink). Such stocks should be discarded if not used after three days. After the phenol crystals have dissolved, half of the lower phenolic layer is removed and added to an equal volume of chloroform/iso-amyl alcohol. The phenol/chloroform settles out as the lower of two layers and is left to settle overnight. The solution is best used within a week.

2. To separate the upper (aqueous) and lower (phenolic) layers the samples were centrifuged in a bench-top microcentrifuge at 14000rpm for 5 minutes. Precipitates such as proteins and

cellular debris were retained at the interface whereas soluble nucleic acids remained in the aqueous layer.

3. The supernatant was transferred to a sterile pre-labelled eppendorph tube using a cut 1 ml plastic pipette tip. Care was taken so as not to disrupt the interface layer, thus minimising the transference of precipitates.

4. Stages 1-3 were repeated twice, substituting phenol for chlorophorm. For the first repetition an equal volume of 1:1 phenol:chloroform (approx. 100µl of each) was added to the transferred aqueous solution but centrifuged for only 5 minutes. To the next aqueous sample, an equal volume of chloroform (approx. 200µl) was added and centrifuged for 2 minutes.

Note that if the sample is required for restriction enzyme digestion (DNA fingerprinting or cloning) it is essential that no precipitates are visible after the phenol extraction stage. If this is so, then the above three steps should be repeated. If the sample is required for PCR, then the phenol extraction should only be repeated if excessive precipitates are visible.

5. The resultant aqueous layer was transferred to a sterile eppendorf tube. To precipitate the DNA two volumes of cold (-20°C) 2M ethanol was added to the aqueous solution plus 0.1 volume sodium acetate, then shaken vigorously by hand and placed in a freezer for thirty minutes. The DNA precipitate was visible as a white "stringy" mass.

6. To pellet the DNA, the DNA precipitate was centrifuged at 15000g for eight minutes in the microcentrifuge. The ethanol was poured off, any excess being removed with a disposable pipette tip, and the pellet then dried on a hot block. After drying, approximately 400µl of 70% ethanol was added to remove salts, then shaken gently and centrifuged at 14000rpm for 3 minutes then poured off and dried again.

7. Once dry, the pellets were resuspended in approximately 20-50µl of sterile TE buffer (0.01 M Tris, 0.01M NaCl, and 2 mM sodium-EDTA). Resuspension was facilitated by repeated careful pipetting followed by overnight incubation at 55°C in a waterbath. TE buffer contains EDTA which prohibits the action of nucleases by binding to Mg ions. DNA polymerase during PCR requires the presence of Mg ions and it was therefore necessary to dilute 10 fold twice.

8. The resuspended DNA solutions were then stored in a refrigerator (4°C) until required for PCR.

Amplification of DNA by polymerase chain reaction

The W version of the CHD gene contains more base pairs and thus has a higher molecular weight than that of the Z version. Hence, chick sex can be ascertained based on the size of a fragment of the gene where the difference occurs. In this part of the process, DNA primers are used to mark the appropriate fragment of the CHD gene, the section is then cleaved using a restriction enzyme and finally amplified by means of the polymerase chain reaction (PCR).

A 'Technone genius' was used for the PCR amplification of genomic DNA. Reaction volumes (10ul) were made up of the following master mix in a sterile fume cupboard: 1.0μ Promega Taq Buffer (1x is 50mM KCL, 10mM Tris.HCL, 1.5mM $MgCl_2$, 0.1% Triton X-100), 0.8ul each of the two primers P2 and P8 (P2: 5'-TCTGCATCGCTAAATCCTTT), 0.075μ Taq DNA polymerase, 0.8μ dNTP's, and 5.525μ double distilled, sterile H_2O. Added to this was 1.0μ black guillemot DNA solution.

In addition to the black guillemot reaction volumes, 3 control reaction volumes were also made up: a negative DNA control (i.e. a sample of the extraction process undertaken without the DNA sample) to ensure no contamination occurred during DNA extraction; a positive DNA control consisting of a chicken sample of known sex to show that the PCR worked; and a negative master-mix control to ensure that no contamination was present in the master-mix.

The PCR reaction was run as follows.

Prog. 2:	1'30s @ 94°C	*1 cycle	to denature DNA
Prog. 3:	30s @ 50°C		
	30s @ 72°C	*32 cycles	denature & synthesis
	20s @ 94°C		
Prog. 4:	60s @ 50°C	*1 cycle	5'00 @ 72°C 30
Prog. 1:	1 soak file @ 4°C		

The PCR products were subsequently electrophoresed in agarose gel containing ethidium bromide using 1xTBE buffer at 80V and photographed under UV light. Female samples produced two DNA fragments of different molecular weight (W and Z fragments), whereas males produced only one (the Z fragment).

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CHAPTER 3:

TEMPORAL PATTERNS OF REPRODUCTION: ANNUAL AND SEASONAL EFFECTS

INTRODUCTION

In his pioneering work almost fifty years ago, Lack (1966, 1968) suggested that many aspects of avian reproduction are adapted to ecological circumstance, such that parents produce the maximum number of offspring. Moreover, Lack also postulated (Lack 1947, 1954, 1966) that, in those species restricted to a single breeding episode per year, timing of breeding should correspond with the most favourable period, primarily when resources are most available. Even at tropical latitudes, many birds show pronounced seasonal breeding, and it is generally accepted that most species predict and prepare for a forthcoming breeding opportunity in order to maximise reproductive potential. To initiate breeding at the appropriate moment, birds use environmental cues: day-length, affecting hormonal cycles is the primary proximate cue for the initiation of breeding (Murton and Westwood 1977), with environmental factors and nutritional condition causing small-scale adjustments within the broad pattern set by inheritant responses to day-length. However, although several components of fitness are increased by early breeding in temperate areas, many species appear not to lay at the most appropriate date, exhibiting a marked decline in breeding success as the season progresses. For instance, clutch size often declines as the season progresses, as do other reproductive parameters, such as egg size, hatching success, growth rates, fledging success and offspring recruitment. Such date effects on reproductive success have been reported for many seabird species (e.g procellariformes: Harris 1969, Ollasson and Dunnet 1978; gulls: Weidmann 1956, Coulson and Porter 1985; terns: Ashmole 1962, Morris 1976, Lanham 1974; alcids: Nettleship 1972, Harris 1980, Birkhead and Nettleship 1982) and, more specifically, for the black guillemot (Petersen 1981).

In addition to seasonal effects, annual differences in breeding parameters are also common among seabirds (Coulson and Thomas 1985, Cairns 1987, Monaghan 1996). Changes in prey availability, or inclement weather are likely to be the major determining factors influencing yearly variations in breeding success in temperate regions. Climatic conditions may affect prey availability and/or the energy budgets of parents so that more energy is expended on self-maintenance than on breeding effort.

Recent thinking has attempted to adapt and incorporate Lack's ideals within the context of life-history theory (Cody 1966, Klomp 1970, Hussell 1972, Roff 1992, Stearns 1976, 1992). Optimal life histories are based on the idea of a trade-off between the costs and benefits of competing activities, the outcome of which varies according to ecological circumstance and individual quality. Since temporal variation in breeding strategy is an important component of seabird behavioural and evolutionary ecology, the residual variation induced by temporal fluctuations in environmental conditions is of particular interest as it may be informative as to how birds respond to environmental change. Determining how a population responds to differing constraints and environmental conditions reveals how and at what stage in the breeding episode any temporal costs are manifest, and highlights the potential trade-offs selected to maximise reproductive success in the face of such costs. Furthermore, if such relationships exist, then they might indicate which elements of reproduction are likely to prove most useful for future research into the causal nature of such variation.

In this chapter I have examined the consequences of both annual and seasonal variation in black guillemot breeding success over four successive years, from 1994 to 1997.

METHODS

Method followed those in the general methods chapter, sections 1-4 inclusive.

TERMINOLOGY AND ANALYSIS

Within two-egg clutches, each egg is referred to as a- or b-egg according to the order of laying. Correspondingly, chicks hatching from two-egg clutches are referred to as a- or b-chick. This was related to hatching order in asynchronous broods (a-chick hatches first in broods where the hatching interval is ≥ 1 day), and to the egg from which the sibling hatched in synchronous broods (a-chick hatches from a-egg). Clutches where laying or hatching order were not determined were excluded from analyses. Eggs and chicks from single-egg clutches are referred to as s-eggs and s-chicks, respectfully. Laying date for the clutch is defined as the laying date of the a-egg (in Julian days) which in turn was standardised across years by subtracting the date of clutch initiation from the median laying date of that year.

Data from all four years (1994 to 1997 inclusive) were used for the egg and incubation analyses in this chapter. However, as a consequence of the experimental manipulation of chicks in 1995 by a former PhD student of Glasgow University, chick growth and survival data from 1995 were excluded from analyses. Appropriate parametric statistics were applied where data met the parametric assumptions of normality and homoscedasticity, otherwise relevant non-parametric tests were used. Arcsine transformed data were used for proportional data but means etc. for such data are expressed in the original data units. Normality was tested for using the Kolmogorov-Smirnov goodness-of-fit test and all probabilities given are two-tailed.

Growth was investigated both in terms of the mass instantaneous growth rate during the period of maximum growth (age 5–25d) and in terms of asymptotic weight. Instantaneous growth rate was calculated according to the following equation:

$$SGR = 100 \times \frac{(\ln(W_2) - \ln(W_1))}{t_2 - t_1}$$

where ln is the natural logarithm, and W1 and W2 are the growth parameter (mass), measured at the respective ages t1 and t2. Relationships between growth rate and year were performed for each chick type independently using one-way ANOVA models. Only broods containing surviving siblings on the day of measuring were used in growth analyses. If mortality occurred, then that brood was deleted from analysis from that date onwards. To ascertain whether chick mortality was starvation related, I compared chick weight in the days prior to death with mean chick weight of that chick type (see chapter 5).

I analysed hatching success and postnatal mortality as a function of year using logistic regression analysis. Nestlings were categorised as "fledged" if they survived to 30d (by which age chicks have attained asymptotic mass and mortality by means other than predation was never evident). Since all ratios between explained deviance and the degrees of freedom (d.f.) were close to one, significance tests were based on the χ^2-distribution (Crawley 1992).

Analyses were undertaken using SPSS for Windows release 7 and all tests were as described by Zar (1996).

RESULTS

1. Clutch size

From 1994 to 1997, we measured a total of 477 eggs in 264 clutches: 49 eggs in single-egg clutches and 430 eggs in 215 two-egg clutches. Overall, two egg clutches represented 81.4% of all clutches and the overall mean clutch size was 1.814 eggs ± 0.02 S.E., n=264.

Year effects

No between year difference was evident in the proportion of one-egg and two-egg clutches in the population (χ^2=1.775, df=3, n=264, n.s.), and consequently no yearly difference was found in the number of eggs laid per nest (Kruskal-Wallis test: χ^2=2.47, df=3, n=264, n.s.; table 3.1).

Table 3.1. Mean clutch size, and percentage of single-egg clutches laid during the years 1994 to 1997.

	1994	1995	1996	1997
Mean number of eggs	1.79	1.78	1.87	1.83
S.E.	0.05	0.05	0.04	0.05
(n)	(70)	(72)	(62)	(60)
Percentage single-egg clutches	21.4%	22.5%	12.9%	16.7%

Seasonal effects

Two-egg clutches were laid significantly earlier in the season than one-egg clutches (two-way ANOVA: $F_{1,197}$=21.251, p<0.001). There was no significant interaction between clutch size and year ($F_{3,197}$=0.345, n.s.).

Potentially, loss of the first-laid egg might result in a clutch being mistakenly registered as a single-egg clutch, thereby artificially exaggerating the laying date for that clutch and also increasing the median laying date for single-egg nests. However, egg loss, either through parental neglect or predation, was clearly evident in the majority of cases, and it is therefore unlikely that such an error was made. Over all four years, a small number of breeding pairs (n=12) laid replacement clutches.

2. Laying date

Over all four years, date of clutch initiation ranged between 13 May and 21 June for two-egg clutches, and between 20 May and 16 June in single-egg clutches. The same colour-ringed female laid the first egg of the colony in the same nest in each of the four years. In all years, those birds laying two-egg clutches generally initiated laying earlier than those laying single-egg clutches. The seasonal distribution of laying dates differed among years (see table 3.2 for median laying dates and ranges for each respective year).

Year effects

Table 3.2 shows the median laying date of the three egg-types for each of the four years. For two-egg clutches, laying was earlier in 1994 and 1996, both in terms of date of clutch initiation and median laying date. A one-way ANOVA reveals that the mean date of clutch initiation for two-egg clutches differed significantly between years ($F_{3,161}=7.598$, $p<0.001$). Post-hoc Tukey tests showed that the median laying date in 1994 was significantly earlier than in 1995 and in 1997, and that in 1996 laying was earlier than in 1995 (1994-95 $p=0.001$, 1994-97 $p=0.039$, 1995-96 $p=0.001$). No such difference was found for single-egg clutches, despite the relatively large range in median laying date across years (Kruskal-Wallis test: $\chi^2=6.214$, df=3, n=41, n.s.).

Table 3.2. Date of clutch initiation for single-egg and two-egg clutches during the years 1994 to 1997.

		1994	1995	1996	1997
Two-egg clutches	**Median** **Range** **(n)**	27 May 19 May - 5 June (31)	2 June 20 May – 21 June (47)	28 May 13 May – 17 June (46)	31 May 21 May – 16 June (41)
Single-egg clutches	**Median** **Range** **(n)**	31 May, 30 May - 5 June (8)	8 June, 20 May – 21 June (16)	4 June 25 May - 20 June (8)	31 May, 27 May - 16 June (9)

Note: Sixteen single-egg clutches were laid in 1994 but date of laying was known only for eight. It is probable that a disproportionate number of the early laid nests were missed, thus the observed median laying date in 1994 is probably an overestimate.

3. Laying interval of two-egg clutches

Year effects

Table 3.3 shows the interval (days) between the laying of two eggs in terms of the proportion of each laying interval, and the mean laying interval, for each year. No significant difference was evident between years in the interval between laying the first and second egg in two-egg clutches (Kruskal-Wallis test: $\chi^2=6.51$, df=3, n=161 n.s.). However, the proportion of laying intervals (1,2,3,4 days and 5 days plus) varied significantly with year (G test: G=23.9, df=9, n=162, $p=0.004$), with laying intervals tending to be more evenly dispersed in 1994 than in other years.

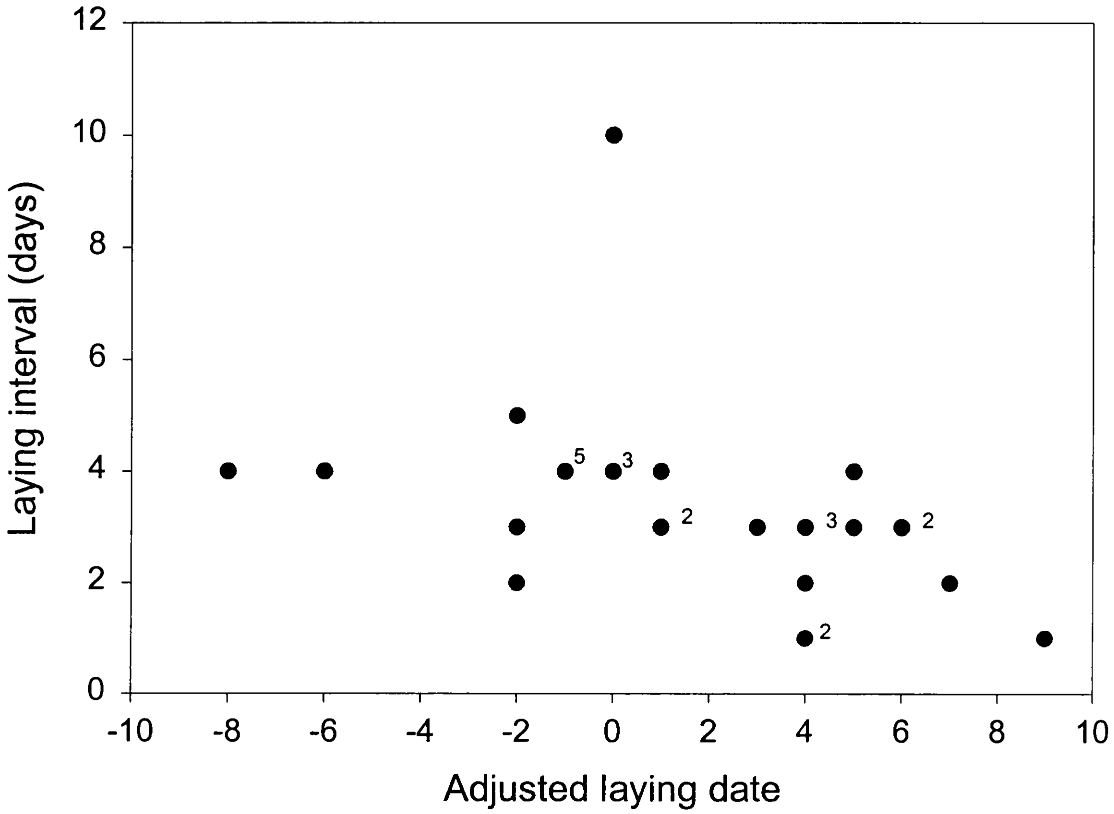
Table 3.3: Interval between the laying of eggs in two-egg clutches for the years 1994 to 1997. This is shown as both the proportion of each laying interval and the mean laying interval.

	Laying Interval (days)					
	1	**2**	**3**	**4**	**5+**	**Mean laying interval (days) ±S.E. (n)**
1994	10%	10%	33%	40%	7%	3.2 ±0.20 (30)
1995	0%	21%	57%	12%	9%	3.21 ±0.18 (47)
1996	2%	23%	61%	11%	3%	2.91 ±0.38 (43)
1997	0%	4.9%	76%	12%	7%	3.24 ±0.11 (41)

Seasonal effects

The egg laying interval was negatively correlated with laying date in 1994 (Spearman's rank correlation $r_s=-0.547$, $p=0.002$, $n=30$; fig 3.1), but no such correlation was evident during the following three years, 1995-1997 inclusive (1995: $r_s=-0.154$, n.s., $n=47$; 1996: $r_s=-0.298$, $p=0.052$, $n=43$; 1997: $r_s=-0.028$, n.s., $n=41$), although the data for 1996 were close to statistical significance at the 5% level.

Figure 3.1. The relationship between laying date (standardised) and the interval between the laying of eggs in two-egg clutches for 1994. Numbers refer to repeated data points.



4.Egg size

Year effects

Table 3.4 shows mean the mean egg mass and volume for single-egg and two-egg clutches in each year. There were no significant differences in mean egg volume between years for either a-eggs or b-eggs in two-egg clutches, or for single-egg clutches. Neither did the mass of single eggs vary between years. However, mean a- and b-egg mass differed significantly between years. Tukey post-hoc comparisons reveal that both a- and b-egg mass was significantly higher in 1994 than in all other years ($p<0.01$ in all cases). One-way ANOVA models reveal that egg-size disparity did not change with year either in terms of egg volume (b-egg volume/a-egg volume effect year: $F_{3,161}=1.56$, n.s.) or egg mass (b-egg mass/a-egg mass: $F_{3,161}=1.68$, n.s.; table 3.5).

Table 3.4. Egg size parameters for years 1994 to 1997. Kruskal-Wallis tests and one-way ANOVA statistics are provided for between-year comparisons.

		1994	**1995**	**1996**	**1997**	**Statistics**
a-egg mass (g)	**Mean**	54.24	48.85	49.05	49.47	$F_{3,161}=17.19$, $p<0.001$
	± S.E.	3.73	3.41	4.06	3.55	
	(n)	(33)	(46)	(45)	(41)	
b-egg mass (g)	**Mean**	51.85	47.12	47.94	47.62	$F_{3,165}=14.928$, $p<0.001$
	± S.E.	3.44	3.56	3.50	3.26	
	(n)	(33)	(45)	(45)	(41)	
s-egg mass (g)	**Mean**	53.5	49.63	48.48	49.5	$F_{3,33}=2.539$, $p=0.073$
	± S.E.	3.46	3.63	2.76	4.53	
	(n)	(8)	(16)	(4)	(9)	
a-egg volume (mm^3)	**Mean**	43.71	43.15	43.39	44.64	$F_{3,162}=1.13$ n.s.
	± S.E.	3.31	3.15	3.58	5.60	
	(n)	(33)	(46)	(46)	(41)	
b-egg volume (mm^3)	**Mean**	42.75	41.91	42.64	42.33	$F_{3,165}=0.64$ n.s.
	± S.E.	3.27	3.12	3.13	2.93	
	(n)	(34)	(48)	(46)	(41)	
s-egg volume (mm^3)	**Mean**	42.54	43.55	42.26	43.03	$\chi^2=0.967$ n.s.
	± S.E.	3.90	3.13	2.07	4.72	
	(n)	(10)	(16)	(5)	(10)	

Note: instead of using one-way ANOVA, 2-way ANOVA with egg 'type' as the additional factor could have been used for the analysis in table 3.4, thereby reducing the number of individual tests. However, the very high P values obtained for egg mass suggest that the results of a 2-way ANOVA would also have been significant.

Table 3.5. b-egg size as a percentage of a-egg size for years 1994 to 1997.

	1994	**1995**	**1996**	**1997**
Mean b-egg volume as a % of a-chick volume ± S.E. (n)	97.68 ±0.73 (33)	97.27 ±0.57 (46)	98.41 ±0.56 (46)	96.23 ±1.06 (40)
Mean b-egg mass as a % of a-chick mass ± S.E. (n)	95.72 ±0.83 (33)	96.56 ±0.77 (46)	97.90 ±0.62 (45)	96.36 ±0.57 (41)

Seasonal effects

In two-egg clutches, neither a-egg nor b-egg size (volume and mass) showed a relationship with laying date (table 3.6). However, egg volume in single-egg clutches was positively correlated with laying date for all years apart from 1997 (Figure 3.2; table 3.6). There was no relationship between egg size disparity within a clutch and laying date for all years (table 3.6). It should be noted that repeating correlation analysis in this way for each year increases the probability of committing a Type I error. One method of reducing such error is to test each correlation using the Bonferroni correction, i.e. using a significance level of $\alpha'=\alpha/k$ (Sokal and Rohlf 1995). However, this method is extremely conservative, would have been unlikely to reveal any true biological differences, and was therefore deemed inappropriate for such analysis.

An analysis of covariance with year as factor and laying date as covariate showed that a-egg mass was significantly affected by year but not by laying date (ANCOVA effect year: $F_{3,155}=13.58$ $p<0.0001$; effect covariate date: $F_{1,155}=0.19$, n.s.; interaction n.s.).

Figure 3.2. The relationship between egg volume and laying date for eggs laid in single-egg clutches

a) 1994

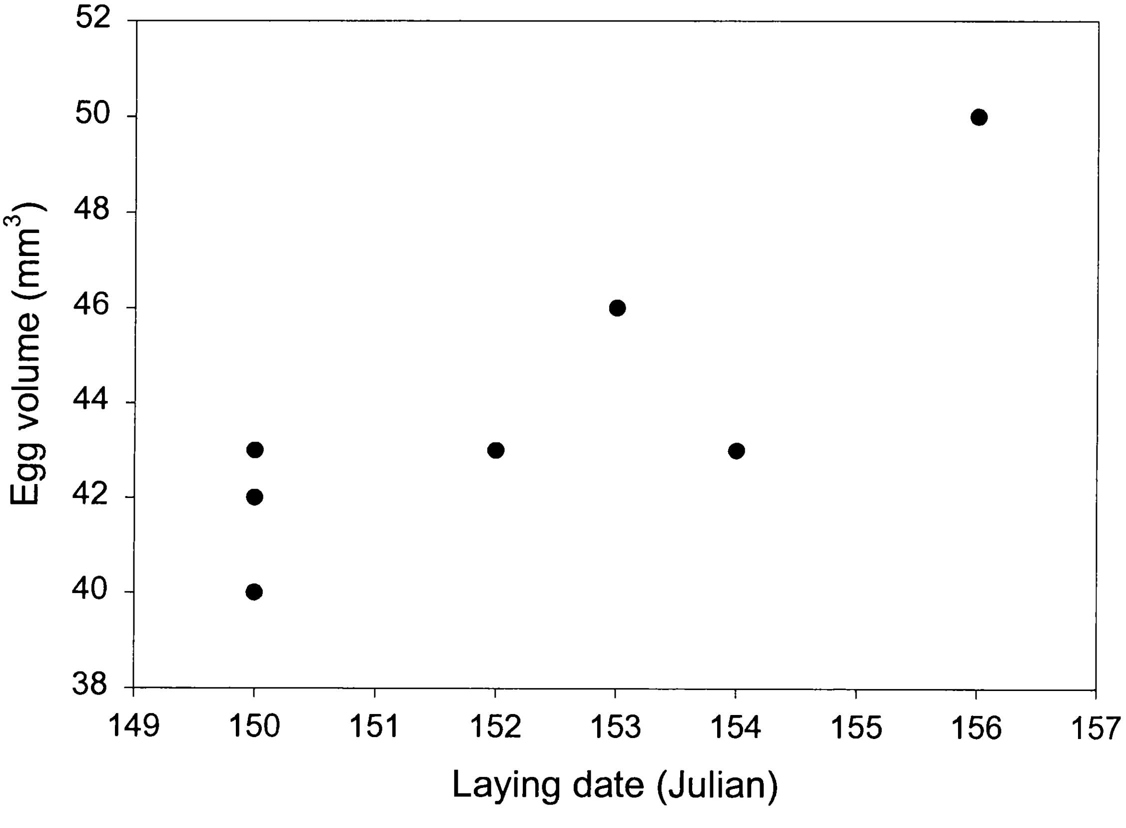


Figure 3.2. The relationship between egg volume and laying date for eggs laid in single-egg clutches

b) 1995

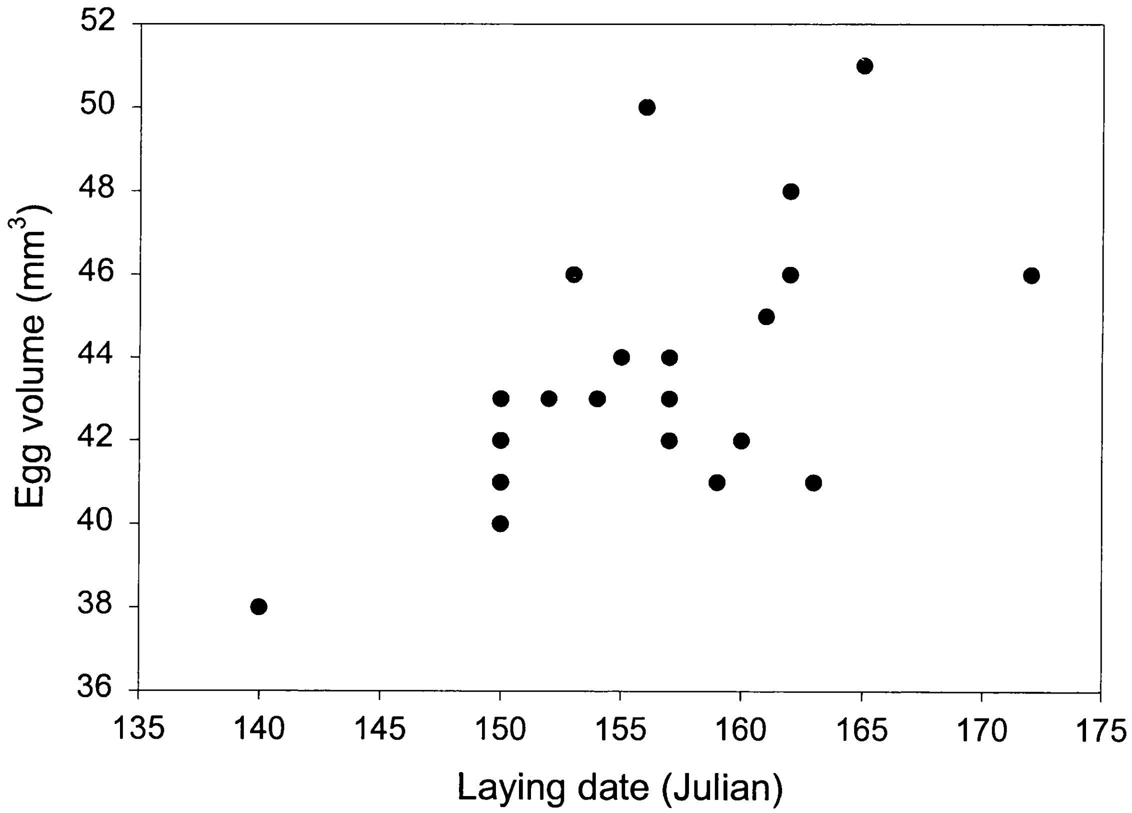


Figure 3.2. The relationship between egg volume and laying date for eggs laid in single-egg clutches

c) 1996

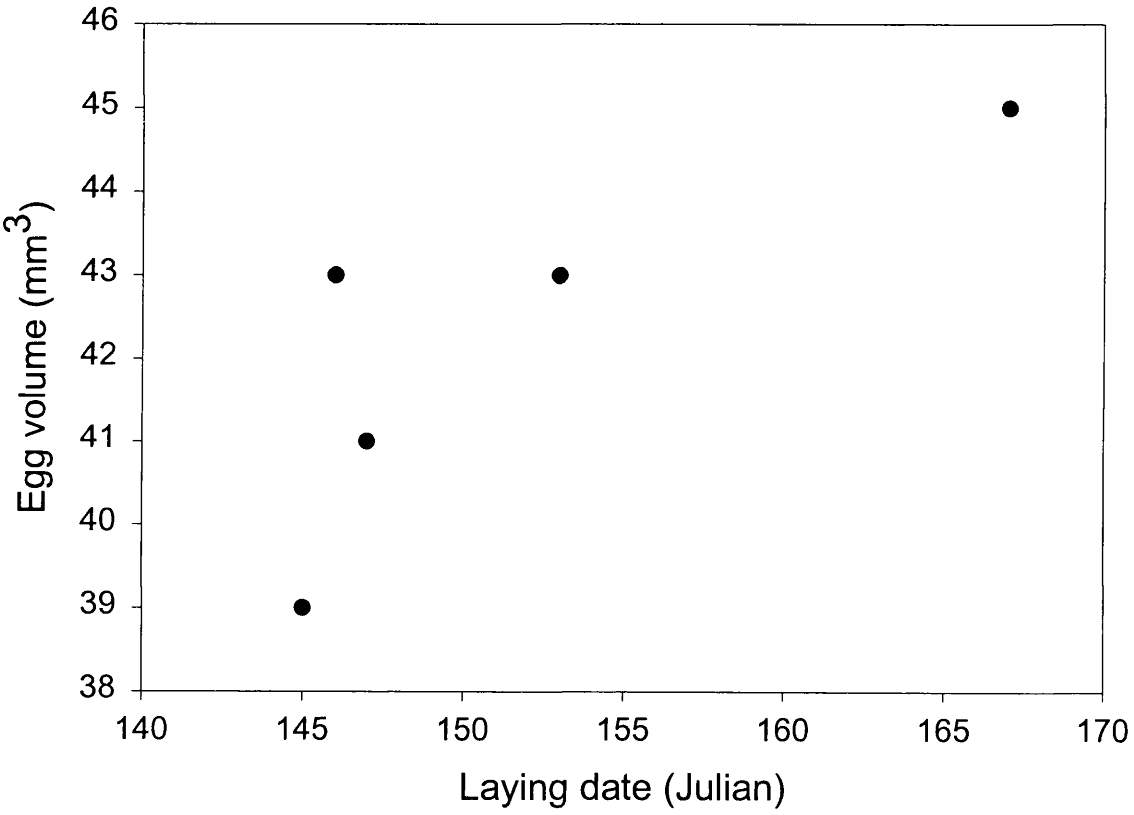


Figure 3.2. The relationship between egg volume and laying date for eggs laid in single-egg clutches

d) 1997

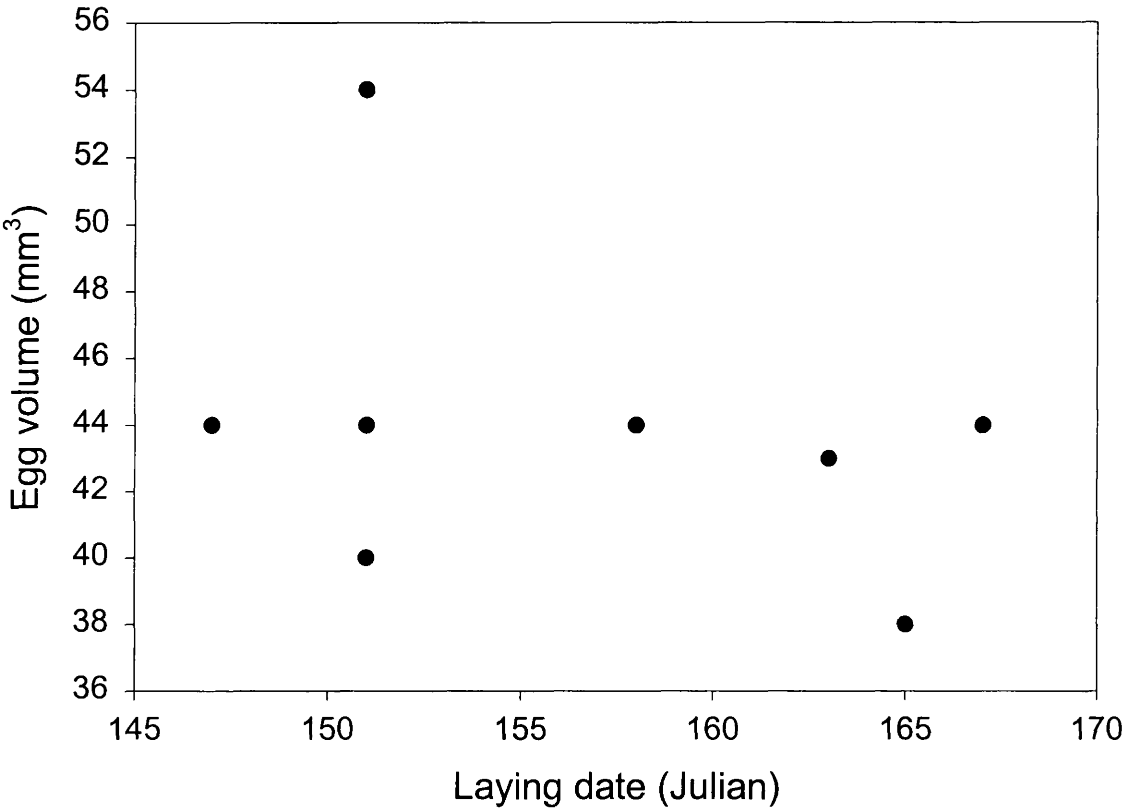


Table 3.6. Correlation coefficients (Pearsons and Spearmans) with significance levels for the relationships between egg size and laying date.

	1994	1995	1996	1997
a-egg mass	r=0.102 n=31 n.s.	r=-0.059 n=46 n.s.	R=-0.06 n=45 n.s.	r=-0.093 n=41 n.s.
a-egg vol	r=0.131 n=30 n.s.	r=-0.159 n=46 n.s.	R=-0.065 n=46 n.s.	r=-0.093 n=41 n.s.
b-egg mass	r=-0.124 n=31 n.s.	r=-0.61 n=47 n.s.	r=-0.141 n=46 n.s.	r=-0.13 n=41 n.s.
b-egg vol	r=0.074 n=33 n.s.	r=-0.187 n=48 n.s.	r=0.072 n=43 n.s.	r=-0.129 n=41 n.s.
b/a mass	r=-0.297 n=31 n.s.	r=-0.24 n=46 n.s.	r=-0.191 n=45 n.s.	r=-0.053 n=41 n.s.
s-egg volume	rs=0.786 n=8 p=0.021	rs=0.551 n=16 p=0.027	rs=0.90 n=5 p=0.037	rs=-0.209 n=9 n.s.

Egg mass differences between one-egg and two-egg clutches

Mann-Whitney U-tests were used to compare egg size (mass and volume) of eggs in two-egg clutches with those in single-egg clutches (Table 3.7). Mean egg mass in single-egg clutches did not differ significantly from those of a-eggs in two-egg clutches. However, s-egg mass was consistently higher than b-egg mass, but significantly so only in 1995. The sample size was relatively high in 1995 and it is possible that the paucity of significant results for the remaining three years was a consequence of Type II errors.

Table 3.7. Mann-Whitney U-test statistics for size comparisons between egg types for the years 1994 to 1997.

	1994	1995	1996	1997
a-egg vs s-egg mass	$U_{8,41}=159$ n.s.	$U_{16,46}=322$ n.s.	$U_{4,51}=88.5$ n.s.	$U_{9,45}=191.5$ n.s.
b-egg vs s-egg mass	$U_{8,41}=109$ n.s.	$U_{16,48}=238$ p=0.023	$U_{4,52}=90.5$ n.s.	$U_{9,45}=150$ n.s.
a-egg vs s-egg volume	$U_{8,41}=110.5$ n.s.	$U_{16,48}=364$ n.s.	$U_{4,51}=86$ n.s.	$U_{9,45}=157.5$ n.s.
b-egg vs s-egg volume	$U_{8,41}=147$ n.s.	$U_{16,48}=288$ n.s.	$U_{4,51}=107$ n.s.	$U_{9,45}=194$ n.s.

5. Hatching success

From 237 nests where clutch fate was known for each egg, 430 eggs were laid and 309 (71.8%) hatched: 273 hatching from two-egg nests and 36 hatching from one-egg nests.

Year effects

Hatching success (number of eggs hatched from the number of eggs laid) did not differ between years for single-egg clutches or two-egg clutches (tables 3.8 and 3.9).

Table 3.8. Hatching success (number of eggs hatched divided by the number of eggs laid). Kruskal-Wallis test statistics provide between-year comparisons.

		1994	**1995**	**1996**	**1997**	**statistic**
one-egg clutches	**Mean** **±S.D.** **(n)**	0.80 ±0.041 (15)	0.75 ±0.45 (16)	1.00 0 (8)	0.63 ±0.52 (8)	χ^2=3.49 n.s.
two-egg clutches	**Mean** **±S.D.** **(n)**	0.81 ±0.35 (48)	0.74 ±0.37 (48)	0.69 ±0.37 (48)	0.78 ±0.36 (37)	χ^2=3.35 n.s.

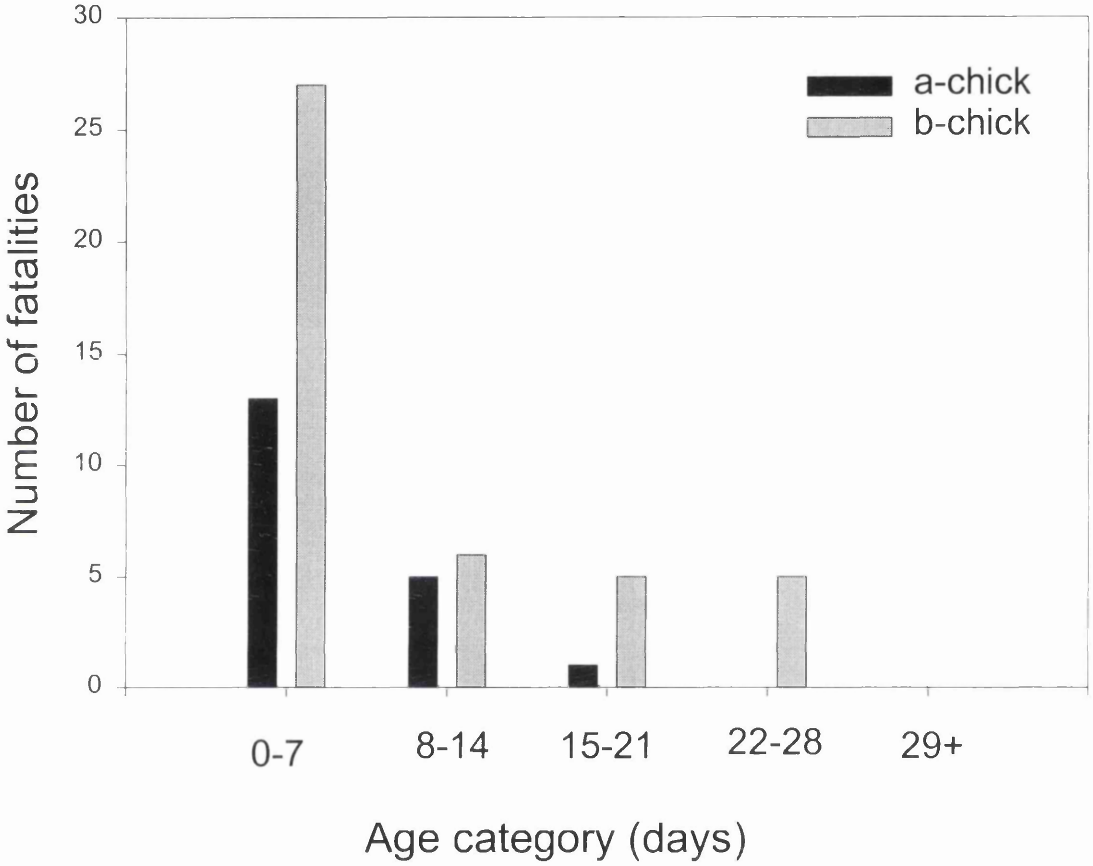
Seasonal effects

Egg hatching success was examined in relation to laying date (Julian) for both a- and b-eggs independently using logistic regression analysis. When data from all years were pooled, neither the hatching probability of the a-egg (χ^2=0.184, d.f.=1, n=163, n.s.), nor the b-egg (χ^2=0.140, d.f.=1, n=163, n.s.) were affected by the date of laying.

6. Chick survival

From two-egg clutches 171 chicks fledged, and 18 fledged from one-egg clutches. Approximately 54% of all nestlings died before fledging in this study population, with full losses occurring in 22.8 % of broods and partial losses occurring in 23.4 %. The greatest mortality occurred during the first 14 days post-hatching (Fig. 3.3).

Figure 3.3. Non-predation related mortality in two-chick broods according to chick age.



Year effects

There was no effect of year on fledging success (number of chicks fledged by the number of eggs laid) for two-egg clutches (χ^2=6.33, n=150, df=6, n.s.) nor for one-egg clutches (G-test: G=6.503, n=39, df=3, n.s.). (I used the G-test in preference to the Chi-squared test for single-egg nests because for a number of cells the observed minus the expected count was smaller than the expected [see Williams 1976; from Zar 1996].)

Table 3.9. Breeding performance.

	1994		1995		1996		1997	
Clutch size	1	2	1	2	1	2	1	2
Number nests (%)	15 (21.4)	55 (78.6)	16 (22.2)	56 (77.8)	8 (12.9)	54 (87.1)	10 (16.7)	50 (83.3)
Number eggs laid	15	110	16	112	8	106	10	100
Number of nests of known fate	15	48	16	48	7	48	8	37
Number of eggs laid	15	96	16	96	7	96	8	74
Number of eggs hatched	12	78	12	71	7	66	5	58
Number of chicks fledged	7	53	5	37	5	46	1	35
Eggs hatched per nest		1.62		1.48		1.38		1.57
Eggs hatched/eggs laid	0.80	0.813	0.75	0.739	1.0	0.688	0.625	0.783
Chicks fledged/eggs hatched	0.583	0.679	0.42	0.521	0.714	0.697	0.2	0.603
Chicks fledged/eggs laid	0.47	0.552	0.313	0.385	0.714	0.479	0.125	0.473
Chicks fledged per nest		1.10		0.77		0.96		0.95

Seasonal effects

Chick fledging success was examined in relation to laying date (Julian) for both a- and b-chicks independently using logistic regression analysis. When data from 1994, 1996 and 1997 were pooled, the hatching probability of the a-chick showed no relationship with laying date (χ^2=0.349, d.f.=1, n=60, p=0.55), but the b-chick demonstrated a significant decrease in survival probability as the season progressed (χ^2=16.58, d.f.=1, n=49, p<0.001).

7. Chick growth

Year effects

Table 3.10 shows the mean instantaneous growth rates (g/day) and asymptotic weights (g) for all chick types in the years 1994, 1996 and 1997. Growth rates of both a- and b-chicks varied significantly between years, although no difference in s-chick growth rate was found between 1994 and 1996 (insufficient growth data were available in 1997). Surprisingly, the asymptotic mass of a-chicks varied between years yet those of b-chicks remained relatively constant. This may have been the result of poorer quality b-chicks dying before sufficient growth data were collected and hence the data being biased towards higher 'quality' b-chicks.

Table 3.10. Instantaneous growth rates (g/d) and asymptotic weights (g). Mann-Whitney U-tests and one-way ANOVA statistics provide between year comparisons.

		1994	**1996**	**1997**	**statistics**
Instantaneous growth rate a-chick	Mean ±S.E.	8.252 ±0.14	7.64 ±0.16	8.45 ±0.15	$F_{2,82}=6.893$, p=0.002
Instantaneous growth rate b-chick	Mean ±S.E.	7.645 ±0.21	6.843 ±0.26	7.70 ±0.26	$F_{2,57}=3.358$, p=0.042
Instantaneous growth rate s-chick	Mean ±S.E.	8.724 ±0.18	8.151 ±0.24	All die <3days	$U_{8,5}=7.0$, p=0.056
Asymptotic weight a-chick	Mean ±S.E.	427.70 ±10.28	374.75 ±11.03	401.30 ±10.28	$F_{2,63}=6.168$ p=0.004
Asymptotic weight b-chick	Mean ±S.E.	384.41	369.62	358.08	$F_{2,39}=0.892$, n.s.
Asymptotic weight s-chick	Mean ±S.E.	Insufficient Data	Insufficient Data	Insufficient Data	

Data from 1994 and 1996 suggest that s-chick growth is less susceptible to environmental conditions than chick growth from two-chick broods. However, if this is the case, the very high death rate of s-chicks in 1997 is somewhat paradoxical.

Hatching order effects

Instantaneous growth rates in two-chick nests was affected both by laying order and year: chick ($F_{1,144}=21.421$, $p<0.001$), year ($F_{2,144}=11.343$, $p<0.001$), interaction ($F_{2,144}=0.329$, n.s.). As was asymptotic weight: chick ($F_{1,102}=9.151$, $p=0.003$), year ($F_{2,102}=4.418$, $p=0.014$), interaction ($F_{2,102}=1.536$, n.s.). Generally, a-chicks grew faster and reached higher asymptotic weights than b-chicks, but care must be taken when interpreting these results due to the potential problems of pseudo-replication. Relative sibling growth is therefore examined in greater detail in chapter 5.

Seasonal effects

Although instantaneous growth rates of both a- and b-chicks were negatively correlated with laying date (a-chick: Pearson's r=-0.241, p=0.046, n=69; fig. 3.4a; b-chick: Pearson's r=-0.369, p=0.008, n=50; fig. 3.4b), asymptotic mass exhibited no such relationship (a-chick: Pearson's r=-0.252, p=0.074, n=51; fig 3.5a; b-chick: Pearson's r=0.040, n.s., n=33; fig. 3.5b). Age at asymptotic mass was negatively correlated with laying date in a-chicks (Pearson's r=-0.317, p=0.025, n=50; fig. 3.6a), but no correlation was evident in b-chicks (Pearson's r=-0.218, n.s., n=29; fig. 3.6b). Thus, generally speaking, chicks from nests hatching late in the season grew slower and reached asymptotic mass at an earlier age than those hatching earlier in the season. The lack of an effect in b-eggs is likely to be due to the high mortality rate of these chicks at the end of the season.

Figure 3.4 relationship between instantaneous growth rate (g/d) and laying date (standardised for yearly differences in median laying date), a) a-chick

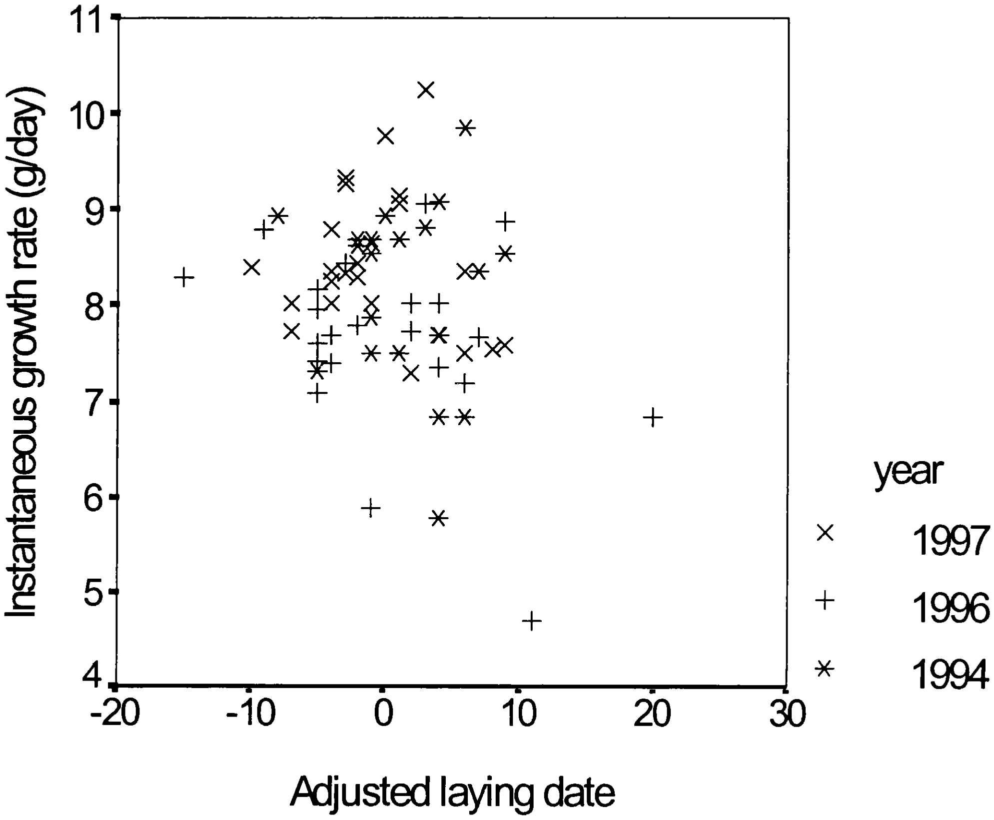


Figure 3.4 relationship between instantaneous growth rate (g/d) and laying date (standardised for yearly mean differences), b) b-chick

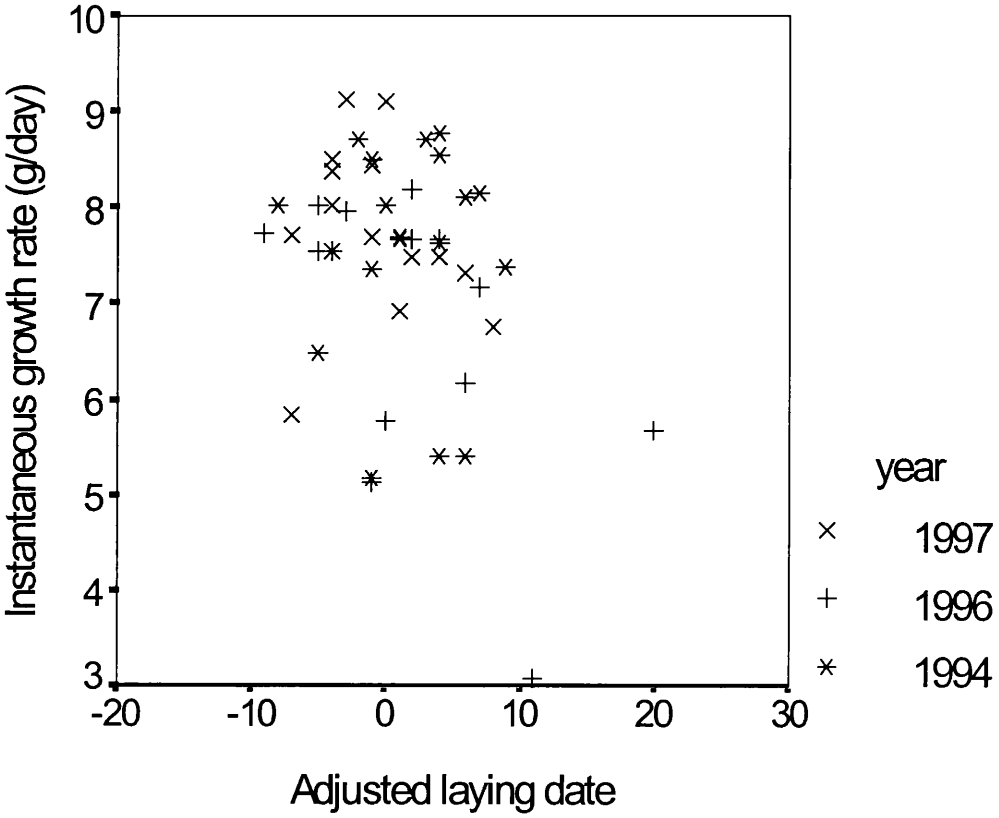


Figure 3.5 relationship between asymptotic weight (g) and laying date (standardised for yearly differences in median laying date), a) a-chick

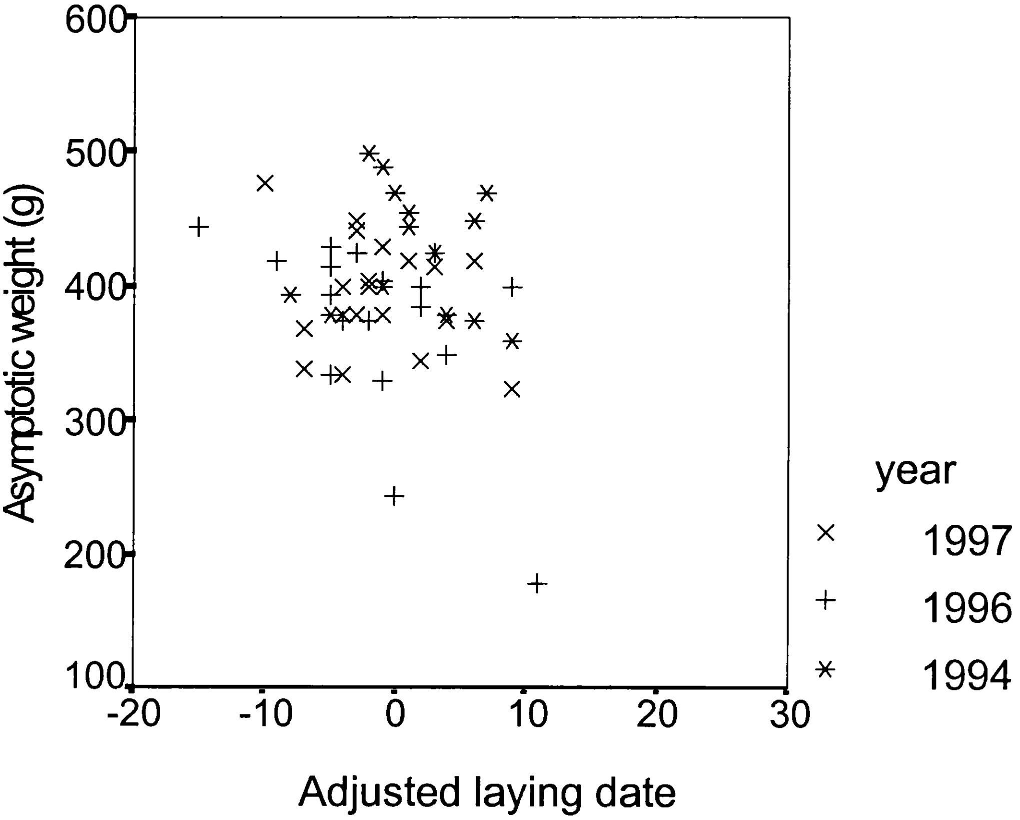


Figure 3.5. Relationship between asymptotic weight (g) and laying date (standardised for yearly differences in median laying date) b) b-chick..

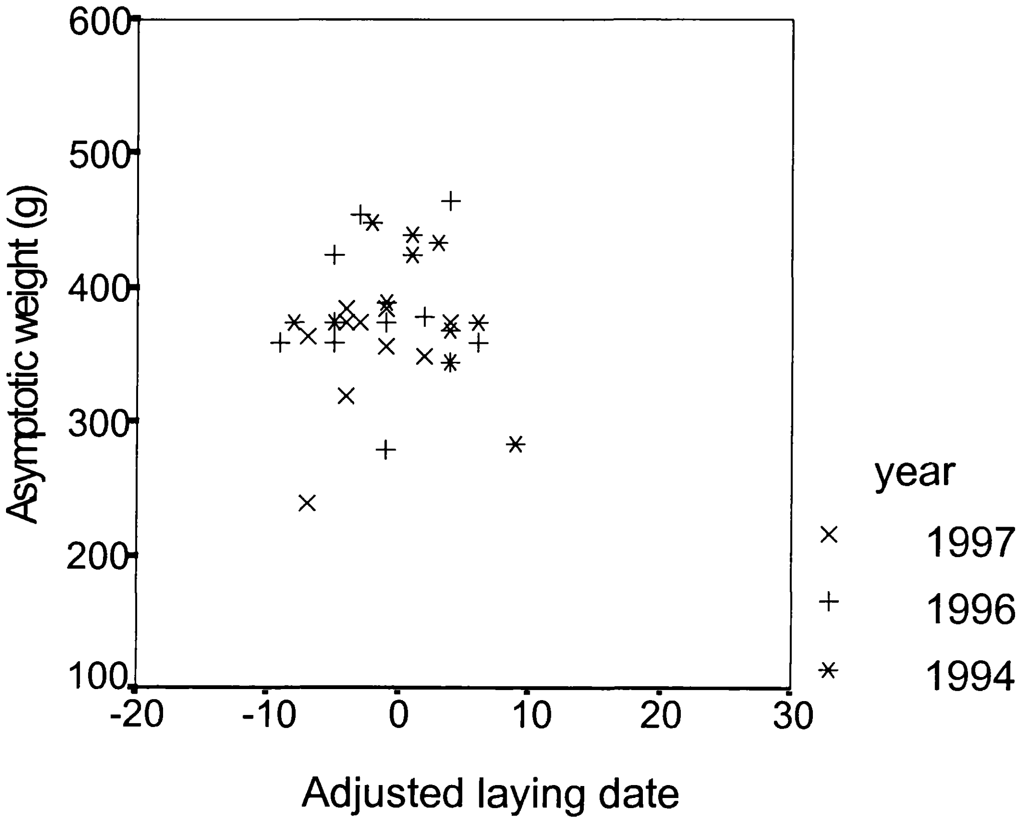


Fig 3.6. The relationship between age at asymptotic weight (days) and laying date (standardised for yearly differences in median laying date) a) a-chick

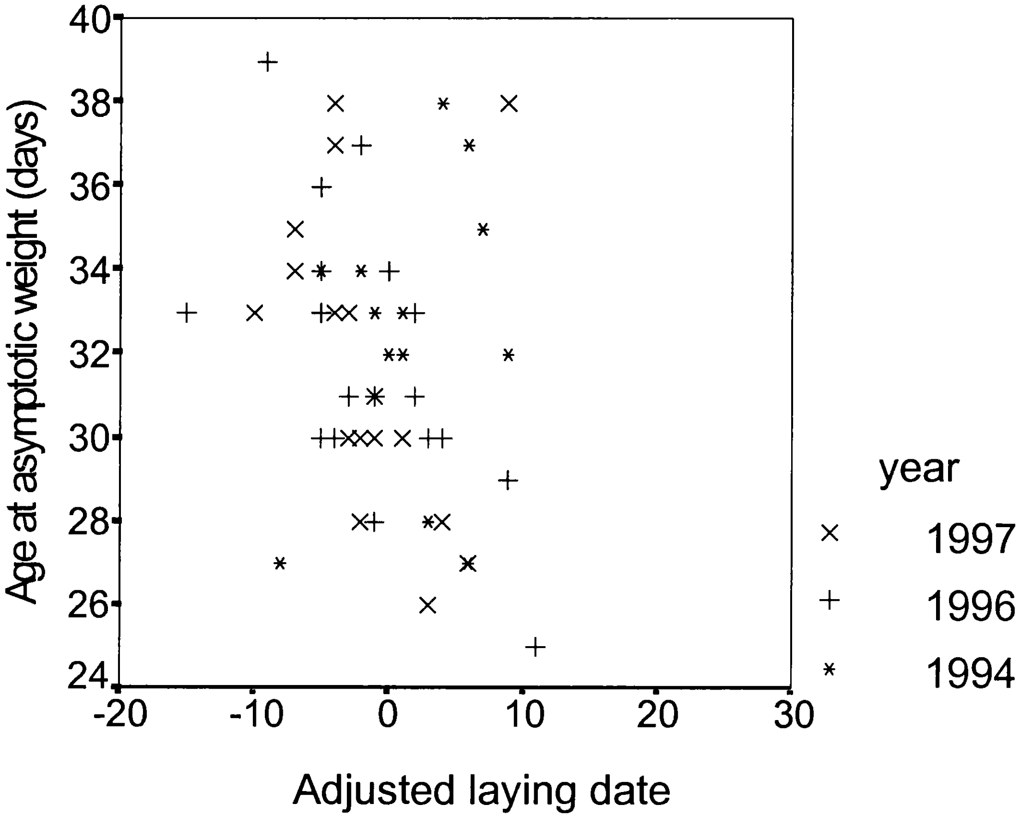
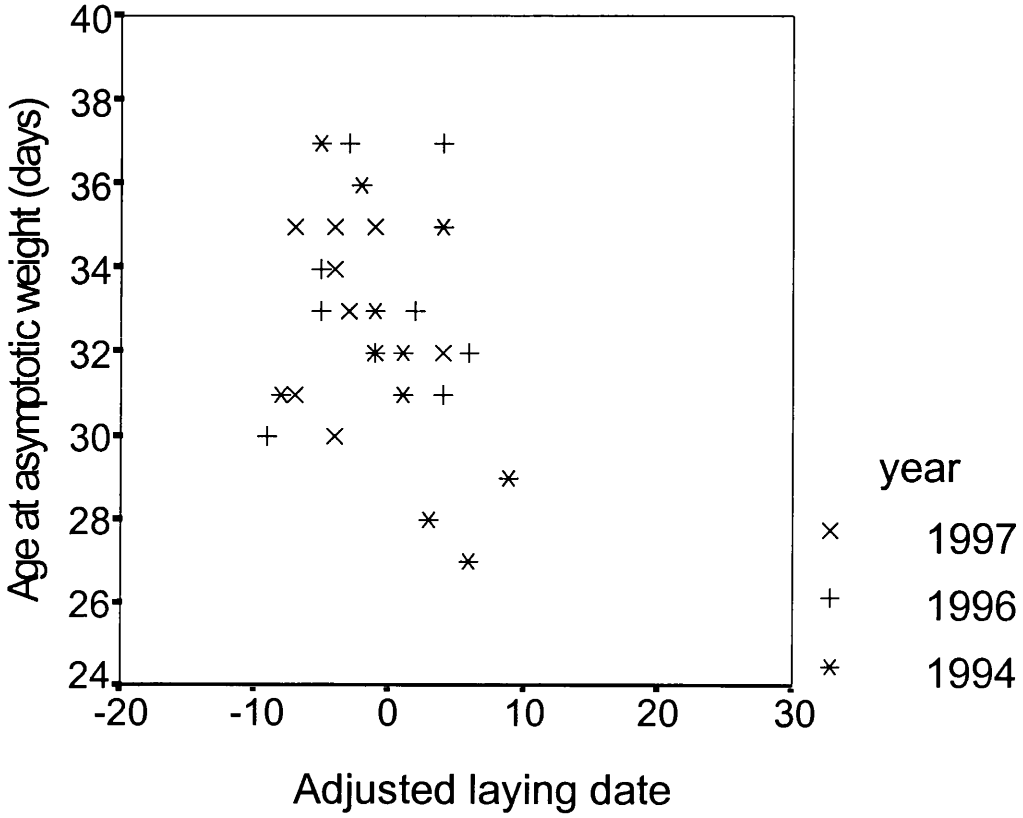


Fig 3.6. Relationship between age at asymptotic weight (days) and laying date (standardised for yearly differences in median laying date)



8. Incubation period

Year effects

Incubation period (defined here as the number of days from laying to hatching) varied significantly between years both for a-eggs (one-way ANOVA: $F_{3,131}=6.567$, $p<0.001$; post-hoc Tukey comparisons: 1995-94, p=0.003; 1995-96, p=0.001, 1995-97, p=0.001) and b-eggs ($F_{3,111}=8.272$, $p<0.001$; post-hoc Tukey comparisons: 1995-94, p<0.001; 1995-97, p=0.001) in two-egg clutches, whereas incubation period of single-egg clutches remained relatively constant during each of the four study years (Kruskal-Wallis test: $\chi^2=4.204$, df=3, n=25, n.s.; Table 3.11).

Table 3.11. Incubation periods (number of days from clutch initiation to hatching) for each year from 1994 to 1997, inclusive.

		1994	**1995**	**1996**	**1997**
Two-egg clutches	**Mean ±S.E.**	29.65 ±0.428	31.60, ±0.369	29.74 ±0.349	29.69 ±0.369
Single-egg clutches	**Mean ±S.E.**	27.21 ±0.316	29.32 ±0.322	28.15 ±0.334	27.63 ±0.301

Seasonal effect

Incubation period exhibited a significant negative relationship with laying date in all years except 1997 (1994: Pearson's r=-3.77, n=26, p=0.050; 1995: Pearson's r=-0.372, n=35, p=0.028; 1996: Pearson's r=-0.293, n=37, p=0.078; 1997: Pearson's r=0.021, n=34, n.s.; all four years combined: Pearson's correlation: r=-0.278, p=0.001, n=132;). Thus, as the season progressed, parents allocated more time to incubating eggs than to other activities.

9. Hatching spread

A Kruskal-Wallis one way ANOVA showed that the mean hatching interval between eggs in two-egg broods was not significantly different across the four years (Table 3.12), nor did the frequency of hatching intervals (0, 1, 2, 3 and 4 days+) differ significantly with year (G-test: G=19.058, df=12, n=131, p=0.087). No significant correlation between hatching spread and laying date was evident ($r_s=-0.042$, n.s., n=108).

Table 3.12. Mean hatching intervals and the percentage of clutches that hatch synchronously (within 24 hours) for each year from 1994 to 1997, inclusive. Kruskal-Wallis statistics are included for between-year comparisons.

	Mean hatching interval ± S.D. (n)	**% of broods hatching synchronously**
1994	1.16 ±1.48 (37)	38.5%
1995	1.0 ±1.11 (30)	40.7%
1996	1.46 ±1.31 (30)	14.8%
1997	1.32 ±1.09 (34)	30%
Kruskal-Wallis ANOVA	$\chi^2 = 2.97$ n.s.	

10. Fledging age

Year effects

Data for fledging age was difficult to obtain because in many cases it was impossible to determine whether chicks had fledged or whether they were depredated in the days immediately prior to fledging. For those nests where chick fate was ascertained (usually through direct observations), I detected no difference in fledging age between the four years for either a- or b-chicks (table 3.12).

Table 3.13. Fledging ages for each year from 1994 to 1997. One-way ANOVA statistics are included for between-year comparisons.

		1994	1996	1997	Statistics
fledging age of a-chick	mean ±S.E (n)	34.58 0.8 (20)	35.0 0.74 (23)	35.45 0.64 (20)	$F_{2,60}=0.324$ n.s.
fledging age of b-chick	mean ±S.E (n)	36.11 0.74 (18)	34.27 1.00 (15)	35.82 0.72 (11)	$F_{2,41}=1.42$, n.s.

Seasonal effects

As with year effects, no significant correlation was exhibited between chick age at fledging and laying date, for either the a-chick (1994: Pearson's r=-0.268, n.s., n=19; 1996: Pearson's r=0.0, n.s., n=23; 1997: Pearson's r=-0.171, n.s., n=20), or the b-chick (1994:

Pearson's r=-0.414, p=0.088, n=18; 1996: Pearson's r=-0.299, n.s., n=19; 1997: Pearson's r=-0.59, n.s., n=11).

DISCUSSION

1. Annual variation

In this study, annual variation in the timing of laying was related to a number of other breeding parameters, indicating important yearly differences in reproductive performance. For two-egg clutches, breeding was earliest in 1994, with a median date of clutch initiation of May 27. The latest year was 1995, with a medium laying date six days later. Single-egg clutches were generally laid later, but the annual pattern of laying date was similar to that of two-egg clutches. The earliest breeding years for single-egg clutches were 1994 and 1997, which both had a median clutch initiation date of May 31, whereas the latest year was 1995, 8 days later. The difference in laying date between clutch types is similar to that recorded in other studies on the black guillemot: for example, over three breeding seasons, Ewins (1989) found that single-egg clutches were laid two, four and five days later than two-egg clutches in respective years, whereas Asbirk (1979) recorded a median difference of eight days between two breeding seasons. In this study, despite exhibiting a similar annual laying phenology to that of two-egg clutches, the observed between year difference in laying date in single-egg clutches was not statistically significant. Thus, unlike females laying two-egg clutches, females laying single-egg clutches did not appear constrained in certain years to lay at a later date. However, small sample sizes, and the fact that laying date of many early nests were missed in 1994, thereby overestimating the median laying date for that year, is likely to account for the lack of year effect on laying date in single-egg nests. A previous study of annual variation in laying date on black guillemots in Mousa, Shetland, found that later laying was associated with lower air temps and stronger winds in the month prior to laying, but no relationship was found with sea temperatures (Ewins 1989).

For two-egg clutches, egg size in terms of egg mass was closely associated with between-year variation in the timing of laying. Mean fresh egg mass was highest in 1994 when breeding was relatively early, and lowest in 1995 when breeding was relatively late. This relationship is highly consistent with the idea that the ease with which females obtain food prior to breeding is responsible for the annual variability in these two parameters. It is conceivable that in years when food is limited, females take longer to build up essential nutrients reserves, and are therefore restricted to laying smaller eggs at a later date. It is interesting to note that although black guillemot egg size in terms of mass varied annually, no corresponding increase in egg volume was evident for either a- or b-eggs, suggesting that important compositional changes were occurring that increased egg density, without the associated change in physical dimensions. This has important implications for studies measuring egg quality. In contrast to two-egg clutches, neither egg mass nor egg volume in single-egg clutches varied significantly between years. This suggests that parents producing single-egg clutches were not constrained to decrease egg size in poor years and thus the primary constraint in clutch production in the black guillemot may thereby be producing the second egg (or chick). Nonetheless, despite the lack of significance in single-egg clutches, the pattern of yearly egg mass variation was similar to that of a-and b-eggs. It is conceivable therefore that the lack of yearly effect is not biological meaningful but an artefact of sample size.

In two-egg clutches, first-laid eggs were significantly larger than second-laid eggs, both at the population and intra-clutch level (chapter 4). The relative intra-clutch size difference between eggs, in terms of both mass (b-egg mass/ a-egg mass) and volume (b-egg volume/ a-egg volume) did not differ between years, indicating that despite potential differences in pre-laying breeding conditions, females did not alter the allocation of resources between the two eggs, and thereby the potential competitive dynamics of the brood with respect to hatching size (see chapter 4). This lack of evidence for a change in clutch asymmetry between years contradicts Kilpi et al. (1996) who suggested that clutch asymmetry should be more sensitive to environmental conditions than clutch volume. However, results similar to that of the current study were also found in the great skua (Catry and Furness 1998).

Black guillemot clutch size on the Holm of Papa Westray remained relatively constant between years with the majority of breeding pairs (range 77.8 - 87.1%) laying a two-egg clutch. This figure is comparable to that reported for other populations (Winn 1950, Cairns 1981, Kuletz 1983), and for that of the pigeon guillemot (Drent 1965). Thus, in spite of annual effects on laying date and egg size, potential yearly differences in breeding condition appeared not to affect clutch size. Ewins (1989) and Asbirk (1979) have shown that black guillemot single-egg clutches are predominantly the product of relatively young, less experienced birds. Although possibly the case in the current study, data from a limited number of colour ringed adults shows that individual clutch size frequently varies with year, independent of age, thereby suggesting that females are capable of adjusting clutch size according to prevailing circumstances. However, if laying date is a reflection of environmental conditions in the pre-laying period, it would seem axiomatic to assume that a corresponding decrease in clutch size would be evident in years when laying date was delayed. Although not significantly different, the number of single egg clutches appeared to be relatively low during 1996 and 1997 in comparison to the previous two years. It is conceivable however that this potential reduction was not due to a smaller proportion of the population laying two eggs, but instead due to a smaller proportion of those adults which would lay a single-egg clutch, failing to breed in these years.

The relationship between egg laying interval and year was also puzzling. The interval between laying the first and second egg was on average 3.1 days, ranging between one and ten days. This is very similar to the pattern recorded elsewhere (e.g. Asbirk 1979, Peterson 1981, Ewins 1989). During the laying interval females obtain important nutrients for the production of the second egg (as is evident from an observed correlation between the duration of the inter-laying interval and the subsequent size of the b-egg relative to the a; the longer the interval, the larger the b-egg tends to be (Ewins 1986). It would seem intuitive to assume therefore that the laying interval in two-egg clutches should also be related to egg size and laying date. Despite finding a significant effect of year on laying interval, this variation did not reflect the yearly variation in laying phenology and egg size. Years of early laying, 1994 and 1996, were associated with relatively large and relatively small laying intervals, respectively. This is in contrast to the results of Ewins (1989) who found that in a bad year the laying interval between eggs was greater than that in a relatively successful year. It appears therefore that factors affecting the laying interval were independent of those that determined egg mass.

Having initiated incubation, most species of bird incubate more or less continuously until hatching is completed. Black guillemots are unusual in that even "experienced" older pairs vary in the onset of incubation relative to clutch initiation and also take extended breaks throughout the incubation period (Petersen 1981). Black guillemot eggs are extremely resistant to chilling (up to 15d of continual neglect; Bergman 1971, quoted in Harris and Birkhead 1985). These adaptations are extreme among birds, and it is likely they are a response to the potential inability of parents to sustain continuous incubation. For two egg clutches, incubation period was longest in 1995, suggesting that parents were constrained to leave incubation duties more often to find food during this year. Cold weather during incubation is likely to affect parental energy demands, thereby increasing metabolic expenditure and the cost of maintaining egg temperature. Indeed, experimental changes in incubation costs alone have been shown to have fitness consequences in seabirds (Heaney and Monaghan 1996, Monaghan and Nager 1997). The cost of extending incubation pauses for food intake, however, would be to expose eggs to an increased risk of chilling. Indeed, for b-eggs at least, there was a higher probability of hatching in larger than in smaller eggs, which may have been due to the fact that smaller eggs have a larger surface area to volume ratio and therefore cool more quickly (chapter 4).

However, despite annual differences in incubation behaviour, the proportion of eggs hatching each year remained relatively constant, corroborating the theory that black guillemot eggs are extremely resistant to neglect. For single-egg clutches, incubation period was comparable to that of two-egg clutches and was also longest in 1995, although the difference between years was not significant (again, possibly due to Type II statistical error). It therefore appears that incubation behaviour in the black guillemot is relatively flexible, and is likely to depend on either parental quality or environmental conditions, or both. Such flexibility has the potential to allow females to replenish reserves lost during egg production and conceivably is an important factor in the ability of this species of auk to lay a two-egg clutch.

Potentially, the degree of hatching asynchrony might also be associated with environmental constraints during egg laying and incubation (the "energy constraints hypothesis" for hatching asynchrony). Parents subjected to poor conditions during laying may need to forage to replenish lost reserves, thus reducing the time available for incubation and causing the clutch to hatch with a greater degree of synchrony than is optimal (Greig-Smith 1985, Slagsvold 1986, Enemar and Arheimer 1989, Moreno 1989). In this study, there was no difference in the level of hatching asynchrony between years (although in 1996 relatively fewer breeding pairs hatched their eggs synchronously). Thus, despite annual differences in environmental condition there was no corresponding effect on brood competitive asymmetry.

For two-egg clutches, variation in environmental condition appeared not to affect hatching and fledging success since no significant effect of year was evident for these parameters; although, as with other measures of breeding success mentioned previously, hatching and fledging rates were higher in 1994. However, an effect of year was evident on mass instantaneous growth rates and asymptotic mass for a-chicks, with both growth parameters being significantly higher in 1994 and 1997 than in 1996 (Unfortunately due to experimental manipulations, I do not have growth or survival data in 1995). For b-chicks, although the same pattern was evident with respect to mass growth rate, no significant between year variation was found for asymptotic mass (although, again, 1994 was the highest). B-chicks therefore appeared to fledge at a relatively constant mass regardless of any potential difference in feeding conditions. Any advantages brought about by improved feeding conditions therefore appeared to be directed primarily toward the a-chick (see chapters 5 and 6 for sibling rivalry effects). For single-egg clutches, hatching success was relatively constant between years and was comparable to that in two-egg clutches. Mass growth rates, however, were generally high for chicks hatching from single-egg clutches, being significantly higher than a-chicks in two-chick broods. This is as expected considering that parents in single-chick broods have only half the number of chicks to feed, and suggests not only that nestlings in two-chick broods were growing below maximal rate, but also that b-chicks in particular were growing far slower than potential. Given the high growth rates in single-chick broods, however, it is surprising that survival was extremely variable between years in these broods. In 1997, for example all save one chick died (80%) within days of hatching, yet in 1996 over 70% of chicks fledged. Nonetheless, due to the limited data on growth and survival in these single-chick broods, care must be taken when interpreting these data.

2. Seasonal variation

Perrins (1970) suggested that low food availability early in the breeding season would act as a constraint on early breeding. He reasoned that much of a population is unable to raise a brood at the optimum time because available resources are not sufficient at the beginning of the season for egg production. The consequence of this is that many breeding pairs are restricted to laying eggs whilst food is increasing and raising offspring when food is decreasing, with the effect that breeding success declines with increasing laying date. Ideally therefore individual females should aim to lay as early as possible, with the seasonal decline in breeding success reflecting either or both a variation in environmental condition and parental quality (Lack 1968, Perrins 1970, Martin et al. 1987, Daan et al. 1989). Other potentially important factors determining breeding success in relation to laying date include seasonal trends in predation rate (Cooke and Findlay 1982, Elridge and Krapu 1988), parasitic load (Moller 1994), or weather condition. However, irrespective of the causal nature of seasonal variability, any differences in reproductive strategy employed between late and early breeders is likely to reveal how and at what stage in the breeding episode the seasonal costs are manifest, and highlight the potential trade-offs selected to maximise reproductive success in the face of such costs. Different breeding strategies are also likely to be informative as to which constraints are likely to be most pertinent to a species.

In this study I have demonstrated that, in addition to annual effects, black guillemots also exhibited a marked effect of season with respect to a number of breeding parameters. Overall productivity was generally higher among earlier nesting birds, with some components of breeding success showing a distinct decline as laying date progressed. In all years except 1997, pairs producing two-egg clutches laid significantly earlier than those laying single-egg clutches, confirming the general finding that black guillemot clutch size decreases with advancing season (Petersen 1981). In addition, a consistent seasonal decline was evident in the mass growth rate of both a- and b-chicks, and the age at which a-chicks attained asymptotic mass. Furthermore, laying date was a significant predictor of b-chick survival, with late hatching b-chicks having a lower probability of survival than those hatching early in the season. Whatever the causal nature of these seasonal effects, it appears that the resulting constraints imposed upon the reproductive strategy of the black guillemot were manifest both at the egg production and chick rearing stages. Importantly, these results also suggest that the fitness returns of producing a two-chick brood decline with increasing laying date, with the effect that on declining below a certain fitness threshold, both parental and offspring fitness will be increased through a reduction in clutch size. This might account for the observed seasonal decline in clutch size in this species.

However, a number of reproductive parameters, despite exhibiting significant annual variability, either showed no seasonal effect or the direction of the response was opposite to that expected according to a seasonal decline in reproductive output. For instance, in contrast to many other studies on alcids which have demonstrated a significant negative effect of laying date on egg size (thick billed murres Gaston and Nettleship 1981, Birkhead and Nettleship 1982, razorbills Lloyd 1976, Atlantic puffins Harris 1980; Ashcroft 1976) including a black guillemot study (Petersen 1981), I found no relationship with laying date in two-egg clutches in this study. Moreover, egg size in single-egg clutches exhibited a significant positive relationship with laying date in three of the four years of this study. The only other report of a seasonal increase in egg size in alcids is that of Gaston and Nettleship (1981) who reported an increase in egg size with laying date in the thick billed murre *Uria Lomvia* during a year of unusually abundant prey. Assuming large eggs take longer to produce than small eggs then it is possible that late breeding black guillemots delayed laying in order to produce a larger egg. Why black guillemots might employ such a strategy however is unclear. Birkhead and Nettleship (1981) point out that chick growth is considerably faster than egg growth and consequently the observed delay in laying in order to produce a larger egg would not be advantageous in terms of fledging the brood earlier. However, these authors also suggest that potentially, the later a chick hatches the more important are its reserves in determining its subsequent growth and survival. Since larger eggs hatch larger and often better quality chicks, and growth and survival is reduced late in the season, it is conceivable that this strategy is necessary in the black guillemot.

Further to egg size, egg laying interval also exhibited seasonal variation in the opposite direction to that expected to be associated with the observed seasonal decline in breeding success. Egg-laying interval significantly decreased with laying date, thereby providing further support for the idea that egg production was not seasonally constrained. However, this relationship was evident only in a year of high breeding success, 1994. Furthermore, the annual variation in laying interval, although significant, appeared not to be related to breeding success.

I have previously shown in part 1 of this discussion that both egg size and the laying interval between eggs varied annually, strongly implying that egg size was sensitive to ecological conditions. Any associated seasonal decline in environmental condition should therefore also reflect a decrease in egg size and an increase in the laying interval. The apparent contradictory results of this study therefore suggest that seasonal effects are not a consequence of a decline in environmental conditions or, alternatively, that egg production was not affected by laying date.

In addition to egg production, the observed decline in incubation duration with laying date suggests that seasonal effects imposed little or no cost during incubation. As with egg size, incubation period varied annually, the observed increase during years of poor breeding success suggesting that environmental condition had a significant effect on incubation behaviour. Theoretically, therefore, if later laying parents were metabolically constrained to forage more often during incubation, then these birds should be expected to have had an extended incubation period relative to those of early nesters. In spite of this logic, the results of the present study contradicted this prediction, with the incubation period becoming relatively shorter towards the end of the season. This observation lends no support to the theory that incubation behaviour in late laying parents is constrained by adverse breeding conditions, indeed, it suggest the opposite. However, since chicks hatching later in the season suffered lower growth rates and survival, it is conceivable that late laying parents expended more effort during incubation in order to hatch their young earlier. This time saving strategy, however, although potentially beneficial for the offspring, is likely to incur additional metabolic costs for the parents.

Other breeding parameters, such as the degree of hatching asynchrony and egg-size disparity exhibited neither annual nor seasonal variability, despite showing considerable individual variation. Phenomena such as hatching asynchrony are likely to excerpt a considerable influence on the competitive dynamics of a brood and it is possible that parents are individually optimising the hatching pattern so as to maximise the number and quality of nestlings at each breeding attempt. However, why such individual variation was evident in this species without a corresponding temporal response, is ambiguous.

Factors influencing such strategies in the black guillemot are clearly complex, and controlled experimental manipulations are required to determine the major constraints affecting reproduction in this species. In addition, it would be pertinent to determine the adaptive nature of strategies such as hatching asynchrony: do parents facultatively adjust the hatching pattern or is the pattern simply a non-adaptive consequence of, for example, energetic constraints impinging upon incubation?

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CHAPTER 4

INTER- AND INTRA-CLUTCH EGG SIZE VARIATION

INTRODUCTION

Recent evidence strongly suggests that avian egg production is considerably more demanding than Lack (1947) originally envisaged in his seminal work on the evolution of clutch size (Monaghan and Nager 1997). Since maternal 'condition' or 'quality' typically differs within populations, the extent to which individuals are constrained during the reproductive bout, and thus in the capacity to meet the high costs of egg production, will vary accordingly. Potentially, these costs can be minimised either through a reduction in clutch size (in those species producing a clutch with more than one egg; Lack 1954, O'Connor 1978) but this inevitably produces a quantum jump downwards in potential fitness benefits. An alternative is a decrease in egg size (Schifferli 1973, Howe 1978, Ricklefs et al. 1978, O'Connor 1979, Birkhead and Nettleship 1982). However, a reduction in egg size may carry also fitness cost in terms of reduced offspring quality, but in a more graded form. For instance, it is generally recognised that chicks hatching from large eggs are heavier and of higher quality than those from smaller eggs (e.g Parsons 1970, Howe 1976, Furness 1983, Stokland and Amundsen 1988, Grant 1991, for review see Williams 1994). Moreover, nestlings from larger eggs often accrue fitness benefits in terms of higher growth rates (Schifferli 1973, Williams 1980, Birkhead and Nettleship 1982, Furness 1983, Magrath 1992) and have a greater probability of survival when ecological conditions deteriorate (Parsons 1970, 1975, Davis 1975, Howe 1976, 1978, O'Connor 1979, Ankney 1980, Birkhead and Nettleship 1982, Rofstad and Sandvik 1987, Davis 1975, Howe 1976, Thomas 1983). These benefits, however, are not ubiquitous among species, and a number of studies have failed to find an effect of egg size on offspring fitness (Ollason and Dunnet 1986, Arcese and Smith 1988, Galbraith 1988, Meathrel et al. 1993, Leblanc 1997). Nonetheless, for species in which chicks hatching from larger eggs do possess an intrinsic advantage of some form, any parental benefit attained through a reduction in egg size is likely to be traded-off against a reduction in offspring fitness.

Variation in egg size is commonplace within most avian species, with the largest egg in the population often being up to twice the size of the smallest (Arcese and Smith 1988, Stokland and Amundsen 1988, Magrath 1992). The adaptive value of this variation has received considerable attention in recent years, both at the level of the population and at the level of the individual clutch. At the population level, egg size variation has been examined with respect to various parameters of offspring fitness (hatching success, chick growth and survival) in relation to parental quality, laying date, locality and clutch size. At the intra-clutch level, egg size variation has been studied with regard to its effects on brood competitive dynamics, largely in association with hatching asynchrony. Slagsvold et al. (1984) posited that the direction of intra-clutch egg size variation, i.e. whether the size of the final egg is smaller or larger than the remainder of the clutch, was related respectively to a parental strategy of either facilitating or counteracting the effects of hatching asynchrony. Conversely, other authors have considered egg size variation to be a non-adaptive consequence of various constraints acting during egg production. For example, several authors claim that such variation might simply reflect a depletion of nutrient or energetic reserves at the end of the laying sequence. This line of reasoning is primarily based on the fact that differences in intra-clutch egg size variation have been found for the same species between localities (Jarvinen and Ylimaunu 1986, Pierotti and Bellrose 1986) or breeding seasons (Ylimaunu and Jarvinen 1987).

Black guillemots are unusual among auks in that the normal clutch size is two eggs rather than one. Since these eggs are comparable in size to those produced by single-egg species, total clutch investment in relation to adult body weight is far higher in the black guillemot. It is conceivable, therefore, that egg investment is relatively costly in this species (See also general introduction for examples of other factors suggesting that reproduction is costly in the black guillemot).

In this chapter I report a correlative study of egg size variation in the black guillemot, collected over all four years of the study period. The shortcomings of such non-experimental studies examining the effects of egg-size variation are well documented (Mueller 1990, Bolton 1991, Williams 1994), the main criticism being that the potential overriding effect of parental quality is not experimentally controlled. However, regardless of the adaptive nature of egg size variation, both at the level of the population and the clutch, the potential exists for such variation to have consequences for the hatchlings. This study is therefore not attempting to disentangle the relative effects of egg size and other attributes such as parental quality on offspring fitness, but rather to determine whether any relationships exist between egg size and other reproductive parameters and, if so, the consequences of such relationships for the chicks. Firstly, I will describe the pattern of egg mass variation, both at the level of the population and the clutch, with the view to determining whether egg production is likely to be constrained in this species. I will also determine the mechanisms that parents use to minimise the costs of egg production and examine the resulting consequences of these actions for the offspring. I will further discuss the adaptive significance of intra-clutch egg size variation and the possible functional significance of such patterns.

METHODS

Methods for collecting data are as described in the general methods chapter, sections 1-4.

TERMINOLOGY AND ANALYSIS

Within two-egg clutches, each egg is referred to as a- or b-egg according to the order of laying. Correspondingly, chicks hatching from two-egg clutches are referred to as a- or b-chick. This was related to hatching order in asynchronous broods (a-chick hatches first in broods where the hatching interval is ≥ 1 day), and to the egg from which the sibling hatched in synchronous broods (a-chick hatches from a-egg). Clutches where laying or hatching order were not determined were excluded from analyses. Eggs and chicks from single-egg clutches are referred to as s-eggs and s-chicks, respectfully. Laying date for the clutch is defined as the laying date of the a-egg (in Julian days) which in turn was standardised across years by subtracting the date of clutch initiation from the median laying date of that year.

Data from all four years (1994 to 1997 inclusive) were used for the egg and incubation analyses in this chapter. However, as a consequence of the experimental manipulation of chicks in 1995 by a fellow PhD student, chick growth and survival data from this year was excluded from analyses. Appropriate parametric statistics were applied where data met the parametric assumptions of normality and homoscedasticity. Where these assumptions were not met appropriate non-parametric tests were used. Arcsine transformed data were used for proportional data but means etc. for such data are expressed in the original data format. Normality was tested for using the Kolmogorov-Smirnov goodness-of-fit test and all probabilities given are two-tailed.

I analysed hatching success and postnatal mortality as a function of year using logistic regression analysis. Since all ratios between explained deviance and the degrees of freedom (d.f.) were close to one, significance tests were based on the χ^2-distribution (Crawley 1992).

Analyses were undertaken using SPSS for Windows release 7 and all tests were as described by Zar (1996).

RESULTS

1. Egg-size variation

Egg size showed considerable variation within this population of black guillemots, both for two-egg and single-egg clutches. Volumes of 166 a-eggs over the four year study period ranged between 34.4 and 72.9 cm^3, with a mean volume of 43.7 cm^3 ±4.03 S.D., coef. variation= 9.2 , whereas the mass of 165 a-eggs ranged between 39 and 61g, with a mean of 50.1g ±4.2 S.D., coef. variation= 8.3. For b-eggs, volumes of 169 eggs ranged between 32.5 and 50.6 cm^3, with a mean volume of 42.38 cm3 ±3.1 S.D., coef. variation= 7.3. The mass of 169 b-eggs ranged between 39 and 58g, with a mean of 48.43g ±3.82 S.D., coef. variation= 7.8 (see also table 3.4, chapter 3). Egg size among single-egg clutches was also highly variable. Mean egg volume for 41 eggs from single-egg clutches was 43.01cm^3 ±3.57 S.D., coef. variation= 8.3, ranging between 35 and 54cm^3. The mean egg mass of 39 eggs was 50.31g ±4.0 S.D., coef. variation= 7.9 ranging between 39 and 58g. Although a part of this variation was a consequence of annual differences in egg mass (chapter 3), no yearly effect was evident for egg volume in two-chick broods, nor for mass and volume in single egg clutches, thereby confirming that within any year, egg size varies considerably.

Egg mass at laying was positively correlated with egg volume (a-eggs: Pearson's correlation $r=0.645$, $n=182$, $p<0.01$; b-eggs: $r=0.824$, $n=186$, $p<0.01$; s-eggs: $r=0.887$, $n=36$, $p<0.01$; fig 4.1). Eggs that were structurally larger therefore also tended to be heavier. However, since it is likely that the same breeding adult was used more than once, this and other data pooled across years cannot be considered as strictly independent. Caution should therefore be exercised when interpreting such results.

Figure 4.1. The relationship between egg mass and egg volume, a) a-egg

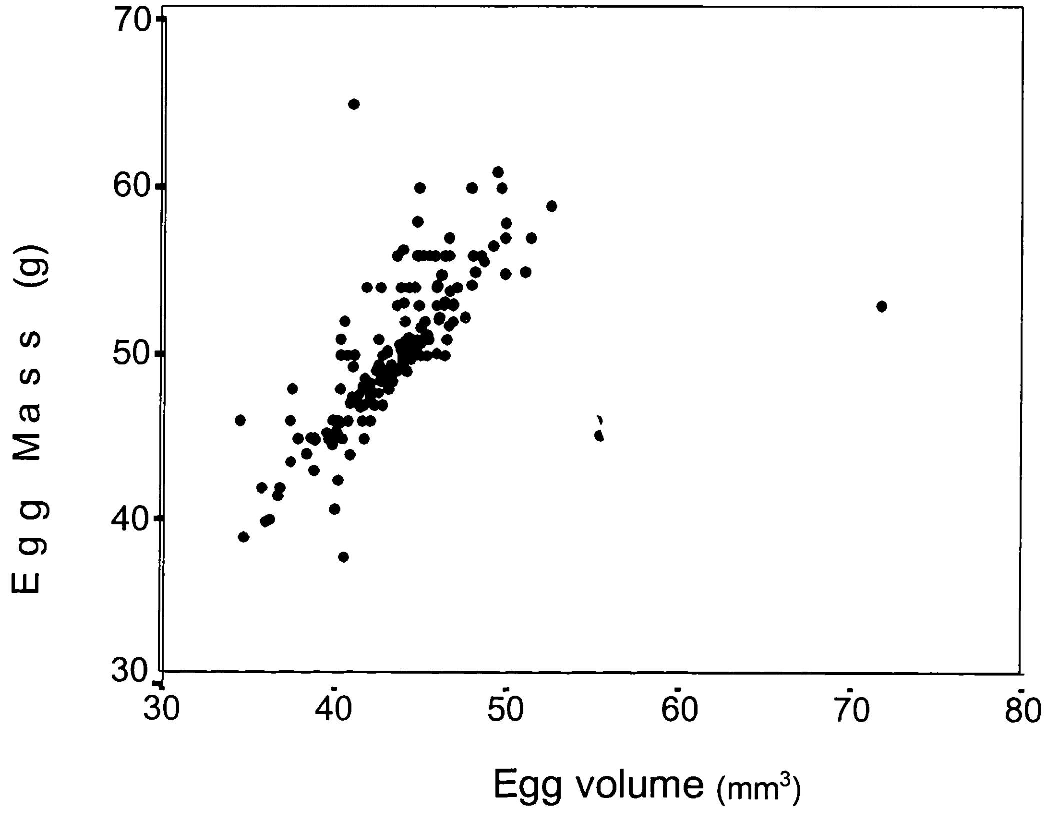


Figure 4.1. The relationship between egg mass and egg volume, b) b-egg

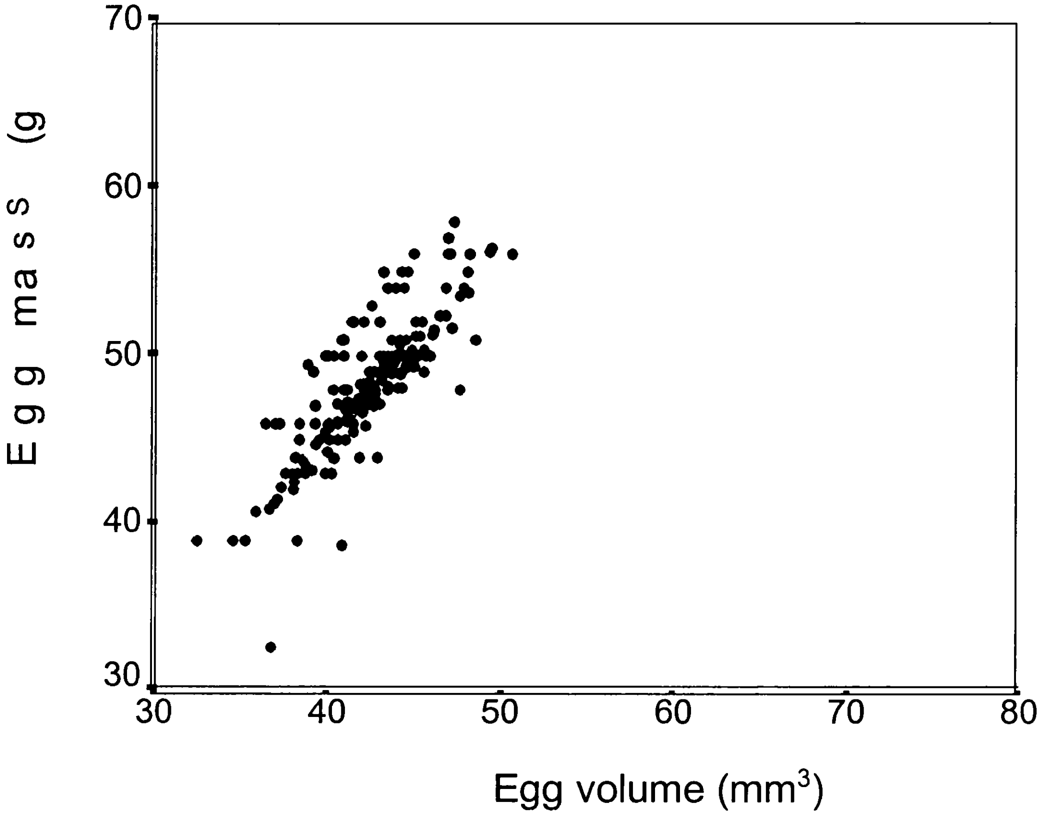
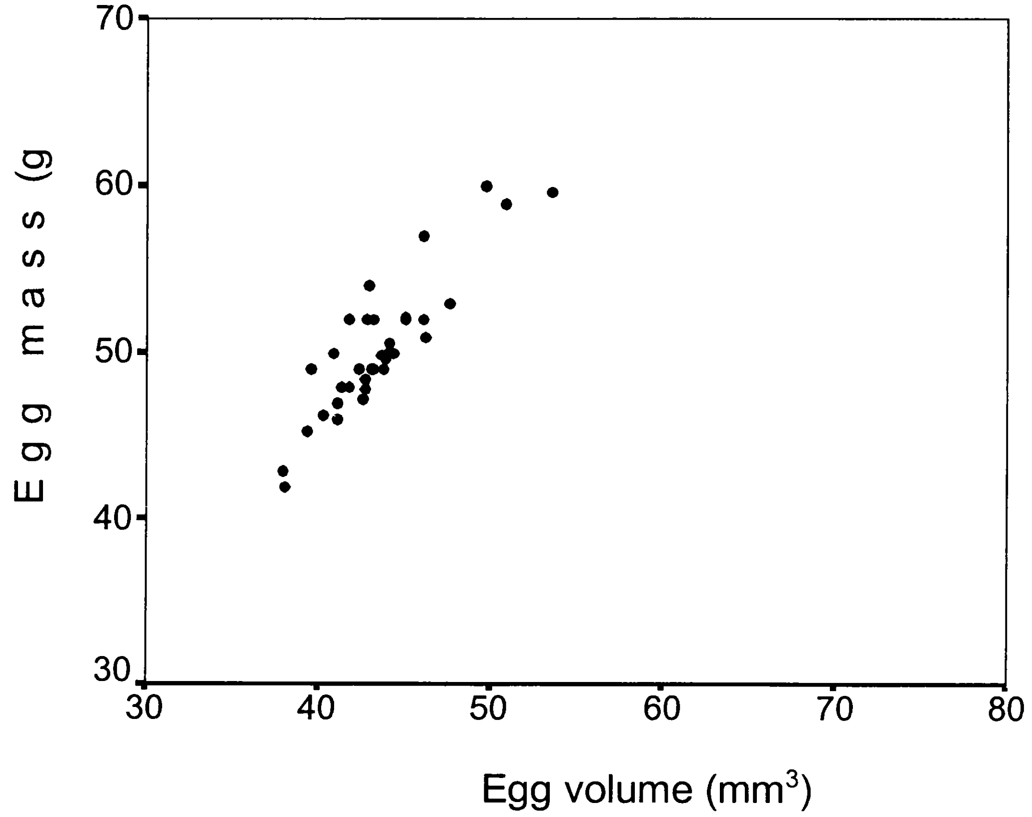


Figure 4.1. The relationship between egg mass and egg volume, c) s-egg



Laying order effects

Within-nest comparisons (paired t-tests) revealed that egg mass and egg volume generally decreased with laying order: first-laid eggs were significantly larger than b-eggs both in terms of mass and volume for all four years (see table 4.1). A linear regression analysis revealed that within clutches, the mass of the a-egg was a significant predictor of the mass of the b-egg for all four years (fig 4.2); 1994: $Y=15.828+0.66x$, $R^2=0.502$, $F_{1,31}=33.2$, p,0.001; 1995: $Y=9.784+0.764x$, $R^2=0.526$, $F_{1,44}=51.02$, $p<0.001$; 1996: $Y=10.73+0.759x$, $R^2=0.767$, $F_{1,43}=146.22$, $p<0.001$; 1997: $Y=8.757+0.786x$, $R^2=0.72$, $F_{1,39}=104.32$, $p<0.0001$). Thus, those females producing large a-eggs also have the capacity to produce large second eggs, strongly implying that egg size is determined by female quality. However, the difference between first- and second-laid eggs was generally small, the mean size of the b-egg being 96.7 % of the a-egg (n=165). Moreover, a significant negative correlation was evident between a-egg size and the within-clutch egg-size disparity (the relative mass of the b-egg to the a-egg; $r=-0.374$, $p<0.01$, n=165 fig. 4.3). That is, as a-egg size decreased, the size of the b-egg relative to the a-egg increased, to the extent that in 17% of clutches the b-egg was heavier than the a-egg. (The problems associated with repeated testing and the possible use of Bonferroni's test are discussed in chapter 3.)

Fig 4.2. The within-clutch relationship between a-egg mass and b-egg mass, a) 1994

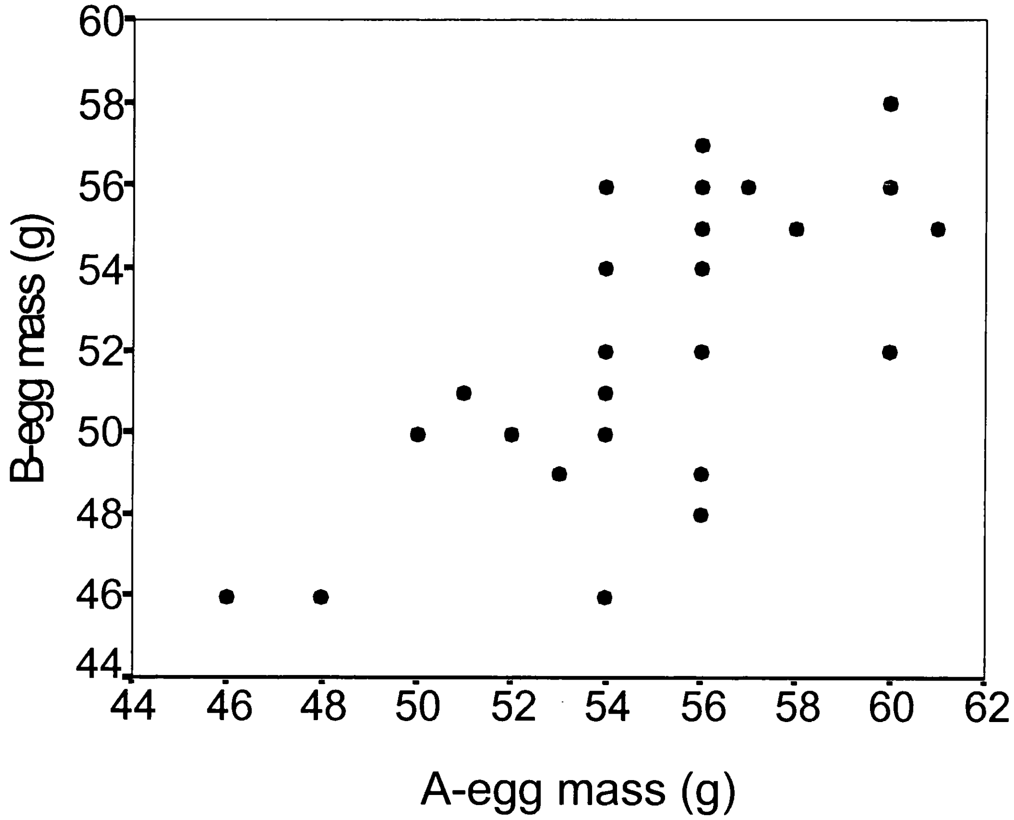


Fig 4.2. The within-clutch relationship between a-egg mass and b-egg mass, b) 1995

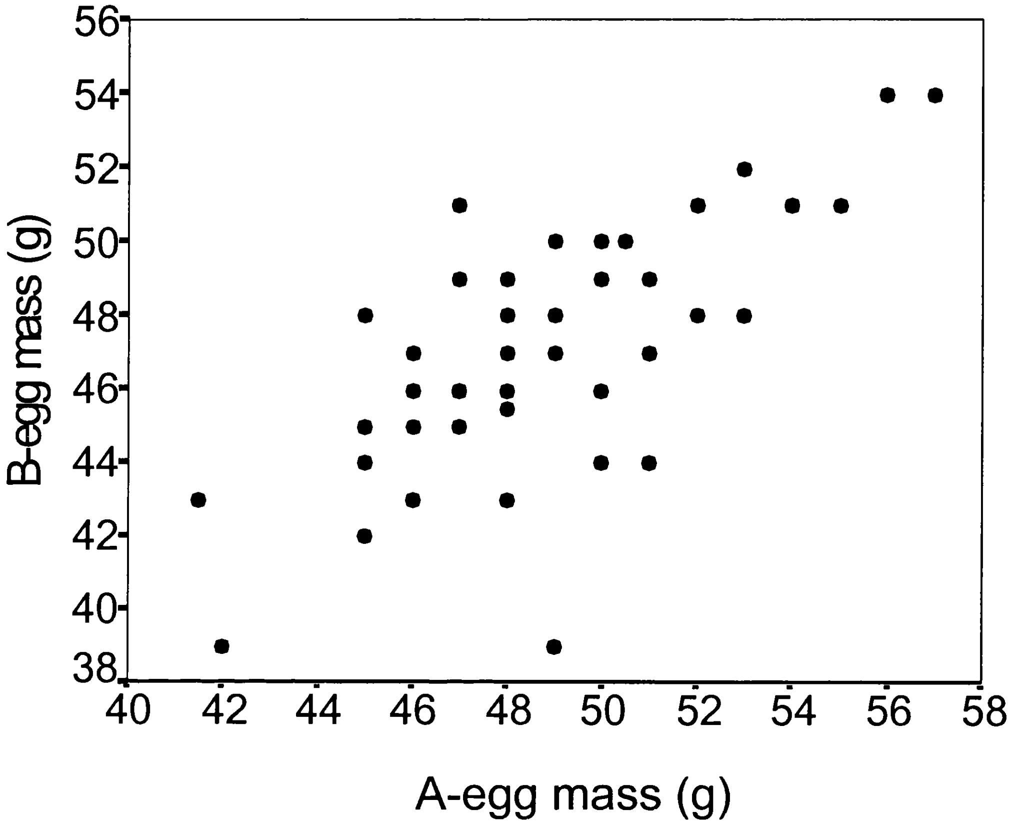


Fig 4.2. The within-clutch relationship between a-egg mass and b-egg mass, c) 1996

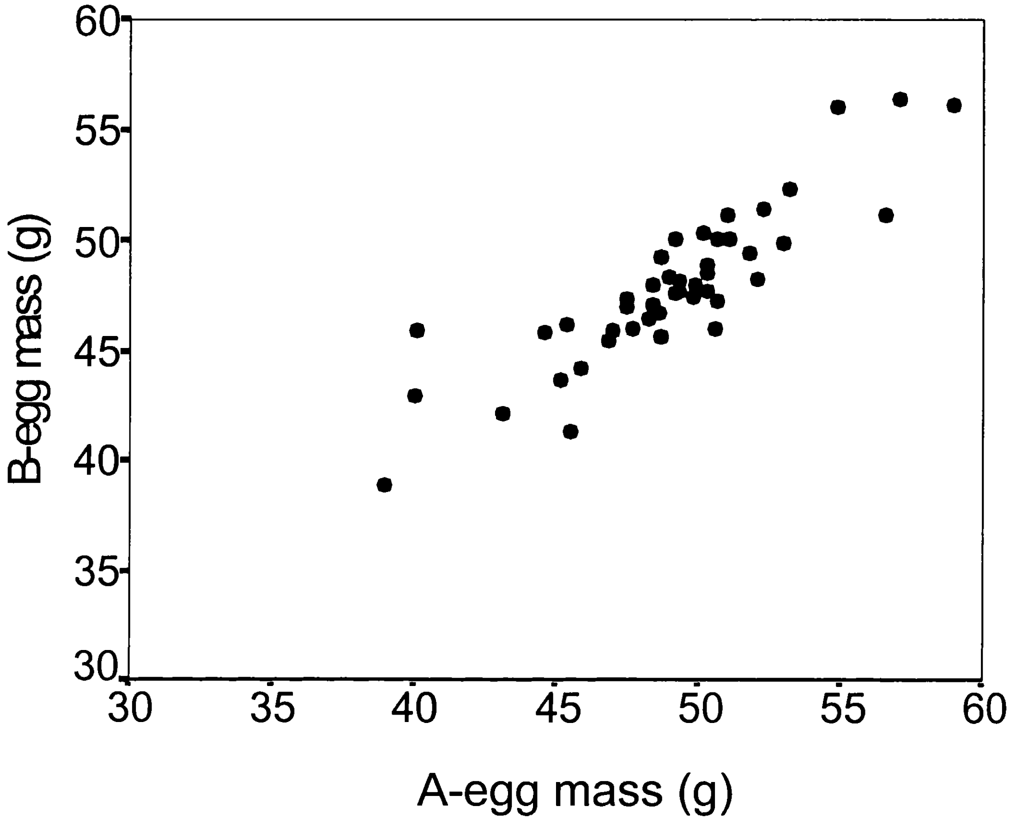


Fig 4.2. The within-clutch relationship between a-egg mass and b-egg mass, d) 1997

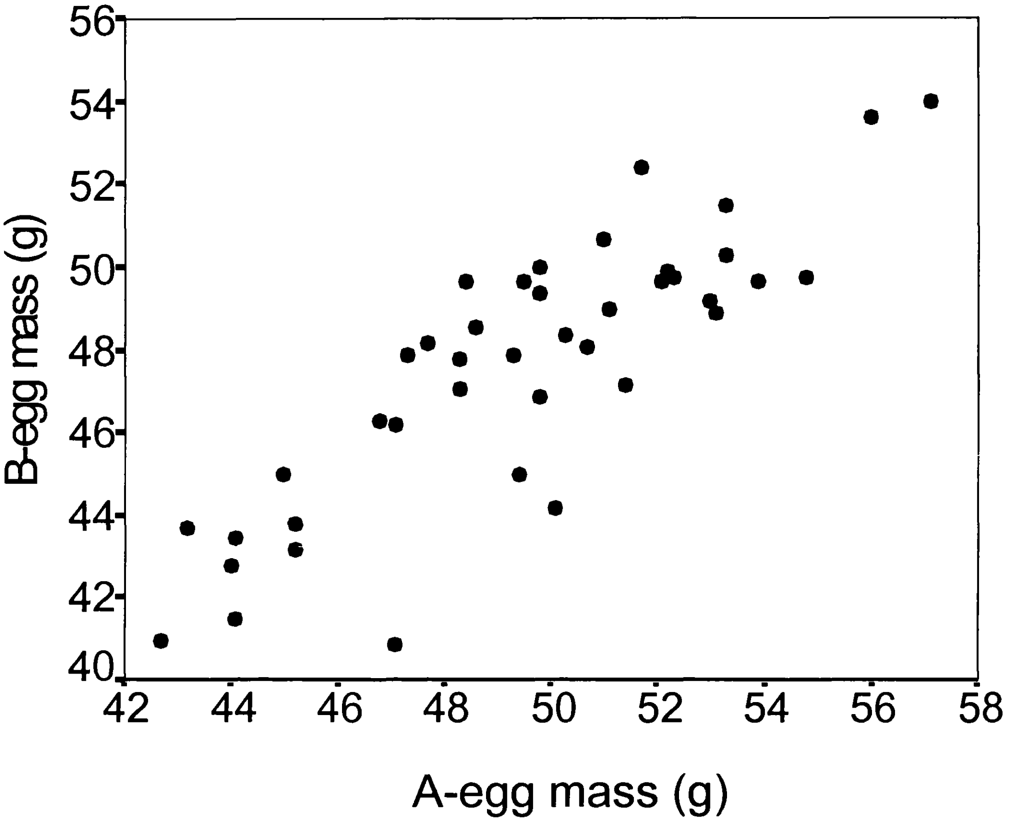
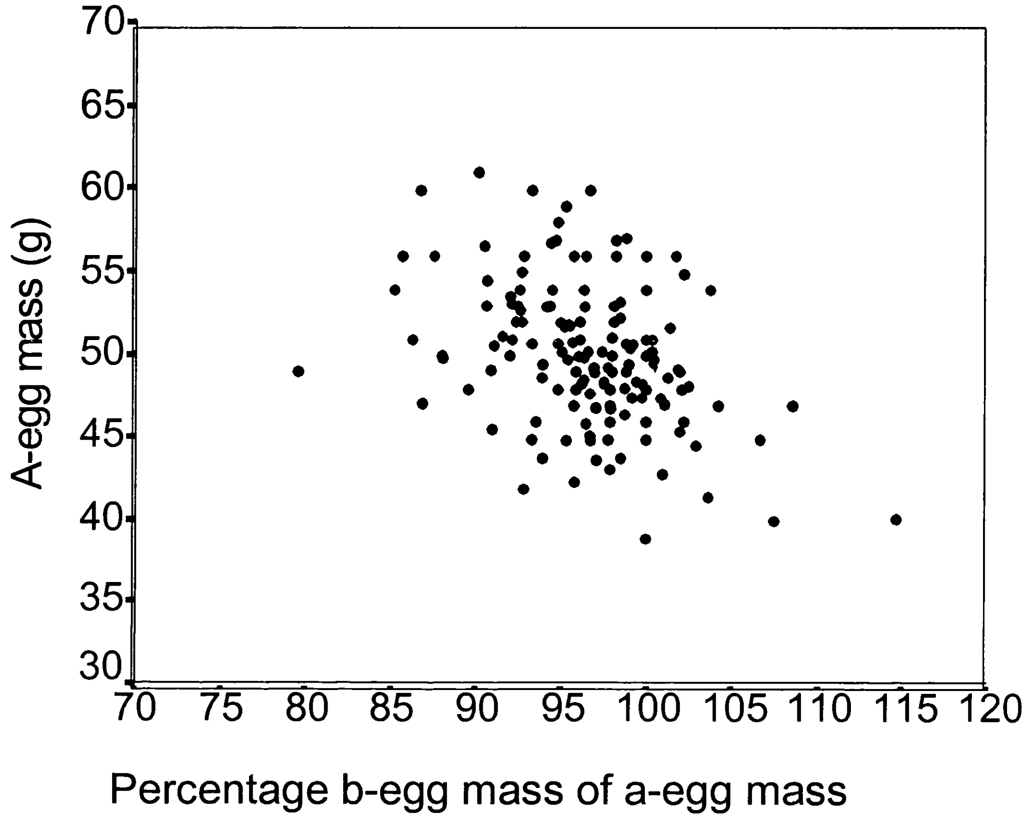


Table 4.1. Egg size (mass and volume) and paired t-test statistics of within-clutch comparisons.

		Egg mass, ±S.E. (n)	**t**	**p**	**Egg volume, ±S.E. (n)**	**t**	**p**
1994	**a-egg**	54.24 3.73 (33)	5.087	<0.001	43.71 3.31 (33)	3.315	0.0023
	b-egg	51.85 3.44 (33)			42.65 3.27 (33)		
1995	**a-egg**	48.85 3.41 (46)	4.591	<0.001	43.15 3.15 (46)	4.879	<0.001
	b-egg	47.12 3.56 (45)			41.94 3.16 (46)		
1996	**a-egg**	49.05 4.06 (45)	3.843	<0.001	43.39 3.58 (46)	3.115	0.0032
	b-egg	47.94 3.50 (45)			42.64 3.13 (46)		
1997	**a-egg**	49.47 3.55 (41)	6.330	<0.001	44.64 5.60 (41)	2.999	0.004
	b-egg	47.62 3.26 (41)			42.33 2.93 (41)		
All years pooled	**a-egg**	50.14 4.21 (165)	9.634	<0.001	43.70 4.03 (166)	5.877	<0.001
	b-egg	48.41 4.21 165			42.37 3.10 (166)		

Fig 4.3 . Relationship between a-egg size and within-clutch egg-size disparity for all four years combined



Relationship between egg size and clutch size

When, therefore, should a female lay a larger single egg rather than two small eggs? A Mann-Whitney U-test was used to assess size differences between eggs in two-egg and one-egg clutches (table 4.2, see also table 4.1 for means etc.). In all years, s-egg volume and s-egg mass did not differ significantly from those of a-eggs or b-eggs (table 4.2), except in 1995 when s-egg mass was higher than b-egg mass. Since the period of yolk deposition is typically longer than the egg laying interval (Carey 1996), it suggests that in single-egg clutches, egg size is conserved at the expense of laying a second egg. Thus, an egg size - egg number trade-off appears to operating, whereby a larger single egg appears to be favoured once the fitness benefits of producing two eggs declines below that of producing a relatively large single egg.

Table 4.2. Mann-Whitney U-test statistics for size comparisons between egg types for the years 1994 to 1997.

	1994	**1995**	**1996**	**1997**
a-egg vs s-egg mass	U=159, p=0.892, n=8,41	U=322, p=0.457 n=16,46	U=88.5, p=0.662 n=4,51	U=191.5, p=0.798 n=9,45
b-egg vs s-egg mass	U=109, p=0.134, n=8,41	U=238, p=0.023, n=16,48	U=90.5, p=0.667, n=4,52	U=150, p=0.223, n=9,45
a-egg vs s-egg volume	U=110.5, p=0.244	U=364, p=0.949	U=86, p=0.358	U=157.5, p=0.358
b-egg vs s-egg volume	U=147, p=0.858	U=288, p=0.137	U=107, P=0.8	U=194, p=0.794

Egg mass and other breeding parameters

For all years combined, incubation period (measured as the number of days from laying to hatching) showed no relationship with egg volume in two-egg clutches (Pearson's correlation: b-egg r=-0.098, p=0.301, n=114), nor in single-egg clutches (s-egg: r=-0.172, p=0.445, n=22).

Egg size showed no significant relationship with the degree of hatching asynchrony (see table 4.3). Furthermore, chick quality at hatching (mass at hatching divided by tarsus length at hatching), for both a- and b-chicks, did not differ between synchronous and asynchronous broods (a-chick: mean synchronous = 1.54 ±0.047 S.E, n=13, mean asynchronous = 1.57, ±0.043 S.E., n=11; t=0.325, p=0.749; b-chick: mean synchronous = 1.57 ±0.039 S.E., n=13, mean asynchronous = 1.53 ±0.05 S.E., n=11; t=-0.569, p=0.575.

Table 4.3. One-way ANOVA statistics comparing various egg size parameters with the degree of hatching asynchrony (0,1,2 and 3 days).

Variable	**F**	**p**
Clutch mass	$F_{3,101}=0.952$	0.418
Clutch volume	$F_{3,116}=1.632$	0.186
b-egg mass / a-egg mass	$F_{3,101}=0.486$	0.693
b-egg volume / a-egg volume	$F_{3,116}=0.530$	0.663
a-egg mass	$F_{3,101}=0.537$	0.658
b-egg mass	$F_{3,108}=1.213$	0.308
a-egg volume	$F_{3,116}=1.775$	0.156
b-egg volume	$F_{3,116}=0.953$	0.417

2. Hatching success

Hatching success did not differ significantly among years (Chapter 3) and data from each year were therefore pooled for all analyses on hatching success. Of the 166 clutches where hatching order was established, 138 (83%) of all a-eggs hatched whereas only 119 (71%) of all b-eggs hatched. Since predation and nest flooding was likely to act randomly with respect to egg size (unless nest site and adult quality are linked), nests that suffered these effects were excluded from further analyses regarding hatching success. (Egg loss by predation occurred respectively in 11 (6.7%) and 13 (7.9%) of a- and b-eggs, largely as a result of otter predation.) After taking these randomly acting factors into account, significantly more b-eggs failed to hatch than a-eggs ($\chi^2=6.54$, n=329 eggs, p=0.011). It is interesting to note that at least one egg in each of these broods hatched, suggesting that embryo mortality was not a consequence of the incubation regime employed.

Laying order and egg size effects

Since a smaller proportion of b-eggs failed to hatch than a-eggs, it is conceivable that egg viability either decreases with egg size (because b-eggs were smaller than a-eggs), decreases due to factors associated with laying order independent of egg size, or that egg viability is affected by a combination of these factors. Table 4.4 shows the mean size (mass and volume) of those eggs that hatched and those eggs that failed to hatch. For b-eggs, eggs that failed to hatch were significantly smaller, both in terms of mass and volume, than those that hatched (table 4.4). This result provides further support for the idea that egg viability decreases once egg size declines below a certain minimum size. Nonetheless, egg size appeared not to affect hatching success in a-eggs, with very little difference in egg size being found between those a-eggs that hatched and those that did not. Thus, although egg size appears to be an important component of b-egg viability, the lack of a size effect in a-eggs suggests that factors other than size, such as egg composition, were also important for egg viability. No significant difference was found in the relative size of the b-egg to the a-egg in those b-eggs that hatched and those that did not, revealing that egg viability was not related to egg size disparity within the clutch.

Egg volume in single-egg clutches that hatched did not significantly vary from those that did not hatch (Mann Whitney U-test: mean volume of hatched single eggs 42.63 mm^3; mean volume of single-eggs that did not hatch 44.35 mm^3; n=36,3 U=21, p=0.133).

Table 4.4. The relationship between egg size parameters and egg hatching success. T-test statistics are used to compare size parameters in eggs that hatch and those that fail to hatch.

Variable	**Mean size of eggs that hatched ±S.D., (n)**	**Mean size of eggs that failed to hatch ±S.D., (n)**	**t**	**p**
a-egg mass (g)	50.08 ± 4.32 (135)	50.58 ±3.75 (28)	0.566	0.572
a-egg volume	43.68 ±4.21 (136)	43.93 ±3.19 (28)	0.301	0.764
b-egg mass	48.80 ±3.81 (118)	46.83 ±3.11 (43)	-2.944	0.004
b-egg volume	42.60 ±3.04 (118)	41.27, ± 2.76 (43)	-2.509	0.013
Relative mass of b-egg (b-/a-egg)	96.55 ±4.54 (116)	96.79, ±4.75 (42)	0.292	0.771
Relative volume of b-egg (b-/a-egg)	97.12 ±4.97 (117)	97.93 ±4.34 (41)	0.924	0.357

It therefore appears that smaller, second-laid eggs were less likely to hatch than first-laid eggs. However, it is possible that this effect was due to position in the laying sequence, rather than to egg size per se. To investigate the effect of egg size on hatching success in greater detail, I performed stepwise logistic regression analysis. The logistic regression procedure assesses the importance of one or more independent variables in predicting a particular outcome on a binary dependent variable (see Bolton 1991). Hatching success over all years was pooled and each egg type was analysed independently with egg size (mass and volume) and laying date used as continuous variables. The analysis revealed that mass was a significant predictor of hatching in the b-egg (χ^2=7.62, d.f.=1, n=163, p=0.006) but not in the a-egg (χ^2=0.32, d.f.=1, n=163, p=0.56)

One-egg versus two-egg clutches

When data for all years were pooled, hatching success (number of eggs hatched/ number of eggs laid) did not differ between one- and two-egg clutches (Mann-Whitney U-test $U_{46,208}$=4373 p=0.256). Therefore, as far as hatching success was concerned, eggs from single-egg clutches appeared to be of similar quality to eggs from two-egg clutches.

3. Egg size and mass of chick at hatching

The size of neonates at hatching was generally positively related to egg size, indicating that large eggs hatched proportionally larger hatchlings than small eggs. Pooling data from all years, a significant positive relationship between egg mass and hatching body mass of the neonate was found for both a- and b-eggs (a-egg: Pearson's r=0.77, n=25, p<0.01; b-egg: r=0.72, n=25, p<0.01). However, although a significant positive relationship between egg mass and hatching tarsus length was evident for b-eggs (Pearson's r=0.511, n=23, p=0.013), the correlation was not significant for a-eggs (Pearson's r=0.311, n=23, p=0.148). A significant correlation between egg mass and chick quality (mass/tarsus) was evident for both a-eggs (r=0.610, n=41, p<0.001) and b-eggs (r=0.422, n=41, p=0.006), showing that chicks hatching from larger eggs were in better condition than those hatching from smaller eggs.

4. Chick growth

No significant correlation was evident between egg size and instantaneous growth for either the a-chick (1994: r=0.294, p=0.145, n=26; 1996: r=0.122, p=0.55, n=26; 1997: r=-0.281, p=0.164, n=26), or the b-chick (1994: r=-0.127, p=0.58, n=21; 1996: r=0.185, p=0.45, n=19; 1997: r=-0.244, p=0.38, n=15). Thus, despite large eggs hatching larger chicks, it appears that chick growth is independent of egg size and is probably determined to a large extent by parental provisioning rates. (I compare relative sibling growth rates in chapters 3 and 5).

5. Chick survival

Since predation is probably a randomly acting process on black guillemot survival, only those nests where predation did not occur were included in the following analysis.

A comparison was made of egg size of chicks that died during the first 14 days and those that survived until this age (Table 4.5). This comparison revealed that egg size had no significant effect on survival during this crucial period. If chick survival is attributable to the relative size disparity between eggs, rather than egg size itself, this infers that laying sequence and competitive dynamics influences mortality. However, there was no effect of relative egg size on the survival of either a- or b-eggs (Table 4.5).

Table 4.5. The relationship between egg size parameters and chick survival. T-test statistics are used to compare egg size parameters in chicks that survive and those that die prior to age 14 days.

	a-chicks that survive ±S.D. (n)	**a-chicks that do not survive ±S.D. (n)**	**t**	**p**
Mean a-egg mass (g)	49.87 ±4.29 (98)	50.64 ±5.15 (17)	0.656	0.513
Mean a-egg volume	43.47 ± 3.39 (104)	43.19 ±3.33 (17)	-0.315	0.571
Mean % b-egg mass of a-egg mass	96.84 ±4.28 (67)	95.51 ±5.07 (21)	-1.18	0.239
Mean % b-egg volume of a-egg volume	97.61 ± 3.97 (67)	97.58 ±3.99 (21)	-0.33	0.974
	b-chicks that survive, ±S.D., (n)	**b-chick that do not survive, ±S.D., (n)**	**t**	**p**
Mean b-egg mass	49.25 ±3.49 (59)	48.52, ±4.08 (38)	-0.942	0.349
Mean b-egg volume	42.59 ±2.81 (64)	42.75, ±3.47 (39)	0.259	0.796
Mean % b-egg mass of a-egg mass	96.75 ±4.75 (46)	95.58 ±4.06 (30)	-1.11	0.27
Mean % b-egg volume of a-egg volume	97.67 ±4.34 (47)	95.93 ±7.18 (30)	-1.32	0.190

DISCUSSION

Inter-clutch egg size variability: is egg production constrained?

As in many other seabird species, inter-clutch egg-size variation was high in this population of black guillemots, with the largest egg being 56% larger than the smallest egg. A number of factors have been shown to affect avian egg size; for example, the genetic and phenotypic components of parental quality, in addition to factors associated with the environment such as energetic constraints prevailing at the time of egg-laying, food intake, and climatic conditions (Ojanen et al. 1981, Arnold 1991, Williams 1994, Dufva 1996). I have previously shown that increased egg size is associated with early laying in a year of relatively high breeding success (i.e. 1994; chapter 3). Therefore, regardless of the potential genetic influence on egg size, an improvement in environmental conditions appears to be expressed as an increase in egg size and, in two-egg clutches, a decrease in the laying interval. Thus, during less favourable years, nutritional or energetic constraints in the black guillemot were manifest as a population wide reduction in egg size. In the same context, egg production at the individual level may be affected not only by environmental conditions, but also by each individual's capacity to obtain essential resources and to assimilate them efficiently. Higher quality individuals should therefore be expected to produce larger clutches with larger, higher quality eggs.

In this study, a-eggs were generally larger than b-eggs, but those females producing large a-eggs also had the capacity to produce large second eggs, strongly inferring that egg size is a function of female quality. Furthermore, as a-egg size declined, the size of the b-egg relative to that of the a-egg increased, such that females producing a small a-egg produced a similar sized b-egg. This relationship might suggest that certain females are unable to produce a relatively large a-egg. Moreover, if egg viability decreases once a certain minimum size threshold is reached, females producing a small a-egg might be compelled to produce a similar sized b-egg because any further reduction in b-egg size would compromise egg viability.

Other studies have demonstrated environmental effects on egg size, the nature of the response varying according to species (Magrath 1992 and references therein). For example, Wiebe and Bortolotti (1995) found that experimental food supplementation increased egg mass independent of laying date in the American kestrel *Falco sparverius*, whereas air temperatures and hence thermoregulatory costs affected egg production in the blackbird, rather than food supply per se. However, Magrath (1992) highlighted the problems of interpreting such data since increased food supply may cause either or both earlier laying and increased egg size, thereby masking any effects of food supply on egg mass.

Some authors have proposed that if the production of large eggs requires more time to amass reserves than smaller eggs, birds producing small eggs will be able to lay earlier which may increase reproductive success (Furness 1983, Birkhead and Nettleship 1984, Redmond 1986, Bolton 1991, Williams 1994, Perrins 1996).

Egg size and chick quality

Black guillemot neonates that hatched from larger eggs weighed more and were heavier for their structural size than those that hatched from smaller eggs. This pattern of increasing neonate mass with egg size appears to be a widespread phenomenon in birds and reflects the results of many other studies showing that chicks hatching from larger eggs are heavier since they are structurally larger and/or carry greater nutrient reserves such as proteins and lipids (Ricklefs et al. 1978, Galbraith 1988, Reid and Boersma 1990, Bolton 1991). Yolk content can increase either allometrically or isometrically with increasing egg size and it has been argued that larger eggs will generally have absolutely more nutrients than small eggs (Ankney and Bisset 1976). The benefits that neonates accrue from increased egg size have been considered mainly with respect to nutrient reserves. Because nestlings from larger eggs have larger yolk reserves remaining at hatching (either proportionally or disproportionally larger), they will be able to withstand periods either of inclement weather or food shortage, or both, for longer. Larger nestlings also tend to be better insulated and have a lower surface area to volume ratio. It is therefore conceivable that female black guillemots that laid larger eggs provided them with more nutrients than were found in smaller ones. This decrease in chick quality with decreasing egg size further suggests that nutrients were limited and thus that egg production may have been more costly for certain individuals.

Consequences of egg size

I have shown that egg size varies considerably within this population of black guillemots, and that egg size is reflected in chick size and quality. What, therefore, were the consequences of egg size per se on nestling fitness, and how does the degree of within-clutch egg size disparity affect the respective siblings within a brood?

Embryo survival was higher in first- than in second-laid eggs, with significantly more a-eggs hatching than b-eggs. Further, second-laid eggs that survived to hatching were larger than those that failed to hatch, but no such difference was evident in first-laid eggs. This higher embryo survival and lack of an effect of egg size in a-eggs suggests that additional factors associated with laying order, independent of egg size, were important in determining hatching success. However, the fact that egg size was a significant predictor of hatching success in the b-egg is highly indicative of a decrease in egg viability once a minimum critical size is reached.

An effect of egg size on hatching success has been demonstrated for a number of other species (e.g. Morris et al. 1968, Birkhead and Nettleship 1982, Weimeskirch 1990, Croxall et al. 1992, Magrath 1992), although such effects are far from universal and a number of both experimental (Moss et al. 1981, Reid and Boersma 1990, Bolton 1991) and correlational studies (Lloyd 1979, O'Connor 1979, Bancroft 1984, Ollason and Dunnet 1986) have failed to find such an effect. Some authors have considered hatching failure to be a result of temporary egg neglect (e.g. Weimeskirch 1990). As egg size decreases, the surface area to volume ratio increases, meaning that smaller eggs lose heat faster and succumb to proportionately greater water loss through evaporation (Drent 1970), and at a relatively higher rate (Carey et al. 1983). During periods when parents are not incubating, smaller eggs will thus cool faster than larger ones and are more at risk of dropping below the physiological critical temperature. However, in the black guillemot, no relationship was evident between incubation period and egg size, suggesting no difference in the degree of egg neglect between clutches containing large eggs and those containing small eggs. Indeed, egg neglect is a common feature of the black guillemot, with eggs being attended on average for only 84% of the incubation period (Cramp 1985). In addition, these eggs are capable of withstanding considerable neglect in comparison to most other species: up to 15 consecutive days without incubation (Bergman 1971, quoted in Harris and Birkhead 1985). Since any detrimental effects of egg neglect would be independent of laying order after clutch completion, it is likely that all eggs in the clutch would be equally affected. However, over all four years of this study, all clutches save those that were either depredated or flooded, hatched at least one egg. It is thus highly unlikely that incubation behaviour had a profound effect on hatching success in the black guillemot.

A number of studies have revealed that avian egg size affects nestling fitness, both in terms of growth (Furness 1983, Magrath 1992a) and survival (Moss et al. 1981). However, a major failing of many studies claiming an effect of egg size on chick fitness is the lack of experimental control for the potentially confounding effects of parental quality or age (see Birkhead and Nettleship 1982, Amundsen and Stokland 1990, Mueller 1990, Bolton 1991, Reid and Boersma 1990). Indeed, more recent studies controlling for such effects show that little unequivocal evidence exists in support of the positive relationship between egg size and chick fitness in birds (Severinghaus 1983, Quinn and Morris 1986, Davis 1975, Schreiber et al. 1979, Bolton 1991, see Williams 1994 for review). Moreover, very few studies have considered the effect of egg size on the ultimate level of fitness, survival until recruitment (Williams et al. 1993, Price and Boag 1987). In spite of these limitations, more convincing evidence now suggests that large eggs have important fitness consequences for the nestling during the immediate post-hatching stage (see Amundsen and Stokland 1990).

With these studies in mind, it would be reasonable to assume that the relatively heavy and better quality chicks hatching from large eggs would be more successful during the crucial post-hatching period when avian nestlings appear to be most at risk from starvation. Surprisingly, however, although non-predation related mortality in black guillemot nestlings occurred primarily during the first two weeks post-hatching, I found no significant effect of egg size on either growth or survival in black guillemot chicks during this early post-hatching period. It is possible that relatively benign conditions during the chick rearing period might have masked the effects of egg size on growth and survival, but the significant effect of year on egg size, chick growth rates and other breeding parameters (chapter 3), strongly implies that rearing conditions were variable over the study period, thus any effects of egg size should have been manifest.

Alternatively, with respect to chick growth, it is possible that parental effort during the nestling phase may have overridden any effects of egg size. For instance, unlike chick mortality, it is possible that the small size and potentially slow growth rate of hatchlings from small eggs can be compensated for later in the chick-rearing period (Ojanen 1983). Providing that individuals from smaller eggs survive the critical post-hatch period, size differentials may disappear and have little effect on the survival of older nestlings. For example, despite the fact that a high percentage of herring gull chicks from last-laid eggs died shortly after hatching, those that survived exhibited similar mortality rates to those hatching from larger eggs (Parsons 1970). This parental effort effect is potentially relevant to many seabird species with relatively long nestling periods, including the back guillemot. It is possible therefore that parental attributes might indeed account for the lack of observed egg size effects on chick growth in this study.

It is also possible that the positive relation between egg size and breeding success is not a linear one, with eggs that are too large incurring various disadvantages. Large eggs and neonates may be at a metabolic disadvantage and increased egg production may only be pertinent when provisioning conditions are good. Under poor conditions, small eggs might be advantageous because chicks hatching from them would require less food, grow more slowly and thus be less likely to starve than larger chicks.

Finally, it is also worth bearing in mind recent evidence suggesting that egg size may not always be a suitable indicator of egg quality, since important compositional changes (e.g. fat and protein content) are not always a function of egg size (P. Monaghan, pers.com.).

Within-clutch egg size patterns

In two-egg broods of the black guillemot, first-laid eggs tended to be larger than second-laid eggs. This within-clutch asymmetry, however, was relatively small in comparison to the between-clutch egg-size variation, the a-egg on average being only 3% larger than the b-egg. Black guillemots also exhibited a relationship such that a large a-egg was associated with a large b-egg, suggesting that females having the capacity to produce large a-eggs were of better quality since overall they produced a higher quality clutch. However, although the mass of the b-egg in most clutches was smaller than the a-egg, the relative size disparity decreased with decreasing a-egg mass. Thus, those clutches with the largest and highest quality eggs exhibited the greatest degree of size disparity. Conversely, those clutches containing smaller eggs either displayed little size disparity or even produced a relatively large b-egg

Many avian studies have shown that within-clutch egg size variation is repeatable, and it is often inferred that even minor within-clutch disparities are adaptive and evolved in association with hatching asynchrony (Slagsvold et al. 1984). In this context, depending on whether the size of the final egg is respectively smaller or larger than the remainder of the clutch, egg size disparity might be considered as a means either of facilitating brood reduction or, conversely, to counteract the effects of hatching asynchrony (Slagsvold et al. 1984). Along a similar line of reasoning, Quinn and Morris (1986) suggested that the competitive disparity brought about by hatching asynchrony results in the last-laid egg having a lower reproductive value than that of the remainder of the clutch and thus the female gains a fitness benefit by investing relatively less in the last egg. By contrast, other authors have suggested that within-clutch differences of only a few percent are of minor significance with regard to establishing a size hierarchy within the brood and that hatching asynchrony plays the greatest role in promoting competitive asymmetry (Bancroft 1984, Stockland and Amundsen 1984, Magrath 1990, 1992). Indeed, some studies have even questioned the adaptive significance of intra-clutch egg size variation, claiming that such variation is largely an inconsequential artefact of other forces impinging on egg size; mainly fluctuating food levels available to the mother during egg formation, although the effects of hormone levels and uterine shrinkage have also been posited (Mead and Morton 1985, Leblanc 1987). At the population level, the degree of within-clutch asymmetry may therefore be a consequence of food availability during laying, rather than a mechanism to counteract potential future food shortages. Evidence for this has been found in a number of gull species (Coulson et al. 1982, Pierotti and Bellrose 1986, Hiom et al. 1991, Kilpi et al. 1986). At the individual level, however, nutritional constraints during egg production may not only be affected by environmental conditions, but also by the ability of each bird to use them efficiently. More efficient foragers, for example, might be expected to produce larger eggs and clutches with less asymmetry. For instance, in an experiment designed to determine the relative effects of egg size and parental quality, Bolton (1991) found that birds that laid larger eggs also raised more chicks, and concluded that egg size represents a useful measure of parental quality. In the same context, it would be expected that those females more constrained by nutritional resources would lay a more asymmetric clutch and take longer to produce a b-egg.

Since egg size in the black guillemot is positively related to hatching size and quality, it seems probable that the largest egg in the clutch hatched a nestling that was generally heavier and of higher quality than its sibling from the smaller egg. (Unfortunately, the absence of sufficient biometric data at hatching for both siblings precluded statistical confirmation of this. Nonetheless, I believe the strong correlation between egg mass and chick hatching size for both a- and b-chicks justifies this derivation of a sibling size disparity at hatching from egg size disparity.) The possible consequence of this disproportional maternal investment is a size hierarchy within the brood whereby chicks hatching from larger eggs have a competitive advantage over their sibling. Moreover, the sibling that hatched from the larger egg was likely to have relatively higher reserves and hence was more likely to withstand periods of reduced food supply, at least during the immediate post-hatching period. These ideas are supported to some extent by the fact that a-chicks grew faster, reached higher asymptotic weights and had a significantly higher fledging rate than b-chicks (chapter 3).

In many of the studies so far conducted on the functional significance of within-clutch egg size variation, the direction of the deviation in egg size for any given species is constant: that is, egg size changes predictably with laying order either upwards or downwards. This contrasts with the current study where, the largest egg in the clutch could be either of the two eggs, largely depending on mean clutch egg-size. Given that the hatching pattern is primarily determined by laying order in the black guillemot (but see chapter 7), b-eggs from clutches containing large a-eggs (high quality clutches) hatched after and produced lighter nestlings than those of a-eggs, regardless of the degree of hatching asynchrony. Thus, under unfavourable environmental conditions, relatively small b-chicks from these broods were potentially more likely to suffer brood reduction due to their competitive inferiority to the larger sibling.

Conversely, b-chicks hatching from broods where the a-egg was small hatched either at a similar or larger size than the chick from the a-egg, thereby potentially counteracting any effects of hatching asynchrony.

At first sight it appears that these results provide evidence that within-clutch egg-size variation has an adaptive function in the black guillemot: a facultative brood reduction strategy appears to be operating in clutches made up of large eggs whilst, conversely, a brood survival strategy appears evident in broods containing small eggs. However, these results are paradoxical in a number of respects. The most obvious point being that those clutches with the smallest degree of size disparity, i.e. those that contained a larger b-egg than a-egg, were generally those made up of smaller, lower quality eggs. Since these clutches were probably laid by poorer quality parents and thus potentially most likely to suffer food limitation, it would seem intuitive to assume that they should also exhibit the greatest degree of size disparity in order to facilitate brood reduction. However, the results of this study contradicted this prediction, with broods containing small eggs appearing to counteract any effects of hatching asynchrony, and vice versa. Furthermore, if clutch asymmetry was selected in order to induce a competitive hierarchy among siblings, then one would expect those broods that hatched synchronously, and thus with little temporal competitive asymmetry, to exhibit the largest egg-size disparity (Slagsvold et al. 1984). However, clutch asymmetry in the black guillemot exhibited no such relationship with the degree of hatching asynchrony. These results therefore suggest that egg size disparity in the black guillemot is not adaptive in the context of within brood competitive asymmetry.

The annual variation in mean egg mass and laying date implies that black guillemots were energetically constrained at the egg laying stage (chapter 3). However, despite a higher mean egg mass in 1994, no corresponding change was evident in the mean egg-size disparity in that year, contradicting the theory that nutritionally constrained females should lay clutches with a greater degree of size disparity (Slagsvold et al. 1984). It appears unlikely therefore, that clutch asymmetry in the black guillemot is a consequence of food limitation. The fact that b-eggs can be larger than a-eggs also suggests that hormonal effects or a decrease in uterus size, were not responsible for the general decrease in egg size with laying order in this species.

Regardless of the ultimate and proximate factors determining egg size disparity, the consequence is a hatching-size disparity and competitive hierarchy within the brood that will interact with the degree of hatching asynchrony. This is reduced in poor 'quality' clutches where probable egg-viability constraints require that b-eggs are similar in size to a-eggs.

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CHAPTER 5

INTRA-SPECIFIC VARIABILITY IN HATCHING ASYNCHRONY

INTRODUCTION

In many avian species eggs hatch asynchronously, producing an age and size hierarchy within the brood (Clarke and Wilson 1981, Magrath 1992). Numerous disparate hypotheses have been put forward to account for the significance of asynchronous hatching (for latest review see Stoleson and Beissinger 1995), although current opinion now holds that no single hypothesis is likely to provide a comprehensive explanation for the phenomenon and the observed variation in hatching pattern is probably the outcome of trade-offs between various constraints and selection pressures (Mock 1984, Magrath 1990, Amundsen and Slagsvold 1991a,b, Stoleson and Beissinger 1995, Amunsden and Slagsvold 1996).

Regardless of the underlying selective pressures determining hatching patterns, the consequence of asynchronous hatching for many species is a competitive disparity among concurrent siblings. This often has a profound effect on chick fitness, and many studies have demonstrated that reduced growth and survival is sustained by the youngest and smallest offspring (non-seabird species: O'Connor 1977, Bryant and Tatner 1990, Viega 1990, Wiebe 1996, Bengtsson and Ryden 1983, Poole 1989, Kacelnik et al. 1985; seabird species: Langham 1972, Parsons 1975, Lundberg and Vaisanen 1979, Grieg-Smith 1985, Quinn and Morris 1986, Bollinger et al. 1990, Stouffer and Power 1990). Despite parental control of the hatching spread, largely via incubation behaviour (but see chapter 7), initial size hierarchies among siblings are often perpetuated or even increased during the nestling period due to the older chick or chicks receiving a disproportionate share of parental resources. This is usually attained either through aggressive dominance or passively through scramble competition. Moreover, in certain circumstances, fitness costs may fall on younger nestlings regardless of food availability (Bryant 1978, Werschkul 1979, Nilsson and Svensson 1996, Stoleson and Beissinger 1997, Mock et al. 1987). However, despite a wealth of studies demonstrating fitness costs for last-hatched nestlings, it appears that such costs are not incurred in all species. For example, a number of studies have demonstrated that later-hatched siblings can grow equally as fast as their siblings (e.g. Amundsen and Stokland 1988), even in a species with an exceptionally large hatching spread (Krebs 1999). Therefore the competitive environment of the brood, and hence how resources are distributed within the brood, are likely to vary according to phylogenetic background and ecological circumstance.

In many species exhibiting asynchronous hatching the pattern of hatching is relatively invariable among individual breeding pairs. Hence the opportunity to investigate the extent to which natural variation in hatching asynchrony affects competitive interactions, and thus the fitness consequences for respective siblings, is limited. In some species, however, hatching spreads vary considerably between individual pairs, yet few studies have attempted to investigate the causes and consequences of this variation (Wiebe 1995, Stenning 1996). Furthermore, most studies have investigated hatching asynchrony with a view to ascertaining its adaptive benefits, with few focusing on its potential costs (Stoleson and Beissinger 1995). Therefore, comparisons between broods showing different degrees of hatching asynchrony may yield important information on the consequences of intra-specific differences in breeding strategy.

In the current chapter I examine natural variation in the level of hatching spread and its relationship with chick survival in two-chick broods of the black guillemot. Evidence that nestling black guillemots hatching from single-egg nests grow faster, and reach higher asymptotic weights than chicks hatching from two-egg broods (Chapter 3), indicates that nestlings rarely grow at their physiological-determined maximum rate (see also Gard and Bird 1992). This suggests that food supplies to two chick broods are frequently limited, and siblings are likely to have to compete for limited parental resources. Being the competitively superior first-hatched nestling therefore probably confers advantages in the form of faster growth and larger final mass. The main objective is to determine how variation in the degree of hatching spread between broods affects the level of agonism among siblings, and the result of the ensuing dominance hierarchies on chick growth and survival.

METHODS

Hatching asynchrony, growth and mortality

The degree of hatching asynchrony, growth and mortality was determined as described in chapter 2. For this study, data from the years 1994, 1996 and 1997 were used to investigate growth and mortality, whereas behavioural data were used from 1997 only.

Chick behaviour

Full methods pertaining to behavioural observations and a detailed account of behavioural terminology and aggressive interactions are described in chapter 2. Preliminary video recordings in 1995 enabled attack behaviour by the aggressor to be categorised into low intensity and high intensity aggression (hereafter called LI and HI aggression, respectively). LI aggression was employed by either sibling and entailed the aggressor facing its sibling and inflicting insubstantial, non-injurous pecks, usually about the bill. HI aggression was employed exclusively by the larger sibling and involved several bouts of violent pecking and tousling of the smaller chick, predominantly about the nape of the neck, face or back of the head. The recipient chick tended to assume a submissive posture and never attempted to retaliate or evade its attacking sibling. Submission from HI attacks was characterised by crouching as low as possible, any attempt to raise the head often being met with further bouts of aggression.

Aggressive behavioural interactions among siblings were recorded during focal nest watches during June and July, 1997, using the CCTV system described in chapter 3. (Limited data were also collected in 1996 but to avoid potential annual effects and problems associated with pseudoreplication, analysis is restricted to that collected in 1997.) I observed behaviour in a total of 36 broods: 7 synchronous broods, and 16, 10 and 3 asynchronous broods with respective hatching intervals of 1, 2 and 3 days. Due to very small sample sizes, broods with a hatching interval greater than 3d were excluded from the analysis (n=1 for both 5d and 6d broods). All safely accessible nests within the colony containing two surviving siblings were included in the study.

Preliminary analysis from 1996 and 1995 revealed that chick aggression occurred primarily during the first two-weeks post-hatching (fig. 5.1). Hence I attempted to control for possible effects of age-related agonistic behaviour by restricting observations to broods with a-chicks aged between 6-12 days old (by which age chicks were also capable of thermoregulation and rarely brooded by their parents). Data from 1996 also demonstrated that feeding rates and chick aggression appeared not to vary diurnally (see also chapter 6).

On any one day, each brood was observed for a continuous three-hour period, either between 0600 and 0900hrs or between 0900 and 1200hrs, and where possible each nest was observed on three days over a six-day period. During any observation period, behavioural data from a maximum of six nests were collected: four broods were observed directly by two observers from four monitors, and behaviour from two broods were recorded on video for later analysis. I also attempted to control for any seasonal effects and daily variation in weather condition by observing, during any one day, nests containing a range of hatching intervals (from 0 to 3 days). Indeed, using the Julian date of the second observation day of each nest, mean observation dates were similar for each level of hatching asynchrony (0d broods: mean=186.21, se=3.15, n=7; 1d broods: mean=193.13, se=6.56, n=16; 2d broods: mean=190.1, se=2.59, n=10; 3d broods: mean=181.67, se=3.76, n=3; one-way ANOVA: $F_{3,32}$=2.53, n.s.). In total, 243 hours of behavioural data were collected. The mean number of hours recorded per nest were similar for each level of hatching interval (0d broods: mean=6.43 hrs/nest, se=0.76, n=7; 1d broods: mean=6.56 hrs/nest, se=0.62, n=16; 2d broods: mean=7.20 hrs/nest, se=0.80, n=10; 3d broods: mean=7.0 hours, se=2.0, n=3; one-way ANOVA: $F_{3,32}$=0.185, n.s.).

I also attempted to collect parental delivery rate and food allocation data, but data were not of sufficient quality and quantity for statistical analysis.

TERMINOLOGY AND ANALYSIS

Within each brood, each sibling was referred to as a- or b-chick. This was related to hatching order in asynchronous broods (a-chick hatches first in broods where the hatching interval is ≥ 1 day), and to the egg from which the sibling hatched in synchronous broods (a-chick hatches from a-egg). Broods with different levels of hatching spread (days) are referred to as 0, 1, 2 and 3 day broods.

I used non-parametric tests to examine behavioural data. Pairwise comparisons of aggression rates between a- and b-chicks were tested using Wilcoxon signed rank tests, and the relationship between aggression and hatching interval was tested independently for each chick type (a- or b-chick) using Kruskal-Wallis ANOVAs.

Growth was investigated in terms of the instantaneous growth rate (see chapter 3 for equation) during the period of maximum growth (age 5–25d), in addition to asymptotic weight, and weight at 12 days post-hatching. Paired t-tests were used for pairwise growth comparisons between a- and b-chicks. Relationships between growth rate and hatching interval were performed for each chick type independently using one-way ANOVA models, except on growth parameters confounded by seasonal effects where an ANCOVA (general linear model procedure) with adjusted laying date as covariate was used. Laying dates for each of the three years were standardised by subtracting the date of clutch initiation from the median laying date of that year. Due to relatively small sample sizes, growth rate data from all years were pooled for these analyses and I was thus unable to account for potential annual effects on growth rates. Only broods containing surviving siblings on the day of measuring were used in growth analyses. If mortality occurred, then that brood was deleted from analysis from that date onwards. To ascertain whether chick mortality was starvation related, I compared chick weight in the days prior to death with mean chick weight of that chick type (see chapter 3).

I analysed postnatal mortality as a function of hatching sequence and the degree of hatching spread using stepwise logistic regression, starting with the highest-order interaction.

Since all ratios between explained deviance and the degrees of freedom (d.f.) were close to one, significance tests were based on the χ^2-distribution (Crawley 1993).

Appropriate parametric statistics were applied where data met the parametric assumptions of normality and homoscedasticity. Normality was tested for using the Kolmogorov-Smirnov goodness-of-fit test and all probabilities given are two-tailed. Analyses were undertaken using SPSS for Windows release 7 and all tests were as described by Zar (1996).

RESULTS

1. Natural variation in hatching asynchrony

Contrary to many other species exhibiting asynchronous hatching, the natural degree of hatching spread in the black guillemot is relatively variable among individual broods. Over all four years of this study, hatching interval ranged between 0 and 6 days, with by far the majority of clutches hatching eggs between 0 and 3 days apart (mean 1.24 +-1.26sd, n=131). The degree of hatching spread changed relatively little between years, and for all years no relationship was evident with laying date (see chapter 3 and tables 5.1 and 5.2).

Table 5.1. Proportion of each hatching interval (days) for years 1994 to 1997

Hatching interval	1994	1995	1996	1997
0	38.5%	40.7%	11.1%	30.0%
1	30.8%	29.6%	51.9%	30.0%
2	19.2%	11.1%	25.9%	23.3%
3	7.7%	18.5%	3.7%	13.3%
4				3.3%
5			3.7%	
6	3.8%		3.7%	

Table 5.2. Mean hatching intervals and the percentage of clutches that hatch synchronously (within 24 hours) for each year from 1994 to 1997, inclusive. Kruskal-Wallis statistics are included for between-year comparisons.

	Mean hatching interval ± S.D. (n)	**% of broods hatching synchronously**
1994	1.16 ±1.48 (37)	38.5%
1995	1.0 ±1.11 (30)	40.7%
1996	1.46 ±1.31 (30)	14.8%
1997	1.32 ±1.09 (34)	30%
Kruskall-Wallis ANOVA	$\chi^2 = 2.97$ n.s.	

2. Nestling behaviour

Sibling aggression and age

Sibling aggression occurred primarily in the first two-weeks post-hatching (Figure 5.1).

Figure 5.1. Mean sibling aggression in relation to age. Error bars represent ±1 S.E., sample sizes in parentheses.

a) high intensity attacks

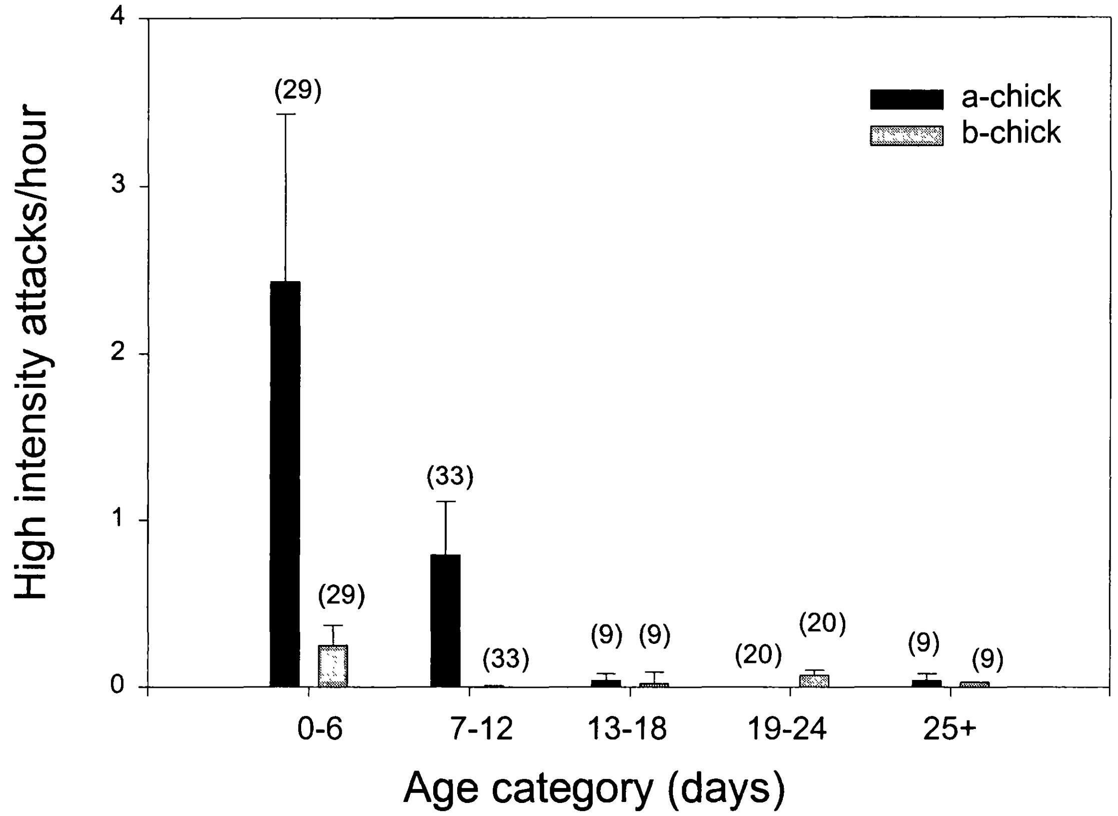
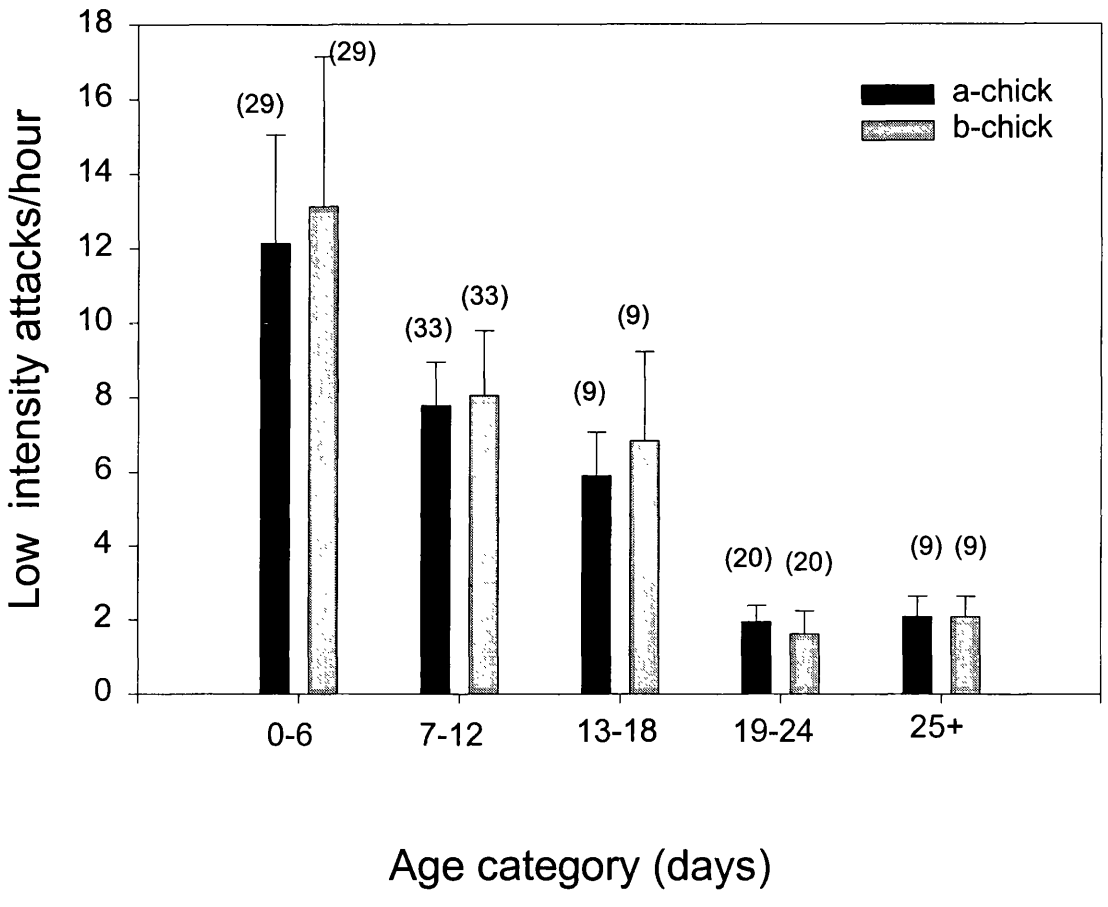


Figure 5.1. Mean sibling aggression in relation to age. Error bars represent ±1 S.E., sample sizes in parentheses.

b) low intensity aggression



Chick aggression and hatching asynchrony

LI aggression was observed in 28 of the 36 broods studied, whereas HI aggression occurred in 19 broods. Kruskal-Wallis ANOVA comparisons reveal that HI attacks showed no relationship with the degree of hatching asynchrony for either a- or b-chick (a-chick: χ^2=4.90, df=3, p=0.920, n=36; b-chick: χ^2=4.05, df=3, p=0.256, n=36; fig. 5.2). However, for all hatching intervals combined, a-chick HI aggression was significantly higher than that of the b-chick (Wilcoxon signed rank test: Z=-3.66, p=0.0002, n=36). Kruskal-Wallis ANOVA comparisons also show no difference in the degree of LI aggression with the level of hatching asynchrony for either sibling (a-chick: χ^2=0.47, df=3, p=0.20, n=36; b-chick: χ^2=0.42, df=3, p=0.94, n=36; fig 5.2). However, LI aggression was consistently higher in the b-chick than in the a-chick (Wilcoxon signed rank test: Z=-2.06, p=0.039, n=36).

Thus, in all nests where aggression was evident, a-chicks pecked their siblings more frequently than vice versa. With respect to HI aggression, the b-chick always responded to a-chick attacks with a submissive posture and never reciprocated aggression. In the few cases where b-chicks initiated HI-aggression, the a-chick retaliated with a prolonged bout of aggression resulting in the immediate submission of the b-chick. No ties were observed with respect to HI-aggression (i.e, where a- and b-chicks exhibited equal numbers of fights during any one bout). Unfortunately, these behaviours were too infrequent to warrant meaningful statistical analyses with the current observational set-up (but see next chapter). It appears, therefore, that HI-aggression led to the formation of stable dominance hierarchies within the brood. On the few occasions that a parent was present during a HI aggressive bout, the parent never attempted to thwart the aggressor.

Figure 5.2. The relationship between sibling aggression (mean attacks per hour ±S.E) and hatching asynchrony. Numbers by bars are sample sizes.

a) a-chicks

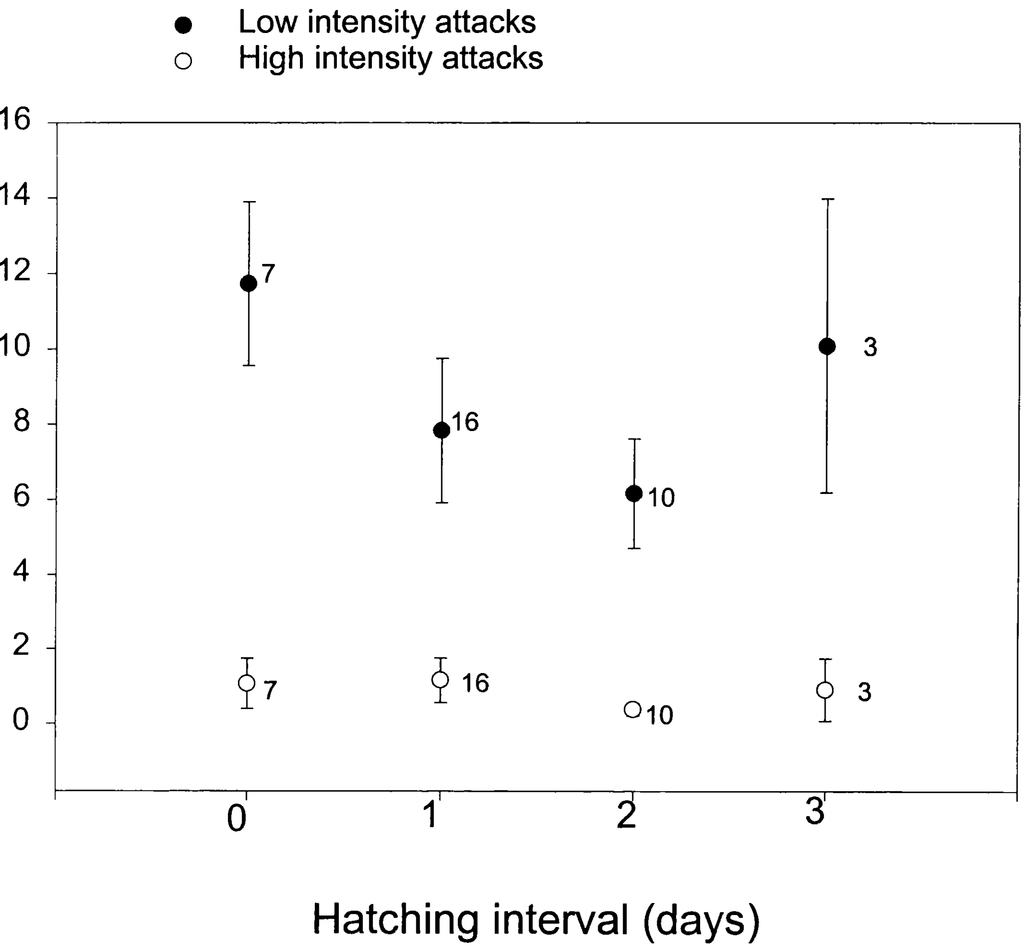
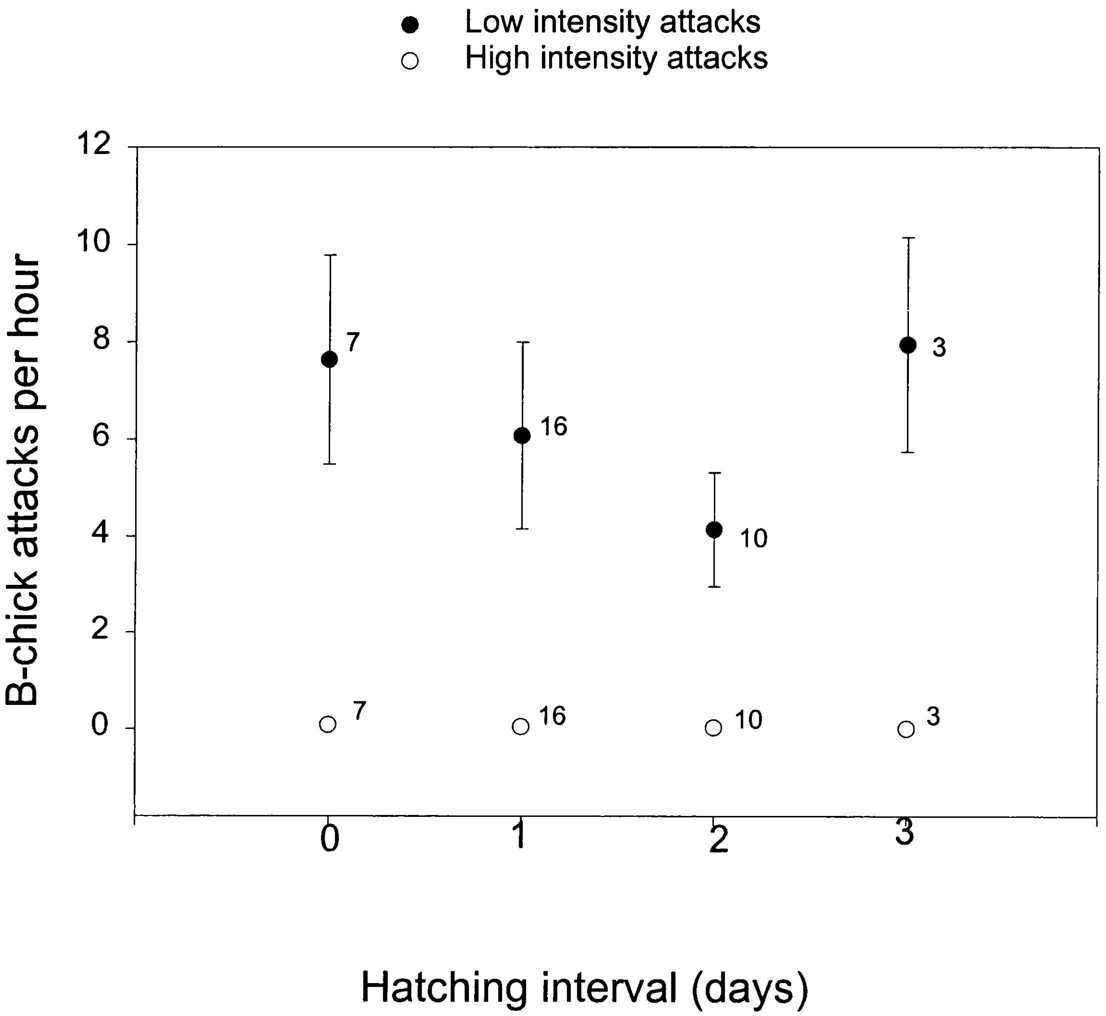


Figure 5.2. The relationship between sibling aggression (mean attacks per hour ±S.E) and hatching asynchrony. Numbers by bars are sample sizes.

a) b-chicks

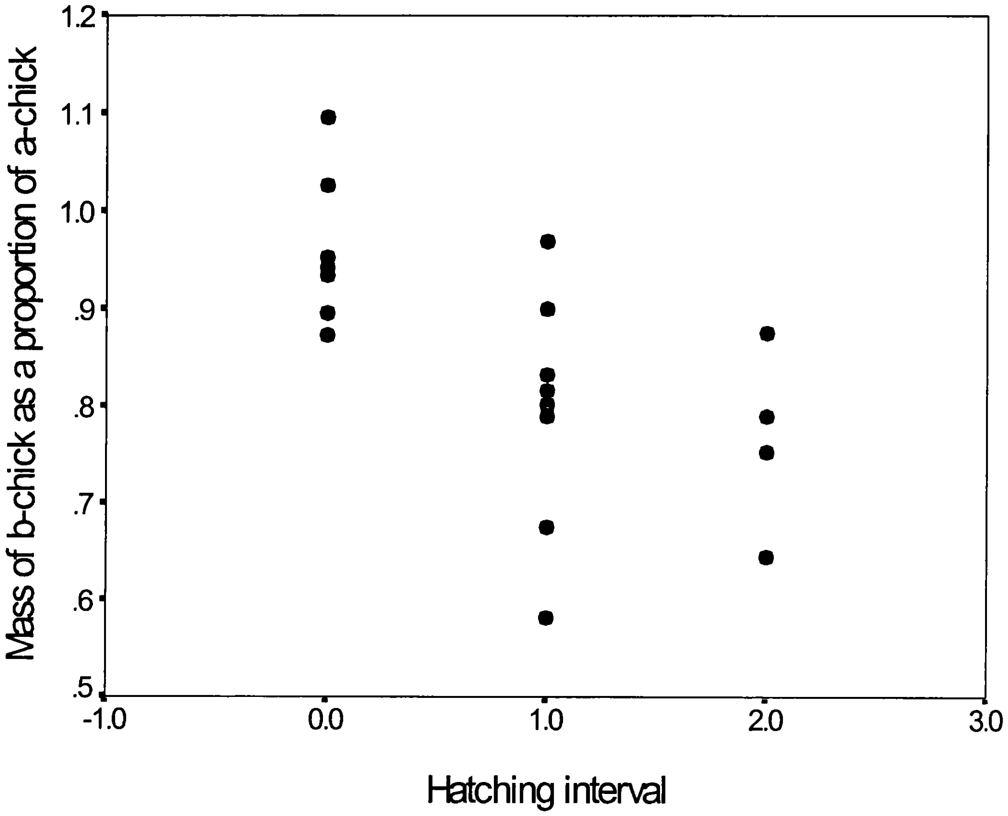


3. Chick growth

Mass and size hierarchies

The within-brood size disparity on the day of hatching of the b-egg is expressed as b-chick mass as a percentage of a-chick mass. Chick mass was rarely obtained on the day of hatching (due to the increased risks of chick mortality, see general methods chapter) and thus sample sizes were limited and no data were obtained for 3d broods. Nonetheless, relative b-chick mass significantly decreased with increasing hatching interval, ranging from c.60% of the a-chick in 2d broods to c.110% in 0d broods (fig. 5.3). Ideally, I would have liked to have determined the relative effect of egg size disparity on sibling size disparity, but sample sizes were too small.

Fig 5.3. Chick size disparity (b-chick mass divided by a-chick mass) on the day of the b-chick hatching in relation to the level of hatching asynchrony (days).



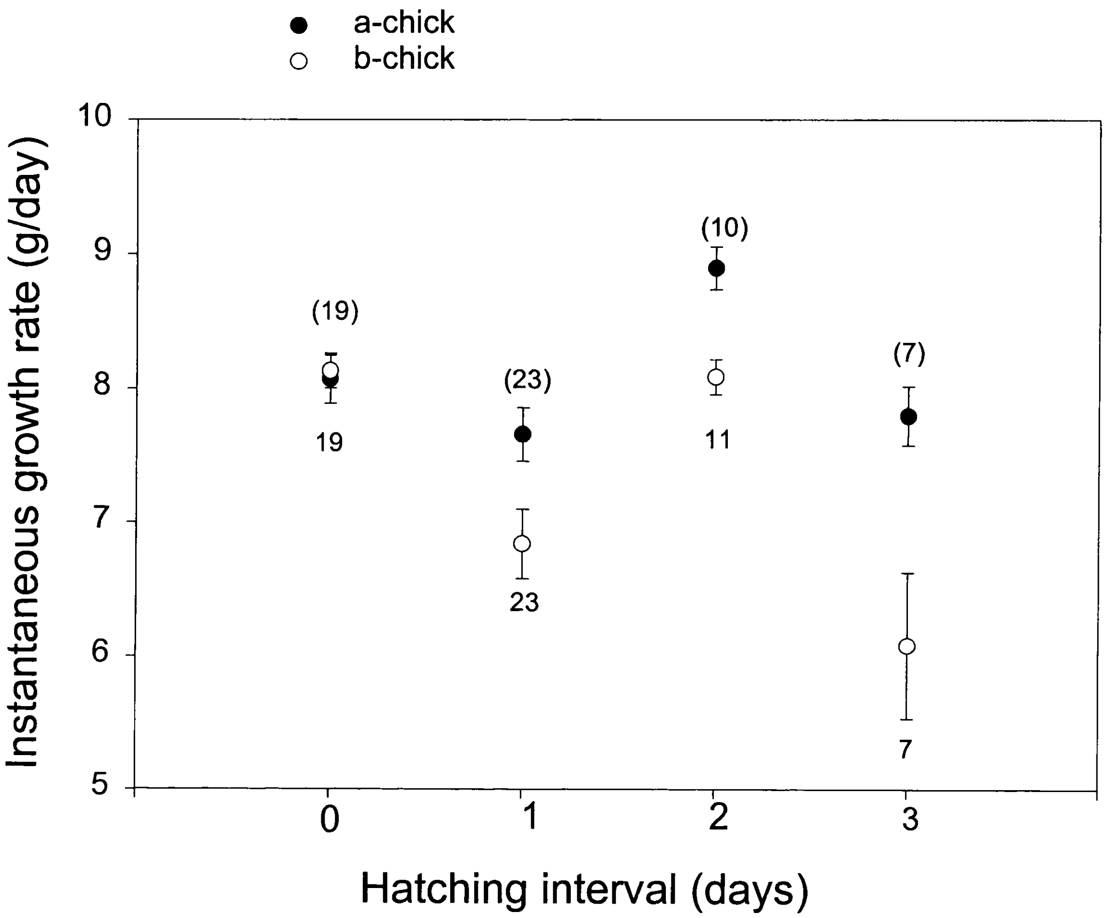
Body mass and hatching asynchrony

Within nest comparisons revealed that a-chicks not only grew faster than b-chicks (t_{58}=5.05, p<0.001) but also reached a higher asymptotic mass (t_{35}=3.74, p=0.001). However, age at asymptotic weight did not vary between siblings (t_{32}=0.529, p=0.601).

The body mass of nestlings at 12 days old did not vary significantly according to the level of hatching asynchrony for either a-chicks (one-way ANOVA $F_{3,63}$=0.35, p=0.79) or b-chicks ($F_{3,53}$=0.048, p=0.99). However, mass instantaneous growth rate (ANCOVA a-chick, main effect hatching interval: $F_{3,37}$=3.68, p=0.02, covariate adjusted date $F_{3,37}$=0.46 n.s., interaction $F_{3,37}$=1.44, n.s.; b-chick: main effect hatching interval $F_{3,38}$=4.055, p=0.014, covariate adjusted date $F_{3,38}$=0.487,n.s, interaction $F_{3,38}$=1.35, n.s.; Fig. 5.4) and asymptotic mass (one-way ANOVA a-chick $F_{2,35}$=4.78, p=0.015; b-chick: $F_{2,36}$=4.14, p=0.024;) exhibited a similar and significant pattern of variation with hatching asynchrony. Note that the sample size for asymptotic mass in 3d broods was too low for statistical comparison and was therefore excluded from the model. Post-hoc Tukey tests reveal that instantaneous growth rates and asymptotic weights of 0d and 2d broods were generally higher than 1d and 3d broods, for both a- and b-chicks. Mean instantaneous growth rates of a-chicks in 2d broods was significantly higher than in 1d and 3d broods, and the difference in growth rate between 0d and 3d broods was close to significance (2d vs 1d p=0.056; 2d vs 3d p=0.034; 0d vs 1d p=0.056). A similar difference in growth rate was evident for b-chicks; growth rates in both 0d and 2d broods were higher respectively than in 1d and 3d broods (0d vs 1d p=0.001; 0d vs 3d p<0.0001; 2d vs 1d p=0.007; 2d vs 3d p=0.001). Asymptotic weights in 0d and 1d broods were significantly higher than in 1d broods for both a- and b-chicks (a-chick: 2d vs 1d p=0.01, 0d vs 1d p=0.04; b-chick: 2d vs 1d p=0.06, 0d vs 1d p=0.04).

Hence, growth appeared to be particularly high in broods with a 2d hatching interval but also relatively high in synchronously hatching broods. Broods hatching at an interval of 1 day or 3 days fared relatively badly. Incidentally, all those broods hatching more than 3 days apart failed to fledge.

Figure 5.4. The relationship between mass instantaneous growth rate (mean ± S.E.) and hatching interval. Sample sizes with and without parentheses represent n for a- and b-chicks, respectively.



5. Fledging success

Mortality rates in this population were comparable to those of other studies, with non-predation related mortality occurring predominantly in the first two weeks post-hatching (chapter 3). Table 5.3 shows the relative fates of a- and b-chicks for all broods with a hatching interval between 0 and 3d during the years 1994 and 1996-97. To determine whether chick mortality was a result of starvation, the last-measured weight of the dead nestling was compared with that of the mean mass of all chicks of the same age, hatching order and degree of hatching asynchrony during that year. Body condition was also examined during a post-mortem examination. In all cases, chicks were significantly lighter than their peers ($p<0.05$, Sign test). Further, a number of emaciated b-chicks revealed peck wounds on the head and neck demonstrating that sibling aggression was often associated with starvation related mortality. Predation rates, despite demonstrating a slight but consistent increase, did not significantly vary according to the level of hatching spread, occurring in 12% (0d), 16.7% (1d), 19% (2d) and 28.6% (3d) of the broods (G-goodness of fit). Great black-backed gulls were the principal predators.

Table 5.3. Relative fates (n and (%)) of a-and b-chicks for all broods with hatching intervals ranging between 0 and 3 days, during the years 1995, 1996 and 1997.

	a-chick	**b-chick**
Fledged	60 (67.4%)	49 (55.1%)
Non-predation related mortality	8 (8.8%)	26 (29.2%)
Depredated	11 (12.4%)	5 (5.6%)
Unknown	10 (11.2%)	9 (10.1%)
Total	89	90

Chick survival was analysed in relation to position in the hatching sequence and the degree of hatching asynchrony using stepwise logistic regression (results given in table 5.4). Discounting all chicks that were depredated and where chick fate was unknown, a significantly greater proportion of b-chicks failed to fledge than a-chicks. However, the level of hatching asynchrony was not a significant predictor of chick mortality, and the lack of a significant interaction suggests that the relative survival probability of a- and b-chicks remains constant with the level of hatching asynchrony. In accordance with these results, there was no difference in the number of chicks fledging per brood according to the level of hatching asynchrony (one-way ANOVA $F_{3,63}=2.86$, $p=0.414$; table 5.5). For a breakdown of chick fate in relation to hatching spread see Table 5.5.

Table 5.4. Results of multiple logistic regression in which chick survival was examined in relation to hatching sequence (a- or b-chick) and the degree of hatching asynchrony (0, 1, 2 and 3 days).

	Deviance	χ^2	d.f.	*P*
Null model	159.20		135	
Final model	148.16		132	
Hatching sequence		11.046	1	0.0009
Hatching interval		0.471	1	0.493
Interaction		0.505	1	0.477

In those broods where both nestlings died of non-predation related mortality (n=6), death always occurred in order of hatching, with the smallest and youngest succumbing first.

Table 5.5. General fledging success in relation to the degree of hatching asynchrony (days).

	Hatching interval			
	0	1	2	3
Fledge 0	3 (15%)	2 (8%)	1 (6%)	0
Fledge 1	0	10 (40%)	7 (39%)	2 (50%)
Fledge 2	17 (85%)	13 (52%)	10 (55%)	2 (50%)
Numbers fledge **± S.E.** **(n)**	0.85 0.074 (20)	0.72 0.066 (25)	0.75 0.078 18	0.75 0.166 4
Total no. broods	20	25	18	4
% of a-chicks fledging	17/20 (85%)	22/25 (88%)	16/18 (88.9%)	4/4 (100%)
% of b-chicks fledging	18/20 (90%)	17/25 (68%)	10/18 (55.6%)	4/4 (100%)

Although victim longevity increased slightly with increasing hatching asynchrony, the age of b-chick mortality did not differ according to the level of hatching asynchrony (Kruskal-Wallis ANOVA: χ^2=0.482, df=3, p=0.92; table 5.6).

Table 5.6. Age of b-chick mortality in relation to the degree of hatching asynchrony.

Hatching interval (days)	Mean age of b-chick mortality ±S.E. (n)
0	5.2 1.8 (6)
1	8.6 2.7 (11)
2	8.0 2.7 (7)
3	13.0 11 (2)

DISCUSSION

Hatching asynchrony in this population of black guillemots was extremely variable in comparison to many species, ranging between 0 and 6 days, with an average interval of 1.2 days. Positively associated with the degree of hatching asynchrony was a size and competitive hierarchy between siblings, such that the greater the temporal difference in hatching, the larger the size and competitive disparity between siblings. Hence siblings from synchronously hatching broods (i.e., siblings hatching on the same day) were of a similar size and competitive ability, whereas the relative size and potential competitive superiority of the a-chick increased with increasing asynchrony.

Aggression

In general, sibling aggression rates during 1997 were probably low (fig.5.2) since they did not appear to equate with the injuries on dead nestlings previously observed from this population (P.Walton, pers. com.). Nonetheless, despite relative clemency during this year, I observed a consistent within-brood pattern of aggressive behaviour across all levels of hatching interval, whereby a-chicks attacked their younger siblings significantly more frequently than vice versa. This difference between nest-mates was evident at both intensities of aggression, but was particularly pronounced with regard to HI aggression; b-chicks only very rarely partaking in HI attacks and always behaving submissively to a-chick HI aggression.

According to the logic of asymmetrical contests (Maynard Smith and Parker 1976) evenly matched competitors (e.g., siblings in synchronously hatching broods) should fight more frequently and at higher intensities than those in which the question of dominance is unequivocal (e.g., siblings in asynchronously hatching broods). This pattern of behaviour was reflected to some extent in the current study, but overall the results were inconclusive. For instance, although LI aggression was highest in synchronous broods and declined with increasing asynchrony until reaching its lowest level in 2d broods, the observed decrease was not statistically significant and, paradoxically, attack rates were relatively high in 3d broods. An even less convincing pattern was evident with respect to HI aggression; again aggression rates were lowest in 2d broods but broods hatching at 0,1 and 3d intervals all had similar aggression rates. Conceivably, additional factors might have influenced black guillemot sibling aggression in this study, thereby potentially confounding any effects of competitive asymmetry. For example, studies investigating the proximate control of sibling aggression have found that chick nutritional condition (Drummond et al. 1986) and the relative monopolisability of parental food deliveries (Mock 1984b, Mock et al. 1987), in addition to competitive asymmetry (Machmer and Ydenberg 1998), are important in effecting avian sibling aggression. Indeed, in the next chapter I experimentally demonstrate the relative roles of food amount and competitive asymmetry in black guillemot sibling aggression. It is therefore possible that food amount had a confounding influence on the results of the current correlational study, particularly with respect to 3d broods for which sample sizes were limited (n=3 nests, 27 hours). Nonetheless, increased aggression rates at extreme levels of hatching asynchrony have been reported in experimentally-doubled asynchronous broods of the blue-footed booby, despite relatively low levels of aggression typifying the natural level of asynchrony (Osorno and Drummond 1995). It is not inconceivable therefore that increased aggression rates in 3d broods of the black guillemot could be a biologically real phenomenon.

A number of studies have demonstrated that strong dominance hierarchies form in experimentally synchronised broods, despite the absence of an obvious size and competitive hierarchy (Bengtson and Ryden 1983, Drummond et al. 1986, Slagsvold 1986, Mock and Ploger 1987, Wiebe 1995). Accordingly, aggressive supremacy was achieved by one sibling in synchronously hatching broods of the black guillemot. Of particular interest, however, was the fact that in all synchronous broods, chicks hatching from the a-egg exhibited the HI aggression, were most aggressive overall and ultimately were the dominant chick of the brood. The seemingly equally matched b-chick in these broods behaved in a similar fashion to b-chicks in asynchronous broods; that is, rarely exhibiting HI aggression and always behaving submissively to a-chick HI attacks. If siblings in synchronous broods were competitively evenly matched, then one would expect b-chicks to exhibit comparable levels of aggression to that of the a-chick, at least until the question of dominance was resolved. This implies that factors in addition to relative sibling size and possibly factors related to egg laying order were important in effecting competitive interactions (see next chapter for further discussion). Thus, a dominance hierarchy was established in synchronous broods in favour of the chick hatching from the a-egg, regardless of the degree of hatching asynchrony, and even in broods where the size disparity was minimal, advantages often accrued to one of the siblings.

In general, first-hatched black guillemot nestlings appeared to be sensitive to the competitive abilities of their siblings, dedicating more effort to domination and competition in situations where the issue of superiority remained unclear, whilst devoting less effort in situations where siblings were competitively inferior. The consequence of this was the establishment of a dominance hierarchy within the brood whereby b-chicks were submissive to a-chicks, regardless of the degree of hatching asynchrony. A 2d hatching interval, appeared to facilitate the formation of a stable dominance hierarchy with relatively little overt aggression, whereas in other brood types greater levels of aggression appeared to be necessary.

Growth and survival

Although Amundsen and Stokland (1988) demonstrated that sibling shags *Phalacrocorax aristotelis* grow at comparable rates despite hatching at 1d intervals (see also Krebs 1999), equal growth rates and fledging success among concurrent siblings have seldom been recorded in asynchronously hatching species (Slagsvold 1986, Magrath 1989, 1991, Stouffer and Power 1990, Blanco et al. 1996). Last hatched nestlings often suffer relatively higher fitness costs compared to the remainder of the brood since initial size disparities rarely decline post-hatching (O'Connor 1975). These costs may be manifest as poorer growth and reduced survival because parents do not provide sufficient food for the entire brood and older chicks sequester a greater proportion of parental provisions (Ploger and Mock 1986, Forbes and Ankney 1987, Osorno and Drummond 1995). This uneven distribution of parental resources may even persist when feeding conditions are favourable. For example, Stoleson and Beissinger (1997) showed that supplementary feeding had little effect on the survival of penultimate green-rumped parotlet (*Forpus passerinus*) nestlings. These authors posited that the considerable size and age asymmetries within these broods meant that parents had little control over the distribution of resources.

In many species of bird with nidicolous young, the parental strategy is to feed the first nestling it encounters or the one that has positioned itself closest to the feeding area (Ryden and Bengtsson 1980, Grieg-Smith 1985). This strategy is believed to be the most effective since other allocation strategies such as preferential feeding of certain nestlings incur time costs (Stamps et al. 1985). Provisioning black guillemot parents appeared to exhibit such a feeding allocation system, releasing the delivered prey item to the first nestling encountered on entering the nest chamber. Thus food allocation in the black guillemot was determined largely through competitive scrambles, with smaller, last-hatched chicks in asynchronous broods being at a potential competitive disadvantage in conflicts over parental food deliveries.

Indeed, one consequence of the competitive hierarchy brought about by staggered hatching in black guillemot broods was a difference in asymptotic weight and mass growth rate between siblings, strongly implying that resources were unequally distributed among nestmates. Siblings in asynchronous broods reached asymptotic mass at a similar age, but a-chicks grew faster and thus attained a higher maximum weight than their younger siblings.

However, no significant difference between siblings was found for chick weight at 12 days old, implying that the observed difference in growth rate occurred after this age. Initially this seems paradoxical, since it is at this age that chicks appear most susceptible to non-predation induced mortality. However, if maintaining aggressive dominance is energetically expensive for the a-chick, a cost of dominance may well be a reduction in chick weight at this particularly vulnerable age. If this is so, then aggression in black guillemots is likely to be very costly to the antagonist, which may thus account for the relatively low levels of aggression in this species. Further studies examining chick energetic expenditure in relation to hatching asynchrony and aggression should shed more light on the cost-benefit relationships of aggression in this species.

In accord with growth rates, b-chicks in asynchronous broods were also considerably more at risk of non-predation related mortality than a-chicks, strongly implying that b-chicks were more prone to starvation during periods of food shortage. Furthermore, the consequence of a low asymptotic mass is probably a low fledging mass, which in turn is often associated with reduced post-fledging survival. The relationship between fledging mass and post-fledging survival is far from straightforward (Magrath 1991), although a positive relationship has been found for some species (Perrins 1965, Magrath 1990, 1991, Newton and Moss 1986). Thus, b-chicks from asynchronous broods will probably face a higher risk of mortality or lower reproductive success after fledging compared with their older and heavier nest-mates.

In contrast to broods with a size and age hierarchy, dominance conferred no significant growth advantages for a-chicks in synchronous broods. Chicks hatching from b-eggs did not grow more slowly, nor reach lower asymptotic weights than chicks hatching from a-eggs. Furthermore, although survival rates were slightly lower for chicks hatching from b-eggs in synchronous broods, the difference between chicks was not significant. Thus, regardless of aggressive a-chick dominance in synchronous broods, b-chicks appeared to maintain an equal competitive status in disputes over parental food supplies. Comparable growth rates among siblings in synchronous broods strongly infers that lower b-chick growth rates in asynchronous broods were not a consequence of innate differences in growth rate according to laying order, but were more likely a result of a-chick dominance in competitions over parental food deliveries.

The major conclusion derived from experimental studies on hatching asynchrony is that asynchronous broods are no more successful than synchronous broods (Amundsen and Slagsvold 1991, Stoleson and Beissinger 1995, Mock and Parker 1997), even when post-fledging mortality is accounted for (Lessells and Avery 1989, Magrath 1989, Harper et al.

1992). Generally speaking, this also appeared to be the case for the black guillemot, although the relationship between growth rate and hatching interval appears to be more complex in this study. On average, growth rates were highest in 2d broods for both a- and b-chicks, with both siblings growing faster and reaching higher asymptotic weights than a-chicks in broods of all other hatching levels. However, both a- and b-chicks in synchronous broods also exhibited relatively high growth rates and maximum weights compared to 1d and 3d broods. Thus relatively high growth rates appeared to be achieved in broods with contrasting degrees of sibling size disparity and aggression levels. Why 1d and 3d broods suffered reduced growth rates is unclear, but it strongly suggests that chick growth rate is determined primarily by parental quality effects rather than the degree of competitive asymmetry. Survival rates of a-chicks were relatively consistent across all levels of hatching spread, although b-chick survival was slightly, although not significantly, higher in synchronous broods than in 1d, 2d and 3d asynchronous broods. Thus, despite higher aggression, hatching synchrony might have slightly increased the frequency of whole brood survival relative to asynchronous broods, contrary to Hahn's (1981) experimental comparison of synchronous versus asynchronous broods of the laughing gull *Larus atricilla*. Thus, in comparison to other brood types, 2d brood aggression rates were lowest and growth rates highest but b-chick mortality rates were slightly lower than that of synchronous broods. It therefore appears that increased competitive asymmetry led to reduced aggression rates in these broods, and although parental investment was skewed slightly towards the a-chick, this was not to the detriment of the fitness of the smaller chick.

Despite the observed differences, the level of asynchrony appeared to have only minor consequences for black guillemot nestlings. It is possible that conditions were not severe enough during these years to demonstrate a marked difference in the various parameters studied. Indeed, Pijanowski (1992) has demonstrated mathematically that the parental strategy of inducing a competitive hierarchy within the brood (through hatching asynchrony), and thus promoting brood reduction, can be selected for even when 'poor' breeding years occur infrequently. Alternatively, parents might have been optimising the level of hatching asynchrony or, conversely, adjusting provisioning rates to compensate for the increased demands of certain brood types.

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CHAPTER 6

EFFECTS OF SHORT-TERM HUNGER AND COMPETITIVE ASYMMETRY ON FACULTATIVE AGGRESSION IN NESTLING BLACK GUILLEMOTS *CEPPHUS GRYLLE.*

In press as:
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Effects of short-term hunger and competitive asymmetry on facultative aggression in nestling black guillemots *Cepphus grylle*
Behavioral Ecology

ABSTRACT

Siblings in a diversity of species are facultatively aggressive, yet the proximate control of the aggressive response and the ecological conditions selecting for such systems are poorly understood. In this study, we investigated the effects of food amount (food amount hypothesis) and competitive asymmetry on sibling aggression in black guillemot broods. Parental provisioning rates were experimentally manipulated in broods comprising a range of hatching intervals over a twelve-hour period. Aggression became evident only after parental provisioning rates were experimentally reduced. When parental provisioning resumed, adults did not increase their feeding rate to compensate for the induced food deficit and the result of sibling rivalry was a change in the allocation of parental deliveries from one of equality to one in favor of the dominant chick. Food deprived chicks from synchronous broods were more aggressive than those from asynchronous broods, suggesting that one benefit of hatching asynchrony in the black guillemot is to establish an efficient competitive hierarchy among siblings which minimizes the need for costly aggressive interactions. On the following day, sibling aggression ceased and chicks regained an equal share of parental feeds. Our results provide the first evidence that short-term food shortage per se acts as an initial trigger for aggression, yet also reveal that the aggressive response is complicated by factors associated with hatching and laying order.

INTRODUCTION

Systems in which sibling competition is manifest through overt aggression provide quantifiable measures of selfish behavior among close genetic relatives (Drummond and Garcia Chavelas, 1989). In addition, they offer the opportunity to elucidate the underlying proximate and ultimate factors driving sibling conflict and brood reduction (Mock and Parker, 1997). It is widely recognized that, in bird species where sibling aggression is facultative, the level of parental provisioning may be an important proximate cue. This putative relationship between provisioning and aggression has been formalized in the "food amount hypothesis" (Mock et al., 1987). According to this hypothesis, dominant siblings become more aggressive during periods of food shortage, thereby obtaining a disproportionate share of total available parental resources. If this is reversible then, on resumption of favorable food supplies, aggression rates will return to base-line levels.

Despite the logic underlying these predictions, only two studies have experimentally demonstrated a proximate link between parental provisioning level and sibling aggression in birds (blue-footed boobies *Sula nebouxii*, Drummond and Garcia

Chavelas, 1989; ospreys *Pandion haliaetus,* Machmer and Ydenberg, 1998*).* This paucity of experimental data makes it difficult to identify the ecological conditions that favor the evolution of the facultative response itself and of the particular environmental cues that may trigger it.

The functional significance of facultative sibling aggression is likely to be dependent on the magnitude of competitive asymmetry between siblings. In most species the competitive dynamics of a brood are largely determined by the timing of the onset of incubation in relation to egg laying patterns (Amundsen and Stokland, 1988; Fujioka, 1985; Hahn, 1981; Magrath, 1992; Slagsvold et al., 1984). For many avian species that initiate incubation before clutch completion, the degree of hatching spread is relatively constant between individuals. Hence the opportunity to investigate the extent to which realistic variation in the degree of competitive asymmetry within broods influences the pattern of sibling aggression is very limited. However, in some species, the degree of hatching spread varies considerably between pairs, but few studies have investigated the level of sibling aggression associated with this variation.

In this study we examine sibling aggression in broods of the black guillemot (*Cepphus grylle*), a seabird species that exhibits considerable inter-pair diversity in the degree of hatching asynchrony (in our study population, ranging between 0 and 6 days; unpublished data). While the majority of breeding pairs lay a two-egg clutch, black guillemots differ markedly in the inter-egg interval and in the timing of the onset of incubation in relation to egg laying (Petersen, 1981). The resulting variation in hatching patterns creates a wide range of size disparities between siblings. In this paper, the results of an experiment investigating the response of black guillemot siblings to reduced parental food deliveries are reported. The study had two objectives: firstly, to determine experimentally whether food amount is a proximate cue for sibling aggression, and secondly, to investigate how the pattern of aggression varies in relation to the highly variable degree of competitive asymmetry within broods.

METHODS

General

Data presented here were collected on the Holm of Papa Westray, Orkney, Scotland (59° 22'N, 2°53'W). The Holm is approximately 0.8 x 0.3 km, uninhabited by humans and free from mammalian predators. Adult black guillemots breed in loose aggregations, nesting predominantly within boulder caves on rocky shores but also among cracks in cliffs and occasionally in disused rabbit burrows. Because direct observations of behavior inside the nest cavity were not possible, we developed a system of miniature CCD charge-couple cameras with infra-red LED illuminators linked to CCTV and Sony-walkman® video recorders to monitor chick behavioral interactions and parental provisioning. The colony comprised approximately 65 breeding pairs of black guillemots in 1997, from which we collected a total of 252 hours of observations, made on 20 nests.

Suitable nest sites (i.e. those that were in range of the CCTV system and safely accessible) were located and the camera system installed before egg laying commenced. To determine egg laying dates, each nest was checked daily during the period of low adult attendance (approx. 12.00 to 17.00 hours; Cairns, 1987) and each egg was marked according to laying order. On completion of the clutch, nests were undisturbed until a few days before estimated a-egg hatching date (eggs are incubated for c. 28 days; Ewins, 1986), whereupon daily nest checking was resumed to determine hatching dates and hatching interval.

Within each brood, siblings were referred to as either A- or B-chick. This was related to hatching order in asynchronous broods (A-chick hatches first in broods where the hatching interval ≥ 1day; mean hatching interval: 1.64days ±0.24 SE, range 1-3days) and to tarsus length in synchronous broods (A-chick being the larger in broods where chicks hatch on the same day). The A-chick in each brood was marked on the head with a small streak of correcting fluid (Tipp-Ex®) to facilitate sibling identification. This mark looked similar to faecal spots that regularly appeared on A- and B-chicks. To determine sibling size disparity for each brood all chicks were weighed and measured on the day prior to behavioral observations. Chick body mass was measured to the nearest 1g using an Ohaus 200g electronic balance or 500g spring-balance, depending on chick mass, and tarsus length was measured to the nearest 0.1mm using Vernier callipers. All broods were measured at approximately the same time of day (between 11.00 and 13.00 hours) and no chick measurements were taken during the food reduction experiment.

Food reduction experiment

Observations of nestling behavior in response to changing food availability were recorded between 2 and 31 July 1997. To control for potential age-related differences in agonistic behavior, observations were confined to broods with A-chicks aged 6-12 days (by which age chicks were capable of thermoregulation, rarely brooded by their parents and sibling aggression rates were highest; unpublished data). Although this age category represents a potential age disparity of up to six days between broods, it represents only a small proportion of the nestling period (black guillemots fledge between 30-40 days: Ewins, 1986; Harris and Birkhead, 1985: Petersen, 1981), and significant age-related changes in behavior over this age range are unlikely. Incidentally, A-chick age did not differ significantly between treatment groups (experimental broods: mean age=9.0 days ±0.57 SE, n=15; control broods: mean age= 8.8 days ±1.16 SE, n=5; Mann-Whitney test, U=33.5, p=0.72) nor between synchronous and asynchronous experimental broods (synchronous broods: mean age=9.0 days ±1.47 SE, n=4; asynchronous broods: mean age=9.0 days ±0.62 SE, n=11; Mann-Whitney test, U=21, p=0.90). On reaching the appropriate age, designated broods were randomly assigned to either the experimental or control treatment.

Each of 15 experimental nests was observed continuously for twelve hours, partitioned into three periods, during which parental provisioning rates were manipulated. During period 1, three hours from 6am to 9am, broods were observed under conditions of natural parental provisioning. During period 2, six hours from 9am to 3pm, parental provisioning was prevented in the experimental nests by placing an adult scaring device - either balloons with painted eyes or a fiberglass great black-backed gull - near the entrance of the nest and in view of the returning adult. Deployment of a scaring device outside the nest provided a non-invasive means of preventing parents from entering the nest chamber and provisioning the young without directly affecting chick behavior. At the start of period 3, the final period lasting from 3-6pm, the scaring device was removed and parental provisioning quickly resumed (a feed was recorded at all nests within 30 minutes). We also observed five control nests, at which parental deliveries remained undisturbed, over the same twelve-hour period as experimental broods.

A team of four observers recorded behaviors from the monitors during the twelve-hour period, two pairs each working a continuous six-hour shift. During any one twelve-hour period, four nests were observed directly from the monitors and two were recorded using the Sony-walkman® video recorders and analyzed later. To ensure inter-observer consistency, observers independently analyzed the same sample of video footage (nine hours), both at the start and end of the season. In both cases, behavioral scoring and recordings by the four observers was identical for 89% and 96% of the behavioral events, respectively. We attempted to control for possible effects of hatch date and daily variations in weather condition by observing, during any one twelve-hour period, experimental nests comprising a range of hatching intervals (from 0 to 3 days) and at least one control nest.

Preliminary studies during the 1996 breeding season revealed that aggression was employed exclusively by the larger sibling and usually involved several bouts of violent pecking and tousling of the smaller chick, usually about the nape of the neck, face or back of the head. The recipient chick tended to assume a submissive posture and rarely attempted to retaliate or evade its attacking sibling. Submission was characterized by crouching as low as possible, any attempt to raise the head often being met with further bouts of attacks. B-chicks frequently died in unmanipulated nests where parental provisioning was poor, although the cause of death, whether directly through aggression or indirectly through starvation, could not be established. (For the 1996 and 1997 breeding seasons combined, 21 B-chicks from 69 broods (30.4%) succumbed to non-predation related fatalities, whereas only 7 A-chicks (10.1%) suffered a similar fate.) For the 1997 study, specified behavioral events were recorded directly on to data sheets using the focal-animal sampling technique (Altmann, 1977). Aggression was quantified in terms of the number of attacks (violent pecks, jabs or grasps) that each chick directed at its sibling, each individual attack being recorded as a discrete event. Adult black guillemots transport a single whole prey item to the nest crosswise in their bills, and on entering the nest chamber food allocation is determined largely by scramble competition; the sibling that first reaches the provisioning parent generally receives the prey item. Thus, only one sibling is fed during each parental delivery. A record was made of the total number of parental deliveries to each nest and the recipient chick of each feed.

To investigate the potential reversibility of the aggressive response we observed five experimental nests that had exhibited high rates of aggression for three hours on the day following the manipulation of parental provisioning rates.

Although this experiment was conceived primarily with the aim to exacerbate sibling aggression in black guillemot broods, our experimental design was such that periods of stress for the chick were short and well within the range experienced under natural conditions.

Statistical analysis

We used parametric statistics when data met the parametric assumptions of normality, homoscedasticity, and in the case of repeated measures ANOVA, sphericity. (Where sphericity was not met we used the Greenhouse-Geisser adjustment whereby both the numerator and denominator degrees of freedom were multiplied by epsilon [Zar, 1996; SPSS for Windows release 7]; *F* values were then calculated using the adjusted degrees of freedom.) Appropriate non-parametric statistics were applied where these assumptions were not met. For proportional data, we used an arcsine square-root transformation to normalize data. Normality was tested for using the Kolmogorov-Smirnov goodness-of-fit test and all probabilities given are two-tailed. Analyses were undertaken using SPSS for Windows release 7 and all tests were as described by Zar (1996).

RESULTS

Control broods

Siblings generally behaved non-aggressively in the control broods throughout the entire twelve-hour observation period: no B-chick aggression was observed and although a very low rate of A-chick aggression (attacks/hour) was noted in two of five control broods, no change in A-chick aggression rate occurred from period 1 through to period 3 (Repeated measures ANOVA, effect of period: $F_{2,8}=0.64$, $p=0.55$; Figure 1). Hence, we detected no evidence of any diurnal pattern of aggression in black guillemot A-chicks. Likewise, parental delivery rates (feeds/hour) at each control nest did not differ significantly during the three periods (Repeated measures ANOVA, effect of period: $F_{2,8}=1.92$, $p=0.21$; Figure 2), with A-chicks receiving similar proportions of parental deliveries during each period (Repeated measures ANOVA, effect of period: $F_{2,8}=0.10$, $p=0.90$; Figure 3).

Experimental broods

The period prior to food restriction (period 1)

During the period prior to food restriction (period 1), parental delivery rates (Mann-Whitney test, $U_{15,5}=24$, $p=0.24$; Figure 2) and the proportion of deliveries received by each sibling in experimental nests (Mann-Whitney test, $U_{15,5}=36$, $p=0.89$; Figure 3) did not differ significantly from those of the control group. In addition, sibling aggression rates in experimental broods were extremely low during period 1 and no significant difference in attack rate was evident between treatment groups during this period (Mann-Whitney test, $U_{15,5}=35$, $p=0.77$; Figure 1).

Food deprivation and post food deprivation (periods 2 and 3)

Aggression

Sibling aggression became evident in experimental broods following the restriction of parental food deliveries, occurring in 13 of 15 broods during periods 2 and 3. No attempt was made by parents to interfere in sibling aggression during period 3, despite the presence of one or more adults at the nest during a number of aggressive bouts. Aggression was performed exclusively by A-chicks in ten broods, exclusively by B-chicks in one brood, and by both siblings in two broods. In those cases where B-chicks were agonistic, bouts were of relatively short duration and did not elicit a submissive response from A-chicks. Thus, since A-chicks were the predominant aggressors and never subordinate, aggression was analyzed with respect to these chicks. Figure 1 shows the mean number of attacks by siblings in the experimental group during the three periods. During periods 2 and 3, A-chicks in experimental broods became aggressive, significantly increasing their attack frequency relative to period 1 (Repeated measures ANOVA, effect period: $F_{1.3,18.3}=7.67$, $p=0.008$, with Greenhouse-Geisser adjustment). A-chick aggression rates in experimental broods were significantly higher than in control broods during both period 2 (Mann-Whitney test, $U_{15,5}=17.50$, $p=0.049$) and period 3 (Mann-Whitney test, $U_{15,5}=10.0$, $p=0.014$).

Figure 1

Mean (±SE) number of black guillemot A-chick attacks per hour during each period of the twelve-hour observation in control (n=5) and experimental (n=15) broods.

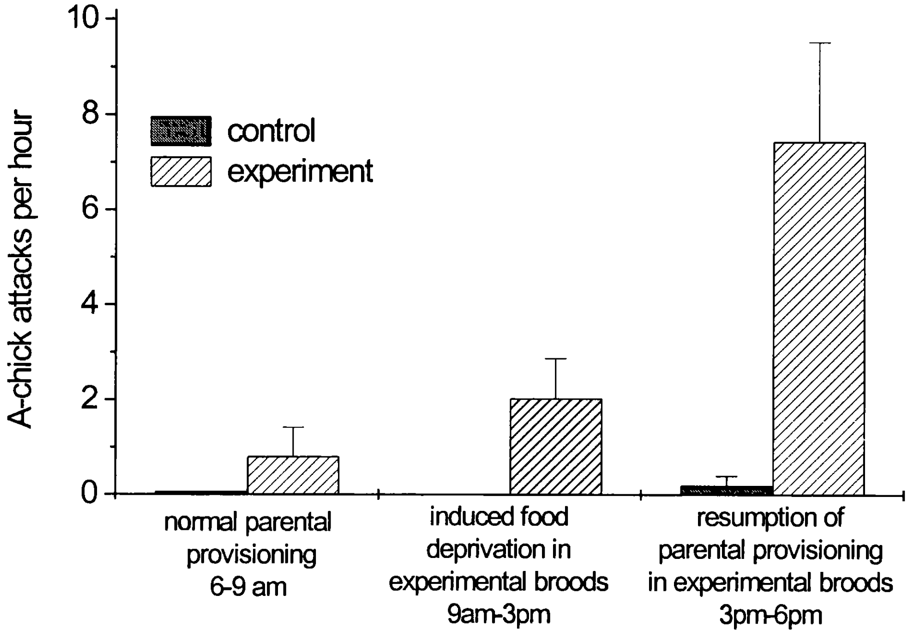


Figure 2

Mean (±SE) number of black guillemot parental feeds per hour during each period of the twelve-hour observation in control (n=5) and experimental (n=15) broods.

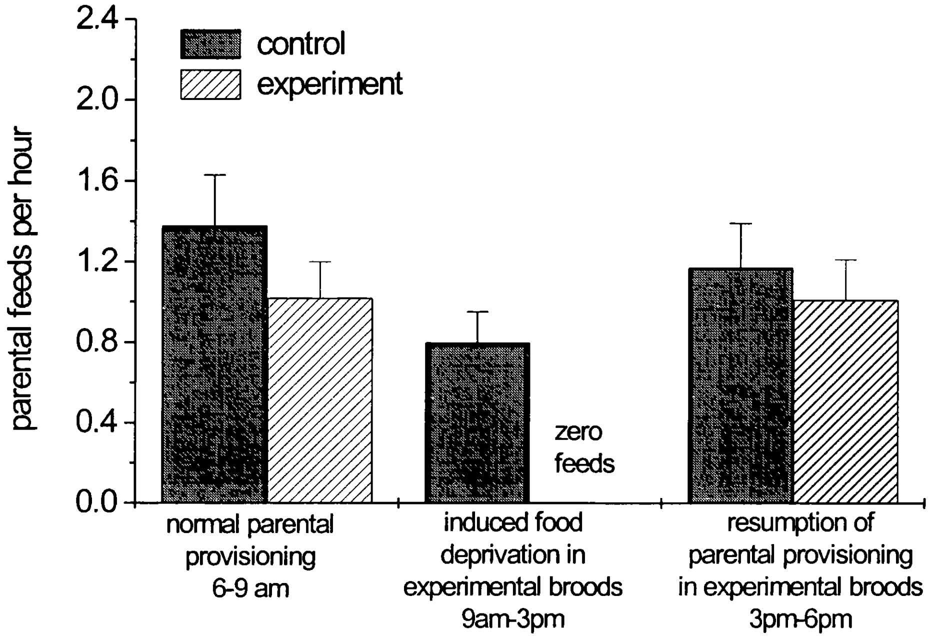
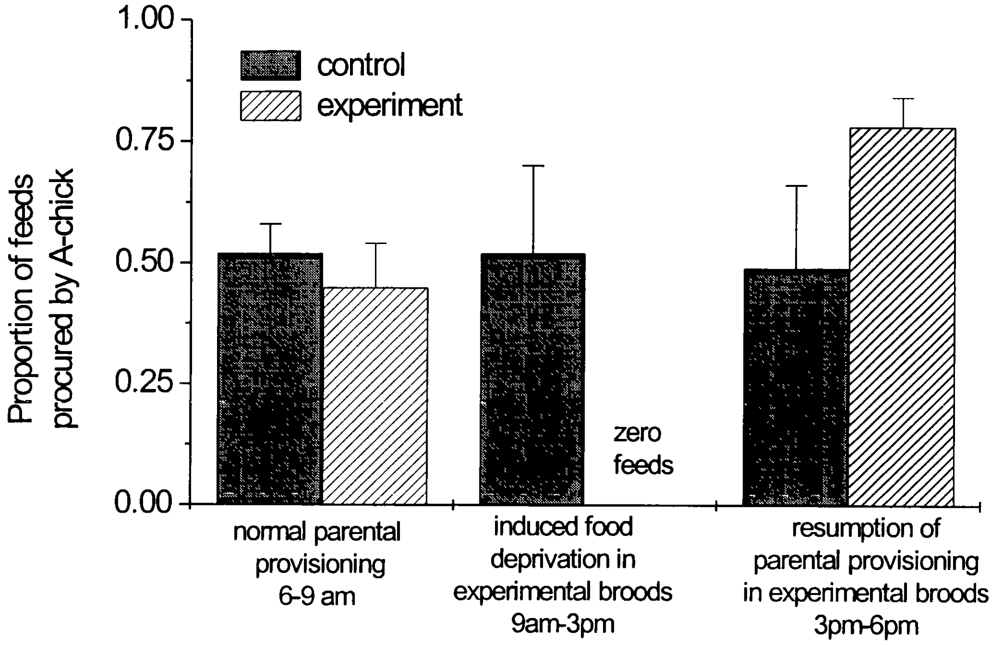


Figure 3

Mean (±SE) proportion of feeds procured by black guillemot A-chicks during each period of the twelve-hour observation in control (n=5) and experimental (n=15) broods.



Parental provisioning

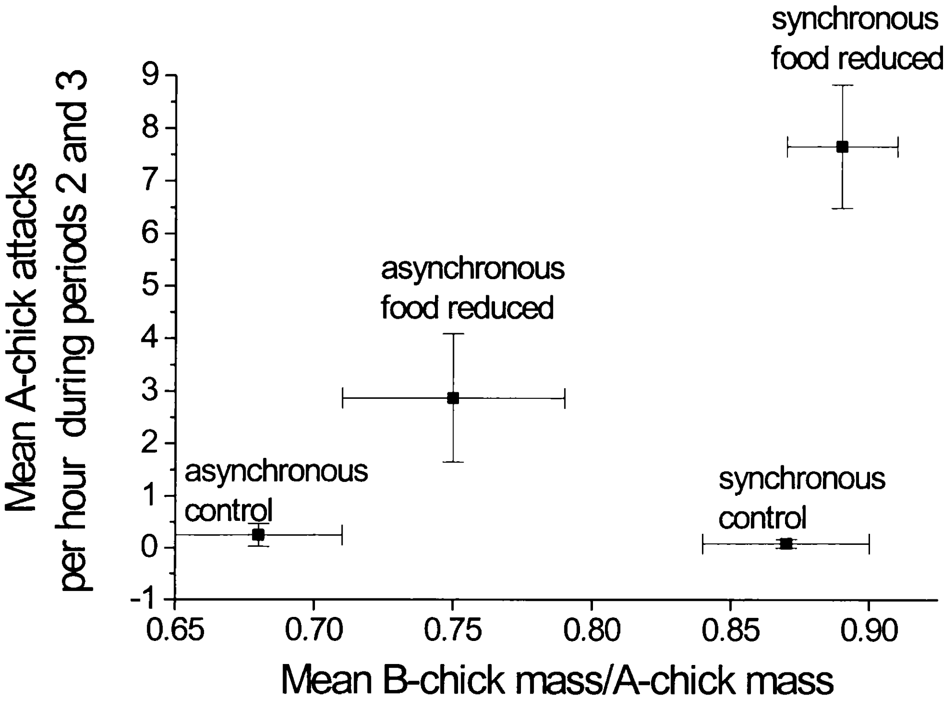
If hunger is the underlying proximate cause of aggression, the "food amount hypothesis" predicts that A-chick aggression will be associated with a corresponding increase in the share of available parental deliveries. Figure 3 shows the mean proportion of feeds received by siblings during each period. As in control nests, parental delivery rates to each experimental nest remained constant between period 1 and period 3 (paired t-test, t=0.21, n=15, p=0.84; Figure 2). The mean proportion received by each chick in experimental nests was similar during period 1 (A-chick 0.45, B-chick 0.55; Wilcoxon matched-pairs signed-rank test, z=0.05, n=15, p=0.96). However, after food deprivation, A-chicks received relatively more feeds than their sibling did (A-chick 0.75, B-chick 0.25; Wilcoxon matched-pairs signed-rank test, z=-2.87, n=15, p=0.004; Figure 3), the proportion received significantly increasing from period 1 to period 3 (paired t-test, t=-2.26, n=12, p=0.045; Figure 3). Parental delivery rates were similar between treatment groups during period 3 (Mann-Whitney test, $U_{15,5}$=28.0, p=0.41), with the effect that the total amount of food received by the experimental broods during the twelve-hour period was lower than that of control broods.

Competitive asymmetry and aggression

As expected, size disparity between siblings was significantly higher in asynchronous than in synchronous broods, both in terms of mass (Mann-Whitney test, $U_{11,4}$=7.0 p=0.049) and tarsus length (Mann-Whitney test, $U_{10,4}$=3.0, p=0.016). Figure 4 shows the relationship between hatching interval, sibling mass disparity and the mean number of A-chick attacks per hour in experimental broods in the periods during and following food deprivation (i.e., periods 2 and 3). Hatching interval had a significant effect on the level of aggression within the nest, with A-chicks from synchronously hatching broods attacking their sibling considerably more often than those from asynchronous broods (Mann-Whitney test, $U_{4,11}$=6.0, p=0.036). Aggression rates of A-chicks in asynchronous broods during periods 2 and 3 were significantly higher than during period 1 (Wilcoxon matched-pairs signed-rank test, z=-2.24, n=11, p=0.025). In synchronous broods, aggression rates increased in all broods during periods 2 and 3 relative to period 1 but the small sample size precluded statistical testing (Figure 4).

Figure 4

Mean (±SE) total number of black guillemot A-chick attacks during periods 2 and 3 for synchronous (n=4) and asynchronous broods (n=11) in the experimental group, in relation to mean (±SE) sibling size disparity (expressed as mass of B-chick divided by mass of A-chick).



Reversibility

An implicit assumption of the 'food amount hypothesis' is the reversibility of the system; aggression should decrease to base-line levels on resumption of satiation, with siblings thereafter receiving an equal share of resources. Behavioral data obtained from five experimental nests the day after manipulation supported this assumption: aggression rates in these nests declined considerably, with sibling interactions being largely passive, and the previous skew in the distribution of parental food deliveries towards the A-chick returned to unity. Both sibling aggression rates (aggression rate [attacks/hr] period 1: mean=1.88 ±1.80 SE; aggression rate after: mean=0.36 ±0.23 SE; Wilcoxon matched-pairs signed-rank test, z=-0.54, n=5, p=0.60) and the allocation of parental food deliveries (proportion of feeds received by A-chick during period 1: mean=0.52 ±0.036 SE; proportion feeds received by A-chick after: mean=0.50 ±0.045 SE; Wilcoxon matched-pairs signed-rank test, z=-0.37, n=5, p=0.72), did not differ significantly from those recorded during period 1 of the experiment.

DISCUSSION

A-chick aggression in black guillemot broods was elevated only after parental provisioning rates were experimentally reduced. When parental provisioning resumed, adults did not adjust their feeding rate in response to changes in their offsprings' requirements and the consequence of the inter-sibling aggression was a skew in the distribution of food received by siblings in favor of the dominant A-chick. Moreover, the system was reversible; on the day following manipulations, A-chick aggression rates returned to pre-food-deprived levels and B-chicks regained an equal share of parental feeds. Thus, by mediating aggression in accordance with changing nutritional requirements, A-chicks ensured a feeding advantage over their sibling when parental provisioning rates were reduced.

A further factor influencing aggression in food deprived broods was competitive asymmetry. Food deprived A-chicks from synchronous broods were significantly more aggressive than those from asynchronous broods, suggesting that fights were more common in situations where the dominance status of siblings was ambiguous. These results are consistent with game theoretical approaches to animal contests (Maynard Smith and Parker, 1976) and support empirical studies which showed that artificially synchronized broods were more aggressive than natural asynchronous broods (Fujioka, 1985; Mock and Ploger, 1987; Osorno and Drummond, 1995). An alternative explanation for higher aggression rates in synchronous broods is related to the relative ability of siblings to respond to, and sequester, parental feeds. Parents return to the entrance of the nest chamber with a single prey item, prompting a scramble competition among siblings from which the successful chick receives the entire delivery. In asynchronous broods, older, more developed chicks might gain greater access to parental deliveries without the need for aggressive domination by virtue of superior locomotory skills. In synchronous broods, however, because both chicks are competitively similar in feeding scrambles, the A-chick is likely to gain a greater proportion of feeds by being aggressive. Although a multitude of hypotheses exist for the functional significance of hatching asynchrony (see Stoleson and Beissinger, 1995 for review) the results of our experiment suggest that one benefit of asynchrony in the black guillemot is to establish an efficient competitive hierarchy among siblings which minimizes the need for costly aggressive interactions (see Hahn, 1981; following Hamilton, 1964). Such a system is also believed to operate in certain species of Ardeidae (Fujioka, 1985; Mock and Ploger, 1987), Sulidae (Anderson, 1989; Osorno and Drummond, 1995), and various raptors (Forbes, 1991; Machmer and Ydenberg, 1998; Viñuela, 1999; Wiebe and Bortolotti, 1994). However, Amundsen and Slagsvold (1991) point out that although moderate degrees of asynchrony could be adaptive, relatively large hatching intervals have the potential to promote unnecessary brood reduction, regardless of environmental conditions. For example, in the facultatively siblicidal black kite moderate hatching asynchrony reduces sibling rivalry, yet younger chicks in broods with extreme asymmetries suffered the highest mortality rate and most serious injuries (Viñuela, 1999). In the current study, broods with hatching intervals greater than 3 days were unavailable for experimental manipulation and thus the effect of extreme asymmetry on black guillemot sibling aggression has yet to be established.

The influence of competitive asymmetry on black guillemot nestling aggression suggests that the proximate response is controlled not only by food shortage but also by factors associated with laying and hatching order. The observed aggressive disparities among siblings, particularly those in synchronously hatching broods where size and age differences were negligible, imply an inherent capacity for aggression. Schwabl (1993) postulated that such within-brood variation may be a consequence of differential concentrations of maternal hormones secreted into the yolk during egg maturation. Indeed, a correlation between sibling social rank and testosterone content of the eggs from which they hatched has been demonstrated in some avian species (Schwabl, 1993; Schwabl, 1996; Schwabl et al., 1997). It is also possible that nestlings are visually sensitive to size asymmetry within the brood and base the decision to elevate aggression rates on the competitive ability of their sib. Clearly, further data are required to establish a comprehensive picture of the proximate causal pathway of sibling aggression. Nonetheless, our evidence that aggression in the black guillemot increases in both synchronous and asynchronous broods when food is reduced, strongly implicates food shortage as the initial and primary stimulus for the aggressive response.

A fundamental prerequisite for the evolution of sibling rivalry is the existence of a resource deficit that has fitness consequences for the offspring (in terms of maintenance, growth and survival) and ultimately creates a competitive environment within the brood (Mock and Parker 1997; based on inclusive fitness theory, Hamilton 1964). However, the use of overt aggression between competing sibs has been documented in relatively few avian taxa (Mock and Parker 1997), with most species relying on passive forms of dominance such as relative begging vigor or the monopolization of favorable positions within the nest (Bengtsson and Rydén 1981, Gottlander 1987, Stamps et al. 1989). The reason for this dearth of violent interactions is straightforward: sibling aggression is costly (in terms of energy expenditure, risk of injury etc.) and for selection to favor aggression in competitive avian sibships, the long-term fitness benefits of fighting must outweigh the potential costs (Lamey and Mock 1991). In this context, it might be argued that since the induced deprivation in the current study was short-term, the resulting effects were potentially reversible and thus had little effect on chick fitness. Therefore, comparable short-term effects under natural conditions (for example, due to weather conditions or kleptoparasitism) would not provide the selection pressure necessary for the evolution of an expensive aggressive response. Nonetheless, several lines of circumstantial evidence suggest that food amount may be an important ultimate cause of black guillemot sibling aggression. For example, the fact that non-predation related mortality rates are considerably higher in the first two weeks post-hatching than during the remainder of the nestling period (17 of 21 B-chicks (80.9%) that suffered non-predation related mortality in 1996 and 1997, died prior to 12d), implies that even short-term reductions in parental delivery rates may confer significant fitness consequences for relatively young black guillemot chicks. Moreover, sibling aggression in black guillemots is highest in young broods (unpublished data), peaking between 4 and 12 days post-hatching and declining rapidly with age thereafter. This close association between chick age, aggression and mortality lends some support to the notion that significant fitness benefits may be accrued through fighting. An additional factor, which may determine the cost-effectiveness of sibling aggression, is the defensibility of the food delivered to the brood. Mock (1994, 1985) formalized the relationship between prey defensibility and aggression with the "prey-size hypothesis", positing that sibling aggression is more likely to be selected for in species where parentally delivered food arrives in monopolizably small units. Parent black guillemots deliver a single, economically defendable, prey item to the brood, with the result that during any one delivery, only a single chick is fed. Such monopilizability in black guillemot broods may allow fighting to be relatively cost-efficient, since submissiveness on behalf of the B-chick can significantly skew parental investment towards the A-chick.

For food amount per se to be a reliable proximate cue in the regulation of aggression it is necessary for short-term parental provisioning rates to be predictable; that is, periods of inadequate current provisioning must correlate with future food-shortages that affect brood fitness (Mock et al., 1987, Mock and Parker, 1997). If this assumption is not met then either a more reliable proximate cue should be employed or, if no forecast of future costs can be made, a strategy of obligate aggression (Mock and Parker, 1997). In contrast to the Atlantic puffin (*Fratercula arctica*), where provisioning rates were found to vary in response to offspring requirement (Cook and Hamer, 1997; Harris, 1983), we observed no increase in black guillemot provisioning rate to compensate for the lack of prey supplied during the experimental period of induced food restriction (Figure 2). Thus, under natural conditions, it is possible that adults supply food to the brood at an intrinsically set rate and any reduction in provisioning rate (e.g. due to inclement weather conditions) may not be subsequently compensated for. The implication that inflexible provisioning rates are a selective pressure in the evolution of the agonistic response is therefore supported by our observation that periods of poor provisioning appeared to be associated with a predictable forthcoming energy deficit to the brood, regardless of future feeding conditions. However, the time-scale over which parental responses to chick need were recorded may have been too short to detect any compensatory increase in provisioning. Furthermore, although the rate of food supply to black guillemot broods did not appear to be regulated by adjustments in feeding frequency, it is possible (although unlikely) that regulation occurred through prey size adjustments. Further studies are required to verify the inflexible nature of parental provisioning and to examine in greater detail the relationship between current and future food supply.

Two previous studies have experimentally demonstrated a proximate link between food supply and aggression in nestling birds, yet important differences exist between the three study species in the mechanism regulating hunger mediated aggression. Drummond & Garcia Chavelas (1989) revealed that dominant blue-footed booby nestlings exhibit a base-line level of aggression regardless of current provisioning rates, increasing attack frequencies in response to their nutritional condition rather than to short-term fluctuations in food supply. By contrast, aggression rates in ospreys, although influenced by hunger, are largely determined by the degree of size asymmetry within the brood (Machmer and Ydenberg, 1998). In black guillemots, while aggression levels were higher in synchronous broods, sibling aggression increased in response to food deprivation regardless of the degree of hatching spread. This study is therefore the first to demonstrate that short-term food reduction per se can function as an initial trigger for aggression among avian siblings.

More studies on other species are necessary so that further inter-specific comparisons of the aggressive response can be made. By identifying the ecological conditions and biological features that favor these different strategies, a greater understanding of the evolution of sibling aggression will be achieved.

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CHAPTER 7

PROXIMATE DETERMINANTS OF HATCHING ASYNCHRONY: EFFECTS OF SEX-SPECIFIC EMBRYO DEVELOPMENT RATES

Submitted to American Naturalist as:
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Proximate determinants of hatching asynchrony: effects of sex-specific embryo development rates

ABSTRACT

Before the functional significance of hatching asynchrony within any avian species can be resolved, it is first necessary to determine the proximate mechanisms controlling the pattern of hatching. To date, most studies have tacitly assumed that hatching patterns are effected predominantly by parental incubation behavior, yet such assumptions have received little empirical investigation. In this study we compared incubation periods of male and female black guillemot (*Cepphus grylle*) embryos to ascertain whether development rates are a function of embryo sex and, if so, the effects of clutch sex-composition on hatching pattern. We determined chick sex using a molecular DNA technique based on the CHD gene. Laying date and egg mass had no significant effect on incubation period, but eggs containing male embryos developed significantly faster than those containing females. The onset of incubation in relation to clutch completion is variable in black guillemots. Thus, in mixed-sexed clutches where the first-laid embryo is male, hatching asynchrony was attained regardless of the incubation regime employed. These results clearly show that mechanisms in addition to incubation behavior are important in establishing avian hatching patterns.

INTRODUCTION

The competitive dynamics amongst siblings within avian broods can have a profound effect on chick growth and survival, and thereby also on parental fitness. Elucidating the proximate factors that determine these dynamics is therefore a key component of avian behavioral and evolutionary ecology. Parent females can potentially control brood dynamics through the manipulation of certain breeding parameters, with factors such as hatching asynchrony (Viñuela 1996), egg size dimorphism (Parsons 1970; Slagsvold et al. 1984; Quinn and Morris 1986), egg composition (Williams 1994), egg additives (Schwabl 1993) and embryo gender (Bradbury and Griffiths 1997), all having the potential to influence brood dynamics. It is generally considered, however, that the temporal effect of hatching asynchrony is of particular importance in determining brood dynamics (Bryant 1978; Howe 1978; Bancroft 1984; Ricklefs 1984; Stokland and Amundsen 1988; Magrath 1992; Lamey 1990), by virtue of the size, age and competitive hierarchy it establishes within the brood. The functional significance of hatching asynchrony has attracted considerable attention over the past five decades and many disparate adaptive hypotheses have been posited to account for the evolution of this phenomenon (for reviews, see Clark and Wilson 1981; Amundsen and Stokland 1988; Magrath 1990; Stoleson and Beissinger 1995). However, two fundamental aspects of hatching asynchrony have largely been ignored: first, the underlying proximate mechanisms that control hatching spread (Stoleson and Beissinger 1995); and second, the assumption on which all adaptive hypotheses are based, that the degree of asynchrony is parentally controlled (Magrath 1992). Most studies of hatching asynchrony in non-precocial species tacitly assume that eggs within a clutch develop at identical rates for a given effort of incubation and, as a consequence, that parental control of hatching is mediated via the timing of effective incubation in relation to laying sequence (e.g. Drent 1975; Clark and Wilson 1981). Although some support for these assumptions is derived from studies where clutches hatch according to laying order (Cargill and Cooke 1981; Inoue 1985; Magrath 1992), the role of incubation as the sole determinant of hatching spread has received only modest empirical scrutiny (Viega and Viñuela 1993; Stoleson and Beissinger 1995). Indeed, general consensus has been that the ability to control hatching patterns independently of incubation regime is restricted to precocial species, in which synchrony is achieved either through accelerated hatching of eggs laid towards the end of the laying sequence (Vince 1964; Davies and Cooke 1983) or retardation of development in more advanced embryos (Vince 1968). However, recent studies have shown that a number of non-precocial species also have the ability to influence hatching patterns by means other than incubation regime. In these studies, factors such as egg-size, laying order and laying date affected embryo development rates (St Clair 1996: Viñuela 1997). Moreover, circumstantial evidence suggests that these disparate development rates may be more prevalent in non-precocial species than were previously considered (Viñuela 1997). Thus, although incubation regime is undoubtedly an important element in determining hatching patterns in non-precocial birds, these studies highlight the importance of investigating the proximate control of hatching patterns before inferences as to the adaptive significance of incubation and hatching patterns are made.

One potentially important factor that may influence avian hatching patterns is embryo sex. If development rate differs between male and female embryos, then the laying order of the two sexes could have an important influence on hatching asynchrony. Not only are some avian species capable of facultatively adjusting the sex ratio of the brood with respect to laying order (Bortolotti 1986; Bednarz and Hayden 1990), but following recent advances in DNA based sexing techniques (Griffiths and Tiwari 1993; Griffiths et al. 1996; Ellegren and Sheldon 1997), it has come to light that some species may also be able to differentially allocate egg resources according to embryo sex (Anderson et al. 1997). Moreover, an increasing amount of evidence from a number of taxa suggests that egg composition can affect embryo development rates (reptiles: Sinervo 1990; birds:

Burke 1992). It is therefore possible that embryo development rate is a function of gender in some avian species. If this is so, then brood sex composition will have a significant influence on brood competitive dynamics through its effect on the level of hatching asynchrony. Although such gender mediated effects on hatching patterns have been reported in captivity, whilst also accounting for egg-size effects (Davies and Payne 1982; Burke 1992), such effects in a wild population have yet to be demonstrated.

The black guillemot (*Cepphus grylle*) is a sexually monomorphic seabird that exhibits marked inter-pair diversity in a number of breeding parameters. Although the majority of breeding pairs lay a two-egg clutch, considerable variation is evident in the egg laying interval, the incubation period and the degree of hatching asynchrony (Asbirk 1979; Ewins 1989). In our study population, egg laying interval ranges from 1 to 10 days; incubation duration ranges from 26 to 39 days for the a-chick, and from 24 to 36 days for the b-chick; hatching asynchrony varies from 0 to 6 days. Furthermore, hatching patterns in this species do not always equate with incubation regime; for example, some pairs that start incubation on the first-laid egg hatch a synchronous brood, whereas other pairs have a hatching interval greater than the laying interval (unpublished data). With these data in mind, this study examined embryo development rates with respect to sex in two-egg clutches of the black guillemot.

METHODS

Study site

Data presented here were collected on the Holm of Papa Westray, Orkney, Scotland (59° 22'N, 2°53'W), between May and August in the years 1996 and 1997. The Holm is approximately 0.8 x 0.3 km, uninhabited by humans and free from mammalian predators. During the 1990's the population of this colony has remained relatively constant at approximately 65 breeding pairs.

Egg laying and development rate

Nests were checked daily for the presence of an egg so that the laying date of each egg and laying interval for each clutch was obtained. On the day of laying, eggs were weighed to the nearest 0.1g using a 200g electronic balance and each egg was marked according to its laying sequence using a permanent marker pen. On completion of the clutch, nests were undisturbed until a few days before estimated a-egg hatching date (eggs are incubated for c. 28 days; Ewins 1986), whereupon daily nest checking resumed to determine hatching dates, hatching interval and incubation period. In those nests where we considered clutches might hatch synchronously, we undertook more frequent checks to ensure that the egg origin of each chick was ascertained. (Since the first signs of hatching can be seen at least two days before the chick emerges from the shell (Preston 1968, from Harris and Birkhead 1985), we were able to approximate the level of hatching asynchrony prior to hatching). All eggs hatched according to laying order and a note was made of the egg from which each chick hatched. Within each brood, siblings were referred to as either a- or b-chick. To facilitate sibling identification, the a-chick in each brood was regularly marked on the head with a small streak of correcting fluid (Tipp-Ex®). Since the egg origin of each chick was established, we therefore knew the laying order, sex and development time for each egg laid.

Initiation of incubation relative to clutch completion in the black guillemot is highly variable. Full or partial incubation of the first egg begins before the second egg is laid in some broods, whereas in others full incubation starts on clutch completion (Asbirk 1979; pers. obs.). Thus, the onset of incubation is variable with respect to a-eggs but b-eggs are fully incubated from laying. Accordingly, we used the incubation period of b-eggs to compare male and female embryo development rates.

Identification of sex

Chick sex was ascertained using a molecular DNA technique based on the presence or absence of the highly conserved W-linked CHD1 gene (chromodomain-helicase-DNA- binding protein W-linked) using genomic DNA isolated from blood (live chicks) or muscle samples (dead chicks; after Griffiths et al. 1996).

Blood samples for molecular sexing of live chicks were obtained under U.K. Home Office license from 5-20d old nestlings by means of tarsal veinipuncture using a sterile hypodermic needle. Approximately 50ul of blood was transferred via capillary tube to an equal volume of BLB buffer (2% sodium dodecyl sulphate (SDS), 50mM EDTA, 50mM Tris (pH8)). Blood samples were kept cool in the field, placed in a refrigerator within five hours of collection and returned to the laboratory for sexing. No chick mortality occurred within three days of blood sampling.

The extraction of DNA from black guillemot blood was based on Wetton (1990), with slight modifications. After digestion of a small portion of the blood sample by proteinase K digestion for 12 hours, DNA was extracted using phenol/chloroform and recovered by ethanol precipitation. For chicks that died before blood samples were obtained, muscle tissue from the corpse was used as a source of DNA. The protocol for the extraction of muscle DNA followed that of the blood samples but muscle tissue required at least 48 hours in proteinase K for complete digestion.

The PCR amplification of DNA followed that of Griffiths et al. (1996). The PCR products were subsequently electrophoresed in agarose gel and photographed under UV light.

ANALYSIS

Since data met the parametric assumptions of normality and homoscedasticity, appropriate parametric statistics were applied. To examine the influence of embryo sex on incubation period, we used an ANCOVA (general linear model procedure) with egg mass and laying date as covariates. Laying dates for each of the two years was standardized by subtracting the date of clutch initiation from the median laying date of that year. Normality was tested for using the Kolmogorov-Smirnov goodness-of-fit test and all probabilities given are two-tailed. Analyses were undertaken using SPSS for Windows release 7 and all tests were as described by Zar (1996).

RESULTS

During the breeding seasons of 1996 and 1997, we examined 59 b-eggs in 42 nests. For 17 of these nests, data were collected in successive years. To avoid pseudoreplication, we randomly assigned each of these nests to one of the two years, and used only data from the allocated year in the analysis.

Egg mass did not differ between years (1996: mean mass=47.7g, n=19, SD=3.9; 1997: mean mass=47.6g, n=23, SD=3.1; $t_{40}=0.11$, n.s.).

Figure 1 shows the mean development period in days for male and female b-eggs.

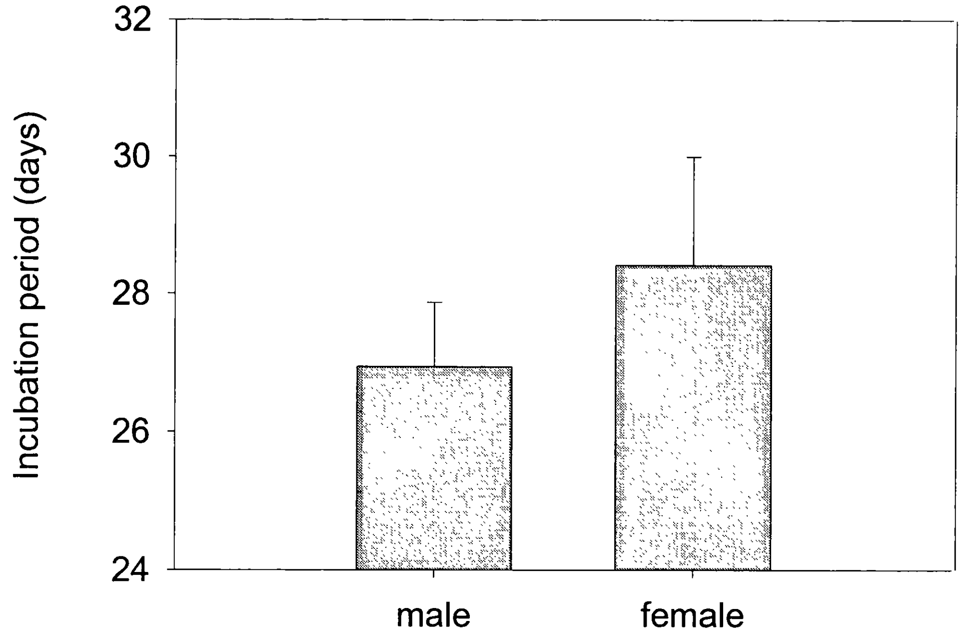
Incubation period was not influenced by egg mass or laying date; however, there was a highly significant difference between the incubation period of male and female eggs, with the mean male-egg incubation period being shorter (ANCOVA of b-egg incubation period, effect of sex: $F_{1,40}=11.61$, $p<0.002$; effect of egg mass: $F_{1,39}=0.98$, n.s.; effect of laying date: $F_{1,38}=0.75$, n.s.; all interactions n.s.).

Sex-specific development rates should result in predictable patterns of hatching according to clutch sex composition, independent of incubation regime. In clutches where both eggs are the same sex, embryos will develop at similar rates and thus the degree of hatching should be independent of gender and determined entirely by incubation regime. In mixed sexed clutches on the other hand, either the male (M-F clutch) or female (F-M clutch) may be laid first. M-F clutches should hatch only asynchronously in the order of laying (i.e., M-F broods) because first laid male eggs develop relatively faster than the second laid females; in these clutches, initiation of incubation prior to clutch completion would serve only to exacerbate hatching asynchrony. In

Table 1. The hatching pattern of black guillemot clutches in relation to the sex of the first and second laid egg (M-M: all male clutch; F-F: all female clutch; M-F: male laid first in mixed-sexed clutch; F-M: female laid first in mixed-sex clutch,), shown as the percentages of each clutch type that hatched synchronously (siblings hatch on the same day), or asynchronously (siblings hatch over a period greater than one day).

	M-M	F-F	M-F	F-M
Percentage hatch synchronously	33.3	66.6	0	0
Percentage hatch asynchronously (a-egg hatches first)	66.7	44.4	100	100
Percentage hatch asynchronously (b-egg hatches first)	0	0	0	0
N	9	9	17	7

Figure 1. Mean (+SE) incubation period in days for male and female second-laid eggs. The numbers of eggs for each sex are given in parentheses.



F-M clutches, by contrast, because first-laid female eggs develop at a slower rate than second-laid males, a range of brood hatching patterns are possible: asynchronous F-M broods would hatch if incubation starts soon enough after clutch initiation to give the relatively slow developing female embryo a sufficient developmental head-start over the male; whereas asynchronous M-F broods would result if the time-scale between initiating incubation on the first-egg and laying the second-laid egg is short enough to enable the faster developing male to hatch first; finally, synchronous hatching would occur if incubation is initiated at the appropriate intermediate stage between laying the first and second egg. The observed pattern of hatching in the black guillemot is summarized in Table 1 and reflects that predicted by a sex-specific pattern of development, i.e. all M-F clutches hatched asynchronous M-F broods.

DISCUSSION

The possibility that hatching asynchrony may be attained through means other than incubation regime has seldom been considered. In this study, we demonstrate that male embryos require a shorter incubation period and thus develop at a faster rate than equivalent female embryos. Other factors that might potentially influence embryo development rates in birds are egg size and laying phenology, yet in the black guillemot neither of these factors had a significant effect. Rahn and Ar (1974) demonstrated a clear positive relationship between egg mass and incubation period among avian species, but the results of studies investigating such effects within species are equivocal (Viñuela 1997). For example, while some studies are in accord with the results of Rahn and Ar (1974), others have failed to show a significant effect of egg mass (see Parsons 1972; Drent 1975; Runde and Barrett 1981; Ricklefs and Smeraski 1983; Martin and Arnold 1991; Kattan 1995). Variation in the degree of mass change within a species, and the possibility of interacting egg compositional changes, may account for these inconsistent results. With respect to laying date, a reduction in incubation period as the season progresses has been demonstrated for a number of species (Parsons 1972; Runde and Barrett 1981; St. Clair 1996), although interpreting such effects is often statistically problematical due to the confounding effects of other breeding parameters and environmental factors which also vary with date (St. Clair 1996). Given that the black guillemot is unusual among temperate and arctic seabird species in that relatively few breeding parameters are affected by season (unpublished data), it is not surprising that date of clutch initiation had no effect on incubation duration in this species.

For this study, we used b-egg incubation period to ascertain development rates since we could not accurately determine the onset of incubation of a-eggs. Egg size generally decreases with laying order in the black guillemot (Ewins 1986, 1989; unpublished data), but since development rates appeared not to be a function of egg size, it is likely that gender differences in development rate will also apply to a-eggs. This is supported by the pattern of hatching in M-F clutches, which hatched only asynchronous M-F clutches, as predicted (Table 1). Furthermore, the pattern of hatching in single sex clutches (M-M and F-F) provide additional evidence that sex-specific development rates operated in both eggs. The embryos of single-sex clutches would be expected to develop at the same rate, and since these clutches hatched both synchronous and asynchronous broods (Table 1), it thus infers that the timing of the onset of incubation in the population is variable. In light of this, if hatching patterns were determined solely by incubation regime, one would expect M-F clutches to hatch synchronous broods at a frequency approximating those of single-sex broods, i.e. about fifty percent, and not the observed one hundred percent asynchronous M-F broods. This is particularly pertinent considering the high proportion of M-F clutches in the population (40.5%). The observed pattern of hatching therefore strongly suggests that first-laid male embryos develop relatively faster than second-laid females, as observed for second-laid eggs. Assuming this is the case, parent females have the potential to control hatching patterns, not only through the onset of incubation in relation to egg laying (e.g., Amundsen and Stokland 1988) but also through sex-ratio adjustment.

Thus hatching patterns will be influenced not just by parental incubation behavior, but also by embryo sex. While this may constrain parental control of the hatching pattern, it is also possible that parents can influence the degree of hatching asynchrony by controlling offspring sex. Indeed, the capacity to facultatively adjust the production of male and female offspring in line with prevailing conditions has been demonstrated in a number of bird species (see Sheldon 1998 for review). Given the likely benefits of asynchronous hatching in black guillemot broods (Cook et al. in press), it is conceivable that the functional basis for such a pattern of development hinges on the trade-off between staggered hatching and the potential costs of the early onset of incubation (i.e., incubation before clutch completion). For many species, potential physiological, social and environmental constraints operating during the laying period are likely to play a key role in determining hatching patterns, and to attain asynchrony in the absence of disparate embryo development rates, incubation must be initiated before clutch completion. However, egg formation and incubation can be relatively demanding processes and these concurrent activities may not be possible due to nutritional or energetic constraints on the female (Nilsson 1993; Monaghan and Nager 1997). Indeed, a number of recent studies have demonstrated that experimental increases in these costs carry fitness consequences (Heaney and Monaghan 1995, 1996; Monaghan et al. 1995; Oppliger et al. 1996). Thus, in the absence of sex- specific development rates, the extent to which incubation and egg formation can occur simultaneously may significantly affect the level of asynchrony observed. The ability of black guillemots to induce asynchronous hatching through sex-related development rates, without the constraints of the early onset of incubation, may therefore represent an efficient cost-reducing mechanism.

Sex-specific embryo development as a means of inducing asynchrony is clearly limited to mixed-sexed clutches; in single-sex clutches, eggs develop at similar rates and incubation prior to clutch completion is required to attain asynchrony. Thus, in a population where the empirically observed sex-ratio approximates fifty percent sons and the frequency of clutch sex-compositions follow the expected binomial distribution, only half of all clutches will be mixed sexed-broods and have the potential to hatch asynchronous broods through sex-specific development. Moreover, in this study, the pattern of hatching exhibited by F-M clutches suggests that additional constraints or selective factors were operating on the parents of these clutches which necessitated the early onset of incubation. If parents laying F-M clutches employed sex-specific development rates as a cost-effective means of attaining asynchrony, then initiation of incubation in these clutches should have occurred after laying the second, faster developing male egg, thereby producing an asynchronous M-F brood. Yet all F-M clutches hatched in the order they were laid, i.e. hatching F-M broods, indicating that incubation started relatively soon after clutch initiation and prior to laying the second egg. The reason for this early onset of incubation is unclear but it is possible that hatching the clutch in the order of laying provides a higher fitness return than the energy saved from delaying incubation until after clutch completion. Ultimately, with a relatively low number of suitable brood types in the population, it appears unlikely that the promotion of asynchronous hatching provides the definitive explanation for sex-specific development. Indeed, a more cost-effective means of promoting asynchrony would be laying-order specific development rates: if a-eggs were to develop faster than b-eggs then hatching asynchrony could be achieved in all broods, independently of incubation regime. Such laying order effects have been found in a number of species, although in all cases so far it is the final egg that develops relatively faster, thereby reducing the level of hatching asynchrony within the brood (Brown 1988; St. Clair 1996; Viñuela 1997). It is conceivable, therefore, that hatching asynchrony in the black guillemot is a non-adaptive consequence of differential embryo development rates rather than the selective pressure promoting such a pattern of development.

The potential mechanisms controlling such a pattern of development are equally as intriguing as the possible adaptive function. One aspect of avian egg physiology that is receiving increased attention of late is yolk hormone concentration. Recent studies have revealed that avian mothers may influence brood dynamics by bestowing differential concentrations of hormones, most notably testosterone and its derivatives, in the yolk of successive eggs within the clutch (Schwabl 1996, 1997). Although no studies have yet investigated the physiological effects of maternal testosterone in the embryo, its effects on the behavior and physiology of the neonate have been demonstrated in a number of species (Schwabl 1997). For example, yolk testosterone has been shown to increase protein synthesis and neuronal differentiation as well as influencing chick behavior (Schwabl 1996, 1997). Evidence of these maternal effects, in addition to recent support for facultative sex adjustment in birds, is highly consistent with the idea that yolk testosterone levels affect embryo development rates, and highlights the need for further studies investigating yolk hormone levels in relation to development rates, gender and reproductive strategy.

Although this study does not provide the likely functional explanation for the observed gender differences in development rate, it clearly demonstrates that mechanisms other than incubation regime are important in determining avian hatching patterns and hence the competitive dynamics of the brood. Not only may the determinants of embryo development rate provide clues as to the potential constraints operating during incubation but also, until these determinants are established, designating an adaptive function to a species' hatching pattern should be treated with caution. More studies are required to determine the general applicability of sex-specific development rates and their role in avian breeding strategies.

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CHAPTER 8

EFFECTS OF BROOD SEX COMPOSITION ON SIBLING GROWTH & SURVIVAL

INTRODUCTION

It was Darwin (1871) who first pointed out that offspring sex ratios are likely to have important and compelling effects on parental fitness. However, our understanding of sex-ratio evolution progressed considerably when Fisher (1930) hypothesised that total parental expenditure in offspring of each sex should be equal at the end of parental care period. Thus, if one sex is relatively more costly to produce than the other, selection should lead to a reduction in the number of the more costly sex. Fisher's ideas have been confirmed both mathematically (Charnov 1982) and, in numerous taxa, empirically (Cronmiller and Thompson 1980, Bancroft 1984, Teather and Weatherhead 1988). However, as Williams (1979) highlights, Fisher's theory applies only to sex ratio allocation at the population level. It is theories concerning maternal manipulation of offspring sex ratio (e.g. Trivers and Willard 1973), broadly known as sex allocation theory (Charnov 1982), that provide the conceptual framework behind strategies at the level of the individual. Sex allocation theory asserts that when the reproductive value of males and females differ with respect to a variable (e.g. environmental quality), it would be adaptive for parents to adjust parental investment in their offspring in response to that variable (Trivers and Willard 1973, Charnov 1982, Frank 1990).

Although theory pertaining to relative investment in male and female offspring has received support from empirical studies on plants, invertebrates and certain vertebrate taxa, documentation of sex allocation bias in birds has traditionally remained scarce (Clutton-Brock 1991, Frank 1990). Nonetheless, due to the recent development of simple molecular sexing techniques for birds (Griffiths and Tiwari 1995, Griffiths et al. 1996, Ellegren and Sheldon 1997), the past few years have witnessed an escalation of publications demonstrating non-random association between offspring sex and laying/hatching order. Many ecological circumstances could affect facultative manipulation of sex allocation and a diversity of adaptive benefits have been suggested. For example, maternal adjustment of offspring sex ratio may vary according to maternal condition (Trivers and Willard 1973; see Wiebe and Bortolotti 1992, Ellegren et al. 1996, Nager et al. 1999) or the perceived quality of her mate (Burley 1986). Alternatively, brood sex ratio may systematically change with hatching date (Dijkstra et al. 1990, Olsen and Cockburn 1991, Daan et al. 1996), or according to a combination of social and environmental conditions (Gowaty and Lennartz 1985, Komdeur et al. 1997). Ultimately, however, any ecological variation that predictably results in more grandchildren through the production of one sex or the other should result in maternal adjustment of progeny sex allocation. Manipulation may occur either prior to ovulation or during the post-natal period.

Regardless of the causal nature of differential sex allocation, if relative parental investment in one sex over the other is favoured (e.g. due to sexual size dimorphism effects), the potential exists to influence brood competitive dynamics (Lack 1954, Edwards and Collopy 1983) and thus offspring fitness in a manner similar to that of hatching asynchrony and egg size-dimorphism (Clutton-Brock et al. 1985). Indeed, sex related differences in postnatal mortality have been reported in a number of sexually dimorphic species (Clutton-Brock et al. 1985, Howe 1977, Roskaft and Slagsvold 1985, Griffiths 1992). However, the effects of sexual size dimorphism on sibling survival vary according to species. For example, in certain species it is the larger sex that enjoys higher survival rates due to its superior competitive ability to attain parental deliveries (Bednarz and Hayden 1991, Bortolotti 1986, Dhondt 1970). In these species, allocation of sex according to position in the laying sequence is likely to be important in determining the competitive hierarchy of the brood and thus the relative fitness of individual nestlings. In other species, however, sex-specific mortality appears to be related to relative food requirements, with lower survival rates of the larger sex often being attributed to the assumption that a large body mass requires comparatively greater resources to sustain it (but see Torres and Drummond 1999). Either way, parents may manipulate the sex ratio of their brood to achieve some optimal combination of sexes in order to maximise their fitness.

However, sex differences in life-history strategies are not necessarily restricted to sexually dimorphic species, yet by far the majority of studies testing sex allocation theory have been carried out on species in which the male is the larger sex. Very few studies have examined differential investment and relative survival in species with reversed sexual size dimorphism and fewer still have examined differential sex effects in a species exhibiting no detectable sexual size dimorphism or difference in growth rate (see Sheldon et al. 1998 for an exception).

In the final results chapter of this thesis I investigate pre-laying maternal investment according to progeny sex and its consequences for chick growth survival in the sexually monomorphic black guillemot. I also examine how such effects determine the possible facultative manipulation of progeny sex ratio in this species.

METHODS

The methods used in this study follow those of the general methods chapter, sections 1 to 4 inclusive, and section 7.

TERMINOLOGY AND ANALYSIS

Within two-egg clutches, each egg is referred to as a- or b-egg according to the order of laying. Correspondingly, chicks hatching from two-egg clutches are referred to as a- or b-chick. This was related to hatching order in asynchronous broods (a-chick hatches first in broods where the hatching interval is ≥ 1 day), and to the egg from which the sibling hatched in synchronous broods (a-chick hatches from a-egg).

Data from 1996 and 1997 were used for the analyses in this chapter, but no data from single-egg clutches or from two-egg clutches where only a single egg hatched were included in the analysis. Appropriate parametric statistics were applied where data met the parametric assumptions of normality and homoscedasticity. Where these assumptions were not met, I used equivalent non-parametric tests. Normality was tested for using the Kolmogorov-Smirnov goodness-of-fit test and all probabilities given are two-tailed.

Growth was investigated both in terms of the instantaneous growth rate during the period of maximum growth (age 5–25d). Relationships between growth rate and sex were performed for each chick type independently using t-tests.

The distribution of sex compositions for two-chick broods was compared with the binomial distribution using the log-likelihood goodness of fit test. To control for variation in the ratio of males to females, the expected binomial distribution should be calculated based on the empirically observed sex ratio, rather than the hypothetical 50 percent sons (Gowaty 1996). The above analyses were undertaken using SPSS for Windows release 7 and all tests were as described by Zar (1996).

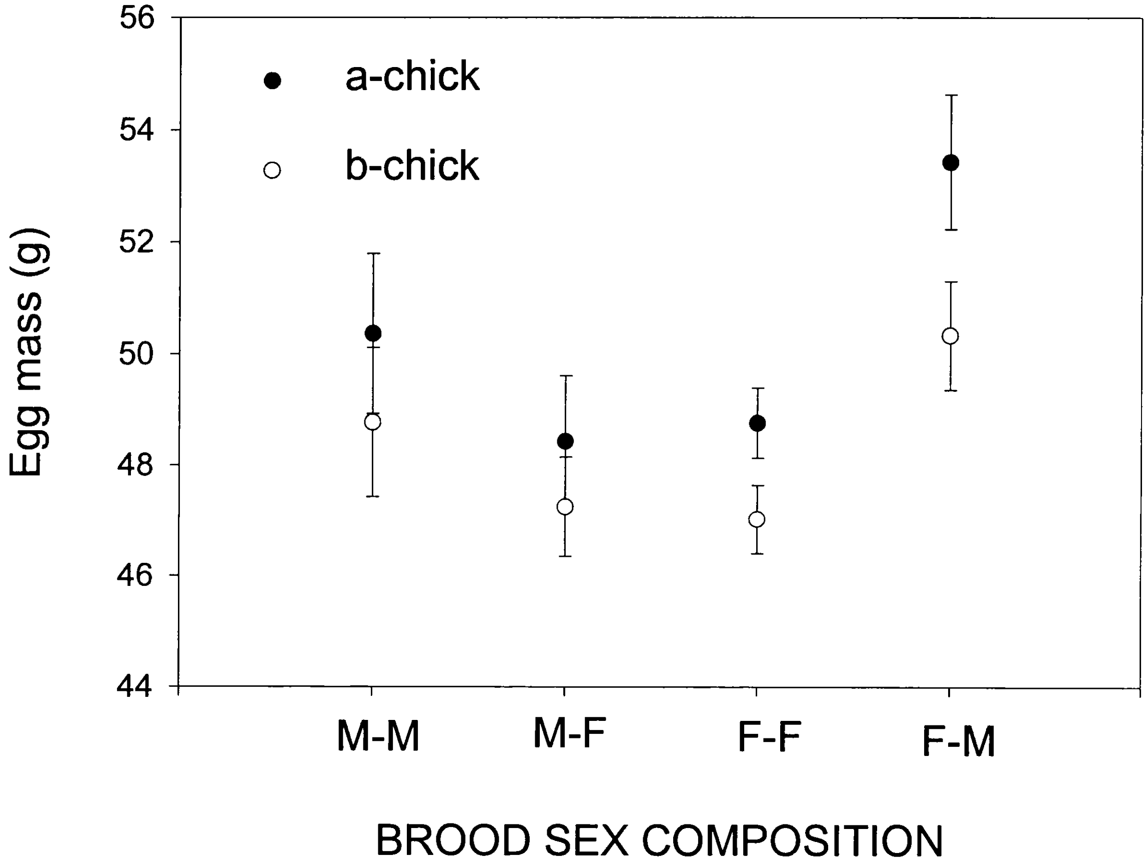
I analysed fledging success in relation to various parameters using Generalized Linear Models (GLIM, version 4, Royal Statistical Society, 1992) with binomial errors and logit-link function, starting with the highest order interaction. A nestling was categorised as "fledged" if it survived to 30d (by which age chicks have attained asymptotic mass and mortality by means other than predation was never evident). Survival was examined both in terms of the entire brood (number of chicks surviving to 30d per brood) and for individual chicks in relation to hatching sequence (proportion of a-chicks and b-chicks surviving to 30d). Explanatory variables tested in the models included: hatching asynchrony (synchronous or asynchronous), year (1996 and 1997) and brood sex composition, which encompassed both sex and hatching order of the two chicks (M-M, M-F, F-M and F-F). The statistical significance of chick survival in relation to explanatory variables was determined from the change in deviance (D) when that variable was excluded from the model (Crawley 1993). Where the ratio between explained deviance and d.f. were approximately 1, significance tests were based on the Chi-square distribution. However, where the data were overdispersed (i.e. the ratio did not approximate 1), significance was based on the F-ratio; the ratio of scaled deviance to degrees of freedom. Parameter estimates remain the same using this latter technique, but the standard errors are increased, resulting in a more conservative test (Crawley 1993).

RESULTS

Egg mass and sex

Egg mass varied significantly with laying order, with a-eggs being heavier than b-eggs, but males did not hatch from larger eggs overall than females (two-way ANOVA dependent variable fresh egg mass, effect laying order: $F_{1,109}$=4.94, p=0.028; effect sex: $F_{1,109}$=0.14, p=0.71; fig. 8.1). The interaction of these effects was also significant ($F_{1,112}$=4.84, p=0.03), suggesting a relationship between clutch mass and brood sex composition. Such a relationship was indeed evident (one-way ANOVA dependent variable total clutch mass, effect sex-composition: $F_{3,54}$=3.32, p=0.027), a post-hoc Tukey-test revealing that the sex-composition with the heaviest mean clutch-mass (F-M broods) was significantly heavier than the lightest (M-F broods; p=0.026, other comparisons n.s.). Thus the effect of laying order on egg mass appeared not to be the same for the different brood sex compositions. To further examine the relationship between maternal investment and embryo sex, I compared egg mass in eggs containing male and female embryos for both a- and b-eggs. Although no sex difference in egg mass was evident for a-eggs (male: mean mass =49.07g ±0.63 S.E.; female: mean mass =50.40g ±0.96 S.E.; t_{55}=-1.220, p=0.23), male b-egg mass appeared to be higher than that of the female b-egg; however, this difference was not quite significant at the 5% level (male: mean mass =49.05g ±0.89 S.E.; female: mean=47.20g ±0.51 S.E.; t_{54}=1.954, p=0.056). It is therefore conceivable that eggs producing sons were relatively constant in size, regardless of their position in the laying sequence, whereas female a-egg mass was maintained and comparable to male a-egg mass, but female b-eggs were reduced in size. Relative mass difference between first- and second-laid eggs (b-egg mass divided by a-egg mass) remained constant between brood sex compositions (one-way ANOVA dependant variable egg mass difference, effect brood sex-composition: $F_{3,54}$=1.064, p=0.372), suggesting that the general pattern of allocation of resources into a- and b-eggs did not vary among sex-compositions.

Figure 8.1. The relationship between egg mass and brood sex composition. Error bars represent ± S.E., n=55 broods (F-F=13, M-F=23, F-M=8, M-M=11).



Brood sex composition and nestling survival

From the 52 broods where I was certain of chick fate and hatching interval, 33 chicks died before 30d post-hatching: 27 of these through non-predation related mortality (or possibly inadequate brooding), and 6 through predation. Starvation related chick mortality occurred predominantly within the first week post-hatching (a-chick: mean age of death =3.67days ±2.25 s.d., n=6; b-chick: mean age of death 6.33 days ±0.96 s.d., n=21), whereas some predation occurred throughout the nestling period (both chicks combined: mean age 18.7 ±7.71 s.d., n=6).

The number of chicks fledging per brood was significantly related to brood sex-composition but not to hatching interval (synchronous or asynchronous) nor to year (table 8.1). The latter two variables were therefore excluded from subsequent models. Since maternal fitness is generally related to clutch mass, and consequently clutch quality, it seems intuitive to assume that the brood sex composition with the greatest average clutch mass should fledge the highest number of chicks per brood. Surprisingly, our evidence ran contrary to this prediction. Despite hatching from the heaviest clutches, F-M broods appeared to produce the lowest mean number of fledglings, the remaining three sex compositions fledging higher and approximately equal mean numbers of chicks (fig. 8.2).

Table 8.1. Results of Generalized Linear Model in which the number of chicks fledged per brood was examined in relation to brood sex composition, hatching interval and year.

	Deviance (Δ)	Df (Δ)	F	P
Null model	61.84	51		
Final model	55.68	48		
Hatch interval		1	0.048	Ns
Year		1	0.27	Ns
Brood sex composition		3	6.16	<0.005
Interactions				n.s.

Given that F-M broods appeared particularly prone to post-natal mortality, and that M-M, M-F and F-F broods fledged on average similar numbers of offspring (fig. 8.2), I compared F-M broods with the combined fledging data of the remaining three sex-composition types.

Since any effect of brood sex composition on individual chick fledging success was likely to differ with hatching order, I ran separate models for a- and b-chicks. Although no difference in survival between the two groups was evident for a-chicks (table 8.2), fledging success of b-chicks in F-M broods was significantly lower than that of the combined group (table 8.3).

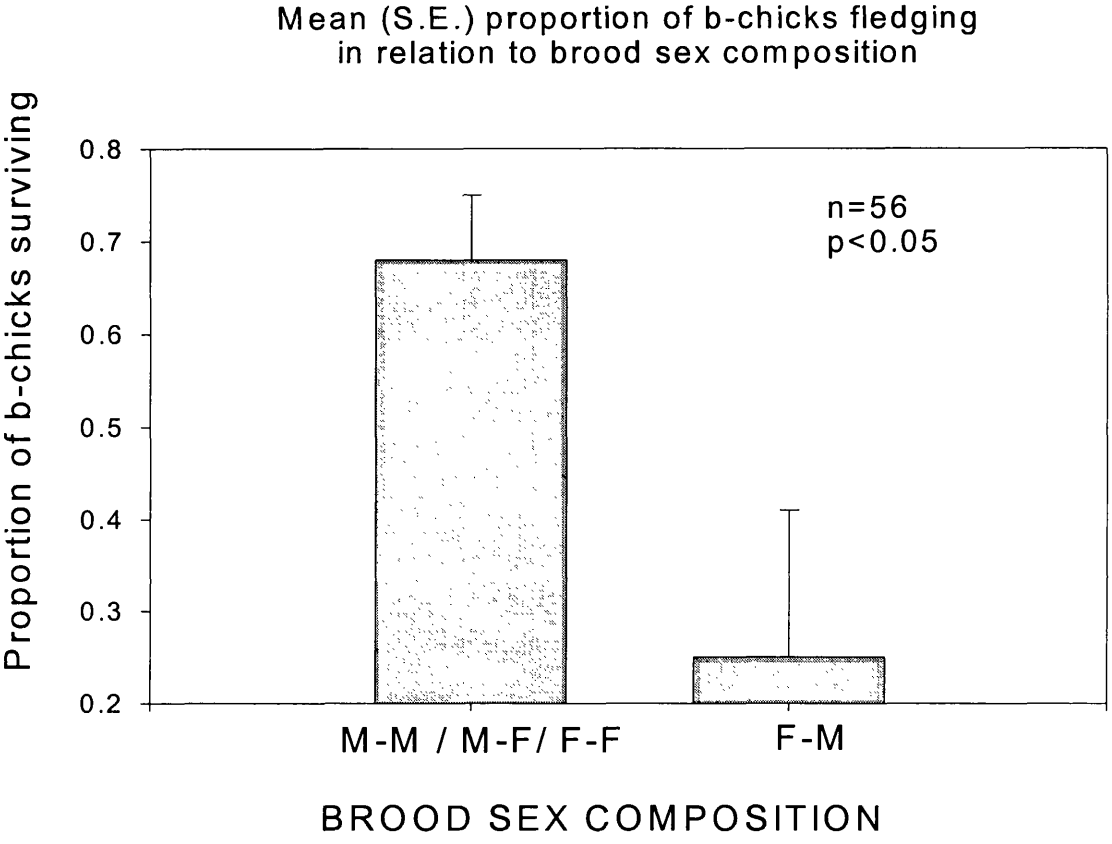
Table 8.2. Results of Generalized Linear Model in which the probability of the a-chick fledging was examined in relation to brood sex composition.

.	Deviance (Δ)	Df (Δ)	χ^2	P
Null model	44.65	51		
Final model	41.74	50		
Brood sex composition		1	2.91	Ns

Table 8.3. Results of Generalized Linear Model in which the probability of the b-chick fledging was examined in relation to brood sex composition.

	Deviance (Δ)	Df (Δ)	F	P
Null model	67.96	51		
Final model	62.81	50		
Brood sex composition		1	4.10	<0.05

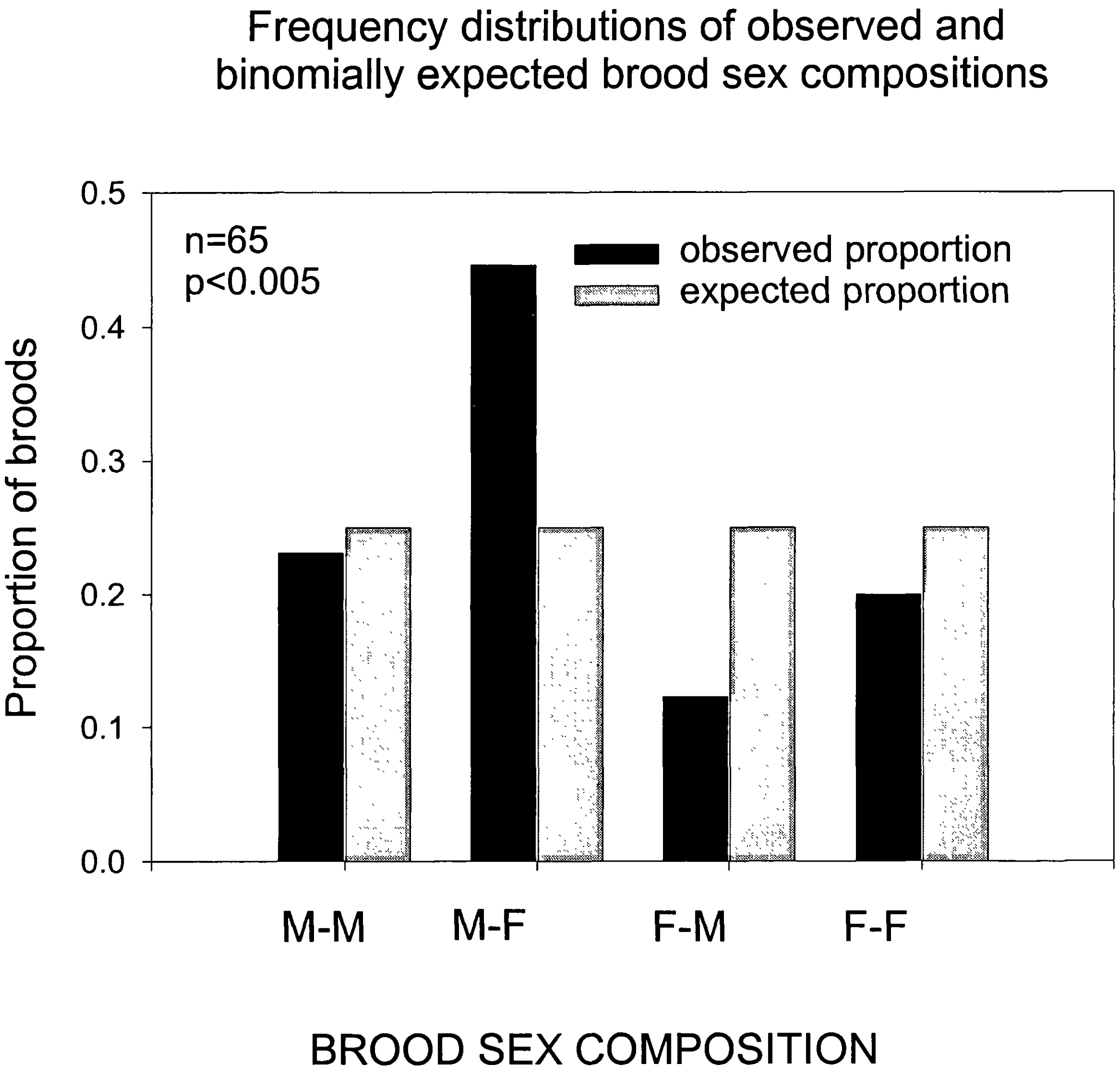
Figure 8.2 Mean (±S.E.) proportion of b-chicks fledging in relation to brood sex composition.



Sex composition at hatching

Given that certain brood sex-compositions are less successful than others, we predicted that female parents should attempt to maximise reproductive output by facultatively manipulating brood sex-composition. As stated earlier, to control for variation in the ratio of males to females, the expected binomial distribution should be calculated based on the empirically observed sex ratio, rather than the hypothetical 50 percent sons. When both years were combined, the observed frequency at hatching for this population was in fact 50 % sons (males: 34 in 1996, 31 in 1997, total = 65; females: 36 in 96, 29 in 97, total = 65). The distribution at hatching of M-M, mixed sex and F-F broods conformed to a binomial distribution (ratio: F-F 0.23, mixed 0.57, M-M 0.20; $G=1.36$, $n=65$, $df=2$, $p>0.5$). However, when mixed broods were segregated into M-F and F-M broods, brood sex composition at hatching differed significantly from the binomial (ratio: F-F 0.23, M-F 0.45, F-M 0.12 M-M 0.20; $G=14.1$, $n=65$, $df=3$, $p<0.005$), with M-F broods being significantly more numerous than F-M broods (binomial test: $p<0.05$ fig. 8.3). Thus, the sex composition at hatching with the poorest success was avoided.

Figure 8.3. Frequency distribution of observed and binomially expected brood sex compositions at hatching



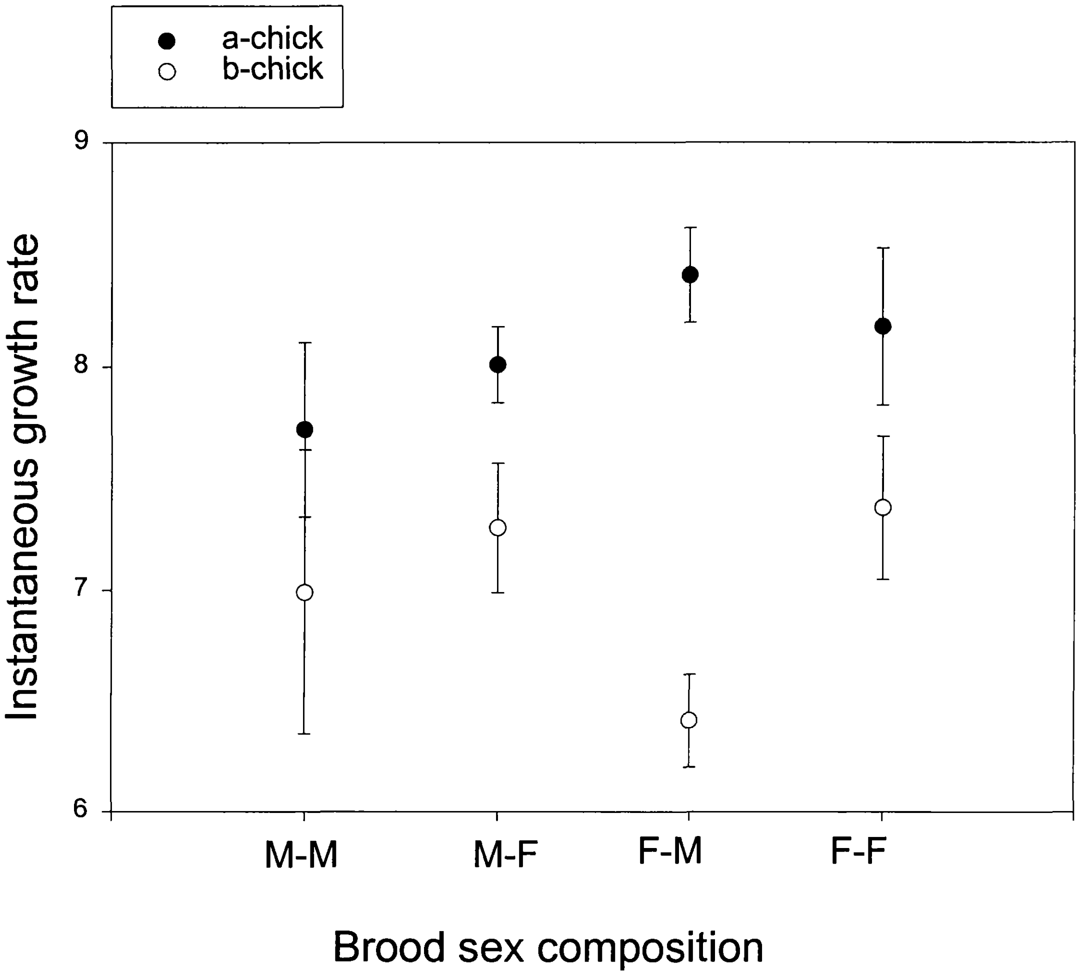
Sex composition at laying

To ascertain whether the observed sex-composition bias was evident pre-hatching and not, for example, a consequence of sex-specific embryo development rates, we reanalysed the data using brood sex-composition at laying. Although we could not identify embryo sex directly, egg sex and hence the pre-hatching sex ratio was established for those broods where the laying sequence and egg origin of each chick was known. As at hatching, sex composition at laying differed significantly from the binomial (observed sex ratio of 50.09% sons; n=58, df=3, G=14.02, $p<0.005$), with M-F broods predominating amongst mixed-sex broods (binomial test: $p<0.01$). Nonetheless, sex-composition bias might have occurred through differential embryo mortality (e.g. Clutton-Brock, 1986). Indeed, hatching failure during both years was relatively high; 23 of 53 clutches (43.4%) and 12 of 50 clutches (24%) respectively hatched only one or no eggs in 1996 and 1997. However, although we cannot statistically discount sex-specific embryo mortality as a source of bias, at least half of all egg failures were due to stochastic effects such as nest flooding or egg-predation rather than to embryo quality per se. It is therefore likely that the observed bias in M-F broods existed prior to egg laying and hence was a consequence of maternal facultative adjustment.

Chick growth

Mean standardised laying date (Julian) did not vary according to brood sex-composition (Kruskal-Wallis test: $\chi^2= 5.153$, d.f.=3, n=55, p=0.161). Hence, any seasonal effects on growth rate were unlikely to confound the results of this study.

Fig 8.4. The relationship between instantaneous growth rate and brood sex composition (mean ± S.E. g/d; n=55: F-F=13, M-F=23, F-M=8, M-M=11)



Although instantaneous growth rates of females were on average higher than those of males, the differences were not significant for either a-chicks (mean male growth rate =7.91g/d ±0.16 S.E., mean female growth rate =8.25g/d ±0.22 S.E.; t=-1.23, df=47, p=0.23; fig 8.4) or b-chicks (mean male growth rate=6.83g/d ±0.46 S.E., mean female=7.32g/d ±0.21 S.E.; t=-1.103, df=35, p=0.28; fig 8.4). However, since growth rates varied according to the level of hatching asynchrony (chapter 5), which in turn was related to brood sex composition (chapter 7), it is possible that any effects of sex on growth rate are confounded by hatching interval effects. Unfortunately, sample sizes were not large enough to statistically control for these effects.

DISCUSSION

Pre-laying maternal investment

Although no sex effect on egg mass was found for the pooled sample of black guillemot eggs, the significant effect of laying order, and the interaction between embryo sex and laying order on egg mass, suggests a linkage between egg mass, embryo sex and laying sequence. This association could have been manifest in one or more of the following ways.

Firstly, maternal investment in male and female black guillemot embryos may have varied according to laying order. Such pre-laying allocation of resources according to sex has rarely been reported in birds, generating scepticism that birds are capable of such manipulations (Charnov 1982, Clutton-Brock 1991). However, a recent study on the American kestrel *Falco sparverius* has demonstrated that, after controlling for laying order, eggs producing sons were heavier than eggs producing daughters (Anderson et al. 1999). A similar, although less marked pattern of pre-laying maternal investment was found with respect to the black guillemot, with maternal investment being on average 6% higher in male b-eggs than in female b-eggs. Although this relationship fell just short of statistical significance at the 5% level, the fact that b-chick mass in M-M and F-M broods was consistently higher than in both F-F and M-F broods infers that maternal investment was maintained in male but reduced in female b-eggs (fig. 8.1). No preferential direction of resources according to offspring sex was found for a-eggs, suggesting that any differential fitness benefits of producing sons and daughters were restricted to second-laid eggs.

A second possible manifestation of the interaction between laying order and sex on egg mass was differential egg size disparity according to brood sex composition. Since egg mass was the primary determinant of hatching mass in this species (chapter 4), within-clutch egg size disparity could potentially affect brood competitive dynamics (chapter 6). Accordingly, any variation in egg size disparity associated with brood sex composition could effectively play an important role in brood competitive interactions. Given that brood sex composition was associated with hatching interval in this species (chapter 7), it is conceivable that brood sex composition is a parental strategy either to mitigate or to enlarge the size and age-related disadvantages imposed by the hatching pattern. However, the degree of within brood egg mass disparity, and hence the relative allocation of resources according to laying order, remained similar between brood sex compositions. Thus, independent of any effects of hatching asynchrony, no difference in the degree of within-brood hatching size disparity was likely to be evident between the different sex compositions. Of course, I cannot discount the possibility that maternally derived factors in addition to size disparity may have affected competitive ability.

A final possible effect of the interaction between sex and laying order was a difference in total clutch mass with respect to brood sex composition. This was indeed found to be the case, with F-M broods being significantly heavier than the lightest brood sex composition, M-F broods.

Although the relationship between egg mass, laying sequence and gender in the black guillemot is not yet fully understood, the results of this study nonetheless suggest that females are capable of differentially allocating resources according to progeny sex and brood sex composition. It is possible that factors in addition to those examined in this study may play a proximate role in the female's decision to differentially allocate resources among her offspring and more studies are required to determine such factors.

Chick survival and growth

Sex differences in nestling mortality have been well documented in avian species, with the majority of studies demonstrating that mortality is greater among males than among females, particularly during periods of poor environmental condition (e.g. Howe 1977, Roskaft and Slagsvold 1985, Teather and Weatherhead 1989, Griffiths 1992). Two main explanations have been proposed to account for this observation in birds. First, sexual size dimorphism: in species where the male is the larger sex, males may require a greater amount of nutrients then females to attain the same viability. Second, higher male mortality may be a consequence of the effects of the male phenotype; for example, development of the male reproductive system requires the production of androgens that may also have deleterious effects on other aspects of physiology such as cell-mediated immunity (e.g. Zuk 1990). Since most studies have examined differential mortality rates with respect to a key assumption of Fisher's (1930) sex ratio theory (i.e. that offspring of the larger sex are more expensive to rear to independence than that of the smaller sex), questions regarding sex-related differences in offspring mortality and growth have largely been addressed using size-dimorphic species in which the male is larger than the female (but see Sheldon et al. 1998). Such differences have been examined both in terms of gender differences in food needs and competitive ability. For example, Roskaft and Slagsvold (1985) showed that starvation related mortality was higher in experimentally enlarged all-male broods of rooks *Corvus monedula* than in enlarged broods of smaller females. Conversely, in the red-winged blackbird *Agelaius phoeniceus*, larger male nestlings use their height advantage to sequester a greater proportion of parentally delivered food than their smaller sisters (Teather 1992).

In the current study, the number of black guillemot chicks fledging per nest did not vary between broods that hatched synchronously or asynchronously, nor did it vary according to year. However, brood sex composition had a significant effect on brood fledging success: a-chick survival rates were relatively similar across brood sex compositions, but the probability of male b-chicks in F-M broods surviving to 30 days post-hatching was considerably lower than in other brood types. Thus survival probability depended not only on the sex of the individual and its position in the laying sequence, but also on the sex of the sibling. Given the relatively fast development rates of male embryos (chapter 7), it is conceivable that sex-related differences in metabolic activity, which in turn might have been a result of increased androgen levels, may explain the higher male mortality rate. However, this does not account for the fact that male survival was markedly reduced only in broods where the older sibling was female, and it is therefore possible that males and females were not equally affected by competitive interactions with a sibling of the opposite sex. Thus, if any differences in male susceptibility were evident, they were likely to be compounded by the presence of an older sister. It is therefore likely that a combination of these factors contributed to the observed pattern in mortality. Since the black guillemot is sexually monomorphic and no obvious difference in growth rate was found with respect to gender, it is improbable that differential mortality was dependent on sex-related size effects.

Despite this reduced survival rate of male b-chicks in F-M broods, I found no evidence of a difference in mass growth rate between male and female b-chicks. However, given that male growth data collected from F-M broods were extremely limited due to high mortality rates, any sex related differences in growth rate as a result of reduced male quality in F-M broods would not have been evident. Effectively, therefore, b-chick growth analysis in this chapter primarily compared female b-chick growth in M-F and F-F broods to male b-chick growth in M-M broods. However, the fact that no difference was evident between b-chick males and females in these brood types, nor between sexes in a-chicks, suggest that mass growth pattern in male and female black guillemot chicks were not inherently different.

Ultimately, it appears that the cost of rearing offspring in this monomorphic species is likely to be affected by the sex composition of the brood, both in terms of relative pre-laying maternal investment in the clutch, and with regard to the survival probability of respective brood members. However, the fact that reduced survival rates were associated with greater maternal investment in terms of increased egg mass is somewhat perplexing.

Sex ratio adjustment

Until the mid 1990's, facultative maternal adjustment of the sex ratio in birds was considered rare (Clutton-Brock 1991). Nevertheless, the development of new molecular techniques for rapidly ascertaining avian sex (Griffiths and Tiwari 1995, Griffiths et al. 1996, Ellegren and Sheldon 1997) are now bringing new and compelling examples to light, demanding a new assessment of both the adaptive nature and the mechanisms behind sex ratio adjustment (Heinsohn et al. 1997). In the black guillemot, I demonstrate that although the overall sex ratio at both laying and hatching was not significantly different from parity, females responded to the decline in survival prospects of male offspring in F-M broods by dramatically skewing the frequency of mixed sexed broods. The result was a marked paucity of F-M broods which was almost exactly offset by an increase in M-F brood. The dyadic combinations of single sex broods (M-M and F-F broods), on the other hand, were present at their expected frequencies. This sex related hatching sequence is likely to be adaptive since it avoided the brood sex composition in which mortality was most likely to occur, thereby providing the highest fitness return for the mother.

Regardless of the ultimate function of these patterns, the data presented in this chapter demonstrates that parental manipulation of the sex ratio and hatching spread (and possible egg compositional effects) has significant effects on avian offspring. The interactions between these variables are complex, however, and further experimental manipulations are required to determine their relative importance for chick fitness.

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CHAPTER 9

GENERAL DISCUSSION

In this thesis, my primary focus has been to examine intraspecific variation in the reproductive strategy of the black guillemot in terms of consequences for offspring fitness. Since the core of the project is largely correlational in approach, the adaptive significance of such variation has generally been inferred rather than demonstrated. Such an approach was not ordained through design but instead was a consequence of the considerable logistical problems of working on a seabird species breeding on a remote, uninhabited island. Moreover, even within the limitations of this correlational approach, many more hypotheses could have been tested, given a larger colony and thus larger sample sizes. As it stands, however, this study has provided an interesting insight into the behavioural ecology of this species. While some aspects of black guillemot breeding ecology were as expected, and in accord with current theoretical and empirical studies (e.g. reduced aggression rates in asynchronous compared to synchronous broods, chapter 6), other results clearly were not anticipated (e.g. sex-related development rates, chapter 7), and hopefully such results will provide important new avenues of research into complex and unresolved aspects of reproductive ecology, such as the phenomenon of hatching asynchrony. In order to understand further the adaptive consequences of the diversity of reproductive strategies employed by the black guillemot, I advocate the use of an experimental approach; more specifically, studies of fitness trade-offs should be coupled with studies examining the proximate mechanisms effecting sibling competitive disparities. In the general discussion, I consider some of the areas of research that might provide important developments to our understanding of the significance of the variation observed in this species, and hence to our overall understanding of behavioural ecology.

Consequences for surviving siblings (both short- and long-term effects)

Although many studies have examined the consequences of differential parental investment in offspring until fledging, the longer-term effects on fitness are difficult to ascertain and thus less understood. In nests where brood reduction occurs, the immediate effect is a reduction in the number of nestlings for the parents to feed, and potentially more available food for the remaining siblings, either of which have the potential to provide long-term fitness benefits. Theoretical support for such benefits has been provided both for parents (Konarzewski 1993, Mock and Forbes 1994), and for surviving siblings (Temme and Charnov 1987, Pijanowski 1992). For example, it has been demonstrated that the parental strategy of inducing a competitive hierarchy within the brood (through hatching asynchrony), and thus promoting brood reduction, can be selected for even when 'poor' breeding seasons occur infrequently (Pijanowski 1992). With respect to empirical studies, a clear relationship has been demonstrated between fledging mass and recruitment into the breeding population (Perrins 1965, Magrath 1990, 1991, Newton and Moss 1986), but to date, few have directly examined the consequences of brood reduction in overburden broods on the survival probability of the respective family members (Husby 1996).

Brood reduction is also likely to have short-term effects. O'Connor (1978) contended that brood reduction is likely to effect the fitness of remaining family members in different ways, some of which may incur costs. Indeed, the death of a sibling may affect the thermal properties of the brood, change the risk and probability of predation and alter parental effort and attendance in ways that are not immediately apparent. Indeed, if the dominant sibling is sequestering a disproportionately large share of parental resources prior to brood reduction (i.e a share of the doomed sibling's food), and parents adjust brood size in a precise 1:1 manner, then the surviving sibling will ultimately receive a reduced supply after brood reduction because it will no longer receive its sibling's share (Mock and Lamey 1991). Alternatively, if brood reduction does not induce a reduction in parental effort, the remaining siblings receive a greater share of resources (e.g. Graves et al. 1984). Chick removal studies will provide an insight into how parents respond to a reduction in brood size and the potential costs and benefits incurred by the surviving sibling.

Costs and benefits of hatching asynchrony

Variation in hatching spread in black guillemot broods suggests that the costs and benefits associated with a particular degree of hatching asynchrony vary between individuals and the optimal level of hatching asynchrony depends on multiple factors. This may be due to factors associated with maternal quality, age or factors associated with mate quality. Several other studies have proposed that optimal breeding strategies may vary between individuals. For example, with respect to hatching asynchrony, Wiebe and Bortolotti (1994b) have shown that females alter the degree of hatching asynchrony according to male provisioning rates at the time of egg laying. However, the only study which has specifically tested whether individual optimisation explained the variation in hatching asynchrony observed within a population, found that individual house wrens *Troglodytes aedon* did no better or worse when hatching asynchrony was experimentally altered (Harper et al. 1994). Black guillemot hatching asynchrony, and thereby within-clutch size hierarchy, showed no relationship with laying date or year implying that parents did not alter the degree of size disparity according to environmental condition. However, the variability in hatching pattern in black guillemot broods showed that the onset of incubation varied considerably between individual pairs. Further experimental manipulations of hatching asynchrony in black guillemots would be useful to determine the relative costs and benefits of hatching asynchrony in this species.

Moreover, when experimental studies were first conducted on the adaptive significance of hatching asynchrony, authors did not anticipate a parental provisioning response to experimentally synchronised broods (see Amundsen and Slagsvold (1991). However, all recent studies simultaneously examining parental feeding effort provide evidence that parents dramatically increase the quantity of food delivered to synchronous broods (Fujioka 1995a, Hebert and Barclay 1986, Mock and Ploger 1987, Gibbons 1987, Osorno and Drummond 1992, Hebert and Sealy 1994, Wiebe and Bortolotti 1994b, Machmer and Ydenberg 1999). Although, I attempted to gain provisioning data in this study, the data was not sufficient enough in both quality or quantity to warrant statistical analysis. Further studies examining parental provisioning between brood types would therefore be informative, especially those coupled with doubly-labelled water techniques, thereby simultaneously investigating potential differences in chick energetic expenditure.

Sex ratio

Sex-ratio trends within clutches have been reported for many species (Howe 1976, Ryder 1983, Olsen and Cockburn 1991). However, only a limited number of studies have examined the interaction between offspring gender, hatching order and sibling competition, and with the exception of (Sheldon 1998) all such studies have been conducted on sexually dimorphic species. Although it was Lack (1954) who first suggested that gender differences in growth rate might affect brood competitive dynamics, Edwards and Collopy (1983) were the first to consider how certain gender/rank combinations might affect sibling aggression and brood success. Basing their ideas on sexually dimorphic raptor species, these authors suggested that if the sibling of the faster growing sex hatched second, then the dominance of the slower growing first hatched chick may be usurped shortly after hatching. Depending on the cost-benefit relationship of the resulting competitive interactions, the slow growing first hatched sibling may be under strong selective pressure to kill its smaller sibling as soon as possible, lest its own fitness be reduced. Such reversals in sibling size may therefore entail increased sibling rivalry costs for the parents in comparison to other brood sex compositions. However, Edwards and Collopy (1983) also posited that hatching the sexes in the reverse order (i.e. hatching the sibling with the fastest growth first) might induce premature brood reduction and thus could also be maladaptive. Recent studies examining these ideas have produced very mixed results (e.g. Bortolotti 1996b, Edwards et al. 1988, Bednarz and Hayden 1991, Drummomd et al 1991). For example, in contrast to the current study, Bortolotti (1986b) reported a significant paucity of M-F broods in the bald eagle *Haliaetus leucocepalus*, whereas all other sex compositions were found at their expected frequency. Adult male bald eagles are about 25% smaller than females, yet males grow considerably faster than females during the immediate post-hatching period. Males in M-F broods are thus considerably larger than their younger sisters for the first few weeks. It was therefore suggested that a reduction in the frequency of M-F broods was an adaptive parental manipulation for avoiding the sex composition with accentuated sibling rivalry. In the golden eagle, on the other hand, it is the male in F-M broods that are more prone to brood reduction. However, growth patterns in this species take a different form to that of the bald eagle (Edwards et al. 1988). The most detailed study of brood gender combination is that on the facultatively aggressive blue footed booby by Drummond et al. (1991), a species in which adult females are considerably heavier than males, and younger sisters in M-F broods do overtake their brothers despite a 4-day hatching interval. In spite of the size reversals in these broods, no corresponding dominance reversal was evident, and gender appeared to play no role in aggressive interactions. The continual domination of smaller males in M-F broods may persist because the benefits of aggressive dominance for the younger sister may not outweigh the associated costs. This in turn may be a function of fighting qualities other than size that improve with maturation and are reinforced by early social experience (Drummond and Osorno 1992). Slagsvold (1990) suggests that the degree of hatching asynchrony in these species may have evolved to prevent such reversals taking place. Indeed the association between hatching asynchrony brood sex composition and chick survival probability in the current study suggests that this may also be the case for the black guillemot. Here, however, any differences in competitive ability are likely to be a result of factors independent of relative sibling size. Empirical research is required to ascertain the relative competitive abilities of males and female siblings, and to examine further the interplay of hatching interval, brood sex composition and survival probability in this species.

Egg additives and compositional effects

Studies on differential maternal investment have concentrated mainly on the temporal effects of hatching asynchrony (see Stoleson and Beissinger 1995), but much less is known about alternative factors that could translate maternal condition into phenotypic differences among her offspring. A recurring question in this thesis has concerned the potential role of key egg constituents (e.g. maternally derived hormones) on traits as diverse as egg development rates, propensity for aggression and probability of fledging. There is an increasing amount of evidence that females invest variable concentrations of testosterone into eggs of the same clutch (Schwabl 1997), thereby affecting the fitness value of, and relationships between siblings (Winkler 1993). While there is no documentation of the physiological fate of maternal hormones in the embryo, the potential fitness costs and benefits of testosterone are well documented in nestling birds (Schwabl et al. 1997). As well as its role in the differentiation of the sexes (Balthazardt and Ball 1995), testosterone has been shown to increase development of both the musculature and neural system, increase aggressiveness and begging vigour, and ultimately increase competitive ability and social ranking (Norris 1985, Hauser and Torand-Allerand 1989, Schwabl 1993, 1996). Associated with these advantages, however, is an increase in resource requirement, such that during less favourable conditions testosterone increases mortality risk (Clutton-Brock et al. 1985). Testosterone has also been shown to have a negative effect on nestling cell-mediated immunity and thus is likely to increase susceptibility to infection (Grossman 1985, Folstad and Karter 1992). However, it is not yet known whether these effects on offspring phenotype have an adaptive significance under field conditions. With respect to adult females, it has recently come to light that mothers can allocate testosterone concentrations according to laying order: one species has been shown to deposit higher titres in the yolk of last-laid eggs, whilst in another species it is the first-laid egg which receives the lions share (Schwabl 1993, 1996, Schwabl et al. 1997). It has also been suggested that increased circulatory levels of testosterone in the female incur similar costs to those of nestlings Hogstad 1987, Ketterson et al. 1996), and circumstantial evidence suggests that the resulting levels deposited in the yolk may depend on maternal condition (R. Nager pers. com.).

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