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On sex-ratio and sibling competition:
an insight into reproductive decisions
in the black-legged kittiwake (*Rissa tridactyla*)

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« Il n'est pas fou celui qui perd ce qu'il ne peut garder pour gagner ce qu'il ne peut pas perdre »

« He is no fool who gives what he cannot keep to gain that which he cannot lose »

Jim Elliott

Avant propos

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Une petite histoire de mouettes ...

Le mâle REO/BS (R = rouge; E = vert clair; O = orange; B = bleu; S = métal) est né en 2003 sur le nid I10 de la tour expérimental de Middleton Island. Comme tous les poussins qui se sont envolés cette année là, il a été bagué avec une bague rouge et une bague métal avec un numéro unique. Il a été recapturé en 2011 sur le nid T12 (non loin du nid I10 où il est né !) et bagué avec la combinaison qu'il possède sur la photo. En 2013, il s'est reproduit avec la femelle YS/GRR (qui elle par contre n'est pas né sur un nid qui a été suivi au cours des années, on ne sait donc pas d'où elle vient) et ils ont élevés deux poussins qui se sont envolés en fin de saison. Peut-être le début d'une longue histoire entre ces deux oiseaux ... En effet, après une première reproduction réussie, il est commun de revoir les deux mêmes parents l'année suivante (et les suivantes) ensemble à nouveau. Des belles histoires comme celle-ci, il y en a plein à Middleton ...



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Introduction

What is a ‘decision’ in behavioural ecology?

Throughout their life animals face decisions that can influence their fitness. In behavioural ecology, the term decision does not necessarily refer to a complex cognitive process but rather describes the fact that an animal is regularly exposed to several alternatives differing in their consequences in terms of fitness (Danchin et al. 2008). For instance, in spatially heterogeneous environments habitat choice likely impacts access to resources such as a safe reproduction site, food or mates for example. Individuals able to choose the habitat that maximises their fitness (survival and reproductive success) should then be favoured by natural selection. It is important to notice that the characteristics (biotic and abiotic) of the chosen habitat may not maximise fitness in every environment. Hence, individuals able to take the decision maximising fitness according to current conditions (i.e. ‘plastic’ individuals) should be advantaged over individuals unable to adapt their decision to changes in conditions.

Focus on some reproductive decisions

In my thesis, I chose to focus on a few reproductive decisions and I tried to test whether individuals are able to choose¹ according to the current conditions. First, I investigated whether individuals adjust the sex of their offspring in order to maximise their own fitness or that of their offspring (Chapter 1A, p. 41 and 1B, p. 65). Second, I studied two decisions potentially influencing sibling competition patterns: the onset of incubation, which affects

¹ Again, the term ‘choice’ is meant here, and throughout this thesis, in the evolutionary sense, i.e. changes in traits (behavioural, life history or other traits) that maximise fitness in the current conditions.

hatching asynchrony (Chapter 2A, p. 97 and 2B, p. 123) and egg yolk composition, which I studied indirectly by manipulating food availability (Chapter 3, p. 145).

In this three-part introduction, I give an overview of what is already known about these reproductive decisions. At the end of each part I give details concerning the study species by focusing on the characteristics making it interesting in regard to the concerned decision.

Throughout the introduction and in the general discussion I will use the personal form, as these present my personal perspective, although this work is obviously not only mine and all the ideas presented in this thesis emerged from discussions with many people and especially with my two PhD supervisors.

Study species: the black-legged kittiwake (*Rissa tridactyla*)

Black-legged kittiwakes (*Rissa tridactyla*) are small pelagic gulls (Laridae) (Photo 1A-B-C), with a circumpolar distribution in the northern hemisphere. They have been widely studied in different part of their range (from Alaska to Norway and France), which is explained by their ubiquity, the fact that they breed in colonies and their ease of observation (in their natural sea cliff habitat or on buildings) (more details in Hatch et al. 2009). Kittiwakes may breed from the age of three years but first breeding usually occurs later (Wooller & Coulson 1977). They are long lived and can live over twenty years in the Pacific part of their range. Parents almost equally share tasks such as nest construction (Cullen 1957), incubation (Coulson & Wooller 1984) and chick rearing (Roberts & Hatch 1993). The modal clutch size is two (Photo 1C) and chicks hatch after twenty-seven days of incubation on average (Hatch et al. 2009). Chicks are semi-precocial: mobile with open eyes and body covered with down (Starck & Ricklefs 1998) (Photo 1D-H). They usually fledge after forty days (Hatch et al. 2009) (Photo 1I) and spend almost all their time at sea until they first come back to breed.



Photo 1. **A:** An adult kittiwake flying. **B:** Close-up view of an adult. **C:** Adults and their eggs. **D:** A newly hatched chick. **F:** Two chicks a few days old. **E:** A parent and its two chicks. **G:** A young chick being fed. **H:** A chick begging for food. **I:** A fledgling in flight.

Field site: Middleton Island, Alaska, USA

All the work presented in this thesis has been conducted on Middleton Island, Alaska, USA (59° 26'N, 146° 20'W) (Photo 2A-B). This island is an abandoned US Air Force base dating from the cold war, where black-legged kittiwakes (*Rissa tridactyla*) come to breed in spring and summer. As their natural habitat (i.e. cliffs) deteriorated, kittiwakes began to breed on the abandoned buildings and especially on an abandoned radar tower (Photo 2C). This tower is a twelve-wall polygon where the walls have been converted into artificial cliffs by Scott A. Hatch in the 1990s (Photo 2D). There are more than three hundred breeding sites occupied by kittiwakes. One-way window glasses allow us to observe parental and chick behaviour from inside the building (~20 cm) (Photo 2E; for more details see Gill & Hatch 2002) and also to capture eggs and chicks repeatedly throughout the season. A small gap below the window allows us to capture the adults with a hook and then we bring them inside through the window. For some nest sites, there is also a small tray near the window that slides through the wall and allow us to experimentally feed the birds (Photo 2F; for more details see Gill & Hatch 2002). All breeders are individually marked by a five-ring combination (four colour rings and one US metal ring) and fledglings are also ringed with one metal ring and one colour ring (the colour changes for each cohort).

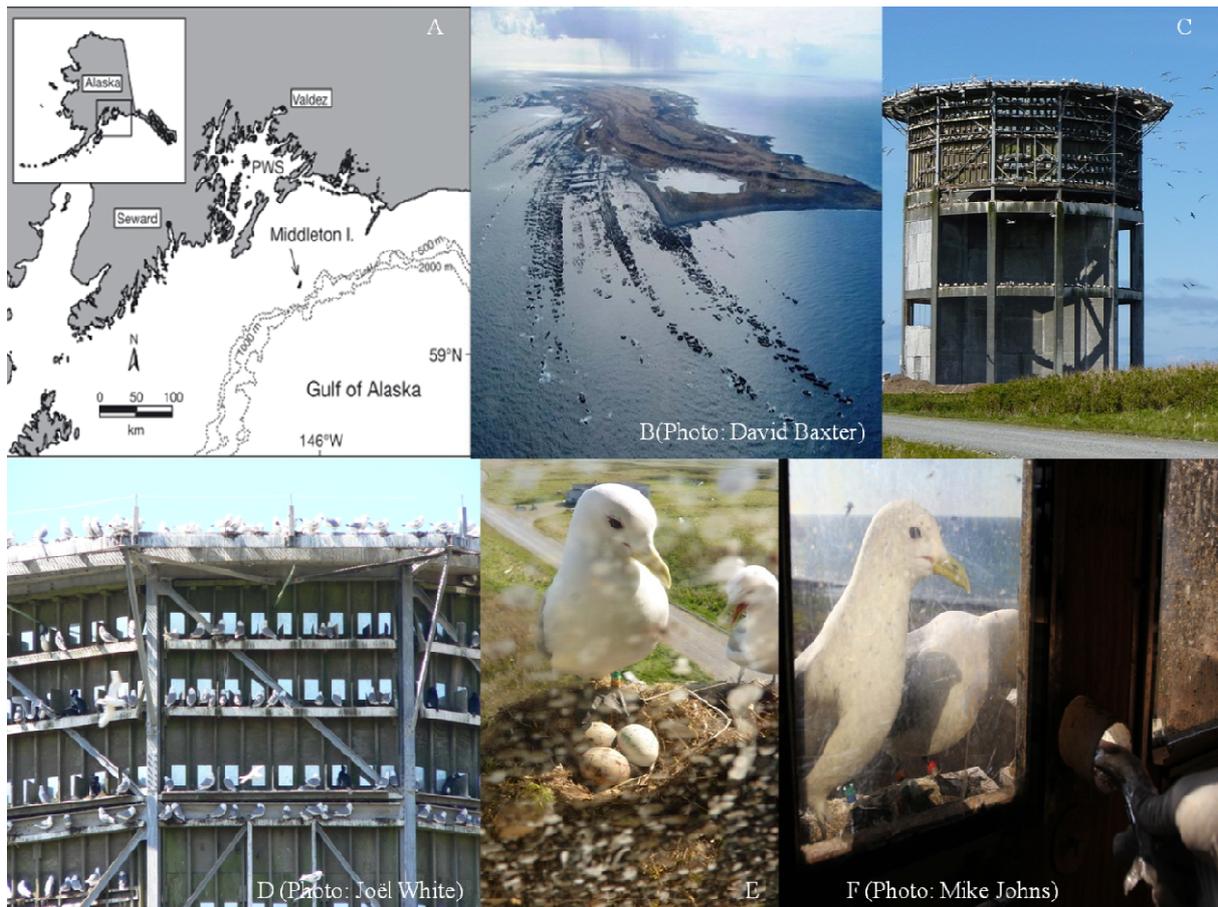


Photo 2: **A:** Location of Middleton Island in the Gulf of Alaska. **B:** An aerial view of Middleton Island. **B:** The abandoned radar tower renovated as artificial cliffs for breeding kittiwakes. **C:** Closer view from the artificial breeding sites. **D:** Kittiwakes can be observed from within the tower through one-way mirror glasses. **E:** Supplemental food is given on a tray that slides through the window.

Part 1: Sex allocation theory: which sex to invest in?

“... I now see that the whole problem is so intricate that it is safer to leave its solution for the future” (Darwin 1874). The problem Darwin referred to was sex allocation. Almost 150 years later, sex allocation theory (Charnov 1982; Frank 1990), and in particular sex ratio variation, is one of the most influential, yet controversial, theory in evolutionary biology. It refers to the way parents invest in male and female functions, with an expected bias toward offspring of the sex providing the higher fitness return under current conditions (Charnov 1982; Frank 1990). Although selection is theoretically expected to act on sex allocation (i.e. parental investment according to sex), most of the studies have assumed that sex-ratio (i.e. the proportion of males) should reflect sex allocation (Sheldon 1998), because sex allocation is usually harder to measure (but see Hewison et al. 2005). Here, I will mostly refer to sex-ratio rather than to sex allocation. Trivers and Willard (1973) were among the first to propose that a sex-ratio bias might be adaptive in certain conditions. They predicted that, in polygynous species such as most ungulates, if maternal condition is correlated to offspring condition and if differences in offspring condition endure until adulthood, mothers in good condition should be selected to bias their sex-ratio towards sons, as they would benefit more from being in good condition when adults as compared to daughters (but see Leimar 1996). Despite strong evidence of adaptive sex-ratio adjustment in almost all taxa (reviewed in West 2009), findings are more consistent among insects, and hymenoptera especially (reviewed in Godfray 1994), than among birds and mammals for instance (reviewed in Cockburn et al. 2002). This was first explained by the difference of control on sex determination between these taxa: haplodiploidy for hymenoptera and chromosomal sex determination for birds and mammals (Maynard-Smith 1978; Williams 1979). However, West and Sheldon (2002) showed that

when the fitness benefits of sex-ratio adjustment are high and easily predictable, adaptive shift in sex-ratios are observed regardless of the mechanism of sex determination.

Striking results among parasitic hymenoptera

All hymenoptera species are haplodiploid and females have full control over sex determination. They store sperm in a spermatheca and during laying, they can either release stored sperm to fertilise the egg or not. Sex is determined by the number of chromosomes: a fertilised egg will give a diploid female, whereas an unfertilised egg will give a haploid male. Among hymenoptera, solitary parasitic wasp species have been widely studied in relation to sex ratio variation. Females lay a single egg in an insect larva in which it will hatch and the wasp larvae will feed from inside the host. Size at emergence is strongly correlated to host size and females should benefit more from a larger size in terms of fitness as larger females are able to lay more eggs, whereas male size does not influence fitness (Charnov et al. 1981; Godfray 1994; Figure 1). Hence, females are expected to bias offspring sex in relation to host size by laying female eggs in large larvae and male eggs in small larvae. This prediction has been supported in many parasitic wasp species (reviewed in Godfray 1994; Figure 1). Interestingly, the precision of sex-ratio adjustment to host size is higher in species killing the host in which they lay an egg (idiobionts) than in species which do not kill the host (koinobionts) (West & Sheldon 2002). This result suggests that sex-ratio adjustment depends on the predictability of the fitness benefits: in koinobiont species, the host can still grow after the wasp has laid, hence it is more difficult for the mother to predict the quantity of resources which will be available to her offspring.

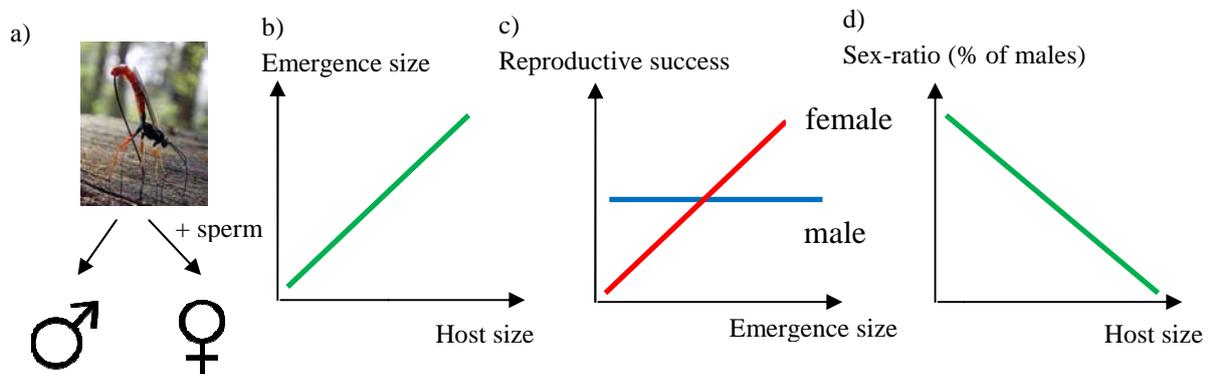


Figure 1. Parasitic wasp can adaptively adjust offspring sex ratio to host size. a) Females come from fertilised eggs, and males from unfertilised eggs; b) Relationship between host size and emergence size; c) Relationship between emergence size and reproductive success in relation to offspring sex.; d) Sex-ratio (proportion of males) according to host size.

Less consistent results in birds and mammals

A potentially costly mechanism?

While parasitic wasps show precise control over sex determination, meiosis is supposed to be random and should thus lead to a mean sex-ratio of 0.5 in birds and mammals. This first led people to think that the observed sex-ratio bias were just due to sampling error or publication bias (Williams 1979; Clutton-Brock 1986; FestaBianchet 1996) or had a purely physiological rather than adaptive basis (Krackow 1995). It is obvious now that birds and mammals are able to overcome sex determination constraint to adaptively adjust sex ratio according to the different selection pressures (e.g., Gomendio et al. 1990; Komdeur et al. 1997; Komdeur 1998; Badyaev et al. 2002; Pryke & Griffith 2009a; Thogerson et al. 2013). Nevertheless, the underlying mechanism remains unknown (reviewed in Pike & Petrie 2003; Alonso-Alvarez 2006). As in mammals the male is the heterogametic sex (XY) and in birds it is the female (ZW), it is likely that the mechanism will differ between these two groups. Moreover, there is no reason that the mechanism should be unique within a group given the wide range of species and life-histories in which adaptive sex-ratio adjustment has been demonstrated (Pike & Petrie 2003). Knowing the mechanism is of importance because, depending on the timing

of the adjustment, it might be costly in time and/or resources and this cost might balance the benefit of adjusting sex-ratio or not (Alonso-Alvarez 2006). For example, if female birds could reabsorb an ovum containing an unwanted sex-chromosome and reovulate until the wanted sex-chromosome is produced, it would delay laying and potentially increase predation risk and/or hatching asynchrony (Emlen 1997). Moreover, as classical sex allocation theory predicts that sex-ratio adjustment should be cost-free (Maynard-Smith 1980), the confirmation of a cost could change the predictions (but see Komdeur et al. 2002 for an example with no apparent cost of sex-ratio adjustment). Investigations on the mechanisms of sex-ratio adjustment have mostly been done on bird species and the proposed hypotheses range over every stage of the egg production (reviewed in Pike & Petrie 2003; Alonso-Alvarez 2006). There is also increasing evidence for a role of maternal hormones at the time of laying on chick sex (e.g., Veiga et al. 2004; Correa et al. 2005; Bonier et al. 2007), but their exact role remains unclear. In relation to these findings, the most recent hypothesis to date in birds states that during the first meiotic division the segregation of the polar body could be blocked due to hormonal effects, which in turn would lead to a chimeric embryo with cells of both sexes (Tagirov & Rutkowska 2013). Then the hormonal environment would cause one cellular type to decline and determine the sex of the egg (Tagirov & Rutkowska 2013). Interestingly, this could also potentially allow spermatozooids to specifically fertilise a cell with a given sex chromosome: contrary to common thought, males (who have ZZ sex chromosomes) may also influence sex determination. However, despite more effort put into seeking the proximate mechanism(s) in the last decade, we are still lacking a mechanism and we can therefore not exclude potential fitness costs in sex-ratio adjustment among birds and mammals. Hence, as long as this question remains unsolved, we might expect sex-ratio adjustment to occur only when the fitness benefits are high and thus probably higher than the fitness costs.

Many factors can influence the fitness return of sons and daughters

There are now many examples of adaptive sex-ratio adjustments in mammals and birds (reviewed in Cockburn et al. 2002; Komdeur 2012), thereby strongly suggesting that, at least in some species, the benefits can outweigh the potential costs. Logically, striking adaptive sex-ratio adjustments are found when there is one strong selection pressure with a differential fitness effect on male and female offspring. For example, in gouldian finches (*Erythrura gouldiae*), there are two colour morphs and chicks hatched from pairs made up of two birds of the same morph (i.e. compatible) have a higher chance to reach sexual maturity than chicks hatched from pairs with two birds of a different morph (i.e. incompatible) (Pryke & Griffith 2009b). Moreover, the fitness costs of incompatible mating are higher among female chicks (80% mortality rate before sexual maturity) than among male chicks (40% mortality rate before sexual maturity) (Pryke & Griffith 2009b). Hence, females mated with a genetically incompatible male adaptively produced a male-biased primary sex-ratio, whereas the same females adjusted their sex-ratio towards daughters when mated with a genetically compatible male (Pryke & Griffith 2009a).

However, as birds and mammals have more complex life-histories than parasitic wasps for instance, there are many hypotheses and corresponding selection pressure that have been proposed to influence sex-ratio adjustment in birds and mammals (reviewed in Cockburn et al. 2002; see Box 1, p. 24). Moreover, it is often unlikely that only one selection pressure will influence the fitness benefits of producing sons and daughters. It is therefore important to identify the different selection pressures acting on a species and to try to disentangle between them in order to better make predictions and interpret the data. The ideal case is when the different selection pressures act in the same direction. The sex-ratio adjustment may then be striking, as showed in red deer and macaques by Gomendio et al. (1990). There were more fitness benefits to produce sons for dominant females than for subordinate females in red deer

(Clutton-Brock et al. 1984) (the narrow sense Trivers-Willard hypothesis, see Box 1, p. 24), whereas the reverse was true for macaques (Silk 1983) (the local resource competition hypothesis, see Box 1, p. 24). Additionally, the rearing of a son (a daughter) led to a reduced probability of reproduction the next season for subordinate females than the rearing of a daughter (a son) in red deer (in macaques), whereas offspring sex did not influence the mother's probability of reproduction in dominant females (the cost of reproduction hypothesis, see Box 1, p. 24). Hence, the relative fitness benefits and the relative fitness costs of producing sons or daughters for dominant and subordinate females were complementary and led to a high selection on females to bias offspring sex accordingly, which has been found (Gomendio et al. 1990).

In the case of the red deer, Gomendio et al. (1990) considered the narrow-sense Trivers-Willard hypothesis and the cost of reproduction hypothesis and clearly checked whether the different underlying assumptions concerning these hypotheses held, thereby allowing them to test sex-ratio predictions. However, this is often not the rule. To test the cost of reproduction hypothesis, for example, one need to confirm that the larger sex is more costly, but people often make the assumption that, in sexually dimorphic species, the larger sex is more costly to rear without testing it.

Although, it is sometimes true (e.g., Northern elephant seal: Reiter et al. 1978; red deer: Clutton-Brock et al. 1981; American kestrel: Anderson et al. 1993; fallow deer: Birgersson 1998; wandering albatross: Weimerskirch et al. 2000; European sparrowhawk: Vedder et al. 2005; common murre: Cameron-MacMillan et al. 2007; brown songlark: Magrath et al. 2007), other studies did not find any evidence of a higher rearing cost of the larger sex (e.g., blue-footed booby: Torres & Drummond 1999; brown falcon: McDonald et al. 2005; Nazca booby: Townsend et al. 2007) or at least to a lesser extent than suggested by sexual dimorphism (Anderson et al. 1993; Krijgsveld et al. 1998).

Box 1. Several hypotheses proposed in the context of adaptive sex-ratio adjustment in birds and mammals (for a more complete review see Cockburn, Legge, and Double 2002).

Individual characteristics:

- ❖ Narrow sense Trivers-Willard hypothesis (Trivers & Willard 1973): In polygynous mammals, males commonly have a higher variance of reproductive success than females. (1) If maternal condition is correlated to offspring condition at the end of the period of parental investment; (2) if differences in offspring condition continue into adulthood and (3) if a small advantage in condition increase males' more than females' reproductive success, mothers in good condition should preferentially produce sons and mothers in poor condition should preferentially produce daughters.
- ❖ Wide sense Trivers-Willard hypothesis (Burley 1981; Leimar 1996; Hewison & Gaillard 1999): Parents should invest more in the sex that gains most benefits either from an extra investment or from any heritable characteristic. For example, if daughters inherit their mother's social rank, high ranking mothers should bias their sex-ratio towards daughters.
- ❖ Cost of reproduction hypothesis (Myers 1978): If one sex is energetically more costly to produce or to rear, mothers in poor condition should avoid fitness costs by producing the cheaper sex in order to increase their survival and/or future reproductive success.
- ❖ Maturation time hypothesis (Dijkstra et al. 1990; Pen et al. 1999): If the outcome of the first breeding event of one sex is more dependent on its birth date than for the other sex, parents should adjust the sex of their offspring to the laying date. Hence, this hypothesis leads to a seasonal trend in sex ratio.

Social environment:

- ❖ Local resource competition (Clark 1978; Silk 1983): Parents more susceptible to resource competition should bias their sex ratio towards the dispersing sex in order to avoid competition between relatives, whereas parents in better environment should be able to pay the cost of producing the philopatric sex.
- ❖ Local resource enhancement (or repayment model) (Emlen et al. 1986; Lessells & Avery 1987; Pen & Weissing 2000): If benefits from helpers-at-the-nest vary with a certain environmental variable, parents who gain the most of producing helpers will bias their sex ratio towards the helping sex and conversely for parents who have either no (or less) benefits or more costs.

Hence, before testing predictions of the cost of reproduction hypothesis, people should probably first test whether the larger sex is indeed more costly to rear, especially in species with a slight sexual dimorphism. The absence of differential cost does not mean that sex-ratio adjustment should not be expected, as other selection pressures could still occur. For example, even without any differential cost, one sex could still benefit more from a higher parental investment or from any characteristic inherited from its parents in term of future reproductive success (wide sense Trivers-Willard hypothesis).

Why is the kittiwake interesting in this regard?

Different behavioural and life-history characteristics indicate that we might expect adaptive sex-ratio adjustment according to the cost of reproduction hypothesis and/or to the wide sense Trivers-Willard hypothesis. During my thesis, I examined whether their assumptions were met and studied whether sex-ratio varied according to these hypotheses.

Males are larger than females as adults (body mass: 10.2%, tarsus length: 3.5%; wing length: 2.8%: Helfenstein et al. 2004a), but nothing is known about sex-specific growth during chick rearing in kittiwakes, although chick growth has already been studied (e.g., Suryan et al. 2002; Jodice et al. 2008). Hence, I first investigated whether sexual dimorphism takes place during chick rearing (Chapter 1A, p. 41), as found in other species (Richner 1991). Nevertheless, as sexual dimorphism is slight in this species as compared to others, it is not obvious that males are more costly to rear than females. I thus also studied a sex-specific cost and I found some evidence for it. I further tested whether it influenced sex-ratio adjustment (Chapter 1B, p. 65).

Not only are adult males larger than adult females, but they also arrive earlier than females on the breeding sites and compete intensely for them (Cullen 1957; Coulson 1968; Wooller & Coulson 1977). The outcome of such competition may impact the onset of reproduction and

the length of the rearing period, a key determinant for chick post-fledging survival and future reproductive performance (Cam et al. 2003). Accordingly, within a breeding season, males starting their reproduction earlier appear to be heavier and better competitors (Coulson 1968; Wooller & Coulson 1977; Coulson & Thomas 1985). Moreover, nest characteristics affect reproductive success (Coulson 1968; Regehr et al. 1998; Massaro et al. 2001) and male-male competition has been reported to be all the more intense at the most attractive nesting sites (Wooller & Coulson 1977). The male's physical ability to defend the nest may also be important during chick rearing (see Moe et al. 2002; Leclaire et al. 2010), when prospecting behaviour (Cadiou & Monnat 1996; Cadiou 1999) and/or predation are common (Massaro et al. 2001), as suggested by the higher nest attendance of males observed during this period (Leclaire et al. 2010). Altogether, given that (1) rearing conditions determine body mass and structural size at fledging (Gill et al. 2002) and given that (2) these characteristics are further expected to have long-term fitness consequences in this species (Cam et al. 2003), these observations suggest that rearing conditions may be more important determinants of male than female subsequent reproductive success (see also Albrecht & Johnson 2002 for a similar fitness return pathway), which accords with the observed sexual dimorphism (Helfenstein et al. 2004a). Hence, selection pressures for optimal rearing conditions, such as larger size and body mass at fledging, might be higher among males. This led me to the prediction that sons may benefit more in terms of future reproductive success than females from a higher parental ability to care (wide sense Trivers-Willard hypothesis, Box 1, p. 24). I used data from a long-term feeding experiment to compare hatching sex-ratios between fed and unfed pairs over three breeding seasons and found that overall unfed pairs produced relatively more daughters than fed pairs (Chapter 1A, p. 41). However, our results could also be coherent with the cost of reproduction hypothesis (see above and Chapter 1B, p. 65). In our case, the underlying assumption of the wide sense Trivers-Willard hypothesis is that male chicks born on fed nests

should have a higher reproductive success as adults than male chicks born in unfed nests, whereas there should be no difference between females. In order to test that, I used the long-term data from the feeding experiment (Box 2, p. 93).

Also, as kittiwakes are genetically monogamous with no extra-pair offspring (Helfenstein et al. 2004b), paternity analysis were not necessary in my studies.

Part 2: Hatching asynchrony: when to begin incubation?

Female birds cannot lay all their eggs at the same time. In species laying more than one egg, there is usually a time lag of at least one day between each egg. Females (or parents, in species sharing the incubation task) thus have to choose whether they begin to incubate assiduously their eggs just after laying or whether they wait until more eggs are laid. A general observation is that hatching asynchrony is common in many bird species (Magrath 1990), which suggests that incubation usually starts before clutch completion. This phenomenon has attracted a lot of attention among ornithologists and many hypotheses have been proposed (reviewed in Amundsen & Slagsvold 1991; Stoleson & Beissinger 1995; Stenning 1996). For example, hatching asynchrony has been proposed to minimise predation risk (Clark & Wilson 1981), to preserve egg viability in harsh (cold or warm) environments (Arnold et al. 1987), to create ‘insurance offspring’ in case the older chick fails to hatch (Cash & Evans 1986) or to allow chicks’ peak food demand to occur at different time (Bryant 1978). However, David Lack was the first to propose an explanation for hatching asynchrony (Lack 1947, 1954). His first observation was that, in unpredictable environments, females usually lay more eggs than the number of chicks the pair would be able to fledge under average environmental conditions (Lack 1954; Ricklefs 1965). He thus hypothesised that, if food appeared to be insufficient to fledge all chicks, hatching asynchrony would create a within-brood hierarchy allowing parents to preferentially feed the older chick(s) and would thus facilitate a quick elimination of the younger chick(s) when needed, whereas hatching synchrony would not allow this preferential feeding, thereby making all chicks starving and leading to an overall reduced fledging success (the facultative brood reduction hypothesis: Lack 1947, 1954). Moreover, if food appears to be plentiful, hatching asynchrony should not be a disadvantage, as despite the within-brood hierarchy parent would be able to feed and

fledge all their chicks. This hypothesis has been widely tested in many species but with inconsistent results: while some studies found support for it (e.g., Magrath 1989), others did not (e.g., Amundsen & Stokland 1988). Amundsen and Slagsvold (1991) reviewed thirty experimental studies on hatching asynchrony and concluded that there was no global support for Lack's hypothesis because synchronous hatching led to a higher fledging success than asynchronous hatching. Interestingly, however, synchronous chicks were found to be generally lighter at fledging and therefore maybe less likely to survive.

Hahn (1981; following Hamilton 1964) completed Lack's hypothesis by proposing that hatching asynchrony would be a way to minimise sibling competition and therefore to reduce energy wastage, leading to a more efficient food allocation (the sibling rivalry hypothesis). Species showing overt aggression should be good candidates to test this hypothesis, as chicks can waste energy through begging (as other species) but also through aggression. In support to this, Mock and Ploger (1987) found that, in cattle egrets (*Bubulcus ibis*), synchronous hatching led to lower fledging success with higher sibling competition and higher parental feeding rates than asynchronous hatching. Hence, not only did hatching synchrony reduce fledging success (coherently with the facultative brood reduction hypothesis) but it also increased parental energy expenditure, thereby potentially reducing their subsequent survival and/ or reproductive success (Mock & Ploger 1987). Chick growth was not measured in this study and one can argue that maybe surviving synchronous chicks were in better condition at fledging due to their parents' higher feeding rates. However, Mock and Ploger's results have been confirmed in species exhibiting aggression or not and none of these studies found a growth advantage for synchronous chicks (Fujioka 1985; Wiebe & Bortolotti 1994a; Osorno & Drummond 1995; Gilby et al. 2011). Creating a dominance hierarchy through hatching asynchrony reduces sibling competition and leads to a more efficient food allocation, but a too large hatching asynchrony seems to be deleterious as it increases brood reduction (e.g.,

Amundsen & Stokland 1988; Osorno & Drummond 1995; Viñuela 1999). Hence, parents should find the best trade-off in order to reduce sibling competition and energy wastage as found in synchronous broods and high chick losses as found in very asynchronous broods. A few studies have investigated whether the natural magnitude of hatching asynchrony led to this pattern and indeed found it in both species showing overt aggressions (e.g., Mock & Ploger 1987; Osorno & Drummond 1995) and begging scramble (Gilby et al. 2011). According to the facultative brood reduction hypothesis (Lack 1947, 1954) and the sibling rivalry hypothesis (Hahn 1981), hatching asynchrony magnitude should influence sibling competition both at the brood (e.g., higher aggression rates in synchronous broods) and individual level (e.g., higher older chick aggression rate with increasing hatching asynchrony) and in terms of growth and survival. However, authors rarely investigated all these aspects together within a study.

How should hatching asynchrony magnitude influence chick stress?

Within this framework it is also easy to predict that the magnitude of hatching asynchrony should influence both parents' and chicks' physiology and stress hormones are probably good candidates. There are many studies in adults showing that a social or nutritional stress like having an unattractive mate (Griffith et al. 2011) or enduring reduced food availability (Kitaysky et al. 1999a) can increase baseline stress hormone levels. To my knowledge, no study has investigated whether the magnitude of hatching asynchrony would influence parental stress hormone levels or any other physiological traits, although it can be predicted that the higher feeding rates, and therefore foraging activity, found in synchronous broods should lead to higher stress hormone levels (Angelier et al. 2007a). Among chicks, as hatching asynchrony usually leads to an unequal allocation of food between chicks, younger chicks may be under nutritional stress and are thus expected to have higher baseline

nutritional stress than their older siblings. However, results are inconsistent: some studies confirmed these expectations (e.g., Tarlow et al. 2001; Eraud et al. 2008), others found no effect of rank (e.g., Blas et al. 2005; Poisbleau et al. 2010) or even the reverse pattern (e.g., Schwabl 1999; Love et al. 2003). Moreover, aside from one study on a captive population of American kestrels (Love et al. 2003), no study has investigated whether the magnitude of hatching asynchrony influences baseline stress hormone levels.

Can parents manipulate hatching asynchrony according to environmental conditions?

If hatching asynchrony is adaptive in a way that it can help parents to have control over brood reduction, we would expect that the optimal magnitude of hatching asynchrony would change with environmental conditions. Many studies found that a large hatching asynchrony magnitude led to a higher frequency of brood reduction (e.g., Mock & Ploger 1987; Amundsen & Stokland 1988; Osorno & Drummond 1995), thus if the environmental conditions are expected to be poor during chick rearing, parents may want to increase hatching asynchrony by an earlier onset of incubation. Wiebe and Bortolotti (1994b) measured hatching asynchrony over four years in American kestrels and showed that hatching asynchrony was lower in good food years than in poor food years. They also showed that food supplemented parents had more synchronous broods than non supplemented pairs (Wiebe & Bortolotti 1994b). Moreover, Wiebe (1995) showed mathematically that the previous result is all the more expected when food supply during chick rearing is predictable from food supply at the time of laying (see also Wellicome 2005). However, there are also studies finding that food supplementation led to a higher hatching asynchrony (Nilsson 1993; Eikenaar et al. 2003), but, interestingly, in these two species (marsh tits *Parus palustris* and Australian reed *Phragmites australis*) only the females incubate the eggs, whereas in kestrels both sexes

incubate. Indeed, Nilsson (1993) argued that, in the former case, experimental feeding reduced the need of females to feed intensively after they laid their first egg and before they begin to incubate, thereby leading to a larger hatching asynchrony. Facultative manipulation of hatching asynchrony may thus depend on food predictability and whether females are constrained to feed before beginning to incubate. Hence, species where both parents share the incubation task should be useful in this regard as the energetic constraint is less likely.

Why is the kittiwake interesting in this regard?

The black-legged kittiwake is a facultatively siblicidal seabird (Braun & Hunt 1983) with a modal brood size of two chicks. Both parents share the incubation and chick-rearing tasks (Coulson & Wooller 1984). The first-egg hatches on average 1.35 days before the second one (Gill et al. 2002). The older chick systematically attacks its younger sibling, who respond by a submissive posture (Braun & Hunt 1983). Sibling aggression intensity is dependent on food availability (Irons 1992; White et al. 2010) and potentially on female feeding effort (Leclaire et al. 2010). The adaptive benefits of hatching asynchrony have been less studied in siblicidal species than in species where chicks compete via begging. Moreover, as there are two potential sources of energy wastage in sibling competition, begging and aggression, this species is interesting to study in regard to the sibling rivalry hypothesis (Hahn 1981). I experimentally manipulated hatching asynchrony magnitude to create experimental broods whose hatching asynchrony ranged from zero to three days. I subsequently observed chicks' and parents' behaviours (i.e. aggression, begging and feeding) both at the brood and individual levels and measured chick growth and survival in order to get a more complete picture of the influence of hatching asynchrony magnitude on these variables (Chapter 2A, p. 97). My results corroborated previous findings concerning the sibling rivalry hypothesis and confirmed that the natural hatching asynchrony magnitude might be optimal. Then, as no

study has yet studied how hatching asynchrony magnitude influences chick baseline corticosterone (i.e. the major stress hormone in birds) levels in a wild bird, I used the same experiment to investigate it (Chapter 2B, p. 123). Finally, I also used the long-term feeding experiment to test whether parents can manipulate hatching asynchrony according to environmental conditions (Box 3, p. 143).

Part 3: Egg yolk composition: how much hormones to put in each egg?

The initial finding that bird mothers can transfer substances such as hormones and antioxidants to their egg yolk and that it could influence chick behaviour (Schwabl 1993) led to extensive research in the last twenty years. This phenomenon falls into the area of maternal effects, which are non-genetic modifications of offspring phenotype caused by the environment provided by the mother during development (Mousseau & Fox 1998). While they were initially thought to be maladaptive, evidence that they provide a mechanism for adaptive transgenerational phenotypic plasticity are accumulating (Mousseau & Fox 1998; Marshall & Uller 2007) and cover a wide range of taxa (plants: Roach & Wulff 1987; insect: Mousseau & Dingle 1991; birds: Groothuis et al. 2005a; mammals: Maestriperi & Mateo 2009). Most of the research concerning maternal effects in birds focused on androgen hormones and especially on testosterone, but some studies also looked at antioxidants (e.g., Royle et al. 2001; Saino et al. 2003) and antibodies (e.g., Gasparini et al. 2007). Besides egg yolk substances, hatching asynchrony can also be regarded as a (indirect) maternal effect (or parental effect, in species sharing the incubation task) (Royle et al. 2001), as parents can influence the within-brood size hierarchy and thereby offspring phenotype (see Introduction Part 2, p. 28 and Chapter 2A, p. 97).

Positive effects of yolk androgens on chick fitness

Many studies have found positive effects of maternal yolk androgens, making no doubt that they can indeed influence offspring behaviour and subsequent survival (reviewed in Groothuis et al. 2005a). Maternal androgens have been found to increase chick competitiveness by triggering earlier hatching (e.g., Eising et al. 2001; Eising & Groothuis 2003) and begging (e.g., Schwabl 1996; Eising & Groothuis 2003; Noguera et al. 2013;

Ruuskanen & Laaksonen 2013). Coherently with these findings, authors found that maternal androgens led to an increased growth (e.g., Groothuis et al. 2005b; Navara et al. 2005; Müller et al. 2010a) and survival rates (e.g., Eising & Groothuis 2003; Pilz et al. 2004; but see Sockman & Schwabl 2000). There are even some lines of evidence that yolk androgens can have long-term effects on offspring fitness (Ruuskanen et al. 2012). To my knowledge, there is only one study showing that yolk testosterone can increase siblicidal aggression (Müller et al. 2012), which is surprising given that testosterone is supposed to mediate aggressive behaviours. Results were sometimes inconsistent which has been proposed to reflect species specific differences (Groothuis et al. 2005a), but positive effects of yolk hormones on pre- and post natal development rates are overall more common than negative effects (reviewed in von Engelhardt & Groothuis 2011).

Negative effects of yolk androgens on chick fitness

Maternal yolk androgens have also been found to entail costs to offspring fitness, leading to the idea that mothers would face a trade-off between the benefits and the costs of bestowing eggs with yolk androgens (Groothuis et al. 2005b). Among these negative effects the most commonly found is immunosuppression (e.g., Andersson et al. 2004; Müller et al. 2005; Navara et al. 2005; Sandell et al. 2009; but see Tschirren et al. 2005). Further studies also found that increased yolk hormones concentrations can lead to increased energy expenditure (Tobler et al. 2007; but see Eising et al. 2003) and reduced antioxidant capacity (Tobler & Sandell 2009).

Yolk hormones within-clutch variation: an adaptive explanation across species?

Maternal effects impact offspring fitness, but they should have been selected to increase the mother fitness (Müller et al. 2007; Marshall & Uller 2007). Interestingly, yolk hormones levels are often found to vary within clutch, which suggests that mothers may manipulate offspring competitive abilities differently according to their position in the hatching sequence in order to increase their own fitness. Authors have found that yolk hormone levels can either increase (e.g., Schwabl 1993; Müller et al. 2012) or decrease (e.g., Schwabl 1997) over laying sequence. This has been suggested to illustrate two alternative maternal reproductive strategies: brood reduction or brood survival (reviewed in Müller & Groothuis 2013). In the former case, mothers would be willing to enhance sibling size asymmetries caused by hatching asynchrony by giving more yolk androgens to the older sibling(s) in order to facilitate brood reduction (Schwabl 1997). In this context, last eggs would be produced as an insurance against hatching failure of the earlier laid eggs (Forbes 1990). In the latter case, mothers would be willing to decrease sibling size asymmetries caused by hatching asynchrony and to boost their younger chicks' competitive abilities by giving them more yolk androgens in order to facilitate the survival of the whole brood (Schwabl 1993; Lipar et al. 1999). However, experimental work showed that increased yolk androgens in younger chicks did not allow them to fully compensate the large handicap due to hatching asynchrony (Schwabl 1996; Eising et al. 2001). It has been predicted that mothers should manipulate different maternal effects in concert in order to increase their own fitness (Marshall & Uller 2007). For instance, they should manipulate hatching asynchrony and yolk androgens together. In a brood survival strategy, they should favour small hatching asynchrony magnitude with increasing yolk androgens in the laying sequence. Müller and Groothuis

(2013) recently confirmed this prediction using a comparative analysis: they showed that yolk testosterone compensation in late eggs was negatively correlated with hatching asynchrony, size difference at hatching and growth rate, thereby confirming that mothers can adaptively manipulate different maternal effects together in order to increase their own fitness by favouring brood survival or not.

Should environmental conditions influence yolk hormones deposition?

Although within-clutch variation in yolk androgens seems systematic within a species, its extent may still vary according to environmental conditions. Hence, mothers in species with a brood survival strategy should favour their younger chick's survival especially when food availability is high and the younger chick survival more likely. Food quality has been manipulated in three studies on captive passerine species and two of them found a steeper increase of yolk testosterone across laying sequence in high quality diet broods (Sandell et al. 2007; Vergauwen et al. 2012), but mothers on high quality diets also laid larger clutches which may have had some confounding effects. In the lesser black-backed gull (*Larus fuscus*), supplementary fed mothers put less androgens into their eggs regardless of laying rank (Verboven et al. 2003). In the blue-footed booby (*Sula nebouxii*), mothers put proportionally more androgens in their second eggs during a poor food year than during a good food year (Dentressangle et al. 2008). Hence, in study where food availability did not modify clutch size, mothers seem to respond to high food availability by decreasing yolk androgens deposition in the last egg as compared to the low food availability situation. If food availability is so high that all chicks should easily survive, mothers may reduce the potential fitness costs of increased yolk androgens deposition in their last egg(s). Interestingly, Benowitz-Fredericks et al. (2013) compared yolk androgen contents in supplementary fed and control black-legged kittiwakes (*Rissa tridactyla*) between two years with contrasting food

availability. They found that mothers deposited relatively more testosterone in their second egg than in their first one when survival prospects of the younger chick were intermediate. Their results thus suggested that mothers avoided the potential costs of increased androgens when the younger chick was very likely to survive and also favoured siblicide when its survival prospects are very low. Indeed, when food availability is very high or very low, the mother and the older chick may agree over the younger chick fate: survival or death, respectively (Fig. 2). However, when food availability is intermediate, the mother may increase her fitness by fledging two chicks, whereas the older chick may benefit more from the death of its younger sibling, thus resulting in a parent-offspring conflict over brood reduction (Fig. 2). Yolk androgens deposition is one way the mother could increase her chance of winning this conflict.

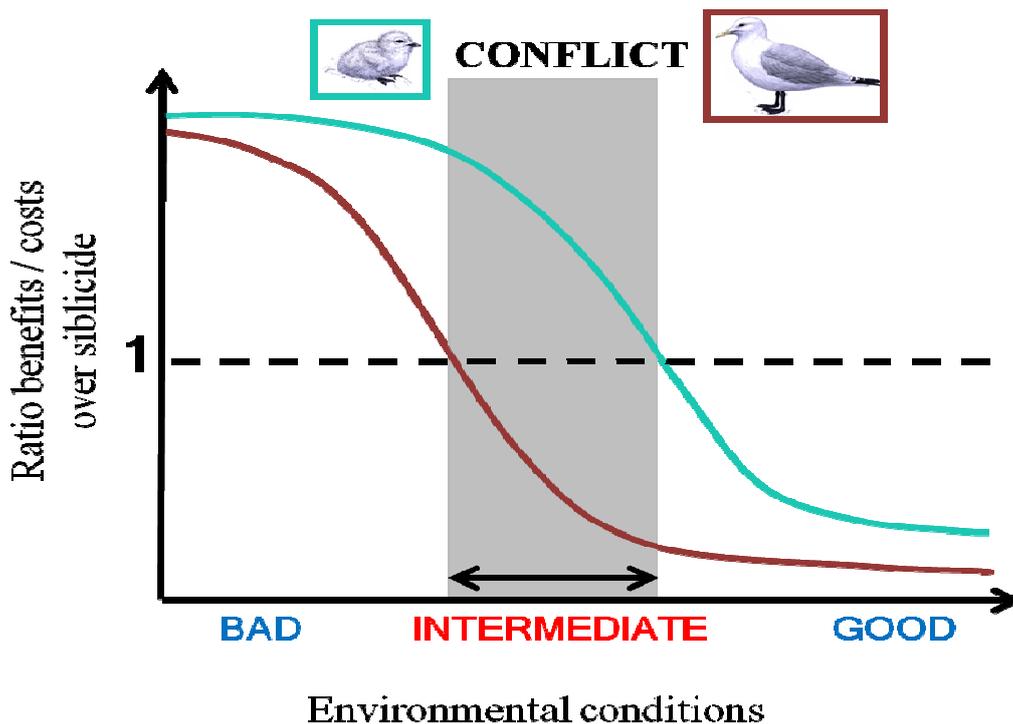


Figure 2. Ratio benefits on costs over siblicide according to environmental conditions for the senior chick (blue curve) and for the mother (red curve). There is a conflict (grey area) when it is more costly than beneficial for the senior chick to keep its sibling, whereas it is the reverse for the mother. (Used with the authorisation of Charlotte Perrot).

Why is the kittiwake interesting in this regard?

There are three findings, from three different kittiwake populations including mine, that second-laid eggs contain more testosterone than first laid eggs (Müller et al. 2012; Vallarino et al. 2012; Benowitz-Fredericks et al. 2013) and also three studies finding the same pattern for androstenedione (i.e. another androgen) (Gasparini et al. 2007; Müller et al. 2012; Benowitz-Fredericks et al. 2013). These patterns are thus in line with a brood survival strategy (Müller & Groothuis 2013), which fits with the fact that kittiwakes are facultatively siblicidal (Braun & Hunt 1983): the younger chick survival prospects are higher when food availability is high (White et al. 2010). Carotenoids, however, seem to be higher in first-laid eggs (Vallarino et al. 2012), as already found in another gull species (Royle et al. 2001). Moreover, there seem to be no differences in antibodies (Gasparini et al. 2007), lipids, proteins or corticosterone concentrations (Vallarino et al. 2012) according to laying order. Chick aggression is enhanced by higher androgen concentrations (Müller et al. 2012), but clearly this advantage given to the younger chick is never enough to compensate the initial competitive disadvantage due to hatching asynchrony (Braun & Hunt 1983; White et al. 2010). Moreover, mothers seem to manipulate the amount of yolk testosterone according to chick survival prospects and food availability (Benowitz-Fredericks et al. 2013): they may put more testosterone into their second egg when its survival would benefit them, but not the older chick. I thus wanted to test whether food availability differentially affected the younger chick's competitiveness, as suggested by these previous results. To do so, I did an experiment where younger chicks, coming from parents either fed or not before laying, were put in a dominant position in order to let them fully express the effects of maternal androgens (Chapter 3, p. 145). I expected younger chicks from unfed parents to exhibit more aggressiveness in a dominant position than those from fed parents and than older chicks from

unfed parents as well. It has to be noticed that I did not directly manipulate yolk androgen levels, but the aim of the experimental design was that our results could only be explained by a difference in yolk constitution.

Chapter 1A: food availability and offspring sex

Food availability and offspring sex in a monogamous seabird: insights from an experimental approach

Behavioral Ecology 23 (4): 751-758

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Disponibilité en nourriture et sexe du descendant chez un oiseau marin monogame: aperçu à partir d'une approche expérimentale

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RÉSUMÉ

La théorie de l'allocation au sexe prédit que les parents devraient favoriser les descendants du sexe qui leur procurera le plus grand retour en valeur sélective (i.e. le sexe qui apportera le plus de petits-enfants). Malgré un nombre croissant de preuves suggérant que les vertébrés sont capables de surmonter la contrainte de la détermination chromosomique du sexe, le patron général reste équivoque, indiquant ainsi un besoin d'études expérimentales. Sur trois saisons de reproduction, nous avons utilisé des données d'une expérience de nourrissage pour étudier l'allocation au sexe chez des mouettes tridactyles (*Rissa tridactyla*). La compétition entre mâles pour l'accès aux sites de reproduction est commune chez cette espèce où les mâles sont plus gros et grands que les femelles. Ainsi, nous avons fait l'hypothèse que les parents produisant des poussins en meilleure condition que la moyenne à l'envol, comme cela devrait être le cas pour des parents recevant de la nourriture supplémentaire, devraient augmenter leur retour en valeur sélective en produisant des fils. Inversement, produire des filles devrait être une meilleure tactique pour les parents non nourris expérimentalement. Nous avons donc prédit que les parents nourris expérimentalement devraient produire plus de fils que les parents non nourris. Cette prédiction devrait être particulièrement attendue si le dimorphisme sexuel apparaît dès la période d'élevage des jeunes, ce qui suggérerait de fortes pressions de sélection pour un développement optimal des mâles. Nos résultats montrent que (1) les mâles étaient plus lourds et grands que les femelles avant l'envol et que (2) les parents nourris expérimentalement produisaient relativement plus de poussins mâles que les parents

non nourris. Nous interprétons ces résultats dans le contexte d'un processus de type Trivers-Willard. De plus, nos données ont aussi montré que les parents non nourris produisaient significativement plus de poussins femelles, alors que la sex-ratio était équilibrée chez les parents nourris expérimentalement. Puisque les trois saisons de reproduction considérées correspondaient à des années avec peu de nourriture naturellement disponible, les parents non nourris expérimentalement ont pu produire plus de filles pour éviter le coût à la reproduction potentiellement supérieur d'élever un fils.

Mots clés: allocation au sexe; croissance de poussins; dimorphisme sexuel; génétiquement monogame; hypothèse du coût à la reproduction; hypothèse de Trivers-Willard; mouette tridactyle; nourrissage expérimental; sex-ratio.

ABSTRACT

Sex allocation theory predicts that parents should favor offspring of the sex that provides the greatest fitness return. Despite growing evidence suggesting that vertebrates are able to overcome the constraint of chromosomal sex determination, the general pattern remains equivocal, indicating a need for experimental investigations. We used an experimental feeding design to study sex allocation during three years in black-legged kittiwakes (*Rissa tridactyla*). Intense male-male competition for securing a breeding site is common in this species in which males are heavier and larger than females. Hence, we hypothesized that parents producing fledglings in better than average condition, as supplementarily fed pairs do, would increase their fitness return by producing sons. Conversely, producing daughters would be a better tactic for unfed parents. Hence, we predicted that fed parents produce more sons than unfed parents. This prediction is particularly expected if sexual dimorphism arises as early as during chick rearing, suggesting strong selective pressures for optimal male development. Our results showed that (1) males were heavier and larger than females prior to fledging and that (2) fed parents produced relatively more male hatchlings than unfed parents. We interpret this result in terms of a Trivers-Willard-type process. Further, our data revealed that unfed parents significantly overproduced female hatchlings whereas offspring sex ratio was balanced among fed parents. Because the three reproductive seasons we considered were particularly poor food years, unfed parents may have overproduced daughters to avoid the apparent higher reproductive costs of raising sons.

Keywords: black-legged kittiwake; chick growth; experimental feeding; genetically monogamous; reproductive cost hypothesis; sex allocation; sex ratio; sexual dimorphism; Trivers-Willard hypothesis.

INTRODUCTION

Sex allocation is one of the most compelling, yet controversial, theories in evolutionary biology. It refers to the way parents should invest in male and female functions, with an expected bias towards offspring of the sex providing the higher fitness return under current conditions (Frank 1990). As an example, in species where body condition impacts the reproductive success of males more than females, and assuming that some females are better able to produce offspring in good condition than others (condition enduring into adulthood), such females are expected to bias their offspring sex-ratio towards males (Trivers & Willard 1973). Whereas sex allocation predictions have been confirmed by empirical studies in some taxa such as parasitic Hymenoptera (reviewed in Ode & Hunter 2002), the factors shaping those patterns remain confused for others, such as birds and mammals (Cockburn et al. 2002).

In birds, the availability of molecular sex identification techniques (Griffiths et al. 1998; Fridolfsson & Ellegren 1999) has led to a growing number of studies reporting adaptive sex ratio biases (reviewed in Alonso-Alvarez 2006; Blanchard et al. 2007), suggesting an ability of some birds to overcome the constraint imposed by chromosomal sex determination (West et al. 2002). However, inconsistent results are also commonly reported, including contradictory results within the same species (e.g., Rosivall et al. 2004; Maddox & Weatherhead 2009), lack of sex-ratio bias when theoretically expected (e.g., Leech et al. 2001; Cockburn & Double 2008) and counterintuitive biases (e.g., Doutrelant et al. 2004; Dietrich-Bischoff et al. 2006). Hence, as highlighted by West et al. (West et al. 2002), further investigations are required to improve our understanding of sex ratio patterns. In particular, because most published studies are based on correlational studies and focus on species with polygynous mating systems or with helpers at the nest, in line with classical theoretical expectations (Cockburn et al. 2002), there is a clear need for new studies using experimental approaches (Komdeur & Pen 2002) and targeting species with contrasting mating systems.

In this paper, we describe an experiment aimed at investigating sex ratio patterns in a genetically monogamous seabird (Helfenstein et al. 2004a), the black-legged kittiwake (*Rissa tridactyla*). Several life-history and behavioral characteristics of this species comprise potential selective pressures that may have led to the evolution of adaptive sex allocation tactics. Males typically arrive at the breeding colonies earlier than females and compete intensely for the best nesting sites (Cullen 1957; Coulson 1968; Wooller & Coulson 1977). The outcome of such competition may impact the onset of reproduction and the length of the rearing period, a key determinant for chick post-fledging survival and future reproductive performance (Cam et al. 2003). Accordingly, within a breeding season, males starting their reproduction earlier appear to be heavier and better competitors (Coulson 1968; Wooller & Coulson 1977; Coulson & Thomas 1985). Moreover, nest characteristics affect reproductive success (Coulson 1968; Regehr et al. 1998; Massaro et al. 2001) and male-male competition has been reported to be all the more intense at the most attractive nesting sites (Wooller & Coulson 1977). The male's physical ability to defend the nest may also be important during chick rearing (see Moe et al. 2002; Leclaire et al. 2010), when prospecting behaviour (Cadiou & Monnat 1996; Cadiou 1999) and/or predation are common (Massaro et al. 2001), as suggested by the higher nest attendance of males observed during this period (Leclaire et al. 2010). Altogether, given that (1) rearing conditions determine body mass and structural size at fledging (thereafter referred to as "condition" and considered as proxies of a male ability to obtain / defend a breeding site) (Gill et al. 2002) and given that (2) these characteristics are further expected to have long term fitness consequences in this species (Cam et al. 2003), these observations suggest that rearing conditions may be more important determinants of male than female subsequent reproductive success (see also Albrecht & Johnson 2002 for a similar fitness return pathway). Indeed, adult male kittiwakes are heavier and larger than females (body mass: 10.2%, tarsus length: 3.5%; wing length: 2.8%, Helfenstein et al. 2004b),

with large heavy males likely favored by sexual selection (as expected in other seabird species with terrestrial displays and between-males competition; Serrano-Meneses & Székely 2006).

Pacific populations of kittiwakes have been experiencing chronic breeding failure at many colonies since the late 1970's (Gill & Hatch 2002). In our population, a supplemental feeding experiment has been carried out since 1996 on a sample of birds to determine whether food availability limits productivity (Gill & Hatch 2002). This hypothesis was strongly supported by the difference in breeding parameters between supplemented (provided with *ad libitum* food through the entire breeding season) and non-supplemented pairs (Gill & Hatch 2002; Gill et al. 2002), as expected given that the chick-rearing stage is highly food limited (Gill & Hatch 2002). In 1996-1997, annual productivity averaged only 0.48 chicks per nest while it reached 1.15 when birds were given extra food (Gill & Hatch 2002). More specifically, chicks from fed parents had greater mass gain and wing chord, attained heavier peak mass, and survived better until fledging than chicks from unfed parents (Gill et al. 2002). Hence, fed parents are better able to provide offspring with good rearing conditions, and ultimately to produce fledglings in good condition, than unfed parents in this food-restricted population.

In this theoretical context, we tested two predictions. Because natural and sexual selection are expected to favor large and heavy adult males, we first predicted that (1) sexual dimorphism arises during chick rearing, with males being larger and heavier than females close to fledging, as commonly reported in dimorphic seabirds (blue-footed booby: Torres & Drummond 1999; wandering albatross: Weimerskirch et al. 2000; common tern: Becker & Wink 2003). Although several previous studies have analyzed chick growth in kittiwakes (Coulson & Porter 1985; Suryan et al. 2002; Jodice et al. 2008), none have compared the growth of male and female chicks. Further, in the context of the supplemental feeding experiment that allowed us to increase inter-pair variability in the ability to provide care (a key determinant of sex allocation in a Trivers-Willard context, e.g. Hewison et al. 2002;

Blanchard et al. 2005), and assuming that condition at fledging impacts adult condition (Trivers & Willard 1973), as expected in this species (Cam et al. 2003), we predicted that (2) fed parents should produce a greater proportion of male chicks than unfed parents. Indeed, we expected fed parents to increase their fitness return by producing high quality, competitive sons (Gill et al. 2002), whereas daughters would confer a higher fitness gain to unfed parents, whose sons would probably become poorer competitors (Trivers & Willard 1973; Albrecht & Johnson 2002). This second prediction is all the more expected if prediction (1) is met, that is, if selective pressures for an increase in male size and body mass and, more generally, for optimal rearing conditions for males, are stronger.

To test these predictions, we (1) compared the growth parameters of male and female chicks (from nests that were not part of the feeding experiment) from hatching to close to fledging and (2) compared the sex ratios of clutches at hatching between nests where parental ability to invest in their chicks (Gill et al. 2002) was experimentally increased by food-supplementing during three breeding seasons (from several weeks prior to fertilization until fledging) with those of non-supplemented nests, using molecular techniques (Fridolfsson & Ellegren 1999) and controlling for laying order.

MATERIALS AND METHODS

Study site and years

The study was carried out from 2006 to 2009 on a population of black-legged kittiwakes (*Rissa tridactyla*) nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59° 26'N, 146° 20'W), Gulf of Alaska. The tower is a twelve-walled polygon where artificial nest sites have been created on the upper walls, permitting observations from inside the tower through sliding one-way windows (Gill & Hatch 2002) allowing us to capture and easily monitor the breeders and their chicks. Each year, nests were checked twice daily during the

entire breeding season to determine nest content (i.e. egg / chick numbers). Laying date was recorded and each egg was individually marked (A for the first-laid egg and B for the second egg, two being the typical clutch size) with non-toxic waterproof ink within twelve hours of laying. Chicks were also marked on the head at hatching with non-toxic marker to identify their hatching rank.

For the growth monitoring, we measured chicks from another part of the colony which were not involved in the experimental setting. We further restricted our analyses to 2008, the only good year regarding environmental conditions and thus providing enough B-chicks that survived until fledging. This allowed us to get a complete understanding of the pattern given that chick rank is known to impact growth parameters in this species (Gill et al. 2002). Nevertheless, similar patterns were found in the other years when focusing on A-chicks only.

For the experiment aimed at investigating the influence of parental supplemental feeding on chick sex, four years were initially considered: 2006 to 2009. However, we excluded the year 2008 from our analyses because only eight and ten chicks were sexed from unfed and fed pairs respectively, in that year.

Measuring chick growth

Chicks were measured every five days from the day of hatching until they were thirty-five days old. Although fledging usually occurs at an older age (mean fledging age = 41.4 days in 2008; see also Mulard & Danchin 2008), we did not manipulate chicks after thirty days old to avoid precocious fledging. We weighed chicks to the nearest 0.1g using an electronic scale and measured tarsus length to the nearest 0.1 mm with a caliper. Chicks were banded and blood-sampled at twenty-five days ($N=116$; A-chicks: $N=66$; B-chicks: $N=50$) from the brachial vein with a syringe and a sterile needle for molecular sexing (see below). Blood samples were kept in Longmire buffer (Longmire et al. 1988).

We used hatching body mass (BM), hatching tarsus length (TL), maximum BM and maximum TL (i.e. maximum recorded measurements for a given chick: this usually occurs at thirty-five days, but possibly at thirty days) as growth parameters. One chick was excluded from the analyses related to TL as it was not measured. We further used well established growth functions to extrapolate two other reliable parameters: maximum growth rate (maximum slope of the growth curve) and asymptotic value for both BM and TL (where the slope of the growth curve reaches zero) (Richards 1959; Ricklefs 1968). Chick growth patterns are best characterized by a sigmoid equation (Richards 1959). Many special cases have been developed to fit growth data, such as the Gompertz, logistic or Richards equations (Ricklefs 1968). We fitted a growth curve from these three growth functions for each individual chick using a non-linear least squares technique. Then the best fit was assessed based on the AIC model selection procedure and growth parameters were extracted from the best model. This procedure has been automated in the *grofit* R library (Kahm et al. 2010). The logistic model provided the best fit overall (for BM: logistic: 54% of the individual chicks considered, Gompertz: 32% and Richards: 14%; for TL: Richards: 43%, logistic: 41%, Gompertz: 16%), in agreement with previous studies on kittiwake chick growth (Suryan et al. 2002). Therefore, we chose to force the procedure to fit only the logistic model to allow inter-individual comparisons (Weimerskirch et al. 2000). Performing the analysis with the parameters estimated from the best model for each chick led to similar results (not shown).

For some chicks, the slope of the growth curve did not reach zero at thirty-five days, leading to an overestimation of asymptotic values. For this reason, we excluded one and eight chicks for BM and TL analyses, respectively, because the difference between the estimated asymptotic value and the maximum value was more pronounced than for the other chicks. We based our decision rule on the breaks in the histogram of the differences between estimated

asymptotic and maximum measured values plotted for all chicks. Results were similar when analyses were performed on the entire data set (not shown).

Experimental feeding design for sex ratio analysis

As mentioned above, a feeding experiment has been carried out since 1996 on a sample of kittiwakes (Gill & Hatch 2002) divided into two treatments: fed (i.e. the experimental group, all nests on three walls) and unfed (i.e. the control group, all nests on two walls). The two walls of unfed birds alternated with the three walls of fed birds so that environmental conditions (e.g. wind, rain and sun exposure) were similar for both treatments. Moreover, the artificial nesting ledges are the same on the five plot walls and thus do not covary with feeding treatment.

Experimental birds were fed with capelin (*Mallotus villosus*) three times a day (at 9:00, 14:00 and 18:00) from inside the tower. During each feeding session, fish were given singly through a plastic tube passing through the wall at each nest site. Feeding continued until the parent(s) present at the nest were satiated.

Our feeding treatment began in early May in 2006 and 2007, in April in 2009 (i.e. at least twenty days before fertilization) and lasted until chicks fledged (or pairs failed to nest, or lost their eggs or chicks). Upon hatching, eggshells were collected from as many nests as possible, including both fed and unfed pairs. The presence of blood vessels in the egg membrane allowed us to sex chicks molecularly in order to estimate the sex ratio. We were only able to sex one unhatched egg, because usually no blood vessels were apparent in their egg membrane and/or unhatched eggs were more frequently lost or depredated. Hence, we thereafter consider sex ratio at hatching and not primary sex ratio (i.e. at fertilization). Sample sizes for each year in relation to egg rank and treatment are presented in Table 1.

Chick sexing

Chick sex for growth analysis was determined from blood samples ($n=116$). Total DNA was extracted from these samples ($\approx 25\mu\text{l}$ of blood in conservation buffer) with the DNeasy® Blood and Tissue Kit protocol (QIAGEN group) following the supplier's guidelines. We performed two elutions with $100\mu\text{l}$ of AE Buffer. DNA concentrations ranged between 0.5 and $15\text{ ng}/\mu\text{l}$. We used the polymerase chain reaction (PCR) to amplify a part of the CHD1 gene including an intron of different size on the W-chromosome and on the Z-chromosome. We used the universal primers MSZ1R (ATCCATCAAGTCTCTAAAGAG; Sehgal et al. 2005) and 2550F (GTTACTGATTCGTCTACGAGA; Fridolfsson and Ellegren 1999). Twenty microlitres reaction mixture contained 2-30ng of genomic DNA, 0.5 units of GoTaq® DNA Polymerase (Promega), $4.0\mu\text{l}$ of 5X Green GoTaq® reaction Buffer (Promega), $200\mu\text{M}$ of each dNTP (Promega) and $0.4\mu\text{M}$ of each primer. The thermal profile consisted of denaturation at 95°C for 3 min, followed by 36 cycles of 95°C denaturation for 30s, 50°C annealing for 45s, and 72°C elongation for 45s. Then a final elongation at 72°C for 5 min was performed. PCR products were visualized on 1% agarose gels stained with ethidium bromide and visualized under UV light. We checked the reliability of the PCR protocol for sex determination on blood samples from adults of known sex (two males and two females). As expected, heterogametic females (ZW) had upper and lower bands ($\approx 650\text{ bp}$ and $\approx 450\text{ bp}$), whereas homogametic males had a single upper band ($\approx 650\text{ bp}$). When in doubt (i.e. PCR bands not clearly visible), we used a second set of primers (P2 and P8; Griffiths et al. 1998), which are less easy to use (only ten bp differences between bands), for cross checking. We were able to determine the sex of all chicks from the blood samples.

Chick sex for the feeding experiment was obtained from eggshells following the modified QIAGEN extraction protocol of Bush et al. (Bush et al. 2005), with an overnight incubation for years 2007 and 2009. For year 2006, we used a salt-extraction protocol following Aljanabi

and Martinez (1997). We collected a total of 333 eggshells over the four years (including 2008); however we were unable to sex eighty of them because of low quality material (see Table 1)

Treatment	Egg rank	2006	2007	2009
Fed	A	31	14	23
	B	30	14	26
Unfed	A	39	9	19
	B	23	7	11
Total		123	44	79

Table 1: Sample sizes for each year according to feeding treatment and egg rank

Statistical analyses

In both the chick growth and the sex ratio analyses, we had broods comprising two chicks with either one or two sexed chicks. In a first step, for every dependent variable, we began by testing the significance of nest identity as a random effect (i.e. whether within-nest variance was high), focusing only on complete broods (i.e. two sexed chicks), using either a simulation-based likelihood ratio test (20,000 simulations) with the *RLRsim* package (Scheipl et al. 2008) for growth parameters or a simulation-based bootstrap approach (4000 bootstrap replicates) with the *glmmML* package (Bröström 2009) for sex ratio analysis. When the random effect was significant, we restricted our analyses to those nests with a complete brood, including nest identity as a random effect ($N = 54$ for hatching BM and BM maximum growth rate; $N = 52$ for maximum TL and asymptotic TL), because there is obviously no within-nest variance for one-sexed chick broods. However results were similar when we used the whole dataset without a random effect (not shown). When the random term was not significant, we used the complete dataset, without including a random effect ($N = 116$ for

maximum BM; $N = 115$ for asymptotic BM; $N = 115$ for hatching TL and TL maximum growth rate; sex ratio analysis: $N = 253$).

For the growth parameters analysis, we accounted for the effect of laying order as this is known to affect chick growth (Velando et al. 2002). We found a significant random effect of nest identity for hatching BM, maximum BM growth rate, maximum TL and asymptotic TL. We thus investigated the relationships between chick sex, rank and their interaction with growth parameters using mixed effects models in the *lme4* package (Bates et al. 2011) with a maximum likelihood estimator, with nest identity as a random term. We first fitted the complete model (i.e. with an interaction between chick rank and sex) and then removed each term successively, starting with the interaction. The significance of a term in the model was determined by assessing the change in deviance after removal of that term (likelihood-ratio test, LRT), using a chi-square test with the appropriate degrees of freedom, as deviance differences are chi-square distributed. For the other parameters (i.e. maximum BM, asymptotic BM, hatching TL, TL maximum growth rate), we ran ANOVA using the same procedure (with F-tests) to assess the effects of sex, rank and their interaction.

In all analyses, we checked for normality and homoscedasticity of data and residuals. When at least one of the assumptions was not met, we used Box-Cox transformation (Box & Cox 1964) in the *MASS* package (Venables & Ripley 2002). This method was designed to find the value of λ in the function of the observed data with: $f(y) = (y^{-\lambda} - 1) / \lambda$ if $\lambda \neq 0$, that maximises the likelihood of the function. The resulting λ s were: hatching BM: $\lambda = -1.1$, asymptotic BM: $\lambda = 7.2$ and maximum TL growth rate: $\lambda = 0.2$. When the random effect was significant, we also tested for normality of intercepts. We then used the transformed variable with the procedure mentioned above.

For the feeding experiment, we investigated the effect of parental feeding treatment on chick sex at hatching, accounting for year and chick rank. Because nest identity was not

significant as a random effect ($P = 0.53$), we investigated the relationship between parental feeding treatment and chick sex (specifying a logit function with 0= female and 1= male) with a generalized linear model (GLM), following the same procedure as for growth parameters. As data were not overdispersed, we did not have to control for this (i.e. estimated scale parameter = 1). Hence, we compared the deviance between models with and without a term (LRT) using a chi-square test with the appropriate degrees of freedom (Wilson & Hardy 2002), as differences of deviances are chi-square distributed. As a possible confounding effect could arise from the same walls being fed and unfed across years, we tested the wall effect on sex ratio using a GLM model with sex as the binary dependent variable and year and wall as fixed effects for each treatment. There was no wall effect in either treatment (fed: difference in deviance = 4.89, difference in df = 1, $P = 0.09$; unfed: difference in deviance = 0.05, difference in df = 1, $P = 0.82$).

Sex ratio deviations were tested against the null expectation of 0.5 using a chi-square goodness-of-fit test. Results are shown with mean \pm standard error (SE) and significance is evaluated against a 5% threshold. All tests were run with R 2.12.1 (R Development Core Team 2012).

RESULTS

Chick sexual dimorphism

Hatching body mass and tarsus length

There was no effect of sex or of the interaction between sex and rank on BM or on TL at hatching (all $P > 0.11$). However, A-chicks were significantly heavier and larger than B-chicks (BM: A-chicks 39.19 ± 0.88 g, $N = 27$; B-chicks 35.44 ± 0.70 g, $N = 27$, $\chi^2_1 = 24.746$, $P < 0.0001$; TL: A-chicks 19.23 ± 0.15 mm, $N = 66$; B-chicks 18.74 ± 0.17 mm, $N = 49$, $F_{112,113} = 5.0072$, $P = 0.027$).

Chick growth: body mass

Maximum growth rate was higher in males, and this was especially true for B-chicks (interaction sex*rank: $\chi^2_1 = 4.99$, $P = 0.025$). Hence, male B-chicks had a higher maximum growth rate than all other types of chicks (21.48 ± 0.81 g.day⁻¹, $N = 14$ as compared to male A-chicks: 20.26 ± 0.71 g.day⁻¹, $N = 15$; female A-chicks: 19.15 ± 0.86 g.day⁻¹, $N = 12$; female B-chicks: 17.28 ± 1.17 g.day⁻¹, $N = 13$).

Males also reached a significantly higher BM asymptotic value than females (447.74 ± 3.31 g, $N = 54$ as compared to 416.94 ± 3.86 g, $N=61$ respectively; $F_{112,113} = 32.093$, $P < 0.0001$; Figure 1). Further, maximum measured BM was 7.7% higher in males (450.51 ± 3.35 g) than females (418.39 ± 3.82 g; $F_{112, 113} = 39.14$, $P < 0.0001$; Figure 1). There was no effect of rank or of its interaction with sex (all $P > 0.16$) on the asymptotic or maximum BM.

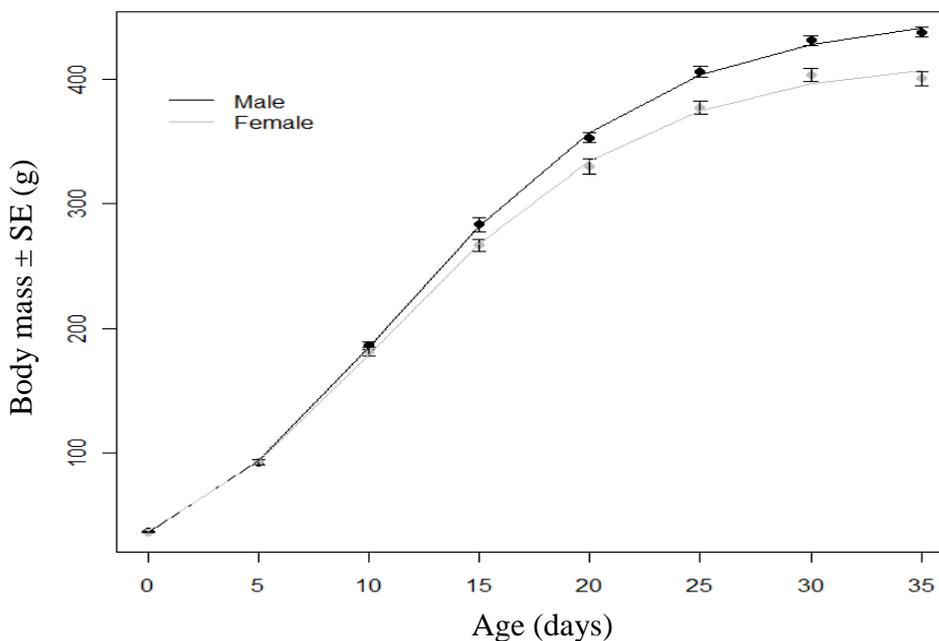


Figure 1. Male and female mass growth curves (logistic model) (males: black curve; females: grey dashed curve) and field data (males: black points; females: grey points) in relation to sex. Males reached a higher mass than females close to fledging. As the random effect of nest identity was not significant, we considered the complete dataset (see text for statistical details).

Chick growth: tarsus length

Maximum growth rate tended to differ in relation to chick sex, with male chicks (1.60 ± 0.04 mm.day⁻¹, $N = 54$) showing a higher tarsus maximum growth rate than female chicks (1.52 ± 0.04 mm.day⁻¹, $N = 61$; $F_{112, 113} = 3.56$, $P = 0.061$). There was no effect of the interaction or of chick rank (all $P > 0.19$).

Males had a significantly higher asymptotic TL than females (respectively 37.99 ± 0.36 mm, $N=28$ and 36.56 ± 0.35 mm, $N= 24$; $\chi^2_1 = 5.82$, $P= 0.016$; Figure 2), a difference that averaged 3.9%. There was also a significant effect of rank on asymptotic TL (A-chicks: 37.74 ± 0.39 mm, $N=26$; B-chicks: 36.93 ± 0.36 mm, $N= 26$; $\chi^2_1 = 7.39$, $P= 0.006$; Figure 2). A-chicks had tarsi that were on average 2.2% longer than those of B-chicks. The sex*rank interaction was not significant ($P > 0.4$).

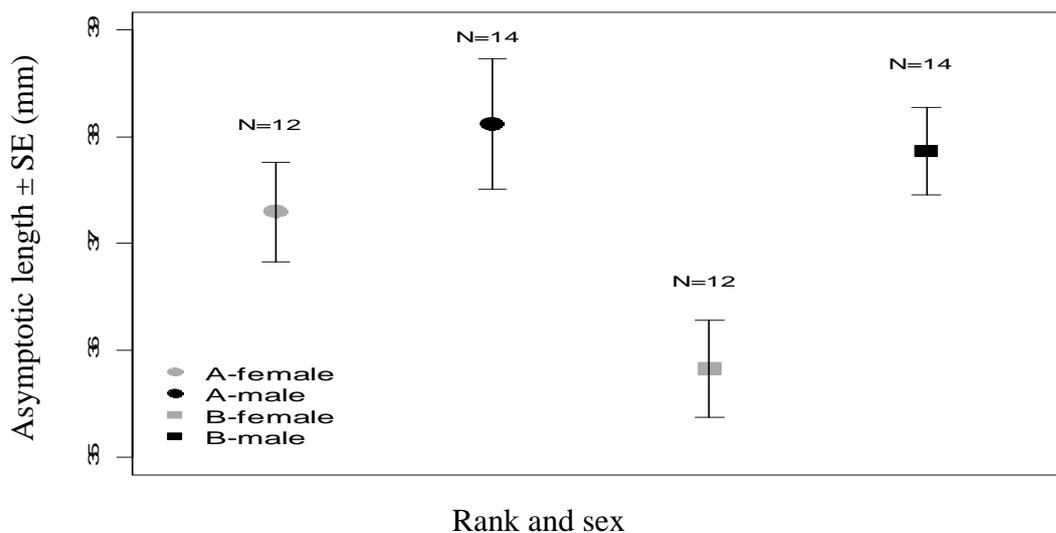


Figure 2. Asymptotic tarsus length in relation to chick rank and sex. Males had longer tarsi than females, and A-chicks had longer tarsi than B-chicks. The interaction between sex and chick rank was not significant. We considered only nests with two chicks, because nest identity was a significant random factor in the model.

Males had a longer maximum TL than females, but the degree of difference depended on chick rank (sex*rank interaction: $\chi^2_1 = 3.84$, $P = 0.05$): maximum TL did not differ significantly between sexes among A-chicks (males: 37.70 ± 0.43 mm, as compared to

females: 37.36 ± 0.50 mm), whereas B-females had significantly lower maximum TL compared with B-males (respectively 35.62 ± 0.46 mm *versus* 37.83 ± 0.37 mm).

Sex ratio and the feeding experiment

As predicted, chick sex at hatching was significantly related to feeding treatment: the probability of producing a male hatchling was higher for fed parents than for unfed parents (difference in deviance = 5.41, difference in df = 1, $P = 0.02$; Figure 3). Egg rank ($P = 0.38$), year ($P = 0.51$), and all interactions (*all* $P > 0.23$) were not significantly related to chick sex. Overall, 70 of 138 chicks from the fed parents were males *versus* 34 of 98 from the unfed parents. The overall male/female ratio was 1.94 times higher among fed parents.

Within treatments, chicks born from fed parents had a balanced sex ratio (all years together: number of males/total number of chicks = 0.507, $\chi^2_1 = 0.03$, $P = 0.86$; all three $P > 0.31$ when each year is considered independently) while chick sex ratio from unfed parents differed from parity: they produced significantly more females than males (all years together: number of males/total number of chicks = 0.347, $\chi^2_1 = 9.18$, $P = 0.002$; when each year is considered independently: 2006: $P = 0.069$; 2007: $P = 0.045$; 2009: $P = 0.068$).

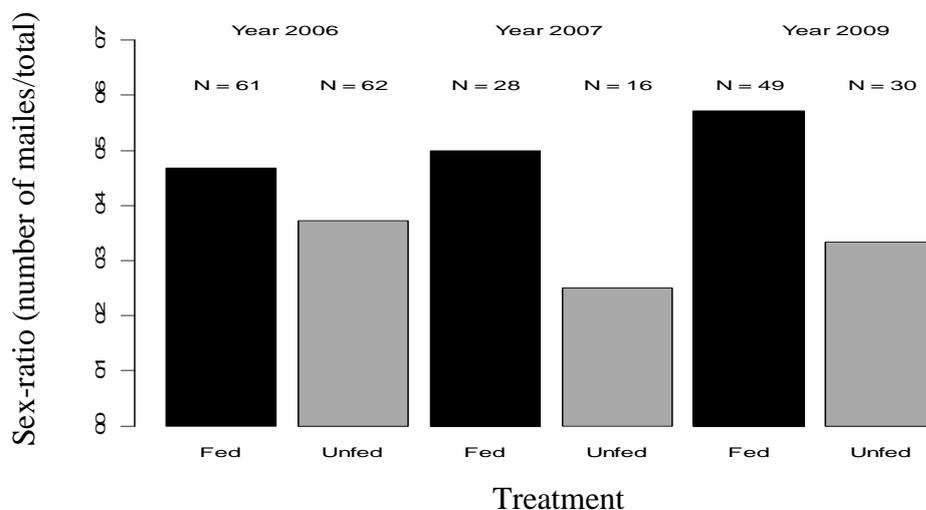


Figure 3. Sex ratios (number of males / total number of chicks) within treatment for each year. Sample sizes are shown above each bar.

DISCUSSION

Sex allocation theory predicts that parents should produce more offspring of the sex with the highest fitness return given their own phenotypic characteristics and their environment (Frank 1990). In birds, a growing number of studies have now found adaptive sex ratio biases (reviewed in Alonso-Alvarez 2006), but general patterns remain equivocal. Here we used an experimental approach to investigate sex ratio variation in the black-legged kittiwake, a genetically monogamous gull (Helfenstein et al. 2004a). In this species, the documented adult sexual dimorphism (Helfenstein et al. 2004b) as well as several life history and behavioral traits led us to expect a stronger effect of body mass and structural size on fitness in males than in females. Accordingly, we first expected sexual size dimorphism (with males bigger than females) to arise during rearing. Our results confirmed this first prediction. In particular, male chicks reached a higher maximum and asymptotic body mass (BM) and tarsus length (TL) than females. Following on from these first results, because pairs that are able to produce better than average condition fledglings should increase their fitness return by producing sons (Trivers & Willard 1973), we predicted that experimentally fed parents, which produce better condition chicks than unfed parents (Gill & Hatch 2002; Gill et al. 2002), should produce relatively more sons at hatching than unfed parents. Our data revealed that the probability of producing a male hatchling was significantly higher for fed parents, controlling for egg rank, thereby supporting our second prediction.

Chick growth and sexual dimorphism: prediction (1)

Our data showed that males were significantly heavier and larger than females close to fledging (for both maximum and asymptotic measures – except for maximum TL for A-chicks). Body mass (BM) increased faster in males than females, especially among B-chicks. This likely buffered the significant effect of rank on body mass at hatching as chicks from

second laid eggs were on average lighter than first laid chicks, leading to the observed sex difference in body mass close to fledging. Males were on average 7.7% heavier than females close to fledging, which is less than the expected 8.8% sex difference in mass among adults in our study population the same year (our unpublished data), suggesting that sexual dimorphism continues to increase after this stage.

Tarsus length (TL), which constitutes a good estimator of overall body size (Rising & Somers 1989), tended to grow faster in males than females. Unlike for BM, the rank effect on TL found at hatching remained significant close to fledging. Thus, we found that A-chicks reached a significantly higher asymptotic TL than B-chicks and that males reached a significantly higher asymptotic TL than females. This rank-related dimorphism close to fledging could potentially affect post-fledging survival, which has been shown to be lower in B- than A-chicks (Cam et al. 2003). As for BM, the degree of sexual dimorphism in TL in chicks close to fledging (3.9%, this study) was lower than the one found in adults in the same population and year (5%, our unpublished data).

It therefore seems, as expected, that most of the sexual dimorphism observed among adults develops during the chick rearing phase in kittiwakes. Our results are in line with previous studies on dimorphic species where sexual dimorphism (in BM and TL) has been shown to develop during the nest stage in a wide array of species, independently of which sex is bigger (for a review see Richner 1991).

Our results suggest that selective pressures favor larger size in kittiwake males, probably as a result of male-male competition. Hence, assuming that large males do comparatively well in terms of reproductive success, parents that are able to increase their investment in males by providing them with more (Anderson et al. 1993) or higher quality food (Magrath et al. 2004) should be favored. Alternatively, and non-exclusively, an advantage may be conferred on male chicks that increase their begging rate (Kitaysky et al. 2001a). However, chick

dimorphism may not fully reflect the differences in food received (Anderson et al. 1993; McDonald et al. 2005). Chick dimorphism may even occur without detectable sex-biased provisioning (e.g., Torres & Drummond 1999). In kittiwakes, however, as chick growth rate has been previously shown to be impacted by meal delivery rate (Jodice et al. 2008), it seems reasonable to expect males to receive more food than females (see also Chapter 1B, p. 65).

Feeding treatment and chick sex

Fed parents produce more male hatchlings than unfed parents: prediction (2)

Whatever the underlying mechanism leading to chick sexual dimorphism, parents producing higher than average quality chicks, as fed pairs do (Gill et al. 2002), are expected to increase their fitness return by producing more sons. Accordingly, we found that the probability of producing a male hatchling was significantly higher in experimentally fed than in unfed pairs, regardless of year and rank, thereby confirming our second prediction. As chick survival from hatching to the age of 35 days was not related to sex, this pattern hold for chicks close to fledging (our unpublished data).

Experimental feeding has already been used to study sex ratio variation in previous studies and notably in an experiment on a closely related species, the lesser black-backed gull (*Larus fuscus*) (Nager et al. 1999). In this species, the survival prospect of male offspring hatching from less well provisioned eggs, such as those laid in compensatory clutches, is lower than for females. Accordingly, the authors reported that unfed females, but not experimentally fed females, adaptively overproduced daughters with increasing overall egg production, induced by egg removal.

The next step toward confirmation of the adaptive value of the pattern revealed by our second prediction would clearly be a long-term study to assess the reproductive output of individuals born from both fed and unfed parents in relation to sex. If our understanding of the

underlying selective pressures is correct, we predict that males born to fed parents should show on average a substantially higher reproductive output than males born to unfed parents whereas such a difference should be much less marked in the case of females.

Within treatment: unfed pairs produce more female than male hatchlings

Our second and main prediction dealt with the relative difference between feeding treatments in the probability of producing a male, in line with models predicting a role of relative parental rearing capacities in shaping sex allocation patterns (Trivers & Willard 1973; Frank 1990; see also Hewison et al. 2002; Blanchard et al. 2005). However, in addition to these Trivers-Willard type selective pressures, the magnitude of the sex ratio deviation within unfed pairs we report, with a substantial bias towards females, is intriguing in itself.

Because hatching success was quite low for unfed parents (2006: 66%; 2007: 50%; 2009: 46%), we cannot rule out the possibility that the bias toward females in hatchlings sex ratio in that group was the result of higher embryo mortality for males. When males are the larger sex, their greater sensitivity, exacerbated by poor environmental conditions, is classically attributed to their higher nutritional requirements (Clutton-Brock et al. 1985). Here, however, we did not find any sexual dimorphism at hatching. Although size-independent traits may also lead to an increased sensitivity in males (Fairbairn et al. 2007), this explanation seems unlikely as our data reveal comparable mortality rates for both sexes during rearing (females: 75%, males: 70%).

Alternatively, given that from an energetic costs point of view, “the earlier the adjustment, the better” (Trivers & Willard 1973), the bias toward females within unfed pairs may instead have been generated at fertilization. Unbalanced sex ratios in poor years (or for poor quality parents) have been explained as the result of the differential costs of producing one sex or the other, with individual parents biasing their primary sex ratio toward the cheaper sex to diminish reproductive costs that may impact their own survival and/or subsequent

reproduction (i.e. the “cost of reproduction hypothesis”, Myers 1978; Wiebe & Bortolotti 1992, see also chapter 1B, p. 65). Sex ratio biases towards the less costly sex have been reported to correlate with poor environmental conditions (Weatherhead 2009). The Trivers-Willard and the reproductive cost hypotheses are not mutually exclusive. The former, as originally stated, focuses on expected offspring reproductive success in relation to parental capacity to invest (and may explain the relative difference between treatments, as suggested above in accordance with our second, main, prediction) while the latter focuses on the parental residual reproductive value (and may explain the strong bias towards females among unfed parents). The three years included in our sex ratio analyses were clearly poor years in terms of environmental conditions. As proxies of environmental conditions, we calculated fledging success and B-chick survival, and both were much lower during these three years than in 2008 (not included in the present analyses because too few chicks were sexed, see Materials and Methods): fledging success: 2006: 43%; 2007: 66%; 2008: 81%; 2009: 29%; B-chick survival: 2006: 13%; 2007: 38%; 2008: 87%; 2009: 44%). If unfed parents avoid producing males during poor years to limit reproductive costs, the skew towards females in sex ratio at hatching should be reduced during good years. Accordingly, although the sample size in 2008 is too low to allow any firm conclusions, the sex ratio at hatching was balanced in that year (four males, four females). Further studies are needed to confirm that prediction and to investigate the cost to parents of producing offspring of either sex in relation to their ability to provide care as well as annual variation in food availability.

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Chapter 1B: reproductive costs

Reproductive costs and offspring sex-ratio bias in a seabird

In preparation

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Coûts à la reproduction et biais de la sex-ratio chez un oiseau

marin

En préparation

RÉSUMÉ

Il est généralement admis que le sexe le plus lourd est plus coûteux à élever chez les espèces sexuellement dimorphiques. D'après l'hypothèse du coût à la reproduction, les parents en pauvre condition devrait éviter de produire ce sexe. Cependant, chez les oiseaux en particulier, il semble qu'être plus lourd ne veut pas nécessairement dire plus coûteux. Donc, pour pouvoir interpréter les patrons observés de sex-ratios, il est important de vérifier, chez une espèce donnée, si le sexe le plus lourd est effectivement plus coûteux. Chez la mouette tridactyle (*Rissa tridactyla*), une espèce avec un léger dimorphisme sexuel où les mâles sont plus lourds, nos précédents résultats concernant la sex-ratio pouvaient être interprétés comme résultant d'une pression de sélection de type coût à la reproduction et/ou de type Trivers-Willard. Dans le but de possiblement séparer ces deux possibilités, nous avons testé si les mâles étaient plus coûteux à élever que les femelles en utilisant deux jeux de données différents. Dans le premier, nous avons mesuré une hormone thyroïdienne (fT3) (i.e. un proxy du taux métabolique de base) et la dépense énergétique journalière (DEJ) au milieu de la période d'élevage des poussins et nous avons trouvé que, en fonction de la taille de ponte, une sex-ratio biaisée en faveur des mâles entraînait une augmentation de DEJ ou une réduction de fT3 chez les mères mais pas chez les pères, suggérant ainsi que les fils étaient plus coûteux à élever pour les mères uniquement. Dans le deuxième jeu de données, les poussins ont été échangés entre nids pour contrôler pour la qualité des parents. En accord avec les précédents

résultats, une sex-ratio biaisée vers les mâles conduisait à plus de dommages oxydants chez les mères mais pas chez les pères. Nous avons trouvé que le taux basal de corticostérone augmentait avec la sex-ratio quelque soit le sexe. Comme les fils semblaient être plus coûteux, du moins pour les mères, nous avons regardé si elles évitaient de produire des fils sur deux années consécutives. En effet, les couples qui n'avaient que des fils à l'envol en 2011 ont produit moins de fils que ceux qui avaient au moins une fille.

Mots clés : dépense énergétique ; dimorphisme sexuel ; dommages oxydants ; hypothèse du coût à la reproduction ; mouette tridactyle

ABSTRACT

It is generally admitted that in sexually dimorphic species the larger sex is energetically more costly to rear. According to the cost of reproduction hypothesis, parents in poor conditions should avoid producing this sex. However, in birds especially, there is some evidence that larger does not necessarily mean more costly. Hence, in order to interpret observed sex-ratio patterns, it is important to check whether the larger sex is indeed more costly in a given species. In the black-legged kittiwake (*Rissa tridactyla*), a slightly sexually dimorphic species where males are larger, we previously found sex-ratio patterns that could be interpreted as resulting from a cost of reproduction type pressure and/or a Trivers-Willard type pressure. In order to possibly disentangle between these two possibilities, we tested whether males are more costly to rear than females using two different datasets. In the first, we measured a thyroid hormone (fT3) (i.e. a proxy of basal metabolic rate) and daily energy expenditure (DEE) during mid chick rearing and found that, depending on brood size, a male-biased sex-ratio led to higher DEE or lower fT3 among mothers but not among fathers, thereby suggesting that sons were more costly to rear for mothers only. In our second dataset, chicks were cross-fostered to control for parental quality. In line with the results on the first dataset, a male-biased sex-ratio led to more oxidative damages among mothers but not among fathers. We also found that baseline corticosterone increased with sex-ratio in both sexes. As sons seemed to be more costly, at least for mothers, we investigated whether they avoided producing sons in consecutive years. Indeed, pairs that fledged only sons in 2011 had fewer sons the next year than those that fledged at least one daughter.

Keywords: black-legged kittiwake; cost of reproduction hypothesis; energy expenditure; oxidative damage; sexual dimorphism

INTRODUCTION

Sex allocation theory predicts that parents should invest differentially in male and female functions according to the sex providing the highest fitness return under current conditions (Charnov 1982; Frank 1990). Its empirical tests are some of the most striking examples in evolutionary biology, especially in insects (reviewed in Godfray 1994). Among birds and mammals, recent studies also provided evidence for extreme adaptive sex-ratio manipulation (e.g., Komdeur et al. 1997; Pryke & Griffith 2009; Thogerson et al. 2013), especially when fitness benefits are high (West & Sheldon 2002). Generally, however, results are more inconsistent probably because life histories are more complex and many selective pressures have been identified (reviewed in Cockburn et al. 2002), leading to either opposite (e.g., Hewison & Gaillard 1999) or identical predictions (e.g., Gomendio et al. 1990) concerning sex-ratio bias. One important challenge is thus to identify the different selective pressures acting on a given species, or even population (see Badyaev et al. 2002), as well as their relative importance in determining sex-ratio manipulation decisions.

In sexually dimorphic species, for example, two non-mutually exclusive hypotheses may apply: the cost of reproduction hypothesis (Myers 1978) and the Trivers-Willard hypothesis (Trivers & Willard 1973). In a species where males are larger, the former states that females in poor condition should avoid the production of males in order to lessen the impact of reproduction on their reproductive value (Cockburn et al. 2002). The latter, however, focused on the offspring reproductive value and predicts that females in good condition would gain more in terms of fitness return by overproducing sons, while the reverse would be true for females in poor condition. Most studies usually assume that the larger sex necessarily demand more parental energy expenditure, which has been found in numerous species with various extent of sexual dimorphism (e.g., Northern elephant seal: Reiter et al. 1978; red deer: Clutton-Brock et al. 1981; American kestrel: Anderson et al. 1993; fallow deer: Birgersson

1998; wandering albatross: Weimerskirch et al. 2000; European sparrowhawk: Vedder et al. 2005; common murre: Cameron-MacMillan et al. 2007; brown songlark: Magrath et al. 2007), but not in others with consequent sexual dimorphism (e.g., blue-footed booby: Torres & Drummond 1999; brown falcon: McDonald et al. 2005; Nazca booby: Townsend et al. 2007) or at least not to the same extent as sexual dimorphism would suggest (Anderson et al. 1993; Krijgsveld et al. 1998). Given these discrepancies, among bird species especially, and the weak or null support for a relationship between sexual dimorphism and hatching sex-ratio depending on the factors considered (Benito & Gonzalez-Solis 2007), it seems important to quantify parental energy expenditure according to offspring sex in order to be able to interpret the presence or absence of any potential sex-ratio bias in relation with the two hypotheses.

Contrary to the initial hypothesis that parental energy expenditure should be physiologically constrained (Drent & Daan 1980), most studies have found that energy expenditure was restricted by extrinsic factors such as food availability (e.g., Jodice et al. 2006; Welcker et al. 2009) or in response to a trade-off between energy expenditure and survival (e.g., Bryant 1991; Daan et al. 1996; Golet et al. 2004). If parents in good condition are better foragers, they might be able to increase their energy expenditure when rearing the more costly and/or the more advantageous sex without necessarily paying any fitness costs. There is, however, recent evidence that energy expenditure is intrinsically constrained in some seabird species and that individuals operate close to their energetic ceiling (Welcker et al. 2010; Elliott et al. 2013a), potentially making it hard (or impossible) for them to increase energy expenditure when rearing the more costly sex. One proposed mechanism to increase energy available for reproduction activities when facing a high demand would be to decrease self-maintenance, basal metabolic rate and/or other costly activities (Welcker et al. 2010; Elliott et al. 2013a).

Here, we wanted to test whether sons are equally costly than daughters or not in the black-legged kittiwake (*Rissa tridactyla*). This species is interesting in this regard for three reasons: (1) sexual dimorphism takes place during chick-rearing (males being 7.7 % heavier and 3.9 % larger than females near fledging: Merklings et al. 2012, p.34), but its extent is lower than in other bird species for which no sex-specific energy requirements have been found (e.g., Torres & Drummond 1999; McDonald et al. 2005), (2) there is evidence that parents operate close to an intrinsic energetic ceiling (Welcker et al. 2010; Welcker et al., submitted) and (3) sex-ratio bias according to parental condition (experimentally manipulated through supplementary feeding) has been found (Merklings et al. 2012, p.34), but we were unable to discriminate between the cost of reproduction and Trivers-Willard hypotheses. Hence, a higher rearing cost of males is not obvious to assume according to the first and second reason. If both sexes require the same energy expenditure from their parents we could thus reasonably discard the cost of reproduction hypothesis as an explanation of our previous results.

To investigate whether sex-specific costs exist or not in kittiwakes, we used two sets of data. The first set came from a brood size manipulation experiment among experimentally fed and unfed birds. Daily energy expenditure (hereafter called 'DEE') and plasma concentrations of free (unbound) triiodothyronine (hereafter called 'fT3') were measured during mid-chick rearing (Welcker *et al.* submitted). fT3 is a good proxy of basal metabolic rate, as shown in two seabird species including kittiwakes (Elliott et al. 2013b; Welcker et al. 2013) and it avoids additional handling of birds in a respiratory chamber. We thus predicted that, if males are more costly to rear, parents should increase DEE and/or decrease fT3 levels with brood sex ratio. In the second dataset, from another breeding season and different birds, we cross fostered chicks (i.e. to control for parental quality) and investigated whether brood sex ratio influenced oxidative damage to lipids (levels of malondialdehyde, hereafter called 'MDA', in plasma and red blood cells) and baseline corticosterone levels (hereafter called 'CORT'). We

predicted that, if males are more costly to rear, parental metabolism should increase with brood sex ratio and therefore lead to increased oxidative damage and CORT levels. Finally, as our results suggested a higher rearing cost for sons than for daughters, we tested a prediction that could be made in line with the cost of reproduction hypothesis: parents should avoid producing males in consecutive years, as found in some mammal species (Bérubé et al. 1996; Monard et al. 1997), but never in birds.

MATERIALS AND METHODS

Study site

The study was conducted in 2011 and 2012 in a population of individually marked black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska. This tower is a twelve-walled polygon where artificial nest sites have been created on the upper walls. Nest sites are fitted with one-way mirror glass window panes and hence can conveniently be accessed from within the building (for more details see in Gill & Hatch 2002). This setup facilitated observations, rapid capture and handling of both adult birds and their offspring. Nests were checked twice daily during the entire breeding seasons to document events such as laying, hatching and chick mortality. We considered chicks as fledged if they actually fledged or reached thirty days, as chick mortality is very low after this age (our unpublished data). Kittiwakes usually lay two eggs and throughout the paper, we will refer to first-hatched chicks as A-chicks and to second-hatched chicks as B-chicks.

Brood size manipulation: Protocol

To investigate the energetic cost of rearing sons versus daughters, we used data from a brood size manipulation experiment conducted in 2011 on Middleton Island (for more details see supplementary material). Pairs were either allowed to rear their two chicks ('Control' group) or were given an extra chick to rear ('Enlargement' group). In each group half of the nests were also part of a supplemental feeding experiment (see below). In total we considered fifty-six parents from twenty-eight nests (twelve unfed and sixteen fed nests).

Brood size manipulation: Feeding treatment

Supplementally fed birds were offered food *ad libitum* three times a day (at 09:00, 14:00 and 18:00 local time) starting prior to egg-laying and continuing throughout the experiment (for more details see supplementary material). All the pairs used in 2011 and 2012 remained at the same nest site and thus at the same feeding treatment.

Brood size manipulation: sampling and physiological measures

All chicks ($N = 71$) were blood sampled at hatching ($\sim 50\mu\text{l}$) from the tarsal vein for molecular sexing (for more details see supplementary material).

All adult birds were sampled for DEE (estimated by the doubly-labeled water: Lifson & McClintock 1966; Butler et al. 2004) and plasma concentrations of fT3. Sampling started when the A-chick of a nest reached the age of eight days. Both parents were caught within a time lag of one day. Sampling was completed when chicks were approx. twelve days old. Chick age at capture did not differ between parents (Wilcoxon test: $W=396$; $p = 0.95$).

DEE measure was done by isotope ratio mass spectrometry at the University of Aberdeen as described in Speakman and Krol (2005). Further details concerning the method used to determine daily energy expenditure are given in Welcker et al. (submitted).

fT3 levels were determined based on a commercially available radioimmunoassay kit (MP Biomedicals) optimized for our study species (for more details see supplementary material).

Brood size manipulation: sex of the chick produced the subsequent year

Among the twenty-eight pairs considered from the brood size manipulation experiment eighteen bred together in 2012 again. There was a significant effect of feeding treatment on pair bonding: unfed birds were less likely to be sighted with the same partner (either because of divorce or survival) than fed birds (GLM binomial: $\chi^2_1 = 4.77$, $P = 0.029$). Birds resighted together in 2012 and six other pairs initially part of the ‘Enlargement’ group in 2011 but not caught because they lost one chick before adult measurements, reared at least one chick until fledging, thus enabling us to have information on the fledging sex ratio in 2011. We did not consider pairs that successfully reared three chicks to fledging in 2011 as they were only fed birds and we also discarded data concerning single and third-hatched chicks as they were too few. We took a blood sample for each chick at hatching ($N = 36$) using the same method as in 2011 (see ‘Sampling’ section). Moreover, we also considered a larger dataset including the previous chicks plus all the chicks from pairs of the feeding experiment that were not included in the brood size manipulation experiment in 2011 but reared one or two chicks to fledging and were resighted in 2012 ($N = 66$ chicks).

Physiological cost: sampling

This part of the study was conducted in 2012 among a separate set of nests that were not included in the brood size manipulation experiment. A random selection of these birds was also supplementally fed from our arrival to the laying of their second egg (see ‘Feeding treatment’ section). We captured male and female parent prior to laying (range: 0 – 27 days prior to laying; mean \pm SE: 11.15 ± 0.87 days; $N = 51$ birds) and/or during late chick-rearing (chick age range: 21 – 36 days; mean \pm SE: 28.38 ± 0.34 days; $N = 61$ birds). All birds caught

prior to laying were also recaptured during chick rearing. At capture, we took a blood sample from the alar vein. Blood samples were taken within three minutes of capture in order to estimate baseline hormone levels and avoid an effect of handling stress (Romero & Reed 2005). Blood was centrifuged, plasma separated from red blood cells and both were frozen at -20°C until analysis to determine baseline CORT levels, MDA levels in plasma (hereafter called 'plasma MDA') and in red blood cells (hereafter called 'RBC MDA').

Physiological cost: physiological assays

CORT analyses for the 2012 samples were performed at the Centre d'Etudes Biologiques de Chizé (for more details see supplementary material). We determined CORT for thirty-three individuals both before laying and during chick-rearing and we had forty-four individuals caught at least during chick-rearing.

Estimation of plasma MDA and RBC MDA levels was performed at the Institute of Biology of the University of Neuchâtel (for more details see supplementary material). We determined MDA plasma levels for forty-six individuals both before laying and during chick rearing while we had fifty-eight individuals caught at least during chick-rearing. For MDA RBC levels, numbers of individuals were forty-two and fifty-seven, respectively. We completely removed from further analyses two outliers with MDA RBC levels at second capture higher than 800 nmol/ml.

Statistical analyses

For all analyses, as a proxy of parental effort, we calculated the cumulative number of days that parents reared their chicks, hereafter called 'ChickDays', during the period considered (e.g. two chicks reared during fifteen days = thirty days). We also determined the proportion of these days which were concerned by male chicks, hereafter called 'PropDaysMale' (e.g.

fourty 'ChickDays', one male during fifteen days and one female during twenty-five days = $15/40 = 0.375$ PropDaysMale).

We always started with a complete model and successively removed each term beginning with the terms of the highest degree. We then compared the change in deviance after removal of that term using a χ^2 test for linear mixed models (LMM) and generalized linear mixed models (GLMM) ran with *lme4* package (Bates et al. 2011). In all models, nest was included as a random effect to control for the non independence of individuals within the same nest. Pair breeding experience was also included as a covariate (only reported when significant; for more details see supplementary material), All analyses were conducted with R 2.14.2 (R Development Core Team 2012). Mean values are shown \pm standard error (SE).

ChickDays was divided into two categories for analyses concerning the brood size manipulation experiment (for more details see supplementary material). In order to meet model assumptions (residuals normality and homoscedasticity), ft3 was log-transformed. As body mass affected DEE, we calculated mass-independent DEE as the residuals of the regression of DEE on body mass (for more details, see Welcker et al., submitted). The complete model contained all three-way interactions between ChickDays, PropDaysMale, parental sex and feeding treatment.

For the analyses concerning a physiological cost of rearing more males in the 2012 data, we did two sets of analyses: first with the difference between second and first capture for all three response variables (MDA plasma, MDA RBC and CORT) and second with only the value of the second capture (i.e. at late chick rearing). In these analyses, PropDaysMale was divided into three categories (for more details see supplementary material). In order to meet model assumptions (residuals normality and homoscedasticity), all variables at recapture were log-transformed. The complete model contained the three-way interaction between

ChickDays, PropDaysMale and Parental Sex (for information concerning covariates see supplementary material).

For analyses concerning the sex produced in 2012 according to 2011 fledging sex ratio, we calculated ChickDays and PropDaysMale during the whole chick rearing period and considering forty ChickDays for chicks for which fledging was not sighted but very likely (i.e. chicks still alive when we left the island). We used a GLMM with a binomial error distribution and a logit link function. The complete model contained the three two-way interactions between PropDaysMale and ChickDays, egg rank and feeding treatment, respectively. We did exactly the same with fledging sex ratio in 2011 instead of PropDaysMale and number of fledgings instead of ChickDays for the same dataset and for a larger dataset considering all pairs from the feeding experiment present in 2011 and 2012.

RESULTS

Brood size manipulation: Energy expenditure parameters

fT3 levels were significantly affected by the interaction between ChickDays and PropDaysMale ($\chi^2_1 = 7.78$; $p = 0.005$). Across treatments, fT3 levels did not change with PropDaysMale in 2-chick broods ($\chi^2_1 = 1.2$; $p = 0.27$; Figure 1a), whereas it significantly decreased with PropDaysMale in 3-chick broods ($\chi^2_1 = 4.53$; $p = 0.03$; Figure 1b). Fathers had also significantly higher levels of fT3 than mothers ($\chi^2_1 = 7.08$; $p = 0.008$) and unfed birds significantly lower levels of fT3 than fed birds ($\chi^2_1 = 9.04$; $p = 0.002$). Moreover, fT3 levels decreased with pair experience ($\chi^2_2 = 6.38$; $p = 0.041$).

Interestingly, mass-independent DEE was also affected by the interaction between ChickDays and PropDaysMale ($\chi^2_1 = 7.77$; $p = 0.005$), but in a different way than fT3 levels. Within ChickDays classes, mass-independent DEE significantly increased with PropDaysMale in two-chick broods ($\chi^2_1 = 6.35$; $p = 0.012$; Figure 1c), whereas it did not

change with PropDaysMale in three-chick broods ($\chi^2_1 = 0.50$; Figure 1d). Mass-independent DEE was also affected by the interaction between parental sex and PropDaysMale ($\chi^2_1 = 4.59$; $p = 0.032$): it increased with PropDaysMale among mothers ($F_{25,26} = 8.51$; $p = 0.007$) but did not among fathers ($F_{25,26} = 0.02$; $p = 0.89$). Finally, mass-independent DEE was affected by the interaction between parental sex and ChickDays ($\chi^2_1 = 4.47$; $p = 0.035$): it increased with ChickDays among mothers ($F_{25,26} = 7.85$; $p = 0.01$) but not among fathers ($F_{25,26} = 0.01$; $p = 0.98$).

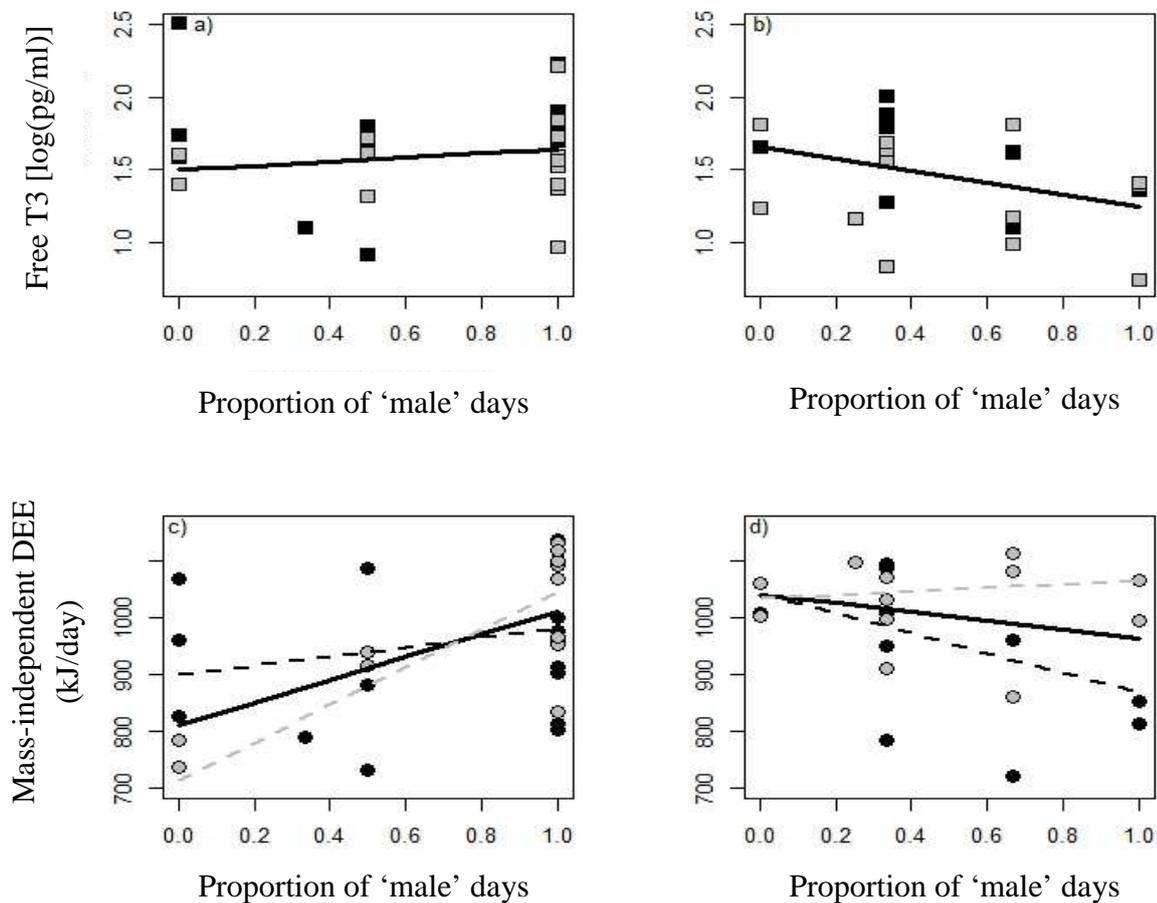


Figure 1. Free T3 levels [log(pg/ml)] and DEE (kJ/day) according to parental sex, ChickDays and PropDaysMale. Black and grey squares represent free T3 levels data in a) 2-chick broods and b) 3-chick broods for males and females respectively. Black and grey circles represent DEE data in c) 2-chick broods and d) 3-chick broods for males and females respectively. Black lines are the linear mixed model predictions. As parental sex significantly interacted with PropDaysMale and ChickDays, we also represent the linear mixed model predictions for males (black dashed line) and females (grey dashed line) in (c) 2- and (d) 3-chicks broods.

Physiological cost

MDA plasma difference between captures significantly depended on the interaction between parental sex and PropDaysMale ($\chi^2_1 = 7.08$; $p = 0.008$). Model assumptions were better met and the interaction was even more significant when removing one outlier with a very low difference ($\chi^2_1 = 18.75$; $p < 0.0001$). For mothers, MDA plasma difference between captures increased with PropDaysMale (with outlier: $F_{24,25} = 1.46$; $p = 0.24$; without outlier: $F_{23,24} = 10.19$; $p = 0.005$; Figure 2), whereas it decreased for fathers (both with and without outlier: $F_{18,19} = 4.90$; $p = 0.043$; Figure 2). The more the first capture was done away from laying the more MDA plasma difference between captures was high (with outlier: $\chi^2_1 = 10.33$; $p = 0.001$; without outlier: $\chi^2_1 = 13.21$; $p = 0.0002$). Moreover, pairs breeding for the first time together had a significantly higher MDA plasma difference between captures than the others, but only when removing the outlier (without outlier: $\chi^2_2 = 11.02$, $p = 0.004$; with outlier: $\chi^2_2 = 1.86$; $p = 0.39$).

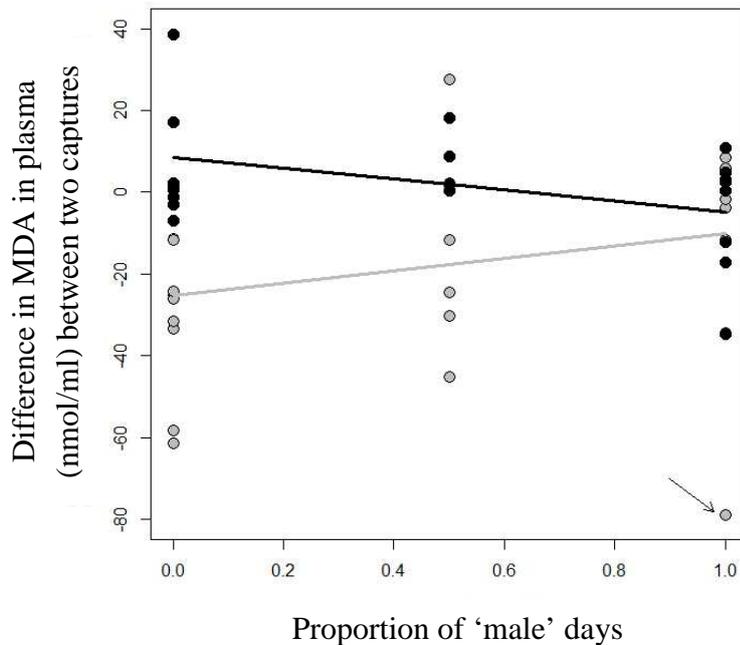


Figure 2. Difference in MDA plasma (nmol/ml) in relation with PropDaysMale and parental sex. Circles represent data and lines represent the linear mixed model predictions (black: males; grey: females). The outlier is shown by the arrow.

For MDA RBC difference between captures, no interactions or single terms were significant (all $p > 0.11$).

CORT difference between captures significantly increased with PropDaysMale ($\chi^2_1 = 5.26$; $p = 0.021$; Figure 3), independently of parental sex.

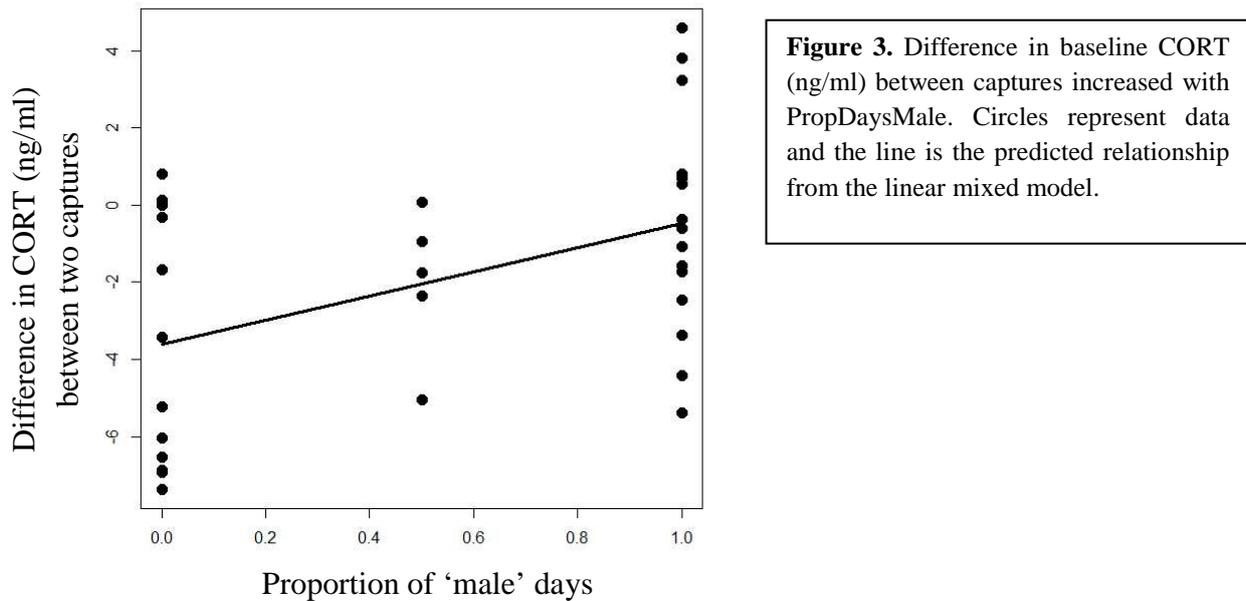


Figure 3. Difference in baseline CORT (ng/ml) between captures increased with PropDaysMale. Circles represent data and the line is the predicted relationship from the linear mixed model.

MDA plasma at recapture significantly increased with ChickDays ($\chi^2_1 = 4.03$; $p = 0.044$). MDA plasma at recapture was also significantly higher in pair that first bred together in 2012 as compared to more experienced pairs ($\chi^2_2 = 9.24$; $p = 0.01$). The interaction between parental sex and PropDaysMale tended to be significant ($\chi^2_1 = 3.13$; $p = 0.077$): MDA plasma at recapture tended to increase in females, but not in males.

For MDA RBC at recapture, the interaction between ChickDays and PropDaysMale tended to be significant ($\chi^2_1 = 2.94$; $p = 0.086$), with a positive effect of ChickDays only in all-male broods.

CORT at recapture significantly increased with PropDaysMale ($\chi^2_1 = 3.81$; $p = 0.051$). Moreover, the interaction between PropDaysMale and ChickDays was marginally significant ($\chi^2_1 = 3.23$, $p = 0.072$).

Brood size manipulation: Sex of the chick produced the subsequent year

The probability of producing a son in 2012 tended to decrease with an increasing PropDaysMale in 2011 ($\chi^2_1 = 2.95$; $p = 0.086$). When considering fledging sex ratio in 2011 instead of PropDaysMale, the effect was significant ($\chi^2_1 = 4.65$, $p = 0.031$; Figure 4). In the larger dataset considering all pairs included or not in the brood size manipulation experiment, the effect of fledging sex ratio in 2011 was even stronger ($\chi^2_1 = 5.21$, $p = 0.022$). Moreover, when we considered only nests where both chicks hatched, fledging sex ratio in 2011 tended to be significant ($\chi^2_1 = 3.0$; $p = 0.083$).

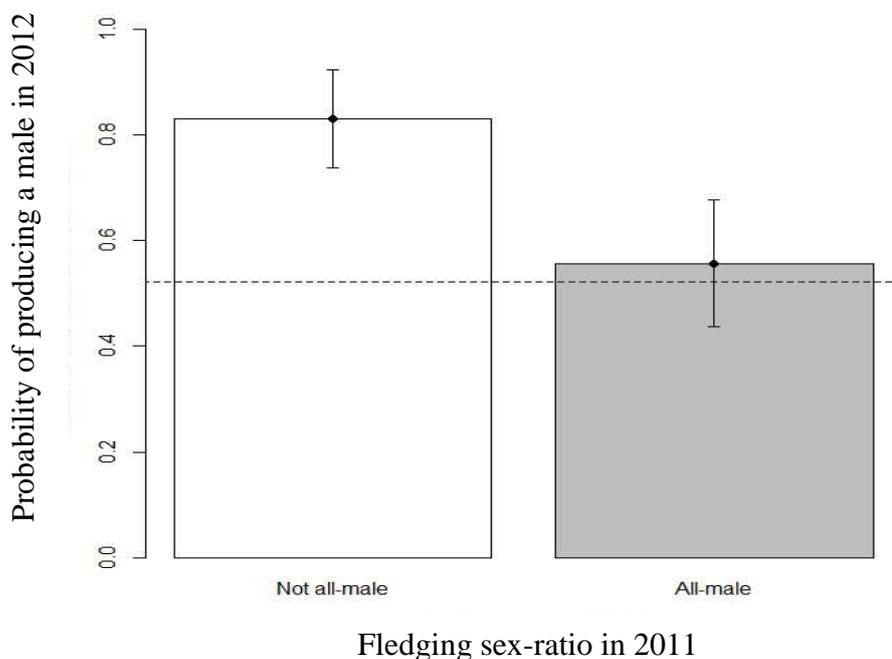


Figure 4. Probability of producing a male in 2012 (\pm SE) in relation to fledging sex ratio in 2011. The white bar represents broods composed of at least one female in 2011 and the grey bar represents broods with only males in 2011. The dashed line is the population sex ratio in 2012 in the nests involved in the supplemental feeding experiment.

DISCUSSION

Our aim was to investigate whether sons are more costly to rear than daughters in a species operating close to its energetic ceiling and with a slight sexual dimorphism in order to better interpret previously found sex ratio patterns. Our results suggest a higher energetic cost of rearing sons for mothers especially and it is expressed either by increased DEE or reduced fT3 (i.e. a proxy of basal metabolic rate), as well as by increased CORT levels and oxidative damage to lipids. We can therefore not exclude the cost for reproduction hypothesis (Myers 1978) and the possibility that unfed birds overproduced daughters as compared to fed birds in response to the higher reproductive cost of sons, as previously found in these species (Merkling et al. 2012, p.34). Moreover, we also provide evidence that pairs avoid the production of sons when they fledged only males the previous year as compared to pairs fledging at least one female.

Reproductive cost

Among the five physiological variables we considered to investigate sex-specific reproductive cost, three of them suggested that sons were more costly to rear, but only for mothers. Specifically, we found that DEE increased with PropDaysMale in two-chick broods, while it remains high and constant in three-chick broods (i.e. when parental effort was increased). We also found that mothers increased DEE with PropDaysMale independently of ChickDays, but fathers did not. They increased DEE with ChickDays independently of PropDaysMale as well, contrary to their mates. Indeed, representing DEE within brood size and by sex (see Fig 1) suggested that mothers increased DEE with PropDaysMale more than fathers in two-chick broods, whereas mothers remain at a high constant DEE and fathers decreased their DEE in three-chick broods. The three-way interaction between parental sex, PropDaysMale and ChickDays was not significant however ($p = 0.53$), but the interpretation of the three two-way

interactions within it suggest that with more statistical power it could have become significant. We suggest that sons were more costly to rear than daughters for mothers in three-chick broods also, because despite the high constant DEE they had reduced FT3 levels with increasing PropDaysMale. Mothers thus seem to reduce their basal metabolic rate to increase the energy available for reproduction and for their sons especially, most likely because they reached their intrinsic energetic ceiling (Welcker et al. 2010). Contrary to mothers, fathers seem to respond less strongly to PropDaysMale in two-chick broods and not at all in three-chick broods. Indeed, in three-chick broods they seemed to decrease their DEE with increasing PropDaysMale, but they decreased their basal metabolic rate concomitantly, maybe in order to keep a constant amount of energy available for reproduction. One possible explanation for this pattern among fathers is that operating close to the energetic ceiling is more costly than decreasing metabolic rate. Moreover, our results also suggest that unfed birds allocated more energy to reproduction than fed birds as they exhibited a lower basal metabolic rate and there was no difference in DEE according to feeding treatment. The absence of interaction between PropDayMale and feeding treatment for FT3 and DEE indicates that sons were not more costly to rear for unfed birds than for fed birds, contrary to what we could have expected.

Interestingly, our results on oxidative damage on lipids, yet on other individuals and another breeding season, corroborate the higher reproductive cost of sons for mothers and not for fathers. Indeed, we found that MDA plasma increased with PropDaysMale between laying and chick-rearing for mothers, whereas it decreased for fathers. It seems that the increased energy expenditure required to rear sons led to oxidative stress among mothers. In line with this, recent studies found that an increased energy expenditure can lead to increased oxidative stress among fathers (Losdat et al. 2011a; van de Crommenacker et al. 2011; Heiss & Schoech 2012) and mothers (Fletcher et al. 2013), but no studies has to our knowledge investigated

whether brood sex-ratio can influence oxidative balance and we suggest that manipulating brood sex-ratio could be another way to measure the oxidative cost of reproduction (Metcalf & Monaghan 2013). Yet, we have to be cautious concerning our findings, as we did not measure antioxidant defences or repair (Metcalf & Monaghan 2013) and our time lag was quite long and transient effect have already been demonstrated (Losdat et al. 2011a). Interestingly, it has been shown that, during chick rearing, male kittiwakes had higher levels of total plasma carotenoids, which comprise antioxidant molecules, than females (Leclaire 2010). Hence, high antioxidant activity and lower energy expenditure might explain their lower MDA plasma levels.

The increase in DEE with PropDaysMale is most likely due to an increase in foraging activity to get more food for sons. Corticosterone is known to be related to foraging behaviour (e.g., Chastel et al. 2005; Angelier et al. 2007a). Therefore the positive relationship we found between CORT levels and PropDaysMale seems logical, but for mothers only. Among fathers, this increase might indicate that males behaviourally responded to sex-ratio but in a way that we could not identify.

Altogether, our results suggest that the reproductive cost of rearing sons was mostly borne by mothers. Such a sex-specific response to offspring sex has already been demonstrated, but in wandering albatrosses (*Diomedea exulans*) males seem to invest heavily in both sexes, whereas females invest less than their partner but more in sons than in daughters (Weimerskirch et al. 2000). In zebra finches (*Taeniopygia guttata*), females biased their investment towards sons, whereas males invested equally in both sexes (Mainwaring et al. 2011). Our results might thus be the consequence of a sexual conflict of interest between parents on how much care to provide to offspring according to their sex (Lessells 2002a, 2002b), but more data are needed on sex-specific feeding behaviour according to offspring sex in order to get more insight into this possibility (Mainwaring et al. 2011).

The main aim of this study was to get more knowledge about the selection pressures acting on the species concerning sex allocation. Our results do not allow us to discard the cost of reproduction hypothesis (Myers 1978) as an explanation to our previous results (Merkling et al. 2012, p.34). The female biased sex-ratio among unfed pairs could thus be a response to the higher energy expenditure needed to rear sons by mothers. This study has been conducted during two seasons with likely higher food availability than the seasons (2006, 2007 and 2009) considered in our previous study (Hatch 2013). It is therefore possible that rearing a son was even more costly for mothers during poorer food years, which should have increased the selection pressure towards the avoidance of the costly sex among unfed birds. Interestingly, it seems that unfed birds were almost as likely to produce males as fed birds during better food year (our unpublished data).

Sex of the chick produced the subsequent year

As our results did not allow us to discard the cost of reproduction hypothesis (Myers 1978), we decided to further test its validity in kittiwakes. Examples supporting the cost of reproduction hypothesis (Myers 1978) can be divided into two categories: higher reproductive cost of one sex can impact (1) the current breeding event or (2) the survival and/or subsequent breeding events. The first category can be illustrated by a study on American kestrels (*Falco sparverius*) showing that females in poor condition or during poor food years overproduced males (i.e. the smaller sex in this species) (Wiebe & Bortolotti 1992) or by the fact that southern elephant seal mothers (*Microunga leonina*) always produced pups of the smaller sex (i.e. females) when they weighed less than 380 kg (Arnbom et al. 1994). The second category can be illustrated by the fact that in bighorn sheep (*Ovis canadensis*) ewes were more likely to produce a daughter when they weaned a son the year before and lamb survival was lower when born the year after a son than after a daughter (Bérubé et al. 1996). To our knowledge, there are no such findings in birds; hence we provided here the first evidence that birds can

bias offspring sex according to the sex fledged the year before. Indeed, we found that pairs that fledged only males in 2011 produced relatively less sons in 2012 than pairs that fledged at least one female. As the effect remains, not significantly though, when considering only nests where both chicks hatched, we can reasonably discard the hypothesis of a higher mortality of male embryo. The observed sex-ratio bias could thus have occurred at conception and be adaptive, but further studies should confirm this pattern.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Brood size manipulation: protocol

The data we used here came from a brood size manipulation experiment conducted in 2011 on Middleton Island (for more details, see Welcker *et al.* submitted). This study comprised sixty-five nests randomly allocated to three experimental groups: 'Removal', where the whole brood was removed after hatching, 'Control', where parents reared their own brood and 'Enlargement', where parents had to rear three chicks (i.e. one more than the modal brood size). In the 'Enlargement' group, foster chicks were not allowed to be either older or younger than the A- or B-chick of the foster nest, respectively. In kittiwakes, fostering is facilitated by the absence of parent-young recognition in young chicks (Storey *et al.* 1992). Here, we obviously focused only on nests from the 'Control' and 'Enlargement' groups as birds in the 'Removal' group did not rear any chicks. We also excluded nests where the two chicks did not hatch to avoid a potential parental quality bias and only considered nests having at least two chicks at the time of capture (see below) as we had too few data for one-chick brood nests.

Brood size manipulation: feeding treatment

Supplemental food consisted of thawed Atlantic capelin (*Mallotus villosus*), a species similar to naturally preferred prey species of kittiwakes at the study site (Hatch 2013). Food was offered through a plastic tube at the nest sites inaccessible to neighboring nests (see more details in Gill & Hatch 2002), and a feeding event continued until the parent(s) present at the nest stopped taking fish. Although not all individuals were present at all feeding events all occupants of fed nests generally accepted offered fish. Supplementally fed kittiwakes

continue to forage at sea and the amount of food consumed varies with natural food availability (Gill & Hatch 2002; SA Hatch, unpublished data).

Brood size manipulation: sampling

Chick blood sampling was done using a capillary tube and a sterile 25-gauge needle (for a detailed molecular sexing protocol see Merklings et al. 2012, p.34). Samples were kept in Longmire buffer (Longmire et al. 1988) and stored at -20°C until analysis.

Adult birds were captured at the nest with a metal hook or nylon noose. They were then weighed and intraperitoneally injected with a dose of 1.25ml DLW containing 65.15 atom percent excess (APE) oxygen-18 (^{18}O) and 36.5 APE deuterium (^2H). We then placed birds in a cloth bag for one hour to allow for complete equilibration of isotopes with the body water of the injected animal. Prior to release, birds were re-weighed and a blood sample was taken from the alar vein in order to estimate initial enrichment of isotopes. Blood was collected into several 75 μl glass micro-capillaries which we flame-sealed immediately. In addition, we took blood samples of twelve unlabeled kittiwakes to determine mean background level of isotopes in the study population (Speakman & Racey 1987: method C).

We attempted to recapture all individuals three days after injection (mean \pm SD: 70.0h \pm 4.7) as extended measurement periods reduce the error due to high day-to-day variation in energy expenditure (Speakman et al. 1994; Berteaux et al. 1996). Upon recapture birds were weighed again and a second blood sample taken as described above in order to estimate final enrichment of isotopes and to determine plasma concentrations of fT3.

fT3 concentrations were estimated at the Institute of Arctic Biology, Fairbanks. The dose-response curve of pooled kittiwake plasma was parallel with standard curves of the assay kit. All hormone samples were analyzed in duplicate. Inter-assay variation was less than 4.6% and intra-assay variation was less than 1.9%.

Physiological cost: sampling

At twenty-five days after laying, eggs were put in an incubator (Compact S 84 MP GTFS, Grumbach Brutgeraete GmbH, Asslar, Germany, set at 37.4°C and 63% of humidity) to closely monitor hatching, which usually occurred at twenty-seven days after laying (Hatch et al. 2009). A blood sample was taken for all their chicks at hatching using the same method as described above (see previous ‘Sampling’ section). Parents were given foster chicks for which hatching asynchrony was manipulated by creating three types of broods: ‘Synchronous’, where both chicks hatched within twenty-four hours; ‘Control’, where the A-chick hatched more than two days before the B-chick and ‘Reversed’, where the B-chick hatched more than two days before the A-chick. However, data analyses revealed that CORT and MDA levels were not affected by the interaction between hatching asynchrony treatment and the proportion of male in the brood (all $p > 0.07$) and we thus present here results without taking into account this variable. However, results were similar when adding it as a covariate.

Physiological cost: CORT assays

CORT plasma concentrations were determined in one assay following methods described in Lormée et al. (2003). The detection limit was 0.3 ng/mL, and the intra-assay coefficient of variation was 6% (n=5 duplicates).

Physiological cost: oxidative damage to lipids

Malondialdehydes (MDA) are formed by the β -scission of peroxidized fatty acids. MDA concentrations in plasma and red blood cells were assessed using HPLC with fluorescence detection, as described previously (Losdat et al. 2011b) with some modifications. Blood was centrifuged upon collection. 5 μ l of red blood cells were diluted in 45 μ l of phosphate buffer saline (PBS), sonicated for 10 minutes in an ice-bath, homogenized using a mixer mill and a

tungsten bead at 30 Hz for 1 minute, and centrifuged for 10 minutes at 10'000 rpm and 4°C. All chemicals were HPLC grade, and chemical solutions were prepared using ultra pure water (Milli-Q Synthesis; Millipore Corporation, Billerica, MA, USA). Sample derivitization was done in 2 ml capacity conical-bottom screw-top microcentrifuge tubes. To a 5 µl aliquot of sample (plasma or red blood cell homogenate supernatant) or standard (1,1,3,3-tetraethoxypropane, TEP; see below) 5 µl butylated hydroxytoluene (BHT) solution (0.05% w/v in 95 % ethanol), 40 µl phosphoric acid solution (0.44 M), and 10 µl thiobarbituric acid (TBA) solution (42 mM) were added. Samples were capped, vortex mixed for 5 seconds, then heated at 100°C for exactly 1 hour in a dry bath incubator to allow formation of MDA-TBA adducts. Samples were then cooled on ice for 5 minutes, before 100 µl n-butanol was added and tubes were vortex mixed for 20 seconds. Tubes were then centrifuged at 12'000 rpm and 4 °C for 3 minutes. A 70 µl aliquot of the epiphase was collected and transferred to an HPLC vial for analysis. Samples (5 µl) were injected into a Dionex Ultimate 3000 Rapid Separation LC system (Dionex Corporation, California, USA) fitted with a GL Sciences Inc. (Tokyo, Japan) Inerstil 2µ ODS-4 2.1 x 100 mm column maintained at 37°C. The mobile phase was methanol-buffer (30:70, v/v), the buffer being a 50mM anhydrous solution of potassium monobasic phosphate at pH 6.8 (adjusted using 5M potassium hydroxide solution), running isocratically over 6 min at a flow rate of 0.3 ml.min⁻¹. Data were collected using a fluorescence detector set at 515 nm (excitation) and 553 nm (emission). For calibration, a standard curve was prepared using a TEP stock solution (5 µM in 40% ethanol) serially diluted using 40% ethanol. TEP standards were assayed in quintuplicate and showed very high repeatability ($r = 0.996$, $P < 0.0001$, $n = 13$). Repeatability assessed using plasma samples was very high ($r = 0.90$, $P < 0.0001$, $n = 12$). FH did all analyses blindly with respect to treatments.

Statistical analyses

Pair breeding experience was separated into three classes: '0' for first breeding attempt together, '1' for less than or two previous breeding attempts together and '3' for at least three previous breeding attempts.

For analyses concerning the physiological cost of rearing more males in the brood size manipulation experiment, we calculated ChickDays and PropDaysMale for the three days between captures. Hence ChickDays ranged between six and nine. There were more all-males broods in the two-chick broods resulting in a significant colinearity between ChickDays and PropDaysMale (Spearman's correlation test: $\rho = -0.29$; $p = 0.029$). To take this into account we divided ChickDays into two categories: '6' for six-seven ChickDays (i.e. mostly two-chick broods) and '9' for eight-nine ChickDays (i.e. mostly three-chick broods).

In the dataset concerning the physiological cost of rearing a male in 2012, mixed-sex broods at recapture had significantly higher ChickDays than single-sex broods (Kruskal-Wallis test: $\chi^2_2 = 20.78$; $p < 0.0001$). This is normal as we recaptured parents raising at least one chick: mixed-sex broods at this time could only occur when the two chicks were still alive, leading to a higher ChickDays number than other nests which could have one or two chicks alive at the time of recapture. To take this colinearity into account, we separated PropDaysMale into three classes: '0' when PropDaysMale was lower or equal to 0.3; '0.5' when PropDaysMale was between 0.3 and 0.7 included; '1' when PropDaysMale was higher than 0.7. For the models with the difference in MDA plasma, MDC RBC and CORT between captures, we added feeding treatment, date of first capture and time before laying as covariates (only reported when significant). For the models with MDA plasma, MDC RBC

and CORT at second capture, we added feeding treatment and date of second capture as covariates (only reported when significant).

Box 2: Is it reasonable to apply the Trivers-Willard hypothesis to the kittiwake?

Some behavioural and life-history traits of the kittiwake suggest that a Trivers-Willard type pressure could apply (see Introduction Part 1, p. 18 and Chapter 1A, p. 41). Our hypothesis was that males should benefit more in terms of reproductive success than females from a high parental ability to care. Hence, according to this hypothesis we expected males born on fed nests to have a higher lifetime reproductive success as adults than males born on unfed nests, whereas the difference should be lower or nonexistent among females. A bird lifetime reproductive success can be influenced by the location where it recruits, by its survival or by the number of breeding events for example. Here, I used the data of the long-term feeding experiment to investigate where the chicks born on these experimental nests recruited and how many fledglings they produced according to their parents' feeding treatment.

Since 1996, there are only one hundred and forty-one birds (thirty-one females and one hundred and ten males) that were reared on a nest of the long-term feeding experiment and subsequently recruited into one of these nests. For each of these birds, I knew when and where they were born, where and when they recruited and at which age, how many times they bred (i.e. laid at least one egg) and how many fledglings they produced during this period. Vincenzi et al. (2013) already showed that chicks born on fed nests were more likely to recruit into fed nests and conversely for chicks born on unfed nests. This is especially true for males, but not for females (Fig. 1). Further analyses revealed that males recruited in the same panel of the tower that they were reared on ($\chi^2_3 = 61.5, p < 0.0001$), thereby showing strong philopatry, whereas females avoided the panel where they were born ($\chi^2_3 = 8.03, p = 0.045$). Hence, fed parents fledging a male have higher chance that it recruits on a fed nest than unfed

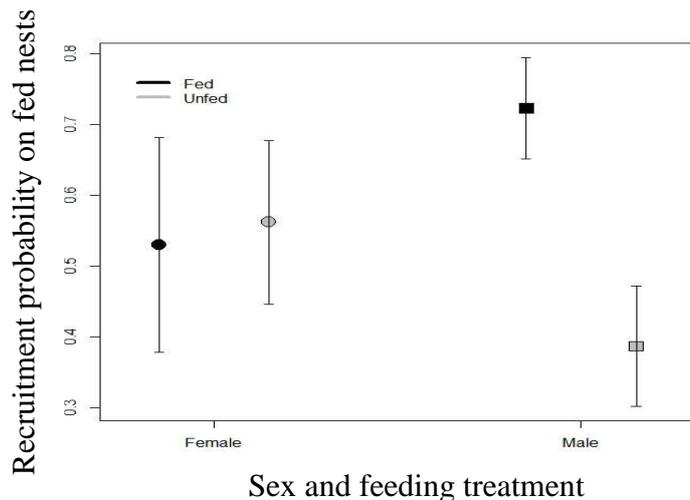


Figure 1. Recruitment probability on fed nests according to sex and feeding treatment as a chick. Circles represent females and squares represent males. Black symbols are for chicks born on fed nests and grey symbols for chicks born on unfed nests.

parents and may thus gain benefits in term of fitness return. As females born on both treatment are equally likely to recruit on either fed or unfed nests, producing a female might a better bet for unfed parents.

Furthermore, I also looked at the reproductive success of these birds according to their sex, their original feeding treatment and the treatment where they recruited. I used Poisson generalized linear mixed models with the year of first breeding as a random effect and the number of breeding events recorded as a covariates to investigate this. The statistical analyses revealed that the total number of fledglings was significantly affected by 2 interactions. First, the interaction between sex and feeding treatment at recruitment was significant ($\chi^2_1 = 4.02$, $p = 0.045$): males recruiting in fed nests fledged more chicks than those recruiting in unfed nests, whereas recruitment treatment did not influence the total number of fledglings among females (Fig. 2). Second, the interaction between feeding treatment as a chick and at recruitment was also significant ($\chi^2_1 = 5.65$, $p = 0.017$): unfed chicks recruiting in fed nests had more fledglings than those recruiting in unfed nests, whereas recruitment treatment did not influence the total number of fledglings among fed chicks (Fig. 3).

As males born on fed nests were more likely to recruit on fed nests and as males which recruited on a fed nest had a higher reproductive success than those recruiting on an unfed

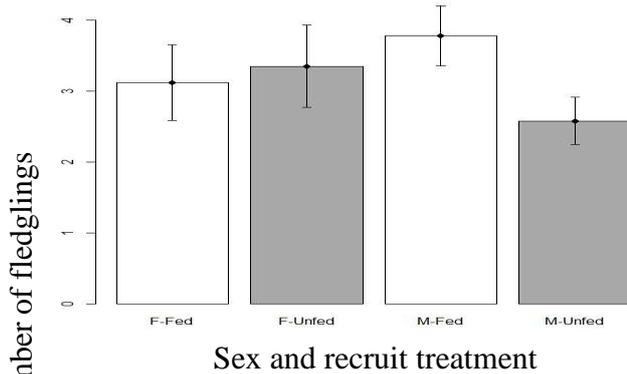


Figure 2. Total number of fledglings produced (means \pm SE) from first breeding to 2013 according to sex and recruitment feeding treatment. White bars represent individuals which recruited in fed nests and conversely for the grey bars.

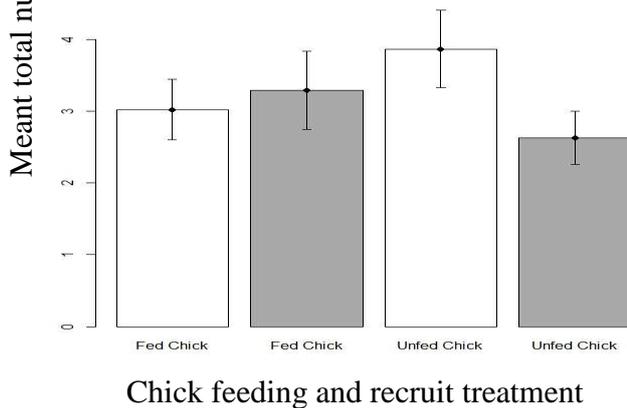


Figure 3. Total number of fledglings produced (means \pm SE) from first breeding to 2013 according to chick and recruitment feeding treatment. White bars represent individuals which recruited in fed nests and conversely for the grey bars.

nest, producing a male could lead to a higher fitness return for fed parents. However, the results also suggest that an unfed chick achieving to recruit on a fed nest may have a higher reproductive success than a fed chick on the same site (Fig. 3). Hence, there is no clear pattern supporting our hypothesis of a differential fitness return (see Discussion also), but it should be kept in mind that fitness return should be calculated with the cumulative lifetime reproductive success of all the offspring produced by an individual (Komdeur 2012). As fed adults usually fledge more chicks and in better condition, but especially among females though (Vincenzi et al. 2013), it is therefore possible that more of their fledglings would secure a fed nest site and breed than fledglings from unfed nests. Nevertheless, these results have to be taken with caution, because we do not know whether all the other fledglings that we did not resight died before sexual maturity or whether they recruited elsewhere. The latter is a likely hypothesis as kittiwakes are not highly philopatric (Coulson & Coulson 2008). It will thus probably be impossible to have a good estimate of an individual's fitness return.

Chapter 2A: hatching asynchrony and sibling competition

Is natural hatching asynchrony optimal? An experimental investigation of sibling competition patterns in a facultatively siblicidal seabird

In press for Behavioral Ecology and Sociobiology

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L'asynchronie d'éclosion est-elle optimale? Une investigation expérimentale des patrons de compétition dans la fratrie chez une espèce à fratricide facultatif

Sous presse pour Behavioral Ecology and Sociobiology

RÉSUMÉ

Dans les environnements imprédictibles, toute tactique qui permettrait aux parents d'ajuster la taille de leur nichée, et donc leur dépense énergétique, aux conditions environnementales devrait être favorisée. L'asynchronie d'éclosion, qui apparaît lorsque l'incubation commence avant que la ponte soit terminée, pourrait être une telle tactique. Par exemple, l'hypothèse de la rivalité dans la fratrie prédit que la hiérarchie entre poussins, concomitante à l'asynchronie d'éclosion, devrait à la fois faciliter l'ajustement de la taille de ponte en fonction des conditions environnementales et réduire certaines composantes de la compétition dans la fratrie, à l'échelle de la nichée et à l'échelle individuelle, en comparaison avec une éclosion synchrone. Nous avons donc prédit que les taux d'agressions, de quémandage et de nourrissage dans la nichée devraient diminuer avec une augmentation de l'asynchronie d'éclosion et que la supériorité du poussin aîné devrait augmenter avec l'asynchronie d'éclosion, les conduisant à une meilleure croissance et survie. Suivant ces prédictions, nous avons testé les effets d'une manipulation expérimentale vers le haut et vers le bas de l'amplitude de l'asynchronie d'éclosion sur le comportement, la croissance et la survie de poussins de mouette tridactyle (*Rissa tridactyla*). Comme prédit par l'hypothèse de la rivalité dans la fratrie, à l'échelle de la nichée, nous avons trouvé qu'une éclosion synchrone augmentait le taux d'agression et avait tendance à augmenter le taux de nourrissage des parents. Cependant, le taux de quémandage augmentait avec l'asynchronie d'éclosion,

contrairement à nos prédictions. A l'échelle individuelle, le poussin cadet était attaqué et quémendait proportionnellement plus souvent quand l'asynchronie d'éclosion augmentait, ce qui entraînait une croissance plus lente et une mortalité plus grande que son aîné. L'occurrence de coûts énergétiques plus élevés pour les parents et les poussins dans les nichées synchrones, ainsi que le faible taux de croissance et la forte mortalité du poussin cadet dans les nichées fortement asynchrones suggèrent que l'asynchronie d'éclosion naturelle est optimale.

Mots-clés: agression; approche expérimentale; asynchronie d'éclosion; fratricide facultatif; hypothèse de la rivalité dans la fratrie; mouette tridactyle.

ABSTRACT

In unpredictable environments, any tactic that enables avian parents to adjust brood size, and thus energy expenditure, to environmental conditions should be favoured. Hatching asynchrony (HA), which occurs whenever incubation commences before clutch completion, may comprise such a tactic. For instance, the sibling rivalry hypothesis states that the hierarchy among chicks, concomitant to HA, should both facilitate the adjustment of brood size to environmental conditions and reduce several components of sibling competition as compared to synchronous hatching, at both brood and individual levels. We thus predicted that brood aggression, begging and feeding rates should decrease and that older chick superiority should increase with HA increasing, leading to higher growth and survival rates. Accordingly, we investigated the effects of an experimental upward and downward manipulation of HA magnitude on behaviour, growth and survival of black-legged kittiwake (*Rissa tridactyla*) chicks. In line with the sibling rivalry hypothesis, synchronous hatching increased aggression and tended to increase feeding rates by parents at the brood level. Begging rates, however, increased with HA contrary to our expectations. At the individual level, as HA magnitude increased, the younger chick was attacked and begged proportionally more often, experienced a slower growth and a higher mortality than its sibling. Overall, the occurrence of energetic costs triggered by synchronous hatching both for parents and chicks, together with the lower growth rate and increased mortality of the younger chick in highly asynchronous broods suggest that natural HA magnitude may be optimal.

Keywords: aggression; facultative siblicide; experimental approach; hatching asynchrony; kittiwake; sibling rivalry hypothesis

INTRODUCTION

In unpredictable environments, female birds often lay more eggs than the number of chicks the pair would be able to fledge under average environmental conditions (Lack 1954; Ricklefs 1965). This strategy allows pairs to produce more chicks than average when environmental conditions turn out to be good. These occasional fitness gains are supposed to exceed costs of caring for extra chicks doomed to die under average or poor environmental conditions (the facultative brood reduction hypothesis: Lack 1947, 1954). Hence, any tactic favouring the facultative elimination of the weakest chick(s) at low costs according to environmental conditions should be advantageous for parents.

Hatching asynchrony (HA) is common among birds. It occurs whenever incubation commences before the clutch completion, and may have evolved, for instance, in response to the threat of nest predation (Hussell 1972; reviewed in Stenning 1996). However, other hypotheses have focused on its importance as a reproductive tactic *per se* (reviewed in Magrath 1990; Stenning 1996), by emphasising that size differences among nestlings arising from HA allow parents to adjust food allocation and thus chicks' fate according to environmental conditions. Among these hypotheses, the sibling rivalry hypothesis (Hahn 1981) states that the natural hierarchy establishment, concomitant to HA, should minimise sibling competition, thereby reducing energy wastage (Hamilton 1964) and allow parents to preferentially feed the older chick when needed. Accordingly, variation in HA magnitude should impact parental fitness through its consequences on chick behaviour at both brood and individual levels.

At the brood level, natural HA is thought to reduce the potential for escalated conflict among hatchlings, whereas synchronous hatching would minimise size differences and lead to wasted energy among chicks through increased aggression and begging rates, in accordance with game theory models (Maynard-Smith 1982). As parents typically feed their chicks below

their maximum capacity (e.g., Mock & Ploger 1987; Gilby et al. 2011), they may be able to increase their feeding effort in response to an unexpected increase in brood demand (as predicted in synchronous broods) (Ostreiher et al. 2012), as long as it does not jeopardise their subsequent survival and reproduction (Williams 1966; Drent & Daan 1980; Stearns 1992). The increased feeding effort may, however, not be sufficient to overcome energy wastage in synchronous broods (Gilby et al. 2011). Consequently, the average chick growth and survival in synchronous broods should be lower than in asynchronous broods, for a given set of environmental conditions (Hahn 1981).

At the individual level, as HA leads to an early disparity in chick fighting abilities, a dominance hierarchy develops among siblings. Hence, theoretically, the higher the magnitude of HA, the more the smaller chicks should be submissive (Maynard-Smith & Parker 1976). When environmental conditions are too poor for parents to fledge all chicks, they are expected to preferentially feed older sibs, with smaller/younger sibs eventually dying from starvation and stress (Lack 1947), thereby shortening period of energetic wastage for parents. Under good environmental conditions, however, food would be sufficient for smaller/younger sibs to fledge as well. Thus, HA could be advantageous for parents in all environmental conditions.

Several studies have provided experimental evidence for the sibling rivalry hypothesis (Hahn 1981) both in species where chicks compete via begging (i.e. “sub-lethal sibling competition”) or via overt aggressions (i.e. “lethal sibling competition”: Roulin & Dreiss 2012). In particular, aggression and/or begging rates were found to be higher in experimentally synchronous broods than in naturally asynchronous broods (Fujioka 1985; Mock & Ploger 1987; Osorno & Drummond 1995; Viñuela 1999; Gilby et al. 2011). When HA was experimentally increased as compared to natural asynchronous conditions, begging rate further decreased (Mock & Ploger 1987). At first sight, the same pattern could have been expected for aggression rate as older sibs could display less aggressive behaviours to establish

their dominance in highly asynchronous broods (Forbes 1991). Surprisingly, most studies reported that brood aggression rate was higher for experimentally increased HA than for controls, leading to a U-shaped curve between aggression rate and HA, with a minimum intensity for natural values of HA (Osorno & Drummond 1995; Viñuela 1999). Furthermore, contrary to predictions, parents responded to a synchronous brood's higher demand by feeding their chicks more often, which nevertheless conferred no growth advantage (Fujioka 1985; Mock & Ploger 1987; Gilby et al. 2011). At the individual level, as the magnitude of the experimental HA increased, the eldest chick became proportionally more aggressive, begged more and was fed more often than its sibling(s) (Mock & Ploger 1987; Osorno & Drummond 1995; Gilby et al. 2011). However, results relating HA magnitude to chick growth and survival are quite inconsistent (e.g., Fujioka 1985; Royle & Hamer 1998; Viñuela 2000; Gilby et al. 2011; Podlas & Richner 2013), except that subordinate chicks suffer higher mortality in broods with HA magnitude higher than natural (Osorno & Drummond 1995; Viñuela 2000). As sibling competition entails two possible modes of energy wastage, begging and overt aggressions, species exhibiting both might be particularly suitable to test the sibling rivalry hypothesis (Hahn 1981). Yet, no study investigated the influence of upward and downward manipulation of HA on chick and parental behaviour, both at the brood and individual levels, along with its consequences on chick growth and survival.

Here we performed such a study in the black-legged kittiwake, *Rissa tridactyla*, a facultatively siblicidal seabird (Braun & Hunt 1983). Females usually lay 2 eggs hatching asynchronously (mean: 1.35 days in our study population, Gill et al. 2002). The first-hatched chick (A-chick) becomes dominant over the second one (B-chick) in few hours via overt aggression and the latter responds submissively ("aggression-submission dominance relationship": Drummond 2006; see also Braun & Hunt 1983). Aggression is related to food availability (Irons 1992; White et al. 2010) and female feeding effort (Leclaire et al. 2010).

Moreover, in natural conditions, A-chicks beg more and are fed more often than B-chicks (Braun & Hunt 1983; White et al. 2010), promoting faster growth (Merkling et al. 2012). We experimentally manipulated HA magnitude from 0 to 3 days. We then recorded chick behaviour (aggression, begging and probability of being fed) and monitored chick growth and survival. Following the sibling rivalry hypothesis (Hahn 1981) and pertinent experimental studies, we predicted that, at the brood level, begging and feeding frequencies would decrease with increasing HA (from 0 to 3 days), while the relationship would be quadratic for aggression, with minimal frequencies for intermediate values of HA (i.e. close to natural conditions), as found in previous studies (e.g., Osorno & Drummond 1995). At the individual level, we predicted that A-chick superiority in terms of aggression, begging, feeding probability and growth would increase with HA magnitude. Finally, we expected A-chicks to have a lower survival in synchronous broods than in more asynchronous broods, whereas B-chicks would suffer higher mortality in highly asynchronous broods than in synchronous or naturally asynchronous broods.

MATERIALS AND METHODS

Study site

The study was carried out from mid-May to mid-August 2011 in a population of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59° 26'N, 146° 20'W), Gulf of Alaska. The tower is a twelve-walled polygon where artificial nest sites have been created on the upper walls. Observations (from a distance of twenty cm) from inside the building through one-way window glass allow us to easily monitor the breeders and their chicks (for details, see Gill & Hatch 2002). Besides behavioural monitoring (see 'Behavioural observations' below), nests were checked twice daily (9:00 and 18:00) throughout the breeding season to record events such as laying, hatching and chick mortality.

Experimental design

Each egg (two being the typical clutch size) was individually marked (A for the first-laid egg and B for the second egg) with non-toxic waterproof ink within twelve hours of laying. Twenty-four days after laying (i.e. three days before the expected hatching date: Hatch et al. 2009), eggs from pairs with two-egg clutches ($N = 307$) were put in an artificial incubator (Compact S 84 MP GTFS, Grumbach Brutgeraete GmbH, Asslar, Germany, set at 37.4°C and a humidity of 63%) to control hatching time. They were replaced in the nests by hen eggs of similar size that had been warmed in the incubator and had been artificially marked to mimic the natural temperature and pigmentation of kittiwake eggs. Parental incubation resumed immediately when the surrogate eggs were placed.

Hatching occurred in the incubator and its timing was assessed to the nearest two hours between 6:00 and 22:00 and to the nearest six hours at night. We then weighed each chick to the nearest 0.1g with an electronic scale, measured head-bill and tarsus lengths to the nearest 0.1 mm with a calliper and measured wing length to the nearest mm with a wing ruler. Chicks were marked on the head with a non-toxic marker to identify their original rank.

To control for parental effects, we then put each chick in a foster nest to create experimental broods of two unrelated chicks, an A- and a B-chick. By choosing chicks according to their hatching date, we experimentally set the magnitude of HA (defined as B-chick hatching date minus A-chick hatching date) from synchronous (i.e. zero days interval) to highly asynchronous broods (i.e. three days interval) (mean \pm SE: 1.3 ± 0.09 days, $N = 107$), with the A-chick having hatched before or at the same time as the B-chick in all but one nest. The mean natural hatching interval (for pairs that had their two eggs hatched in the incubator) was 1.64 days (SE \pm 0.07 days, $N = 117$), which is slightly higher than previously reported in the same colony: mean \pm SE: 1.35 ± 0.09 days (Gill et al. 2002). Our experimental

manipulation thus encompassed the mean natural hatching interval and entailed a wide, but biologically plausible, range around it.

Behavioural observations

We arbitrarily assigned a number to each nest and randomized the order of observations. Instantaneous scan sampling (Altmann 1974) was performed for each nest three times a day (at 10:00, 14:00 and 18:00). For each chick, we recorded whether it was begging (i.e. pecking its parent's bill), being aggressive toward its sibling (i.e. pecking any part of its sibling's body), being fed, or exhibited none of those three behaviours. Nests were observed from the day the B-chick was put in the nest until it was twenty days old (i.e. the period when most aggressiveness occur : White et al. 2010; Leclaire et al. 2011) or until one of the chicks died. In total 8618 observations were performed on 107 nests.

Measuring chick growth

Chicks were measured every five days from day five to thirty-five. Although fledging usually occurs at an older age (after forty days: Hatch et al. 2009), we did not handle chicks after thirty-five days old to avoid premature fledging. The same measurements were taken as those described for the hatching stage (see 'Experimental design' above).

We ran a principal component analysis on wing, tarsus and head-bill lengths on all ages. We then considered the score of each individual on the first principal component (94% of total variance explained) as a measure of its structural size at a given age (e.g., Blanchard et al. 2007). We calculated the mass gain and the structural size growth rate between zero and ten days (instead of between zero and twenty days in order to maximise sample size), by estimating the slope of the linear regression between body mass and age and between structural size and age, respectively. We also considered maximal body mass and structural size for chicks that survived until at least thirty days.

Statistical analyses

For each analysis (behaviour, growth, and survival), we started with a complete statistical model and successively removed terms beginning with those of the highest degree. We compared the change in deviance after removal of a term, using a χ^2 test with the appropriate degrees of freedom (likelihood ratio test). When an interaction was tested, the corresponding main effects were kept in the model. All analyses were conducted with R 2.14.2 (R Development Core Team 2012). Mean values are shown \pm standard error (SE).

Behavioural observations

To investigate whether the patterns observed were true overall or specific to a period, we analysed behavioural observations using the whole observation period and by sub-periods containing the first ten days or the last ten days.

At the brood level, we looked at the effect of HA on behaviour using generalized linear mixed models (GLMM) with a binomial error distribution and a logit link function as the response variable was binary (i.e. absence or presence of one of the studied behaviours). We used a maximum likelihood estimator (lme4 package: Bates et al. 2011) with nest and individual (nested within nest) included as random effects to account for the non independence of observations from the same nest and from the same individual within a nest. Based on our predictions, we were only interested in the quadratic and the linear models. Hence, for each behaviour and period, we first considered the quadratic model containing HA, HA² and chick age and B-chick hatching date (i.e. to correct for a potential seasonal effect) as covariates (reported only when significant). When HA² was significant, we kept HA regardless of its significance. Otherwise, we removed HA² from the complete model for further comparisons.

Behaviour were then analysed at the individual level. We wanted to consider the interactions between all explanatory variables and chick rank but it led to GLMM convergence problems due to the small occurrence of aggression and feeding behaviours. To circumvent them, we used the rate at which A-chick displayed each behaviour as a response variable (e.g.: 1 = the A-chick was the only one seen exhibiting a given behaviour; 0 = B-chick only) in a GLM (aggression: $N = 26$ nests; begging: $N = 62$; feeding: $N = 29$). This led to more simple models without interactions or random terms. The complete quadratic model contained HA^2 , HA and B-chick hatching date as a covariate (reported only when significant).

Chick growth

For the four chick growth parameters we considered as dependent variables, the complete linear mixed model contained the interactions between rank and HA^2 and between rank and HA . It also contained B-chick hatching date as a covariate (reported only when significant), and nest as a random effect. To meet model assumptions (normality and homoscedasticity of data and residuals), we used Box-Cox transformation (Box & Cox 1964) in the *MASS* package (Venables & Ripley 2002) on mass gain ($\lambda = 1.7$) and structural size growth rate ($\lambda = 2.4$) (A-chicks: $N = 98$; B-chicks: $N = 83$) as well as on maximal body mass ($\lambda = 2.5$) and maximal structural size ($\lambda = 4.9$) (A-chicks: $N = 83$; B-chicks: $N = 58$). Due to chick mortality, sample sizes were larger for analyses of mass gain and structural size growth rate than for analyses of maximal values.

Chick survival

Birth date was known for every chick, but death date was not known for chicks that were still alive when we left the field. Hence, we used either fledging age ($N = 87$) or age at our departure from the field for unfledged individuals ($N = 50$) as the age of last sighting of the individuals. Age for unfledged individuals still alive when we left ranged between 34 and 50

days. Because survival at this age is very high (Fig.5, see also Barrett & Runde 1980), we are confident that those chicks fledged after our departure and could therefore be considered as survivors. As survival was not independent for chicks in the same nest, we used Cox proportional hazards mixed regression models (CPH mixed) in the *coxme* package (Therneau 2012), with nest included as a random effect when considering both ranks (A-chicks: $N = 104$; B-chicks: $N = 102$). We tested the only interaction between rank and HA.

RESULTS

Influence of HA on aggression, begging and feeding at the brood level

Before ten days, the quadratic relationship between aggression frequency and HA was significant (Table 1), with synchronous and highly asynchronous broods having a higher aggression frequency than broods with a medium HA magnitude (Fig. 1). No significant relationship between aggression frequency and HA or HA^2 was found after ten days, while in the entire set of observations, the negative linear relationship between aggression frequency and HA was marginally significant (Table 1). Aggression frequency decreased with chick age both overall and before 10 days (Table 1). It also decreased with hatching date but only after ten days (Table 1).

Begging frequency increased linearly, although not significantly, with HA before ten days but not overall or after ten days (Table 1). It also increased with chick age both overall and before ten days (Table 1).

Feeding frequency decreased, although not significantly, with HA overall and after ten days but not before ten days (Table 1). It also decreased with chick age and hatching date both overall and before ten days (Table 1).

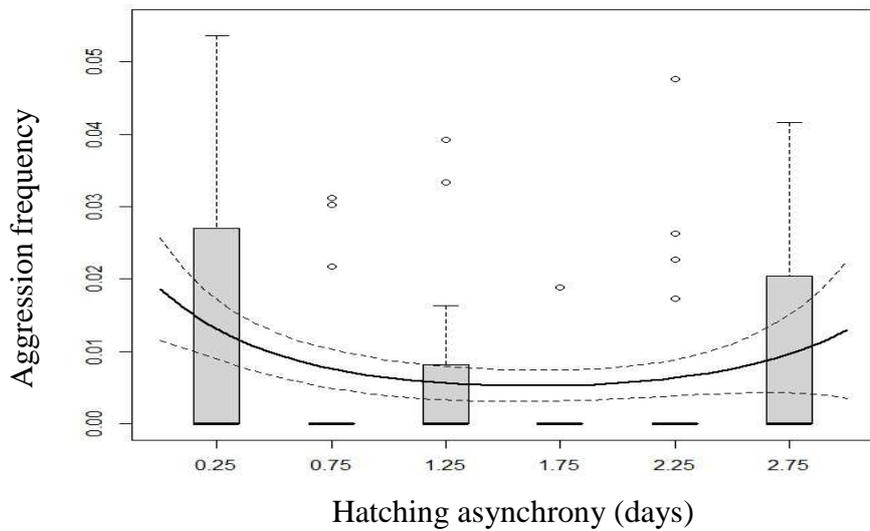


Figure 1. The relationship between aggression frequency at the brood level and hatching asynchrony (HA) during the ten first days after B-chick hatching. Frequencies were obtained from 8618 scan samplings on 107 nests. We grouped the data by class of 0.5 days of HA (i.e. 6 classes) for illustrative purposes. Observed data are represented per class by a grey boxplot and outliers above but one outlier at 0.07 in the fourth class is not shown to reduce the scale of the figure. Solid and dashed lines are GLMM predicted values and SE, respectively, with chick age (i.e. a significant covariate) set at 2 days. Nest and individual within nest were included as random terms in the model.

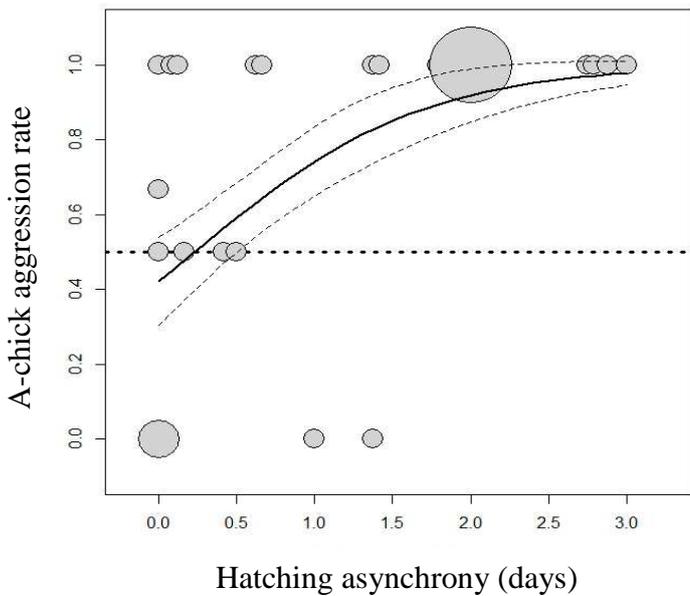


Figure 2. The relationship between hatching asynchrony and A-chick aggression rate during the first ten days after B-chick hatching. Shaded dots represent observed data (dot size proportional to the number of observations) from twenty-six nests where at least one chick was seen behaving aggressively toward the other. Solid and dashed lines are GLM predicted values and SE, respectively, whereas the dotted line represents a 50% rate

Variable removed from the complete model	Before 10 days			After 10 days			Overall		
	χ^2	<i>df</i>	<i>p</i>	χ^2	<i>df</i>	<i>p</i>	χ^2	<i>df</i>	<i>p</i>
Aggression:									
HA ²	3.62	1	0.05	0.11	1	0.74	2.59	1	0.11
HA	2.16	1	0.14 ^a	1.59	1	0.21	3.28	1	0.07
Hatching date	0.14	1	0.71	0.99	1	0.32	0.08	1	0.78
Age	3.98	1	0.046	1.40	1	0.24	28.26	1	<0.0001
Selected model:									
		β	SE					β	SE
(Intercept)		- 5.09	0.54				(Intercept)	-6.52	0.30
HA ²		0.48	0.28				Age	-0.98	0.23
HA		-1.57	0.76 ^a						
Age		-0.71	0.37						
Begging:									
HA ²	0.04	1	0.85	0.38	1	0.54	0.03	1	0.85
HA	3.12	1	0.08	1.58	1	0.21	0.08	1	0.77
Hatching date	0.02	1	0.87	0.35	1	0.56	0.62	1	0.43
Age	8.70	1	0.003	2.13	1	0.14	14.33	1	0.0001
Selected model:									
		β	SE					β	SE
(Intercept)		-3.11	0.19				(Intercept)	-3.37	0.07
Age		0.56	0.20				Age	0.23	0.06
Feeding:									
HA ²	0.23	1	0.63	0.08	1	0.78	0.73	1	0.39
HA	0.23	1	0.63	3.06	1	0.08	3.42	1	0.06
Hatching date	5.04	1	0.02	0.64	1	0.42	4.07	1	0.04
Age	14.18	1	0.0002	0.22	1	0.64	18.90	1	<0.0001
Selected model:									
		β	SE					β	SE
(Intercept)		-6.38	0.52				(Intercept)	8.6	8.4
Hatching date		-0.49	0.23				Hatching date	-0.08	0.05
Age		-1.45	0.41				Age	-0.72	0.19

Table 1. Generalized linear mixed model selection to explain variation in aggression, begging and feeding rates at the brood level according to hatching asynchrony (HA), HA², hatching date and chick age for the three different time periods

Significant terms are highlighted in bold, while marginally significant terms are highlighted in italic (but not retained in the selected model).

^a: when HA² was significant, HA was retained in the model even if not significant.

χ^2 = Chi-square value (change in deviance), *df* = difference in degrees of freedom between the two models compared, *p* = p-value, β = estimated coefficient, SE = standard error of the estimated coefficient

Influence of HA on aggression, begging and feeding at the individual level

A-chick aggression rate increased with HA overall as well as before ten days but not after ten days (Table 2). B-chicks were more aggressive than A-chicks for HA magnitude smaller than 0.5 days, while A-chicks were clearly more aggressive when HA magnitude exceeded 1 day (Fig. 2). A-chick aggression rate increased, although not significantly, with hatching date before ten days (Table 2).

A-chick begging rate tended to have a quadratic relationship with HA before ten days (Table 2, Fig. 3). A-chicks begged more than their siblings for HA magnitude between approximately 0.5 days and 1.8 days, while B-chicks begged more when HA exceeded approximately 2.2 days (Fig. 3). No other significant effects were found overall or after ten days (Table 2).

No significant terms were found concerning A-chick feeding rate (Table 2).

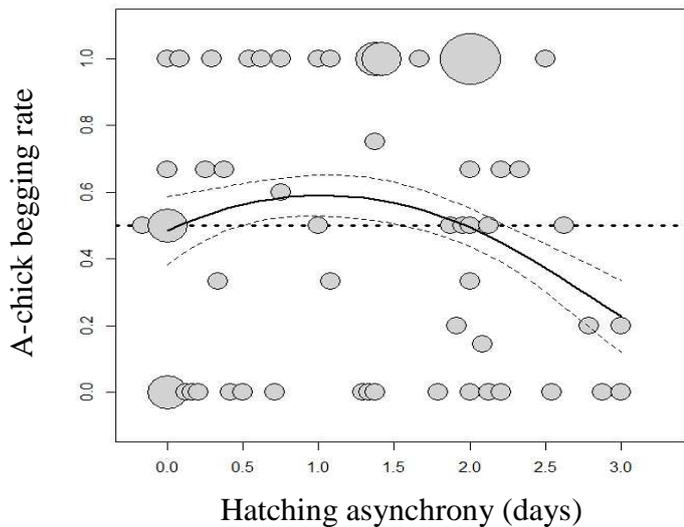


Figure 3. The relationship between hatching asynchrony and A-chick begging rate during the first ten days after B-chick hatching. Shaded dots represent observed data (dot size proportional to the number of observations) from sixty-two nests where at least one chick was seen begging. Solid and dashed lines are GLM predicted values and SE respectively, whereas the dotted line represents a 50% rate

Table 2. Generalized linear model selection to explain the variation in the rate at which the A-chick displayed aggression, begging or feeding behaviours according to hatching asynchrony (HA), HA² and hatching date for the three different time periods

Significant terms are highlighted in bold, while marginally significant terms are highlighted in italic (but not retained in the selected model).

^a: when HA² was significant, HA was retained in the model even if not significant.

χ^2 = Chi-square value (change in deviance), df = difference in degrees of freedom between the two models compared, p = p-value, β = estimated coefficient, SE = standard error of the estimated coefficient

Variable removed from the complete model	Before 10 days			After 10 days			Overall		
	χ^2	df	p	χ^2	df	p	χ^2	df	p
Aggression:									
HA ²	1.53	1	0.22	0	1	1	0.91	1	0.34
HA	11.55	1	0.0007	0	1	1	8.58	1	0.003
Hatching date	3.25	1	0.07	0	1	1	0.0008	1	0.97
Selected model:									
		β	SE					β	SE
(Intercept)		-0.32	0.48				(Intercept)	-0.04	0.45
HA		1.37	0.58				HA	1.33	0.57
Begging:									
HA ²	3.65	1	0.05	0.02	1	0.88	1.26	1	0.26
HA	1.93	1	0.16 ^a	0.39	1	0.53	1.56	1	0.21
Hatching date	0.52	1	0.47	0.006	1	0.93	0.03	1	0.87
Selected model:									
		β	SE					β	SE
(Intercept)		-0.06	0.40						
HA ²		-0.40	0.24						
HA		0.83	0.68 ^a						
Feeding:									
HA ²	0.17	1	0.68	0.65	1	0.42	0.39	1	0.53
HA	0.0008	1	0.98	0.06	1	0.80	0.08	1	0.78
Hatching date	2.44	1	0.12	0.08	1	0.78	1.77	1	0.18

Chick growth

HA significantly influenced structural size growth rate in relation to rank: it decreased linearly with HA for B-chicks but not for A-chicks (LMM; interaction HA \times rank: $\chi^2_1 = 8.61$, $p = 0.003$, Fig. 4). For mass gain however, only chick rank was significant, with A-chicks gaining significantly more mass than B-chicks (A-chicks: 14.26 ± 1.44 g.day⁻¹, B-chicks: 13.2 ± 1.45 g.day⁻¹, LMM; rank: $\chi^2_1 = 9.00$, $p = 0.003$, HA: $\chi^2_2 = 0.002$, $p = 0.96$; all other $p > 0.23$). Chick rank was also the only significant variable explaining variation in chicks' maximal structural size and body mass (all other $p > 0.27$). A-chicks reached a significantly higher maximal structural size and body mass than B-chicks (A-chicks: maximal structural size: 5.29 ± 0.58 PCA units.day⁻¹, maximal body mass: 408.75 ± 44.86 g; B-chicks: maximal structural size: 5.15 ± 0.68 PCA units.day⁻¹, LMM; rank: $\chi^2_1 = 10.51$, $p = 0.001$, maximal body mass: 389.63 ± 51.16 g, LMM; rank: $\chi^2_1 = 4.80$, $p = 0.028$). Hatching date had a negative effect on mass gain and maximal structural size (LMM; mass gain: $\chi^2_1 = 3.71$, $p = 0.054$; maximal structural size: $\chi^2_1 = 4.39$, $p = 0.036$).

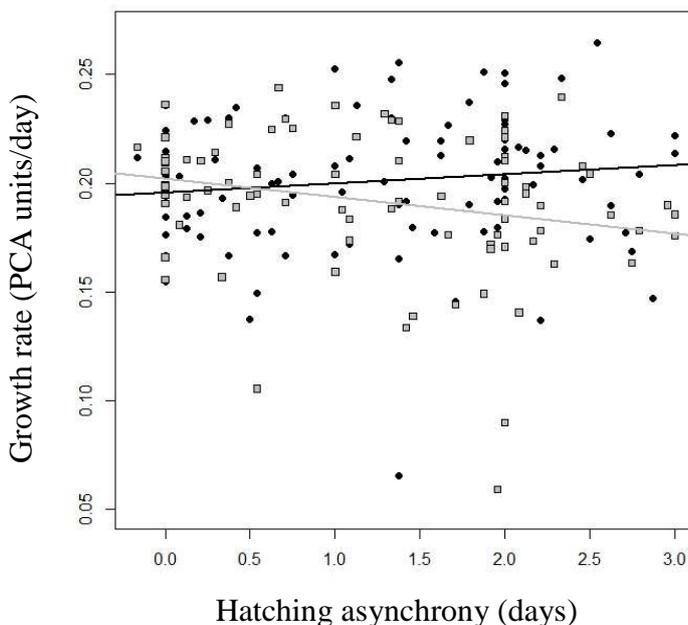


Figure 4. Structural size growth rate between zero and ten days (PCA units/day) for A- (black dots, $N = 98$) and B-chicks (grey squares, $N = 83$), according to hatching asynchrony. Lines are linear regression predictions. Results were not affected when the four outliers with an extreme growth rate below 0.11 were removed

Chick survival

The interaction between rank and HA was significantly related to chick survival (CPH mixed; $\chi^2_1 = 6.43$, $p = 0.011$, Fig. 5). The effect of HA on chick survival was reversed for A- and B-chicks. With HA increasing, B-chicks survival prospects decreased significantly whereas there was a slight increase of A-chicks survival (CPH; A-chicks: HA: $\chi^2_1 = 2.14$, $p = 0.14$; B-chicks HA: $\chi^2_1 = 4.67$, $p = 0.03$, Fig. 5). Chicks born later in the season were also more likely to die (CPH mixed; $\chi^2_1 = 9.25$, $p = 0.002$).

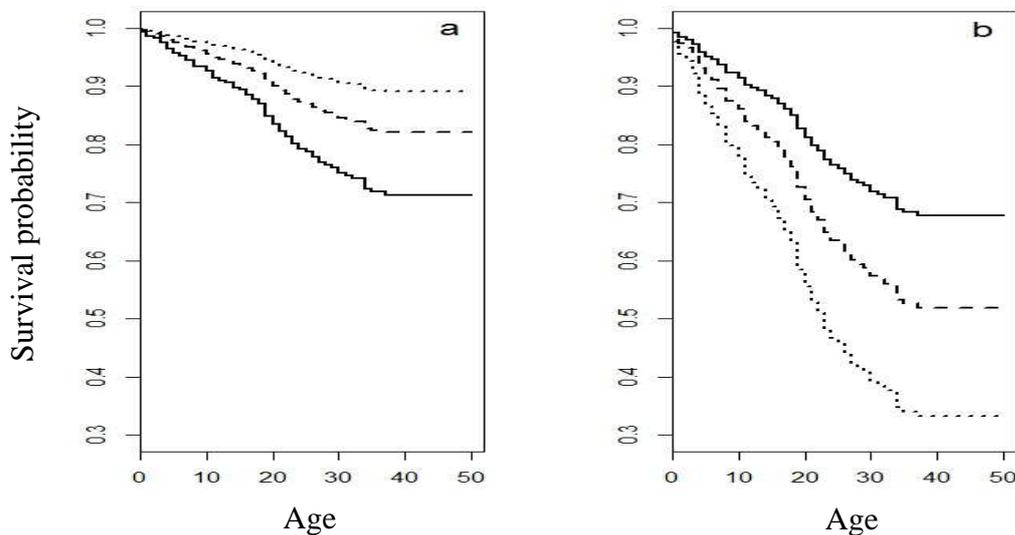


Figure 5. Survival probability according to chick age and hatching asynchrony (HA) (solid lines: HA = 0 days; dashed lines: HA = 1.5 days; dotted lines: HA = 3 days) for (a) A-chicks and (b) B-chicks. Although HA was analysed as a continuous variable (see text), it is displayed here as a categorical variable for illustrative purposes. B-chick hatching date (i.e. a significant covariate) was held at its average value

DISCUSSION

Among the hypotheses proposing adaptive benefits of HA in birds (Magrath 1990; Stenning 1996), the sibling rivalry hypothesis (Hahn 1981) has received among the more convincing experimental support (e.g., Mock & Ploger 1987; Gilby et al. 2011). This hypothesis states

that HA facilitates the establishment of a within-brood dominance relationship, thereby helping to reduce energy wastage in sibling competition. Here we experimentally manipulated HA magnitude both downward and upward in a facultatively siblicidal species to evaluate its consequences from hatching to fledging.

Overall, our experimental manipulation of HA magnitude seemed to confirm the sibling rivalry hypothesis (Hahn 1981). Brood-level competition (in terms of aggression, but not begging, see Gilby et al. 2011) generally decreased when the magnitude of HA increased and parents tended to feed synchronous broods more actively, although no benefits concerning maximal structural size or body mass were observed. Synchronous hatching may thus lead to higher costs and energy wastage for both chicks and parents, as shown by previous studies (e.g., Osorno & Drummond 1995; Gilby et al. 2011). At the individual level, our results confirm that A-chick aggression rate increased with the magnitude of HA, leading to an exaggerated B-chick mortality in highly asynchronous broods. Yet, contrary to previous studies (Mock & Ploger 1987), experimentally increased HA led to an increased begging frequency among B-chicks. Overall, energetic costs triggered by synchronous hatching and increased B-chick mortality in highly asynchronous broods together with lower aggression rate in broods with natural HA suggest that the natural situation may be optimal for parents. However, further studies are needed to confirm whether this is always true or dependent on environmental conditions (Lack 1947; Hahn 1981; see also Wiebe & Bortolotti 1994). Particular focus should be put on the long term fitness consequences for chicks and parents of the different magnitudes of HA (Mock & Forbes 1994; see also Mainwaring et al. 2012).

Chick behaviours

Our experimental manipulation of HA indicated that aggression frequency at the brood level had a quadratic relationship with HA before 10 days, with more aggressions occurring in very synchronous broods, as expected (e.g., Osorno & Drummond 1995; Viñuela 1999; Gilby et al.

2011) and, surprisingly, in highly asynchronous broods (Osorno & Drummond 1995; Viñuela 1999; but see Forbes 1991). This latter result echoes patterns observed in species with obligate siblicide, where HA is usually larger (e.g., Anderson 1989). However, aggression frequency tended to be linearly negative across the whole dataset, which mean that aggression was very scarce in highly asynchronous broods after 10 days. Hence, once the dominance relationship was established, A-chicks probably reduced their aggression rates, in line with the decrease we found in aggression frequency with chick age (see also Nathan et al. 2001). Sibling competition was thus higher in synchronous broods because both chicks were very aggressive toward each other during the whole period, suggesting an escalated conflict between size-matched individuals as predicted by game theory models (Maynard-Smith 1982). Interestingly, our analysis at the individual level highlighted that B-chicks were slightly more aggressive than A-chicks for small HA magnitudes, whereas A-chicks were clearly more aggressive with increasing HA magnitude. Previous studies also found an increase of aggression frequency in synchronous broods (e.g., Mock & Ploger 1987; Osorno & Drummond 1995), but A-chicks were always more aggressive than B-chicks. In cattle egrets (*Bubulcus ibis*), A-eggs contain more androgens (Schwabl 1997) and in blue-footed boobies (*Sula nebouxii*) there are no differences in androgen concentrations according to rank (Drummond et al. 2008). In kittiwakes, however, B-eggs contain more androgens than A-eggs (e.g., Vallarino et al. 2012; Benowitz-Fredericks et al. 2013) and these hormones have been shown to experimentally increase chick aggression and dominance (Müller et al. 2012). This might explain B-chick behaviour when HA was very close to 0, despite their smaller size at hatching (Merkling et al. 2012). Altogether these results confirm that asynchronous hatching favours within-brood hierarchy establishment and that A-chicks need a substantial age/size advantage to become dominant, in accordance with the sibling rivalry hypothesis (Hahn 1981).

Begging frequency tended to increase with HA before 10 days at the brood level, contrary to the sibling rivalry hypothesis (Hahn 1981) and previous studies (Fujioka 1985; Gilby et al. 2011). For the same period, analyses at the individual level showed a significant quadratic relationship between HA and A-chick begging rate. Consistent with previous findings in kittiwakes (Braun & Hunt 1983), A-chicks begged slightly more than B-chicks for HA magnitudes approximating the natural range. Yet, for HA magnitudes above about 2.2 days, B-chicks begged significantly more than their siblings. Hence, the more B-chicks faced a low growth rate, the more they begged, coherently with theoretical predictions of begging scramble models (Parker et al. 2002). As we did not record behavioural sequences, we were not able to determine whether B-chick begging led to A-chick aggression, as previously reported (e.g., Forbes 1991) and as it would be expected if A-chicks were selected to increase their share of food available. However, our personal observations seem to corroborate these findings. Although high B-chick begging could signal their higher hunger level to parents, we did not find any effect of HA magnitude on A-chick feeding rate, in contradiction with previous studies (e.g., Osorno & Drummond 1995; Gilby et al. 2011). This may suggest that B-chicks needed to beg substantially more than A-chicks to get the same amount of food, and that begging became more costly for B-chicks with increasing HA (Godfray 1995; Parker et al. 2002).

Moreover, in agreement with our predictions, we found that feeding frequency at the brood level tended to decrease with HA overall and after 10 days. In line with previous studies (e.g., Fujioka 1985; Mock & Ploger 1987; Wiehn et al. 2000), it seems that the increase in sibling competition, through aggression but not begging, arising from synchronous hatching forced parents to increase their feeding effort. Yet, parental compensation was delayed, as we did not detect any effect of HA on feeding frequency before 10 days. This increase in parental feeding frequency in synchronous broods as compared to more asynchronous broods could

also be coherent with the non-mutually exclusive peak load reduction hypothesis (Hussell 1972; Mock & Schwagmeyer 1990). It states that HA could enable parents to reduce the maximum level of the brood's daily food requirements by offsetting the chicks' demand curves. However, no study found strong support for this hypothesis despite many trials (e.g., Wiebe & Bortolotti 1994b; Siegel et al. 1999; Smiseth & Morgan 2009) and the long chick-rearing period in kittiwakes might prevent these selective pressures to occur in our species (Mock and Schwagmeyer 1990). Future studies should investigate the long-term effects on parental survival and reproduction of this increase in parental effort arising from synchronous hatching (Mock & Ploger 1987; Mock & Forbes 1994). Indeed, we would expect long-lived species, such as kittiwakes, to favour their survival and future reproductive attempts over current offspring and therefore to be reluctant to increase parental effort (Drent & Daan 1980; but see: Leclaire et al. 2011).

Chick growth and survival

In accordance with our predictions, we found that the difference between A- and B-chicks in structural size growth rate increased with the magnitude of HA. A-chicks grew at the same rate regardless of HA magnitude, whereas B-chick growth was impaired by increasing HA. These results suggest that, contrary to previous findings (Viñuela 2000; Gilby et al. 2011), increased aggression in synchronous broods did not affect chick growth. However, asynchronous hatching, either natural or experimentally increased, negatively affected B-chick structural size growth rate compared to synchronous broods, as previously found (Fujioka 1985; but see Osorno & Drummond 1995). It is unlikely that feeding differences were involved in the decline of B-chick structural size growth rate, as we did not find any differences in A- and B-chicks feeding rates or frequencies before 10 days. Nonetheless, B-chicks in asynchronous and highly asynchronous broods may have faced a trade-off between growth and other activities. Indeed, a high begging frequency combined with the high

frequency of aggression received may have been physiologically costly (Noguera et al. 2010; see also Parker et al. 2002). Regardless of the mechanisms explaining these differences in structural size growth rate during the first 10 days, HA magnitude did not alter the body size chicks reached before fledging. This is probably because structural size growth rate negatively affected B-chick survival probability until fledging (CPH; $\chi^2_1 = 4.17$, $p = 0.04$), thus masking differences in growth rate found during the first 10 days.

Mass gain did not depend on HA magnitude in either A- or B-chicks, but the former gained more weight than the latter during the first 10 days, despite similar rates of A- and B-chicks feeding (see also Leclaire et al. 2011; but see White et al. 2010). In highly asynchronous broods, B-chicks may gain less weight than A-chicks because of the costs associated with their higher begging rate and aggressions they received. In experimentally synchronous broods, however, A- and B-chicks had similar levels of aggression and feeding, while the difference in mass gain held true. One possible explanation is that parents fed A-chicks larger meals than B-chicks. A-chicks came from larger eggs (Braun & Hunt 1983; our unpublished data) and were thus naturally slightly larger than B-chicks at hatching, even in synchronous broods. Parental favouritism toward the largest nestling in term of meal size has been experimentally demonstrated in zebra finches (Gilby et al. 2011). The difference in mass gain between ranks held true until fledging — A-chicks were significantly heavier than B-chicks, contrary to previous findings in natural broods (Merklings et al. 2012) — and no influence of HA magnitude was found. The combined results concerning maximal structural size and body mass confirm previous findings that HA magnitude does not influence fledging size and mass in species with a long rearing period (e.g., Osorno & Drummond 1995; Viñuela 1999).

HA magnitude did affect chick survival before fledging, in line with our prediction: A-chick survival increased slightly with HA magnitude, whereas B-chick survival significantly decreased with HA magnitude, as previously found (Mock & Ploger 1987; Osorno &

Drummond 1995; Viñuela 1999). B-chicks that were heavily attacked and begged frequently (i.e. in highly asynchronous broods) were more likely to die than B-chicks in other brood types. Increased sibling competition in synchronous broods was deleterious to A-chicks, as they were almost as likely to die as their siblings, in accordance with the sibling rivalry hypothesis (Hahn 1981). Survival costs thus increased more rapidly for B chicks than they decreased for A chicks with increasing HA. This is coherent with the asymmetric sibling rivalry hypothesis (Forbes & Glassey 2000), which states that the phenotypic handicap imposed upon subordinate chicks via HA should buffer dominant chicks from adverse conditions, as shown in red-winged blackbirds (*Agelaius phoeniceus*) (Forbes et al. 1997; Forbes 2011). Our results are also coherent with Lack's explanation that natural HA should facilitate the elimination of the weakest chick when needed (Lack 1954). However, the question remains whether parents are able to intervene in sibling competition to facilitate or to prevent siblicide. Most studies (e.g., Drummond et al. 1986; Mock & Parker 1997) have reported that parents did not interfere in chick aggression to change the outcome of sibling competition, but not all (e.g., Viñuela 1999; Wiebe & Bortolotti 2000). Kittiwake parents appear at times to interfere physically in sibling aggression by sitting on the chicks (authors personal observations), but an adaptive role of this behaviour has still to be proved. As A-chicks were highly aggressive and B-chicks had poor survival prospects in highly asynchronous broods, it is possible that a greater than natural HA magnitude can lead to a parental loss of control over brood reduction through siblicide (as defined by Mock 1994). Hence, the natural magnitude of HA may be adaptive in part because it moderates sibling competition and chick losses, as shown in our study, but also because it may give parents greater control over the outcome of sibling competition.

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ETHICAL STANDARDS

This experiment was conducted under the approval of the USGS Alaska Science Center Animal Care and Use Committee, the IPEV Ethical Committee, in accordance with United States laws and under permits from the U.S. Fish and Wildlife Service and the State of Alaska. Any use of trade names is for descriptive purposes only and does not imply endorsement of the U.S. Government.

Chapter 2B: hatching asynchrony and corticosterone

Physiological and fitness correlates of experimentally altered hatching asynchrony magnitude in wild seabird chicks

In revision for General and Comparative Endocrinology

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Effets sur la physiologie et sur la valeur sélective d'une manipulation expérimentale de l'asynchronie d'éclosion chez des poussins d'un oiseau marin

En révision pour *General and Comparative Endocrinology*

RÉSUMÉ

La compétition dans la fratrie est souvent intense chez les espèces où les poussins restent au nid et dépendent entièrement de leurs parents pour la nourriture. L'asynchronie d'éclosion, qui résulte du début de l'incubation avant la fin de la ponte, facilite l'établissement d'une hiérarchie au sein du nid où le cadet est sujet à un taux de nourrissage et un taux de croissance plus faibles. Comme les stress sociaux et nutritionnels influencent les taux basaux d'hormones de stress chez les oiseaux, on s'attend à ce que les poussins cadets aient des concentrations plus élevées de corticostérone (i.e. la principale hormone de stress chez les oiseaux) que leurs aînés. Etant donné que des études précédentes ont montré que l'amplitude de l'asynchronie d'éclosion influence le déroulement de la compétition dans la fratrie, cela devrait aussi influencer les taux basaux de corticostérone. Nous avons mesuré le taux basal de corticostérone à cinq jours chez des poussins de mouettes tridactyles (*Rissa tridactyla*) dans trois types de nichées expérimentales : synchrones, asynchrones et fortement asynchrones. Comme le dimorphisme sexuel se met en place pendant la période d'élevage des jeunes et pourrait aussi influencer le taux basal de corticostérone, nous avons inclus le sexe des poussins dans nos analyses et nous avons suivi la croissance et la survie des poussins. Le taux basal de corticostérone ne différait pas entre poussins aînés, mais il était plus élevé chez les poussins cadets des nichées fortement asynchrones par rapport aux autres types de nichées, en accord avec la présumée augmentation de stress nutritionnel. Dans les nichées asynchrones,

les poussins aînés avaient un taux basal de corticosterone plus élevé que leurs cadets, contrairement à nos attentes. Nous interprétons ce résultat comme reflétant un coût à la dominance chez les aînés. De manière cohérente avec de précédentes études, le gain de masse était négativement corrélé au taux basal de corticosterone. Nous avons aussi trouvé que le taux basal de corticostérone au jour cinq prédisait la survie d'un individu de manière sexe-dépendante. Quelque soit le rang d'éclosion, les mâles avec un taux basal de corticosterone souffrait d'une mortalité plus élevée, suggérant ainsi que les mâles étaient plus sensibles à des stress élevés indépendamment de la cause.

Mots-clés: agression; asynchronie d'éclosion; compétition dans la fratrie; corticosterone; dimorphisme sexuel

ABSTRACT

Nest-bound chicks depend entirely on their parents for food, and consequently sibling competition is often intense. Asynchronous hatching, resulting from the onset of incubation before clutch completion, facilitates the establishment of within-nest hierarchy, with younger chicks being subject to lower feeding and growth rates. Because social and nutritional stresses affect baseline stress hormone levels in birds, younger chicks are expected to have higher levels of corticosterone than their siblings. As previous studies showed that hatching asynchrony magnitude influenced the course of sibling competition, it should also affect baseline corticosterone. We measured baseline corticosterone at age five days in nestling black-legged kittiwakes (*Rissa tridactyla*) in three types of experimental broods: synchronous, asynchronous, and highly asynchronous. Sexual dimorphism takes place during chick-rearing and might also influence baseline corticosterone, we thus included chick sex in our analyses and also monitored chick growth and survival. Baseline corticosterone did not differ among A-chicks, but was higher in B-chicks from highly asynchronous broods compared with the other brood types, in line with the presumed increase in nutritional stress. In asynchronous broods, A-chicks had higher baseline corticosterone than their siblings, contrary to our expectations. We interpret that result as a cost of dominance among A-chicks. In line with previous studies, mass gain was negatively correlated with baseline corticosterone levels. We found that baseline corticosterone predicted survival in a sex-specific way. Regardless of hatching rank, males with higher baseline corticosterone suffered higher mortality, suggesting that males were more sensitive to high level of stress, independently of its cause.

Keywords: aggression; corticosterone; hatching asynchrony; sexual dimorphism; sibling competition

INTRODUCTION

When vertebrates face conditions that are unpredictable and stressful—such as predation or severe weather events, for example—secretion of stress hormones (i.e. glucocorticoids) typically increases (Wingfield 1994; Sapolsky et al. 2000). Such stress responses are adaptive, because they lead to physiological and behavioural changes favouring immediate survival over non-vital activities (reviewed in Wingfield et al. 1998; Sapolsky et al. 2000). Upon removal of the threatening condition, stress hormone levels return to baseline levels.

Vertebrates also face mild chronic stresses, such as social or nutritional challenges, during which their immediate survival is not jeopardised. Situations such as reduced food availability (Kitaysky et al. 1999a), a change of mate (Angelier et al. 2007b), mate incompatibility (Griffith et al. 2011) or any change in the social environment (Pryke et al. 2007) can lead to increased baseline levels of stress hormones. Chastel et al. (2005), for example, showed that chick-rearing black-legged kittiwakes (*Rissa tridactyla*) have higher levels of corticosterone (the major stress hormone in birds) than failed breeders, probably in response to increased foraging activity and the energy cost of chick rearing (Golet et al. 1998, 2000).

Similarly, chicks are exposed to social and nutritional stresses. Chicks from altricial and semi-precocial species are nest-bound, substantially developing after hatching and dependent on parental food provisioning (Starck & Ricklefs 1998). In polytocous species, food shortage typically leads to sibling competition and potentially to the unequal allocation of food between chicks (Mock & Parker 1997). That outcome is especially common when hatching asynchrony, resulting from the onset of incubation before clutch completion, leads to the establishment of a within-brood hierarchy (Magrath 1990). The older chick's age and size advantage allows it to dominate through begging and/or overt aggression toward its siblings, thereby receiving a disproportionate share of food from the parents (Mock & Parker 1997; Cotton et al. 1999). Facing reduced feeding rates and growth (e.g., Hébert 1993; Gilby et al.

2011) and a greater likelihood of starvation (e.g., Drummond et al. 1986; Hébert 1993), younger chicks are expected to have higher baseline levels of corticosterone than their older siblings. While some studies have confirmed those expectations (e.g., Nuñez-de la Mora et al. 1996; Tarlow et al. 2001; Eraud et al. 2008), others have found no effect of rank on baseline corticosterone (e.g., Ramos-Fernandez et al. 2000; Blas et al. 2005; Poisbleau et al. 2010) or even the reverse pattern. The latter results (e.g., Schwabl 1999; Love et al. 2003) occurred in captive populations, with scramble competition within the brood mediated via begging.

Beyond simple hatching order, the magnitude of hatching asynchrony influences sibling competition (e.g., Mock & Ploger 1987; Osorno & Drummond 1995; Gilby et al. 2011), and may affect social and nutritional stress levels among chicks. Several studies, in species competing via begging or via overt aggression, found that synchronous (simultaneous) hatching, led to an unstable hierarchy, an increase of sibling competition through an escalated conflict, with comparatively equal feeding and growth rates among chicks (e.g., Mock & Ploger 1987; Osorno & Drummond 1995; Gilby et al. 2011). Thus, in synchronous broods, all chicks are expected to show equal levels of baseline corticosterone. Conversely, increasing hatching asynchrony magnitude experimentally further disadvantages a younger chick, which is heavily attacked and more rarely fed, is growing slowly and is more likely to die than in naturally asynchronous broods (e.g., Mock & Ploger 1987; Osorno & Drummond 1995). The social and nutritional stress of being subordinate should thus increase with greater hatching asynchrony, and younger chicks should exhibit higher baseline corticosterone levels than in the natural situation. The response in older siblings, however, would not change necessarily.

To our knowledge, no study has investigated in a wild bird population the effect of hatching asynchrony magnitude on baseline corticosterone levels. We studied this relationship in black-legged kittiwakes (*Rissa tridactyla*), a seabird usually having two chicks hatched asynchronously (mean: 1.35 days apart in our study population, Gill et al. 2002). We showed

recently that experimental manipulation of hatching asynchrony magnitude influences the course of sibling competition (Chapter 2A, p. 97). In synchronous broods, both chicks behaved almost identically and had similar growth rates, whereas greater hatching asynchrony led to increased attacks on B-chicks (i.e. second-hatched chicks), with higher begging intensity and reduced growth and survival prospects in that group. Here, using the same dataset, our aim was to investigate how baseline corticosterone levels vary with birth rank and magnitude of hatching asynchrony. Males, which grow faster and reach a larger size than females near fledging (Merkling et al. 2012, p.34), are expected to be more sensitive to social and nutritional stresses. We therefore included chick sex in our analyses. We predicted that synchronous hatching would lead to equal baseline corticosterone levels in A and B-chicks (with higher levels in males), whereas differences between siblings would increase with increasing hatching asynchrony magnitude. Asynchronously hatched B-chicks, especially males, should have higher baseline corticosterone levels than A-chicks.

Additionally, we explored relationships among body condition, growth patterns, hatching asynchrony magnitude, rank and baseline corticosterone levels. We expected a negative correlation between chick body condition and/or growth and baseline corticosterone (e.g., Kitaysky et al. 1999b; Sockman & Schwabl 2001; Eraud et al. 2008; Müller et al. 2010b; Rensel et al. 2011). Finally, we predicted that high baseline corticosterone levels (i.e. in chicks experiencing high social and nutritional stress) would be associated with low survival (Blas et al. 2007) upon controlling for rank, chick sex, sibling sex, and hatching asynchrony magnitude.

MATERIALS AND METHODS

Study site

The study was conducted from from mid-May to mid-August 2011 in a population of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59° 26'N, 146° 20'W), Gulf of Alaska. The tower is a twelve-walled polygon with artificial nest sites that have been added to the upper walls, which allowed us to monitor easily the breeding adults and their chicks from inside the building (for more details, see Gill & Hatch 2002). Nests were checked twice daily (9:00 and 18:00) throughout the breeding season to document events such as laying, hatching, and chick mortality.

Experimental design

The dataset is the same as in Chapter 2A (p. 97). Each egg (two being the typical clutch size in this population) was individually marked (A and B for the first- and second-laid egg respectively) with non-toxic waterproof ink within twelve hours of laying. Twenty-four days after laying (i.e. three days before the expected hatching date: Hatch et al. 2009), eggs from two-egg clutches ($N = 307$) were placed in an incubator (Compact S 84 MP GTFS, Grumbach Brutgeraete GmbH, Asslar, Germany, set at 37.4°C and 63% of humidity) to control for hatching time. They were replaced in the nests by hen eggs of similar size that had been warmed in the incubator and artificially marked to mimic the natural temperature and pigmentation of kittiwake eggs. Parental incubation resumed immediately after the surrogate eggs were placed.

Hatching occurred in the incubator and its timing was assessed to the nearest two hours between 6:00 and 22:00 and to the nearest six hours at night. Chicks were marked on the head with a non-toxic marker to identify their original rank. We then weighed each chick to the nearest 0.1g with an electronic scale, measured head-bill and tarsus lengths to the nearest 0.1

mm with a caliper and measured wing length to the nearest 1 mm with a wing ruler. Each chick was also blood sampled for molecular sexing from the brachial vein (~ 50 μ l) using a sterile 25-gauge needle and a capillary tube (for a detailed molecular sexing protocol see Merklings et al. 2012, p.34). Samples were kept in Longmire buffer (Longmire et al. 1988) and stored at -20°C until analysis. No sex ratio bias was found in relation to hatching order (forty-four females and thirty males among A-chicks, thirty-three females and forty males among B-chicks, $\chi^2_1 = 2.44$, $P = 0.12$).

To control for parental effects, we put each chick in a foster nest, creating broods consisting of an A-chick (born from a first-laid egg) and an unrelated B-chick (born from a second-laid egg). By choosing chicks according to their hatching date, we experimentally created three types of broods with different magnitudes of hatching asynchrony. The first type contained two chicks hatched less than one day apart (mean \pm SE: 0.28 ± 0.05 days, $N = 31$, hereafter called ‘synchronous broods’), the second contained two chicks hatched between one and two days apart (1.48 ± 0.07 days, $N = 26$, hereafter called ‘asynchronous broods’), while the third contained two chicks hatched more than two days apart (2.31 ± 0.07 days, $N = 26$, hereafter called ‘highly asynchronous broods’). The mean natural hatching interval (for pairs that had their two eggs hatched in the incubator) was 1.64 days (SE \pm 0.07 days, $N = 117$), which is slightly higher than previously reported in the same colony: 1.35 ± 0.09 days (Gill et al. 2002). Our experimental manipulation thus encompassed the mean natural hatching interval and entailed a wide, but biologically plausible, range around it.

At five days of age, chicks were blood sampled within three minutes of capture to determine their baseline corticosterone level (Romero & Reed 2005). Blood samples were collected from the alar vein with a 21-gauge needle and capillary tubes (maximum volume collected: 225 μ l). Sampling occurred between 11:00 and 16:00 to minimise the effect of

diurnal fluctuations on plasma corticosterone levels (Breuner et al. 1999; Romero & Remage-Healey 2000; Tarlow et al. 2003).

Chick growth

Chick's weight and measures were taken every five days from day five to thirty-five (i.e. close to fledging: Hatch et al. 2009). Measurements were the same as at hatching (see section 2.2). We ran a principal component analysis on wing, tarsus and head-bill lengths on all ages and considered the score of each individual on the first principal component (94% of total variance explained) as a measure of its structural size at a given age (e.g., Blanchard et al. 2007). We calculated mass gain and growth in structural size between zero and ten days, by estimating the slope of the linear regression between body mass and age and between structural size and age, respectively. We also estimated chick body condition at the stage when corticosterone levels were assessed (i.e. at day five) by calculating residuals of the linear regression between chick mass and structural size on that day.

Corticosterone assays

All hormonal analyses were performed at the Centre d'Etudes Biologiques de Chizé. Plasma concentrations of corticosterone were determined in one assay following methods described in Lormée et al. (2003). The detection limit was 0.3 ng/mL, and the intra-assay coefficient of variation was 6% (n=5 duplicates). We chose not to assess free corticosterone and corticosteroid-binding globulin concentrations on the premise that total corticosterone concentration is the more biologically relevant estimate of corticosterone levels (Schoech et al. 2013). Baseline corticosterone levels were assessed for one hundred and fifty chicks for which we had information on sex and sibling sex, but three outliers were removed from statistical analyses because their corticosterone concentrations were above 20 ng/ml (i.e. above baseline corticosterone levels found in kittiwake chicks in other studies, Kitaysky et al.

2001a). We speculate that in those chicks the stress response had already begun, because sampling time was close to three minutes (Romero & Reed 2005) or because they were otherwise stressed in the nest before we captured them. Corticosterone levels were considered to reflect the baseline level in all remaining chicks ($N = 147$) (Chastel et al. 2005) and statistical results were unaffected if the three outliers were included.

Statistical analyses

For each analysis, we started with a complete model and successively removed each term beginning with the terms of the highest degree. We compared the change in deviance after removal of a term using a χ^2 test for linear mixed models (LMM) and Cox models. When an interaction was tested, the corresponding main effects were kept in the model. Post-hoc Bonferroni correction for multiple comparisons were done using the *multcomp* package (Hothorn et al. 2008).

To meet model assumptions (normality of data and residuals and homoscedasticity), baseline corticosterone levels ($\lambda = 0.35$) were transformed using Box-Cox transformation (Box & Cox 1964) in the *MASS* package (Venables & Ripley 2002). As some chicks died before ten days and we were unable to calculate their mass gain, structural size growth rate or body condition, we ran separate sets of models with and without these variables. We began with a complete model containing rank, hatching asynchrony, sex, sibling sex, and all three-way interactions containing rank and two-way interactions within them using LMM, with nest as a random effect to account for the non-independence of chicks within a nest. Because sex and sibling sex did not seem to affect baseline corticosterone levels (see first section of Results) and our sample size decreased when considering mass gain, structural size growth rate and body condition ($N = 138$), we did not consider sex and sibling sex when running analyses with those other variables. As structural size growth rate, mass gain and body condition were inter-correlated (Spearman correlation: all $p < 0.01$), we did not consider them

in the same model, but ran three sets of models. The complete model always contained the interactions among rank, hatching asynchrony, and the growth/condition variable. For models with structural size growth rate, we present results without three outliers with very low growth rates, but considering them led to similar results.

For survival analyses ($N = 147$), we used mixed effects Cox proportional hazards models (i.e. frailty models) in the *coxme* package (Therneau 2012) with nest as a random effect. We started with a complete model including three three-way interactions in addition to the main effects—baseline corticosterone levels, rank, hatching asynchrony, sex, and sibling sex.

All analyses were conducted with R 2.14.2 (R Development Core Team 2012). Results are shown with mean \pm standard error (SE).

RESULTS

Effects of hatching asynchrony, rank, sex and sibling sex on chick corticosterone levels

Baseline corticosterone levels were significantly affected by the interaction between rank and hatching asynchrony ($\chi^2_2 = 6.11$, $p = 0.047$, Fig.1). Nevertheless, due to a lack of statistical power, post-hoc Bonferroni correction for multiple comparisons did not reveal any significant pairwise differences (all $p > 0.2$). To reveal differences and to better interpret this interaction, we created a greater contrast between hatching asynchrony classes by removing nests with hatching asynchrony magnitude close to class edges (1 ± 0.1 days and 2 ± 0.1 days ($N = 29$)). The same interaction was then more pronounced ($\chi^2_2 = 8.18$, $p = 0.017$), which allowed us to conduct multiple comparisons. B-chicks in asynchronous broods had significantly lower baseline corticosterone levels than their sibling in the same broods ($p = 0.014$; Fig. 1). They also tended to have lower baseline corticosterone levels than A-chicks in highly asynchronous

broods ($p = 0.074$; Fig. 1). Moreover, there were no differences across treatments for A-chicks (all $p = 1$), whereas baseline corticosterone levels were greater among B-chicks in highly asynchronous than among B-chicks in synchronous and asynchronous broods ($p = 0.046$ and $p = 0.011$ respectively; Fig. 1). No other interaction or single term was significant (all $p > 0.15$). No significant sex ratio bias in relation to hatching asynchrony magnitude and rank was found ($\chi^2_6 = 2.92$, $p = 0.82$) suggesting that our manipulations were not biased in relation to chick sex.

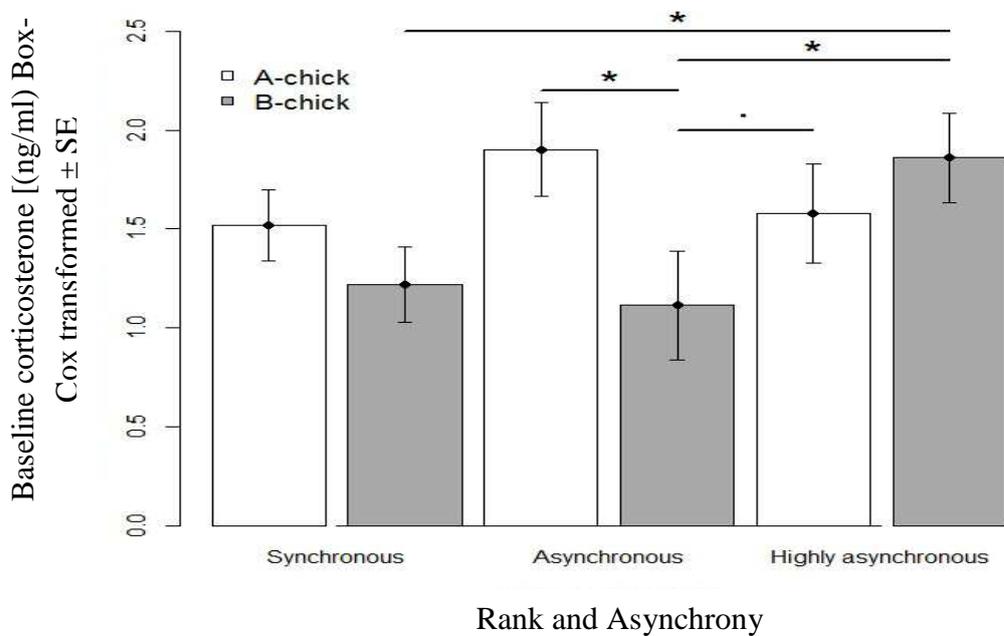


Figure 1. Baseline corticosterone levels (mean \pm SE; Box-Cox transformed ng/ml) according to rank and hatching asynchrony. White bars represent A-chicks; grey bars represent B-chicks. Significant ($p < 0.05$) and marginally significant ($p < 0.1$) pairwise comparisons (with post-hoc Bonferroni correction for multiple comparisons) are indicated by asterisks and points, respectively. The degree of significance was assessed from a dataset comprising the greatest contrast between hatching asynchrony classes (see section 3.1 for explanations).

Corticosterone levels and growth/condition variables

Mass gain was negatively correlated with baseline corticosterone levels ($\chi^2_1 = 8.90$, $p = 0.0028$, Fig. 2), while the negative correlation between structural size growth rate and baseline corticosterone levels was only marginally significant ($\chi^2_1 = 2.91$, $p = 0.088$). We found no correlation between chick body condition and baseline corticosterone levels. Apart from the interaction between rank and hatching asynchrony magnitude (see above), none of the other interactions were significant (all $p > 0.30$).

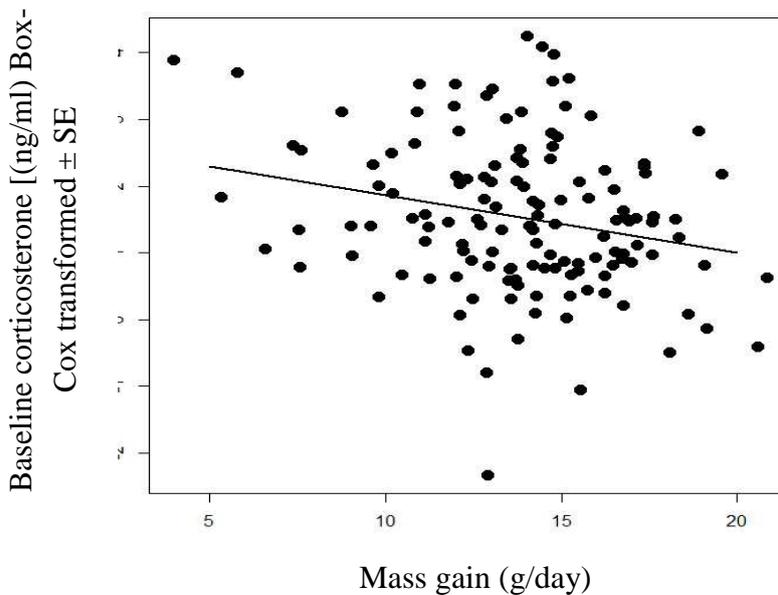


Figure 2. Correlation between baseline corticosterone levels (Box-Cox transformed ng/ml) and mass gain (g/day) during the first ten days. Dots represent data points and the fitted line is the predicted linear model.

Corticosterone levels, chick sex, sibling sex, egg rank and survival

Chick survival was related to the interaction between baseline corticosterone levels and chick sex, regardless of rank ($\chi^2_1 = 5.32$, $p = 0.021$, Fig. 3). Male survival was negatively related to baseline corticosterone (Cox model: $\chi^2_1 = 3.79$, $p = 0.051$, Fig. 3), whereas no similar relationship was detected among females (Cox model: $\chi^2_1 = 1.74$, $p = 0.19$, Fig. 3). A-chicks

had higher survival than B-chicks ($\chi^2_1 = 9.27, p = 0.002$, Fig. 3) and chicks with a female sibling tended to be more likely to die than those with a male sibling ($\chi^2_1 = 3.71, p = 0.054$).

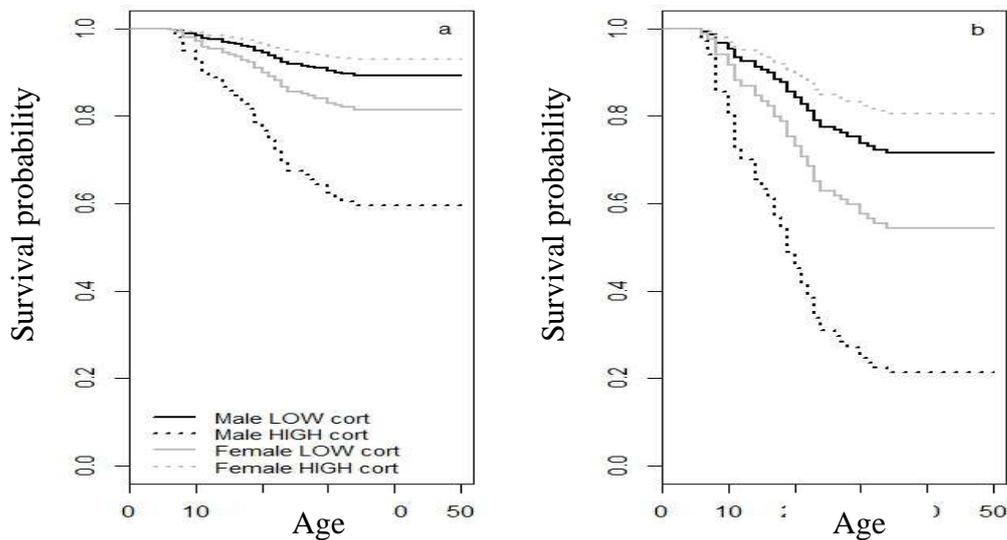


Figure 3. Chick survival probability according to baseline corticosterone levels (solid lines: low baseline corticosterone; dotted lines: high baseline corticosterone) and chick sex (black lines: males; grey lines: females) for a) A-chicks and b) B-chicks. Although baseline corticosterone levels were analysed as a continuous variable (see section 3.3), they are displayed here as a discrete variable for illustrative purposes.

DISCUSSION

Effects of hatching asynchrony, rank, sex and sibling sex on chick corticosterone levels

This is the first experimental investigation of the links between hatching asynchrony magnitude and chick baseline corticosterone in a wild population. The observed pattern depended on chick rank in the brood. Only in B-chicks did the magnitude of hatching asynchrony affect baseline corticosterone levels. Corticosterone was significantly higher in highly asynchronous broods than in the other types of brood. This is consistent with previous findings reporting higher social and nutritional stress for B-chicks in highly asynchronous

broods, where they were heavily attacked, grew more slowly, and were less likely to fledge (Osorno & Drummond 1995; Chapter 2A, p. 97). In our study, B-chicks of highly asynchronous broods were also significantly more likely to beg than in other brood types, probably signalling their higher hunger level (Chapter 2A, p. 97). Their higher baseline corticosterone levels might thus have facilitated their higher begging rates (Kitaysky et al. 2001a). However, contrary to our predictions and despite slower growth and more attacks received (Chapter 2A, p. 97); B-chicks in asynchronous broods (i.e. close to the natural situation) had lower baseline corticosterone levels than B-chicks in synchronous broods. Our results differ from those in a captive population of American kestrels (*Falco sparverius*), which is the only study investigating the role of hatching asynchrony magnitude on corticosterone levels (Love et al. 2003). In that study, increased hatching asynchrony led to higher baseline corticosterone levels among older chicks than among younger chicks, as we found in asynchronous broods but not in highly asynchronous broods. Among younger chicks, Love et al. (2003) found no effect or a negative effect of hatching asynchrony on baseline corticosterone levels in accordance with our results in synchronous and asynchronous B-chicks, but not with highly-asynchronous B-chicks. Parents were fed *ad libitum* in their study and kestrel chicks do not attack each other. Therefore, younger chicks probably did not endure the same social and nutritional stress as in our study, which might explain the different outcomes for large hatching asynchrony.

Surprisingly and contrary to our predictions, A-chicks in asynchronous broods (i.e. approximating the natural situation) had significantly higher baseline corticosterone levels than their siblings. It is unlikely that differences in baseline corticosterone levels in asynchronous broods came from differences in yolk corticosterone, as A- and B-egg yolk corticosterone contents do not differ in black-legged kittiwakes (Vallarino et al. 2012). A previous study on kittiwakes found no differences between A- and B-chicks' baseline

corticosterone levels in natural broods. However, chicks in the study were twelve to fifteen days old (i.e. after most of chick mortality occurred), which may have biased patterns (Brewer et al. 2010). Sockman and Schwabl (2001) found that yolk androgens can influence neuroendocrine development and lead to higher plasma baseline corticosterone levels in chicks. In kittiwakes, B-eggs have higher concentrations of androgens than A-eggs (Müller et al. 2012; Vallarino et al. 2012). We would thus expect B-chicks to exhibit higher corticosterone levels, in line with our prediction, but found the contrary. Our result could be viewed as a cost of being dominant for A-chicks in asynchronous broods. Indeed, dominant individuals usually have higher stress hormone levels than subordinates in cooperative breeders, which has been considered as a cost of dominance (Gesquiere et al. 2011; reviewed by Creel 2001), although the pattern seems inconsistent in birds. In kittiwakes, A-chicks are highly aggressive during the first several days after hatching, when their dominance is established (Braun & Hunt 1983; White et al. 2010; Leclaire et al. 2011). Comparable to the situation in cooperative breeders perhaps, this may be stressful for A-chicks, providing an explanation for the discrepancy between our prediction and results. In support of this, baseline corticosterone levels were the lowest for A-chicks in synchronous broods (i.e. where there was no hierarchy), albeit not significantly so.

Altogether, our results suggest stress associated with dominance in A-chicks when a hierarchy is established and substantial stress from being subordinate among B-chicks only when the latter are heavily attacked and growing very slowly. Contrary to our predictions, chick sex seemed not to influence baseline corticosterone levels, either alone or in interaction with hatching asynchrony magnitude. As between-sex differences in growth increase with chick age (Merkling et al. 2012, p.34), it is reasonable that differences at five days of age were insufficient to affect baseline corticosterone levels.

Corticosterone levels and growth/condition variables

We found no correlations between chick body condition and baseline corticosterone levels regardless of rank, contrary to previous studies, which often report that chicks in a lower nutritional state (i.e. in poor condition) exhibit higher baseline corticosterone levels (e.g., Kitaysky et al. 1999b; Sockman & Schwabl 2001; Eraud et al. 2008; Müller et al. 2010b). However, prior studies used different means of estimating chick body condition (e.g., body lipids: Kitaysky et al. 1999b; furcula fat score: Müller et al. 2010b). This may explain why we found no correlation with our body condition index (i.e. residuals of the linear regression between mass and structural size) despite correlations with mass gain and structural size—those two variables might have better represented chick “condition” in this study. Chicks gaining less weight and growing slowly during the first ten days had higher baseline corticosterone levels, regardless of hatching asynchrony magnitude. Their higher baseline corticosterone levels may indicate nutritional stress caused by lower feeding rates or lower-energy food (Kitaysky et al. 2001b) and may have facilitated food-begging (Kitaysky et al. 2001a).

Corticosterone levels, chick sex, sibling sex, egg rank and survival

Although chick sex did not influence baseline corticosterone, survival probability depended significantly on the interaction between sex and corticosterone. Regardless of rank, males with more baseline corticosterone were less likely to survive than males with less corticosterone, while baseline corticosterone was not correlated with females’ survival prospects. We found previously that male chicks grow faster and reach a larger size near fledging than females (Merklings et al. 2012, p.34), and presumably have higher energy requirements, as found in other species (Clutton-Brock et al. 1985; Cameron-MacMillan et al. 2007; Chapter 1B, p. 65; but see Torres & Drummond 1999). Survival probability did not

depend on the interaction between chick sex and mass gain, however (unpublished data)—the higher mortality of males with high baseline corticosterone seemed independent of their growth. In our study, high baseline corticosterone seemed to reflect a cost of dominance among A-chicks and nutritional stress among B-chicks. Our results suggest that male survival is relatively sensitive to stress, irrespective of its cause. Interestingly, Goutte et al. (2010) found that adult male kittiwakes with a corticosterone implant were less likely to survive during the next two years as compared to control birds. The authors did not measure females, however, and the corticosterone implants produced a higher corticosterone concentration than the baseline level we measured here.

Many studies have investigated, in sexually dimorphic species, whether the viability of the larger sex is more sensitive to poor conditions during early life (e.g., Nager et al. 2000; Kalmbach et al. 2005), but meta-analyses suggest that the pattern is inconsistent (Raberg et al. 2005; Jones et al. 2009). Here, we found no evidence that subordinate males were more likely to die than dominant males, as suggested by the absence of interaction between sex and rank. Hence, our results do not support the idea that the larger sex is more sensitive to poor environmental conditions.

CONCLUSIONS

Our data show that experimentally increased hatching asynchrony magnitude influences baseline corticosterone in B-chicks, but not in A-chicks. We interpret the observed rise of corticosterone levels in highly-asynchronous B-chicks as an indication of nutritional stress. Contrary to our predictions, however, A-chicks in asynchronous broods (i.e. similar to the natural situation) had higher baseline corticosterone levels than B-chicks—a possible cost of being aggressively dominant, as found in some cooperatively breeding species. This

hypothesis should be tested in other siblicidal species and under additional controls, where for example the same chicks are alternatively in dominant and subordinate positions. Regardless of rank, male survival probability was negatively correlated with high baseline corticosterone early in life. However, slow growth did not affect male survival more than female survival, which therefore suggests that our result is not a consequence of higher male sensitivity to poor conditions early in life, as suggested by previous studies (Nager et al. 2000; e.g., Kalmbach et al. 2005), but rather a consequence of higher male sensitivity to stress, which could arise simply from sexual dimorphism. Measuring stress-induced corticosterone and/or experimental manipulation using corticosterone implants (Goutte et al. 2010) may help to elucidate the patterns we found.

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Box 3: Do kittiwakes manipulate hatching asynchrony in relation to food availability?

During good food conditions, parents should be willing to rear their two chicks to fledging and should consequently reduce hatching asynchrony in order to lessen the size disadvantage of their younger chick (Wiebe & Bortolotti 1994b). Using data from the long-term feeding experiment (from 2003 to 2013), I predicted that hatching asynchrony should not vary between years of good and bad environmental conditions among fed birds, whereas unfed birds should increase hatching asynchrony when conditions are bad. I categorized years as ‘good’ or ‘bad’ based on the finding that 2008 marked a shift in the ocean regime (Hatch 2013): years before 2008 were considered ‘bad’ and years from 2008 to 2013 considered as ‘good’. Hatching asynchrony was measured to the nearest day only and was thus a discrete variable. Across the ten years, I had data available from 742 nests. According to the prediction, I tested the interaction between feeding treatment and year quality using a Poisson GLM. Neither the interaction nor any single factor was significant (all $p > 0.3$). However, graphically the data seem to confirm the prediction (Fig. 1). Hence, maybe by using another proxy of hatching asynchrony such as chick size dimorphism at hatching, I might be able to find a significant interaction.

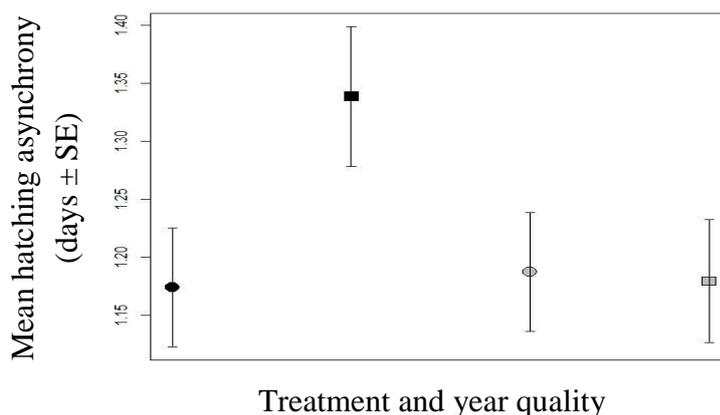


Figure 1. Hatching asynchrony according to Year quality and feeding treatment. Circles represent fed nests and squares unfed nests. Black is for ‘bad’ years and ‘grey’ for good years.

Chapter 3: maternal effects

Differential influence of maternal effects on chick aggression depending on pre-laying food availability

In preparation

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Influence différentielle des effets maternels sur l'agressivité du poussin en fonction de la disponibilité en nourriture avant la ponte

En préparation

RÉSUMÉ

Chez les espèces d'oiseaux à siblicide facultatif, un conflit entre les parents et le poussin aîné peut survenir quand les parents sont capables d'élever la totalité de la ponte, alors que pour le poussin aîné, les bénéfices associés à la mort de son frère cadet sont supérieurs aux coûts en termes énergétique dû à son agressivité et en termes de réduction de la fitness inclusive. Dans ce contexte, on s'attend à ce que les femelles manipulent le phénotype de ses poussins en déposant plus d'androgènes dans le second œuf, augmentant ainsi les capacités compétitrices du poussin cadet. Une étude précédente montre une telle allocation différentielle d'hormones chez la mouette tridactyle (*Rissa tridactyla*), mais les investigations sur les corrélations avec les comportements des poussins restent manquantes. Le but de notre étude est d'examiner expérimentalement, les patterns d'agressivité des poussins, ainsi que leur croissance et leur survie en fonction de leur rang et de l'occurrence d'un conflit. Nous avons (1) manipulé la disponibilité en nourriture avant la ponte afin de mimer une situation où il devrait y avoir conflit (groupe contrôle) et une situation où il devrait être absent (groupe nourris) et (2) créer des nichées artificielles avec une séquence d'éclosion inversée, permettant ainsi une détection plus aisée des différences de comportements du poussin initialement cadet en fonction de la disponibilité en nourriture avant ponte. En accord avec nos prédictions, nos données ont révélé que dans un contexte de conflit, l'agressivité du poussin né en second a été augmentée, suggérant fortement que les femelles peuvent manipuler la compétitivité entre les poussins en

fonction des conditions environnementales. Cependant, nous n'avons trouvé aucun effet de cette tactique sur la croissance et la survie des poussins, ce qui suggère que l'allocation différentielle d'hormones seule n'est pas suffisante pour diminuer la probabilité de siblicide dans un contexte de conflit.

Mots-clés : agression; conflit parent-enfant; effets maternels; fratricide; mouette tridactyle

ABSTRACT

In siblicidal species, a conflict between parents and the senior chick can occur when parents may be able to raise the whole brood, whereas for the senior chick the benefits associated to the death of its junior sib exceed the inclusive fitness and energetic costs arising from its aggressive behaviour. In this context, females are expected to manipulate offspring phenotype via depositing more androgens in second laid eggs, thereby improving the competitive abilities of the junior chick. A previous study reported such a differential hormone allocation in the black-legged kittiwake (*Rissa tridactyla*), but investigations of chick behaviour correlates are missing. Here, we aim to experimentally investigate chick aggression rate along with growth patterns and survival according to hatching rank and occurrence of a conflict. We (1) manipulated food availability before laying in order to mimic a situation where a conflict is expected to occur (control group) or not (supplemented group) and (2) created artificial broods with reversed hatching sequence, allowing an easier detection of differences in the second hatched chick behaviour according to food availability before laying. In line with our predictions, our data revealed that in a context of a conflict, aggressiveness of second hatched chicks was increased, strongly suggesting that females can modulate between-chick asymmetry in competitiveness according to environmental conditions. However, we did not find any evidence that this tactic influenced chick growth and survival, thereby suggesting that differential hormone allocation is not sufficient alone to decrease the probability of siblicide in a context of conflict.

Keywords: aggressiveness; kittiwake; maternal effect; parent-offspring conflict; siblicide

INTRODUCTION

A fundamental tenet of Darwin's (1859) theory of evolution by natural selection is that individuals compete for limited resources. Interestingly, this also holds true for related individuals such as chicks within a brood, known to compete for parental resources such as food (Mock & Parker 1998). Most of the time, this competition is nonviolent, involving begging displays and scrambling (Wright & Leonard 2002). In some species however, competition can be direct via overt aggressive attacks sometimes leading to siblicide (Mock & Parker 1998). Siblicide relates to the death of a chick caused by its sibling via pecking or ejection from the nest (Mock 1994). This behaviour is facilitated by hatching asynchrony, which leads to a size asymmetry facilitating the establishment of a dominance hierarchy (Lack 1947, 1954), the junior chick typically being the victim during aggressive confrontations.

Siblicide behaviour occurs either in "obligate siblicidal species", in which competition nearly always results in the death of a chick, or in "facultative siblicidal species", where the death of the junior chick is conditional to food abundance (Mock et al. 1990). In the latter species, aggression level by the senior chick increases when the amount of food delivered by parents decreases (Drummond 2001). In poor environmental conditions, the concomitant loss of one or more competitive "hungry mouths" in the nest would result in more food available for the remaining senior chick and thus, in a higher growth rate and fledging prospects. However, aggressive behaviour could be energetically costly for the senior chick and killing its sibling would reduce its inclusive fitness (O'Connor 1978). Broodmate aggression is thus the result of a balance between associated costs and benefits, regulated in part by environmental conditions (Mock et al. 1990). From the point of view of the parents, the situation is different. If the environmental conditions could allow them to raise the whole brood, the loss of a chick would reduce their fitness return. Conversely, in poor environmental conditions, a single chick might be a better bet than many weak (or dead) costly chicks.

Hence, depending on environmental conditions, a conflict between parents and senior chick(s) may occur over brood reduction (Lack 1947, 1954). More precisely, this conflict is expected to occur under moderate environmental conditions as parents may be able to raise the whole brood while at the same time, the benefits related to the death of the junior chick (in terms of growth rate / survival prospects) for the senior chick are expected to exceed the inclusive fitness / energetic costs arising from its aggressive behaviour. In this context of a parent-offspring conflict over siblicide, any tactic allowing parents to increase junior chick competitiveness should be favoured (Benowitz-Fredericks *et al*, 2013).

One way parents could favour the junior chick is by reducing hatching asynchrony. Indeed, Wiebe and Bortolotti (1994b) found that hatching asynchrony was lower during good food years (or among good condition individuals) than during poor food years (or among poor condition individuals). Parents may also modulate chick competitiveness according to environmental conditions through differential allocation of hormones in eggs (Hackl *et al*. 2003). Indeed, as elevated yolk androgens are known to promote faster pre- and post-natal development and increase competitive ability (Groothuis *et al*. 2005a), it could be used as a way to manipulate competitive asymmetry between chicks (Sockman *et al*. 2006). However, exposure to a high concentration of androgens may also have negative effects on the immune system (e.g., Müller *et al*. 2005; Sandell *et al*. 2009), on adult behaviour (Müller *et al*. 2008) or an antioxidant activity (Tobler & Sandell 2009). Parents would thus have to balance between the benefits and costs of high deposition of yolk hormones depending on the conditions (Groothuis *et al*. 2005b). When no conflict is expected, females should limit the amount of androgens in their eggs in order to avoid the associated costs, as the younger chick should survive anyway. Conversely, when facing a conflict, they are expected to increase yolk androgen concentrations in the second egg to stimulate the junior chick competitiveness. Several authors investigated the pattern of androgen allocation along the laying sequence

(e.g., Schwabl 1997; Drummond et al. 2008; Müller et al. 2012), but only one study experimentally tested whether this pattern depended on environmental conditions (Benowitz-Fredericks et al. 2013). In this study, the authors reported that in black-legged kittiwakes, *Rissa tridactyla*, mothers deposited relatively more androgens in their second egg when the younger chick had intermediate survival prospects.

In order to confirm that this observed yolk hormones within-clutch variation in relation to environmental conditions is adaptive in a context of sibling competition (Müller & Groothuis 2013), the next step would be to investigate how it impacts chick aggressive behaviour, growth and survival. While the aggressiveness of the senior chick should be easy to measure through its high expected aggression rate, the aggressiveness of the junior chick proves to be quite difficult to assess as, being younger and smaller than its sibling, it is expected to display very few aggressive behaviours (White et al. 2010). A solution would be to create experimental broods in which B-chicks are older than A-chicks, allowing an easier detection of differences in B-chicks behaviour according to environmental conditions.

In this study, we aimed to measure aggressiveness, growth rate and survival according to rank and environmental conditions. As theoretically expected (Benowitz-Fredericks et al. 2013), we considered environmental conditions as a proxy of the occurrence of a conflict between the senior chick and the parents over siblicide. We studied the same population of black-legged kittiwakes, *Rissa tridactyla*, as Benowitz-Fredericks et al. (2013). This allows us to precisely predict when the younger chick should be behaviourally boosted. Black-legged kittiwake is a facultatively siblicidal seabird (Braun & Hunt 1983) in which females usually lay two eggs hatching asynchronously (Gill et al. 2002). The first-hatched chick (A-chick) becomes dominant over the second one (B-chick) in few hours via overt aggression and the latter responds submissively (“aggression-submission dominance relationship”: Drummond 2006; see also Braun & Hunt 1983). We created two experimental brood groups: (1) “control”

broods, composed of two non related nestlings that hatched with an asynchrony of about two days and for which the laying order was not changed in their foster nest (i.e. A-chick hatched two days before B-chick); (2) “reversed” broods, composed of two non related nestlings that hatched with an asynchrony of two days and for which the laying order was reversed (i.e. B-chick hatched two days before A-chick). As in the study of Benowitz-Fredericks *et al.*, (2013), we experimentally manipulated environmental conditions in order to create two groups of parents for each type of experimental brood: the “pre-laying fed” group where pairs were provided with additional fish until females laid their second egg and the “pre-laying unfed” group where pairs were not fed. We assumed that the pre-laying fed group matched a situation where there was no parent-offspring conflict as food availability was excellent and predictable and that the pre-laying unfed group matched a situation with conflict because during this breeding season, environmental conditions were moderate (see Appendix 1). We stopped feeding at laying so that chick behaviour could only be affected by yolk hormone deposition. We then recorded chick aggression rate, begging rate (see Appendix 2) and feeding rate (see Appendix 3), as well as chick growth and survival.

Regarding aggression rate, we predicted that pre-laying unfed senior chick in “reverse” broods should be more aggressive than pre-laying unfed senior chick in “control” broods, as the pre-laying unfed situation is supposed to mimic a family conflict and females should have boosted their B-egg with yolk androgens. Among pre-laying fed chicks, however, we expected this difference to be lower, as females should not have boosted their B-egg too much in order to avoid the potential negative effects of increased yolk hormones.

We also looked at chick growth and survival. As senior chicks should receive more food, as junior chicks should be subject to costly attacks and as elevated yolk androgens should promote faster growth (e.g., Groothuis *et al.* 2005b) we predicted that (1) overall, senior chicks should grow faster than junior chicks and should have a higher survival rate. However,

for clutches with no supplemented parents: (1) in “control brood”, as junior chicks (i.e. B-chick) should receive more androgens, they should grow faster than other juniors, (2) in “reversed brood”, junior chicks (i.e. A-chicks) should have a lower growth and survival rate than all other chicks because of the level of their sibs’ aggressiveness, while the senior chicks (i.e. B-chicks) should have a faster growth and a higher survival rate because, besides being in dominant position, they should receive more androgens.

MATERIALS AND METHODS

Study site

The study was carried out in 2012 from mid-May to mid-August in a population of black-legged kittiwakes *Rissa tridactyla* nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59° 26’N, 146° 20’W), Gulf of Alaska. The tower is a twelve-walled polygon where artificial nest sites have been created on the upper walls, permitting observations (from a distance of ~20 cm) through one-way window glass from inside the tower and allowing us to easily monitor the breeders and their chicks (for more details see: Gill & Hatch 2002).

Experimental procedure

Upon our arrival, breeding pairs were randomly allocated into two experimental groups: ‘pre-laying fed’ for parents which were food supplemented (N=30) and ‘pre-laying unfed’ for non food-supplemented parents (N=37). Pre-laying fed males and females were provided with capelin *Mallotus villosus* three times daily (08:00, 14:00 and 18:00 h) until satiation was reached. Food supplementation began on April 20 (i.e. 41.7 days before the laying of the first egg, mean \pm SE: 41.7 \pm 6.3 days), and was stopped after the second egg was laid.

Nests were checked twice daily (9:00 and 18:00) throughout season to record event such as laying or chick death. Laying date was recorded and each egg was individually marked (A for the first-laid egg and B for the second egg, two being the typical clutch size) with non-toxic waterproof ink. Eggs were put in an incubator (Compact S 84 MP GTFS, Grumbach Brutgeraete GmbH, Asslar, Germany) set at 37.4°C and 63% of humidity twenty four days after laying (i.e. three days before expected hatching date; mean incubation period: twenty-seven days; Hatch et al. 2009). They were replaced by hen eggs (of similar size and previously put in the incubator) artificially marked to mimic the natural pigmentation of kittiwake eggs. No change in parental behaviour was observed when incubating the fake eggs.

The time of chick hatching in the incubator was assessed within two hours between 6:00 and 22:00 and within six hours during the night. Each chick was then weighed to the nearest 0.1g with an electronic scale and measured for head-bill and tarsus length to the nearest 0.1 mm with a calliper and for wing length to the nearest mm with a wing ruler. Chicks were marked on the head with a non-toxic marker to identify their origin rank (blue for A-chicks and green for B-chicks).

Following hatching, each chick was put in a foster nest to create two types of experimental broods, each containing one A-chick and one B-chick. The first type (“control broods”) contained two chicks hatched two days apart (51.4 ± 2.5 hours, $N= 37$) and whose position in the laying sequence was maintained (i.e. A-chick had hatched before B-chick). The second type (“reverse broods”) contained two chicks hatched two days apart (57 ± 1.7 hours, $N= 30$) but whose position in the laying sequence was reversed (i.e. A-chick had hatched after B-chick so that, unlike natural situation, A-chick is smaller and younger than its sib when put in their foster nest). Chicks from pre-laying fed parents and pre-laying unfed parents were equally distributed among these two types of brood.

Behavioural observations

To observe chick behaviors we used fifteen minutes focal sampling (Altmann 1974). Each nest was observed at least once a day and in a random order. A total of 1668 observations (25025 min) were performed on sixty-seven nests during the season. For each chick, we recorded whether it was being aggressive towards its sibling, whether it pecked its parent beak to beg for food (see Appendix 2) and whether it was fed (see Appendix 3). Nests were observed from the day the younger chick was put in the nest until it was ten days old (the period when most aggressive acts occur : White et al. 2010; Leclaire et al. 2011) or one of the chicks died.

Measuring chick growth

Chicks were measured every five days from day zero to thirty-five. Although fledging usually occurs at an older age (after forty days: Hatch et al. 2009), we did not manipulate chicks after thirty-five days old to avoid precocious fledging. Measurements were the same as at hatching (see 'Experimental procedure' section). We ran a principal component analysis on wing, tarsus and head-bill lengths with all ages together in order to create a synthetic morphological variable and kept the score of each individual for each of the three ages, on the first principal component (89% of total variance explained). We calculated the mass gain and the body growth rate between zero and ten days ($N = 110$) with the slope of the linear regression between body mass and age and between body size (i.e. ACP scores) and age, respectively. We also considered maximal body mass and body size for chicks that survived until thirty days at least ($N = 96$).

Statistical analyses

For each analysis, we followed the same procedure. We started with a complete model and successively removed each term beginning with the terms of the highest degree, we then compared the change in deviance (in mixed models with a maximum likelihood estimator using *lme4* package (Bates et al. 2011) after removal of that term, using a χ^2 test. When an interaction was tested, the simple effects within the interaction were always kept in the model too. We used presence/absence data to analyse aggression rate because the percentage of focal including these behaviour was low.

Behavioural observations were analysed using generalized linear mixed models (GLMM) with a binomial error distribution and logit link function (i.e. response variable was binary: 0 or 1 for each behavior concerned). We first assessed the effect of the food treatment, the origin rank (A or B) and the new status of chicks in their new brood (senior, junior) and their interaction. In order to control for the potential effect of seasonality and of the age of chicks on their behaviour, we included hatching date and chicks' age as covariates in all models. The food treatment of adoptive parents was also included in models in order to control for an eventual behaviour bias. In each model, individual identity was nested within foster nest as a random effect to avoid pseudo-replication, as well as individual identity nested within biological nest. We also included observation date and observer identity as random effects.

We used Box-Cox transformation (Box & Cox 1964) in the *MASS* package (Venables & Ripley 2002) on mass gain ($\lambda = 2.02$), body growth rate ($\lambda = 1.97$), and maximal body size ($\lambda = 4.93$) to meet model assumptions (data and residuals normality and homoscedasticity) in the growth analyses. For all variables, we assessed the effects of food treatment, origin rank, new status and their interactions using linear mixed models with nest as a random effect. We included the hatching date as a covariate to control for the seasonality and as well as food treatment of adoptive parents.

For survival analysis, we used right-censored data as birth date was known for every chick, but death date was not known for chicks still alive when we left the island. Hence, we used the age of thirty-five days in models for these chicks. As survival is not independent for chicks coming from the same nest we used Cox proportional hazards mixed regression models (CPH mixed) in the *coxme* package (Therneau 2012). Food treatment of biologic and adoptive parents, origin rank, status and hatching date were included as fixed predictors in models, and the foster nest as random factor to avoid pseudo-replication issues due to interdependence of siblings.

All analyses were conducted with R 2.14.2 (R Development Core Team 2012). Results are shown with mean \pm standard error (SE).

RESULTS

Aggression

Aggression rate depended on the interaction between food treatment of biological parents, original rank and status (interaction: rank * food treatment * status: $\chi^2_1 = 5.04$, $P = 0.024$, Fig. 1). Senior chicks from pre-laying unfed parents in reversed broods had a higher aggression rate than the same chicks in control broods. In line with our key prediction, this pattern was not found among pre-laying fed chicks, as senior chicks were more aggressive than junior chicks irrespective of their origin rank (Fig.1). Moreover, as predicted, senior chicks from pre-laying unfed parents in control broods had the same level of aggressiveness than senior chicks from pre-laying fed parents. No differences were found among junior chicks. Aggression rate decreased already significantly with age (age: $\chi^2_1 = 7.24$, $P = 0.007$), while hatching date and food treatment of adoptive parents were not significant alone (hatching date: $\chi^2_1 = 2.73$, $P = 0.10$; food treatment of adoptive parents: $\chi^2_1 = 0.68$, $P = 0.41$).

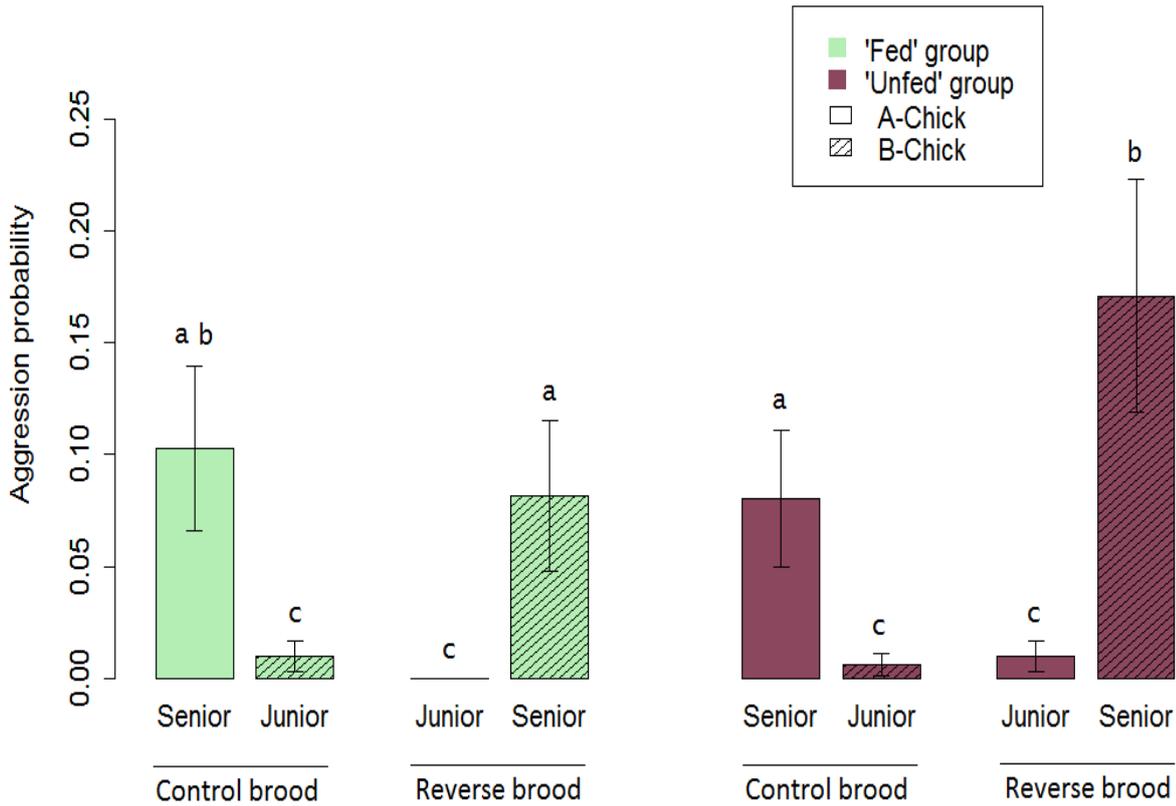


Figure 1. Predicted aggression rate (mean \pm SE) according to chicks' status (Senior, Junior), original rank (A and B) and pre-laying food treatment (Fed and Unfed). Bars with the same letter do not differ significantly.

Chick growth

Interactions between food treatment of biological parents, origin rank and status had no influence on mass gain (all > 0.09), but mass gain depended on chick status (status: $\chi^2_1 = 6.52$, $P = 0.011$, Fig.2a) with senior chicks displaying a higher mass gain than junior chicks. We did not find any effect of food treatment of biological and adoptive parents, origin rank, and hatching date on mass gain (all $P > 0.34$). Maximal body mass did not depend on food treatment, origin rank, status and their interaction.

Body growth rate depended also on status and followed the same pattern than mass gain (status: $\chi^2_1 = 7.69$, $P = 0.006$, Fig.2b). Interactions between food treatment, origin rank and status and all single factors within them were not significant (all $P > 0.07$). Body growth rate depended on hatching date (hatching date: $\chi^2_1 = 11.49$, $P < 0.001$) with a lower rate when

chicks hatched later in the reproductive season. Similarly to maximal body mass, we found no effect on maximal body size (all $P > 0.42$) except for hatching date (hatching date: $\chi^2_1 = 3.82$, $P = 0.05$), with maximal body size being smaller when chicks hatched later.

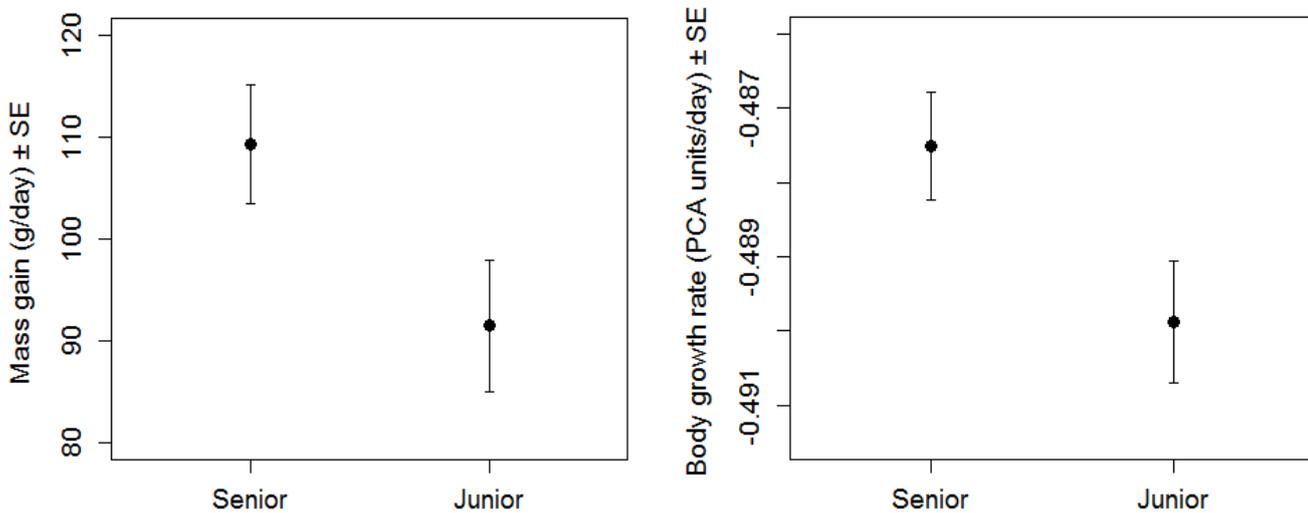


Figure 2. **a)** Predicted mass gain (g/day; mean \pm SE) according to chick status. **b)** Predicted body growth rate (PCA unit/day; mean \pm SE) according to chick status.

Survival

Survival did not depend on any interactions involving origin rank, status and food treatment. Origin rank and food treatment alone had no influence on survival neither (origin rank: $\chi^2_1 = 1.28$, $P = 0.26$; food treatment: $\chi^2_1 = 1.13$, $P = 0.29$). Nevertheless, survival was influenced by status (status: $\chi^2_1 = 5.86$, $P = 0.016$) with a higher mortality rate for junior chicks as compared to senior chicks (Fig. 3). Hatching date and food treatment of adoptive parents had no effect on survival probability (hatching date: $\chi^2_1 = 0.08$, $P = 0.78$; food treatment: $\chi^2_1 < 0.001$, $P = 0.99$).

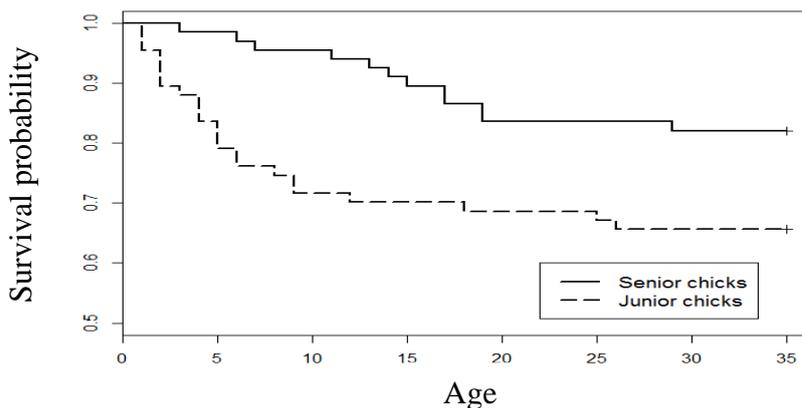


Figure 3. Survival probability according to chick status (solid lines: Senior chicks; dashed lines: Junior chicks)

DISCUSSION

When environmental conditions are good, both the parents and the senior chick should agree over the junior chick survival. However, in intermediate environmental conditions, parents and senior chick are expected to be in conflict over brood reduction, with parents wanting the junior chick to survive contrary to the senior chick. In this context, females are expected to boost their second hatched chick by allocating more androgens to this egg, as androgens can increase chick aggressiveness and growth (Groothuis et al. 2005a). Here we manipulated environmental conditions via supplemental feeding. As predicted, we found that a B-chick from an unfed nest that we put in a senior position exhibited a higher aggression rate than the others senior chicks, as expected from the yolk testosterone patterns found by Benowitz-Fredericks (2013) in the same population.

More precisely, we found that whatever the type of brood, senior chicks shown a higher aggressiveness than junior chicks confirming that the age difference between the chicks due to hatching asynchrony is determinant to obtain dominance within the brood. Further, one of our main results is that in situation of conflict (pre-laying unfed parents), senior chicks coming from B-eggs were more aggressive than senior chicks coming from A-eggs, whereas this pattern was not found when biological parents had been fed. Pre-laying unfed females thus probably increased yolk androgens concentrations to reduce between-chick asymmetry in competitiveness in a situation of parent/senior chick conflict, as previously found (Benowitz-Fredericks et al. 2013).

However for the group with unfed parents, we expected junior chicks hatching from a B-egg to show higher aggressiveness than junior chicks hatching from an A-egg. But as already mentioned earlier, even if a junior chick is more aggressive compared to other juniors, detecting this effect could be difficult since the behaviour of junior chicks seems rapidly inhibited by its senior sib. One may then ask why females would increase aggressiveness of

its junior chick if it cannot express it. One likely reason would be that testosterone increase aggression but also other components involved in competitiveness. For example, in red-winged blackbird, *Agelaius phoeniceus*, direct injections of testosterone into egg yolk resulted in an increase in hatching muscle mass responsible for breaking the shell during hatching (Lipar & Ketterson 2000). As it has been demonstrated in the same population that females increased the amount of testosterone with laying sequence (Lipar et al. 1999), this strategy could allow females to reduce the effect of hatching asynchrony by enabling a faster hatching of the younger chicks. Moreover, in Japanese quails, *Coturnix japonica*, testosterone increased the boldness and activity level (Niall Daisley et al. 2005). Hence, female kittiwakes, by increasing testosterone in B-eggs, may favour the emergence of a “cocktail” of competitive abilities in junior chicks, such as aggressiveness, higher activity and musculature to reduce hatching asynchrony effects. This could allow B-chicks to better respond to the attacks of its sib, or to better avoid it thanks to a better locomotion and alertness.

Concerning chick growth and survival, the hierarchy within the brood seemed to be decisive. Indeed we found that senior chicks had better growth and lower mortality than junior chicks. This difference in growth and survival between chicks would not result from a preferential feeding of parents because chicks seem to be fed in the same way (our unpublished data). Aggressiveness of senior chicks should be responsible of this pattern. Junior chicks would pay a cost in terms of growth and survival of being frequently aggressed. Additionally, we did not find any effect of status on maximal body mass or maximal body size, although senior chicks grew better than junior chicks. This result suggests that junior chicks with a low growth rate between 0 and 10 days did not survive and that only chicks in good condition reached fledging, thereby explaining the higher mortality of junior chicks.

Then, if females were trying to strengthen the junior chick's competitive abilities in situation of conflict, B-chicks from pre-laying unfed parents should have a better growth and

survival rate than B-chicks from pre-laying fed parents. It was not the case in our study and several reasons can explain this result. First, testosterone may not impact growth in black-legged kittiwake as suggested by Müller et al. (2012). Indeed, in their study, experimentally injected androgens had no effect on chick growth. But these chicks were fed *ad libitum* which may induce a strong growth for all individuals (both chicks from androgen-injected eggs and control chicks), and would thus conceal the effect of androgens. The second reason might be methodological. Artificial broods were created with a hatching asynchrony of approximately two days, whereas the natural asynchrony in this population is about 1.35 days (mean: 1.35 days, Gill et al. 2002). Our hatching interval increased size asymmetry between chicks and probably reinforced the senior chick's dominance. Indeed, when hatching asynchrony is increased, the junior chick is totally disadvantaged and usually has a slower growth and a higher mortality rate, as found in other species (e.g., Mock & Ploger 1987; Osorno & Drummond 1995). Consequently, even if unfed females increased the competitiveness of junior chicks via maternal effects, the gained advantage for junior chick might not have been sufficient to counteract the excessive asymmetry between chicks. Hence, to increase their second chick survival prospects, females should not only increase yolk hormones concentrations but also reduce hatching asynchrony concomitantly, as proposed by Müller et al. (2004). There are some evidence that hatching asynchrony can be manipulated depending on environmental and individual condition (Wiebe & Bortolotti 1994b), but to our knowledge no study investigated whether females can manipulate both variables together according to environmental conditions. Thus, it would be relevant to investigate whether in our population, there is a difference in incubation behaviour and, ultimately, in hatching asynchrony between fed and unfed females depending on natural food availability.

To our knowledge, our study is the first to suggest a manipulation of chick aggressiveness by females according to environmental conditions in a context of parent-offspring conflict

over siblicide. Moreover, this manipulation seems to be realized via testosterone, since our results are in line with Benowitz-Fredericks et al. (2013). However to confirm this, we need experimental studies to test the influence of testosterone on chick competitive abilities other than aggressiveness. For example, we could inject testosterone in eggs and look at its effects on chick locomotion, alertness and general activity level. Next it could be interesting to repeat our experiment, but in year where the environmental conditions would be unfavourable. In this case, parents should be in moderate condition before laying if food supplemented and in poor condition if not. In poor conditions parents might not be able to fledge two chicks, so the death of the younger chick would be beneficial for both sides and no conflict should occur. Hence, females should not increase the competitive abilities of the junior chicks via maternal effect. Conversely, pre-laying fed females should manipulate chick behaviour and increase competitive abilities of junior chicks such as aggressiveness, mobility, alertness.

In conclusion, our results suggest that female kittiwakes can manipulate chick aggressiveness according to environmental conditions, probably via the allocation of testosterone in eggs in order to improve their fitness return. In situation of parent-offspring conflict, they seem to increase the aggressiveness of the junior chick in order to increase their fledging success.

ACKNOWLEDGEMENTS

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States laws and under permits from the U.S. Fish and Wildlife Service and the State of Alaska. Any use of trade names is for descriptive purposes only and does not imply endorsement of the U.S. Government.

SUPPLEMENTARY DATA

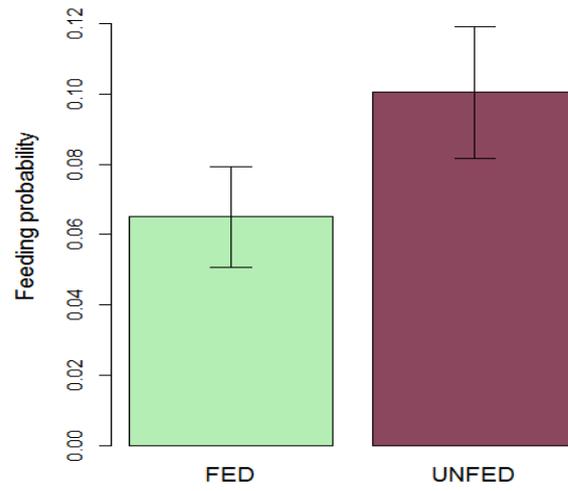
As proxies of environmental conditions, we calculated fledging success and B-chick survival from nests which were not manipulated. Comparing our results with fledging success and survival of previous years (2006, 2007, 2008, 2009), we can estimate the quality of the season 2012. In 2012 fledging success and B-chick survival were intermediate as compared to 2008 which was a very good year and to 2009 which was a very bad year. Hence, in 2012 birds were probably facing moderate environmental conditions.

	Fledging success	B-chick survival
2006	43%	43%
2007	66%	66%
2008	81%	87%
2009	29%	44%
2012	70%	68%

Appendix 1. Fledging success and B-chick survival until fledging in years 2006, 2007, 2008, 2009, 2012 proxy of environmental conditions

Appendix 2. Summary of the different terms for which significance was tested for concerning begging rate and feeding rate.

	Chisq	df	P
Begging			
Food treatment * Origin rank * Status	2.852	1	0.091
Food treatment * Origin rank	0.051	1	0.822
Food treatment * Status	0.062	1	0.803
Origin rank * Status	0.013	1	0.909
Food treatment (biological parents)	0.183	1	0.669
Origin rank	7.934	1	0.164
Status	2.340	1	0.126
Age	0.159	1	0.691
Hatching date	0.007	1	0.932
Food treatment (adoptive parents)	0.395	1	0.53
	Chisq	df	P
Feeding			
Food treatment * Origin rank * Status	2.142	1	0.143
Food treatment * Origin rank	0.079	1	0.778
Food treatment * Status	0.026	1	0.872
Origin rank * Status	0.113	1	0.737
Food treatment (biological parents)	5.749	1	0.017
Origin rank	0.097	1	0.756
Status	1.778	1	0.183
Age	0.734	1	0.392
Hatching date	0.207	1	0.649
Food treatment (adoptive parents)	0.821	1	0.365



Appendix 3. Predicted feeding probability (mean \pm SE) according to food treatment of biological parents.

General discussion and perspectives

Parents face several reproductive decisions and should have been selected to make the choice maximising their fitness. Among these decisions are, for example: Which mate should I choose? When should I begin to breed? How much should I invest in this breeding attempt? In this thesis, I chose to study sex allocation, hatching asynchrony and egg yolk composition, three reproductive decisions taken before or at the time of egg laying.

How could sex-ratio patterns be explained?

One of my main goals concerning sex allocation was to try to disentangle between the different selection pressures potentially influencing the fitness benefits of producing sons or daughters. The main finding concerning sex-ratio was that unfed birds produced relatively more daughters than fed birds over three breeding seasons with poor environmental conditions (Chapter 1A, p. 41). The behavioural and life-history characteristics of the study species, the black-legged kittiwake, suggested that this result could be explained by a cost of reproduction type pressure and/or by a Trivers-Willard type pressure (Box 1, p. 24). I tried to get some insights into the strength of each of these potential selection pressures.

Males grew faster and got bigger close to fledging than females (Chapter 1A, p. 41): sons might be more costly to rear. However, being larger does not necessarily mean being more costly (e.g., Torres & Drummond 1999). I thus wanted to test whether sexual dimorphism meant differential rearing cost in the kittiwake. Sons were indeed more costly to rear than daughters (Chapter 1B, p. 65): a male-biased sex ratio led to either higher daily energy expenditure or reduced basal metabolic rate (i.e. a way to redirect energy from self-

maintenance to reproduction) and to higher oxidative damages, but only among mothers. Brood sex-ratio had less influence on the energy expenditure of fathers: they did not seem to expend more energy in rearing sons. The only variable negatively impacted by brood sex-ratio in both sexes was baseline corticosterone (Chapter 1B, p. 65). Moreover, contrary to wandering albatrosses (*Diomedea exulans*) (Weimerskirch et al. 2000), males did not seem to invest heavily in reproduction, but if anything invested less than females. Hence, there is some evidence supporting the occurrence of a cost of reproduction type pressure, but only in females. In mammals, rearing sons might lead to a lower survival (Gomendio et al. 1990; Helle et al. 2002) or a reduced or delayed investment in reproduction the following year (Hogg et al. 1992; Bérubé et al. 1996; Monard et al. 1997). In birds, however, such sex-ratio related inter-seasonal effects are rarer, but in long-lived birds, such as the kittiwake, energetically demanding activities are known to decrease future survival (Golet et al. 1998, 2004; Jacobsen et al. 1995; but see Weimerskirch 1992) and future reproductive success (Wernham & Bryant 1998; Hanssen et al. 2005). As rearing males seem more costly for mothers, a male-biased sex-ratio could potentially influence their subsequent survival or reproductive success, but this remains to be tested. In line with the cost of reproduction hypothesis, mothers should thus be willing to avoid having only males on consecutive years because of the potentially high fitness costs and indeed they avoided producing a son when they already fledged sons the previous year as compared to birds that fledged at least one daughter (Chapter 1B, p. 65). To my knowledge, this type of results has only been found in mammals (e.g., bighorn sheep: Bérubé et al. 1996; horse: Monard et al. 1997).

To my opinion, for a Trivers-Willard type pressure to occur, a differential cost is not necessary. In a species with no differential cost according to sex, the larger sex could still benefit more from an extra investment of its parents in terms of future reproductive success. Hence, I would not have excluded the Trivers-Willard type pressure if my results concerning

the cost of reproduction would have been negative. Data of the long-term feeding experiment gave me the possibility to test whether a Trivers-Willard type pressure could occur in kittiwakes. According to this hypothesis, I expected sons from fed birds to have a higher reproductive success as adults than sons from unfed birds, whereas feeding treatment would have less impact on female reproductive success. The expected interaction between feeding treatment as a chick and sex on total number of fledglings was not significant, but I did find that this interaction significantly influenced recruitment probability on fed nests (Box 2, p. 93). Males born on fed nests were more likely to recruit on fed nests than males born on unfed nests, whereas females feeding treatment as a chick did not influence where they recruited. Moreover, within sex it seems that males recruited in the same panel where they were born, whereas females avoided it (Box 2, p. 93). For now, there is low evidence for a Trivers-Willard type pressure. Indeed, fed parents could have overproduced sons as compared to unfed parents, as their sons would be more likely to recruit on a fed nest, where reproductive success is higher. On the contrary, overproducing daughters would be a better bet for unfed parents, as females avoided recruiting where they were born. This kind of selection pressure could be similar to the 'advantaged daughter hypothesis' (Hiraiwa-Hasegawa 1993), which states that, if mothers transmit their rank in the hierarchy to their daughters but not to their sons, high-ranking mothers should overproduce daughters, whereas sons would be a better bet for low-ranking mothers. This hypothesis has been proposed for primate species, where daughters remain within their mothers' group while sons disperse. However, in kittiwakes, fledglings do not seem to stay with their parents (Hatch et al. 2009), hence the mechanism through which the position in the colony could be 'transmitted' is unknown. I can think of at least two possibilities: (1) males try to settle where they were born (philopatry) or (2) sons of fed parents fledge in better condition and are still in better condition as adults than sons of unfed parents and are therefore better competitors and able to secure a better nest site (i.e. a

fed nest). As male-male competition is intense (Cullen 1957), it is unlikely that males could settle on an attractive nest site without being a strong competitor, hence the first possibility is more unlikely to me. However, I do not really have data to support the second possibility either.

As for now, it seems that the sex ratio patterns are best explained by a cost of reproduction type pressure. Regardless of the underlying selection pressure, the observed sex-ratio bias (either in Chapter 1A, p. 41 or 1B, p. 65) were admittedly quite weak, although significant, as compared to other studies (e.g., Komdeur et al. 1997; Pryke & Griffith 2009a). This is probably due a low benefits/costs balance: (1) sons are more costly than females, but only for mothers and effects are not striking (Chapter 1B, p. 65), which might lead to weak fitness benefits of sex-ratio adjustment, as compared to other studies (e.g., Gomendio et al. 1990; Komdeur 1998) and (2) I have no evidence that the underlying mechanism is not costly, contrary to what has been suggested in other species (Komdeur et al. 2002; see also Tagirov & Rutkowska 2013).

How can parents influence sibling competition before hatching?

In this thesis I have also investigated two means that parents (or mothers only, for egg yolk composition) could use to influence the course and outcome of sibling competition and to maximise their fitness according to environmental conditions: hatching asynchrony and egg yolk composition.

First, I have showed that the natural magnitude of hatching asynchrony was optimal for kittiwakes, as found in other species (e.g., Mock & Ploger 1987; Osorno & Drummond 1995; Viñuela 1999). Indeed, synchronous hatching led to increased sibling competition, expressed by a higher brood level aggression rate, and to higher parental effort, expressed by a higher brood level feeding rate (Chapter 2A, p. 97). Despite the fact that synchronous junior chicks

grew faster than the other junior chicks, at the time of fledging hatching asynchrony did not influence chick size and mass. A high magnitude of hatching asynchrony led to high aggression probability towards junior chicks, which consequently had low survival prospects. Hence, intermediate hatching asynchrony magnitude allowed parents to minimise the costs due to synchronous and highly asynchronous hatching, but senior chicks seemed to be stressed by their dominant position, which might be an inherent cost of this position (Chapter 2B, p. 123). Overall these findings corroborated the facultative brood reduction hypothesis (Lack 1947, 1954) and the sibling rivalry hypothesis (Hahn 1981) and they may explain why hatching asynchrony has been selected to be in an intermediate range, but they do not demonstrate that parents can adjust hatching asynchrony in order to maximise their fitness. Indeed, it would be expected that parents increase hatching asynchrony when conditions would not allow them to fledge the whole brood. I tried to investigate this hypothesis using data from the long-term feeding experiment, but despite results graphically corroborating it, I did not find any significant interaction between year quality and hatching asynchrony (Box 3, p. 143). The measure of hatching asynchrony was not very accurate, which may explain the non significant result. To further confirm or not whether kittiwakes can adjust hatching asynchrony to environmental conditions, as shown in American kestrels (*Falco sparverius*) (Wiebe & Bortolotti 1994b), food availability should be experimentally manipulated before laying and, once incubation started, eggs should be put in an incubator to closely monitor hatching.

Second, I indirectly studied whether mothers manipulated their egg yolk content, androgens especially, according to the survival prospects of their junior chick. The results suggested that when the mother and her senior chick may not agree over the survival of the junior chick, mothers increased their junior chick's competitiveness (Chapter 3, p. 145). Indeed, junior chicks from unfed parents before laying were more aggressive when put in a

dominant position than senior chicks from the same parents, whereas no difference was found among fed parents. Though I cannot be sure that the observed effect was due to yolk androgens because I did not directly manipulate them, it is likely given the known increase of yolk androgens with laying sequence in kittiwakes (e.g., Gasparini et al. 2007; Müller et al. 2012; Vallarino et al. 2012) and their apparent ability to adaptively adjust yolk testosterone to environmental conditions (Benowitz-Fredericks et al. 2013).

Hence, these results taken together suggest that mothers should use these two means in a complementary manner (Müller et al. 2007). Accordingly, in a comparative analysis, Müller and Groothuis (2013) found a negative correlation between hatching asynchrony and the magnitude of yolk androgen compensation. They also suggested that this pattern should be observed within species and dependent on environmental conditions. They predicted that in good environmental conditions parents should provide juniors with more yolk androgens. However, to my opinion this should not necessarily be the case if there is no conflict, as suggested by results from Benowitz-Fredericks et al. (2013). Instead, in the absence of conflict (i.e. when environmental conditions are good), mothers should avoid the costs of high androgen concentrations, because both chicks are very likely to survive anyway. This hypothesis is supported by two studies finding that experimentally fed mothers (Verboven et al. 2003) or mothers enduring good food conditions (Dentressangle et al. 2008) put less androgens into their last egg as compared to control mothers or mothers enduring poor food conditions. I illustrated how hatching asynchrony and yolk androgen compensation should vary with environmental conditions according to this hypothesis in Figure 1.

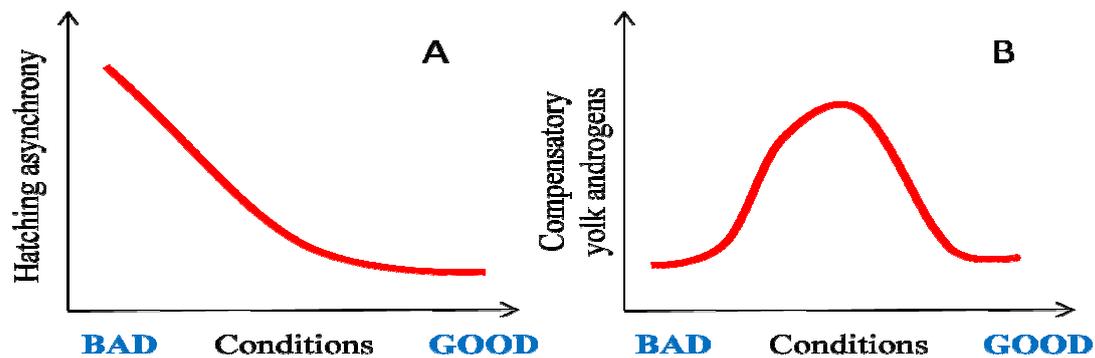


Figure 1. A: Hatching asynchrony according to environmental conditions. The better the conditions the lower the hatching asynchrony should be. **B.** Compensatory yolk androgens (difference in yolk androgens between the junior and the senior chick) according to environmental conditions. Mothers should avoid bestowing their last egg when conditions are good to avoid the negative effects of yolk androgens.

To my knowledge, no study experimentally investigated whether and how hatching asynchrony, yolk androgens covary with environmental conditions. Researchers (me included) usually use only conditions considered as good and bad, mostly because of logistical reasons, but here I suggest that it would be important to have at least three different types of conditions. One way to do it could be to have fed (i.e. good conditions), control (i.e. intermediate conditions) and handicapped (i.e. bad conditions, either through feather clipping or by adding a weight on the back of the bird) parents. Handicap increases the cost of flying and foraging and decreases body condition (e.g., Velando 2002; Leclaire et al. 2011), hence mothers should not favour their junior chicks when being handicapped. These treatments should be applied ahead of laying in order to influence parental decisions such as egg yolk composition and hatching asynchrony. At laying, eggs should be sampled for yolk androgens and put back in the nest and after incubation began, eggs should be placed in an incubator to estimate hatching asynchrony accurately.

Are reproductive decisions taken before laying sufficient to control sibling competition outcome?

The two reproductive decisions I studied occur at laying time but should influence sibling competition occurring a month later. I showed that these decisions should be influenced by environmental conditions, but such a pre-planning one month ahead should be efficient only if environmental conditions are, to some extent, predictable. Seabirds' food availability seem to be more predictable than initially thought (Weimerskirch 2007). However, many things can happen in a month and small changes in food availability between the laying and the chick rearing stage could likely change (increase or decrease) the benefits of the previously made reproductive decisions. Hence, it would probably not be advantageous for parents to rely only on decisions taken before laying to control sibling competition outcome. Moreover, when hatched chicks should also make decisions to maximise their fitness and their evolutionary interests might differ from those of their parents. For instance, chicks should have been selected to demand a higher level of resources than parents may be willing to provide in the context of their long-term interests, thereby resulting in a parent-offspring conflict over parental care (Trivers 1974; Kilner & Hinde 2012). This topic has mainly been studied in the context of begging behaviour from chicks and parental provisioning response (e.g., Hinde et al. 2010), but less in the context of chick aggression (but see O'Connor 1978). From a behavioural ecology point of view, the main questions are: who has control and how is the conflict resolved? In canaries (*Serinus canaria*), recent work from Hinde and colleagues suggest that mothers have control and feed their brood close to their optimum, in particular through the use of maternal effects (Hinde et al. 2009, 2010). These findings are opposed to what is often assumed in facultatively siblicidal species: chicks are on control and parents passively feed the dominant chick and do not interfere in sibling aggression (Mock & Parker 1997; Drummond 2001). There is however some findings suggesting that parents may not

simply accept the outcome of sibling competition. In great egrets (*Ardea alba*), mothers seem to be able to preferentially feed their second-hatched chick, when chicks are physically separated (Ploger & Medeiros 2004) and preferential feeding of smaller chicks by mothers has been reported in different species (although non siblicidal) (reviewed in Slagsvold 1997; Lessells 2002a). In kittiwakes, previous studies found that older chicks were fed more often than younger chicks (Braun & Hunt 1983; White et al. 2010), but I did not find this result neither in 2011 (Chapter 2A, p. 97) nor in 2012 (Chapter 3, p. 143). This difference might be due to a parental preferential feeding of the younger chick in good years (as were 2011 and 2012) in order to favour its survival. Moreover, there are some species in which parents have been seen physically interfering in sibling aggression (reviewed in Wiebe & Bortolotti 2000), whereas other authors claimed that they have never seen parents interfering (Mock & Parker 1997; Drummond 2001). This difference could potentially be explained by the absence of parent-offspring conflict in the latter case: parents and senior chicks did maybe agree over siblicide in these cases. In kittiwakes, however, we have seen parents interfering in sibling aggression by either doing false alarm calls or by sitting on the chicks. Interestingly, people who have worked on Middleton during poor food years said that they have not seen parents interfering (White J. personal communication). Hence, I hypothesise that parents could adaptively interfere in sibling aggression to influence sibling competition outcome and maximise their fitness. In order to test this hypothesis I did an experiment during the 2013 breeding season where parents were either fed, unfed or handicapped after they laid their two eggs (i.e. eggs should not differ in yolk content). Chick and parental behaviours were then recorded before and after chicks were taken out of the nest for six hours with no food. The aim of the fasting was to increase the conflict between parents and the older chicks. I predicted that the more difficult it would be for parents to find food (i.e from handicapped to fed), the lower the rate of interference should be. Such a finding would clearly demonstrate

that parents do not rely only on pre-hatching decisions to influence sibling competition outcome.

Conclusion

During my thesis I focused on some reproductive decisions such as sex-ratio, hatching asynchrony and egg yolk composition and I provided some evidence that kittiwakes were able to make decisions maximising their fitness under the current conditions. Fitness should be calculated over an individual lifetime, but here I mostly considered one or two breeding seasons for each aspects. As kittiwakes are long-lived seabirds (Hatch et al. 2009), these reproductive decisions and their fitness consequences should ideally be examined in a long-term perspective (future adult survival and reproductive success), as highlighted by Mock and Forbes (1994) concerning hatching asynchrony. For instance, it would be interesting to see if sex-ratio influences future survival and how individuals adjust their sex-ratio throughout their life (see Gomendio et al. 1990 for an example). We now have several years of sex-ratio data from the long-term feeding experiment, which could give us some insight about whether and how parents adjust their sex-ratio according to their age and their previous breeding experience for example. In line with this, there is to my knowledge no evidence that synchronous hatching may lead to long-term fitness consequences such as a reduce adult survival and/or future reproductive success, as previously suggested (Mock & Ploger 1987). Long-term experiment and long-term individual monitoring could definitely help to answer this question and many others about how individual maximise their fitness, as recently highlighted (Clutton-Brock & Sheldon 2010).

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Abstract

AUTHOR: Thomas MERKLING

TITLE: On sex-ratio and sibling competition: an insight into reproductive decisions in the black-legged kittiwake (*Rissa tridactyla*)

PhD SUPERVISORS: Etienne DANCHIN et Pierrick BLANCHARD

PLACE AND DATE OF DEFENCE: Université Toulouse III, Paul Sabatier, Toulouse (Schwartz Amphitheater) - Thursday 7 November 2013 at 14:30

ABSTRACT: During a breeding event, parents face several decisions that may impact their fitness. In this thesis, I have been interested in sex-ratio and sibling competition patterns in the black-legged kittiwake (*Rissa tridactyla*). First, I showed that parents in poorer condition overproduced daughters, which seems to be due to their lower reproductive cost and, possibly, to their higher fitness return for parents as compared to sons. Then, my results suggested that the natural magnitude of hatching asynchrony minimised the costs for parents and younger siblings, while older siblings seemed to be stressed by their dominant position. Finally, mothers favoured their younger chick's competitiveness only when in conflict over brood reduction with their older chick. This thesis thus highlighted some means through which parents could maximise benefits or reduce costs associated with their reproduction.

KEYWORDS: behavioural ecology; black-legged kittiwake; competition; reproduction; sex-ratio; siblicide.

DISCIPLINE: Ecology, Biodiversity & Evolution

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TITRE : Sexe-ratio et compétition dans la fratrie : un aperçu de décisions prises lors de la reproduction chez la mouette tridactyle (*Rissa tridactyla*)

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LIEU ET DATE DE SOUTENANCE : Université Toulouse III, Paul Sabatier, Toulouse (Amphithéâtre Schwartz) - Jeudi 7 novembre 2013 à 14h30

RÉSUMÉ : Au cours d'un événement de reproduction, les parents font face à plusieurs décisions qui peuvent influencer leur aptitude. Dans cette thèse, je me suis intéressé à la sexe-ratio et à la compétition dans la fratrie chez la mouette tridactyle (*Rissa tridactyla*). Tout d'abord, j'ai montré que les parents en moins bonne condition ont produit plus de filles que les autres, ce qui semblait dû à leur coût énergétique plus faible et peut-être aussi à leur plus grand retour en investissement en termes de valeur sélective pour les parents comparativement aux fils. Ensuite, mes résultats ont suggéré que l'asynchronie d'éclosion naturelle était celle qui minimisait les coûts pour les parents et pour les poussins cadets, alors que les poussins aînés semblaient être stressés par leur position dominante. Enfin, les mères ont favorisé la compétitivité de leur poussin cadet seulement lorsqu'elles étaient en conflit avec leur poussin aîné à propos de la réduction de taille de nichée. Cette thèse a donc mis en évidence certains moyens par lesquels les parents peuvent maximiser les bénéfices ou réduire les coûts associés à leur reproduction.

MOTS-CLÉS : compétition ; écologie comportementale ; fratricide ; mouette tridactyle ; reproduction ; sexe-ratio.

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