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Sibling competition in common tern *Sterna hirundo* chicks: underlying hormonal and behavioural patterns and mechanisms

Diplomarbeit

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Common tern siblings competing for an anticipated feeding (photo A. Maywald).

Abstract

In avian young that are dependent on parental food provisioning, brood size and corresponding sibling competition are key environmental factors affecting present performance, future developmental trajectories, and ultimately fitness. Within-brood competition for nutritional resources as well as the energetic costs of social competition may have a general impact on the sibship, but often also differently affect chicks within brood hierarchies based on hatching rank and sex. Testosterone (T) and corticosterone (CORT), steroid hormones associated with aggression and stress response, respectively, are focal candidates for proximately mediating sibling conflicts and the physiological consequences. I studied the effect of sibling competition on chick condition and possibly underlying behavioural and hormonal patterns and mechanisms in the common tern *Sterna hirundo*, a semiprecocial bird species exhibiting hatching asynchrony, slight sexual size dimorphism, and profound effects of chicks' mass growth on their fitness.

First, I conducted an observational study on sex-, hatching rank-, and brood size-specific patterns in chick condition, success in competitive behaviour, and T and CORT levels. I hypothesized T and CORT levels to vary with hatching order and sex of the chicks. Condition was better in senior than junior chicks and as a tendency also better in male than female chicks. In contrast, neither competitive success nor hormone levels varied with hatching rank or sex. However, T levels were affected by the interaction of the sex of the senior chick in a brood with hatching order: Male seniors had lower and female seniors had higher T levels than their junior siblings, respectively. Correspondingly, with respect to condition male seniors tended to be more superior to their younger brood mates than did female seniors.

Second, to investigate the effect of sibling competition on chick condition, feeding rates, and T and CORT levels, I experimentally varied the extent of within-brood competition by a one-day removal of the senior siblings from two-chick broods. I tested the hypotheses that the junior chick remaining in the colony will in response show increased feeding rates, ameliorated condition, and decreased T and CORT levels. In the absence of their siblings, juniors' condition indeed improved. Their CORT levels covaried with condition and tended to drop, and significantly rose again upon the senior's return. In contrast, the experiment did neither affect juniors' feeding rates nor T levels.

Overall, the results show that sibling competition imposes limits on chick condition, at least in junior chicks, and underline the role of elevated CORT levels as a response to connected energetic stress. T seems to especially play a role for female senior chicks as a means to impose their dominance in the brood hierarchy in spite of a hardly superior physique, but the proposed general link between sibling competition and endogenous T does not hold in common terns. Hatching rank- and sex-related condition patterns do not appear to be governed by baseline endogenous steroid hormones, but could instead be influenced by short-term level elevations or maternal yolk steroids.

Contents

1 Introduction

Environmental conditions experienced early in life may not only have immediate effects on individual performance, but also substantial long-term consequences for life-history-trajectories and fitness (reviews in Lindström 1999, Metcalfe & Monaghan 2001, Mangel & Munch 2005, Ludwigs & Becker 2006). In avian young that are dependent on parental food provisioning, brood size and corresponding sibling competition are key environmental factors in this context (Mock & Parker 1997). Crucial traits of young may be negatively affected by within-brood competition for nutritional resources (Leonard *et al.* 2000, Stoehr *et al.* 2001, Magrath *et al.* 2007) and also by social competition, i.e. begging and aggression (Leonard *et al.* 2000, Neuenschwander *et al.* 2003), and related energetic costs (Kilner 2001, Rodriguez-Girones *et al.* 2001, Chappell & Bachman 2002). Hence, especially chicks in larger broods often suffer reduced growth, condition and survival (review in Martin 1987; de Kogel 1997, Sanz & Tinbergen 1999, Naguib *et al.* 2004, Bogdanova & Nager 2008, Gil *et al.* 2008).

Sibling rivalry, however, does not only have effects on sibships as a whole, but also differentially affects their single members, often within a hatching rank hierarchy resulting from hatching asynchrony (reviews in Stoleson & Beissinger 1995, Stenning 1996, Mock & Parker 1997). The developmental head start of first-hatched siblings brings about superior size and weight (Stenning 1996) and, consequentially, superior competitive abilities (Ploger & Medeiros 2004, Smith *et al.* 2005). Hence, the disadvantaged younger siblings show impaired growth, recruitment and survival (e.g. Bollinger 1994, Cam *et al.* 2003, Gonzalez-Solis *et al.* 2005, Eraud *et al.* 2008).

In addition to hatching rank, chicks' sex may also affect their traits, as has frequently been shown for growth patterns in birds with sexual dimorphism in adults (e.g. Anderson *et al.* 1993b, Weimerskirch *et al.* 2000, Badyaev 2002, Müller *et al.* 2007a). The larger sex, which in most cases is the male, has been shown to be competitively superior in some cases (e.g. Anderson *et al.* 1993a, Price *et al.* 1996), but on the other hand, due to higher energy demands (Anderson *et al.* 1993b, Krijgsveld *et al.* 1998, Vedder *et al.* 2005), also more costly to rear (Weimerskirch *et al.* 2000, Cameron-MacMillan *et al.* 2007) and more vulnerable and exhibiting higher mortality, especially under unfavourable conditions (e.g. Nager *et al.* 2000, Gonzalez-Solis *et al.* 2005, Benito & Gonzalez-Solis 2007). These sex asymmetries are not only a basis for biased sex ratios (review in Dhondt & Hochachka 2001), but also for more complex patterns of within-brood competitivity and consequentially post-hatching mortality (Uller 2006). Indeed, in a number of studies the performance of individual chicks or the entire sibship has been found to be dependent

on sex of certain, mostly first-hatched chicks (Bednarz & Hayden 1991, Carranza 2004, Martinez-Padilla *et al.* 2004, Blanco *et al.* 2006) and on the interaction of sex and hatching rank (Bortolotti 1986, Velando *et al.* 2002, Becker & Wink 2003).

Evolutionary origins of the described patterns of sibling competition and its short- and long-term effects have received much attention (reviews in Stenning 1996, Mock & Parker 1997, Lindström 1999, Dhondt & Hochachka 2001, Badyaev 2002). A necessary complement to insight into these ultimate causes is the detection of proximate mechanisms of sibling competition. The endocrine system has been a focus of research in this field, as it can interpret environmental variation to induce plastic individual reactions on the physiological and behavioural level (Dufty *et al.* 2002). Endocrine modifications are typically short-term; in developing organisms, though, even ephemeral changes in early hormone patterns may permanently change the phenotype and yield fundamental effects on their life-history trajectories (reviews in Clark & Galef 1995, Lindström 1999, Dufty *et al.* 2002, Groothuis *et al.* 2005b, Müller *et al.* 2007b). In birds, steroid hormones, in particular testosterone (T), an androgen, and corticosterone (CORT), a glucocorticoid, are focal candidates for mediating sibling conflicts and the physiological consequences.

T has long been known to regulate aggression and to covary with social competition levels in adults (e.g. Harding & Follett 1979, Wingfield *et al.* 1987). Endogenous T is already present in nestlings at hatch (Adkins-Regan *et al.* 1990) and there is accumulating evidence that T also promotes nestling aggression (Groothuis & Meeuwissen 1992, Sasvari *et al.* 1999, Ros *et al.* 2002, Ferree *et al.* 2004, Groothuis & Ros 2005; but see Quillfeldt *et al.* 2007b). Similarly, chicks' begging intensity has been found to be positively influenced by T (Groothuis *et al.* 2005b, Quillfeldt *et al.* 2006, Goodship & Buchanan 2006, 2007; but see Groothuis & Meeuwissen 1992, Groothuis & Ros 2005, Quillfeldt *et al.* 2007b). In the long term, however, elevated T levels involve both benefits (e.g. Schwabl 1996, Groothuis *et al.* 2005a,b, Goodship & Buchanan 2006) and substantial costs (Ros 1999, Naguib *et al.* 2004, Groothuis *et al.* 2005a, Fargallo *et al.* 2007, Sockman *et al.* 2008) with respect to development and survival.

Elevation of levels of CORT, the principal glucocorticoid in birds (Wingfield *et al.* 1997, Palme *et al.* 2005), triggers an array of physiological and behavioural responses allowing the animal to cope with stressful situations, including food shortage (Sapolsky 1992, Kitaysky *et al.* 1999), both in adults (e.g. Cockrem *et al.* 2006) and young (Holmes *et al.* 1992, Saino *et al.* 2003, Quillfeldt *et al.* 2006, 2007a). These responses include mobilization of energetic resources (Wingfield *et al.* 1997, Kitaysky *et al.* 1999) as well as increased begging intensity, aggression

and consequentially food intake (Kitaysky *et al.* 2001b, 2003, Quillfeldt *et al.* 2006, Loiseau *et al.* 2008). On the other hand, elevation of CORT levels, especially when chronic, has considerable costs including low growth efficiency, reduced immunocompetence and compromised cognitive abilities later in life (reviews in Wingfield *et al.* 1997, Kitaysky *et al.* 2003, Padgett & Glaser 2003).

In the present study, I investigate the effect of sibling competition on chick condition and possible relations to competitive behaviour and steroid hormones in the common tern *Sterna hirundo*. This species is an appropriate model for the following reasons: Long-term consequences of early development have been demonstrated: Fledging mass relates positively to recruitment probability (Ludwigs & Becker 2006). The semiprecocial chicks grow at very high rates (Langham 1983) and exclusively depend on parental feeding until fledging (Becker & Ludwigs 2004), brood reduction (Lack 1954) occurs frequently (Gonzalez-Solis *et al.* 2005), and starvation is in many cases, including the study colony, the principal cause of death (Langham 1972, Becker 1998). Chicks hatch asynchronously (Langham 1972) and sexes are slightly dimorphic (Becker & Ludwigs 2004, Gonzalez-Solis *et al.* 2005). T and glucocorticoid metabolites are present and detectable in chicks (Braasch 2005, Sprenger 2007), and begging and sibling competition for food are distinct and well observable (Braasch 2005, Smith *et al.* 2005). For older nestlings it has been suggested that competition between brood mates rather than parental favouritism is decisive for food allocation (Becker & Wink 2003, Braasch 2005, Smith *et al.* 2005; cf. Fargallo *et al.* 2003, Ploger & Medeiros 2004), which facilitates interpretation of feeding rates in terms of competitivity.

First, I conducted an observational study on the effect of chick sex and hatching rank within the brood and of brood size on chick condition. To identify possibly related behavioural and hormonal mechanisms, I looked for similar patterns in competitive success scores as well as in T and CORT levels. In a study on common terns, Becker & Wink (2003) surprisingly found that male chicks, especially in the last hatching position, had superior peak and pre-fledging masses, measures interpreted as indicators of condition (Becker & Wink 2002, 2003). This pattern contrasts with the general view of competitive and physical superiority of senior chicks (e.g. Langham 1972). In several bird species, exposure to androgens has been shown to promote competitivity and growth of chicks (see above), and positive covariation of androgen levels with laying sequence has been found (review in Groothuis *et al.* 2005b). This pattern thus has the potential to compensate the disadvantages of later-hatched chicks (Eising *et al.* 2001). Inspired by the finding that also in common terns levels of maternal yolk androgens are lower in first- than in later-laid eggs (French *et al.* 2001), Becker & Wink (2003) therefore suggested chicks'

endogenous steroid hormone levels to vary with hatching order as an explanation for the hatching order-specific weight patterns they had documented. I accordingly predicted steroid levels to vary with chick sex and, within the framework of the observational study, tested both hypotheses for T and CORT.

Second, in an attempt to establish proximate connections, I experimentally investigated the effect of sibling competition on chick condition, feeding rates and hormone levels. Practically, I eliminated the within-brood competition experienced by juniors in two-chick broods by short-term removal of their senior siblings. In detail, based on previously documented correlates and effects of brood size variation I tested the following hypotheses on the effect of the sibling removal on the junior chick: As a consequence of decreased competition for dietary resources, I predicted feeding rate to increase (cf. Leonard *et al.* 2000, Stoehr *et al.* 2001, Magrath *et al.* 2007), and as a result of this and decreased social competition, I also predicted an improvement of condition (cf. review in Martin 1987; Bollinger *et al.* 1990, Sanz & Tinbergen 1999, Saino *et al.* 2003, Naguib *et al.* 2004). The reduction of social and resource competition and energetic stress should also reduce the demand for T and CORT triggering competitive behaviour and mediating a stress response. Due to the severe costs of maintaining high levels of these hormones and because avian steroids have biological half-lives of less than an hour (e.g. Norris 1997), I therefore further hypothesized decreasing levels of T (cf. Naguib *et al.* 2004, Sprenger 2007) and CORT (cf. Saino *et al.* 2003, Sprenger 2007, Eraud *et al.* 2008) over the experimental period of one day. Correspondingly, I predicted hormone levels to increase again after the return of the senior siblings.

2 Materials and methods

2.1 Study species

The common tern is a larid exhibiting sexual monomorphism at the adult stage in most measurements including weight (Becker & Ludwigs 2004). Only length of bill, head-bill length (Coulter 1986, Wendeln *et al.* 1997, Craik 1999) and tarsus length (Becker & Ludwigs 2004) are greater in males. In fledglings, correspondingly, the only size measurement reliably found to differ between sexes is head-bill length (Becker & Ludwigs 2004). In addition, Becker & Wink (2003) found higher peak and fledging masses for male chicks.

Common terns are long-lived, monogamous and territorial colony breeders with a modal clutch size of three (Becker & Ludwigs 2004). Food provisioning of chicks is biparental (Becker & Ludwigs 2004). Partial incubation over the laying interval results in asynchronous hatching over a 2- to 3-day period, and consequentially in a size hierarchy among the siblings (Bollinger 1994). Chicks are semiprecocial, i.e. they are highly mobile and thermoregulate independently within a few days of hatching (LeCroy & Collins 1972), but they remain dependent on food provisioning until fledging and several weeks beyond. In the study colony chicks fledge at a mean age of 26-28 days (Becker & Wink 2003). The principal food, small fish, is mainly caught by plunge-diving (Becker & Ludwigs 2004). Common terns are single-prey loaders, i.e. they can only transport one prey item at a time from the foraging site to the colony to feed the young (Hays *et al.* 1973, Orians & Peterson 1979). The item cannot be shared among them, but is monopolized by one nestling. With the chicks becoming increasingly mobile, the arrival of a provisioning parent regularly triggers an array of signalling and physical competition behaviour. The siblings, and regularly also kleptoparasitic young from neighbouring nests, engage in attempts to outrun and outjump the opponents towards the arriving parent, in begging postures and calls, in attacks on the opponents and tug of war for the food item (for detailed descriptions see Braasch 2005, Smith *et al.* 2005). For comprehensive information on the species see Becker & Ludwigs (2004).

2.2 Study site

The field work was carried out between April and August 2007 in a monospecific common tern colony located at the Banter See (53°30'40''N, 8°06'20''E), a brackish lake by the Jade Bay, German North Sea. The colony comprises six artificial islands of equal size, with a total area of about 295 m^2 (for colony details see Becker & Wendeln 1997, Becker *et al.* 2001). Over the studied breeding season, it supported a total of 420 breeding pairs.

2.3 Sample collection

2.3.1 General fieldwork procedures

Over the whole study period, the colony was visited every 1-3 days (daily around peak hatch) in the morning hours to collect basic data such as brood size, hatching date and order, chick weight and fate (for details see Wagener 1998). To this end, nests were individually marked with a stake and chicks were marked with a steel ring as soon as they were 1 day old. Chick age at first encounter after hatching was recorded as 0 days when the plumage still was moist and otherwise as 1 day, unless cracks or a hole in the egg shell registered at the previous visit indicated an even earlier hatch. When more than one newly hatched chick was encountered in the same nest on the same occasion, the size of the yolk sac on the chicks' bellies, which decreases with age, was used to establish the hatching order (Wagener 1998). To obtain data on the average chick body mass at a certain age, on two of the six islands all chicks encountered were weighed with a digital balance (±1g). This data basis was broadened for the ages of special interest for the present study (see below) by weighing all chicks older than approximately 14 days on all islands at each visit.

When the chicks were robust enough, at a mean age of 19 days, 2-5 growing body feathers with blood-filled quills were sampled. Dead chicks were collected and stored at –20° C, and of those that had not been feather-sampled, a muscle tissue sample from the leg was taken instead. Both feather and muscle tissue samples were stored in EDTA-thymol buffer at 4° C for molecular sexing.

2.3.2 Sampling protocol and chick removal experiment

Since hardly any broods with three siblings survived to the age relevant for this study (14 days, see below), I concentrated on sibling competition in two-chick broods. To avoid confounding influences of natural brood size reduction prior to sampling, I only included broods in the sample that had had a stable brood size for at least five days. All sibling pairs included were first- and second-hatched chicks; no first- and third- or second- and third-hatched pair was sampled.

Data were collected from broods at three different sample occasions: before (sample 1), at the end of (sample 2), and a few days after a chick removal experiment (sample 3). These occasions were timed according to brood age, which was defined as the age of the oldest sibling alive in the brood. On the first two occasions behavioural observations, chick weights (digital balance, ±1g) and wing lengths (stopped wing rule, ±1mm) were recorded and blood and faecal samples

collected, on the third occasion only blood and faeces were sampled. Apart from the behavioural observations (see below), this generally happened between 1800 and 2200 hours to control for possible diel patterns (weight: Massias & Becker 1990; *ß*-hydroxybutyrate: Jenni-Eiermann & Jenni 1997, Jenni & Schwilch 2001; T: review in Kempenaers *et al.* 2008; CORT: reviews in Millspaugh & Washburn 2004, Goymann *et al.* 2006, Quillfeldt *et al.* 2007a, but short-term fluctuations tend to be smoothed in faeces compared to blood plasma (Palme 2005)). A second reason for this timing was that I expected the effects of active sibling competition to build up over daytime in the diurnal study species (Massias & Becker 1990). Moreover, with respect to *ß*-hydroxybutyrate, the effect of the obligatory feeding recess at night would possibly even have masked any effects in samples taken in the morning (Jenni & Jenni-Eiermann 1996).

Sample 1 was taken as basis for the observational part of the study. Both siblings were sampled at a time when brood age was 14-18 days, a stage at which I expected relatively strong sibling competition for food for two reasons: First, at this time common tern chicks reach a stage of constantly high energy requirement (Drent *et al.* 1992, Klaassen *et al.* 1992), and second, this is paralleled by a period of increased chick mortality by starvation in larger broods (Becker & Finck 1985). A total of 24 two-chick broods were sampled and 15 of them observed. To be able to compare different natural brood sizes, sample 1 was additionally taken from 21 chicks from single-chick broods of comparable age (15-19 days), 10 of which were observed.

Seventeen of the initially sampled two-chick broods were subsequently included in a chick removal experiment as follows: Right after sample 1 had been taken, the second-hatched, younger sibling (b-chick) remained in the colony, while the first-hatched, older one (a-chick) was removed for 1 day. It was held in a cage and provided with fish *ad libitum*. The older siblings were removed because they tend to be competitively superior to the younger ones (Rossell *et al.* 2000, Smith *et al.* 2005) and I therefore expected this protocol to yield stronger effects. Just prior to releasing its caged sibling on the following evening, sample 2 was taken from the younger sibling only. Sample 3 was taken 3-5 days after siblings had been reunited (brood age 19-24 days), again from the b-chick only, given that both chicks had survived until then (*n*=12). This last sample aimed at exploring the hormonal response to the re-increase of brood size. A control treatment was not employed to keep experimental sample size large (cf. section 4.2). Sample sizes might deviate from those given above because not all variables could be successfully measured in all broods.

2.3.3 Behavioural observations

The behavioural observations aimed at recording feeding rates of and competitive interactions between the chicks. Since in common tern chicks the vast majority of interactions of competitive nature are connected to food provisioning by the parents (Braasch 2005, Smith *et al.* 2005; pers. obs.), I focused the observations solely on these feeding visits.

Few hours prior to the blood and faecal sampling of the same occasion, observations were conducted, by the unaided eye or occasionally using binoculars (8x magnification), from two hides situated 0-12 m from the nests. The maximal number of broods observed simultaneously was nine. Normally the observation duration per sampling occasion and nest was 3 h, while in one case, harsh weather restricted it to 2:15 h, and in few other cases, it was increased to up to 6:45 h, mainly by adding a second session from the previous day to sampling occasion 1. Observation times ranged from 0740 to 2105 hours, a time interval during which time of day could be assumed not to influence feeding rate patterns (Frank 1992, Smith *et al.* 2005). As far as possible the single sessions were timed around low or alternatively high tide, since at these times feeding rates have been found to be highest (Frank 1992).

For individual identifiability the chicks were marked with patches of adhesive fabric tape of different colours and forms on head and back. For every feeding visit at the focal nests the following parameters were recorded:

(1) the single chicks' behavioural reaction to their parent's arrival until the food item had been fed. It was recorded as the chick's most engaged mode of behavior, scored on the following three-step scale in increasing order:

(i) passive, i.e. showing no or hardly any reaction,

(ii) begging, including begging calls, erect begging posture, running and jumping towards the arriving parent,

(iii) fighting, i.e. an aggressive attempt to obtain the food item from a conspecific other than the own parent, frequently including a contest of pulling the food item from each others beaks, often between several juvenile and adult competitors from several nests, and pecking the opponents;

(2) which chick reached the arriving parent first;

(3) whether the feeding was successfully completed, i.e. whether one of the providing parent's chicks swallowed the food item, and if so, which one.

When observing broods with only one chick present in the colony, i.e. natural one-chick broods or experimental broods with one chick removed, the protocol was limited to (3). Regardless of brood size, successful attempts by focal chicks to steal a food item from a nest other than its own were additionally recorded. Since molecular sexing was carried out after the field season, observations were unbiased concerning chick sex. Limitations of the observation methods are discussed in Braasch (2005).

2.3.4 Blood sampling

All chicks of a brood were captured at one time and immediately sampled in random order to minimize stress response effects on plasma hormone levels (cf. Fridinger *et al.* 2007). Sampling duration did not exceed 10 min, so that *ß*-hydroxybutyrate (Jenni-Eiermann & Jenni 1998)and T levels (Wingfield & Farner 1976) of the sampled blood could be assumed baseline and not raised by handling stress. Blood samples (50-300 µl) were collected from the brachial veins, in few cases alternatively from the tibial veins, with a 23- to 27-gauge cannula. A droplet was immediately used to determine the concentration of *ß*-hydroxybutyrate, the rest was taken up in self-sealing heparinized capillaries and cooled. Within 6 h, the samples were centrifuged at 10,000 rpm for 8 min to separate plasma from cells and then stored at –20° C until later analysis of T levels.

2.3.5 Faeces sampling

Faecal sampling is, in contrast to blood sampling, generally considered to be free of immediate feedback on measured steroid levels (Palme 2005). Upon capture each chick was placed in a box compartment with a plastic floor onto which it normally defecated within short time. Floors were rinsed and wiped between uses. Droppings were taken up with tissue papers of uniform weight, which then were stored in tightly sealed containers and cooled. Within 4 h, containers were stored at –20° C for later analysis of CORT metabolite levels.

2.4 Sample processing

2.4.1 Molecular sex identification

DNA was isolated from feather and muscle tissue samples. Molecular sexing was modified based on the principle and methods presented by Kahn *et al.* (1998). Primers used were 2550F

and 2718R (Fridolfsson & Ellegren 1999). The 10 µl PCR mix consisted of 1 µl (approx. 25 ng) total template DNA, 0.8 µl 2550F Primer (10 mM), 0.8 µl 2718R Primer (10 mM), 0.8 µl Nucleotide-mix (equal concentration of all dNTPs, 2.5 mM in total), 1 µl 10x PCR-buffer, 0.06 µl Taq-Polymerase (0.3 units) and 5.54 µl dH_2O. PCR thermal profile was 5 min at 94° C, 1 min at 55° C, 2 min at 72° C; then 33 cycles with 1 min at 92° C, 1 min at 50° C, 2 min at 72° C; and finally 5 min at 72° C and at 4° C thereafter. 3.5-5.0 µl of PCR products were separated by electrophoresis on a 2% agarose gel at 80 V and 15 mA for 45 min. Afterwards gels were placed in a bath with 0.5 µg/ml ethidium bromide for 30 min. Stained bands were visualized under UV light. Samples from male chicks produced one band, those from females two. See Becker & Wink (2003) for intercalibration with other methods.

2.4.2 Body condition index

I obtained an age-independent body condition index (BCI [%]) for chicks by calculating the relative deviation of the measured body mass (M [g]) from the mean body mass for study chicks of the same age (M_{mean} [g]): BCI=(($M-M_{mean}$)/M_{mean})*100% (cf. Stienen & Brennikmeijer 1999, Quillfeldt *et al.* 2006). In case there were two weights for the same chick on the same day, only the one measured later was included in the calculation of the mean to avoid pseudoreplication. While all weights taken in the context of the chick removal experiment were measured in the evening, the mean weight values are mainly based on weights taken during the routine nest controls in the morning, when chicks were generally lighter. This resulted in predominantly positive BCIs for the present study. To obtain meaningful BCI values all study BCIs were therefore standardized to a mean of zero. In not using the alternative correction by a linear body size indicator, I follow Green's (2001) general criticism of mass/length residuals as well as the specific counterargument by Quillfeldt *et al.* (2007b), in short, that since in seabird chicks mass and linear size measures like tarsus or wing length do not grow in a parallel fashion (common tern: LeCroy & LeCroy 1974; other terns: Klaassen *et al.* 1989, Stienen & Brenninkmeijer 2002) and therefore derived residuals are not independent of age.

2.4.3 *ß*-Hydroxybutyrate

As a second, inverse measure of chick condition, I employed *ß*-hydroxybutyrate, an important avian fat metabolite synthesized in fasting situations when carbohydrate stores are used up and body lipids are mobilized (review in Jenni-Eiermann & Jenni 1998). Plasma *ß*-hydroxybutyrate levels are positively related to fasting duration (Totzke *et al.* 1999, Vleck & Vleck 2002) and

negatively related to body mass change, which they predict (Jenni-Eiermann & Jenni 1994, 1998, Jenni & Schwilch 2001). They have been identified as one of the most reliable indicators of the short-term physiological situation in terms of nutritional status and condition in another larid (Totzke *et al.* 1999). Since *ß*-hydroxybutyrate levels react quickly to the nutritional situation (Jenni-Eiermann & Jenni 1998) and indicate dynamics of the physiological state (Williams *et al.* 1999), they are especially suitable to detect the short-term condition changes I predicted for the 1-day chick removal experiment, but might be less advantageous to assess long-term condition patterns (Jenni-Eiermann & Jenni 1998).

The concentration of *ß*-hydroxybutyrate was determined electrochemically (±0.1 mmol/l) in a fresh droplet of venous whole blood using a standard test kit (MediSense® Precision® Xceed™ meter and Precision Xtra™ *ß*-ketone test strips, Abbott Laboratories, Abbot Park, IL, USA). To allow a straight interpretation of higher plasma levels of *ß*-hydroxybutyrate as indicators of increasing severity of food deprivation, it is important to ensure that chicks were not sampled in a state of starvation, when *ß*-hydroxybutyrate levels generally are intermediate between those for less extreme fasting (high *ß*-hydroxybutyrate) and resorptive state (low *ß*-hydroxybutyrate; Jenni-Eiermann & Jenni 1998, Vleck & Vleck 2002). Chicks in obviously weak condition were not sampled. This made it very unlikely for starving chicks to enter the sample, especially given the lack of a particular resistance to starvation shown in adults and especially fledglings and immatures of another larid, the herring gull *Larus argentatus* (Jeffrey *et al.* 1985, Totzke *et al.* 1999). Nevertheless, I excluded one chick from the analysis of the *ß*-hydroxybutyrate data due to (1) initially extremely low BCI ($z < -3.5$) combined with a *ß*-hydroxybutyrate level below average, and (2) marked increases in BCI (+3.6%) and *ß*-hydroxybutyrate levels (+0.5 mmol/l) in response to its sibling's removal, which clearly suggested the chick was initially sampled in a state of starvation.

2.4.4 Behavioural parameters

Based on the recorded behavioural observations, I derived four parameters to analyze chick competitiveness. First, I calculated per chick *feeding rate*, i.e. the number of items swallowed by a certain chick per unit time, including both items provided by its own parents and items obtained at neighbouring nests.

For calculation of the other three parameters, all dealing with direct behavioural sibling competition on a relative within-brood scale and therefore relevant only for broods with more than one chick, I excluded all feeding visits from the data basis during which at least one sibling

was recorded passive. This was to achieve clarity in interpretation, since passivity in this context can reasonably be understood as a result of weakness and generally low attentiveness, i.e. low competitiveness, or of repletion (Braasch 2005, Smith *et al.* 2005), i.e. a result of high competitiveness. In contrast, competitiveness unambiguously is positively related to both begging and fighting, the success of which I describe by the following parameters: *Outrunning success* was calculated as the proportion of a certain chick's first arrivals to all feeding visits with a chick reaching the arriving parent prior to its sibling. *Begging success* of a chick was calculated as the proportion of feeding occasions with both siblings at least begging that ended with this chick securing the food item. This parameter is similar to the feeding rate, but exclusively reflecting direct active sibling competition. *Fighting success* was calculated correspondingly to begging success, but based only on feeding visits in the course of which both siblings engaged in a fight. Broods hatching around peak hatch were confronted with disproportionally many close neighbours and therefore faced an increased risk of suffering from kleptoparasitism. To avoid a hatching date bias of success rates, I therefore based the latter two parameters only on feeding visits successfully completed within the brood.

2.4.5 Testosterone

T levels were determined in plasma samples. Common tern chicks have been shown to have extremely low concentrations of plasma T (Braasch 2005: 6-126 pg/ml; Sprenger 2007: 0.2-17.2 pg/ml). Therefore an ultra sensitive T radioimmunoassay (RIA) was used based on a standard test kit (Testosterone RIA, DSL-4100, Diagnostic Systems Laboratories, Sinsheim, Germany) and modified as described in detail by Hoppen & Niederstucke (2008). The authors report sensitivity of 0.6 pg/ml, intra- and inter-assay CVs of 11.7% and 13.5% respectively, recovery between 97% and 105% and relatively low cross-reactivity.

If the amount of material permitted, as was the case in >80% of samples, duplicate determinations were performed. Volume of aliquots was 50 µl. Duplicate values of samples were used only if CV was within 50%. Otherwise, if possible the incorrect measurement was identified based on additional determinations. In the six assays run, the minimum detectable plasma T level varied between 4.60 and 14.92 pg/ml. For statistical purposes, assay values below half the respective detection limit were assigned this value (cf. Naguib *et al.* 2004), and a T level of 0.05 pg/ml was assigned to all samples measured below 0.00 pg/ml (i.e. the level of the blank control sample), since a minimum of T was to be expected in all samples (H.-O. Hoppen, pers. comm.). The assay in which they were measured had significant effects both on T levels for all

samples taken (Kruskal-Wallis test: $H=73.716$, $P<0.001$, $n=6$) and on the levels of a duplicate control sample with a concentration in the range of the chick samples (exact Kruskal-Wallis test: $H=10.077$, $P=0.005$, $n=6$). Therefore the assay effect was corrected for by adjusting all measurements relative to the deviation of the respective assay's control sample from the mean of the control sample measurements of all assays.

2.4.6 Corticosterone

Enzyme immunoassays (EIAs) were performed to determine the faecal levels of glucocorticoid metabolites as a measure of CORT, the principal glucocorticoid in birds (Wingfield *et al.* 1997, Palme *et al.* 2005). The EIA used was 72(T), a 11-oxoaetiocholanolone-EIA measuring glucocorticoid metabolites with a 5*ß*-3*α*-11-one structure, as described in detail by Möstl *et al.* (2002). This had been the best performing assay in a previous validation experiment on adult common terns, which moreover showed that peak concentrations of blood glucocorticoid metabolites are detectable in faeces with a time lag of 2:30-3:00 h (P. H. Becker & R. Palme, unpubl. data).

Standard samples did not reveal a substantial effect of the respective assay on the metabolite levels measured (R. Palme, pers. comm.). I excluded samples weighing <0.1 g from the analysis (cf. Tempel & Gutierrez 2004) to avoid confounding influences of higher extraction efficiency (Millspaugh & Washburn 2004) and relative water loss by evaporation in small samples or of small variations in the weight of the tissue papers the samples were taken up with. Concentrations are given in ng/g faeces fresh weight.

2.5 Statistical analysis

For all statistics and graphs I used SPSS® 13.0 for Windows® (SPSS Inc., Chicago, IL, USA). Normality was tested using Shapiro-Wilk tests and Kolmogorow-Smirnow tests with Lilliefors significance correction. Homogeneity of variance was tested with Levene tests based on the mean, additionally taking into account the ratio between the biggest and smallest group variance (Field 2005). To make them meet parametric test assumptions, prior to formal analysis *ß*-hydroxybutyrate data were $\log_{10}$-transformed, and raw data (x) for T and CORT were transformed according to the equations $y=\log_{10}(1+x)$ and $y=1-x^{-1/2}$ respectively.

For the condition and hormone variables, patterns in the natural, pre-experimental situation in two-chick broods were generally tested for as follows: First, the effects of individual chicks'

traits on their score for the respective dependent variable were tested with a univariate ANOVA, which included the chick's sex and its hatching position in the brood as fixed factors and the nest as random factor. Second, influences of within-brood hatching position and brood sex composition, i.e. effects on the brood level, were tested for using a repeated measures ANOVA, treating hatching position within the subject brood as the repeated measure (cf. e.g. Sheldon *et al.* 1998, Becker & Wink 2003) and both sex of the a-chick and sex of the b-chick as fixed factors. Finally, I included the data from one-chick broods to test for an effect of natural brood size, employing a univariate ANOVA with nest as random factor nested within the fixed factor brood size.

The immediate effect of the experimental treatment (sample 1 vs 2) on the focal b-chicks, which remained in the colony, was tested for with a repeated measures ANOVA, with sample number being the repeated measure. Both the sex of the chicks and the sex of their removed siblings were included as fixed factors to test for interactions with the experimental treatment. For the analysis of the hormone data only, the effect of the return of the a-chick (sample 2 vs 3) on the b-chick was additionally tested in the same way. A three-level repeated measures design with adjusted post-hoc pairwise comparisons was not employed because the subsamples with valid data for testing sample 1 vs 2 and sample 2 vs 3 differed considerably with respect to the chicks they contained.

I generally used GLM-type ANOVAs with type III sum of squares and always included an intercept. All possible quadratic interactions between fixed factors (both within- and between-subject) were included in the models. All explanatory variables were tested following a backward selection procedure successively removing all factors whose effects were not statistically significant or at least nearly so, as sample sizes were generally small. Removed factors will generally not be specifically referred to and mainly results from the final models only will be presented. The random factor nest was retained in the models irrespective of significance to avoid pseudoreplication. Significant interactions were kept in the model in combination with the respective single effects.

When violations of parametric test assumptions could not be removed, non-parametric tests were employed to follow a test strategy as far as possible parallel to the one described above. This was mainly necessary in analyses of feeding rates and behavioural interactions, since continuousness of the respective data was borderline. Correlations were used to investigate whether hormonal and behavioural parameters were related to (sample 1 data) or covaried with (differences between pre- and immediate post-experimental sample) condition parameters.

The level of significance was set to $\alpha=0.05$. For the tests of within-subject effects in repeated measures ANOVAs, sphericity was assumed throughout since Mauchy's test of sphericity never yielded a significant result. Unless otherwise stated, tests were two-tailed, the general exceptions being the tests of pre- vs post-experimental state (sample 1 vs 2) with the aforementioned directional *a priori* hypotheses. For the effect of the return of the siblings (sample 2 vs 3) on the hormone levels, the directional hypotheses were consequently inverse. In all these cases, tests were one-tailed, i.e., since two categories were tested, *p*-values were halved given the correct direction was predicted, and otherwise $P_{\text{one-tailed}}=1-(P_{\text{two-tailed}}/2)$ (Howell 1996). Tests were performed conditional on a minimum sample size of 5 in all cells. Unless otherwise noted, results are presented as arithmetic means $\pm$ 1 SD. When plotting repeated measures data, between-subject variability was eliminated by normalizing subject means according to Loftus & Masson (1994).

3 Results

3.1 Condition

As expected, the two indicators of condition used, BCI and β-hydroxybutyrate, were negatively related for the entire non- and pre-experimental part of the sample (Spearman rank-order correlation: r_s=-0.313, d.f.=66, P(one-tailed)=0.005). The changes of both variables in b-chicks whose senior siblings were removed from the nest were negatively related on an individual basis as well (Spearman rank-order correlation: r_s=-0.463, d.f.=15, P(one-tailed)=0.035; Fig. 1).
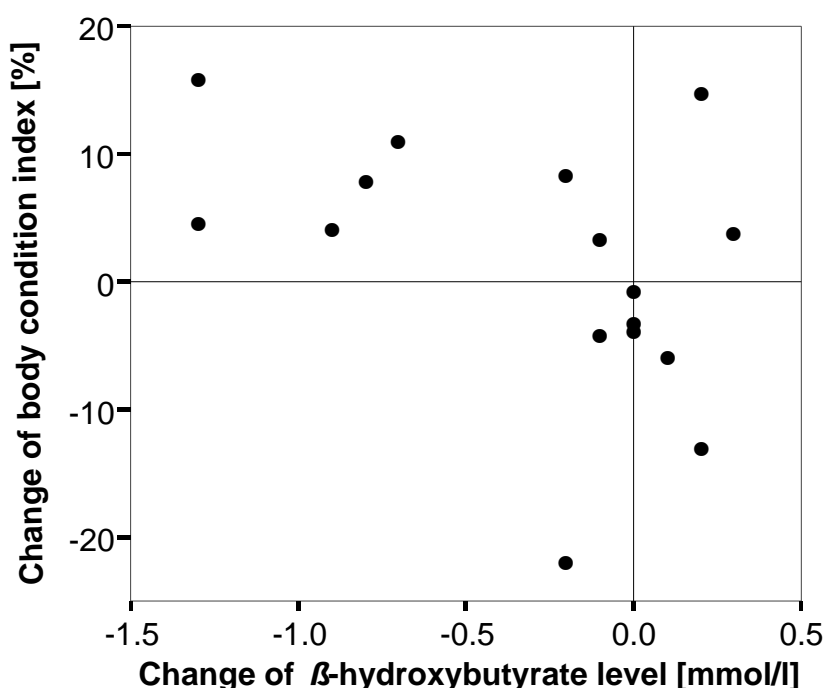

Fig. 1. Relationship between change of β-hydroxybutyrate level and change of body condition index in junior chicks from start to end of the chick removal experiment. Senior chicks were removed from two-chick broods for 1 day.

3.1.1 Body condition index

Pre-experimental BCI varied between -52.2% and 37.1%. In unmanipulated two-chick broods, a-chicks (6.6±13.3%, n=24) had significantly higher BCIs than b-chicks (-1.8±18.4%, n=24; $F_{1,22}$=4.830, P=0.039; Fig. 2). Male chicks (3.8±16.2%, n=23) had BCIs superior to those of females (1.2±16.9%, n=25), but this difference was only marginally significant ($F_{1,22}$=3.771, P=0.065; Fig. 2). The influence of the nest identity was significant ($F_{23,22}$=3.154, P=0.004), the interaction hatching position*sex was not, even though the male advantage only showed in a-

chicks (Fig. 2). The repeated measures ANOVA corroborated the significance of the effect of hatching position on BCI in a within-brood context ($F_{1,22}=5.798$, $P=0.025$), but failed to find significant effects of the sex of the a- or b-chick on the BCI levels in the brood. There were indications of an interaction between hatching position and the sex of the brood's a-chick ($F_{1,22}=2.974$, $P=0.099$): Senior male chicks tended to be more superior in BCI to their younger siblings than female seniors did (Fig. 3).
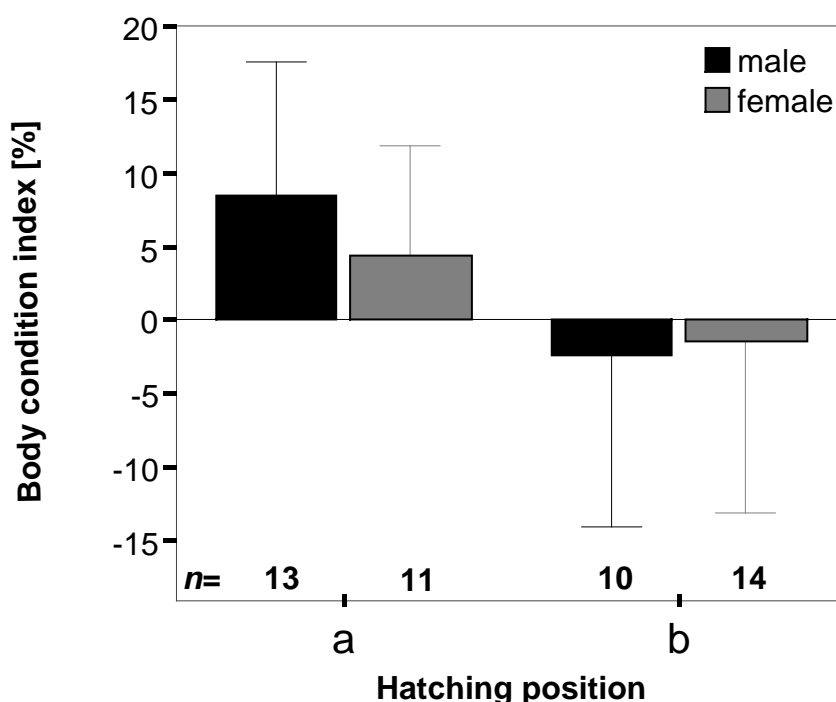

Fig. 2. Body condition index (mean +/- 95% CI) of chicks of different hatching position and sex in unmanipulated two-chick broods.

BCI did not differ between chicks from one- ($1.5\pm13.2\%$, $n=21$) and two-chick broods ($2.4\pm16.4\%$, $n=48$; $F_{1,51.574}=0.047$, $P=0.829$; nest $F_{43,24}=1.665$, $P=0.092$). The experimental removal of sibling competition was followed by a nonsignificant increase of the remaining chicks' BCIs ($F_{1,16}=0.319$, $P=0.290$; Fig. 4a). Sex of junior (Table 1) or senior chick did not interact with the experimental effect.
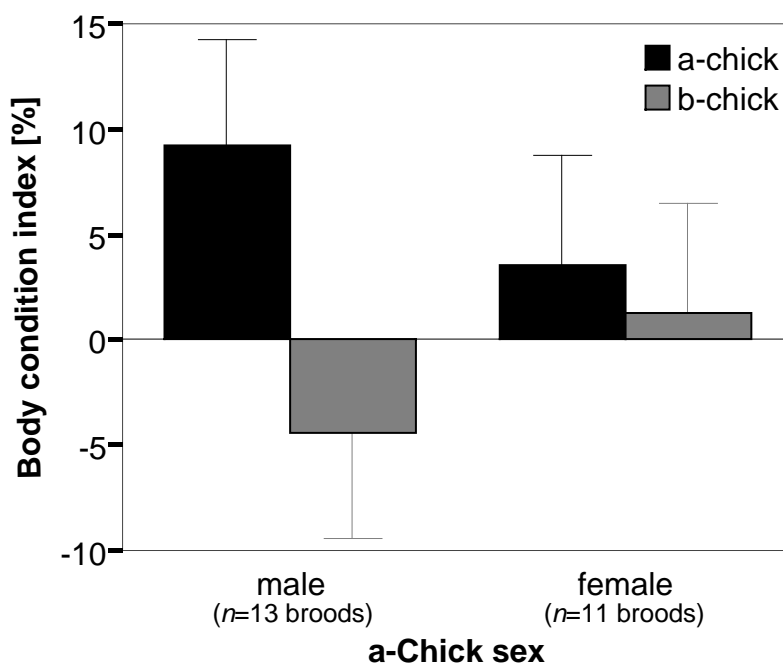
Fig. 3. Body condition index in relation to sex of the senior chick within each brood and to individual hatching position in unmanipulated two-chick broods. Columns indicate means, error bars 95% CIs adjusted by eliminating between-nest variability.

3.1.2 *ß*-Hydroxybutyrate

Natural chick blood levels of *ß*-hydroxybutyrate varied between 0.5 and 2.3 mmol/l. The analysis of *ß*-hydroxybutyrate patterns in unmanipulated two-chick broods did not reveal any significant effects, neither on the individual (Table 2), nor on the brood level. First-hatched chicks (0.96±0.29 mmol/l, n=23), tended to have lower concentrations than their younger siblings (1.15±0.49 mmol/l, n=23; $F_{1,22}$=2.763, P=0.111; Table 2).

ß-Hydroxybutyrate levels of chicks from two-chick broods (1.06±0.41 mmol/l, n=46) were nonsignificantly higher than those of single chicks (0.92±0.23 mmol/l, n=21; $F_{1,51.341}$=1.246, P=0.270; nest $F_{42,23}$=1.334, P=0.232). Similarly, the experimental reduction of brood size was connected to a decrease of *ß*-hydroxybutyrate levels in the focal chicks (sample 1: 1.18±0.54 mmol/l, n=16; sample 2: 0.88±0.21 mmol/l, n=16), but this effect was significant ($F_{1,15}$=4.881, P=0.022; Fig. 4b). There was no interaction of this effect with the sex of the respective b-chick (Table 1) or its removed sibling. Since violation of the assumption of homogeneity of variance for pre- and immediately post-experimental data could not be entirely excluded, the effect of the sibling removal was non-parametrically retested and its significance confirmed (exact Wilcoxon signed ranks test: Z=-1.678, P=0.049, n=16).

Table 1. Development of condition indicators, feeding rate and hormone levels in junior chicks of different sex during the chick removal experiment. Senior chicks were removed from two-chick broods for 1 day. Given are means (± SD) of pre- and immediate post-experimental samples and their differences, and numbers of chicks (*n*).

| Parameter	Sex	Start of experiment	End of experiment	Change over experimental period	*n*
Body condition index [%]	♂	-1.2±18.0	0.5±11.4	+1.7±9.1	8
	♀	-3.5±22.4	-2.5±19.6	+1.0±10.7	9
ß-Hydroxybutyrate [mmol/l]	♂	1.09±0.47	0.91±0.22	-0.18±0.55	8
	♀	1.26±0.62	0.84±0.21	-0.43±0.50	8
Feeding rate [items/h]	♂	0.90±0.52	0.73±0.55	-0.17±0.85	5
	♀	0.39±0.38	0.78±0.72	+0.39±0.43	6
Testosterone [pg/ml]	♂	8.79±5.30	12.24±7.40	+3.45±9.78	5
	♀	7.73±6.43	6.05±1.88	-1.68±5.91	7
Corticosterone [ng/g]	♂	32.36±26.84	18.31±22.64	-14.06±39.00	5
	♀	12.91±7.50	9.40±5.28	- 3.51±3.76	7
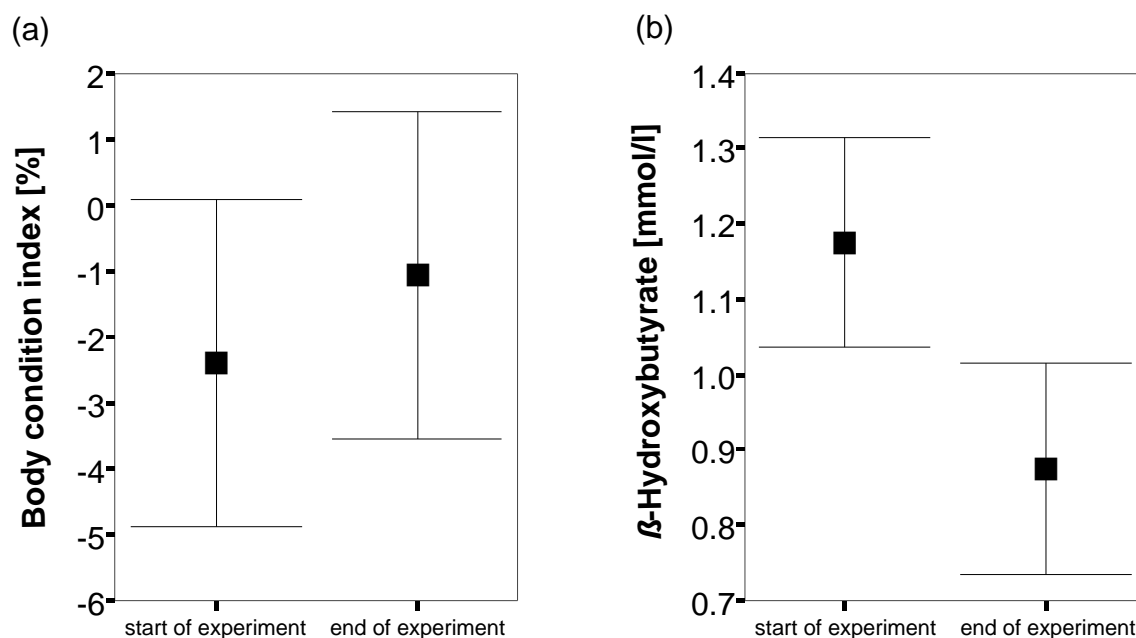
Fig. 4. Change of condition parameters (mean ± 95% CI; (a) body condition index, *n*=17; (b) *ß*-hydroxybutyrate, *n*=16) of junior chicks during the chick removal experiment. Senior chicks were removed from two-chick broods for 1 day. CIs are adjusted by eliminating between-chick variability.

Table 2. *ß*-Hydroxybutyrate level and feeding rate (mean ± SD, *n* in parentheses) of chicks of different sex and hatching position in unmanipulated two-chick broods.

Parameter	Sex	Hatching position a	b
ß-Hydroxybutyrate [mmol/l]	♂	0.97±0.26 (12)	1.10±0.45 (10)
	♀	0.96±0.33 (11)	1.19±0.54 (13)
Feeding rate [items/h]	♂	0.60±0.58 (7)	0.71±0.53 (7)
	♀	0.70±0.51 (8)	0.51±0.39 (8)

3.2 Success in competitive behaviour

In competing with their junior siblings senior chicks could rely on a clearly superior physique: In unmanipulated two-chick broods, they had significantly higher body weight (a-chicks 114±14g, b-chicks 101±19g, *n*=24; repeated measures ANOVA: $F_{1,23}$=12.499, *P*=0.002) and greater wing length (a-chicks 123±15mm, b-chicks 110±20mm, *n*=24; repeated measures ANOVA: $F_{1,23}$=18.347, *P*<0.001).

3.2.1 Feeding rate

Registered feeding rates of chicks from unmanipulated broods were in the range of 0.00-2.67 items/h. The identity of unmanipulated two-chick nests was far from significantly affecting chicks' feeding rates (Kruskal-Wallis test: *H*=11.980, *P*=0.608, *n*=15) and was therefore omitted from further analyses. Hatching position did not affect individual pre-experimental feeding rates in two-chick broods (exact Wilcoxon signed ranks test: *Z*=-0.189, *P*=0.868, *n*=15), and neither did chick sex (exact Mann-Whitney *U* test: *Z*=-0.335, *P*=0.750, *n*=30). The latter was also true both among a-chicks only (exact Mann-Whitney *U* test: *Z*=-0.528, *P*=0.630, *n*=15), where females' rates were slightly superior (Table 2), and among b-chicks only (exact Mann-Whitney *U* test: *Z*=-0.928, *P*=0.379, *n*=15), where males reached highest rates (Table 2). In the entire non- and pre-experimental part of the sample, individual chick feeding rate was significantly and positively correlated with body condition index (Spearman rank-order correlation: r_s=0.349, d.f.=39, *P*=0.027; Fig. 5), but not with *ß*-hydroxybutyrate (Spearman rank-order correlation: r_s=-0.130, d.f.=39, *P*=0.425).

Fig. 5. Relationship between feeding rate and body condition index in individual unmanipulated chicks.

Feeding rates were significantly higher in chicks from one-chick broods (1.17±0.70 items/h, $n=10$) than in those from unmanipulated two-chick broods (0.63±0.49 items/h, $n=30$; exact Mann-Whitney U test: $Z=-2.278$, $P=0.021$, $n=40$). Similarly, the focal chicks' feeding rates slightly increased with the experimental removal of their senior siblings (Fig. 6), but this change was not significant (exact Wilcoxon signed ranks test: $Z=-0.715$, $P=0.258$, $n=11$). The individual changes between the pre- and the immediately post-experimental sample were not significantly affected by the respective chick's sex (exact Mann-Whitney U test: $Z=-1.195$, $P=0.275$, $n=11$), even though feeding rates of male focal chicks slightly decreased during the experiment while those of females increased (Table 1). Feeding rates of chicks whose removed senior sibling was a male increased during the experiment (+0.58±0.40 items/h, $n=4$), while in contrast those of chicks with a female senior removed slightly decreased (-0.12±0.70 items/h, $n=7$). Due to small sample size the effect of the removed sibling's sex was not tested for. Over the experimental period, the junior chicks' feeding rate neither covaried with body condition index (Spearman rank-order correlation: $r_s=-0.164$, d.f.=10, $P=0.629$) nor with β-hydroxybutyrate (Spearman rank-order correlation: $r_s=-0.341$, d.f.=10, $P=0.305$).
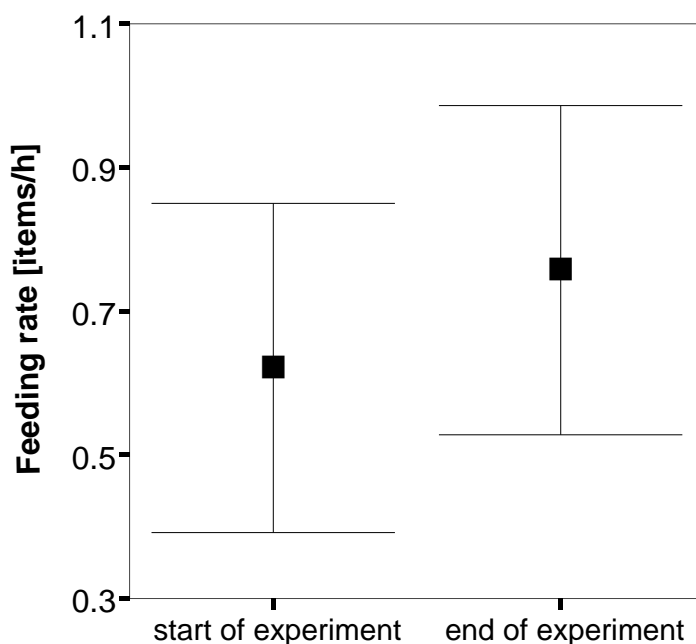
Fig. 6. Change of feeding rate (mean ± 95% CI) of junior chicks (*n*=11) during the chick removal experiment. Senior chicks were removed from two-chick broods for 1 day. CIs are adjusted by eliminating between-chick variability.

3.2.2 Success in competitive behavioural interactions

In total, 62 running and 63 begging duels entered the analyses. Based on a total of 15 interactions only, the results concerning fighting success have to be treated with great caution and are of limited validity.

First- and second-hatched chicks did not significantly differ in any of the competitive interaction success scores (outrunning success: exact Wilcoxon signed ranks test, Z=-0.494, P=0.658, n=14; begging success: exact Wilcoxon signed ranks test, Z=-0.412, P=0.692, n=15; fighting success: exact sign test, P=1.000, n=7). The tendencies were that a-chicks arrived first at the feeding parent slightly more often (54.9±33.4%) and also fought more successfully (57.1±53.5%), while their begging success was slightly inferior (44.8±42.0%) to that of their junior siblings. Sex effects on the three success scores remained untested, because (1) the interdependent nature of the relative within-brood success scores ($score_{a\text{-chick}}+score_{b\text{-chick}}=1$) did not allow testing for the effect of sex in general or of sex of the chick in one hatching position alone, and (2) the effect of chick sex combination in the nest was not testable because of low sample sizes: For the three

success scores, the total number of nests with data was 15 or lower, while the number of two-chick brood sex combinations is 4.

81.5% of the feedings (*n*=81) in unmanipulated two-chick broods went to the chick that reached the feeding parent first, while 9.9% could be secured by the other sibling and 8.6% fell to kleptoparasitic conspecifics. On the individual chick level, outrunning success and feeding rate were closely positively related as well (Spearman rank-order correlation: r_s=0.468, d.f.=27, *P*=0.012; Fig. 7).
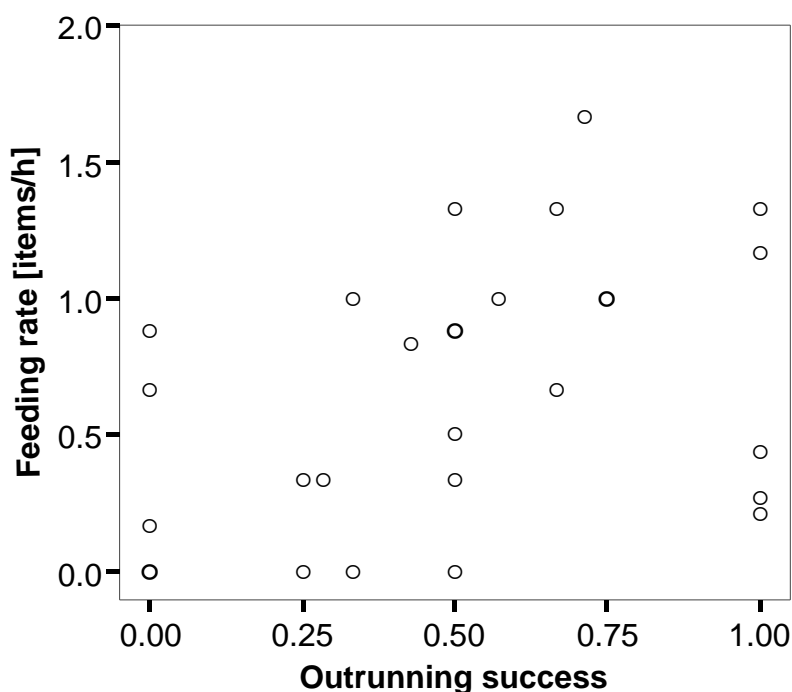
Fig. 7. Relationship between outrunning success and feeding rate in individual chicks from unmanipulated two-chick broods.

Table 3. Plasma testosterone and faecal corticosterone level (mean ± SD, *n* in parentheses) of chicks of different sex and hatching position in unmanipulated two-chick broods.

Parameter	Sex	Hatching position	
		a	b
Testosterone [pg/ml]	♂	6.48±4.44 (8)	8.39±4.31 (8)
	♀	9.93±6.30 (10)	6.12±2.78 (10)
Corticosterone [ng/g]	♂	17.47±12.23 (8)	21.52±18.48 (5)
	♀	18.09±11.48 (8)	13.81±9.37 (11)

3.3 Hormones

3.3.1 Testosterone

Natural plasma T levels ranged from 0.13 to 23.42 pg/ml. No effect of hatching position ($F_{1,15}$=0.002, P=0.964) or sex ($F_{1,15}$=1.312, P=0.270) on the individual chicks' T levels was detected. Both factors, though, tended to interact ($F_{1,15}$=3.107, P=0.098; nest $F_{17,15}$=1.817, P=0.125): While female a-chicks had higher plasma T concentrations than their male counterparts, the difference was inverse in b-chicks (Table 3). In the within-brood context, T levels were independent of main factors sex of a- and b-chick and repeated measure hatching position (all P>0.35), but the interaction between hatching position and sex of the a-chick was significant ($F_{1,16}$=4.561, P=0.049): Plasma T levels of senior female chicks were higher than their younger siblings', while levels of senior males were lower than those of their nest-mates (Fig. 8). No indication was found of a correlation between natural T levels and either of the condition parameters (Spearman rank-order correlations; BCI: r_s=0.056, d.f.=60, P=0.668; ß-hydroxybutyrate: r_s=0.140, d.f.=59, P=0.286).
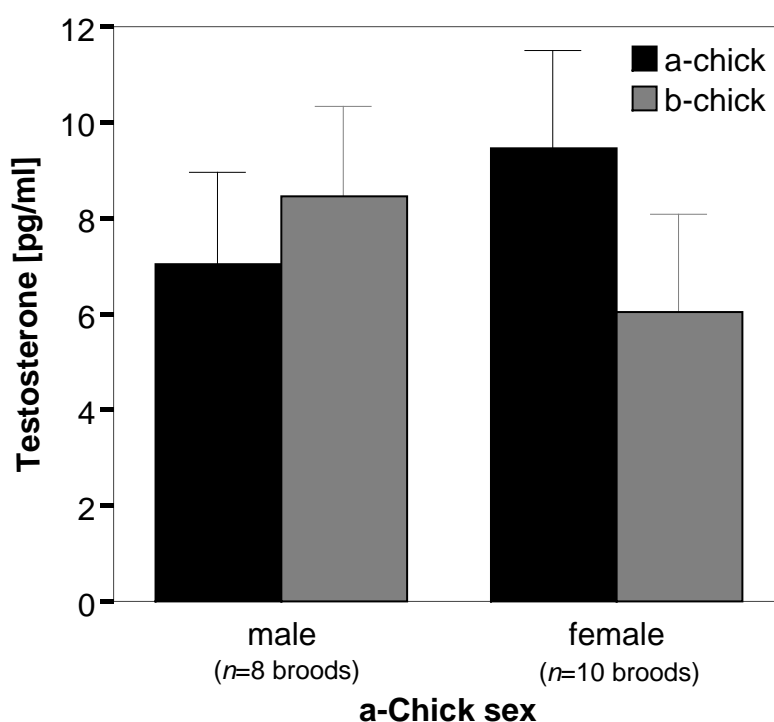

Fig. 8. Plasma testosterone level in relation to sex of the senior chick within each brood and to individual hatching position in unmanipulated two-chick broods. Columns indicate means, error bars 95% CIs adjusted by eliminating between-nest variability.

T levels in chicks from two-chick broods (7.76±4.74 pg/ml, n=36) were nonsignificantly higher than those in single chicks (6.06±2.95 pg/ml, n=20; $F_{1,39.962}$=1.326, P=0.256; nest $F_{36,18}$=1.487, P=0.186). Contrary to expectations, in two-chick broods the experimental removal of the senior siblings was not connected to a general decrease of the b-chicks' plasma T concentrations, instead there was even a slight nonsignificant increase (sample 1: 8.17±5.75 pg/ml, sample 2: 8.63±5.66 pg/ml, n=12; $F_{1,11}$=0.114, P=0.629; Fig. 9a). This change was not significantly affected by sex of a- or b-chick, even though T levels increased in males, while they slightly dropped in females (Table 1).
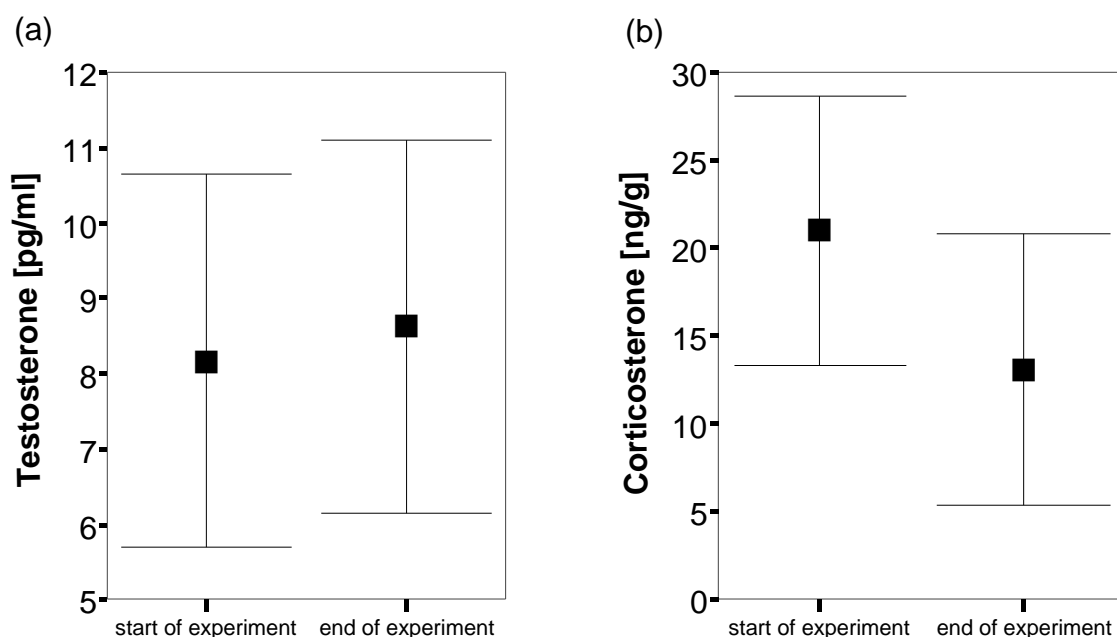

Fig. 9. Change of hormone levels (mean ± 95% CI; (a) plasma testosterone, n=12; (b) faecal corticosterone, n=12) of junior chicks during the chick removal experiment. Senior chicks were removed from two-chick broods for 1 day. CIs are adjusted by eliminating between-chick variability.

There was a marginally significant negative correlation between the individual changes of BCI and T level of the experimental b-chicks from start to end of the sibling removal (Spearman rank-order correlation: r_s=-0.582, d.f.=10, P=0.060; Fig. 10). However, this correlation was only this close to significance conditional on removing a two-dimensional outlier (Fig. 10), representing the chick with the strongest decrease in both T levels and BCI (z=-2.42 for the latter). In contrast, β-hydroxybutyrate and T levels did not covary over the sibling removal period (Spearman rank-order correlation: r_s=-0.208, d.f.=11, P=0.516).
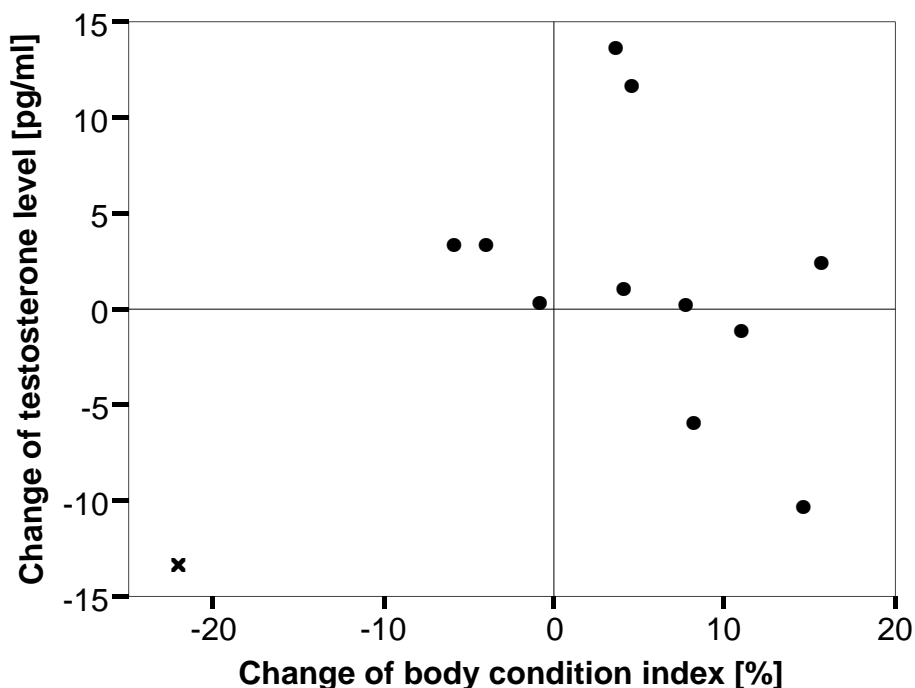
Fig. 10. Relationship between change of body condition index and change of plasma testosterone level in junior chicks from start to end of the chick removal experiment. Senior chicks were removed from two-chick broods for 1 day. The cross marks the two-dimensional outlier excluded from correlation analysis.

The return of the removed senior sibling was, similar to its previous removal, connected to a minute and nonsignificant change of b-chick T levels in the following days ($F_{1,7}=0.423$, $P=0.268$; Table 4). Since sample sizes for male b-chicks ($n=1$) and b-chicks with a male senior sibling ($n=3$) were too small, a test of the influence of sex of a- or b-chick (Table 4) on the effect of the return of the removed a-chick (sample 2 vs 3) was not performed.

3.3.2 Corticosterone

CORT levels measured in faeces of unmanipulated chicks ranged from 2.8 to 174.2 ng/g. For the analyses of this subsample, two outliers, a male b-chick ($z=+4.25$) and a male single chick ($z=+4.14$), were excluded for statistical reasons. No clear patterns were detected in CORT levels on individual (Table 3) or brood level in unmanipulated two-chick broods ($P>0.2$ for all factors). There was a significant negative correlation between faecal CORT level and BCI of individual

unmanipulated chicks (Pearson product-moment correlation: r=-0.385, d.f.=54, P=0.004; Fig. 11). In contrast, CORT and β-hydroxybutyrate level were not correlated (Spearman rank-order correlation: r_s=0.072, d.f.=54, P=0.601).
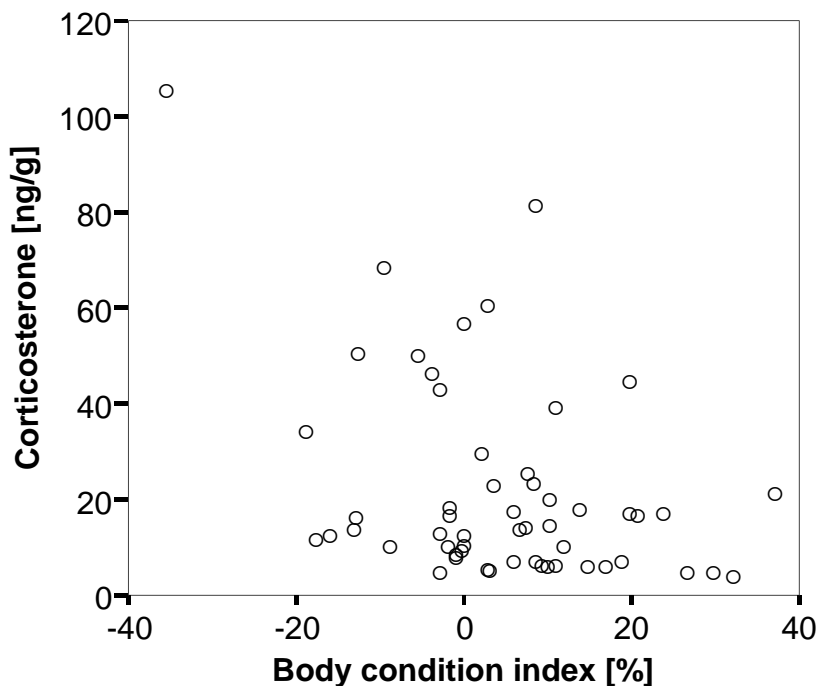

Fig. 11. Relationship between body condition index and faecal corticosterone level in unmanipulated chicks.

CORT levels in two-chick broods (17.00±11.97 ng/g, n=32) were lower than in single chicks (29.36±30.04 ng/g, n=18), but the difference was not significant ($F_{1,35.688}$=0.535, P=0.469; nest $F_{32,16}$=1.337, P=0.273). Over the experimentally induced 1-day period free of sibling competition, focal b-chicks' CORT levels tended to drop (sample 1: 21.02±19.82 ng/g, sample 2: 13.11±14.92 ng/g, n=12; $F_{1,11}$=1.976, P=0.094; Fig. 9b). This effect was independent of sexes of both siblings (both P>0.6), even though in relative and absolute numbers the decrease was considerably stronger in male than in female b-chicks (Table 1). As normality was borderline for the immediately post-experimental subsample, the experimental effect was retested nonparametrically and found to be very close to significance (exact Wilcoxon signed ranks test: Z=-1.647, P=0.055, n=12).

From pre- to immediately post-experimental sample, CORT level did not covary with BCI (Spearman rank-order correlation: r_s=-0.308, d.f.=11, P=0.331). In contrast, changes of CORT and β-hydroxybutyrate level over the experimental period were positively correlated (Pearson product-moment correlation: r=0.639, d.f.=11, P=0.025; Fig. 12). Prior to the latter analysis, quadratic transformation was performed on data (x) for both CORT ($y=(x+100)^2$) and β-hydroxybutyrate ($y=(x+2)^2$) to achieve bivariate normality.
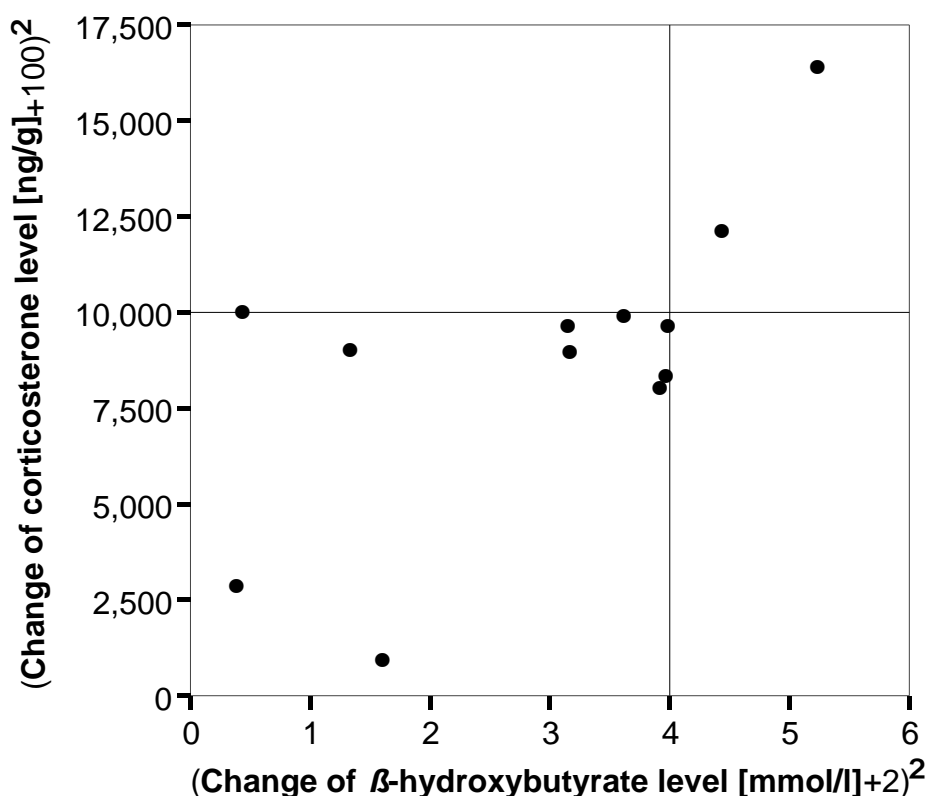

Fig. 12. Relationship between change of β-hydroxybutyrate level and change of faecal corticosterone level in junior chicks from start to end of the chick removal experiment. Senior chicks were removed from two-chick broods for 1 day. Transformations were performed to achieve bivariate normal distribution assumed by Pearson product-moment correlation. Reference lines indicate zero change of the respective parameter over the experimental period.

The highest faecal CORT level in the entire sample (202.9 ng/g) was sample 3 of a female b-chick. This exceptional concentration was attributable to the fact that the sampling was directly preceded by several inclement and stormy days, which had caused starvation in the entire colony. Influences of weather being beyond scope of the present analysis, this extreme value

(z=+2.99) was excluded from the analysis of sample 3. As expected, experimental b-chicks' CORT levels significantly rose again over the days following their siblings' return ($F_{1,10}$=4.698, P=0.028; Table 4). This was unaffected by the sex of the returning chick. An effect of the b-chicks' sex (Table 4) was not tested for because there were only 4 males in the sample.

Table 4. Development of hormone levels in junior chicks of different sex after the chick removal experiment. Senior chicks returned to the nest after they had been removed from two-chick broods for 1 day. Given are means ($\pm$ SD) for the immediate post-experimental and the 3- to 5-day follow-up sample, their differences, and numbers of chicks (n).

Parameter	Sex	End of experiment	Few days later	Change over post-experimental period	n
Testosterone	♂	18.94	8.75	-10.19	1
[pg/ml]	♀	5.49±2.25	6.85±1.69	+ 1.36±2.33	7
	total	7.17±5.19	7.09±1.70	- 0.08±4.62	8
Corticosterone	♂	19.32±25.99	18.31±12.77	- 1.01±21.83	4
[ng/g]	♀	16.65±22.87	26.99±22.98	+10.35±31.52	7
	total	17.62±22.76	23.84±19.62	+ 6.22±27.78	11

4 Discussion

4.1 Observational study

4.1.1 Condition

The fact that in the observational part of the study all at least marginally significant condition patterns were found in the BCI and not in the *ß*-hydroxybutyrate data reflects the more stable, longer-term and integrative nature of BCI as a measure of condition as opposed to *ß*-hydroxybutyrate levels, which indicate short-term condition changes (see section 2.4.3). Nevertheless, the significant correlation found between the two measures confirms their close connection at the individual level.

4.1.1.1 Influence of hatching order

The only studies that to my knowledge documented an effect of hatching rank on condition-related parameters in common tern chicks found that a- and partly b-chicks have higher weight growth rates than c-chicks in three-chick broods (Langham 1972, Bollinger *et al.* 1990, Kikker 1995; in pronouncedly asynchronously hatching broods only: Sorokaite & Budrys 2000) and that pre-fledging mass is higher in a- than in b-chicks in two-chick broods (Kikker 1995). I found that a-chicks have higher BCIs than b-chicks in primary or secondary two-chick broods, and thus broaden evidence of a decrease of condition with hatching rank. This is in accordance with the general view on the effect of hatching asynchrony on weight growth of chicks: Briefly, while seniors enjoy advantages, juniors' development is impaired (e.g. Stenning 1996, Mock & Parker 1997, Saino *et al.* 2001, Eraud *et al.* 2008; for other larids: Lemmetyinen 1972, Barrett & Runde 1980, Nisbet *et al.* 1995). This pattern has generally been assumed for common terns as well, since chick survival was shown to decline with hatching rank (Langham 1972, Becker & Finck 1985, Bollinger *et al.* 1990, Bollinger 1994, Gonzalez-Solis *et al.* 2005) and starvation has been identified to be the principal cause of chick mortality (Langham 1972, Mlody & Becker 1991, Becker 1998). Nevertheless, in contrast to the studies mentioned above several studies on common tern chick development have failed to find an influence of hatching rank (e.g. Robinson & Hamer 2000, Becker & Wink 2003, Braasch 2005, Sprenger 2007). A possible reason is that the latter investigated growth parameters like linear growth rates, peak and pre-fledging mass, which are less direct measures of condition and not exactly age-corrected.

It is especially striking, though, that with respect to hatching position the present study was unable to find condition patterns similar to those in Becker & Wink (2003), because their study

was based on a large sample from the same colony and because the patterns they found led to one of the central hypotheses the present study tested, namely that endogenous chick steroid hormone levels covary with hatching rank. In detail, they found that in two-chick broods, hatching rank did not significantly affect chicks' growth parameters but interacted with the sex of the b-chick in such a fashion that these parameters were superior in male chicks in the last hatching position. The condition-related parameters were measured close to fledging. Sibships were included in the sample conditional on their fledging, as in the other studies cited above that neither found an influence of hatching rank on chick condition. In sharp contrast, I found condition to generally decrease with hatching rank and hatching order not to interact with sex of the b-chick; male junior chicks were the group with the poorest average condition (Fig. 2). Condition was measured at an age of approximately 15 days, i.e. half-way to fledging, and broods were included in the sample regardless of whether they survived to fledging.

Given both samples can be assumed representative and the patterns are unaffected by year effects, a possible explanation for these conflicting results is that junior chicks, and especially the males among them, may generally develop particularly well relative to their senior siblings in the second half of pre-fledging life. Indeed, this is exactly what the growth patterns of chicks that survive to fledging suggest: Even the group with the highest pre-fledging mass, male junior chicks, hatch lighter than their siblings and do not catch up with these in terms of weight relative to age until older than 2 weeks (Becker & Wink 2003). For the entire group of junior chicks, the age at which their weight approaches that of their senior siblings will consequentially be higher than these 2 weeks, the age at which the present study finds junior chicks to be in significantly poorer condition than their siblings. Thus, the seemingly contradictory results described above are actually compatible; and the age at which condition patterns with respect to hatching order are investigated may itself affect the patterns found.

The mechanisms that possibly allow junior chicks to develop this well and reach peak and pre-fledging masses similar to or even higher than their senior siblings' will be discussed below, with a focus on steroids. Independently of the existence of such a mechanism favouring junior chicks *per se*, though, it is important to point out that a more fundamental mechanism may partly or even fully be an alternative explanation for the surprising condition patterns found by Becker & Wink (2003). In common terns, there is a decline of pre-fledging survival with hatching rank (Langham 1972, Becker & Finck 1985, Bollinger *et al.* 1990, Bollinger 1994, Gonzalez-Solis *et al.* 2005), and additionally a mortality bias towards male chicks (Gonzalez-Solis *et al.* 2005). Selection thus acts especially heavy on the same groups that were found to exhibit especially high peak and pre-fledging masses (Becker & Wink 2003). This might in the long term promote

the evolution of special growth mechanisms, but will in the short term selectively eliminate chicks from the population. Since starvation is the main cause of common tern chick mortality (Langham 1972, Mlody & Becker 1991, Becker 1998), dying chicks are likely to be in relatively poor condition (Bollinger *et al.* 1990, Mlody & Becker 1991). Their death will therefore tend to improve the mean condition of the remaining chicks of their hatching rank and also sex. Thus, the apparent general condition advantage especially of male siblings of high hatching rank (Becker & Wink 2003) may actually only be a result of differential mortality in these chicks. In other words, chicks of high hatching rank might generally be in poor condition as the present study shows, but the exceptional few among them that, in spite of the many handicaps they face (cf. Becker & Wink 2003), manage to reach a condition enabling them to survive to fledging, might even surpass the senior siblings.

4.1.1.2 Influence of sex

Common terns show a very limited sexual size dimorphism with males being larger (review in Gonzalez-Solis *et al.* 2005). With respect to weight, they were considered monomorphic until Becker & Wink (2003) surprisingly found male chicks to have superior peak and pre-fledging masses, growth curve characteristics of the last third of pre-fledging life which they interpreted as indicators of condition (Becker & Wink 2002, 2003). My findings with regard to a more direct measure of condition, BCI, support this interpretation: Male chicks have, though only marginally significantly, higher BCIs than female ones. Moreover, I document that sex-specific condition already emerges in the second third of pre-fledging life, i.e. earlier than hitherto found (Becker & Wink 2003, Braasch 2005, Sprenger 2007).

Since in common tern chicks there is a sex-specific chick mortality bias towards males (Gonzalez-Solis *et al.* 2005), it is possible that a selection mechanism as described above (section 4.1.1.1) contributes to the sex-specific condition pattern. Still, there is substantial evidence in favour of viewing common tern chicks as truly sexually dimorphic with respect to weight growth and condition, i.e. respective differences as directly based on sex-specific traits: Even within the same group of fledging chicks, the sex difference consistently gets more distinct from peak to pre-fledging mass (Becker & Wink 2003, Braasch 2005, Sprenger 2007). This longitudinal pattern cannot be explained by mortality between the measurements because there is none in fledging chicks. Instead, it corresponds to the ontogeny in a larid with a more pronounced sexual size dimorphism, the black-headed gull *Larus ridibundus*, where chick mass has been shown to increasingly diverge not until the late pre-fledging phase (Ros 1999).

In birds, the syndrome of sexual dimorphism mostly includes that chicks of the larger sex are more expensive to rear (see section 4.1.2) and more vulnerable, especially under adverse conditions during development, and this often results in a sex ratio bias at fledging towards the smaller, cheaper sex (e.g. Torres & Drummond 1997, Nager *et al.* 1999, 2000, Daunt *et al.* 2001, Velando 2002, Martinez-Padilla *et al.* 2004, Kalmbach *et al.* 2005, Müller *et al.* 2005, Benito & Gonzalez-Solis 2007, Bogdanova & Nager 2008, Dietrich-Bischoff *et al.* 2008). The latter has indeed also been shown in the common tern, where the adverse conditions include poor quality parents (Gonzalez-Solis *et al.* 2005).

4.1.1.3 Interaction of hatching order with sex

There was a non-significant tendency for an interaction between sex of the a-chick and hatching order to affect BCI, indicating that a-chicks had a better condition than their junior siblings most pronouncedly if the former were male (Fig. 3). In other words, condition hierarchies were much more pronounced in broods with a senior chick of the bigger sex. This is not surprising against the background of the sexual dimorphism documented. Notably, though, a corresponding tendency was not found for the sex of the b-chick. This suggests that the clarity of the within-brood hierarchy is predominantly governed by the sex of the senior chick. Usually, a-chicks are initially physically superior through hatching asynchrony (e.g. Morris *et al.* 1991) and thus out-position their siblings, thereby creating a positive feedback loop that helps to maintain or even increase the size and weight hierarchy (Mock & Parker 1997). This process is probably reinforced when the senior chick is of the bigger sex.

An interesting complement is that entire common tern two-chick broods are generally in better condition if the a-chick is male (pre-fledging mass: Becker & Wink 2003; peak mass: Braasch 2005). Hence, there are indications for entire sibships profiting on average in terms of condition in broods with clear condition hierarchies. This does of course not imply advantages for every single brood member, as is underlined by the especially poor condition of junior siblings in broods with a male a-chick (Fig. 3). From the perspective of the entire brood, and hence the parents' reproductive output, though, a pronounced hierarchy may be advantageous, because it allows the dominant chick to impose a stable competitive supremacy based on its superior physique, but without using aggression (Fargallo *et al.* 2003). In contrast, the absence of a strong physical asymmetry tends to intensify social competition and aggression (review in Drummond 2001; Tarlow *et al.* 2001) and possibly also stress (Blanco *et al.* 2006; but see Martinez-Padilla *et al.* 2004, Eraud *et al.* 2008) and thus increases general energetic costs in a sibling competition

context (e.g. Kilner 2001, Rodriguez-Girones *et al.* 2001, Chappell & Bachman 2002, Velando *et al.* 2002, Quillfeldt *et al.* 2006). In a contrasting case, large within-brood size and weight differences, and especially a senior chick of the bigger sex, have been shown to decrease reproductive output in the Harris's hawk *Parabuteo unicintus* (Bednarz & Hayden 1991). This was explained with pronounced hierarchies increasing the probability of siblicide (cf. Bortolotti 1986), a phenomenon unimportant in common terns.

If clear brood hierarchies, i.e. in common terns broods with a male a-chick, were indeed adaptive for the parents, mothers, the heterogametic parents in birds, should be selected to adjust offspring sex accordingly (Carranza 2004), as has been found in several bird species (reviews in Carranza 2004, Blanco *et al.* 2006, Kim & Monaghan 2006). Preliminary results, though, indicate that hatching order in common terns is not sex-biased and especially that the ratio of male to female senior chicks does not differ from parity (M. M. Benito & P. H. Becker, unpubl. data). This might suggest that in common terns mothers are unable to control offspring sex, in marked contrast to the closely related roseate terns *Sterna dougallii* (Szczys *et al.* 2001).

4.1.2 Success in competitive behaviour

In the behavioural data there was generally little evidence for differential competitivity between groups. I did not find an effect of hatching position or sex on a chick's feeding rate. Hence, none of the condition patterns the present study documented could be connected to a corresponding feeding rate pattern. Notably, on an individual chick basis this plausible connection was found, feeding rate and BCI were significantly correlated; the effect size was rather small, though. Both results suggest additional determinants of chick condition to play a role besides the measured food input, possibly affected by competition for nutritional resources. There are at least two influences neglected so far:

First, I measured the number of items fed to a chick per unit time, but did not account for item quality or mass. Mass feeding rate is not necessarily proportional to item feeding rate (cf. Stoehr *et al.* 2001), and neither item quality nor item mass are necessarily constant: Items fed to common tern chicks differ considerably with respect to species (Frank 1992, Braasch 2005, Sprenger 2007) and size (pers. obs.). Furthermore, food species vary considerably in their nutritive value, including caloric value, fat and protein content, and these differences have the potential to affect chick growth rate (Massias & Becker 1990). Thus, energy input was measured very roughly. Moreover, junior common tern chicks have been shown to receive food of lesser

quality compared to their siblings (Kikker 1995). This alone might account for the absence of a parallel to the clear condition disadvantage of b-chicks in the feeding rate data.

Second, energy expenditure might differ between chicks, causing condition differences in spite of similar feeding rates. Average total energy budgets of common tern chicks vary relatively little over the ages investigated in the present study (Drent *et al.* 1992). In a sibling competition context, though, siblings might be differentially affected by the energetic costs of social competition (Kilner 2001, Rodriguez-Girones *et al.* 2001, Chappell & Bachman 2002, Neuenschwander *et al.* 2003). The fact that begging intensity does not vary with hatching order in common tern chicks (Smith *et al.* 2005), however, does not support this idea. On the other hand, in another larid, the black-headed gull, females, the smaller sex, were more persistent in begging displays (Müller *et al.* 2007a). If this was the case in common terns as well, it might point to an increased energy expenditure related to social competition in females, which could explain the inferior condition of females chicks despite similar feeding rates.

Finally, the fact that the number of broods that entered the analysis was markedly smaller for the feeding rates than for the condition parameters might have allowed patterns to become evident in the latter rather than the former. The trends were in fact similar: Average feeding rates were higher for senior than junior chicks and for male than female chicks, respectively (cf. Table 2). These are also the patterns prevailing in literature:

Feeding rates of common tern chicks generally tend to increase with decreasing hatching rank (Heinrichs 2003, Smith *et al.* 2005; but see Sorokaite & Budrys 2000, Sprenger 2007), especially from c-chicks to their senior siblings (Langham 1972, Morris *et al.* 1991, Kikker 1995, Braasch 2005). It has been argued that the general feeding conditions may determine whether and in how far feeding rate differences appear in the brood hierarchy of common terns (Heinrichs 2003). When plenty of food or high quality food is available, even less competitive chicks might get a similar share because dominant chicks are satiated (cf. Safriel 1981, van Heezik & Seddon 1996). The absence of a clear effect of hatching rank on feeding rate in the present study can however not be explained in this context, because mean per chick feeding rate (0.63 items/h, cf. Table 2) was near the lower limits of the hitherto documented range for the colony (e.g. Frank 1992: 0.92-1.20 items/h; Kikker 1995: 0.6 items/h; Heinrichs 2003: 1.2 items/h; Braasch 2005: 0.69 items/h; Sprenger 2007: 1.25 items/h), suggesting comparatively bad food availability.

In sexually dimorphic birds, chicks of the larger sex have often been shown to be more expensive to rear. Several studies documented their greater energetic needs (e.g. Fiala & Congdon 1983, Krijgsveld *et al.* 1998) and some also their greater food intake during

development to independence (e.g. Weimerskirch *et al.* 2000, Fargallo *et al.* 2003, Quillfeldt *et al.* 2007b; but see Torres & Drummond 1999, Müller *et al.* 2007a). Both was even shown in a bird species with only slight sexual dimorphism, the common guillemot *Uria aalge* (Cameron-MacMillan *et al.* 2007). In common terns, though, feeding rates have not been found to significantly differ between chicks of different sex (Heinrichs 2003, Braasch 2005), even though there were indications for males to reach higher rates in one study (Sprenger 2007). Due to the very slight dimorphism in common terns (Gonzalez-Solis *et al.* 2005), however, no strong feeding rate differences are to be expected, and this is presumably the reason why the mentioned studies, which rely on limited sample sizes, did not find any.

None of the parameters derived to characterize success in direct competitive interactions between siblings was even near to revealing a difference between a- and b-chicks. This agrees with results of previous studies of different methodology (Braasch 2005, Sprenger 2007) and corresponds with my findings for the resulting parameter feeding rate. Again, the small sample sizes, especially for fighting success, certainly made it harder to discover possibly existing differences in competitivity between groups. Nevertheless, I was able to confirm that chicks which arrive first at the parent about to provide food get the food item in a vast majority of the cases. This had previously been shown for common terns (Smith *et al.* 2005: 95% of feedings) and similarly for other species with mobile young (e.g. Safriel 1981, van Heezik & Seddon 1996, Müller *et al.* 2007a).

4.1.3 Hormones

Based on the T and CORT data presented in this study I reject the hypotheses that endogenous steroid levels vary with hatching order and sex of chicks. Thus, neither the peak and pre-fledging mass patterns (Becker & Wink 2003) nor the BCI patterns (this study) found in common tern chicks appear to be directly linked to their steroid hormone patterns. On the individual level, though, CORT levels were connected to condition, as indicated by the negative correlation between the two parameters. This result is in accordance with the abundant evidence showing that in birds CORT has a central role in the response to various stressors, including food shortage and declining internal energy stores (e.g. Kitaysky *et al.* 1999, 2001a, Saino *et al.* 2003, Palme *et al.* 2005, Quillfeldt *et al.* 2006, 2007a). With respect to T, the absence of a sex-specific pattern agrees with results of the majority of corresponding bird studies (review in Fargallo *et al.* 2007), including those on common terns (Braasch 2005, Sprenger 2007). Also T level differences between hatching ranks have not been found in this species (Braasch 2005, Sprenger 2007).

It is nevertheless possible that steroid patterns affect chick condition patterns in a way different from the one tested here. The present study measured levels of endogenous steroids at a mean age of just above 2 weeks, hence assuming condition was affected by activational hormone effects (Phoenix *et al.* 1959; review in Elekonich & Robinson 2000) on chick behaviour. This was suggested by the fact that male common tern c-chicks that finally fledge heavier than their older siblings are lighter than these at ages up to 2 weeks, when some mechanism allows them to surpass the older competitors (Becker & Wink 2003; cf. section 4.1.1.1).

At second sight, though, it is similarly, if not more plausible to attribute the course of these growth curves to a general quality characterizing these junior chicks already from the time of hatching on, and maternal yolk hormones may be a candidate for the origin of this quality:

In spite of physical disadvantages caused by hatching asynchrony, junior chicks that survive to fledging, especially males, reach growth rates not much inferior to those of their senior siblings at the same age already soon after hatching (Becker & Wink 2003). More importantly, relative to their size and weight at the same time, i.e. in a competition context, these junior chicks' growth rates may therefore be viewed as equal, if not even superior to those of the senior chicks. Thus, the high quality of these last-hatched chicks does express itself long before they surpass their older siblings in terms of absolute weight. The reasons why this still does not happen earlier probably is that in common terns junior chicks start life in a very unfavourable competitive setting: Compared to their siblings, they hatch from smaller eggs (Becker & Ludwigs 2004) and are lighter at hatch (LeCroy & LeCroy 1974), a difference aggravated by hatching asynchrony (e.g. Morris *et al.* 1991). In addition to their inferior physique, the fact that due to hatching asynchrony they remain dependent on brooding longer than their siblings and thus tend to stay in the nest further reduces their chances to win scrambles for food (LeCroy & LeCroy 1974). This situation is not ameliorated before a brood age of 1 week, when the chicks' developing ability to thermoregulate independently and the consequentially decreasing necessity of brooding allows both parents simultaneously to hunt and provide food. This shift in parental care markedly increases the food availability and decreases the severity of nutritional competition for the entire brood (cf. Langham 1972). The physically and competitively (Smith *et al.* 2005) inferior junior chicks especially profit from this weakening of nutritional restrictions (Langham 1972).

Against this background, it is possible that the proposed steroid hormone effect on competitivity and thus growth and condition is not an activational effect of endogenous steroids, but an organizational effect (Phoenix *et al.* 1959; review in Elekonich & Robinson 2000) originating early in the chick's life or even pre-hatch from maternal yolk hormones. Maternally derived

steroids might influence the *modus operandi* of chicks already at the embryonic stage (Schwabl 1993; reviews in Clark & Galef 1995, Groothuis *et al.* 2005b, Müller *et al.* 2007b), e.g. by causing a long term increase in sensitivity and behavioural responsiveness to later steroid exposure (cf. Ros *et al.* 2002). Maternal steroids, specifically androgens, have been detected in common tern eggs (French *et al.* 2001) and have in other birds, including larids, been shown to increase chicks' begging intensity and promote chick growth (review in Groothuis *et al.* 2005b). Interestingly, maternal steroids, including T, in common terns are lowest in the first-laid egg (French *et al.* 2001), and are therefore a good candidate for promoting competitivity and possibly causing superior weight growth (Becker & Wink 2003) of junior chicks. With respect to sex, yolk hormones in common terns have not been investigated, but in other birds there are indications of sex-specific levels of steroids, including T, possibly also as part of the sex-determining process (review in Groothuis *et al.* 2005b). To sum up in the light of the present study's results, if there are steroid patterns causing hatching rank- and sex-specific condition patterns in common terns at all, I suggest that these are more probably found in maternally derived yolk steroids than in endogenous chick steroids.

In one respect, though, there was an interesting endogenous steroid pattern. T levels were significantly affected by the interaction of the sex of the a-chick with hatching order: While male a-chicks had lower levels than their junior siblings, female a-chicks had higher levels than their nest-mates (Fig. 8). This suggests a connection with the indication for a corresponding interaction on BCI (Fig. 3; section 4.1.1.3): Male a-chicks were pronouncedly superior to their junior siblings in terms of condition and had lower T levels. On the other hand, female a-chicks were only slightly superior to their junior siblings in terms of condition and had clearly higher T levels. Senior chicks of the bigger sex thus enjoyed a clearer physical within-brood hierarchy and invested less in the double-edged sword T, which might enhance competitive abilities such as aggression and begging intensity (e.g. Groothuis & Meeuwissen 1992, Sasvari *et al.* 1999, Groothuis & Ros 2005, Quillfeldt *et al.* 2006, Goodship & Buchanan 2006, 2007), but also entail serious costs in terms of growth, condition, plumage pigmentation, immunocompetence and survival (e.g. Ros 1999, Naguib *et al.* 2004, Fargallo *et al.* 2007). For senior chicks of the smaller sex, the cost-benefit ratio may be different. Their physical advantage over the junior siblings is smaller and thus less reliable. In order to sustain their dominant status, investment in other aspects of competitivity, e.g. by T secretion (cf. Tarlow *et al.* 2001), may therefore be more profitable. This interpretation agrees with existing evidence that aggression escalates with decreasing physical dominance of the senior sibling (e.g. Osorno & Drummond 1995, Cook *et al.* 2000, Nathan *et al.* 2001). Also stress levels, as indicated by heat shock proteins, have been

shown to be elevated in senior chicks of the smaller sex (Blanco *et al.* 2006). A similar pattern in T levels, as documented by the present study, has to my knowledge not been previously shown, and it is thus surprising to just find it in a species with a very slight sexual dimorphism. This and the fact that two other contributions on common terns (Braasch 2005, Sprenger 2007) did not find a similar pattern call for a re-examination of this topic based on a larger sample.

4.2 Chick removal experiment

None of the dependent variables was significantly affected by an interaction of the main experimental effect with the sex of the removed a- or the remaining b-chick. Corresponding to the results of the observational part of the study, such interactions could have been expected especially for the a-chick sex with respect to condition and T. However, effects would have been expected to be small, as the common tern is a species with only a slight sexual dimorphism (review in Gonzalez-Solis *et al.* 2005), and hard to detect, as the duration of the experimental period was 1 day only and the experimental sample size was small. Therefore, the lack of interactions with chick sex was unsurprising. In the discussion I will consequentially concentrate on the general effects of the brood size manipulation on the different parameters, their correlations and their comparison with differences between natural one- and two-chick broods found in the observational part of the study.

Since no control treatment was employed, the general experimental trends may theoretically be confounded, especially by changes of the dependent variables with age. However, substantial systematic changes are improbable for the following reasons: BCI is a measure relative to the mean body mass of chicks of the same age and thus age-correction is immanent. The second measure of condition, *ß*-hydroxybutyrate, covaried with BCI and its concentration in chicken *Gallus gallus* blood has been shown to be stable from an age of 3 to 14 days, when the study ended (Ohtsu *et al.* 2003). Most importantly, in common terns fed *ad libitum* tissue catabolism is not initiated until an age of 22 days (Drent *et al.* 1992), i.e. shortly before fledging. Hence, above baseline levels of *ß*-hydroxybutyrate, originating from mobilization of body lipids, should not be a general phenomenon at ages under 20 days, when samples for this study were taken. Common tern item feeding rates per chick do not significantly differ between ages of 1 and 20 days, and are especially stable from day 10 on (Kikker 1995). Hormone levels were not only followed over the single experimental day, but also up to 5 days longer for a follow-up sample. Therefore, it is especially important that systematic change with age is absent in T and CORT levels. Previous studies indicate this might indeed be the case: T levels did not significantly change from the first

to the second half of the nestling period in the zebra finch *Taeniopygia guttata* (Adkins-Regan *et al.* 1990). CORT levels in precocial species do not significantly change in the first 4 weeks after hatching (review in Holmes *et al.* 1990; Williams *et al.* 2008). CORT levels were also stable in the age between 2 and 5 weeks in a larid, the red-legged kittiwake *Rissa brevirostris* (Kitaysky *et al.* 2001a). Finally, for both hormones the changes I found from sample 1 to sample 2 and from sample 2 to sample 3 were inverse. Therefore they could not even be caused by age-related changes like those shown for CORT in altricial species (Schwabl 1999, Tarlow *et al.* 2001, Quillfeldt *et al.* 2007a), since these are monotonic.

4.2.1 Condition

As predicted, condition of b-chicks improved over the day following the removal of their senior siblings from the colony. This experimental effect was significant in the *ß*-hydroxybutyrate data and similar but less distinct in the BCI data (Fig. 4), as was expected due to the specific suitability of the former for detection of short-term condition changes (Jenni-Eiermann & Jenni 1998). Both parameters also covaried on the individual level (Fig. 1), which corroborated their close linkage.

The experimental effect agrees with abundant and largely consistent evidence of an inverse relation between brood size on the one hand and chick weight, growth and condition on the other hand from brood size manipulation studies across a range of avian taxa with young that are not self-feeding (e.g. review in Martin 1987; Bollinger *et al.* 1990, Sanz & Tinbergen 1999, Saino *et al.* 2003, Naguib *et al.* 2004, Gil *et al.* 2008). The cited studies employed both brood size enlargement and reduction, and especially the latter provides convincing evidence that naturally occurring brood sizes impose limitations on chick performance. This is thus also true for the present study. In several cases, the brood size manipulations did not only affect chick condition and similar parameters, but also weight far beyond fledging (Naguib *et al.* 2004) and, inversely to condition, chick mortality (e.g. review in Martin 1987; Sanz & Tinbergen 1999). In common terns, an observational study has shown increased weight growth in chicks whose siblings had died (Heinrichs 2003). Experimental results confirmed this: C-chicks grew faster after the experimental removal of an older sibling (Bollinger *et al.* 1990), and also in two-chick broods there were tendencies to accelerated weight growth of b-chicks after a-chick removal (Sprenger 2007). The latter is corroborated by the significant result of my study. This combined evidence further justifies to interpret in a sibling competition context the condition patterns found (section

4.1.1) and indicates that parents do not fully compensate for the greater demands of larger broods.

Studies investigating differences between common tern chicks from natural broods of different size indicate the same, though more equivocally: Chicks from larger broods showed lower growth rates (Becker & Finck 1985, Robinson & Hamer 2000; but see Becker & Wink 2003, Sprenger 2007), smaller peak and pre-fledging weights (Becker & Wink 2003, Sprenger 2007), higher fledging ages (Robinson & Hamer 2000; but see Becker & Wink 2003, Sprenger 2007) and higher mortality (Sprenger 2007). These tendencies are largely in accordance with growth and mortality patterns in other non-precocial birds (review in Martin 1987).

In contrast to these findings and the present chick removal experiment, my analysis of natural brood size variation did not reveal condition advantages for single chicks compared with chicks in two-chick broods. This apparent contradiction most probably reflects that the between-brood comparison does, in contrast to the within-brood experiment, not account for differences in parental quality. Accordingly, a natural singleton would receive parental care of lower quality or quantity compared to what two-chick brood parents would provide if they reared one chick only, due to e.g. foraging efficiency and experience or territory quality (review in Martin 1987). In support of this interpretation, many parameters of parental quality in common terns, such as age, mass, and timing, indicate superiority of those rearing three chicks to those rearing two (Gonzalez-Solis *et al.* 2005), and my results suggest that extrapolating this relation to one-chick broods is legitimate.

4.2.2 Feeding rate

In altricial bird species, several lines of experimental evidence lead to the conclusion that the amount of food that parents can provide relative to the energy demands of their young is limited and that this leads to nestlings being food-limited (review in Martin 1987). For instance, nestlings were shown to weigh less in experimentally established large broods unless supplemental food was provided in the territories to help parents meet the extra energy demands of the brood adequately (Crossner 1977). Magrath *et al.* (2007) similarly documented the decline of growth rates and additionally also of per chick provisioning rates in enlarged broods (but see Neuenschwander *et al.* 2003). Like altricial species, common terns are fully dependent on parental food provisioning. Hence, in accordance with the evidence presented, I predicted the feeding rate of the b-chick to increase with the removal of its senior sibling. However, while the mean feeding rate indeed was higher after than before the removal (Fig. 6), this difference was

not significant and the hypothesis is thus rejected. This result is apparently incompatible with the corresponding positive effect of sibling removal on chick condition. The same contrast between results concerning condition and feeding rate was already found in the observational part of the study, and I suggest related possible explanations:

First, items fed to a chick per unit time are probably only a very rough measure of energy input (see section 4.1.2). In the current context, this means that the b-chicks may have achieved a better condition after their sibling had been experimentally removed without an increase of their item feeding rate if parents delivered prey items of greater size or higher quality. The fact that the brood size reduction allowed parents to reduce their total provisioning rate may for instance have given them the opportunity and time to hunt more selectively or to visit higher quality foraging grounds situated further from the colony. At least concerning the size of food items, though, there is counterevidence from another common tern colony: Total rate of parental food provisioning is uncorrelated with the size of the prey items delivered across natural broods of different size (Wiggins & Morris 1987). An identical result was obtained in a study across experimentally varied brood sizes in the great tit *Parus major* (Neuenschwander *et al.* 2003).

Second, the disappearance of the social aspect of within-brood competition and connected energetic costs (Kilner 2001, Rodriguez-Girones *et al.* 2001, Chappell & Bachman 2002) might be the proximate cause for the improvement of condition connected to the sibling removal. The positive trend in feeding rates after sibling removal hints at social competition being an addition rather than an alternative to the explanation based on increase of feeding rate due to the absence of within-brood competition for nutritional resources. The fact that feeding rate covaried with neither of the condition parameters over the experimental period, however, points in the opposite direction. Correspondingly, Neuenschwander *et al.* (2003) found that individual great tit chicks in experimentally enlarged broods received unchanged amounts of food, but increased begging intensity and suffered deteriorating body condition.

Finally, the subset of experimental broods for which behavioural data were obtained might have been too small to reach reliable results. In support of this view, the advantage of absent sibling competition in terms of feeding rate, which was expected to show in the experiment, indeed became apparent in the comparison of chicks from natural one- and two-chick broods, where the sample size of at least the latter was considerably larger. On the grounds of the evidence referred to in the experimental context, the higher per chick feeding rates in one-chick broods were not surprising, although the influence of parental quality decreasing with brood size (Gonzalez-Solis *et al.* 2005) was expected to reduce the effect compared to the experiment.

This is probably also the reason why observational studies obtained equivocal results on the effect of increasing brood size on per chick feeding rate. With respect to common terns, different studies found a slight decrease (Robinson & Hamer 2000), a nonsignificant trend towards a decrease from two- to three-chick broods (Kikker 1995, Sprenger 2007), and no change at all (Wiggins 1989). Also for other non-precocial bird species, studies documenting decreasing per chick feeding rates (e.g. Leonard *et al.* 2000, Stoehr *et al.* 2001) and no effect (e.g. Kalmbach & Becker 2005, Mizuta 2005, Falconer *et al.* 2008) in larger broods form an incoherent picture.

Trends with regard to interactions of the experimental effect with chick sex were nonsignificant, but plausible and compatible with the demonstrated trend to better condition in male than in female chicks: Female b-chicks profited more from the removal of their senior sibling, and b-chicks in general profited more from the removal of a male than of a female a-chick. This suggests that the larger and better conditioned male sex might indeed be superior in competing for food.

4.2.3 Hormones

4.2.3.1 Testosterone

Contrary to predictions, baseline T levels of b-chicks did not decrease following their siblings' removal (Fig. 9a) and neither increased after their return (Table 4). The corresponding hypotheses are thus rejected. Instead, T levels remained rather stable and were also unaffected by natural variation of brood size, which both indicates that in common terns the extent of sibling competition does not affect circulating T.

This is in marked contrast to results of the few similar previous experiments, which however were equivocal already: In the zebra finch, T levels increased with experimental brood size (Naguib *et al.* 2004), while at the same time they decreased in another passerine, the spotless starling *Sturnus unicolor* (Gil *et al.* 2008). Even in an experiment identical to the one presented here, T levels of common tern chicks were reduced by sibling removal (Sprenger 2007), however, without the assay effect being corrected for. Likewise, results of related studies on T correlates are ambiguous: T levels were found to be positively (Quillfeldt *et al.* 2006, Goodship & Buchanan 2006, 2007), but also negatively (Groothuis & Ros 2005) and not (Quillfeldt *et al.* 2007b) related to begging. In other cases, T levels did not vary with the extent of sibling competition expressed by brood size (Tarlow *et al.* 2001) or with induced food shortage or deprivation (Nunez-de la Mora *et al.* 1996, Kitaysky *et al.* 1999), or even decreased with food shortage (Perez-Rodriguez *et al.* 2006). This incoherent picture has been appreciated as evidence

for species-specific differences (Gil *et al.* 2008), but explanatory approaches to date are rather fragmentary than unifying. I suggest to include the following aspects in future attempts to solve this puzzle:

First, different advantageous behavioural reactions to varying degrees of sibling competition might be affected by T in opposite ways. In chicks of the black-headed gull, T implants facilitated sibling aggression, but substantially suppressed begging behaviour (Groothuis & Ros 2005). Even if such a link was generally found in birds (for counterexamples see Kitaysky *et al.* 2003, Quillfeldt *et al.* 2006), it would result in T level reactions to variations in the intensity of sibling competition depending on the species' characteristics. These possibly influential characteristics include the relative importance of scrambling and begging for the decision to whom feedings are directed and the occurrence of pronounced inter-sibling aggression or even siblicide. The absence of the latter in common terns (Braasch 2005) might be the reason for the missing effect of sibling removal on T levels.

Second, the specific conditions in which studies are carried out will affect the cost-benefit ratio of a change in chicks' T levels. A generally poor food availability will enhance sibling competition for food and thus make it more attractive to invest in a T level elevation, which at least in some cases has been shown to promote competitivity (e.g. Groothuis & Meeuwissen 1992, Sasvari *et al.* 1999, Quillfeldt *et al.* 2006, Goodship & Buchanan 2006, 2007). Also, the absolute brood sizes investigated might affect inferences made on the relevance of hormone levels. Kitaysky *et al.* (1999) did not find an elevation of chick T levels in response to food restriction and interpreted this as evidence against a dependence of sibling aggression on T. However, the chicks had been kept separately and thus exerting aggression on siblings was impossible (cf. Naguib *et al.* 2004). Similarly, in the present study the disadvantages from intra-brood social competition, which has been suggested to directly affect T levels (Naguib *et al.* 2004), may have been too small in the original brood size of the experimental two-chick broods to yield a change in T levels when reduced.

Third, instead of (Tarlow *et al.* 2001, Ferree *et al.* 2004) or in addition to (Ros *et al.* 2002) constantly maintaining high baseline levels of T to cope with potentially arising competitive challenges, chicks may, in order to avoid the serious costs entailed by long-term elevated T levels (e.g. Ros 1999, Naguib *et al.* 2004, Fargallo *et al.* 2007), only raise T levels when instantly needed. This general pattern is predicted by the Challenge Hypothesis (Wingfield *et al.* 1990) and has already been shown for chicks in the black-headed gull (Ros *et al.* 2002) and the Nazca booby *Sula granti* (Ferree *et al.* 2004). It is currently under investigation in common terns

(A. Braasch, pers. comm.). The adaptivity of a mechanism up-regulating T on demand might be low in this species due to the low importance of inter-sibling aggression (Braasch 2005), but also high in the context of tug of war for food items. If this mechanism indeed was present in the common terns, it could possibly explain the contrast between the results of the present study and an identical experiment by Sprenger (2007), who found T levels to decrease with sibling removal. Neither of the two studies took into account aggressive challenges in the colony that possibly directly preceded chick sampling. If such coincidences occurred, they could have distorted the results of either study.

While neither T levels nor BCIs were significantly affected by the experiment, both parameters tended to negatively covary over the experimental 1-day period. My results show that T levels are unaffected by a manipulation that indirectly ameliorates chick condition. This suggests that the covariation of T and BCI is rather not driven by the latter, but may reflect the condition costs that T has been shown to impose at least in the longer term (e.g. Ros 1999, Groothuis & Ros 2005, Fargallo *et al.* 2007). The result is however also open to the interpretation that an unknown third parameter governs both T levels and condition.

4.2.3.2 Corticosterone

As hypothesized, the removal of the senior sibling induced CORT levels of junior chicks to drop (Fig. 9b), though only marginally significantly, and the reintroduction of sibling competition led to a significant recovery of CORT levels (Table 10). These results suggest that sibling competition indeed elicited a stress response mediated by CORT. Moreover, on an individual basis I found levels of CORT and *ß*-hydroxybutyrate to positively covary over the day between pre- and immediately post-experimental sampling. This further substantiates the assumption of a positive proximate connection between energetic stress imposed by sibling competition via social stress or competition for nutritional resources on the one hand and CORT levels on the other hand.

These findings are in accordance with the majority of previous studies; there are also deviating results, though. The decrease of CORT levels as reaction to sibling removal in the common tern has already been indicated by a similar experiment (Sprenger 2007), which was however based on a much smaller sample. My results are thus confirmatory in this respect. Also in further bird species whose chicks are not self-feeding, CORT has been shown to positively covary with experimentally varied brood size (barn swallow *Hirundo rustica*, Saino *et al.* 2003; collared dove *Streptopelia decaocto*, Eraud *et al.* 2008), as have other indicators of physiological stress (e.g.

heterophil-lymphocyte ratio: Ilmonen *et al.* 2003, Suorsa *et al.* 2004). However, in other instances CORT levels remained unaffected by experimental brood size variations (pied flycatcher *Ficedula hypoleuca*, Ilmonen *et al.* 2003; spotless starling, Gil *et al.* 2008; blue tit *Cyanistes caeruleus*, Lobato *et al.* 2008). Covariation of CORT and indicators of condition on the individual level has also been previously documented in birds (e.g. in thin-billed prion *Pachyptila belcheri*, Quillfeldt *et al.* 2006; collared dove, Eraud *et al.* 2008). Furthermore, the direct imposition of energetic stress by experimental restriction of food quantity and also quality augmented chick CORT levels in a number of bird species (e.g. blue-footed booby *Sula nebouxii*, Nunez-de la Mora *et al.* 1996; black-legged kittiwake *Rissa tridactyla*, Kitaysky *et al.* 1999; red-legged kittiwake, Kitaysky *et al.* 2001a; barn swallow, Saino *et al.* 2003; red-legged partridge *Alectoris rufa*, Perez-Rodriguez *et al.* 2006). This effect was also reversible (e.g. Nunez-de la Mora et al. 1996). Other species did not mount a CORT response to food restriction, though (zebra finch, Spencer *et al.* 2003; tufted puffin *Fratercula cirrhata*, Williams *et al.* 2008).

There is thus substantial evidence which, in line with conclusions from my findings, points to energetic stress, especially caused by deficiencies in nutritional resources, as the proximate link between sibling competition and CORT levels. A CORT-based stress response entails various costs (review in Wingfield *et al.* 1997), but may help chicks to cope with energetic deficits by mobilization of internal resources (Wingfield *et al.* 1997, Kitaysky *et al.* 1999) and, especially important for chicks that are not self-feeding, by facilitation of begging: Experimentally raised CORT levels were shown to increase begging frequency in black-legged kittiwakes (Kitaysky *et al.* 2001b, 2003) and house sparrows *Passer domesticus* (Loiseau *et al.* 2008). Moreover, CORT levels covary with begging intensity in thin-billed prions (Quillfeldt *et al.* 2006).

Against the background of this solid combined evidence, the mentioned instances of species whose chicks do not mount a CORT-based stress response when faced with intense sibling competition or food restriction are remarkable. Attempts to explain these discrepancies in the results reported have focused on three mutually nonexclusive aspects: First, while the hypothalamic-pituitary-adrenal axis, along which CORT secretion is physiologically initiated, probably becomes functional shortly after hatching in precocial and semiprecocial chicks, it takes longer to fully develop in most altricial chicks (review in Kitaysky *et al.* 2001a). This would explain the deviating results in pied flycatchers (Ilmonen *et al.* 2003), zebra finches (Spencer *et al.* 2003), spotless starlings (Gil *et al.* 2008), and blue tits (Lobato *et al.* 2008). Second, the evolution of a hormonal response to food shortages is more probable in species that are particularly exposed to such events due to their ecology. Insectivorous birds foraging on the wing and also seabirds are generally more likely to regularly experience a lack of food resources

than are omnivorous species (cf. Gil *et al.* 2008; but see Kitaysky *et al.* 2003). This difference may further explain the absence of a CORT response in the omnivorous zebra finch (Spencer *et al.* 2003), spotless starling (Gil *et al.* 2008), and blue tit (Lobato *et al.* 2008), and also its presence in the altricial barn swallow (Saino *et al.* 2003) and collared dove (Eraud *et al.* 2008). The latter species is omnivorous, but nestlings are initially fed on crop milk, the secretion of which is regularly resource-limiting for them (review in Eraud *et al.* 2008). Third, a disadvantageous cost-benefit ratio (review in Wingfield *et al.* 1997) might prevent CORT secretion under certain conditions, e.g. when parents are unresponsive to chick begging. This was probably the case in experimentally food-restricted tufted puffins (Williams *et al.* 2008). The two former, main comparative explanations also hold with respect to my results. The common tern, a semiprecocial seabird regularly exposed to food shortages (e.g. Becker & Finck 1985, Mlody & Becker 1991), mounted a CORT-based stress response.

CORT levels did not differ between chicks from natural one- and two-chick broods, which is in contrast to the experimental brood size reduction lowering CORT levels. This inconsistency can probably be explained by the lower quality of parents of natural one-chick broods (review in Gonzalez-Solis *et al.* 2005) and the consequentially higher stress and thus CORT levels relative to brood size in these broods (cf. section 4.2.1).

4.3 Conclusion and prospects

In summary, my results show that sibling competition imposes limits on chick condition, at least in junior chicks, and underline the role of elevated CORT levels as a response to energetic stress resulting from this constraint, possibly promoting the mobilization of energetic resources (cf. Wingfield *et al.* 1997, Kitaysky *et al.* 1999) or the intensification of begging (cf. Kitaysky *et al.* 2001b, 2003, Quillfeldt *et al.* 2006, Loiseau *et al.* 2008). In contrast, endogenous T, which generally promotes aggressive behaviour in chicks (Groothuis & Meeuwissen 1992, Sasvari *et al.* 1999, Groothuis & Ros 2005; but see Quillfeldt *et al.* 2007b), did not seem to play a general role in sibling competition. However, T was elevated in female senior chicks, which probably could not sustain their within-brood dominance via weight and size alone. This pattern has not been previously documented in birds. The observational part of the study revealed a condition disadvantage for junior chicks, which, interpreted in the light of the experimental result, was probably caused by sibling competition. Furthermore, there was an indication of better condition in male chicks compared to female ones. These condition patterns, though, could not be

attributed to similar patterns in endogenous steroids or competitive behaviour. The results call for follow-up studies especially on the following aspects:

The general relevance of T for mediating sibling competition strategies cannot finally be rejected without further investigation of maternal yolk steroids, which have been detected in common terns (French *et al.* 2001). Their possible organizational effects (Schwabl 1993; reviews in Clark & Galef 1995, Groothuis *et al.* 2005b, Müller *et al.* 2007b) are good candidates for explaining especially the surprisingly distinct sex-specific condition (this study) and growth patterns (Becker & Wink 2003) found. Furthermore, short-term elevation of T levels in situations instantly requiring the corresponding effects (cf. Ros *et al.* 2002, Ferree *et al.* 2004)., as suggested by the Challenge Hypothesis (Wingfield *et al.* 1990), is another way in which T might mediate sibling competition strategies and which was not tested for in the present study.

To answer the question whether condition patterns are caused by differential energy input, i.e. sibling competition for nutritional resources, or differential energy expenditure, e.g. through differential in energetically costly social competition, a closer look at feeding rates will be necessary. This needs to involve a more precise measure of food item energy content via item size and food species (cf. Massias & Becker 1990, Kikker 1995).

I investigated chick condition and its relation to behavioural and hormonal parameters, but not the interrelation of the latter two. However, based on literature, I interpreted my findings assuming certain effects of the hormones investigated on behaviour. These links need to be established before a deeper understanding of sibling competition in the common tern as a model species can emerge. Appropriate studies need to go beyond success scores and focus on parameters describing behavioural effort, e.g. via begging frequency, intensity and persistence. These may directly be governed by hormone levels (e.g. Kitaysky *et al.* 2001b, Quillfeldt *et al.* 2006, Goodship & Buchanan 2006, 2007, Loiseau *et al.* 2008) and, in connection with physical parameters and feeding history, proximately determine competitive success (cf. Godfray 1995, Price *et al.* 1996).

Finally, to come back to the starting point, the longer-term implications of sibling competition for life-history-trajectories and fitness warrant further investigation. If the sex of certain chicks in the hatching sequence affects T levels and possibly also condition in the brood, as my results show, it might as well have consequences on chick mortality. While effects of sex ratio on chick mortality have been studied in the common tern (Heinrichs 2003), sex composition of a brood including the chicks' respective hatching rank is a factor that has hardly been studied (but see Sprenger 2007) and still awaits thorough analysis based on a large sample size. But also later in

life, consequences of pre-fledging sibling competition might become apparent. With respect to common terns, research on long term effects on fitness-related traits has focused on predictors such as hatching order, sex, clutch size, fledging weight and age (Dittmann *et al.* 2001, Ludwigs & Becker 2006), but have neglected endocrine properties of chicks. These may however be of special importance, as studies on long term costs of high CORT levels have shown (Wingfield *et al.* 1997, Kitaysky *et al.* 2003). In the context of compensatory growth (Metcalfe & Monaghan 2001), CORT levels, which are related to energetic deficits (e.g. Kitaysky *et al.* 1999, Quillfeldt *et al.* 2007a), might also more honestly reflect mortgages raised during development than measures of chick growth. Specifically, the present study's finding of elevated baseline CORT levels in response to sibling competition thus raises the question whether this is a transient effect only or one which itself or via related costs lingers into adulthood.

5 Zusammenfassung

Geschwisterkonkurrenz bei Küken der Flußseeschwalbe *Sterna hirundo*:
zugrundeliegende Hormon- und Verhaltensmuster und -mechanismen

Die Leistungsfähigkeit, die weitere Entwicklung und letztlich die Fitneß von Vogeljungen, die von elterlicher Nahrungsversorgung abhängig sind, werden entscheidend von Brutgröße und, damit zusammenhängend, Geschwisterkonkurrenz beeinflußt. Innerbrutliche Konkurrenz um Nahrungsressourcen und die energetischen Kosten sozialer Konkurrenz können die Brut als Ganzes beeinflussen; häufig aber richten sich die Auswirkungen auf die Geschwister nach deren Stellung in der Bruthierarchie, die ihrerseits von Schlupfposition und Geschlecht abhängt. Steroidhormonen, insbesondere Testosteron, das Aggression steuert, und Kortikosteron, das Streßreaktionen reguliert, wird eine wichtige Rolle bei der physiologischen Steuerung von Geschwisterkonflikten und dem Ausbalancieren von deren Folgen zugeschrieben. Die vorliegende Arbeit beschäftigt sich am Beispiel der Flußseeschwalbe mit Auswirkungen der Geschwisterkonkurrenz auf die Kondition von Küken und analysiert, ob Hormon- und Verhaltensmustern und -mechanismen diesen zugrundeliegen. Die untersuchte Art zeichnet sich durch asynchrones Schlüpfen, leichten Sexualdimorphismus und einen wesentlichen Einfluß des Gewichtswachstums von Küken auf ihre Fitneß aus.

In einem ersten Schritt suchte ich nach geschlechts-, schlupfrang- und brutgrößenbezogenen Mustern bezüglich der Kondition der Küken, ihres Erfolges im Konkurrenzverhalten und ihres Testosteron- und Kortikosteronspiegels. Ich testete die Hypothese, daß Testosteron- und Kortikosteronspiegel von Schlupfreihenfolge und Geschlecht der Küken abhängig sind. Die Kondition von Erstgeschlüpften war besser als die ihrer jüngeren Geschwister und die von männlichen Küken tendenziell besser als die von weiblichen. Der Erfolg im Konkurrenzverhalten und die Hormonspiegel waren jedoch unabhängig von Schlupfposition und Geschlecht. Die Interaktion zwischen dem Geschlecht des Erstgeschlüpften einer Brut und der Schlupfreihenfolge hatte jedoch Einfluß auf den Testosteronspiegel: Dieser war im Vergleich zu den jeweiligen Geschwistern bei männlichen Erstgeschlüpften niedriger und bei weiblichen Erstgeschlüpften höher. Hinsichtlich der Kondition waren entsprechend männliche Erstgeschlüpfte ihren Geschwistern stärker überlegen als weibliche Erstgeschlüpfte.

Im zweiten Schritt veränderte ich experimentell das Ausmaß innerbrutlicher Konkurrenz, indem ich das Erstgeschlüpfte für einen Tag aus Bruten mit zwei Küken entfernte, um die Auswirkungen der Geschwisterkonkurrenz auf Kondition, Fütterungsraten sowie Testosteron-

und Kortikosteronspiegel der einzelnen Küken zu untersuchen. Ich testete die Hypothesen, daß sich die Fütterraten des Zweitgeschlüpften infolge des Verschwindens seines Geschwisters erhöhen, seine Kondition verbessert und sein Testosteron- und Kortikosteronspiegel absinken. Die Kondition verbesserte sich tatsächlich. Der Kortikosteronspiegel kovariierte mit der Kondition und sank tendenziell ab, um nach der Rückkehr des Geschwisters signifikant anzusteigen. Dagegen wurden Fütterungsraten und Testosteronspiegel der Zweitgeschlüpften vom Experiment nicht beeinflußt.

Insgesamt zeigen die Ergebnisse, daß Geschwisterkonkurrenz die Kondition zumindest bei zweitgeschlüpften Küken beeinträchtigt. Des weiteren betonen sie die Rolle der Kortikosteronsekretion als Reaktion auf damit verbundenen energetischen Streß. Testosteron scheint insbesondere für weibliche Erstgeschlüpfte, die physisch ihren Geschwistern kaum überlegen sind, bei der Durchsetzung ihrer Dominanz in der Brut relevant zu sein. Die vermutete generelle Verbindung von Geschwisterkonkurrenz und endogenem Testosteron besteht jedoch bei Flußseeschwalben nicht. Offenbar werden die Konditionsunterschiede zwischen Schlupfrängen und Geschlechtern nicht von endogenen Steroiden bestimmt, kurzfristige Konzentrationserhöhungen oder alternativ maternale Steroide im Dotter könnten diesbezüglich aber Einfluß haben.

6 Acknowledgements

7 References

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Erklärung

Hiermit versichere ich, daß ich die vorliegende Diplomarbeit selbständig verfaßt und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Jena, 8. Oktober 2008

(Luis Schmidt)