J Comp Physiol A (2011) 197:1009–1020
DOI 10.1007/s00359-011-0663-4

ORIGINAL PAPER

Body condition, hormonal correlates and consequences for survival in common tern chicks

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Received: 11 March 2011 / Revised: 12 May 2011 / Accepted: 21 June 2011 / Published online: 7 July 2011

Abstract Although sibling competition in avian species has been extensively studied, the proximate mechanisms remain largely unknown. Recent research proposed that steroid hormones, in particular testosterone (T) and corticosterone (CORT), might play a role either in promoting competitive behavioral displays or in response to chronic nutritional stress accompanied by a sustained competitive situation. Here, we examine body condition, endogenous T levels and fecal glucocorticoid metabolites (FGM) as non-invasive measures of CORT in sibling broods of wild common tern chicks (*Sterna hirundo*) during three post-natal developmental stages. In this species, distinct within-brood size asymmetries are imposed by an asynchronous hatching interval, and sexes show slightly different growth patterns. First-hatched (a-)chicks were in better condition than their later-hatched sibling (b-chick). FGM levels inversely covaried with condition and were elevated at the end of pre-fledging development. T levels of a- and b-chicks changed with age, although the direction of the changes differed, with b-chicks eventually having higher levels than their older siblings. Survival to fledging was not associated with FGM but with T levels, which tended to be higher in surviving chicks. Our results are discussed with regard to how plasticity in steroid hormones could be involved in mediating sibling competition in common terns.

Keywords Testosterone · Corticosterone · Fecal glucocorticoid metabolites · Hatching asynchrony · Sibling competition

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Introduction

The fate of vertebrates growing up with siblings is strongly associated with competition among progeny members for limited critical resources provided by their parents. This constitutes the first instance of social conflict an animal encounters after birth. Due to age and size disparities, competitive skills diverge resulting in unevenly distributed resources among siblings (reviewed in Mock and Parker 1997). In birds, hatching asynchrony induced by incubation onset before clutch completion is one such mechanism that leads to distinct age-related size differences among nestmates (Clark and Wilson 1981). This has been proposed, at least for some species, to mitigate competition by establishing an early developmental hierarchy within a brood (laughing gull, *Leucophaeus atricilla*—Hahn 1981). The older and generally larger chicks usually dominate their younger siblings in competition for parentally provided resources. They further enhance their competitive ability through monopolizing relatively more food and, hence, fledge in better condition (Mock and Parker 1997). Hatching asynchrony can therefore have profound effects on growth and survival of offspring that is often detrimental to later-hatched siblings (Stoleson and Beissinger 1995).

The competitive ability of a chick may not only be size related, but may also differ by sex. In species with distinct

sexual size dimorphism, the larger sex usually attains a better performance in scramble interactions (reviewed in Uller 2006). Male and female chicks can further differ in their susceptibility to environmental conditions, such as food availability (e.g., Oddie 2000), reinforcing or diminishing the negative effect on the less competitive sex (Uller 2006). Given competitive asymmetries established by hatching asynchrony, success in competition is expected to depend on the interplay between hatching order, individual sex and the gender composition of the brood (Bonisoli-Alquati et al. 2011).

Physiological and behavioral adaptations may also vary among progeny of the same brood to regulate these competitive asymmetries. Because of their wide range of central and peripheral effects, steroid hormones have been proposed to serve as regulators of a variety of physiological and behavioral traits that play a role in sibling competition (reviewed in Adkins-Regan 2005). Two steroids that behavioral endocrinologists have mainly focused on are testosterone (T) and corticosterone (CORT). Since maintaining elevated levels of both androgens and glucocorticoids may entail costs in terms of immune suppression (Folstad and Karter 1992; but see Martin et al. 2005, 2009; Roberts et al. 2004) and reduced growth (Fargallo et al. 2007; Hull et al. 2007), modifications of the steroid milieu are typically short term. Nevertheless, even short-term exposure to steroid hormones, especially when occurring early in development, may have organizing effects on the phenotype with fundamental consequences for individual life history trajectories. For example, in the black-legged kittiwake (*Rissa tridactyla*), experimentally increased CORT facilitated begging, and chicks solicited more food (Kitaysky et al. 2001). However, chronic elevation of CORT was not only associated with benefits, but also with costs such as low growth efficiency and compromised cognitive abilities later in life (Kitaysky et al. 2003). Furthermore, Blas et al. (2007) have shown that even under natural conditions, variability in the short-term responses to stress during early development can exert long-term consequences on individual fitness.

Previous research has shown that T can mediate sibling competition; results, however, do not reveal a consistent picture. In some species, increased T levels were associated with higher begging intensity and enhanced competitive ability (Goodship and Buchanan 2006; Quillfeldt et al. 2006), while in others no such relations have been detected (Núñez-de la Mora et al. 1996; Gil et al. 2008). Studies of black-headed gull (*Chroicocephalus ridibundus*) chicks have shown that experimentally elevated T levels increased sibling aggression, but suppressed begging intensity (Ros et al. 2002). Further, T-treated gull chicks showed decreased growth rates (Ros 1999), which, together with the previous finding, suggests a T-regulated trade-off between growth and aggression. Many types of sibling competition might also be stressful and thus co-variation of T and CORT should be considered (Ros 2008). Some studies have found that competition within broods may result in higher levels of circulating CORT (Núñez-de la Mora et al. 1996; Tarlow et al. 2001; Eraud et al. 2008; but see Gil et al. 2008). However, plasma CORT levels in samples taken during disturbances may indicate short-term physiological stress rather than chronic stress induced by a sustained competitive situation. Chronic stress may be better reflected by fecal glucocorticoid metabolites (FGM), which recently have been in the focus as non-invasive tools to measure stress responses in both mammals and birds (Möstl and Palme 2002; Möstl et al. 2005). These FGM levels are supposed to be an integration of the circulating CORT since the last defecation. They do not fluctuate as rapidly as plasma glucocorticoid levels and, due to the delay time of FGM excretion, samples taken within a short time span after capture should not be affected by handling stress (Möstl and Palme 2002). This is underlined by findings in Wilson's storm-petrels (*Oceanites oceanicus*), where chicks in poor body condition had increased levels of FGM (Quillfeldt and Möstl 2003).

We studied the relationships between sibling competition, body condition and steroid hormones in the common tern (*Sterna hirundo*). In this species, chicks hatch asynchronously from a modal clutch size of three eggs at 1- to 2-day intervals, and on average only one chick fledges. The semi-precocial young grow up in a relatively short period (fledge on average at 27 days, Becker and Wink 2003), in which competition for food is intense. The young show indirect sibling competition by monopolizing a single non-shareable prey item through scramble competition. Like in many other species with an asynchronous hatching interval, the oldest sibling is usually larger, receives more food and is more likely to survive than later-hatched chicks (Bollinger et al. 1990; Bollinger 1994), suggesting that hatching order reflects competitive ability in this species.

Although being monomorphic in most adult measurements (Becker and Ludwigs 2004), the pre-fledging development of the young shows some interesting sex-specific characteristics: body mass development is sex dependent with sons reaching both higher peak and fledging mass than daughters, which is more pronounced in siblings that hatch last (Becker and Wink 2003). This suggests that even in a monomorphic species, males might be the more expensive sex to rear, thus being more vulnerable to stressful situations such as food shortages or severe environmental conditions. On the other hand, males appear to be the more competitive sex, being more able to monopolize food and hence counterbalance negative effects of within-brood size disparities. One possible explanation may be plasticity in steroid hormone levels that

may vary with sex and/or hatching order and thus could mediate the dominance structure within a brood.

In the present study, we aimed to disentangle possible correlations of body condition and steroid hormone levels with hatching order and sex in sibling broods of the common tern under natural conditions. The objectives were (1) to draw a comprehensive picture of the physical status during the competitive development of siblings, and (2) to investigate the dynamics of sibling steroid hormone secretion through repeated measures of baseline levels of plasma T and FGM during three successive developmental stages. Based on previous findings mentioned above, we predicted that first hatched chicks would be in better condition than their younger siblings, in which sex-related differences would be more pronounced. If this were the case, steroid hormone levels should also vary with hatching order and sex. As a third (3) objective, we analyzed whether body condition, plasma T and FGM could be used to predict survival until fledging.

Materials and methods

Study site and fieldwork procedures

We conducted our study in a monospecific common tern colony situated in the harbor area of Wilhelmshaven, Lower Saxony, Germany (53°30′40″N, 8°06′20″E), during two consecutive breeding seasons, 2006 and 2007. The colony site consists of six equally sized rectangular islands (10.7 × 4.6 m) arranged in a line and 0.9 m apart. The islands are surrounded by concrete walls (60 cm high) that prevent chick losses by drowning and allow us to follow an individual's fate until fledging. Two permanently fixed hides situated within the colony provide suitable sampling facilities. Being the subject of a long-term study, this colony has been under observation since the early 1980s (for details see Becker and Wendeln 1997; Becker et al. 2001).

We individually marked newly laid clutches with numbered stakes and checked their contents daily during egg-laying (until clutch completion) and hatching periods. Freshly laid eggs were marked with a non-toxic, water-resistant marker to indicate laying order. The fate of each clutch was followed during regular checks every 2 or 3 days. Usually, hatching order corresponds with laying order; however, hatching spread is shorter than laying interval (Nisbet and Cohen 1975) and, occasionally, two freshly hatched chicks were found in a nest on the same day. In such cases, information on hatching order could be derived from the size of the remains of the yolk sac on the chick's belly, which diminishes with age (Wagener 1998). On the day a chick was first found, it was banded and its age was designated as 0 if the chick was freshly hatched, otherwise as day 1. First-, second-, and third-hatched chicks were denoted as a-, b-, and c-chicks, respectively. A chick was considered to have fledged successfully when it was neither observed at the colony site nor found dead thereafter (cf. Braasch et al. 2009). The sex of all focal chicks was determined by standard molecular methods (Kahn et al. 1998), with modifications described in Becker and Wink (2003).

Sampling procedure and chick measurements

Sibling broods were sampled at three post-natal developmental stages (age groups 10, 15 and 20 days post-hatching), which was determined according to the age of the a-chick (±1 day) thereby defining the age of the brood (b-chick was on average 0.7 ± 0.1 days, $N = 69$ younger than a-chick; c-chick was on average 1.7 ± 0.2 days, $N = 13$ younger than a-chick). These age groups were chosen in an attempt to cover the span of post-natal development sufficiently, as well as focus on major stages of behavioral development. To meet concerns about stress responses of nestmates (in sibling broods) after initial nest disturbance, we decided to collect all nestmates simultaneously (Fridinger et al. 2007), in the late afternoon assuming that chicks had been fed regularly during the day. Siblings were kept in a subdivided box to prevent contact with each other and transported immediately to one of the two hides. When removing a chick from the box, we strictly followed a standardized sampling protocol: We measured its wing length, weighed it with a digital balance (accuracy 1 g) and took a small blood sample (200 µl). The exact time that elapsed between removing the chick from the box and finishing blood withdrawal was measured. Meanwhile, chicks defecated into plastic bowls covering the bottom of the box, usually shortly after being captured. Immediately thereafter, fresh droppings were collected by means of tissue papers of uniform weight and cooled in tightly sealed plastic containers for transport to the laboratory, where they were stored at −20°C until analysis.

We obtained blood samples by puncturing the superficial brachial wing vein, alternatively the tibial vein, with a sterile needle (23, 24, 27 g depending upon the size and condition of the chick). Blood was collected in self-sealing, heparinized hematocrit microcapillary tubes (capacity 75 µl), kept in a cool bag and directly (within 1 h) transferred to the laboratory, where they were centrifuged at 10,000 rpm for 8 min (Haematokrit 210, Hettich). Plasma samples were then stored at −20°C until proceeding with laboratory analyses at the University of Veterinary Medicine, Hannover, Germany. All samples were assayed within 6 months of collection.

Due to the fact that siblings were collected simultaneously but processed successively, individual T and FGM

levels may be affected by differences in capture time. Based on the experience of a follow-up study, we assume that the time span between collecting the sibling brood and the beginning of bleeding the first chick is constant (1 min). Thus, we calculated a reliable estimate for "capture time" for each sibling by subtracting the end time of bleeding from the time the first sibling was taken out of the box, and accounted for a possible effect of this measure in the analysis of T and FGM levels.

To calculate a condition index, we followed Stienen and Brenninkmeijer (2002) using the formula

$$\mathrm{CI} = \frac{M - M_{\mathrm{exp}}}{M_{\mathrm{exp}}} 100\%$$

The condition index (CI %) obtained is the age-specific ratio of the deviation of the measured body mass (M) from the expected body mass (M_{exp}), which is the arithmetic mean of all chicks of a specific age. We did not correct by structural size as common tern chicks reach peak mass followed by a mass recession prior to fledging; thus, body condition indices relative to age are more appropriate than mass controlled for measures of structural size in this species.

Testosterone assay

Since preliminary results suggested low detectable T-concentrations, we measured T by an ultra-sensitive radioimmunoassay (RIA). The assay procedure is based on a commercially available RIA-Kit (Testosterone RIA [I-125], DSL-4100, Diagnostic Systems Laboratories, Sinsheim, Germany) with modifications described by Hoppen and Niederstucke (2008). Briefly, plasma (25–50 µl) and controls (50–70 µl) were extracted for 30 min with ethyl acetate (1.5 ml), and the aqueous layer was frozen at −20°C. The solvent was decanted in an assay tube and evaporated in a vacuum concentrator (Hetovac VR1, Heto Lab Equipment, Birkerød, Denmark), and the dried extracts were reconstituted in 50 µl of assay buffer. Standard curves were set up in assay buffer containing 1.5–100 pg testosterone/50 µl. Added tracer and antiserum concentrations were reduced to 125 and 25 µl, respectively, which is 25% of the manufacturer's recommendation. Incubation time was increased to 1 h at 37°C and another 2.5 h at room temperature. Precipitating agent (1 ml) was then added, vortexed and, after another 20 min of incubation, all tubes were centrifuged at 4,000g at 4°C for 20 min. The supernatant was decanted and radioactivity was measured in a gamma counter for 300 s (Clinigamma, Wallac—Perkin Elmer, Rodgau, Germany). Standard curves and sample concentrations were calculated using the counter's RIA-CALC software.

When volumes allowed, samples were assayed in 50 µl duplicate volumes (69.5%), otherwise volumes of single samples ranged from 25 to 50 µl. The samples were run in ten assays with an average detection limit of 0.36 pg/ml. Four solvent blanks, which showed no T, were included in each assay, as well as low and high T control samples. According to the manufacturer, the main cross-reactivities were low (6.6% for 5α-dihydrotestosterone, 2.2% for 5-androstane-3β,17β-diol and <2.0% for all other steroids tested), indicating that the antibody used was highly specific for T. The intra- and inter-assay coefficients of variation were 11.7 and 13.5%, respectively (Hoppen and Niederstucke 2008). Those samples falling below the minimal detection limit were assigned half the detection limit value of the respective assay (cf. Naguib et al. 2004). For statistical purposes, we assigned a T level of 0.05 pg/ml to all samples measured 0.00 pg/ml ($N = 3$).

FGM assay

Fecal samples (including the tissue they were taken up with) were extracted with methanol. All samples were run in two different enzyme immunoassays (EIAs) to determine the fecal levels of glucocorticoid metabolites as a measure of CORT, the principal glucocorticoid in birds. In a validation experiment performed on samples of adult common terns, both EIAs detected an increase of FGM levels after capture in individuals of both sexes ($P < 0.05$). Details of the two different EIAs are given elsewhere (Möstl et al. 2002; Rettenbacher et al. 2004). As both assays were highly positively correlated, we decided to present only results for the assay that measure glucocorticoid metabolites with a 3-11-dione structure (Rettenbacher et al. 2004). We excluded samples weighing <0.1 g from the analysis (cf. Tempel and Gutiérrez 2004) to avoid confounding influences of (1) higher extraction efficiency (Millspaugh and Washburn 2004), (2) relative water loss by evaporation in small samples or (3) small variations in the weight of the tissue papers the samples were taken up with. The intra- and interassay coefficients of variation of a pool sample were 17 and 20%, respectively. Concentrations are given in ng/g feces fresh weight. All assays were run at the University of Veterinary Medicine, Vienna, Austria.

Data analyses

Since brood reduction occurred over the course of our study, brood size did not remain constant. The same was true for brood sex composition; e.g., starting with a mixed sister–sister–brother brood at sampling occasion one, after the loss of the c-chick we ended up with a unisex two-chick brood at brood age 15 and 20 days. Because few c-chicks survived to fledging in both study years, herein we concentrate on a- and b-chicks. However, change in brood size

between the subsets of brood age at day 10, 15 and 20, respectively, and thereby a potential influence of a c-chick was accounted for by including brood size (two- or three-chick brood) as a covariate in the statistical analyses.

We used linear mixed effects models (LME) with the restricted maximum likelihood method (REML) for parameter estimation. This procedure can produce unbiased estimates of variance and covariance parameters and can handle complexly structured models containing both repeated measures and random effects. This method proved suitable for our multi-level clustered data set: repeated measures (blood samples, fecal samples, body condition) are nested within individuals (chick-id), and individuals are nested within broods (brood-id). We considered that chicks from the same brood were not independent samples and, hence, treated brood identity as a random effect with a scaled identity covariance structure using a randomized complete block design, in which variation in each chick variable was controlled for nest. We accounted for repeatedly taken measurements on the same individuals by using brood age as a repeated effect with a first-order autoregressive covariance structure in all mixed model analyses. Additionally, brood age was maintained as a fixed effect to explore interactions. Year, hatching order and sex were also fitted as fixed effects, and brood size was entered as a covariate. To control for inter-assay variation, the assay number was additionally fitted as a random term nested in 'year' for analyses of T and FGM. Furthermore, we controlled for capture time in the analysis of T and FGM. Initially, we fitted all main effects and all possible two-way interactions of fixed factors. We used a stepwise backward model simplification procedure by deleting non-significant terms until only significant terms remained in the final model. If interactions were significant, the main effects were kept in the model regardless of their significance. Sample sizes varied between different subsets as data were unbalanced due to missing values in some categories. Intercepts were included for all random effects and we used type III sums of squares. When the response was dichotomous, we used generalized estimating equations (GEE) with robust standard errors, which are an extension of generalized linear mixed models accounting for repeated measures (brood age) and adjusting for clustered hierarchical data (Krackow and Tkadlec 2001). We applied a logit link transformation and a binomial distribution to analyze the effects of the predictor variables on the dichotomous response variable 'survival until colony departure' (0 = No; 1 = Yes). Year, hatching position and sex were fitted as fixed factors; body condition, FGM and T were included as covariates. We adopted the same model selection procedure as in the LME analyses.

When parametric test assumptions were violated, variables were transformed appropriately. Plasma T and FGM were right skewed (Kolmogorov–Smirnov test, $P < 0.05$) on all three sample occasions, and values were therefore log-transformed. Homoscedasticity assumptions were met for all fixed factors included in the mixed models. Post hoc analyses for multiple comparisons were performed by sequential Bonferroni correction. All analyses were performed by means of SPSS 17.0. If not otherwise stated, we present mean values ± standard errors (SE). All tests were two tailed, and the level of statistical significance was set at $P \leq 0.05$; >0.05 to ≤ 0.1 was defined as marginally significant.

Results

Body condition of a- and b-chicks

Our final model contained the expected significant effect of hatching order (Table 1), indicating that a-chicks were in better condition than their younger siblings (post hoc pairwise comparison by Bonferroni adjusted P values: a–b $P = 0.001$). Although the sexes per se did not differ in terms of body condition, sex appeared in the final model through an interaction with brood age (Table 2). While sisters were in better body condition than brothers at brood age 10 days, this sex-specific difference disappeared at brood age 15 days and was even reversed by small amounts shortly before fledging (brood age 20 days, cf. Table 1). The body condition of a- and b-chicks was unaffected by the presence of a c-chick, as brood size was not significant in our model (Table 2). However, there was high among-brood variation as brood identity (random effect) accounted for a statistically significant amount of variability not explained by the fixed effects model (Table 2).

Relationships between body condition and T and FGM were tested by introducing each as a covariate into the final model. We found that T ($\beta = -3.55 \pm 1.71$) as well as FGM ($\beta = -2.11 \pm 0.82$) were significantly and negatively related to body condition (Table 2).

To disentangle potential effects of the sex of broodmates, we analyzed a reduced dataset of a- and b-chicks with at least two measurements. We substituted the factor sex for the four-level factor 'brood sex composition' (refers only to a-, b-chicks) and tested for possible interactions with hatching order and brood age. Again, hatching order was highly significant ($F_{1,24.8} = 11.73$, $P = 0.002$), and we also found a significant effect of brood age ($F_{2,85.5} = 3.48$, $P = 0.035$). Brood sex composition itself had no effect ($F_{3,22.0} = 0.130$, $P = 0.941$); however, it appeared in the final model through a significant interaction term (brood sex composition × hatching position: $F_{3,25.1} = 3.72$, $P = 0.024$). This suggests that the sex of an older or younger broodmate, respectively, might affect the body

Table 1 Body condition of sibling common tern chicks in relation to hatching order (A = first-hatched; B = second-hatched) and sex

Hatching order	Sex	Brood age (days)		
		10	15	20
A	♂	5.6 ± 2.4 (17)	5.8 ± 2.4 (34)	4.0 ± 3.8 (19)
	♀	8.2 ± 3.0 (12)	2.3 ± 2.5 (27)	1.4 ± 5.9 (8)
B	♂	−5.4 ± 5.0 (10)	0.4 ± 3.8 (21)	−2.2 ± 4.0 (8)
	♀	5.5 ± 3.2 (19)	−2.3 ± 2.5 (37)	−2.5 ± 3.3 (19)
Total	♂	1.5 ± 2.5 (27)	3.8 ± 2.1 (55)	2.2 ± 2.9 (27)
	♀	6.6 ± 2.3 (31)	−0.3 ± 1.8 (64)	−1.4 ± 2.9 (27)

Given are values of mean ± SE (*N*)

See Table 2 for statistics

Table 2 Results of a linear mixed model analysis (LME) of body condition of sibling common tern chicks. Brood identity was treated as a random effect

	Final model				Statistics at exclusion		
	Z	*F*	*df*	*P*	*F*	*df*	*P*
Year					2.066	1,66.4	0.155
Brood age		2.349	2,96.4	0.101			
Hatching order		12.315	1,60.0	**0.001**			
Sex		0.616	1,105.1	0.434			
Brood size					0.072	1,160.0	0.789
Brood identity	3.904			**<0.001**			
Brood age × hatching order					0.145	2,121.7	0.865
Brood age × sex		3.216	2,111.4	**0.044**			
Sex × hatching order					2.161	1,71.6	0.146
Testosterone[a]		4.308	1,151.7	**0.040**			
Fecal glucocorticoid metabol[a]		6.658	1,165.7	**0.011**			

Statistics at exclusion of the non-significant terms are reported; significant terms are denoted in bold

[a] Covariates were introduced solitary into the final model

condition of the respective sibling. The greatest differences were found in brother–brother broods, in which the younger brother was in a considerably worse condition at all brood ages (Table 3). In sister–sister broods, however, the b-sisters were in an equal or in an even better condition than their older sibling (Table 3). Mixed broods were intermediate.

Plasma testosterone levels

In general, T levels of a- and b-chicks were low (6.3 ± 0.3 pg/ml, $N = 184$), ranging from 0.05 to 22.8 pg/ml, and did not show sex-specific differences (individual means: brothers: 7.1 ± 0.5 pg/ml, $N = 59$, sisters: 5.9 ± 0.4 pg/ml, $N = 59$; LME: main factor sex: $F_{1,101.4} = 1.068$, $P = 0.304$). Further, T levels in larger (three-chick) broods were not higher than in smaller (two-chick) broods ($F_{1,109.4} = 0.359$, $P = 0.550$). We also found neither the expected effect of hatching order with b-chicks having higher T levels to compensate for competitive asymmetries ($F_{1,129.2} = 0.017$, $P = 0.896$), nor a significant interaction with sex (details not shown). However, using a reduced sample only including chicks with at least two repeated T measurements, we found a significant interaction of hatching order with brood age (for LME statistics see Fig. 1): While a-chicks first had significantly higher T levels at brood age 10 days than their younger siblings (one-way ANOVA: $F_{1,32} = 4.204$, $P = 0.049$), they showed decreasing concentrations of T afterward (Fig. 1). Conversely, those in b-chicks increased with brood age ending up with higher levels at brood age 20 days than in a-chicks (one-way ANOVA: $F_{1,35} = 5.144$, $P = 0.030$; Fig. 1). Sex composition of a brood, disregarding the sex of an optional c-chick, had no effect ($F_{3,19.7} = 0.870$, $P = 0.473$).

Fecal glucocorticoid metabolites

Similar to our T findings, we could not detect an effect of sex ($F_{1,77.6} = 0.292$, $P = 0.591$) on FGM levels, and no significant interaction with hatching order ($F_{1,53.5} = 2.734$, $P = 0.104$) or brood age ($F_{2,93.8} = 0.415$, $P = 0.662$) could be found either. The a- and b-chicks had similar FGM levels (hatching order: $F_{1,85.8} = 0.008$, $P = 0.929$), which were not affected by the presence of a c-chick (brood size: $F_{1,85.5} = 0.016$, $P = 0.900$). Our final model included brood age as marginally significant effect (Table 5), which is attributed to an increase of individual FGM levels from first to second brood age (pairwise comparison with Bonferroni corrected *P* values: brood age 10 vs. brood age 15 days; $P = 0.049$), and afterward

Table 3 Body condition of sibling broods in relation to brood sex composition and hatching order (A = first hatched; B = second hatched)

Brood sex composition	Hatching order	Brood age (days)					
		10		15		20	
♂♂	A	10.4 ± 4.5	(4)	7.7 ± 3.2	(5)	10.7 ± 3.8	(5)
	B	3.4 ± 4.2		−1.4 ± 2.0		0.8 ± 4.6	
♂♀	A	4.9 ± 3.4	(10)	7.2 ± 3.2	(12)	7.6 ± 4.6	(11)
	B	1.8 ± 3.5		−1.4 ± 2.0		−0.7 ± 5.2	
♀♂	A	12.1 ± 6.3	(2)	6.9 ± 12.4	(2)	13.6 ± 19.5	(2)
	B	−3.3 ± 7.0		−1.0 ± 20.1		−3.9 ± 11.9	
♀♀	A	9.6 ± 4.7	(6)	−4.6 ± 5.0	(7)	−2.6 ± 5.2	(6)
	B	11.3 ± 7.0		0.8 ± 4.5		−0.6 ± 2.0	

Given are values of mean ± SE (*N*)

See text for statistics

Table 4 Plasma testosterone concentrations (pg/ml) of sibling common tern chicks in relation to hatching order (A = first hatched; B = second hatched) and sex

Values of mean ± SE (*N*) are presented for individuals with at least two valid testosterone values

See text and Fig. 1 for statistics

Hatching order	Sex	Brood age (days)		
		10	15	20
A	♂	7.8 ± 1.2 (9)	6.5 ± 1.2 (11)	5.6 ± 0.4 (13)
	♀	6.1 ± 2.0 (6)	6.1 ± 1.1 (5)	5.2 ± 1.6 (5)
	Total	7.1 ± 1.0 (15)	6.4 ± 0.9 (16)	5.5 ± 0.5 (18)
B	♂	2.9 ± 0.3 (6)	5.3 ± 0.9 (6)	7.8 ± 1.0 (6)
	♀	5.2 ± 0.6 (13)	4.9 ± 0.8 (17)	7.2 ± 0.8 (13)
	Total	4.5 ± 0.5 (19)	5.0 ± 0.6 (23)	7.4 ± 0.6 (19)

remaining at this level until the brood age 20 days (Bonferroni corrected *P* values: brood age 15 vs. 20 days; n.s.). Random effects statistics revealed significant between-nest variation (brood identity: Table 5). Similar to the method used for T, we analyzed the influence of brood sex composition using a subset of individuals with at least valid FGM measurements ($N = 46$), and again we found no significant effect ($F_{3,15.1} = 0.439$, $P = 0.728$).

Survival until colony departure

Of 140 sampled individuals, 88 fledged successfully and were not found dead prior to leaving the colony (total survival rate: 63.8%; males: 44/65 = 67.7%, females: 44/72 = 61.1%; a-chicks: 48/70 = 68.6%, b-chicks: 40/68 = 58.8%). We intended to find out whether body condition, T and FGM could be used as predictors of survival and controlled for hatching order and sex in our initial model. Due to missing values in some categories, we analyzed a reduced sample of 90 individuals from 55 broods. Survival until colony departure was significantly and positively associated with body condition (Wald $\chi^2 = 7.109$, $df = 1$, $P = 0.008$; Fig. 2). Interestingly, we also found a marginally significant influence of T on survival (Wald $\chi^2 = 3.717$, $df = 1$, $P = 0.054$; Fig. 2), indicating that higher T levels were associated with higher survival probability. Inspecting Fig. 2, the surviving young tended to have higher FGM levels than non-survivors at all brood ages sampled. However, this covariate did not reach statistical significance in our model (Wald $\chi^2 = 0.116$, $df = 1$, $P = 0.733$) and thus was not a viable predictor of survival. We found neither an influence of hatching order (Wald $\chi^2 = 2.817$, $df = 1$, $P = 0.093$) nor a differential survival probability in brothers and sisters (sex: Wald $\chi^2 = 1.826$, $df = 1$, $P = 0.177$). Neither sex nor hatching order interacted with T and FGM (details not shown).

Discussion

Physical disparities among siblings

One of the most prominent factors that generate initial within-brood size asymmetries is position in the hatching sequence. The disadvantage of hatching asynchrony imposed by partial incubation after the first egg has been laid is usually pronounced in last-hatched chicks in terns and gulls and usually leads to their reduced survival (Langham 1972; Bollinger et al. 1990), with starvation being the principal cause for mortality (Mlody and Becker 1991). In the present study, we used an age-corrected condition index to assess physical disparities due to

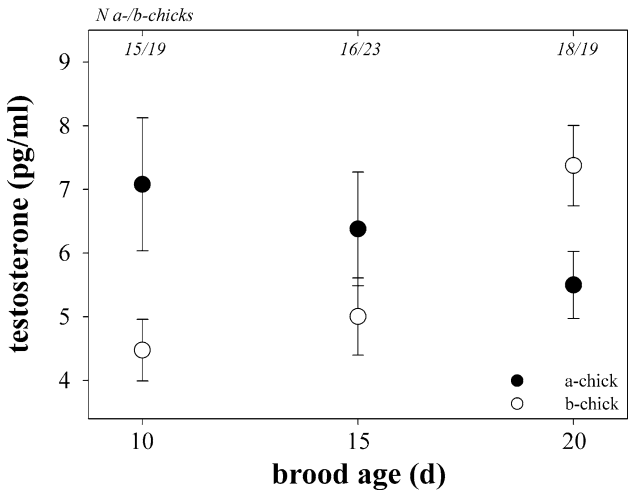

Fig. 1 Testosterone levels (mean ± SE) of common tern a- and b-chicks through pre-fledging development at brood ages 10, 15 and 20 days. *Plotted* are only chicks with at least two valid measurements per individual (cf. Table 4). Linear mixed model (LME): final model included *'brood age'* ($F_{2,74.3} = 0.810$, $P = 0.449$), *'hatching order'* ($F_{1,59.6} = 5.370$, $P = 0.024$), their interaction *'brood age × hatching order'* ($F_{2,69.1} = 4.117, P = 0.020$) and *'capture time'* ($F_{2,84.2} = 5.572$, $P = 0.021$); *'brood identity'* ($Z = 0.085$, $P = 0.933$) and *'assay'* ($Z = 1.301$, $P = 0.193$) were included as random effects

differential mass increment among members of the same brood. Although focused on a- and b-chicks, we found the expected strong influence of hatching order on body condition. As predicted, we also found sex-specific differences in the development of body condition until fledging. While male b-chicks slightly improved their body condition over time, the condition of sisters decreased. This finding corresponds to the results of Becker and Wink (2003) who showed that body mass development of the two sexes increasingly diverged at the end of the pre-fledging period resulting in both higher peak mass and pre-fledging mass of male common tern chicks. During the second half of the pre-fledging period, food demands of offspring increased considerably (Klaassen et al. 1992); thus, competition is assumed to be highest among broodmates at this time and may result in brood reduction if there are food shortages. When a brother has the additional advantage of hatching first, his increased competitive ability coupled with higher food requirements may influence the body condition of his subordinate sibling. Our results partially support this view as we found the largest differences in body condition between chicks of brother–brother broods, although the overall sex composition of the brood had no significant influence on individual body condition (cf. Bonisoli-Alquati et al. 2011). However, the combination of sexes within a brood was interrelated with hatching order and might determine the body condition of the individual offspring. In contrast to the distinct differences in brother–brother broods, unisex sister broods hardly differed.

Do hormone levels mediate intra-brood disparities?

In species, such as the common tern that have semi-precocial young that become active within a few days of hatching, having well-developed locomotor capability is critical. In the context of sibling competition, it could enable superior chicks to successfully outrun their younger siblings. Smith et al. (2005) have shown that "reaching the parent first" determines the outcome of scramble competition in the vast majority of feeding events in sibling common tern broods. We predicted that T might play a role in mediating this kind of competitive interactions and expected to find higher levels in b-chicks. Indeed, there was an interesting developmental pattern of individual T levels: First-hatched siblings showed decreasing levels of T with age, whereas those in b-chicks increased and surpassed their older sibling prior to fledging. This could be associated with a higher degree of competitiveness necessary to catch up with the older and physically larger sibling. However, the absolute differences of T levels were

Table 5 Fecal glucocorticoid metabolites (ng/g) of sibling common tern chicks in relation to hatching order (A = first hatched; B = second hatched) and sex

Hatching order	Sex	Brood age (days)		
		10	15	20
A	♂	163.5 ± 44.6 (12)	290.4 ± 119.8 (26)	300.9 ± 113.3 (16)
	♀	80.8 ± 17.9 (9)	290.9 ± 75.4 (21)	127.0 ± 39.2 (7)
B	♂	104.4 ± 35.3 (7)	333.9 ± 86.0 (16)	255.0 ± 88.4 (7)
	♀	170.7 ± 54.2 (16)	300.3 ± 74.7 (28)	215.4 ± 57.7 (17)
Total	♂	141.7 ± 31.1 (19)	306.9 ± 80.4 (42)	287.0 ± 82.2 (23)
	♀	138.3 ± 35.9 (25)	296.2 ± 53.0 (49)	189.6 ± 42.8 (24)

Given are values of mean ± SE (*N*)

Linear mixed model (LME): final model included *'brood age'* ($F_{2,102.1} = 2.996$, $P = 0.054$), *'capture time'* ($F_{1,121.3} = 0.455$, $P = 0.501$); *'brood identity'* ($Z = 2.177$, $P = 0.029$) and *'assay'* ($Z = 1.194$, $P = 0.233$) were included as random effects

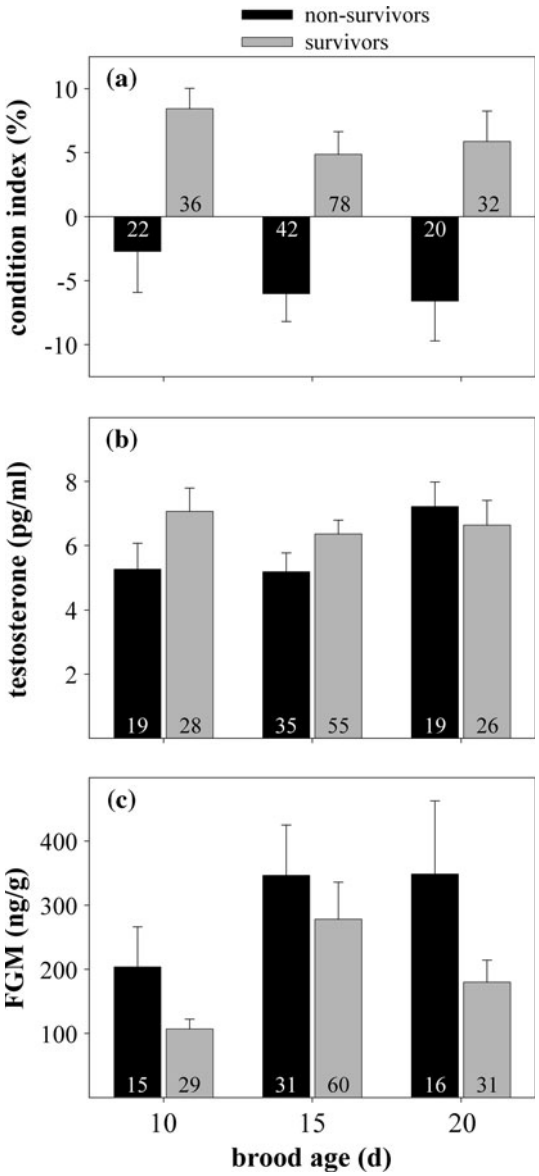

Fig. 2 Mean (±SE) values of **a** body condition, **b** plasma testosterone (T) and **c** fecal glucocorticoid metabolites (FGM) of common tern chicks relative to their survival status until fledging. Sample size is denoted at the *bottom of each bar*. The generalized estimating equations (GEE) revealed a significant effect of body condition (Wald $\chi^2 = 7.109$, $df = 1$, $P = 0.008$) and a marginally significant influence of T on survival (Wald $\chi^2 = 3.717$, $df = 1$, $P = 0.054$). FGM levels were not associated with survival probability (Wald $\chi^2 = 0.116$, $df = 1$, $P = 0.733$)

quite low, thereby challenging their interpretative value. To date, most of the few studies that have measured plasma T under natural conditions in immature birds report low T levels (Verboven et al. 2003; Naguib et al. 2004; Gil et al. 2008), which is in line with the remarkably low baseline T levels we measured in common tern chicks. Moreover, T plasma levels may increase only during periods of high competition as it has been proposed by the Challenge Hypothesis (Wingfield et al. 1990), the validity of which has also been supported by studies in immature birds (Ros et al. 2002; Ferree et al. 2004).

We detected no sex differences in circulating levels of T, which is in line with results from most other studies conducted at nestling stage (reviewed in Fargallo et al. 2007). Also, in other seabird species, such as gulls (e.g., Groothuis and Meeuwissen 1992; Ros 1999; Verboven et al. 2003; Groothuis and Ros 2005) and siblicidal blue-footed boobies (*Sula nebouxii*, Núñez-de la Mora et al. 1996), T levels do not differ between the sexes. These data together with our results do not support the idea that sexual differences in condition early in life can be determined by sexual differences in circulating T levels. Furthermore, the brood sex composition did not affect endogenous T. Thus, male-dominated sibships, assumed to have elevated competition, could not be identified to have generally higher T levels or larger differences in baseline T levels than female-dominated broods.

Many types of sibling competition might be simultaneously competitive and stressful and this might stimulate CORT production (Ros 2008). As FGM are expected to integrate basal, stress-induced and chronic levels of circulating CORT, we predicted finding a positive association between this measure and the strength of sibling competition (Dehnhard et al. 2003). However, FGM levels in common tern chicks were neither affected by hatching order nor brood size (cf. Gil et al. 2008). We found a positive association between FGM and brood age, which corresponds to the results found by Lobato et al. (2008) conducted on both nestling pied flycatchers (*Ficedula hypoleuca*) and nestling blue tits (*Parus caeruleus*). This age-related increase may be due to an age-related increase in food demands resulting in high competition at the end of the pre-fledging period (Klaassen et al. 1992). An alternative however not mutually exclusive explanation for this finding derives from the Developmental Hypothesis (e.g., Schwabl 1999; Kitaysky et al. 2003; Blas et al. 2006), which predicts an association between nestling age and the ability to respond to a given stressor. It is expected that the adrenocortical response to stress increases during postnatal development, eventually reaching adult-like responses near fledging (Blas et al. 2006). For example, Love et al. (2003) have shown in nestling American kestrels (*Falco sparverius*) that stress-induced CORT levels of chicks close to fledging were significantly higher than those of 1-year-old adults. Studies performed on nestling seabirds have shown that CORT elevation facilitates begging and aggression, two forms of adaptive responses useful in dealing with a perturbation such as food stress early in life (Núñez-de la Mora et al. 1996; Kitaysky et al. 2001, 2003). Given that food deprivation is a common scenario in sibling tern broods, our finding that FGM are

negatively related to body condition constitutes conclusive information. In line with our study, age-related changes in plasma CORT in nestling American kestrels were negatively correlated with body condition (Sockman and Schwabl 2001).

Survival until colony departure

As expected, survival until colony departure was predicted by individual body condition, which underpins the close connectivity of body mass increment and survival prospects in terns (e.g., Langham 1972; Stienen and Brenninkmeijer 2002; Braasch et al. 2009). Despite some evidence from the literature, we did not find an effect of hatching order (Bollinger 1994) or sex (González-Solís et al. 2005) on survival, possibly due to parental quality.

Whilst survival until colony departure was not related to FGM levels, we found that plasma T levels, albeit only marginally significant, seemed to have an influence on survival to departure from the colony. At first glance, this indicates a benefit for increased T production in common tern chicks. A few observational and manipulative studies investigating endogenously produced T in nestling birds have identified correlations between T and behavioral traits, such as begging behavior (Quillfeldt et al. 2006; Goodship and Buchanan 2007) and aggression (Groothuis and Ros 2005) that could be potentially beneficial for survival in common terns as well. Furthermore, Goodship and Buchanan (2006) have shown in pied flycatcher nestlings that broods with higher levels of T showed increased fledging success. On the other hand, in chicks of the black-headed gull, T was found to facilitate aggression necessary for territorial defense, but also suppressed growth and begging (Groothuis and Ros 2005), suggesting an inherent trade-off between benefits and costs of endogenous T. Moreover, T is also thought to mediate a number of potential physiological costs such as increased basal metabolic rate (Buchanan et al. 2001) and immunosuppression, as postulated by the immunocompetence handicap hypothesis (Folstad and Karter 1992), which has been suggested to apply for nestlings as well (e.g., Fargallo et al. 2007; Gil et al. 2008). Studies combining measures of immunity and androgens during the nestling stage and relate them to survival prospects are rare. Recently, López-Rull et al. (2011) found in nestlings of the spotless starling (*Sturnus unicolor*) that cellular-mediated immune response (CMI) was a good predictor of post-fledging survival; however, nestling T levels, although negatively related with CMI, were not associated with survival prospects.

More likely, however not mutually exclusive, is a connection with body condition, which was found to significantly covary with T in our study. In addition to immunological costs, T-dependent competitive behavior, such as aggressive sibling interactions, may be costly in terms of energy, which reinforces the importance of body condition for endogenous T production, as only individuals in good body condition could face such energetic costs (Pérez-Rodríguez et al. 2006).

Conclusions and prospects

In the present study, we found rank-related and sex-specific differences in body condition with consequences on pre-fledging survival. Our results show some interesting hormonal patterns that we think could potentially be involved in mediating sibling competition in this species. Drawing causative conclusions clearly needs further investigation based on experimental approaches, particularly with regard to the low T levels found in this study under natural conditions. One possibility would require appropriately timed samples taken shortly after or during phases of sibling competition, e.g., when parents feed offspring. Such studies should also consider recording behavioral aspects of both offspring and parents with regard to the close interrelation of sibling competition and parent–offspring conflict.

Acknowledgments This study would not have been possible without the help of numerous people during field work, especially C. Bauch, J. Riechert, J. Sprenger, L. Schmidt and G. Wagenknecht. We are obliged to R. Nagel and J. Trauernicht for technical assistance and H. Niederstucke for help with testosterone assays. We thank L. Szostek for checking the English. F. Bairlein and two anonymous referees provided useful comments on an earlier draft of the manuscript. Field work was carried out under license of the Conservation Board Wilhelmshaven "*Untere Naturschutzbehörde*, *Wilhelmshaven*" and LAVES, Oldenburg, and complied with the current laws of the country. The study was financially supported by the German Research Association *Deutsche Forschungsgemeinschaft* (BE 916/8).

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