acta ethol (2003) 5:115–122
DOI 10.1007/s10211-003-0074-9

ORIGINAL ARTICLE

Petra Quillfeldt · Erich Möstl

Resource allocation in Wilson's storm-petrels *Oceanites oceanicus* determined by measurement of glucocorticoid excretion

Received: 28 June 2002 / Revised: 17 December 2002 / Accepted: 31 January 2003 / Published online: 27 March 2003

Abstract When resources are limited, life-history theory predicts that long-lived animals should allocate available resources to body maintenance rather than to reproduction in order to maximise their lifetime reproductive success. In the present study, we estimated physiological stress in a small procellariiform seabird, the Wilson's storm-petrel *Oceanites oceanicus*, as a means of understanding how limited resources are partitioned between provisioning parents and their chicks. We analysed adrenocortical activity of Wilson's storm-petrels during the breeding season by measuring glucocorticoid (GC) excretion, using an enzyme immunoassay measuring tetrahydrocorticosterone concentrations in extracts of faeces and urine of chicks and adults. Faecal GC measures were negatively correlated with chick body condition, suggesting that measures of tetrahydrocorticosterone in faeces and urine can be used to assess adrenal activity characteristic for physiological stress in Wilson's storm-petrels. In the breeding season of 1999, the colony was subject to low food availability, and the faecal and urine GC levels of chicks were elevated during these months of chronic starvation. In contrast, adults did not show elevated GC levels. The data thus suggest that Wilson's storm-petrels respond to unfavourable conditions by maintaining their own body condition and reducing provisioning of food to their chicks.

Keywords Enzyme immunoassay · Faeces · Food availability · Procellariiform seabirds · Stress

Communicated by R.F. Oliviera

P. Quillfeldt (✉)
Institut für Ökologie,
Friedrich-Schiller-Universität Jena,
Dornburgerstrasse 159, 07743 Jena, Germany
e-mail: petra.quillfeldt@excite.com
Fax: +44-2920-874305

E. Möstl
Institut für Biochemie,
Veterinärmedizinische Universität,
Josef Baumanngasse 1, 1210 Vienna, Austria

Present address:
P. Quillfeldt, School of Bioscienes,
Cardiff University,
Museum Avenue, Cardiff, CF10 3TL, U.K.

Introduction

The study of physiological stress and its context in free-living birds provides a means for understanding the challenges found in the natural habitat. Food-limiting conditions are regularly experienced stressful events for birds living in the wild, and they demand changes in energy use in order to promote self maintenance, and may require the inhibition of energy-demanding physiological processes such as reproduction. Glucocorticoids (GC) act as physiological mediators in these situations when environmental conditions require modification of behaviours and metabolism, and titres of GC are therefore the most common method of assessing individual responses to stress.

Long-lived birds often accumulate fat and protein as energy reserves for self-maintenance during reproduction (Drent and Daan 1980). As fat reserves are depleted, parents rely more on catabolism of muscle protein (Cherel et al. 1988; Cherel and Groscolas 1999), which is stimulated by the secretion of corticosterone, the main GC in birds. In adults, increased plasma levels of corticosterone facilitate foraging behaviour, and irruptive migration, and have been found to lead to reduced nest attendance (Astheimer et al.1992; Wingfield et al. 1997; Kitayski et al. 2001). These behavioural responses can improve the survival of adult birds during food shortages (Astheimer et al. 1992).

Wilson's storm-petrels *Oceanites oceanicus* are small seabirds with a long lifespan (mean life expectancy 10.4 years, Beck and Brown 1972). They are the smallest endotherm animals breeding in the extreme climatic conditions of the Antarctic, an environment with changeable and patchy food supply. Starvation due to insuffi-

cient food abundance was the main cause for chick mortality in Wilson's storm-petrels in three out of four breeding seasons (Quillfeldt 2001). The overall food availability to Wilson's storm-petrels differed between the years (Quillfeldt 2001). Mean krill biomass density indices (g/1,000 m^3 wet weight) from scientific net sampling surveys in the Elephant Island area during summer were low at 5.61 in 1999 and intermediate at 15.61 in 2000 (Siegel 2003). This is in line with results obtained from feeding rates of Wilson's storm-petrels (Quillfeldt 2001). The very poor food supply in 1999 resulted in chick mortality of 49% by starvation as compared with only 6% in 2000.

According to life history theory, in the year of food shortage parent Wilson's storm-petrels would be expected to allocate the limited resources to body maintenance, and thus to future offspring, rather than to the growth of the present chick.

Former studies have shown that adult body mass cannot be used for studying resource allocation in Wilson's storm-petrels. Adults carry large food loads (up to 13 g, at a body weight of 38 g, Quillfeldt and Peter 2000). Depending on the amount of food carried, the body mass may, therefore, be overestimated by up to 30%, such that weights of provisioning adults are an unreliable measure of body condition in the field. In the present analysis, we therefore suggest the estimatation of excreted GC as a measure of physiological stress in Wilson's storm-petrels.

The aim of this study is to determine whether faecal and urine GC levels of Wilson's storm-petrels reflect physiological stress caused by starvation in the low-food year 1999, and to analyse how limited resources are allocated to either adult maintenance or provisioning of their chicks.

Methods

Subjects

The life cycle and basic biology of Wilson's storm-petrels have been described by Roberts (1940) and Beck and Brown (1972). The birds spend the austral winter and spring (May–October) at sea, many crossing the equator and reaching the northern hemisphere. The species breeds in natural cavities, mainly in rock slopes. At the South Shetlands, Wilson's storm-petrels start visiting the colonies in November (Quillfeldt, unpublished data), when the nest chambers are usually still filled with ice. The ice beneath the rocks which form the breeding cavities melts during November and early December, when the temperatures climb above freezing point most days. The first eggs are laid in mid-December, and in January most of the breeding birds are incubating. Wilson's storm-petrels lay single-egg clutches, and have a monogamous mating system (Quillfeldt et al. 2001) with intensive biparental care during incubation and chick-feeding. Most chicks hatch in the first half of February. Chicks are left unattended in the burrow during the day when only few days old, and they are fed by their parents during nocturnal visits until fledging, without a desertion period. Wilson's storm-petrels prey mainly on Antarctic krill *Euphausia superba* during the breeding season, although some fish and amphipods are also taken (Quillfeldt, unpublished data). Fledging starts in the colony in the second half of March. Former studies suggested that the relative food availability at any given time is reflected in the feeding frequencies of the storm-petrels (number of feeds per night per nest, Quillfeldt and Peter 2000). The feeding rates are used here as a realtime measure of food availability within the foraging range of the petrels.

Study site and sample collection

The field work was carried out in the Tres Hermanos (Three Brothers Hill) colony on King George Island (62°14'S, 58°40'W), South Shetland Islands in the maritime Antarctic from December 1998 to April 2000. About 2,000 pairs breed in this colony (Hahn et al. 1998). Nests in the study colony are marked for ongoing studies of breeding success (Quillfeldt 2001). Faeces samples were obtained from breeding adults and chicks on the nest (Quillfeldt and Peter 2000). At the first control of each nest, incubating adults were captured, measured and ringed. Wilson's storm-petrels have the tendency to desert in response to disturbances during the incubation period (Beck and Brown 1972). To reduce observer disturbance, the hatching dates of eggs were estimated from egg density (Furness and Furness 1981; Quillfeldt and Peter 2000), and nests were not revisited until about 3 days after the estimated hatching date. After hatching, the chicks were taken daily from the nests for measuring and weighed with a digital 100-g balance to the nearest 0.1 g. Chick body condition was calculated as percentage of the population mean of the mass of chicks of similar age: $BC=(m/m_{mean})\times100\%$. The analysis included chicks of 9–61 days of age with a range of body conditions between 65% and 192% of the mean body mass. Feeding frequencies were estimated from daily weighing as described by Quillfeldt and Peter (2000), by estimating meal sizes from daily mass differences, corrected for metabolic mass loss. Meal sizes were defined as representing one feeding for meal sizes up to 13 g and two feedings for meal sizes larger than 13 g (Obst and Nagy 1993; Quillfeldt and Peter 2000). The feeding frequencies have been reported in Quillfeldt (2001).

Faecal samples were collected opportunistically when chicks or adults defecated during capture. Upon capture, the birds were immediately placed over a plastic funnel fitted with a 2-ml Eppendorf tube. Between uses, the funnel was rinsed with water. Urine and faeces were separated by allowing the faeces to sediment and decanting the urine as completely as possible. All faecal samples were taken during the day, between 1100 and 1900 hours. Totals of 36 samples in 1999 and 77 samples in 2000 were obtained from chicks which defaecated instantly upon capture, and totals of 65 samples in 1999 and 24 samples in 2000 were obtained from adults which defaecated immediately. We included only a single sample of each adult, and most chicks were only sampled once, but up to three samples of individual chicks were included if they were collected more than 4 days apart. In this case we treated the samples as statistically independent following the methodology of Furness (1983).

Of the sampled adults from 1999, we determined the breeding stage (i.e. days before or after hatching of the egg) of 24 successfully incubating adults and 23 chick-feeding adults (n=47). The remaining 18 samples came from adults with failed eggs, such that the time to hatching could not be determined.

In order to test for the effect of handling, in addition to the samples immediately after capture some samples were collected at different intervals after capture. As a further reference, additionally to the samples from handled birds, a total of 49 samples were collected from the nest floor of some accessible nests in the season of 2000 (Table 1). A total of 13 samples was collected from chicks which defaecated after weighing and measuring, at about 5 min from capture. In order to obtain samples at a longer time after capture, some chicks were recaptured again 30 or 60 min after initial capture. Only few chicks defaecated during these attempts, and thus only two samples at 30 min and four samples at 60 min were obtained. The urine fraction of one sample at 30 min was lost as the chick defecated into the fresh snow, where faeces but not urine could be collected (Table 1).

Table 1 GC levels in faeces (ng/g) and urine (ng/ml) of Wilson's storm-petrels *Oceanites oceanites* collected at different intervals after handling

	n	Faeces					Urine				
		Median	Min	Max	25%	75%	Median	Min	Max	25%	75%
Season 1999											
Chicks 0 min	36	169.0	68.4	3454.8	116.0	330.9	41.3	16.9	4345.0	22.6	62.2
Adults	65	52.2	0	151.5	35.0	71.8	19.5	4.6	85.3	14.6	29.6
Season 2000											
Nest floor	49	84.4	0.0	874.2	44.3	174.1	–	–	–	–	–
Chicks 0 min	77	127.7	32.0	444.5	91.2	164.5	32.1	5.6	150.3	22.3	41.0
Chicks 5 min	13	148.3	73.4	569.5	102.9	318.3	54.4	12.4	167.5	30.1	71.8
Chicks 30 min	2 (1)	1,669.7	946.9	2392.4	–	–	105.2	–	–	–	–
Chicks 60 min	4	164.6	86.0	386.4	109.3	291.5	44.7	21.6	81.8	23.5	72.9
Adults	24	40.0	17.2	138.4	28.8	61.5	18.1	7.9	58.7	13.2	30.9

Processing of faecal samples

Faeces were stored and transported at −20°C, and stored at −70°C after transfer to the laboratory.

Extraction

Between 0.04 g and 0.1 g of fresh faeces or 50 µl urine were extracted with 4 ml methanol and 0.5 ml double-distilled water. After vortexing for 30 min, the sample was centrifuged (2,500 *g*, 10 min) and the supernatant transferred to a new tube. This methanol extract was used in the enzyme immunoassays (EIA) described below.

Enzyme immunoassays

Corticosterone was not detected in faeces of Wilson's storm-petrels. To measure the corticosterone production an assay for tetrahydrocorticosterone (5β-pregnane-3α,11β,21-triol-20-one, a principal metabolite of corticosterone) was developed. Tetrahydrocorticosterone (Steraloids, Wilton, N.H., USA) was converted in the carboxymethyloxime (CMO) derivative and linked to bovine serum albumin (BSA) as described by Kohen et al. (1975). The antibody was raised in a rabbit. As label, the 21-hemisuccinate derivative of tetrahydrocorticosterone (TH"B") was formed by heating 10 mg TH"B" with 5 mg succinic anhydride in 5 ml benzene for 5 h. Afterwards, 2 ml of saturated sodium hydrogen carbonate were added. After vortexing (2 min) and centrifugation (1,500 *g*, 10 min), the aqueous phase was extracted using a Sep-Pak C_{18} cartridge (Fa. Waters, Milford, Mass., USA). After priming this mini-column (according to the manufacturer's instructions), the diluted sample was passed through the cartridge, which was washed with 5 ml water and the derivative eluted using 4 ml methanol. The methanolic phase was evaporated, dissolved in 300 µl water/methanol (50/80 v/v) and the extract was purified by HPLC (Novapac C_{18} column 0.39×15 cm, Fa. Waters; solvent: water/methanol, linear gradient from 50% methanol to 80% within 30 min; flow 1 ml per min; three fractions per min were collected). The derivative eluted from the column in the fractions 46–48. The biotinylated label was synthesised as described by Möstl et al. (2002). To determine the working dilutions of the antibody and label, a checkerboard titration (Meyer et al. 1990) was performed.

The working dilution of the antibody of 1:40,000 and of 1:8,000 for the label showed best results and were used for the EIA. The standard curve ranged from 0.82 to 200 pg/well; the 50% intercept was about 18 pg. The cross-reactions were as follows: 5β-androstane-3a,11β-diol-17-one, 43.9%; tetrahydrocortisol, 25.7%; cortol, 2.7%. All other steroids tested like cortisol, corticosterone, 5α-pregnane-3β-11β,17a,21-tetrol-20-one, 5β-pregnane-11β,17a,21-triol-3,20-dione, 5β-pregnane-3a,17a-diol-11,20-dione, 5β-androstane-3β,11β-diol-17-one, 5β-androstane-3a-ol-11,17-dione, progesterone or androstenedione showed less than 1% cross-reactions (limited amounts of antibodies and label are available free of charge from E. Möstl).

Determination of immunoreactive substances in faeces

After extraction of the samples, 1 ml of the methanolic phase was diluted in 10 ml of water, and extracted using Sep-Pak C_{18} cartridges as described before. The dried extract was dissolved in 20% methanol and separated by HPLC (Novapac C_{18} column 0.39×15 cm; solvent: water/methanol; 0–5 min: 20% methanol; 5–35 min linear gradient from 20% methanol to 100%; flow 1 ml per min; three fractions per min were collected). All fractions were divided in two halves, evaporated, and the immunoreactive substances were measured before and after enzymatic hydrolysis (Kotrschal et al. 1998).

Observer influence

Chicks

Faeces and urine of Wilson's storm-petrels were collected without any detectable influence on the birds. Chicks were weighed during the day, when nests were not attended by an adult, such that no desertion occurred due to chick weighing. The chicks were caught by hand and got accustomed to regular handling, such that regurgitation of small quantities of stomach oil ceased after the first days of the study. All handled chicks fledged normally.

Adults

In 1999, 24 of 42 eggs of handled birds hatched (57%), which was similar to the overall hatching success of 56% (Quillfeldt 2001). Therefore, no adverse influences on the breeding output due to handling of adults was detected.

Results

The HPLC immunogramms showed no differences between the fractions before and after hydrolysis. Therefore, samples were subsequently analysed without this enzymatic step. The dominant peak eluted from the column in fractions 72–74 (Fig. 1).

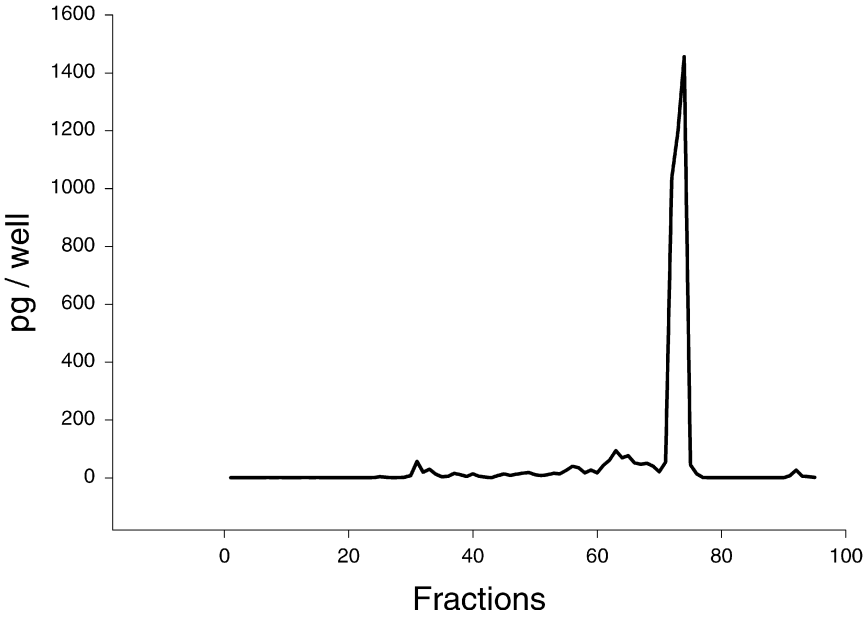

Fig. 1 Elution pattern of GC as measured by EIA for tetrahydrocorticosterone in the methanolic faecal extracts of Wilson's storm-petrels *Oceanites oceanicus*

Table 1 summarises the GC levels measured in faeces and urine of the different sample categories of Wilson's storm-petrels. Because GC data were not normally distributed (Fig. 2), non-parametric tests were applied for all following analyses. We observed a strong correlation between the GC values measured in the faeces and urine fractions of samples (Spearman correlation for chicks: r=0.530, P<0.001, n=113; for adults: r=0.709, P<0.001, n=89).

Influence of handling

Samples collected from the nest floor had significantly lower GC than samples collected immediately after capture in the same season (Table 1, Mann-Whitney test: $U_{49,77}$=1,331.0, P=0.005). In March 2000, seven nests were sampled simultaneously for faeces from the nest floor and faeces from chicks during capture. Samples collected from the nest floor consistently had less GC than samples from handled chicks (paired Wilcoxon test: z=28.0, P=0.016).

The time elapsed after capture influenced the GC titres of faeces (Table 1, Fig. 2, Kruskal-Wallis One-way Analysis of Variance for Ranks of 0, 5, 30 and 60 min: χ^2=8.78, df=3, P=0.033).

Because of the lack of samples from the nest floor from adults, as well as from the breeding season 1999, in the following analyses, we decided to use only samples of handled birds which defecated immediately.

Influence of sampling time, age and chick body condition

GC levels were independent of the age of the chicks (Spearman correlation for faeces: r_s=0.042, P=0.656, N=113; for urine: r=0.080, P=0.399, n=113). The GC values measured in faeces and urine were furthermore

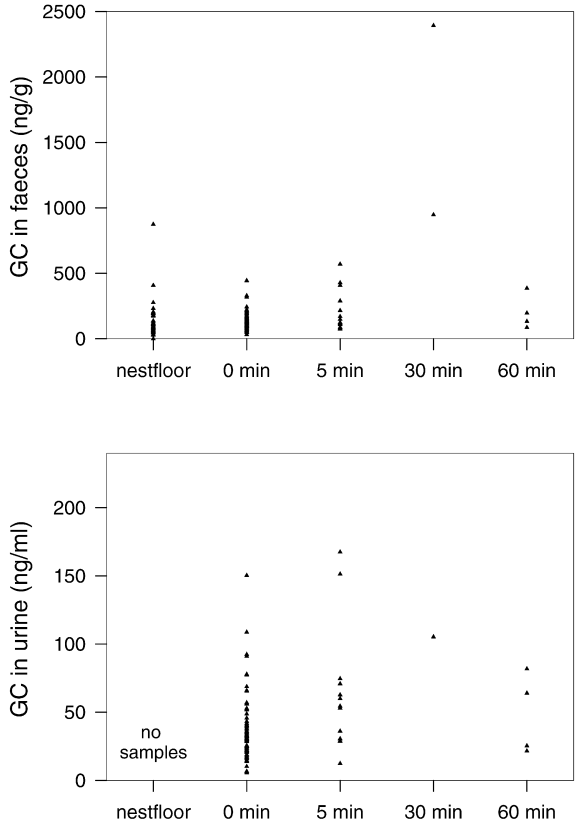

Fig. 2 GC titres of faeces and urine of chicks of Wilson's storm-petrels in 2000. In addition to samples immediately after capture (0 min) some samples were collected at intervals of 5, 30 or 60 min after capture. Additional to the samples from handled birds, 49 samples were collected from the nest floor of some accessible nests. The urine fraction of one sample at 30 min was lost as the chick defaecated into the fresh snow, where faeces but not urine could be collected (n=2 for faeces, n=1 for urine)

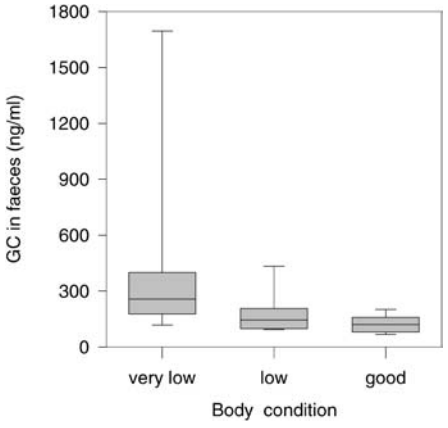

Fig. 3 Median GC levels of chicks of Wilson's storm-petrels measured in faeces of very low body condition (less than 85% of mean body mass for chicks of the same age, n=15), low body condition (between 85% and 100% of mean body mass for chicks of the same age, n=26) and good body condition (more than 100% of mean body mass for chicks of the same age, n=72)

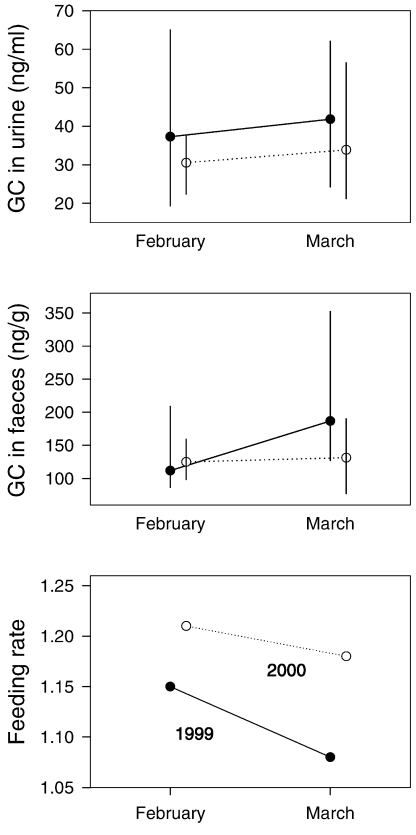

Fig. 4 Median GC levels (with 25% and 75% percentiles) of chicks of Wilson's storm-petrels measured in faeces and urine in 2 years of differing feeding rate (feedings/night)

independent of the time of the day (data of 2000: between 1100 and 1900 hours; Spearman correlation for faeces: r_s=0.137, P=0.171, n=77; for urine: r_s=0.015, P=0.896, n=77).

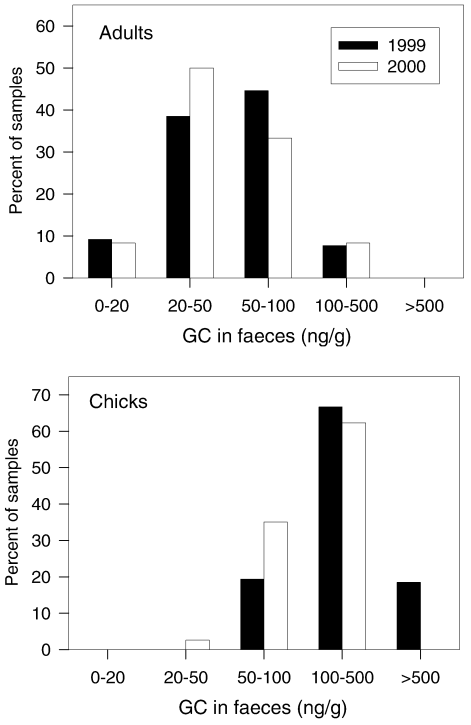

Fig. 5 GC levels measured in faeces of adults (*above*) and chicks (*below*) of Wilson's storm-petrels in 2 years of differing food availability. *Black bars* show GC levels in the breeding season of 1999, a year of low food availability, while white bars show GC levels in the season of 2000

Excreted GC was negatively correlated with chick body condition (Fig. 3, Spearman correlation for faeces: r_s=–0.404, P<0.001, n=113; for urine: r_s=–0.327, P<0.001, n=113), and reflected food availability as measured in chick feeding rates (Fig. 4).

We therefore suggest that measures of tetrahydrocorticosterone in faeces and urine collected from birds immediately after capture can be used to assess adrenal activity characteristic for physiological stress in Wilson's storm-petrels.

Influence of food availability on chicks and adults

The breeding season of 1999 was a year of low food availability, while in the season of 2000 food availability and consequently feeding rates were higher (Fig. 4; Quillfeldt 2001). In both years, chicks had more than threefold GC titres compared to adults (Fig. 5, Mann-Whitney test for 1999: $U_{36,65}$=95.0, P<0.001; for 2000: $U_{24,77}$=148.0, P<0.001).

The mean body condition of chicks was 9.5% better in 2000 compared to 1999 (t-test, t=2.297, df=112, P=0.024). Chicks had higher GC in 1999 than in 2000 (Fig. 5, Mann-Whitney test for faeces: $U_{36,77}$=906.5, P=0.003; for urine: $U_{36,77}$=1063.5, P=0.047), indicating that they were stressed by chronic starvation.

In contrast, adults did not show elevated GC levels in 1999 compared with 2000 (Fig. 5, Mann-Whitney test for faeces: $U_{24,65}$=660.5, P=0.271; for urine: $U_{24,65}$=736.0, P=0.688).

Within the breeding season 1999, GC in faeces of chicks increased over the nestling period (Fig. 4, Spearman correlation for faeces vs date: r_s=0.461, P=0.005, n=36), while the level was constant in 2000 (Fig. 4, Spearman correlation for faeces vs date: r_s=–0.029, P=0.805, n=77) . GC of adults was constant in faeces and decreased slightly in urine in the course of the food-limited breeding season of 1999 (Spearman correlation for faeces of adults vs breeding stage: r_s=–0.143, P=0.335, n=47; for urine vs breeding stage: r=–0.290, P=0.048, n=47). Failed breeding adults (sampled during incubation) did not differ in excreted GC at the time of sampling from successfully incubating adults: failed incubating adults had 55.9±7.23 ng/g GC in faeces and 18.76±2.39 ng/ml in urine, compared with 54.0±6.64 ng/g GC in faeces and 18.74±3.82 ng/ml in urine for successfully incubating adults (Mann-Whitney test for faeces: $U_{18,24}$=201.5, P=0.712; for urine: $U_{18,24}$=166.5, P=0.208). There was no statistical difference in excreted GC between incubating and chick-feeding adults (Mann-Whitney test for faeces: $U_{42,23}$=407.0, P=0.297; for urine: $U_{42,23}$=433.5, P=0.497).

Discussion

Few studies of GC have been carried out in seabirds. These studies suggested that plasma corticosterone levels are negatively correlated with fat reserves, and are elevated in response to chronic starvation and low nutritional quality of food (Kitaysky et al. 1999a, 1999b; Nuñez-De la Mora et al. 1996; Robin et al. 1998; Wingfield et al. 1999). Our results are in line with these results.

In the present study, we estimated physiological stress in Wilson's storm-petrels as a means of understanding how limited resources are partitioned between provisioning parents and their chicks. In the breeding season of 1999, chicks were starving due to low food availability, and correspondingly their faecal and urine GC levels were elevated during these months of chronic starvation. In contrast, adults did not show elevated GC levels, and even showed decreased GC in the course of the breeding season. The data thus suggest that Wilson's storm-petrels respond to unfavourable conditions by maintaining their own body condition and reducing provisioning to their chicks. Heavy investment in current offspring may be associated with reduced chances of survival and future reproductive success (Williams 1966). Therefore, there should be a trade-off between the resources allocated to the offspring and the resources used for adult body maintenance in any breeding season, resulting in a parent-offspring conflict when the resources are limited (Stearns 1989; Bell and Koufopanou 1986). Animals with a short reproductive lifespan are expected to invest more in current offspring, while those with a long reproductive lifespan should allocate more resources to their own survival and thus to future offspring (Trivers 1974). Procellariiformes (storm-petrels, shearwaters, petrels and albatrosses) are long-lived seabirds, and adults should therefore use scarce resources mainly for body maintenance.

In contrast to adult birds, nest-bound chicks are limited in their behavioural responses to food-related stress. A hungry chick can only intensify its begging for food from a parent, and this has recently been shown to be reflected by elevated corticosterone levels in black-legged kittiwakes *Rissa tridactyla* (Kitaysky et al. 2001).

All previous studies of GC in seabirds used blood plasma levels of corticosterone as an index of physiological stress. However, blood samples can be difficult to obtain from free-living birds without influencing the results, because capture and handling lead to rapid and significant increases in circulating GC levels. Recent studies have shown that metabolites of glucocorticoid hormones are detectable in faeces or cloacal fluid of birds and can be used as a non-invasive measure of stress levels (Hiebert et al. 2000; Kotrschal et al. 1998). Measuring excreted GC also has the advantage that faecal GC metabolites provide a measure of adrenal status, an integrated measure for the period after the last defecation rather than a punctual sample. Despite this advantage, immunoassays for faecal glucocorticoid metabolites have been described for very few species of birds. Kotrschal et al. (1998) used an EIA with an antibody against corticosterone-3-CMO in greylag geese *Anser anser*; while various authors used radioimmunoassay for corticosterone (northern spotted owl *Strix occidentalis caurin*a, Wasser et al. 1997; rufous humming-birds *Selasphorus rufu*s, Hiebert et al. 2000). Wilson's storm-petrel are mainly planktivorous birds with a long gut passage time (>12 h, Quillfeldt, unpublished data). Therefore, faecal GC metabolites may provide a measure of adrenal status over a relatively long period. Faeces contain multiple GC metabolites with little native hormone, because corticosterone is rapidly and extensively metabolised before excretion. Species-specific differences in steroid metabolism, gut microflora and diet may cause the GC metabolites to differ between species. As a result, highly specific corticosterone antibodies may have relatively little affinity for the faecal GC metabolites. In fact, corticosterone was not detected in the faeces of Wilson's storm-petrels. Instead, the correlation between excreted tetrahydrocorticosterone and chick body condition indicated that tetrahydrocorticosterone levels in faeces and urine collected from Wilson's storm-petrels immediately after capture is an adequate measure for adrenal activity characteristic for physiological stress in Wilson's storm-petrels. As the dominant peak eluted from the column in fractions 72–74, we conclude that the assay measured TH"B" in an unconjugated form. As in other field studies (Creel et al. 1996; Kotrschal et al. 1998; Hirschenhauser et al. 2001), blood was not sampled simultaneously and thus corticosterone plasma levels are unknown. An

excretion study with domestic geese, which received systemic injections of 3 H corticosterone (E. Möstl, unpublished data), revealed that metabolite excretion started a few minutes after the injection and peaked 1–2 h later. Studies by Cockrem and Rounce (1994; plasma-faeces relationships in domestic fowl, *Gallus gallus domesticus*), by Cook et al. (1996; serum-saliva relationships in swine, *Sus scrofa domestic*a), and by Cavigelli (1999; plasma-faeces relationships in ring-tailed lemurs, *Lemur catta*) showed deterministic relationships between plasma steroids and metabolites in the faeces. We are therefore confident that the urine and faeces analysed contained an integrated, proportional record of the plasma corticosterone levels of minutes to hours before defaecation.

Handling could be shown to influence the GC values. Corticosterone is known to peak from 10 to 30 min after handling in blood, and in sheep, horses and pigs corticosterone was metabolised extremely rapidly, and metabolites were measurable in urine nearly parallel to the increase in blood after the disturbance (Palme et al. 1996). It is, therefore, not surprising that the median GC value for handled birds was higher than that in nest floor samples. However, in the present study most chicks defaecated immediately after capture, and still had higher GC than nest floor samples. It is unlikely that the high values resulted from a very rapid increase in excreted GC after handling within 1 min. Alternatively, chicks might have higher values during the day than at night. Birds have a diurnal rhythm of corticosterone correlated with the activity period rather than the light/dark cycle (Dufty and Belthoff 1997). In nocturnal birds, such as the Wilson's storm-petrels, circulating corticosterone levels are higher during the day (inactive period) than during the night (active period). All samples from handled birds were taken during the afternoon, and thus contained an integrated, proportional record of the plasma corticosterone levels over the daytime hours. In contrast, the samples collected from the nest floor were defaecated before the afternoon nest controls, and thus represented at least partly the hours of nocturnal activity and possibly associated lower levels the plasma corticosterone. Samples from chicks handled at night would be desirable in order to achieve a more complete understanding of the regulation of corticosterone secretion.

In both years, chicks had more than threefold GC titres compared to adults (Fig. 5). If the metabolism of GC in this species is not different between life-history stages (i.e. chicks vs adults), these elevated values indicate that even in a situation of normal food supply, many chicks were exposed to stress. Chicks of Wilson's storm-petrels are left unattended in the burrows when only a few days old, and need to maintain their body temperature despite low ambient temperatures, which may be responsible for the high GC titres (e.g. Wingfield 1984, 1988).

In each breeding season, some Wilson's storm-petrels in the study colony abandoned their eggs before the chicks hatched, and the proportion of desertion was mainly explained by the food availability at the beginning of the season (Quillfeldt 2001; e.g. 56% and 62% of eggs hatched in 1999 and 2000, respectively). We here found that failed breeders did not differ in excreted GC at the time of sampling from successfully incubating adults. In this species, digestion is slow and excretion rates are low. Because adults excreted large amounts of faeces in response to handling, we assume that the measurement of GC during incubation integrates the time from the initiation of an incubation shift until capture. It is possible that there was no difference in excreted GC between failed and successful breeders because all adults start incubation shifts after re-feeding and in relatively good body condition. Analysis of corticosterone in blood samples may have to be carried out in the future to test if corticosterone increases in the course of incubation shifts, and if it may trigger the most extreme decision in resource partitioning between parent and offspring, to abandon the egg.

Acknowledgements We are grateful to A. Kuchar-Schulz who carried out the EIA. We would also like to thank J.F. Masello, K. Arbter and H-U. Peter for their contributions to the work. The manuscript benefited from the comments of A. Kitaysky and two anonymous referees. Logistical support was provided by the Alfred-Wegener Institute for Marine and Polar Research (Bremerhaven, Germany), the National Antarctic Institute of Argentina and Hapag Lloyd Seetouristik GmbH. This study was partly funded by grants provided by the State of Thuringia, Germany (Landesgraduiertenstipendium). The study was carried out with permission of the Environmental Agency (Umwelt-Bundesamt) of Germany, and complied with the current laws for scientific work in the Antarctic.

References

Astheimer LB, Buttemer WA, Wingfield JC (1992) Interactions of corticosterone with feeding, activity, and metabolism in passerine birds. Ornis Scand 23:355–365

Beck JR, Brown DW (1972) The biology of Wilson's Storm petrel, *Oceanites oceanicus* (Kuhl), at Signy Island, South Orkney Islands. Br Antarct Surv Sci Rep 69:1–54

Bell G, Koufopanou V (1986) The cost of reproduction. Oxford Surv Evol Biol 3:83–131

Cavigelli SA (1999) Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. Anim Behav 57:935–944

Cherel Y, Groscolas R (1999) Relationships between nutrient storage and nutrient utilisation in long-term fasting birds and mammals. In: Adams NJ, Slotow RH (eds) Proc Int Ornithol Congr 22:17–34

Cherel Y, Robin J-P, Walch O, Karmann H, Netchitailo P, Le Maho Y (1988) Fasting in king penguin I. Hormonal and metabolic changes during breeding. Am J Physiol 254:R170–R177

Cockrem JF, Rounce JR (1994) Fecal measurements of oestradiol and testosterone allow the non-invasive estimation of plasma steroid concentrations in the domestic fowl. Br Poult Sci 35:433–443

Cook S, Schaefer A L, Lepage P, Morgan Jones S (1996) Salivary vs. serum cortisol for the assessment of adrenal activity in swine. Can J Anim Sci 76:329–335

Creel S, Creel N M, Monfort S L (1996) Social stress and dominance. Nature 379:212

Drent RH, Daan S (1980) The prudent parent: energetic adjustments in avian breeding. Ardea 68:225–252

Dufty AM Jr, Belthoff JR (1997) Corticosterone and the stress response in young Western Screech-owls: effects of captivity, gender, and activity period. Physiol Zool 70:143–149

Furness RW (1983) Variations in size and growth of great skuas *Catharacta skua* chicks in relation to adult age, hatching date, egg volume, brood size and hatching sequence. J Zool 199:101–116

Furness RW, Furness BL (1981) A technique for estimating the hatching dates of eggs of unknown laying date. Ibis 123:98–102

Hahn S, Peter H-U, Quillfeldt P, Reinhardt K (1998) The birds of the Potter Peninsula, King George Island, South Shetland Islands, Antarctica, 1965–1998. Mar Ornithol 26:1–6

Hiebert SM, Ramenofsky M, Salvante KG, Wingfield JC, Gass CL (2000) Noninvasive methods for measuring and manipulating corticosterone in hummingbirds. Gen Comp Endocrinol 120:235–247

Hirschenhauser K, Möstl E, Wallner B, Dittami J, Kotrschal, K (2001) Endocrine and behavioural responses of male Greylag Geese (*Anser anser*) to pairbond challenges during the breeding season. Ethology 106:63–77

Kitaysky AS, Wingfield JC, Piatt JF (1999a) Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Funct Ecol 13:577–584

Kitaysky AS, Piatt JF, Wingfield JC, Romano M (1999b) The adrenocortical stress response of Black-legged Kittiwake chicks in relation to dietary restrictions. J Comp Physiol B 169:303–310

Kitaysky AS, Wingfield JC, Piatt JF (2001) Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. Behav Ecol 12:619–625

Kohen F, Bauminger S, Lindner HR (1975) Preparation of antigenic steroid-protein conjugates. In: Cameron EHD, Hillier SG, Griffiths K (eds) Steroid immunoassay. Alpha Omega, Cardiff, Wales, pp 11-32

Kotrschal K, Hirschenhauser K, Möstl E (1998) The relationship between social stress and dominance is seasonal in greylag geese. Anim Behav 55:171–176

Meyer HHD, Sauerwein H, Mutayoba BM (1990) Immunoaffinity chromatography and a biotin-streptavidin amplified enzyme-immunoassay for sensitive and specific estimation of estradiol –17β. J Steroid Biochem 35:263–270

Möstl E, Maggs JL, Schrötter G, Besenfelder U, Palme R (2002) Measurement of cortisol metabolites in faeces of ruminants. Vet Res Comm 26:127–139

Nuñez-De la Mora A, Drummond H, Wingfield JC (1996) Hormonal correlates of dominance and starvation-induced aggression in chicks of the blue-footed booby. Ethology 102:748–761

Obst BS, Nagy KA (1993) Stomach oil and the energy budget of Wilson's Storm-Petrel nestlings. Condor 95:792-805

Palme R, Fischer P, Schildorfer H, Ismail MN (1996) Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. Anim Reprod Sci 43:43–63

Quillfeldt P (2001) Variation of breeding success in Wilson's storm-petrels: influence of environmental factors. Antarct Sci 13:400–409

Quillfeldt P, Peter H-U (2000) Provisioning and growth in chicks of Wilson's storm-petrels (Oceanites oceanicus) on King George Island, South Shetland Islands. Polar Biol 23:817–824

Quillfeldt P, Schmoll T, Peter H-U, Epplen JT, Lubjuhn T (2001) Genetic monogamy in Wilson's Storm-Petrel. Auk 118:245–251

Roberts B (1940) The life cycle of Wilson's petrel *Oceanites oceanicus* (Kuhl). Sci Rep British Graham Land Exped 1:141–194

Robin J-P, Boucontet L, Chillet P, Groscolas, R (1998) Behavioural changes in fasting emperor penguins: evidence for a "refeeding signal" linked to a metabolic shift. Am J Physiol 274:R746–R753

Siegel V, Bergström B, Mühlenhardt-Siegel U, Tomasson M (2003) Demography of krill Euphausia superba in the Elephant Island area (Antarctic Peninsula) during austral summer 2001 and long-term variability of the stock. Antarct Sci (in press)

Stearns SC (1989) Trade-offs in life-history evolution. Funct Ecol 3:259–268

Trivers RL (1974) Parent-offspring conflict. Am Zool 14:249–264

Wasser SK, Bevis K, King G, and Hanson E (1997) Noninvasive physiological measures of disturbance in the northern spotted owl. Conserv Biol 11:1019–1022

Williams GC (1966) Natural selection, the costs of reproduction, and a refinement of Lacks principle. Am Nat 100:687–690

Wingfield JC (1984) Influences of weather on reproduction. J Exp Zool 232:589–594

Wingfield JC (1988) Changes in reproductive function of freeliving birds in direct response to environmental perturbations. In: Stetson MH (ed) Processing of environmental information in vertebrates. Springer, Berlin Heidelberg New York, pp 121–148

Wingfield JC, Breuner C, Jacobs J (1997) Corticosterone and behavioural responses to unpredictable events. In: Harvey S, Etches RJ (eds) Perspectives in avian endocrinology. Society of Endocrinology, Bristol, pp 267–278

Wingfield JC, Ramos-Fernandez G, Nuñez-De la Mora A, Drummond H (1999) The effects of an "El Niño" Southern Oscillation event on reproduction in male and female blue-footed boobies, *Sula nebouxii*. Gen Comp Endocrinol 114:163–172