Resting and daily energy expenditures during reproduction are adjusted in opposite directions in free-living birds

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Summary

1. Reproduction is energetically expensive, and daily energy expenditure (DEE) often peaks during the period of rearing young. The 'potentiation' hypothesis predicts that high DEE needs to be sustained by a corresponding up-regulation of metabolic machinery; thus, a concomitant increase in the resting metabolic rate (RMR) is expected. Alternatively, the 'compensation' hypothesis predicts that DEE and RMR are regulated independently and animals may maintain low RMR to maximize the energy available for reproduction. This might particularly be the case if DEE was limited, for example, by extrinsic food supply or intrinsic physiological factors.

2. We tested these hypotheses in free-living seabirds by manipulating their energy demands (experimentally reduced or increased brood size) and supplies (providing supplemental food), and simultaneously measuring their DEE and RMR (by the doubly labelled water method and an indirect hormonal proxy, respectively).

3. In support of the 'compensation' hypothesis, metabolic rates were adjusted independently and in opposite directions with an increase in DEE and a decrease in the hormonal proxy for RMR in individuals rearing young compared to birds with removed broods. Energy expenditure of unfed birds with chicks appeared to be limited as experimental brood enlargement did not cause an increase in DEE. Supplemental feeding did not allow DEE to exceed this apparent limitation.

4. We propose that a reduction in the resting metabolism is a strategy to increase allocation of energy to reproduction when DEE is constrained and that this constraint is unlikely to be related to food supply.

Key-words: BMR, body temperature, daily energy expenditure, doubly labelled water, endothermy, field metabolic rate, hypothermia, oxidative stress, resting metabolic rate, thyroid hormones

Introduction

Energy is one of the essential resources that affects all aspects of the life of an animal. Energy is often thought to be a limited resource, and animals are required to allocate it among competing life-history traits to maximize fitness (Stearns 1992). Hence, how much energy an animal allocates to reproduction is one of the central allocation decisions that may have important repercussions for their current and future reproductive performance and survival.

The rate at which an animal expends energy during a specific life-history event constitutes an important part of this allocation process. During breeding, daily energy expenditure (DEE), the overall rate of energy expenditure

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animals sustain over prolonged periods, integrates the energy expended on both reproduction and self-maintenance. The basal or resting metabolic rate (RMR), the minimal energetic cost of living, quantifies the energy expended by a quiescent, post-absorptive animal within its thermoneutral zone (Bech, Langseth & Gabrielsen 1999; McNab 2009) and can therefore be regarded as a large part of the energy that is expended for self-maintenance.

Reproduction is often assumed to be the life-history stage that is energetically most demanding. Accordingly, energy intake and DEE of lactating mammals and birds provisioning chicks usually increase to accommodate the additional energetic needs of offspring (Bryant 1997; Speakman 2008). The extent to which DEE can be raised is likely to be limited (Speakman 2000), and a metabolic ceiling has been demonstrated in several free-living animals (Tinbergen & Verhulst 2000; Welcker *et al.* 2010; Elliott *et al.* 2014). The cause of this limitation is debated. While there is waning support for a direct physiological cost causing a life-history penalty (Selman *et al.* 2012), there is little doubt that DEE is at some point intrinsically limited by physiological factors (Weiner 1992; Hammond & Diamond 1997), for example, by the capacity to dissipate metabolically produced heat (Speakman & Krol 2010). In most natural situations, however, the cause of this limitation is thought to be extrinsic and primarily related to the ability of animals to extract energy from the environment (Tinbergen & Verhulst 2000; Thomas *et al.* 2001; Speakman *et al.* 2003).

It is less clear whether animals can make metabolic adjustments to peak energy demands by changing their RMR. A widespread assumption is that RMR and DEE are intrinsically linked (Daan, Masman & Groenewold 1990; Weiner 1992; Hammond & Diamond 1997), and hence, animals may not be able to modulate RMR independently. As the consequence of such a physiological constraint, an increase in DEE during reproduction would be expected to be accompanied by a concomitant increase of RMR. This notion [the 'potentiation' hypothesis (Speakman *et al.* 2003)] is based on the assumption that the RMR determines the level of DEE an individual can sustain because high DEE and energy intake needs to be facilitated by large and costly metabolic machinery such as the liver, heart or alimentary tract (Drent & Daan 1980; Konarzewski & Diamond 1995; Hammond & Diamond 1997). If RMR was primarily driven by variation in size and metabolic intensity of these organs, a close association between RMR and DEE would be expected. Empirical evidence, however, is equivocal and the nature of the relationship between RMR and DEE remains unclear (Ricklefs, Konarzewski & Daan 1996; Speakman, Krol & Johnson 2004). In support of an intrinsic coupling, a correlation between RMR and DEE has been reported by a number of studies both across (Daan *et al.* 1989) and within species (Nilsson 2002; Tieleman *et al.* 2008; White *et al.* 2011; Careau *et al.* 2013). Several studies, however, have failed to confirm such a relationship in the field (Meerlo *et al.*

1997; Fyhn *et al.* 2001; Speakman *et al.* 2003) or in the laboratory (Johnson, Thomson & Speakman 2001).

However, if an adjustment of RMR was not constrained by a physiological link to DEE, RMR may be expected to be regulated according to energy allocation decisions. Reproducing animals may down-regulate their RMR as such an adjustment would allow them to increase metabolic scope and hence the amount of energy they could allocate to reproduction (Blackmer *et al.* 2005) without the need to acquire more energy. This might be facilitated by a reduction in potentially costly processes such as immune responses (Deerenberg *et al.* 1997) or a decrease in the metabolic intensities of vital organs (Rønning *et al.* 2008). This has been termed the 'compensation hypothesis' (Nilsson 2002) and might particularly be expected in animals operating close to an energetic ceiling constraining possible adjustments of DEE (Welcker *et al.* 2010).

Another mechanism by which birds may achieve a reduction of resting metabolic costs is temporal hypothermia. Down-regulation of body temperature during inactive periods is a widespread strategy among small bird species to conserve energy in situations of energetic constraints (Daan *et al.* 1989; McKechnie & Lovegrove 2002). Even mild hypothermia may result in important energy savings (Reinertsen & Haftorn 1984), and the degree to which body temperature is reduced may mainly depend on the availability of food and the existence of endogenous energy reserves (Nord, Nilsson & Nilsson 2011). This may be less feasible as a strategy in reproducing mammals because of the intrinsic heat production of milk synthesis during lactation (Krol, Murphy & Speakman 2007) and the incompatibility of milk synthesis with lowered body temperature (Wilde, Knight & Racey 1999).

In this study, we experimentally tested whether RMR and DEE are linked or can be adjusted independently. With a brood size manipulation experiment, we determined metabolic adjustments to reproduction in a free-living bird species, the kittiwake (*Rissa tridactyla*). We increased and decreased parental workload by removing whole clutches in one group of nests and enlarging broods in a second group, while non-manipulated nests served as a control. By providing supplemental food to a subset of experimental nests, we evaluated the effect of food supply on metabolic adjustments, particularly with respect to the hypothesis of an extrinsic limitation of DEE by the amount of available food.

Measuring RMR and DEE concurrently in the same individuals in the field is difficult and may lead to spurious results, especially in species sensitive to handling. We estimated DEE by the doubly labelled water (DLW) method (Lifson & McClintock 1966; Butler *et al.* 2004), and to avoid additional handling of birds for RMR measurements in a respiratory chamber, we used thyroid hormone concentrations as an indirect measure of RMR. 3, 3'-Triiodo-L-Thyronine (T3), the primary thyroid hormone, stimulates tissue oxygen consumption with corresponding effects on RMR (Hulbert 2000; Kim 2008) across a broad range of

environmental conditions and physiological states (Zheng, Liu & Swanson 2014: in press). In accordance with this, we have recently shown that thyroid hormones [both bound and unbound (free) T3 fractions] can serve as a reliable proxy for RMR based on a close association of thyroid hormone secretion with RMR in breeding kittiwakes ($R^2 \sim 0.6$) and other bird species (Elliott *et al.* 2013; Welcker *et al.* 2013). Body temperature was measured to determine metabolic adjustments due to hypothermia. In addition, we used the stress hormone corticosterone (CORT), the main avian glucocorticoid, as an indicator of physiological stress associated with metabolic adjustments in the kittiwake (Kitaysky *et al.* 2010).

Materials and methods

STUDY AREA AND ANIMALS

The experiment was carried out in June and July 2011 at a semi-artificial colony of individually marked black-legged kittiwakes on Middleton Island, Alaska, USA (59.4°N, 146.3°W). At the study colony, kittiwakes breed on wooden ledges of an abandoned radar tower. Nest sites are fitted with one-way mirror glass window panes and hence can conveniently be accessed from within the building (see detailed description in Gill & Hatch 2002). This set-up allowed for unobtrusive observations and rapid capture and handling of both adult birds and their offspring throughout the experiment. Kittiwakes are medium-sized (body mass about 400 g), sexually dimorphic (males heavier than females) seabirds that feed predominantly on pelagic fish (Hatch 2013). Males and females share parental duties during both incubation and chick-rearing.

BROOD SIZE MANIPULATION

In 2011, kittiwakes on Middleton Island mostly laid two-egg clutches (mean 1.9, range 1–3, Table 1). We randomly chose 65 nests for the brood size manipulation, half of which were part of the supplemental feeding treatment (see below). Only nests that successfully hatched at least one egg were included and brood manipulations took place immediately after hatching. To determine the exact time of hatching, nests were monitored twice daily.

We created three experimental groups irrespective of natural brood size: (i) 'removals', nests where we removed the complete clutch; (ii) 'enlargements', where the number of chicks was increased to a total of three chicks; and (iii) 'controls', the brood size of which remained unchanged. We randomly assigned nests to the different treatments and foster chicks were not allowed to be either older or younger than the oldest and youngest chick of the foster nest, respectively. Parent kittiwakes do not recognize their own offspring during the first week after hatching and adopt any chick present in their nest.

Not all chicks in 'control' and 'enlargement' nests survived until the end of the experiment at chick age 12 days (see below, Table 1). However, at that time 'enlargements' still contained on average 1.18 and 0.75 more chicks in fed and unfed nests, respectively, compared to control nests (Table 1). None of the 'removals' attempted to lay a replacement clutch, but all of them continued to defend their nest site throughout the experiment.

FEEDING TREATMENT

Supplementally fed birds were offered food *ad libitum* three times a day (at 09.00, 14.00 and 18.00 h local time) starting prior to egg-laying and continuing throughout the experiment. Supplemental food consisted of thawed Atlantic capelin (*Mallotus villosus*), a naturally preferred prey species of kittiwakes at the study site (Hatch 2013). Food was offered at the nest sites through plastic tubes inaccessible to neighbouring nests (see Gill & Hatch 2002 for details), and a feeding event continued until the parent(s) present at the nest stopped taking fish. All occupants of fed nests generally accepted offered fish and were present at the majority of feeding events but continued to forage at sea (Gill & Hatch 2002, S.A. Hatch, unpublished data).

SAMPLING

All adult birds were sampled for body temperature, DEE and baseline plasma concentrations of free (unbound) T3 and CORT. Sampling started when the oldest chick of a nest reached the age of 8 days. We sampled both parents of all nests with a time lag of usually one day. Sampling was completed when chicks were *c.* 12 days old.

Birds were captured at the nest with a metal hook or nylon noose, and body temperature was measured immediately with a commercially available digital thermometer with *c.* 5 s response time. The thermometer was cautiously inserted *c.* 2 cm in the cloacae thus reflecting core rather than peripheral body temperature. Birds were then weighed and intraperitoneally injected with a dose of 1.25 mL DLW containing 65.15 atom percent excess (APE) oxygen-18 (^{18}O) and 36.5 APE deuterium (^2H). We then placed birds in a cloth bag for 60 min to allow for complete equilibration of isotopes with the body water of the injected animal. Prior to release, birds were reweighed and a blood sample was taken from the alar vein to estimate initial enrichment of isotopes. Blood was collected into several 75 μL glass micro-capillaries which we flame-sealed immediately. In addition, we took blood samples of 12 unlabelled kittiwakes to determine mean background level of isotopes in the study population (Speakman & Racey 1987: method C).

Table 1. Brood size and chick loss of fed and unfed kittiwake nests on Middleton Island 2011 that were part of the brood size manipulation experiment. Brood sizes are given for nests prior to the brood size manipulations, at the start (chick age 8 days) and the end of the sampling period (chick age 12 days). In addition, sample sizes are given

	Fed			Unfed		
	Removal	Control	Enlargement	Removal	Control	Enlargement
N_{nests}	10	11	11	11	10	12
Original brood size	1.50	1.64	1.91	1.64	1.80	1.83
Brood size (day 8)	0	1.64	2.82	0	1.60	2.75
Brood size (day 12)	0	1.55	2.73	0	1.50	2.25
Total chicks lost	NA	1	3	NA	3	9

We attempted to recapture all individuals 3 days after injection (mean 70·0 h ± 4·7 SD) as extended measurement periods reduce the error due to high day-to-day variation in energy expenditure (Speakman *et al.* 1994; Berteaux *et al.* 1996). Upon recapture birds were weighed again and a second blood sample taken as described above to estimate final enrichment of isotopes and to determine plasma concentrations of fT3 and CORT. Blood for hormone analysis was centrifuged, plasma separated and frozen at −20 °C immediately after sampling. Out of 127 injected birds, only one individual evaded recapture. During all handling times of adult birds, their chick(s) remained in the nest and their survival was monitored until the sampled birds or their partner returned to the nest. No chick mortality was recorded during sampling of the parent birds.

DAILY ENERGY EXPENDITURE

Analysis of isotopic enrichment of blood was performed by isotope ratio mass spectrometry as described in (Speakman *et al.* 1990; Speakman & Krol 2005). We regarded only final samples >20 ppm above background to be sufficiently enriched to estimate energy expenditure. Two samples did not meet this condition and were subsequently removed from the data set.

We used a single-pool model to calculate rates of CO_2 production as recommended for birds of <1 kg body mass (Speakman 1997). By using equation 7·17 of Speakman (1997), we corrected for fractionation effects assuming a fixed evaporative water loss of 25%. This has been shown to minimize deviations from reference methods (Visser & Schekkerman 1999; Speakman & Krol 2005) and provided the best fit to simultaneous indirect calorimetry in the only validation of the method on a seabird (Shirai *et al.* 2012). We calculated initial body water based on the ^{18}O-dilution space as determined by the plateau method (Speakman 1997). Final body water was inferred from final body mass assuming a constant fraction of body water throughout the experiment. We converted estimates of CO_2 production to DEE (kJ day^{-1}) using a caloric equivalent of 27·64 J mL CO_2^{-1}. This value is appropriate for the protein- and lipid-rich diet of kittiwakes (Welcker *et al.* 2010).

HORMONE RADIOIMMUNOASSAYS

We determined both CORT and free T3 (fT3) concentrations by radioimmunoassay. The CORT assay is described in detail in (Kitaysky, Piatt & Wingfield 2007). Free T3 levels were determined based on a commercially available radioimmunoassay kit (MP Biomedicals, Santa Ana, CA, USA) optimized for our study species. The dose–response curve of pooled kittiwake plasma was parallel with standard curves of the assay kit. All hormone samples were analysed in duplicate. Interassay variation was <4·6% (fT3), and intra-assay variation was <1·9% and 1·2% for fT3 and CORT, respectively.

DATA ANALYSIS

To avoid an effect of handling stress on either hormone concentrations or body temperature, sampling took place within 3 min of capture, as recommended for baseline CORT samples (Romero & Reed 2005). In agreement with this, we did not find a significant increase with handling time in either CORT, fT3 or body temperature (handling time range 0·58–3·17 min, all $P > 0.05$, all $R^2 < 0.03$). However, we found a slight increase in body temperature with time of day ($R^2 = 0.03$, $F_{1,125} = 4.1$, $P = 0.045$). In addition, there was a curvilinear relationship between fT3 and time of day with hormone concentrations reaching a minimum in the early afternoon and being higher in the morning and evening (polynomial regression, $R^2 = 0.09$, $F_{2,122} = 6.03$, $P = 0.003$). However, as correcting for time of day had no effect on results for body temperature and fT3, we report statistics for uncorrected values only.

We also found significant sex differences in all parameters except body temperature ($F_{1,59} = 2.7$, $P = 0.108$). Males were overall about 13·2% heavier than females ($F_{1,61} = 168.4$, $P < 0.001$), and fT3 and CORT concentrations were higher in male kittiwakes (fT3: $F_{1,59} = 6.7$, $P = 0.012$, CORT: $F_{1,60} = 9.96$, $P = 0.003$). In contrast, mass-independent DEE was slightly but significantly lower in males compared to females ($F_{1,58} = 4.16$, $P = 0.046$). However, for none of the above parameters did we find a sex difference in the response to either supplemental feeding or brood size manipulation (likelihood ratio tests; interaction sex × feeding: all $\chi_1^2 < 2.87$, all $P > 0.09$; sex × brood: all $\chi_2^2 = 2.37$, all $P > 0.31$). We therefore omitted 'sex' from all final models and report results for pooled data only.

We tested for effects of brood size manipulation and feeding treatment by fitting linear mixed-effects models with 'nest identity' as a random term to account for the statistical non-independence of partners within a pair. To control for the effect of body mass on DEE (all other parameters were independent of body mass), we included body mass as a covariate in the model with DEE as the response variable.

We used the thyroid hormone T3, specifically the free, unbound fraction (fT3) as an indirect proxy of RMR to minimize stress for the study birds and hence a potential bias in our data. The additional handling procedures involved in the direct measurement of RMR in a respiratory chamber would primarily have required to keep the birds captive for a time period longer than 10 h (Bech, Langseth & Gabrielsen 1999). In concert with the other handling procedures involved in our experiment, particularly the DLW method, this could have severely affected the birds, potentially leading to unreliable results.

There is a large body of evidence demonstrating effects of thyroid hormones on tissue oxygen consumption and a strong link with basal or resting metabolism (Hulbert 2000; Kim 2008). In laboratory studies, the correlations coefficient between T3 and BMR has been shown to be as high as 0·98 (Bobek, Jastrzebski & Pietras 1977). There is also strong support for a close relationship under varying environmental conditions and physiological state of individuals. Several field studies have demonstrated that the relationship between T3 and basal metabolism is stable across a large variety of species and conditions, including different seasons and life-history stages (Chastel, Lacroix & Kersten 2003; Vezina *et al.* 2009; Li, Yan & Wang 2010; Elliott *et al.* 2013; Zheng, Liu & Swanson 2014: in press). In addition, two recent studies on free-living kittiwakes have confirmed a strong relationship between thyroid hormones and RMR at different stages of the breeding period ($R^2 \sim 0.6$; Elliott *et al.* 2013; Welcker *et al.* 2013). Hence, using hormone levels as a proxy for RMR allows for an approximation of individual RMR, without the confounding effect of individually variable responses to the stress of capture and handling, and avoids spurious results in DEE due to such handling.

To evaluate the error introduced by the indirect measure of RMR and hence the robustness of our inference about RMR based on fT3, we used data on RMR and fT3 collected simultaneously from individual kittiwakes at the same study site in 2010 and published by Elliott *et al.* (2013). Based on the relationship RMR[W] = 2·61 + 0·058 * fT3[pg/mL] and associated error (σ = 0·4626) as derived from the data reported in Elliott *et al.* (2013), we generated predicted RMR values for the current data set incorporating the statistical error of the published relationship. We then reran linear mixed models as described above replacing fT3 with simulated RMR data with 10 000 iterations. The 95% confidence intervals of the resulting distribution of parameter estimates of these models showed that effects of brood size manipulation and supplemental feeding were largely

consistent for models based on fT3 and generated RMR data (see Results), and hence, the indirect measure of RMR did not compromise the conclusions.

Data were log-transformed where necessary to meet condition of normality. All statistical analyses were performed using R.2.15.2 (R Development Core Team 2012). Our data are accessible at the Dyrad Data Archive (Welcker *et al*. 2014).

Results

There was a strong response of body mass to both brood size manipulation (Fig. 1a; $F_{2,61} = 8.18$, $P < 0.001$) and feeding treatment ($F_{1,61} = 14.73$, $P < 0.001$). The effect of brood size was mainly due to a significant increase of body mass among 'removals' (Fig. 1a; $t_{61} = 3.13$, $P = 0.003$), whereas there was no significant difference between 'controls' and enlarged broods ($t_{61} = 0.59$, $P = 0.56$). Supplementally fed birds generally increased body mass by about 5.7% independent of brood size (Fig. 1a; interaction feeding × brood size: $\chi^2_1 = 1.62$, $P = 0.44$).

There was also a strong effect of the feeding treatment on body temperature (Fig. 1c). Temperature was *c*. 0.6 °C higher in supplementally fed individuals ($F_{1,59} = 6.7$, $P = 0.012$). However, this difference was only apparent in birds raising chicks (Fig. 1c; 'controls': $t_{59} = 2.4$, $P = 0.020$; 'enlargements': $t_{59} = 2.1$, $P = 0.038$). There was no difference in body temperature in birds with experimentally removed broods ('removals': $t_{59} = 0.02$, $P = 0.98$).

Similarly, fT3 varied significantly with brood size with 'removals' having higher hormone concentrations than 'controls' and 'enlargements' (Fig. 1b; $F_{1,61} = 44.3$,

$P < 0.001$). Additionally, fT3 was significantly higher in fed individuals ($F_{1,61} = 7.7$, $P = 0.007$) but again this was only apparent in birds with chicks (Fig. 1b; 'controls': $t_{59} = 2.30$, $P = 0.025$; 'enlargements': $t_{59} = 2.29$, $P = 0.025$); there was no difference in fT3 concentration in 'removals' ($t_{59} = 0.48$, $P = 0.63$). Models based on simulated RMR values as predicted by fT3 and integrating the statistical uncertainty of the relationship between fT3 and RMR confirmed significant higher resting metabolism of 'removals'. However, as the 95% confidence intervals of the parameter estimates for the feeding treatment effect included zero [−0.22, 0.10], it remains uncertain whether the feeding treatment significantly increased RMR of kittiwakes rearing chicks.

Daily energy expenditure corrected for body mass varied significantly with experimental brood size (Fig. 1d; $F_{2,58} = 10.62$, $P < 0.001$), but there was no overall effect of the feeding treatment ($F_{1,58} = 0.10$, $P = 0.75$). Parameter estimates revealed that while in supplementally fed birds DEE was significantly higher in 'enlargements' compared to 'controls' ($t_{58} = 2.16$, $P = 0.035$), there was no such difference in unfed birds ($t_{58} = 0.21$, $P = 0.84$). However, 'removals' had significantly lower DEE compared to 'enlargements' in both feeding treatments (Fig. 1d, fed: $t_{58} = -3.55$, $P < 0.001$; unfed: $t_{58} = -3.16$, $P = 0.003$).

Corticosterone levels were strongly affected by both the brood size manipulation ($F_{1,59} = 28.1$, $P < 0.001$) and the feeding treatment ($F_{1,59} = 33.6$, $P < 0.001$). While supplemental feeding decreased CORT for all brood sizes, a significant brood × feeding interaction ($F_{2,59} = 3.82$, $P = 0.027$) indicated that variation in CORT among brood
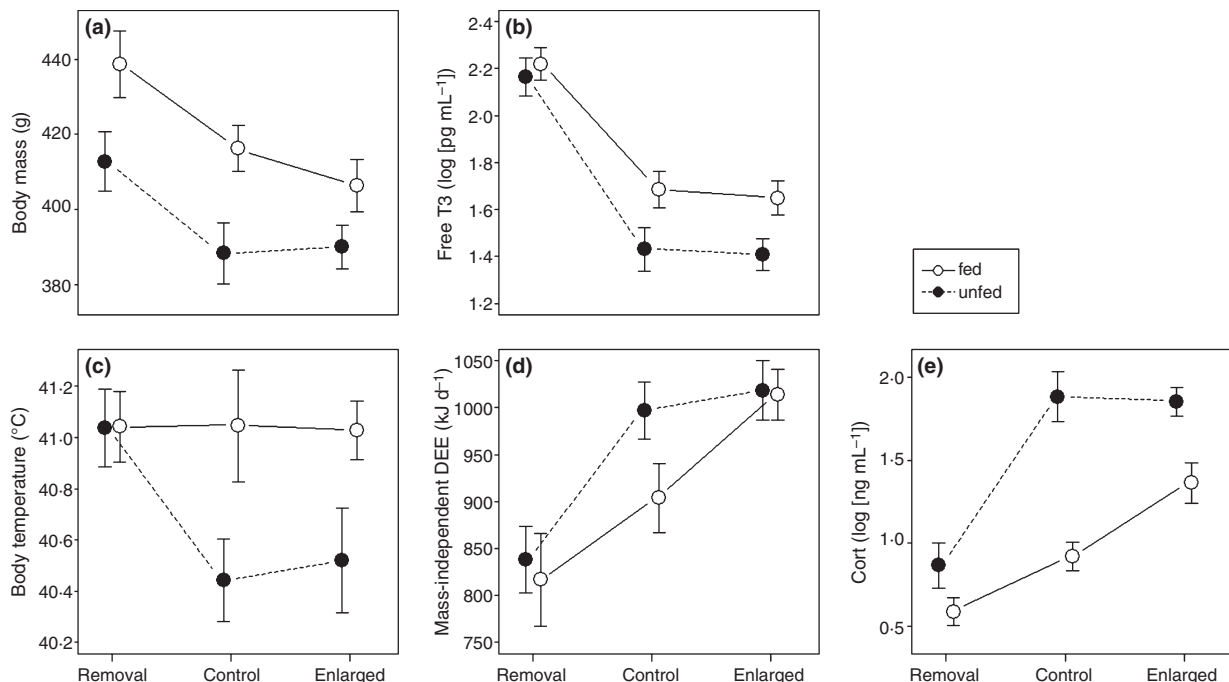
Fig. 1. Effects of brood size manipulation and supplemental feeding in kittiwakes on (a) body mass, (b) the free fraction of the thyroid hormone T3, (c) body core temperature, (d) mass-independent daily energy expenditure (DEE) and (e) the stress hormone corticosterone. Means ± SE are given. Broods of 'removals' were removed shortly after hatching, increased to three chicks in 'enlarged' broods and left unchanged in 'controls'. Supplementally fed individuals were offered fish at the nest *ad libitum* three times a day.

sizes was not parallel for both feeding treatments mainly due to a lack of difference between unfed controls and enlarged broods (Fig. 1e; $t_{59} = 0.15$, $P = 0.88$). CORT levels were lower in 'removals' compared to 'controls' in both feeding treatments (fed: $t_{59} = -1.93$, $P = 0.058$; unfed: $t_{59} = -6.65$, $P < 0.001$).

Discussion

Our results do not support the hypothesis of an intrinsic link between RMR and DEE in breeding kittiwakes. By comparing metabolic rates of chick-rearing individuals with those of experimentally removed broods, our study provides strong evidence that metabolic rates can be adjusted independently and in opposite directions. As expected, DEE increased in chick-rearing birds compared to 'removals'. However, results for fT3 indicate a substantial simultaneous decrease in RMR in chick-rearing individuals, a response incompatible with the hypothesis of a close positive association between RMR and DEE. Hence, contrary to the common view that high DEE needs to be supported by large and energetically costly metabolic machinery, our results suggest a decoupling of RMR and DEE during reproduction in these birds. These results are in line with a previous study that suggested independent changes of RMR and DEE across different stages of the breeding cycle in kittiwakes (Bech *et al.* 2002).

The notion of a functional relationship between DEE and RMR forms the basis of the 'parental care' or 'assimilation capacity' model for the origin of endothermy (Koteja 2000, 2004). This hypothesis posits that the evolution of endothermy was driven by reproductive benefits associated with intensive parental care behaviours, particularly the provisioning of offspring with food. These behaviours would require increased parental DEE and consequently increased food consumption and energy assimilation (Koteja 2000). Selection for high DEE would therefore necessitate morphological and physiological adaptations that require high RMR which in turn would favour the evolution of endothermy. In contrast, we suggest that RMR may be down-regulated in periods of high energy expenditure and assimilation.

Even though some previous empirical studies have confirmed a correlation between RMR and DEE (Daan, Masman & Groenewold 1990; Ricklefs, Konarzewski & Daan 1996), it has been argued that such a relationship might be caused by shared extrinsic factors affecting RMR and DEE simultaneously (Speakman *et al.* 2003; Careau *et al.* 2013). In addition, support for pivotal assumptions underlying an intrinsic link between RMR and DEE is weak, particularly with respect to a link between RMR and maximum energy intake (Speakman, Krol & Johnson 2004), RMR and maximum oxygen consumption (Gebczynski & Konarzewski 2009), and between RMR and organ size (Speakman, Krol & Johnson 2004; Brzek *et al.* 2007).

Chick-rearing kittiwakes adopted a variety of mechanisms to conserve energy. First, they reduced body mass which presumably not only resulted in a reduction of RMR but, mainly due to effects on metabolic costs of flight, also of DEE. Second, the observed decrease in fT3 likely indicates a decrease in RMR in excess of what can be expected from the concurrent decrease in body mass. Concentrations of fT3 were not related to body mass in our study, and they have previously been shown to predict intraspecific changes in RMR independently of body mass (Elliott *et al.* 2013; Welcker *et al.* 2013). Free T3 is therefore likely to reflect changes in RMR due to changes in metabolic intensity and/or the relative size of tissues rather than changes due to the absolute size of those tissues (Zheng, Liu & Swanson 2014: in press). In line with this, previous studies found a disproportionate mass loss and a significantly reduced metabolic intensity of the liver and kidneys in kittiwakes rearing chicks compared to incubating birds, with a concurrent decrease in thyroid hormone concentrations (Langseth *et al.* 2000; Rønning *et al.* 2008). Finally, unfed birds raising chicks showed signs of mild hypothermia when at the nest suggesting a further decrease of energy turnover during phases of rest.

These energy conservation mechanisms in reproducing individuals are in line with the 'compensation hypothesis' (Nilsson 2002) and may have fitness benefits as they may allow birds to allocate a larger proportion of the available energy to their offspring. However, reduced levels of RMR and hypothermia are also likely to incur costs as they reflect reduced energetic investment in self-maintenance which might impair their ability to maintain somatic functions (Kirkwood & Austad 2000; Speakman 2008). This is further supported by the fact that fT3 and body temperature were not further reduced in birds with enlarged broods suggesting that kittiwakes would not adjust their resting metabolism beyond this potential minimum threshold. Similarly, the concurrent increase in DEE of birds raising chicks might be associated with a fitness penalty (Drent & Daan 1980; Williams *et al.* 2010). The modification of metabolic rates may therefore represent a physiological pathway mediating the cost of reproduction (Golet *et al.* 2004). In our study, metabolic adjustments were closely reflected in corresponding changes in stress levels as indicated by plasma concentrations of the stress hormone CORT. Although CORT might not be causally linked to changes in metabolic rates, elevated CORT levels are likely to be associated with a fitness cost in kittiwakes (Kitaysky *et al.* 2010; Satterthwaite *et al.* 2010). The reduction in RMR and increase in DEE of chick-rearing birds compared to 'removals' seem to result in a maximum burden of stress that the birds can or will withstand as CORT was not further increased in the 'enlargement' group.

Several explanations may account for the down-regulation of RMR during reproduction. First, it might be related to natural food constraints limiting the flexibility of DEE. In situations of limited food supply, a reduction in RMR would enable birds to increase the amount of energy

allocated to reproduction without increasing overall food requirements. However, our results suggest that even though food limitation may affect the degree to which RMR is adjusted, it does not fully account for decreased RMR. Supplemental feeding attenuated but did not remove the modification of RMR as indicated by reduced concentrations of fT3 in fed birds raising chicks compared to 'removals'. Secondly, the observed pattern could be the result of an intrinsic limitation of DEE. If kittiwakes operated close to a metabolic ceiling, the only option to increase energetic investment in reproduction, among fed and unfed birds alike, would be to decrease RMR. However, the fact that supplementally fed 'controls' reduced RMR while operating at a level of DEE below that of unfed conspecifics does not lend support to this hypothesis. Hence, we suggest that the modification of RMR might rather be the result of differences in the relative fitness costs and benefits associated with adjustments of metabolic rates. If costs related to a decrease in RMR, especially those pertaining to future reproduction, were lower than potential costs of elevated DEE, a strategy involving down-regulation of RMR might maximize lifetime reproductive success in a long-lived species such as the kittiwake.

In contrast to RMR, DEE increased in birds raising chicks compared to birds with experimentally removed broods. The increase in DEE of chick-rearing individuals was moderate (21%) in comparison with the expected doubling of food requirements of a parent kittiwake raising two chicks (Gabrielsen, Mehlum & Nagy 1987; Gabrielsen, Klaassen & Mehlum 1992), presumably partly due to the energy savings resulting from a reduced RMR and lower body temperature. However, the level of DEE birds were able to sustain appeared to be constrained: challenged birds ('enlargements') did not raise their DEE above the level of 'controls', similar to a study in great tits (*Parus major*) (Tinbergen & Verhulst 2000). With our data, we cannot distinguish whether the apparent limitation of DEE was exerted proximately, that is, whether birds were not able to increase energy expenditure due to a physiological constraint (Speakman & Krol 2010) or ultimately, that is, whether birds were not 'willing' to increase DEE due to a life-history penalty associated with high energy expenditure (Drent & Daan 1980). However, DEE was unlikely to be constrained by limited energy supply which has often been suggested as the primary cause for limitation of DEE in free-living animals (Speakman 2000). As DEE of supplementally fed 'enlargements' did not exceed but was identical to DEE of unfed 'controls' and 'enlargements' the apparent limitation of DEE seemed to be independent of the amount of available food (see also Green *et al.* 2009). Instead of an increase in DEE, additional food allowed challenged birds to maintain higher RMR and body temperature. The apparent limitation of DEE evident in our experimental study confirms empirical data that suggest that energy expenditure in kittiwakes is limited independently of brood size and food availability (Welcker *et al.* 2010).

In conclusion, our experiment suggests that in kittiwakes RMR and DEE are not intrinsically coupled but are adjusted independently and in opposite directions in periods of high energy demands. However, DEE appears to be limited. The nature of this limitation remains to be explored but is unlikely to be related to limited energy supply.

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Data accessibility

Data associated with this article are deposited in the Dryad Data Archive doi:10.5061/dryad.t9q56.

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