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Ecophysiological response of Adélie penguins facing an experimental increase in breeding constraints

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SUMMARY

Foraging strategies play a key role in breeding effort. Little is known, however, about their connection with hormonal and nutritional states, especially when breeding constraints vary. Here, we experimentally increased foraging costs and thus breeding constraints by handicapping Adélie penguins (*Pygoscelis adeliae*) with dummy devices representing 3–4% of the penguins' cross-sectional area. We examined food-related stress (*via* plasma corticosterone concentration) and nutritional state (*via* metabolite levels). Concurrently, we investigated the use of ecological niches *via* the isotopic signature of red blood cells indicating the trophic position ($\delta^{15}N$) and the spatial distribution ($\delta^{13}C$) of penguins. Handicapped birds performed ~70% longer foraging trips and lost ~60% more body mass than controls and their partners. However, corticosterone levels and the nutritional state were unchanged. The isotopic signature revealed that males and females differed in their foraging behaviour: upper trophic levels contributed more in the males' diet, who foraged in more pelagic areas. Handicapped and partner birds adopted the same strategy at sea: a shift towards higher $\delta^{13}C$ values suggested that they foraged in more coastal areas than controls. This change in foraging decisions may optimize feeding time by decreasing travelling time. This may partly compensate for the presumed lower foraging efficiency of handicapped birds and for the energetic debt of their partners who had to fast ~70% longer on the nest. We propose that this flexible use of ecological niches may allow birds facing increased breeding constraints to avoid chronic stress and to minimize the impact on their body condition.

Key words: corticosterone, foraging, handicap, isotopic signature, metabolite, stress.

INTRODUCTION

In an unpredictable environment, breeding constraints may vary between years or within one single reproductive season. To cope with these fluctuating breeding constraints, animals have to be able to adapt and change their behaviour accordingly. One major component of reproductive effort is foraging activity. Several studies have examined whether animals are able to modify their foraging behaviour according to different breeding constraints, in different foraging locations (Wienecke et al., 2000; Tremblay and Cherel, 2003; Lescroël and Bost, 2005), under different environmental conditions (Green et al., 2005; Yoda and Ropert-Coudert, 2007) or at different stages of the breeding cycle (Clarke et al., 1998; Clarke, 2001).

Though changes in foraging behaviour provide worthwhile information on the response of parents when facing variable breeding constraints, understanding of the regulation of animal behaviour can be further enhanced by the examination of a combination of physiological parameters. These may provide useful information on: (1) food-related stress, (2) nutritional condition and (3) the use of ecological niches by experimental animals (Kern et al., 2007; Navarro and González-Solís, 2007; Navarro et al., 2008).

Glucocorticoids play an important role in the regulation of feeding, locomotor activity and energy metabolism (see Landys et al., 2006). For instance, in Adélie penguins (*Pygoscelis adeliae*, Hombron and Jacquinot 1841), baseline corticosterone levels have been correlated with foraging behaviour (Angelier et al., 2008). Moreover, corticosterone is a stress hormone that increases when parents have to work harder (Storey et al., 2006) or when they have to face an unpredictable situation (Pravosudov et al., 2001; Reneerkens et al., 2002). Finally, corticosterone levels have been proposed as a reliable measure of food-related stress and, as a consequence, a direct measure of food availability in free-living birds (Kitaysky et al., 2007).

Changes in foraging decisions may also affect the nutritional state of parents. For this purpose, metabolites can be used as indicators of the nutritional state in free-living animals (Jenni-Eiermann and Jenni, 1998). For example, plasma triglyceride concentration is an indicator of fattening because it increases with the amount of food absorbed and it decreases during heavy endurance exercise. An increase in uric acid levels characterizes the rise in protein breakdown which occurs once a critical threshold has been reached in the depletion of body fuel reserves (see Lindström and Piersma, 1993) or may result from higher muscle activity and from a higher dietary protein fraction. It is also useful to investigate metabolites and hormone levels in parallel as glucocorticoids may increase protein breakdown (Jenni et al., 2000) and decrease plasma triglyceride levels (Remage-Healey and Romero, 2001; Kern et al., 2007).

The measurement of stable isotope ratios is a valuable tool for examining the use of ecological niches by animals (Kelly, 2000; Inger and Bearhop, 2008). The concept of the isotopic method is that animals are constituted by what they consume. For example, as trophic level increases, the quantity of ^{15}N increases, so the ratio $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) indicates the trophic position of the

consumer (Bearhop et al., 2002). The ratio $^{13}C/^{12}C$ (expressed as $\delta^{13}C$) is more stable in marine foodwebs and its variation instead reflects the spatial distribution of consumers (Inger and Bearhop, 2008), with high values being found in coastal foragers and low values in pelagic foragers (Hobson et al., 1994; Cherel and Hobson, 2007). The isotopic signature of the consumer thus reflects the isotopic signature of the consumed prey species.

The main goal in the present study was to enhance the understanding of foraging decisions in Adélie penguins when they face an increase in their breeding constraints. We increased the foraging cost of breeding males and females by equipping them with large dummy devices known to affect the drag of these streamlined animals (Culik and Wilson, 1991; Culik and Wilson, 1992; Watanuki et al., 1992; Miller and Davis, 1993). We thus examined the consequences of this experimental increase in foraging cost on foraging trip duration, body mass loss and the profile of physiological parameters. Handicapped birds were expected to be exposed to a chronic stress due to the presence of the instrument and the difficulty it causes in catching prey efficiently, as well as to an extra foraging cost. In addition, if handicapped birds performed longer foraging trips, their partners were expected to endure longer fasting periods on the nest and consequently to face an additional energetic debt when returning to the sea to feed. For these reasons, we expected corticosterone levels to increase in handicapped and partner birds. Moreover, we expected a decrease in triglyceride levels and an increase in uric acid concentrations in handicapped birds because they would have to make a greater effort (Culik and Wilson, 1991) while being less efficient at catching prey (Ropert-Coudert et al., 2007).

MATERIALS AND METHODS

Study species and area

Fieldwork was carried out during the austral summer 2006–2007 in Dumont d'Urville (66°40′S; 140°00′E), Adélie Land, Antarctica. The Adélie penguin breeding cycle comprises four phases: courtship, incubation [males are in charge of the first incubation shift (~12 days) while females re-feed at sea], guard stage (when the two parents alternate foraging at sea and chick attendance at the nest) and crèche stage (when the two parents forage at the same time leaving the young alone on the colony). This study focused on the incubation and the guard stage, because during the crèche stage it was impossible to precisely monitor the birds.

Thanks to the method of stomach flushing (Ridoux and Offredo, 1989; Kent et al., 1998; Wienecke et al., 2000; Ropert-Coudert et al., 2002; Libertelli et al., 2003), Adélie penguins are known to prey upon two trophic levels: krill (mainly *Euphausia superba* and *Euphausia cristallorophias*) and fish (mainly *Pleuragramma antarcticum*). These prey species have been segregated by their overall isotopic signature in Adélie Land, Antarctica: *Euphausia superba* constitutes a lower trophic level than fish and lives in more oceanic areas (Cherel, 2008). In addition, diet determined by stable-isotope analysis closely mirrors that determined from stomach content (Tierney et al., 2008) and there is a positive relationship between the proportion of fish consumed by Adélie penguins and their $\delta^{15}N$ values (Ainley et al., 2003).

Study protocol

This study was approved by the ethics committee of the French Polar Institute Paul Emile Victor.

Eighty individuals belonging to 40 pairs were followed. A few days before egg laying, the birds were weighed (electronic balance, ±2 g; Ohaus, Pine Brook, NJ, USA) and individually marked for identification with a subcutaneous transponder and a letter painted on their chest with Nyanzol-D, and some of them were handicapped (see below). Sex was determined *a posteriori* by using a combination of parameters including cloacal inspection before egg laying, copulatory position and incubation routine (Taylor, 1962; Kerry et al., 1993).

From the beginning of the incubation period to the crèche stage, we increased the cost of foraging by equipping one bird per pair (N=25 birds) with a large dummy Plexiglas device (25 mm×35 mm×60 mm, 60 g) attached with mastic, cyanoacrylate glue, Tesa tape and cable ties to the middle back feathers (Wilson et al., 1997). When considering the deleterious effects of instrumentation in diving animals, three main parameters have to be taken into account (Bannasch et al., 1994): (1) the shape of the device, (2) the attachment position and (3) the cross-sectional area (CSA) of the device relative to the animal's CSA. The consensus recommends attaching hydrodynamic instruments on the lower back, with a CSA of less than 1% of that of the animals, so as to prevent the generation of extra drag and extra foraging cost (Culik and Wilson, 1991; Bannasch et al., 1994). In the present study, the dummy devices were parallelepipeds (not hydrodynamic), attached to the middle back and their CSA represented 3–4% of the penguins' CSA. An instrument with a CSA 3.5% that of the penguin is likely to produce a drag similar to that of the bird (Bannasch et al., 1994). In addition, Culik and Wilson (Culik and Wilson, 1991) reported that the cost of transport was increased by 25% in penguins equipped with instruments representing ~2% of their CSA. As a result, we can confidently assume that our dummy devices increased the foraging cost of handicapped penguins. However, the size of the dummy device was chosen so as not to be too deleterious for the birds, according to previous studies which used devices of comparable size on Adélie penguins (Culik and Wilson, 1991; Culik and Wilson, 1992; Watanuki et al., 1992; Miller and Davis, 1993).

In total, 15 pairs were assigned to the control group (where neither mate in a pair was handicapped), 12 pairs to the handicapped-female group (where only the females were equipped with the device) and 13 pairs to the handicapped-male group (where only the males were equipped with the device). We distinguished three treatments at the pair level (control, handicapped-female and handicapped-male pairs), therefore resulting in six treatments at the parent level (Table 1).

Foraging trip duration was determined by visual nest observation ranging from every 2 h to continuous. The birds were captured and weighed a second time during the guard stage (40–45 days after egg laying), after a nest relief and just before leaving the colony to forage at sea. Body mass loss was defined as the difference between the first and the second weighing. Blood was collected from the wing vein with a heparinized syringe and centrifuged. Plasma and red blood cells were then quickly stored at –20°C. Because the capture and restraint constitute an acute stress which may influence baseline blood parameters (Jenni-Eiermann and Jenni, 1998; Cockrem et al., 2008), great attention was paid to minimizing the stress for the birds. The pengiun's head was covered by a hood (Cockrem et al., 2008) and handling duration was minimized and measured from the approach of the experimenter towards the nest until the end of blood sampling. A 5 min threshold was chosen as it has been shown that handling durations of less than 5 min had no effect on corticosterone levels in Adélie penguins (Vleck et al., 2000). Blood sampling depended on the bird departure and therefore occurred at any time of the day. Note that in Adélie penguins, no daily rhythm of corticosterone secretion has been reported (Vleck and Van Hook, 2002; Angelier et al., 2008).

Table 1. Foraging trip duration, body mass loss and physiological parameters of Adélie penguins according to their sex and their status (control, handicapped or partner birds)

	Control pairs (*N*=15)		Handicapped-female pairs (*N*=12)		Handicapped-male pairs (*N*=13)	
	Control males	Control females	Partner males	Handicapped females	Handicapped males	Partner females
Foraging trip duration (days)	0.97±0.28	1.02±0.28	1.01±0.31	1.84±0.72	1.62±1.08	1.05±0.29
Body mass loss (g)	504±288	386±260	487±320	696±198	752±274	378±234
[Corticosterone] (ng ml^{-1})	2.49±2.55	1.56±1.34	3.35±2.57	2.14±1.64	2.92±3.24	1.59±1.56
[Triglycerides] (mmol l^{-1})[a]	1.21±0.53	1.14±0.51	1.50±0.71	1.28±0.43	1.60±0.50	1.41±0.49
[Uric acid] (mmol l^{-1})	0.30±0.11	0.34±0.16	0.29±0.15	0.36±0.18	0.31±0.16	0.35±0.16

Data are means ± s.d.
Partner birds formed pairs with handicapped birds.
[a][Triglycerides] corresponds to estimated marginal means obtained by a general linear model with handling time as a covariate.

Laboratory analyses

Analyses of the plasma concentrations of corticosterone, triglycerides and uric acid were carried out at the IPHC-DEPE, France. Corticosterone levels were determined by immunoassay (Assay Pro, AssayMax Corticosterone ELISA Kit, St Charles, MO, USA) and concentrations of triglycerides and uric acid were measured using enzymatic colorimetric tests (Sigma Diagnostic, St Louis, MO, USA). Intra-assay and inter-assay coefficients of variation were between 1% and 3% for metabolite measurements and were 5% and 7%, respectively, for corticosterone measurements.

Tissue isotopic signature mirrors the diet throughout the period of tissue synthesis (Bearhop et al., 2002). For the birds of this study, the period between the first time they fed at sea and blood sampling was 37.6±2.0 days for females and 25.1±3.2 days for males (means ± s.d.). This time corresponds to the turnover of red blood cells (Hobson and Clark, 1993; Haramis et al., 2001; Bearhop et al., 2002). For these reasons, we chose to investigate isotopic signature in red blood cells because it reflects the diet of birds over the whole study period. Before isotopic analyses, red blood cells were lyophilized (48 h) and powdered (Hobson et al., 1997) but were not delipidated (Cherel et al., 2005). Stable carbon and nitrogen isotope assays were carried out at the Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés (CRELA), L'Houmeau, France. Intra-assay coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide were 0.88% and 0.63%, respectively. Inter-assay coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide were 0.42% and 0.24%, respectively. Results are expressed in the standard δ notation (‰) relative to PDB (Peedee belemnite) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

Data analyses

Body mass and body mass changes were compared between groups with general linear models. Comparisons of foraging trip duration were performed using a generalized linear model with a gamma distribution. For these comparisons, we considered only the guard stage period because corticosterone levels and nutritional state measured 40–45 days after egg-laying should better reflect this period.

For plasma parameters, we first checked whether handling duration was correlated with the plasma concentrations of corticosterone, triglycerides and uric acid, using Spearman correlations. Then, to compare these plasma concentrations between the different groups of birds, we used general linear models. If handling duration was correlated with the considered parameter, it was added as a covariate in the model. Normality of residuals was assessed using a Shapiro–Wilk test. When this condition was not fulfilled (corticosterone), we used a generalized linear model with a gamma distribution.

Analyses were conducted using SPSS 16.02 (SPSS, Chicago, IL, USA). Results are expressed as means ± s.d. and significance level was set at α=0.05.

RESULTS

Foraging trip duration

During the guard stage, bird treatment affected foraging trip duration (Wald χ^2=70.33, d.f.=2, P<0.001) with handicapped birds performing longer foraging trips (1.73±0.91 days) than control birds (1.00±0.28 days, P<0.001) and partner birds (1.03±0.30 days, P<0.001). In contrast, foraging trip duration was not influenced by the sex of the bird (Wald χ^2=2.78, d.f.=1, P=0.10) or by the interaction between the sex and the treatment of the bird (Wald χ^2=0.99, d.f.=2, P=0.61, Table 1).

Body mass

Bird body mass was similar during the courtship period between the three treatment groups ($F_{2,66}$=0.42, P=0.66) and between the treatment groups within males and females (interaction sex×treatment, $F_{2,66}$=1.21, P=0.31). However, during the courtship period, males were heavier than females (5.17±0.45 and 4.60±0.27 kg, respectively; $F_{1,66}$=39.13, P<0.001).

Forty to forty-five days after egg laying, handicapped birds had lost ~60% more mass (724±233 g, $F_{2,64}$=8.25, P=0.001) than control birds (445±276 g, P=0.002) and partner birds (432±285 g, P=0.003). Male and female birds lost body mass at the same rate ($F_{1,64}$=2.10, P=0.15) and the interaction sex×treatment was not significant ($F_{2,64}$=0.09, P=0.92, Table 1).

Effects of handling duration

Corticosterone levels and plasma concentrations of uric acid were not correlated with handling duration (Spearman correlations: R=–0.13, P=0.26; R=–0.05, P=0.63, respectively). However, plasma concentrations of triglycerides were correlated with handling duration (Spearman correlation, R=–0.42, P<0.001).

Corticosterone

Corticosterone levels were higher in males than in females (2.92±2.81 and 1.77±1.50 ng ml^{-1}, respectively; Wald χ^2=4.71, d.f.=1, P=0.03) but were not affected by the treatment or the interaction sex×treatment (Wald χ^2=0.86, d.f.=2, P=0.65 and Wald χ^2=0.57, d.f.=2, P=0.75, respectively; Table 1).

Triglycerides

Males and females exhibited similar plasma concentrations of triglycerides (1.43±0.62 and 1.27±0.49 mmol l^{-1}, $F_{1,69}$=1.83, P=0.18). This concentration did not significantly differ between the different treatment groups ($F_{2,69}$=2.52, P=0.09). In addition, the

interaction sex×treatment was not significant ($F_{2,69}$=0.45, P=0.64; Table 1).

Uric acid

Plasma concentrations of uric acid did not differ between males and females (0.30±0.14 and 0.35±0.16 mmol l^{-1}; respectively, $F_{1,79}$=2.37, P=0.13) and between the different treatment groups ($F_{2,79}$=0.01, P=0.91). The interaction sex×treatment was also not significant ($F_{2,79}$=0.05, P=0.95; Table 1).

Isotopic signature

Males had higher values of δ^{15}N (8.93±0.34‰, $F_{1,71}$=11.03, P=0.001) and lower values of δ^{13}C (–24.60±0.24‰, $F_{1,71}$=17.07, P<0.001) than females (δ^{15}N=8.67±0.36‰, δ^{13}C=–24.31±0.38‰). The value of δ^{15}N was not significantly affected by the treatment ($F_{2,71}$=2.36, P=0.10) whereas the value of δ^{13}C differed according to the treatment ($F_{2,71}$=5.22, P=0.008), with control birds presenting lower values than partner birds (P=0.02) and handicapped birds (P=0.01). In contrast, handicapped and partner birds presented similar δ^{13}C values (P=0.84). The interaction sex×treatment was not significant for δ^{15}N ($F_{2,71}$=1.67, P=0.20) or for δ^{13}C ($F_{2,71}$=0.44, P=0.65; Fig. 1).

DISCUSSION

Sex-specific foraging strategies

Our results highlight variable ecophysiological trends according to the sex or the treatment of birds. First, we confirmed the sex-specific foraging behaviour of Adélie penguins (Clarke et al., 1998). Males had higher values of δ^{15}N and lower values of δ^{13}C than females, suggesting that higher trophic levels contributed more to the diet of males than to that of females and that males tended to forage in more pelagic areas than females. Using the model proposed by Tierney and colleagues (Tierney et al., 2008) and the isotopic signature of prey (*E. superba* and *P. antarcticum*) given by Cherel (Cherel, 2008), krill contribution for females' diet was 42% while it was 37% for males' diet. The difference in δ^{15}N values between males and females was small but Ainley and colleagues (Ainley et al., 2003) found a positive relationship between the proportion of fish consumed by Adélie penguins and δ^{15}N values over a relatively small range of δ^{15}N values. δ^{15}N measurement therefore appears to be a sensitive tool able to detect small differences in the diet of animals. Moreover, our results on trophic levels *via* δ^{15}N values confirmed what is known about the diet of Adélie penguins: males and females both feed on krill (lower trophic level) but males feed more extensively on fish (higher trophic level) than females (Clarke et al., 1998; Tierney et al., 2009). This trophic difference between males and females is not exceptional in animals, particularly amongst penguins (Volkman et al., 1980; Forero et al., 2002; Forero et al., 2005; Norris et al., 2005; Bearhop et al., 2006; Awkerman et al., 2007). This may be due to different feeding requirements and/or foraging capacities between males and females and may serve to reduce the intra-specific competition on feeding grounds. Moreover this difference in the use of the habitat between males and females may be modulated by corticosterone levels, which are known to affect feeding behaviour and locomotor activity. The 60% higher corticosterone levels in males may drive them to forage in more pelagic areas. To confirm this hypothesis, further studies should experimentally modulate corticosterone levels and examine simultaneously the consequences in terms of the use of the habitat by males and females.

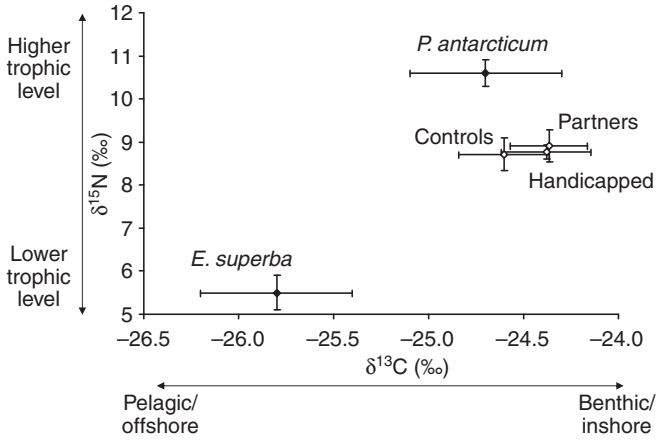

Fig. 1. δ^{15}N and δ^{13}C values of Adélie penguins (open symbols) and their prey (filled symbols). Values for prey come from Cherel (Cherel, 2008). Results are presented as means ± s.d.

Foraging strategies of handicapped penguins and their partners

As shown by prolonged foraging trips in handicapped birds, the handicap affected foraging behaviour. Consequently, we first hypothesized that handicapped and partner birds were exposed to a situation of stress (longer foraging trips for handicapped birds, suggesting a lower foraging efficiency, and prolonged fasting periods for their partners) so corticosterone levels should have increased and nutritional state should have been altered. However, our results show that handicapped and partner birds maintained their corticosterone levels and their nutritional state in a range comparable to that of control birds. Even in handicapped birds, in which body mass loss was increased (Table 1), corticosterone levels and nutritional state remained unchanged. In our study, corticosterone levels were low but comparable to those measured in Adélie penguins by Cockrem and colleagues (Cockrem et al., 2008), just after bird capture, so we can be confident that these values reflect baseline corticosterone levels. This consistency in corticosterone levels was also found in handicapped pied flycatchers *Ficedula hypoleuca* (Kern et al., 2007) and Cory's shearwaters *Calonectris diomedea* (Navarro et al., 2008).

At the beginning of the experiment, the handicap may have elevated corticosterone levels but this increase may have been only temporary (Suedkamp Wells et al., 2003). Several non-exclusive hypotheses may explain why corticosterone levels were not increased several weeks after the beginning of the experiment: (1) the handicap did not represent a significant chronic stress, (2) birds may have habituated to the stressor, (3) birds may have changed their foraging decisions to avoid a chronic stress. Indeed, as the stressor was always the same throughout the experiment, after some time its effects would no longer be unpredictable and thus the handicap probably would not represent a stressor anymore (hypothesis 1). Moreover, animals are expected to avoid situations of chronic stress to remain healthy because chronic stress is associated with physiologically deleterious effects (Sapolsky et al., 2000). To this end, after a repeated or a chronic exposure to a stressor, an animal is expected to habituate (Fig. 2) and to reduce its glucocorticoid response through an acceptation of the stressor and/or physiological feedback (hypothesis 2) (Romero, 2004).

In our study, birds even seem to have coped with the stressor, as they changed their foraging strategies (hypothesis 3, Fig. 2): they still fed on the same trophic levels but foraged in more coastal areas as suggested by the small but significant shift towards higher δ^{13}C

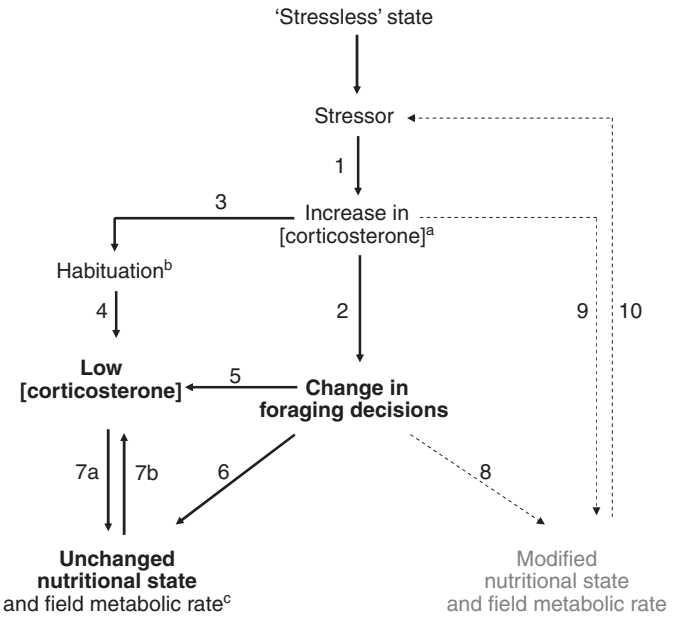

Fig. 2. Schematic view showing the potential ecophysiological responses of Adélie penguins to increased breeding constraints. Superscript letters refer to data from other studies, potentially transposable to our study: [a](Suedkamp Wells et al., 2003); [b](Romero, 2004); [c](Culik and Wilson, 1992). Data in bold were obtained in this study and bold arrows indicate the most probable linkage between these data. In our study, the stressors we considered were the low foraging efficiency due to the large dummy device attached to handicapped penguins and the prolonged periods of fasting for their mates. This may have led to a temporary increase in corticosterone levels (arrow 1) but the birds may have solved this stressful situation by foraging in more costal areas thus optimizing feeding time at the expense of travelling time (arrow 2). This behavioural change, associated with habituation to the stressor, for instance through an acceptation of the stressor and/or physiological feedback (arrow 3), may explain the low corticosterone levels (arrows 4 and 5) and unchanged nutritional state and field metabolic rate (arrows 6 and 7a). Conversely, unchanged nutritional state and metabolic rate may also allow corticosterone levels to remain low (arrow 7b). Though not observed in our study, other responses could have occurred: changes in foraging behaviour could have been insufficient to cope with the stressor (arrow 8) or could have not occurred at all (arrow 9), thus resulting in modified nutritional state and field metabolic rate. Finally, the modified nutritional state and field metabolic rate could be perceived as a potential stressor (arrow 10), thus resulting in a vicious circle, which does not seem viable over a long time scale.

values in handicapped penguins. Interestingly, Navarro and Gonzáles-Solís (Navarro and Gonzáles-Solís, 2007) found that handicapped Cory's shearwaters also modified their spatial distribution in the Atlantic Ocean (although $\delta^{13}C$ values remain constant) but did not change their diet (as suggested by constant $\delta^{15}N$ values). In our study, the difference in $\delta^{13}C$ values might be due to a difference in metabolic rate between handicapped and control penguins, but Carleton and Martínez del Rio (Carleton and Martínez del Rio, 2005) found in birds that an increased metabolism had no effect on the rate of ^{15}N incorporation into red blood cells and had a very small effect on the rate of ^{13}C incorporation. In addition, in our study, partner and control birds that were supposed to present similar metabolic rates, nevertheless exhibited different isotopic signatures. This suggests that the different $\delta^{13}C$ values observed between the experimental groups cannot be (fully) explained by different isotopic incorporation rates. In our study, the difference in $\delta^{13}C$ values between groups was small presumably because our $\delta^{13}C$ values encompassed several consecutive foraging trips, which were not necessarily all coastal. Yet, the resulting average still indicates that the overall $\delta^{13}C$ values were significantly smaller in controls than in other groups. The strategy of foraging in more coastal waters may allow birds to optimize feeding time by reducing travelling phases (back and forth) between the colony and the feeding grounds. The presumably lower efficiency of handicapped birds while travelling, diving and catching prey is likely to explain this change in their foraging behaviour. Culik and Wilson (Culik and Wilson, 1991) reported that instrumented Adélie penguins, swimming in a canal, had a 25% higher swimming metabolic rate than controls but they paradoxically found that the field metabolic rate during one foraging trip at sea was similar to that of controls (Culik and Wilson, 1992). They suggested that this discrepancy was possible if the foraging range was reduced. Our present data may confirm this hypothesis as instrumented penguins foraged in more coastal areas, presumably less distant than offshore areas.

Surprisingly, the partners of handicapped penguins adopted the same strategy as their mates. Because of the prolonged trips of handicapped birds, partners had to fast ~70% longer at the nest than control birds and therefore had an energetic debt when returning at sea to feed. If we consider that penguins lose 50 g per day when fasting (Chappell et al., 1993), the partners of handicapped penguins should have weighed approximately 250 g less than controls when they were weighed during the guard stage. Moreover, considering that the energy cost of fat and protein deposition is 53 kJ g^{-1} and that krill has a metabolizable energy content of 3.5 kJ g^{-1} (Chappell et al., 1993), partner birds would need an additional 3.8 kg of krill to compensate for their prolonged fasting periods. To maintain their body mass constant (Table 1), the alternatives for them could be: (1) to lengthen the duration of their foraging trips to catch more prey items, (2) to reduce the quantity of food given to the chicks or (3) to increase the rate of prey capture per foraging trip. The first strategy was not observed in our study while the other two seem possible. In addition, the data from our study give some support to the third hypothesis: the $\delta^{13}C$ signature showed that partner birds foraged in more coastal waters than control birds thus probably reducing travelling phases and optimizing feeding time per foraging trip. It would be worthwhile examining whether the same foraging strategy is adopted by penguins in natural conditions (i.e. poor food conditions) obliging the penguins to forage for longer and then forcing their partners to fast longer on the nest.

However these results raise a new question: why did control penguins not optimize their foraging trips similarly? One reason may be that they avoided a higher feeding competition in coastal areas by foraging offshore. Another hypothesis explaining the difference between control and handicapped penguins is that coastal waters are more predictable (Weimerskirch, 2007) but less productive than oceanic areas. Handicapped penguins were unable to forage and/or could not 'take the risk' of foraging in unpredictable oceanic waters even though these may be more productive. Partners of handicapped birds adopted a similar cautionary strategy and chose to forage in coastal waters in response to the long trips of their handicapped mates. In contrast, control birds with better foraging ability may be more flexible in exploring their environment and thus may be better able to cope with resource unpredictability and may find oceanic waters to be more productive grounds.

In our study, handicapped penguins opted for changing their foraging behaviour and not abandoning their breeding attempt, while sacrificing their body condition. This suggests that Adélie penguins can tolerate a lower body condition when breeding constraints

increase. However, such a strategy is not expected in long-lived animals that should prioritize their body maintenance. This may be because the body condition of handicapped penguins was not drastically altered. Indeed, Adélie penguins are able to support severe body mass losses (more important than that experienced by handicapped individuals) during their breeding cycle and particularly when they incubate (Cockrem et al., 2006). Even though handicapped individuals lost more body mass than control birds, this mass loss was in the physiological range for this species. In addition, abandoning their breeding attempt would obviously have allowed handicapped penguins to forage only for themselves but it would also have implied negative effects: (1) no breeding success the year they were handicapped and (2) a potentially diminished breeding success the subsequent year. Indeed, information on breeding performance can affect the probability of divorce (Dubois and Cézilly, 2002), potentially altering breeding success in Adélie penguins (Ainley et al., 1983).

Finally, our study could have been extended to the physiological and behavioural responses of handicapped-pair young as they represent the final level of parental investment. Because handicapped parents performed longer foraging trips, handicapped-pair chicks were less frequently provisioned during the guard stage (provisioning rate=1/foraging trip duration). Moreover, handicapped parents may have reallocated energy for their own maintenance and transferred the extra cost induced by the handicap to their offspring. To what extent handicapped-pair young present a lower body mass, an altered nutritional state and higher levels of corticosterone (known to affect begging behaviour) should be considered in future studies.

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