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Metabolic strategies in wild male Barbary macaques: evidence from faecal measurement of thyroid hormone

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Selection is expected to favour the evolution of flexible metabolic strategies, in response to environmental conditions. Here, we use a non-invasive index of basal metabolic rate (BMR), faecal thyroid hormone (T3) levels, to explore metabolic flexibility in a wild mammal inhabiting a highly seasonal, challenging environment. T3 levels of adult male Barbary macaques in the Atlas Mountains, Morocco, varied markedly over the year; temporal patterns of variation differed between a wild-feeding and a provisioned group. Overall, T3 levels were related to temperature, foraging time (linked to food availability) and intensity of mating activity, and were higher in the provisioned than in the wild-feeding group. In both groups, T3 levels began to increase markedly one month before the start of the mating season, peaking four to six weeks into this period, and at a higher level in the wild-feeding group. Our results suggest that while both groups demonstrate marked metabolic flexibility, responding similarly to ecological and social challenges, such flexibility is affected by food availability. This study provides new insights into the way Barbary macaques respond to the multiple demands of their environment.

1. Introduction

Understanding how metabolic strategies allow animals to cope with environmental and social demands is a fundamental goal of evolutionary ecology [1–3]. Consistent individual differences in basal metabolic rate (BMR), a measure of cellular activity of an organism at rest, have been positively associated with fitness enhancing traits such as growth, reproductive output and survival [1]. However, individuals with higher BMRs have higher energy requirements, and might suffer greater oxidative stress [4] and reduced longevity [5]; in situations of limited food availability higher BMRs can have negative effects on growth and survival [1]. Selection is therefore predicted to lead to the evolution of metabolic physiologies that are sensitive to energy availability and flexible to the competing needs of maintenance, growth and reproduction. Our understanding of the flexibility of metabolic strategies in wild animals is limited by the ability to collect data on *in situ* BMR, linked to variation in social and ecological parameters [1,2].

Thyroid hormones, T4 and the biologically more active T3, regulate energy metabolism and thus provide an index of BMR [3,6]. Studies show that variation in BMR due to climate [7], nutrition [8] and reproduction [3] is associated with variation in plasma T3 levels. Newly developed non-invasive methods to assess T3 from faecal samples provide a powerful tool to explore metabolic flexibility among animals in their natural environment [9], allowing for repeated measurements from the same individuals, without the need for capture.

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Table 1. Parameter estimates of the best-supported GLMM models (electronic supplementary material, table S1) explaining T3 levels, for (*a*) whole study period, (*b*) non-mating season, (*c*) mating season. Group = wild-feeding and provisioned, the former used as reference. Mating dyads = number of different mating dyads recorded each day. MinT° = minimum temperature. Foraging and resting time = % of hourly scans per day in these activities.

	estimate (s.e.)	*t*-value	χ^2	*p*-value
(*a*) whole study period				
intercept	271.41 (6.83)	39.77		
group	−14.41 (7.44)	−1.94	3.75	0.0528
MinT°	−2.31 (0.34)	−6.87	47.18	<0.0001
mating dyads	6.42 (1.09)	5.9	34.77	<0.0001
rainfall	1.28 (0.74)	1.73	3.01	0.0830
foraging time	−0.40 (0.16)	−2.47	6.10	0.0135
(*b*) non-mating season				
intercept	293.00 (6.80)	43.10		
group	−25.81 (6.80)	−3.80	14.40	0.0001
MinT°	−3.34 (0.37)	−9.35	87.50	<0.0001
foraging time	−0.46 (0.18)	−2.48	6.15	0.0131
(*c*) mating season				
intercept	239.28 (18.50)	12.93		
group	34.60 (10.89)	3.18	10.09	0.0015
MinT°	2.51 (0.65)	3.87	14.94	0.0001
mating dyads	4.84 (1.31)	3.70	13.66	0.0002
rainfall	2.56 (0.62)	4.10	16.84	<0.0001
foraging time	−1.11 (0.25)	−4.42	19.54	<0.0001
resting time	−0.22 (0.17)	−1.97	3.88	0.0988

In this study, we explored variation in T3 as an index of BMR among wild male Barbary macaques (*Macaca sylvanus*) living in the Atlas Mountains, Morocco. Here, macaques experience marked temperature variation, from +40°C in summer to −5°C in winter, seasonal scarcity of food and water [10] and strong breeding seasonality [11]. We collected data from two groups—one wild-feeding and one receiving food supplementation from tourists—across nine months, allowing us to explore how macaques respond, in terms of their energetic physiology, to food availability, as well as climatic and social challenges.

2. Material and methods

We followed the provisioned and wild-feeding groups for 5 and 2 days a week, respectively, from March to December 2012, and recorded animals' general activity: resting, travelling and foraging time (searching for, handling and consuming food; evidence indicates this measure is inversely related to food availability in this population [10]). We recorded the daily number of different mating dyads (as a measure of the intensity of mating activity), and collected data on temperature and rainfall from a nearby weather station. We assessed dominance rank using David's Scores [12]. Behavioural and climatic data were averaged over a 7 day period as thyroid hormones may take several days to adjust to changes in ambient temperature [13]; to take into account the 2 day T3 excretion lag [9], this period ran from 2 to 8 days before the date of faecal sample collection.

We collected 395 faecal samples and extracted T3, following Wasser *et al*. [9]. We analysed T3 using a total T3 enzyme-immunoassay from IBL International (RE55251).

We used GLMMs to analyse the relationship between climatic and behavioural variables and faecal T3 concentrations. Three sets of analyses were conducted: whole study period, non-mating season and mating season (table 1; electronic supplementary material, table S1).

See the electronic supplementary material for further details on methods.

3. Results

Ambient temperature varied markedly throughout the year, peaking in July–August and being lowest in December–February. Rainfall was also markedly seasonal, mainly falling September–December, with smaller peaks in January and April (figure 1*a*). The first mating seen in the provisioned group was on 15th September, and in the wild-feeding group on 10th October.

Average T3 levels varied seasonally (figure 1*b*). From March to June, levels were relatively constant within each group, but higher throughout in the provisioned one. T3 levels in the provisioned group dropped markedly from June to August; at the end of this period, a moderate decrease was seen in the wild-feeding group. One month before the onset of their mating seasons, both groups showed a clear increase in T3, beginning in August and September in the provisioned and wild-feeding groups respectively (figure 1*b*). T3 levels peaked in October in the provisioned group and at a higher level in November for the wild-feeding group.

Across the whole study period, T3 levels increased as foraging time and minimum temperature decreased—patterns also

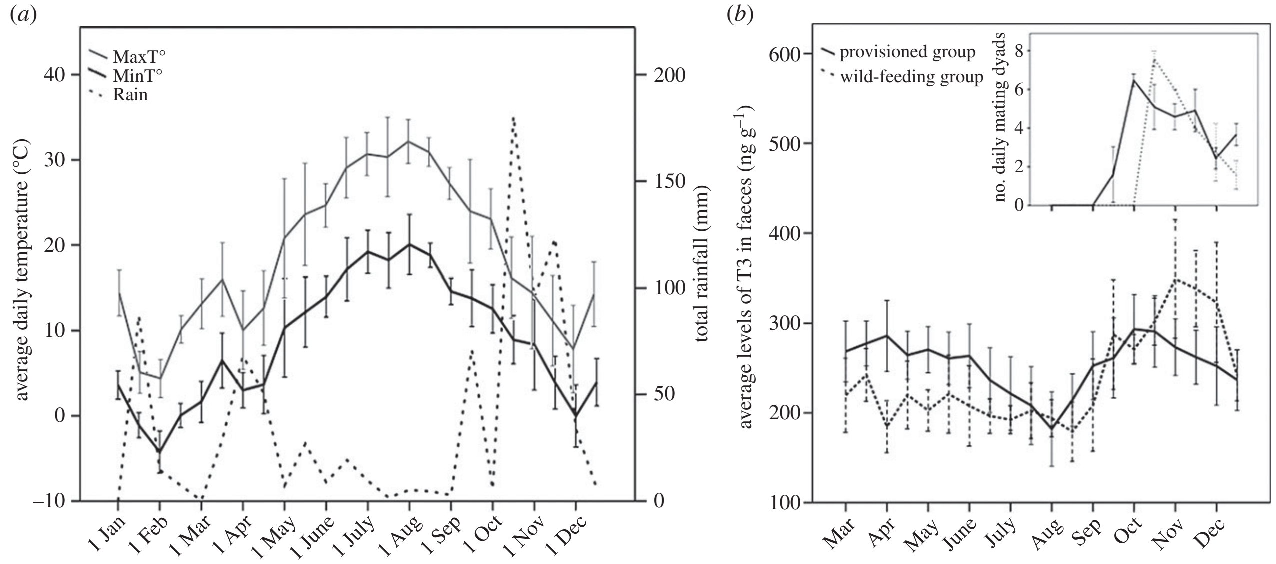

Figure 1. Bi-weekly variation in: (*a*) average (± s.d.) daily maximum (MaxT°) and minimum temperature (MinT°) and rainfall (Rain); (*b*) average (± s.d.) faecal levels of T3 and daily number of mating dyads in the provisioned and the wild-feeding groups; the *x*-axes of the main and inset graphs of (*b*) are aligned.

observed in the non-mating season—and as the number of mating dyads increased (table 1*a*,*b*). In the mating season, T3 levels again increased as foraging time decreased, and also as the number of mating dyads, minimum temperature and rainfall increased (table 1*c*). T3 levels were higher in the provisioned group in the non-mating season, and higher in the wild-feeding group in the mating season; over the whole period levels were higher (though not formally significantly, $p = 0.053$) in the provisioned group (table 1).

4. Discussion

Our analyses of T3 in wild adult male Barbary macaques suggest that BMR varied markedly over the year in relation to both environmental and social demands and that metabolic responses were mediated in part by nutritional status, providing evidence for flexible metabolic strategies in this species. A limitation of this study is that for logistical reasons, data were collected over a single nine month period, and consistency of patterns across multiple seasons and years cannot therefore be assessed. Nevertheless, our findings provide new insights into the adaptations of our study species to its challenging environment.

Across the whole study period and in both the mating and non-mating seasons, T3 levels were negatively related to foraging time, an inverse measure of food availability [10]. Moreover, T3 levels across the whole study were lower in the wild-feeding group. These results support the role of thyroid hormones in the regulation of the energetic physiology of vertebrates that downregulate their secretion in order to reduce BMR and save energy when nutritionally stressed [8].

Across the whole study period and in the non-mating season, levels of T3 showed a negative association with temperature. This is in line with studies demonstrating that endotherms increase BMR in response to lower temperatures, as a mechanism to generate metabolic heat, with this physiological response being mediated by thyroid hormones [7]. The provisioned group maintained higher T3 levels than the wild-feeding group in particular during spring, when temperatures were very low, indicating animals in the latter group were more constrained in their ability to elevate BMR as a thermogenic response at this time, potentially due to having lower fat reserves [14].

T3 levels increased markedly in both groups approximately one month prior to the start of their respective mating seasons (a similar phenomenon is seen in house sparrows [9]). These seasons started around one month apart, indicating the rise in T3 is not strictly tied to environmental cues such as photoperiod. In the mating season itself, increased T3 levels were linked to increases in intensity of mating activity, and to temperature and rainfall. Mating activity is highly energetically demanding in polygynous male primates [15], and Barbary macaque males mate at high rates and experience intense intra-sexual competition during the mating season [16]. An increase in BMR could therefore benefit males by increasing their aerobic capacity [17]. Furthermore, as T3 promotes testis maturation and semen quality [18], the occurrence and timing of the observed increase in T3 may reflect a resumption in testicular activity in preparation for the mating season. Notably, T3 levels dropped in both groups after their respective peaks in mating activity, suggesting there is a limit on how long elevated T3 can be maintained. This drop coincided with temperatures falling, perhaps explaining the unexpected positive correlation between these two variables. The positive link between rainfall and T3 may indicate a high thermoregulatory cost of wet fur.

Macaques' behavioural and dietary flexibility are well recognized as key factors underlying the adaptive radiation of this genus, and species' ability to inhabit a wide range of habitats [19]. Here, working with Barbary macaques, which inhabit one of the most extreme primate environments, we provide evidence that flexibility in energetic physiology is also an integral part of their adaptation, allowing these animals to respond to ecological and social challenges by regulating BMR. Nevertheless, this capacity appears to be constrained by food availability in the marginal, high altitude habitat in which remaining populations of this species are found [20] and where extreme winter conditions have led to significant mortality [10]. Thus, this study of physiological ecology provides new insights into the way Barbary macaques balance the multiple energetic demands of their harsh and highly variable environment.

Ethics. Our research protocol adhered to standards as defined by the European Union Council Directive 86/609/EEC, and the Ethics Committee of the University of Roehampton.

Data accessibility. Data are in the electronic supplementary material.

Authors' contributions. J.C.A., S.S. and A.M. conceived the study, interpreted results and drafted the manuscript; L.M. collected and collated all field data, calculated dominance, and prepared faecal samples; B.M. expedited all data collection at the field site; J.C.A. undertook laboratory work and data analysis; L.M. and B.M. contributed to the interpretation of data and manuscript revisions. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing interests. The authors declare no competing interests.

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