

1 **Metabolic strategies in free ranging male Barbary macaques: evidence from faecal**
2 **measurement of thyroid hormone**

3

4 Jurgi Cristóbal-Azkarate^{1*}, Laëtitia Maréchal^{2,3}, Stuart Semple³, Bonaventura Majolo², Ann
5 MacLarnon³

6

7 ¹University of Cambridge, Cambridge, CB23QY, UK

8 ²University of Lincoln, Lincoln, LN67TS, UK

9 ³ University of Roehampton, London SW154JD, UK

10

11 *Corresponding author: Division of Biological Anthropology, University of Cambridge, Pembroke
12 Street, Cambridge, CB23QG, UK; Tel: (44) 01223761227

13 Email: jca40@cam.ac.uk

14

15

16

17

18

19

20

21

22

23

24

25

26 **Abstract**

27 Selection is expected to favour the evolution of flexible metabolic strategies, in response to
28 environmental conditions. Here we use a non-invasive index of basal metabolic rate (BMR), faecal
29 thyroid hormone (T3) levels, to explore metabolic flexibility in a wild mammal inhabiting a highly
30 seasonal, challenging environment. T3 levels of adult male Barbary macaques in the Atlas
31 Mountains, Morocco, varied markedly over the year; temporal patterns of variation differed
32 between a wild-feeding and provisioned group. Overall, T3 levels were related to temperature,
33 foraging time (linked to food availability), and intensity of mating activity, and were higher in the
34 provisioned than the wild-feeding group. In both groups, T3 levels began to increase markedly one
35 month before the start of the mating season, peaking 4-6 weeks into this period, and at a higher
36 level in the wild-feeding group. Our results suggest that while both groups demonstrate marked
37 metabolic flexibility, responding similarly to ecological and social challenges, such flexibility is
38 affected by food availability. This study provides new insights into the way Barbary macaques
39 respond to the multiple demands of their environment.

40

41 Keywords: eco-physiology; climate; primate; thermoregulation; energetics; food availability

42 **1. Introduction**

43 Understanding how metabolic strategies allow animals to cope with environmental and social
44 demands is a fundamental goal of evolutionary ecology [1–3]. Consistent individual differences in
45 basal metabolic rate (BMR), a measure of cellular activity of an organism at rest, have been
46 positively associated to fitness enhancing traits such as growth, reproductive output and survival
47 [1]. However, individuals with higher BMRs have higher energy requirements, and might suffer
48 greater oxidative stress [4] and reduced longevity [5]; in situations of limited food availability
49 higher BMRs can have negative effects on growth and survival [1]. Selection is therefore predicted
50 to lead to the evolution of metabolic physiologies that are sensitive to energy availability and
51 flexible to the competing needs of maintenance, growth and reproduction. Our understanding of the
52 flexibility of metabolic strategies in wild animals is limited by the ability to collect data on *in situ*
53 BMR linked to variation in social and ecological parameters [1,2].

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

60 In this study, we explored variation in T3 as an index of BMR among wild male Barbary macaques
61 (*Macaca sylvanus*) living in the Atlas Mountains, Morocco. Here, macaques experience marked
62 temperature variation, from +40°C in summer to -5°C in winter, seasonal scarcity of food and water
63 [10], and strong breeding seasonality [11]. We collected data from two groups – one wild-feeding
64 and one receiving food supplementation from tourists – across nine months, allowing us to explore

65 how macaques respond, in terms of their energetic physiology, to food availability, as well as
66 climatic and social challenges.

67 **2. Material and methods**

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

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

80 We used GLMMs to analyse the relationship between climatic and behavioural variables and faecal
81 T3 concentrations. Three sets of analyses were conducted: whole study period, non-mating season
82 and mating season (tables 1, 1S).

83 See supplementary material for further details on methods.

84 **3. Results**

85 Ambient temperature varied markedly throughout the year, peaking in July-August and being
86 lowest in December-February. Rainfall was also markedly seasonal, mainly falling September-

87 December, with smaller peaks in January and April (figure 1a). The first mating seen in the
88 provisioned group was on 15th September, and in the wild-feeding group on 10th October.

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

93

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

101 Across the whole study period, T3 levels increased as foraging time and minimum temperature
102 decreased – patterns also observed in the non-mating season – and as the number of mating dyads
103 increased (table 1a,b). In the mating season, T3 levels again increased as foraging time decreased,
104 and also as the number of mating dyads, minimum temperature and rainfall increased (table 1c). T3
105 levels were higher in the provisioned group in the non-mating season, and higher in the wild-
106 feeding group in the mating season; over the whole period levels were higher (though not formally
107 significantly, $p=0.053$) in the provisioned group (table 1).

108

109

110 Table 1. Parameter estimates of the best-supported GLMM models (table S1) explaining T3 levels,
 111 for a) whole study period, b) non-mating season, c) mating season. Group= wild-feeding and
 112 provisioned, the former used as reference. Mating Dyads= number of different mating dyads
 113 recorded each day. MinT°= minimum temperature. Foraging and Resting Time= % of hourly scans
 114 per day in these activities.
 115

		Estimate (SE)	t value	χ^2	P
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

116

117 4. Discussion

118 Our analyses of T3 in wild adult male Barbary macaques suggest that BMR varied markedly over
 119 the year in relation to both environmental and social demands and that metabolic responses were
 120 mediated in part by nutritional status, providing evidence for flexible metabolic strategies in this
 121 species. A limitation of this study is that for logistical reasons, data were collected over a single
 122 nine month period. Nevertheless, our findings provide new insights into the adaptations of our study
 123 species to its challenging environment.

124

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

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

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

149 falling, perhaps explaining the unexpected positive correlation between these two variables. The
150 positive link between rainfall and T3 may indicate a high thermoregulatory cost of wet fur.

151 Macaques' behavioural and dietary flexibility are well recognised as key factors underlying the
152 adaptive radiation of this genus, and species' ability to inhabit a wide range of habitats [19]. Here,
153 working with Barbary macaques, which inhabit one of the most extreme primate environments, we
154 provide evidence that flexibility in energetic physiology is also an integral part of their adaptation,
155 allowing these animals to respond to ecological and social challenges by regulating BMR.

156 Nevertheless, this capacity appears to be constrained by food availability in the marginal, high
157 altitude habitat in which remaining populations of this species are found [20] and where extreme
158 winter conditions have led to significant mortality [10]. Thus, this study of physiological ecology
159 provides new insights into the way Barbary macaques balance the multiple energetic demands of
160 their harsh and highly variable environment.

161

162 **Ethics.**

163 Our research protocol adhered to standards as defined by the European Union Council Directive
164 86/609/EEC, and the Ethics Committee of the University of Roehampton.

165 **Data accessibility.**

166 Data are in supplementary material.

167 **Authors' contributions.**

168 J.C.A., S.S., A.M. conceived the study; L.M. collected and collated all field data, calculated
169 dominance, and prepared faecal samples; B.M. expedited all data collection at the field site; J.C.A.
170 undertook lab work and data analysis; J.C.A., S.S., A.M. interpreted results and drafted the
171 manuscript; L.M., B.M. contributed to manuscript revisions.

172 **Competing interests**

173 The authors declare no competing interests.

174 **Funding**

175 This research was funded by the Cambridge Humanities Research Grants Scheme.

176

177 **Acknowledgements**

178 We are grateful to Professor Mohamed Qarro (Ecole Nationale Forestière d'Ingénieurs, Morocco)

179 for his support in the field; to the Haut Commissariat aux Eaux et Forêts et à la Lutte Contre la

180 Désertification of Morocco for research permission; to Prof Jolanta Opacka-Juffry for help with

181 radio-recovery experiments; to Alan Rincon, Célia Gobeaut, Laura Martinez-Iñigo, Dorsa Amir,

182 Anna Nesbit and Marcelle Khalil for field assistance; and to Balbir Singh Josen for lab assistance.

183

184 **References**

185

- 186 1. Burton, T., Killen, S. S., Armstrong, J. D. & Metcalfe, N. B. 2011 What causes intraspecific
187 variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B*
188 *Biol. Sci.* **278**, 3465–3473. (doi:10.1098/rspb.2011.1778)
- 189 2. White, C. R. & Kearney, M. R. 2013 Determinants of inter-specific variation in basal
190 metabolic rate. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **183**, 1–26.
191 (doi:10.1007/s00360-012-0676-5)
- 192 3. Chastel, O., Lacroix, A. & Kersten, M. 2003 Pre-breeding energy requirements: thyroid
193 hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus*.
194 *J. Avian Biol.* **34**, 298–306. (doi:10.1034/j.1600-048X.2003.02528.x)
- 195 4. Harman, D. 1956 Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.*
196 **11**, 298–300. (doi:10.1093/geronj/11.3.298)
- 197 5. Ruggiero, C., Metter, E. J., Melenovsky, V., Cherubini, A., Najjar, S. S., Ble, A., Senin, U.,
198 Longo, D. L. & Ferrucci, L. 2008 High basal metabolic rate is a risk factor for mortality: the
199 Baltimore Longitudinal Study of Aging. *J. Gerontol. A. Biol. Sci. Med. Sci.* **63**, 698–706.
200 (doi:http://dx.doi.org/10.1093/gerona/63.7.698)
- 201 6. Hulbert, A. J. & Else, P. L. 2013 Basal metabolic rate: history, composition, regulation, and
202 usefulness. *Physiol. Biochem. Zool.* **77**, 869–76. (doi:10.1086/422768)
- 203 7. Silva, J. 2006 Thermogenic mechanisms and their hormonal regulation. *Physiol. Rev.* **86**,
204 435–464. (doi:10.1152/physrev.00009.2005)

- 205 8. Eales, J. 1988 The influence of nutritional state on thyroid function in various vertebrates.
206 *Am. Zool.* **28**, 351–362. (doi:10.1093/icb/28.2.351)
- 207 9. Wasser, S. K. et al. 2010 Non-invasive measurement of thyroid hormone in feces of a diverse
208 array of avian and mammalian species. *Gen. Comp. Endocrinol.* **168**, 1–7.
209 (doi:10.1016/j.yggen.2010.04.004)
- 210 10. Majolo, B., McFarland, R., Young, C. & Qarro, M. 2013 The effect of climatic factors on the
211 activity budgets of Barbary macaques (*Macaca sylvanus*). *Int. J. Primatol.* **34**, 500–514.
212 (doi:10.1007/s10764-013-9678-8)
- 213 11. Young, C., Majolo, B., Heistermann, M., Schülke, O. & Ostner, J. 2013 Male mating
214 behaviour in relation to female sexual swellings, socio-sexual behaviour and hormonal
215 changes in wild Barbary macaques. *Horm. Behav.* **63**, 32–39.
216 (doi:10.1016/j.yhbeh.2012.11.004)
- 217 12. de Vries, H., Stevens, J. M. G. & Vervaecke, H. 2006 Measuring and testing the steepness of
218 dominance hierarchies. *Anim. Behav.* **71**, 585–592. (doi:10.1016/j.anbehav.2005.05.015)
- 219 13. van der Lans, A. A. J. J. et al. 2013 Cold acclimation recruits human brown fat and increases
220 nonshivering thermogenesis. *J. Clin. Invest.* (doi:10.1172/JCI68993)
- 221 14. Borg, C., Majolo, B., Qarro, M. & Semple, S. 2014 A comparison of body size, coat
222 condition and endoparasite diversity of wild barbary macaques exposed to different levels of
223 tourism. *Anthrozoos* **27**, 49–63. (doi:10.2752/175303714X13837396326378)
- 224 15. Emery Thompson, M. & Georgiev, A. V. 2014 The high price of success: costs of mating
225 effort in male primates. *Int. J. Primatol.* **35**, 609–627. (doi:10.1007/s10764-014-9790-4)
- 226 16. Heistermann, M., Brauch, K., Möhle, U., Pfefferle, D., Dittami, J. & Hodges, K. 2008
227 Female ovarian cycle phase affects the timing of male sexual activity in free-ranging Barbary
228 macaques (*Macaca sylvanus*) of Gibraltar. *Am. J. Primatol.* **70**, 44–53. (doi:DOI
229 10.1002/ajp.20455)
- 230 17. Hayes, J. P. & Garland, T. 2007 The evolution of endothermy: Testing the aerobic capacity
231 model. *Evolution (N. Y.)* **49**, 836–847. (doi:10.2307/2410407)
- 232 18. Wagner, M. S., Wajner, S. M. & Maia, A. L. 2008 The role of thyroid hormone in testicular
233 development and function. *J. Endocrinol.* **199**, 351–65. (doi:10.1677/JOE-08-0218)
- 234 19. Thierry, B. 2007 Unity in diversity: Lessons from macaque societies. *Evol. Anthropol.* **16**,
235 224–238. (doi:10.1002/evan.20147)
- 236 20. Elton, S. & O'Regan, H. J. 2014 Macaques at the margins: The biogeography and extinction
237 of *Macaca sylvanus* in Europe. *Quat. Sci. Rev.* **96**, 117–130.
238 (doi:10.1016/j.quascirev.2014.04.025)

239

240