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Primates

ISSN 0032-8332
Volume 53
Number 1

Primates (2012) 53:31-40
DOI 10.1007/s10329-011-0268-0

Springer

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ORIGINAL ARTICLE

Intergroup variation in stable isotope ratios reflects anthropogenic impact on the Barbary macaques (*Macaca sylvanus*) of Gibraltar

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Received: 10 January 2011 / Accepted: 11 July 2011 / Published online: 1 September 2011
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Abstract Interactions with humans impact many aspects of behavior and ecology in nonhuman primates. Because of the complexities of the human–nonhuman primate interface, methods are needed to quantify the effects of anthropogenic interactions, including their intensity and differential impacts between nonhuman primate groups. Stable isotopes can be used to quickly and economically assess intergroup dietary variation, and provide a framework for the development of specific hypotheses about anthropogenic impact. This study uses stable carbon and nitrogen isotope analysis to examine intraspecific variation in diet between five groups of Barbary macaques, *Macaca sylvanus*, in the Upper Rock Nature Reserve, Gibraltar. Analysis of hair from 135 macaques showed significant differences in $\delta^{13}C$ and $\delta^{15}N$ values between a group with minimal tourist contact and groups that were main tourist attractions. Because we observed no overt physiological or substantial behavioral differences between the groups, feeding ecology is the most likely cause of any differences in stable isotope ratios. Haphazard provisioning by tourists and Gibraltarians is a likely source of dietary variation between groups. Stable isotope analysis and observational data facilitate a deeper understanding of the feeding ecology of the Barbary macaques relevant to the role of an anthropogenic ecology for the species.

Keywords *Macaca sylvanus* · Isotope analysis · Gibraltar · Provisioning · Tourism

Introduction

Traditional socio-ecological models used in assessing primate behavior rely on female distribution, patterns of competition (scramble vs. contest), presence/distribution of predators, resource availability, and forest structure/composition (Kappeler and van Schaik 2002; Strier 2007). These basic ecological constraints that we generally invoke in social-ecological explanations (Lambert 2010; Vogel and Dominy 2010) are directly impacted by human activities. Interactions between humans and nonhuman primates are an increasingly important aspect of primate studies, as more and more primates live in close contact with humans or face alterations of their habitats (Paterson and Wallis 2005; Wolfe and Fuentes 2007). The basic premise that human–nonhuman primate interconnections matter, and that human–nonhuman primate communities make up important components of some primate ecosystems, has gained substantial support over the last decade (Fuentes and Wolfe 2002; Paterson and Wallis 2005; Hockings et al. 2009). Expanding our notion of primate ecological communities to include anthropogenic components expands our understanding of key concepts and details in community ecology (Riley 2006; Hockings 2009; Fuentes and Hockings 2010; Riley and Fuentes 2011).

Many methods are available for determining the most basic aspects of feeding ecology—what primates eat, and how much of it they consume (Vogel and Dominy 2010).

Electronic supplementary material The online version of this article (doi:10.1007/s10329-011-0268-0) contains supplementary material, which is available to authorized users.

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These methods include field observations, macro- and microscopic analysis of gut contents and feces, gut passage times, analyses of dental and masticatory morphology, controlled food choice experiments, and stable isotope analysis of feces or animal tissues. Each method has its advantages and drawbacks. For example, field observations provide a menu of foods that are consumed and the frequency with which they are eaten, but are time consuming (hence expensive) and do not provide direct information about the nutritional contribution of individual foods. Field observations are also difficult to conduct on arboreal and/or semi-arboreal primates. Macro- and microscopic analysis of feces or gut contents can quantify some of the foods eaten, but some extremely important foods (such as meat) may not be detectable. Stable isotope analysis of animal tissues and feces is a very useful method for examining diet in animal populations that are difficult to study by direct observation. Variations in the carbon and nitrogen stable isotope ratios of animal tissues and excrement reflect dietary variation because the isotope ratios reflect the isotope ratios of the types and amounts of foods consumed. For example, stable isotopes have been used to examine such diverse ecological parameters as the geographic origins of animals and their migration patterns (Hobson 1999), niche width and fitness (Darimont et al. 2007), nutritional status (Hobson et al. 1993), and weaning in polar bears (Polischuk et al. 2001).

The stable isotopes of carbon and nitrogen are widely used in ecological studies of many species of animals (including living primates) and to reconstruct the diets of past primates, especially fossil humans (Schoeninger and Moore 1992; Lee-Thorp 2008). The stable carbon and nitrogen isotope ratios of a specific animal tissue (such as hair) are primarily determined by the isotopic composition of the diet. The carbon isotopes of an animal consuming terrestrial plants are determined by the photosynthetic pathways of the plants that are consumed. Plants using the C3 pathway have δ^{13}C values that range between −31 and −23‰ whereas plants using the C4 pathway range from −15 to −11‰ (O'Leary 1988). Plants using crassulacean acid metabolism (CAM) have values intermediate between those of C3 and C4 plants (Kluge and Ting 1978). For a given ecosystem, animal nitrogen isotope ratios are largely determined by the trophic level of the diet so that increased trophic level is correlated with higher values of δ^{15}N (DeNiro and Epstein 1981).

Although they have not yet been applied extensively to nonhuman primates (Sponheimer et al. 2009), the existing literature is diverse and provides a robust foundation for future studies. Stable isotopes have been used to reconstruct aspects of diet and habitat, such as the density of canopy cover (Schoeninger et al. 1998, 1999; Loudon et al. 2007), relative dietary importance of legumes (Schoeninger et al. 1997, 1998), feeding variations by sex and health status (Loudon et al. 2007), solitary versus group feeding (Schoeninger et al. 1998), feeding selectivity (Codron et al. 2005), and to evaluate chimpanzees (Sponheimer et al. 2006) and baboons (Codron et al. 2008) as ecological analogues for early hominin behavior. A recent study of isotopic variation in samples from rhesus macaques (*Macaca mulatta*) demonstrated regional variation in Asian macaque diets from museum specimens, although the sample sizes are relatively small (O'Regan et al. 2008).

Research demonstrates that nonhuman primates affected by human interactions have distinctive isotopic signatures (Loudon et al. 2007; Codron et al. 2008) and that this may be an important part of their ecological profiles. In this paper, we combine field observations of the feeding behavior of the Barbary macaques (*Maccaca sylvanus*) of Gibraltar with the stable carbon- and nitrogen-isotope analyses of their hair. Combining observations of feeding behavior of macaques with stable isotope analyses of their hair allows us to assess the impact of human provisioning on the diets of Gibraltar macaques.

Fossil records of the genus *Macaca* date back to up to 6–7 Mya in Algeria and Egypt (Delson 1980). The maximum distribution of *Macaca sylvanus* occurred during the late Pleistocene when they extended from England to the Mediterranean coast from Spain to Morocco (Delson 1980). Their current distribution is much diminished with remaining populations subsisting in the wild in the cedar and oak forests north of the Sahara Desert in Morocco and Algeria as well as in Gibraltar. *M. sylvanus* has been present in Gibraltar for at least 230 years, possibly much longer (Cortes and Shaw 2006). The Gibraltar macaques have been promoted as a tourist attraction since 1936. Their popularity has grown over the years and they currently serve as a vital source of revenue for the tourist industry of Gibraltar with an estimated 800,000 tourists visiting the reserve each year (Perez and Bensusan 2005). Tourists visit the reserve on guided bus or taxi tours and feed the monkeys human foods. These interactions with humans impact the ranging and activity patterns and the social behavior profiles of the Gibraltar Barbary macaques (Fa 1984; O'Leary and Fa 1993; Shaw and Cortes 2006; Fuentes 2006a; Fuentes et al. 2007a; Unwin and Smith 2010), and create the potential for consumption of unregulated human provisioned foods.

We examined stable isotopes in hair samples collected from 135 sympatric Barbary macaques, *M. sylvanus*, living in five distinct social groups within the Upper Rock Nature Reserve, Gibraltar. All five groups have access to naturally occurring foods as well as to provisioned fruits and vegetables, but vary in level of exposure to tourism. We hypothesize that the macaque groups least exposed to tourism and tourist provided foods will be identifiable via

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differences in $\delta^{13}C$ and $\delta^{15}N$ isotope ratios due to the differential properties of the foodstuffs in the diets.

Methods

More than 230 macaques now reside in at least five groups in the Upper Rock Nature Reserve, Gibraltar. The reserve was officially designated in 1993 and covers 97 ha, making up the majority of the rock of Gibraltar outside the city of Gibraltar and Caleta town (Fig. 1). The reserve contains a network of roads, a cable car station, two restaurants, a theatre and natural cave complex, and several former military installations (Perez and Bensusan 2005). The western side of the rock has a broad area of dense matorral scrub and developing woodland (Cortes 1994) as well as cliff areas. The eastern side is predominantly cliff face. The climate is typically Mediterranean with hot dry summers and cool wet winters, with mean temperatures ranging from 13 to 25°C (Cortes and Shaw 2006).

Our focal macaques resided in five distinct groups: Ape's Den, Prince Phillip's Arch, Royal Anglian Way, Farrington's Barracks, and Middle Hill. The sites have varying exposure to tourist traffic with the highest levels at Ape's Den and Prince Phillip's Arch due to bus and taxi tours, respectively. The Royal Anglian Way and Farrington's Barracks, though not official tour stops, have more limited interaction with tourists. The Middle Hill group lives within a former military base off limits to the public and thus has no exposure to tourist interaction.

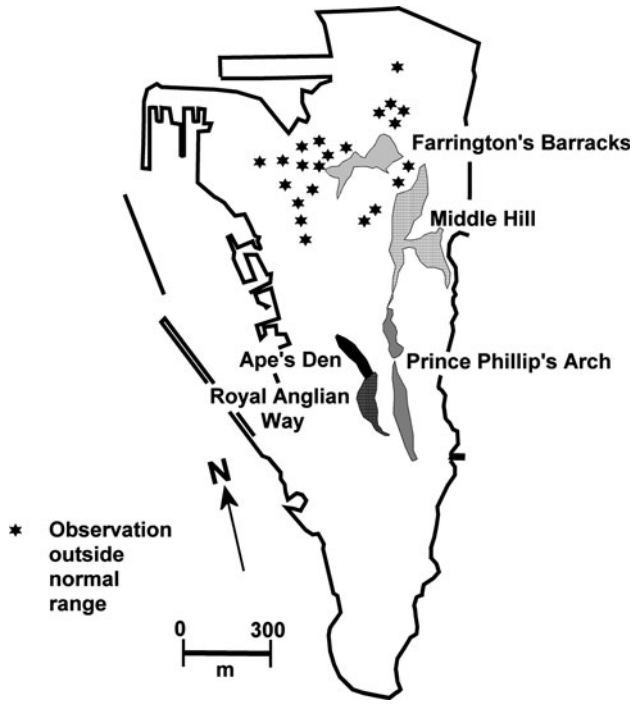

Fig. 1 Range of macaque groups and main interaction locations. Adapted from Perez and Bensusan (2005)

Provisioning of the Gibraltar macaques began in 1918 with the initial intent of keeping monkeys from wandering into nearby urban areas seeking food. From 1999, the responsibility for this provisioning has been contracted by the Gibraltar government to the Gibraltar Ornithological and Natural History Society (GONHS), a Gibraltar-based non-governmental organization. GONHS is responsible for providing food and water at established feeding sites and the cleaning of these sites. They also maintain a database of the population and, in association with the Gibraltar Veterinary Services, regularly trap the animals to tattoo, photograph, and insert micro-chips for identification as well as test for diseases, vaccinate, and collect biometric data from each individual (Cortes and Shaw 2006).

The macaques are provisioned daily with fresh fruits and vegetables (Table 1) in an amount equivalent to approximately 500 g per animal per day. Fruit and vegetables are cut into small pieces and distributed to the groups' feeding sites around 0700 hours every day when fresh water is supplied and sites are cleaned. Later in the day around (1300 hours), wheat and seed mix (commercial birdseed mix, primarily millet) are scattered at feeding sites (Cortes and Shaw 2006).

All macaque groups are provided with the same per animal amount of provisioning by GONHS, but Ape's Den and Prince Phillip's Arch also receive a large amount of food from tourists and taxi drivers throughout the year. Middle Hill receives no foods from tourists or taxi drivers (they do not interact with them at all).

Feeding observations

The Gibraltar government and veterinary services of Gibraltar approved this research as did University of Notre Dame Institutional Animal Care and Use Committee (protocol numbers 06-067 and 11-014). We collected feeding data at the Ape's Den, Prince Phillip's Arch, and Middle Hill sites between 0730 and 1830 hours from June 6 to 23 2006 and June 5 to 25 2009 during 30–120 min follows of a single individuals recorded in 10-min blocks. Fourteen observers recorded feeding incidences, the specific type of food, and whether it was provisioned or naturally occurring. We established inter-observer reliability at a 95% confidence level prior to the initiation of data collection. When a naturally occurring food was not immediately identifiable by an observer in the field, a sample of the plant was collected for later classification. Focal individuals were chosen based on a rotational basis between sexes and age groups.

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Table 1 Breakdown of food (kg/year) provided to the Gibraltar macaques for the year 2000 and 2001, excluding seeds (Cortes and Shaw 2006)

Food	2000	2001
Potato (*Solanum tuberosum*)	9,042.5	4,512.5
Carrot (*Daucus carota*)	6,305.0	7,280.0
Apple (*Malus domestica*)	5,934.5	7,467.0
Orange (*Citrus sinensis*)	5,639.0	2,491.0
Cabbage (*Brassica oleracea*)	3,901.5	5,176.5
Onion (*Allium sepa*)	3,627.0	3,655.0
Pear (*Pyus* sp.)	2,607.0	2,085.0
Cucumber (*Cucumis sativus*)	1,552.5	1,322.0
Tomato (*Solanum lycopersicum*)	1,552.0	4,829.0
Swede/Rutabaga (*Brassica napobrassica*)	1,360.0	
Sweet potato (*Ipomea batatas*)	899.5	67.5
Celeriac (*Apium graveolens*)	818.0	
Banana (*Musa acuminata*)	50.0	
Turnip (*Brassica rapa*)	45.0	
Green Bean (*Phaseolus vulgaris*)	20.0	
Aubergine (*Solanum melongena*)	10.0	
Melon (many genera, fam. *Cucurbitacea*)		3,180.0
Celery (*Apium gravioleus*)		1,300.0
Cauliflower (*Brassica oleracea*)		486.0
Red Cabbage (*Brassica oleracea*)		23.0
Pumpkin (*Cucurbita* sp.)		6.0
Total weight	43,363.5	43,879.5

Stable isotope analysis

We obtained the 135 hair samples used for this study from stored samples collected by GONHS between 2000 and 2006 during routine gathering of biometric data (107 were used in the final analysis). We bundled hair and segmented the bundle into lengths to provide a short-term record of diet averaged over just a few weeks (O'Connell and Hedges 1999; West et al. 2004). We washed and dried bundles of hair with acetone to remove contaminants, weighed them, carefully aligned them at the root end, and wrapped them lengthwise in analytical tin foil (we used the same foil in the standard sample cup for mass spectrometry) such that there was approximately 0.1 mg of hair per mm of sample length. We took three 4-mm samples (approximating 0.4 mg of hair) starting from the root end of each foil wrap. We then enclosed each sample in a tin foil cup to prevent hair loss and to form smooth, spherical samples. We combusted samples in a Carlo-Erba elemental analyzer, and determined stable carbon and nitrogen ratios and elemental abundances using a flow-through inlet system on a continuous flow isotope ratio mass spectrometer (Finnigan Delta Plus). We express the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios as δ values in parts per thousand or per mil (‰) relative to international standards: Vienna PeeDee Belemite (VPDB) for carbon and atmospheric N_2 for nitrogen. We used a working sulfanilamide standard ($\delta^{13}C_{VPDB} = -28.55 \pm 0.14$, $n = 10$; $\delta^{15}N_{AIR} = -2.44 \pm 0.23$, $n = 10$) that was calibrated to National Institute of Standards and Technology standards 8,542: Sucrose ANU (for carbon, $\delta^{13}C_{VPDB} = -10.45 \pm 0.07$) and 8,547: IAEA-N1 ammonium nitrate (for nitrogen, $\delta^{15}N_{AIR} = 0.4 \pm 0.2$) The NIST unfortunately does not offer a single standard that contains both carbon and nitrogen with isotope ratios in the range of biological materials so a working standard is necessary (and more economical). The analytical precisions are ±0.1‰ for carbon and ±0.2‰ for nitrogen. We ran three samples from each animal and report the mean value. We found no evidence for short-term changes in diet within the time span represented by 12 mm of hair. Variation between samples from the same animal appeared random and was similar to the analytical precision. Replicate samples produced mean standard deviations of ±0.4‰ for carbon and nitrogen.

We tested for isotopic differences between the five groups and between males and females within and between groups using analysis of variance (ANOVA) and post hoc least significant difference (LSD) tests in SPSS 16.0. We set statistical significance at $P = 0.05$.

Results

Feeding observations

Feeding records yielded a non-provisioned plant food list including 23 plant families and 31 species (Table 2). Naturally occurring (non-provisioned) foods made up approximately a quarter of feeding records at Ape's Den and Prince Phillip's Arch (23 and 24%, respectively) and more than 50% of the feeding records at Middle Hill. Across the sites, adult male macaques are significantly overrepresented in interactions with humans relative to their proportion in the population and adult female macaques are underrepresented in interactions relative to their proportion in the population (see also Fuentes 2006a). Immature macaques participated in interactions proportionally (52% of interactions, 54% of population). Also, At both Ape's Den and Prince Phillip's Arch sites immature macaques showed more non-provisioned feeding instances (68% at Ape's Den, 69% at Prince Phillip's Arch) than adult female macaques (22, 20%) or adult males (9, 11%).

Stable isotope analysis

The $\delta^{13}C$ values ranged from −22.6 to −20.2‰ and $\delta^{15}N$ ranged from 4.1 to 7.1‰ (Table 3). $\delta^{13}C$ and $\delta^{15}N$ were not

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Table 2 Wild plant species observed as food in 2006 (based on 1,293 feeding records) and previous reports for Gibraltar and Morocco (Fa 1984), grouped by family

Plant	Common name	Gibraltar 2006	Gibraltar 1984	Morocco
Acanthaceae	Acanthus family			
Acanthus mollis	Bear's Breech	X		
Anacardiaceae	Sumac family			
Pistacia terebinthus	Turpentine Tree or Terebinth	X	X	X
Pistacia lentiscus	Mastic tree or Lentisc	X	X	
Apiaceae[a,b]	Carrot family	X		
Ferula tingitana	Giant Tangier Fennel	X	X	
Daucus carota	Queen Anne's Lace or Wild Carrot	X		
Arecaceae	Palm family			
Chamaerops humilis	Dwarf Fan palm			
Aristolochiaceae[a]	Birthwort family			
Aristolochia baetica	Dutchman's Pipe or Pipe Vine	X	X	
Asteraceae[a]	Aster family			
Carduus spp.	Plumeless Thistle	X		X
Asteriscus maritimus	Gold Coin Daisy or Compact Gold Coin	X	X	
Andryala integrifolia	Rabbits Bread (Thistle)	X		X
Urospermum picroides	Prickly Goldenfleece	X		
Boraginaceae[b]	Borage family			
Echium creticum	Cretan Viper's Bugloss	X		
Echium vulgare	Common Viper's Bugloss		X	
Brassicaceae	Mustard/Cabbage family			
Hirschfeldia incana	Shortpod Mustard	X		
Fabaceae[a,b]	Pea family			
Ceratonia siliqua	Locust Tree	X		
Geraniaceae[a,b]	Geranium family			
Erodium chium	Stork's Bill	X		
Liliaceae[a]	Lily family			
Asparagus albus	Wild or White Asparagus	X		
Malvaceae[b]	Mallow family			
Lavatera cretica	Lesser Tree Mallow	X		
Moraceae	Mulberry family			
Ficus carica	Edible Fig	X		
Myrtaceae	Myrtle family			
Eucalyptus globules	Blue Gum	X		
Oleaceae	Olive family			
Olea europaea	Wild Olive	X	X	
Oxalidaceae	Wood Sorrel family			
Oxalis pes-caprae	Bermuda Buttercup or Cape Sorrel	X	X	
Pinaceae[a]	Pine family			
Pinus halepensis	Aleppo Pine	X		
Pinus pinea	Stone Pine or Umbrella Pine	X	X	
Poaceae[a,b]	Grass family			
Lagurus ovatus	Hare's Tail	X		
Melica minuta	Lesser Melick	X		
Primulaceae	Primrose family			
Anagallis arvensis	Scarlet Pimpernel	X		
Ranunculaceae	Buttercup family			
Clematis cirrhosa	Virgins Bower or December Clematis	X	X	

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Table 2 continued

Plant	Common name	Gibraltar 2006	Gibraltar 1984	Morocco
Resedaceae[a]	Mignonette family			
Reseda lutea	Wild Mignonette	X		
Solanaceae	Nightshade family			
Solanum alatum	Orange Nightshade	X		
Ulmaceae	Elm family			
Celtis australis	European Hackberry or Nettle Tree	X		

X reported for that dataset

[a] Other genus/species of same family eaten by macaques in Morocco (Fa 1984)

[b] Other genus/species of same family eaten by macaques on Gibraltar in 1984 (Fa 1984)

Table 3 Summary of mean δ^{13}C and δ^{15}N values of *Macaca sylvanus* hair from the Upper Rock Nature Reserve, Gibraltar by site and sex

Site	*n*	δ^{15}N (‰) ± SD	δ^{13}C (‰) ± SD
Ape's Den	12	5.3 ± 0.8	−21.8 ± 0.6
Females	4	5.4 ± 1.2	−22.0 ± 1.0
Males	8	5.3 ± 0.5	−21.7 ± 0.3
Prince Phillip's Arch	15	5.3 ± 0.5	−21.9 ± 0.4
Females	10	5.3 ± 0.2	−22.0 ± 0.4
Males	5	5.2 ± 0.9	−21.8 ± 0.3
Farrington's Barracks	11	5.0 ± 0.5	−21.9 ± 0.6
Females	4	5.2 ± 0.6	−22.2 ± 0.8
Males	7	4.9 ± 0.5	−21.7 ± 0.5
Royal Anglian Way	29	5.4 ± 0.8	−21.8 ± 0.5
Females	18	5.4 ± .80	−21.9 ± 0.3
Males	11	5.4 ± 1.0	−21.6 ± 0.6
Middle Hill	40	6.2 ± 0.7	−22.3 ± 0.7
Females	20	6.3 ± 0.8	−22.3 ± 0.6
Males	20	6.1 ± 0.7	−22.3 ± 0.8

significantly different between four of the sites: Ape's Den, Prince Phillip's Arch, Royal Anglian Way, and Farrington's Barracks (Table 4; Fig. 2). The Middle Hill group, however, showed δ^{13}C values that were significantly lower ($P < 0.05$) than the Ape's Den and Prince Phillip's Arch groups and δ^{15}N values that were significantly higher ($P < 0.01$) than all four of the other study groups (Table 4; Fig. 2). Within-group comparisons of males and females yielded no significant differences between the sexes for either δ^{13}C or δ^{15}N values (mean values for males and females generally differ by less than one SD; Table 3).

Discussion

Our observational data show that, although provisioned with an assortment of foods, Barbary macaques in Gibraltar still use a wide variety of naturally occurring foods from their environment for at least one-quarter of the observed feeding instances. The stable isotope ratios indicate that dietary and nutritional patterns as reflected in δ^{13}C and δ^{15}N vary significantly between some groups. These variations are likely due to exposure to tourists, an important aspect of the local anthropogenic ecology.

Feeding observations

Of the feeding records, 13 (62%) families and 10 (36%) species overlap with those reported for the Gibraltar population in the early 1980s (Fa 1984), giving a combined dataset of 36 plant families and 64 species used by the Gibraltar macaques for food. The consumption of naturally occurring foraged foods recorded by Fa (1984) and in this study (Table 2) indicate that the Gibraltar macaques also use a wide range of naturally occurring foods to supplement their provisioned diet. While there was substantial overlap between our study and the earlier study, differences in the number of plant species suggest that ecological/floral profiles have changed within the reserve over the course of the past 20 years. The consumption of 36 families of plants is typical for a macaque species that is generally characterized as having varied and opportunistic diets (Thierry 2007). Free-ranging *Macaca sylvanus* in Morocco consumed plants from 39 plant families (Fa 1984), with a total overlap with the Gibraltar-reported food families of 51%, suggesting that there is substantial overlap in food choice patterns and local ecologies between the Moroccan and Gibraltar macaques.

Macaque groups and age/sex classes showed different patterns of consumption of provisioned and non-provisioned foods as well as varied levels of interaction with tourists. Unwin and Smith (2010) show that feeding patterns varied between three groups of macaques in Gibraltar with differing levels of human contact. Fuentes (2006a) shows that, compared to Ape's Den, Prince Phillip's Arch has more interactions per hour, and more aggressive

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Table 4 Least significant difference (LSD) probability comparisons between sites for δ^{15}N

	Apes' Den	Royal Anglian Way	Middle Hill	Farrington's Barracks	Prince Phillip's Arch
Apes' Den	1				
Royal Anglian Way	1	1			
Middle Hill	**0.005**	**0.000**	1		
Farrington's Barracks	0.813	0.610	**0.000**	1	
Prince Phillip's Arch	0.999	0.992	**0.001**	0.886	1

Probabilities of <0.001 are in bold

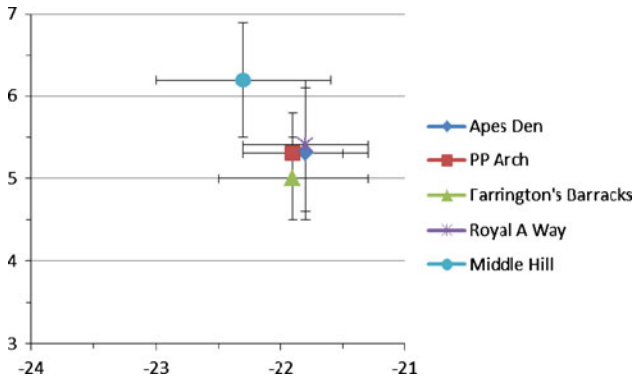

Fig. 2 Plot of the δ^{13}C values (*X* axis) and δ^{15}N values (*Y* axis) for macaques from the five groups in this study. The *symbols* represent the mean values and the *bars* show the standard deviations. See Table 3 for detailed values and *N* for each site

interactions with tourists. Our observational data demonstrate that there are quantitative and qualitative differences in feeding ecology, due to anthropogenic influences, between Ape's Den/Prince Phillip's Arch and Middle Hill sites. The data also suggest that immature macaques consume more non-provisioned forage than adults, with adult males consuming the least non-provisioned food. This is likely reflective of hierarchical structure within the group. Those with a higher social status would be more likely to have access to preferred provisioned foods (Fuentes 2010; Lambert 2010). This is consistent with patterns of behavior observed at the provisioning sites: we frequently saw older adult macaques sitting amongst the provisioned food feeding freely while younger monkeys visited the site sporadically. Access to provisioned foods due to hierarchical status may contribute to nutritional differences (Lambert 2010) and thus some of the variation in stable isotope ratios within the same group. However, between group variations in stable isotope ratios may also be related to the different feeding ecologies between the sites.

Stable isotopes and diet

Stable isotope analysis supports the observational data indicating variation in diet between the sites. The absolute magnitude of the isotopic differences between sites are small (less than 1‰) but statistically significant. While all sites are equally provisioned by the management team of GONHS, additional dietary provisioning linked to tourism at some of the sites may impact the diet of these macaques. The isotope data reflect the different levels and patterns of anthropogenic influence between the Ape's Den and Prince Phillip's Arch sites and the Middle Hill site. We suspect that the Farrington's Barracks and Royal Anglian Way sites lie somewhere between the two extremes in terms of tourist exposure and traffic. The significant differences in average carbon and nitrogen isotope ratios between the relatively isolated Middle Hill site and the high tourist density Ape's Den and Prince Phillip's Arch sites imply different physical and nutritional factors at the sites and support the hypothesis that frequency of exposure to human contact through tourism has a real impact on dietary patterns and nutrition (Fuentes 2006a). However, each group is healthy and tourism is changing the diet but not the overall nutritional status (see below).

The greater consumption of foods supplied by tourists and taxi drivers appears to play a pivotal role in macaque nutrition. Bus and taxi drivers serve as guides and frequently encourage interaction between the macaques and tourists, usually using food to entice the monkeys to approach tourists. They generally provide uncooked pasta or peanuts to the macaques as rewards for interacting with the tourists. The monkeys have come to associate these high calorie foods with humans and thus exhibit begging behavior as well as actively seeking out food to steal out of vehicles, bags, and people's hands (Fuentes 2006a). Monkeys living at the Ape's Den and Prince Phillip's Arch sites have increased exposure to tourist traffic and thus more opportunity for feeding on human foods. The addition of unregulated tourist feeding most likely reduces the amount of non-provisioned forage eaten by the macaques at heavily touristed sites.

The Middle Hill site has no obvious environmental differences from the other sites beyond exposure to tourism and the lack of traffic and roadways bisecting the groups range. Though stress levels have not been studied directly,

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simple density of humans is not a source of stress for the macaques at the heavily touristed sites (Fuentes 2006b). The sites provide ample space to avoid tourist exposure, thus those monkeys that do interact with humans are doing so by choice (Fuentes et al. 2007b). Because the macaques are choosing when to interact, their stress levels should be minimally impacted and differential stress levels are not a likely explanation of differences in isotopic patterns between Middle Hill and the other sites.

The hair of macaques from Middle Hill was slightly depleted in ^{13}C and slightly enriched in ^{15}N compared to those from the other sites. This group relies most heavily on natural forage. Although we did not measure the isotope ratios of the plants consumed by the Gibraltar macaques directly, published inventories of plant photosynthetic pathways can be used to infer the photosynthetic pathways of the plants that were observed being eaten (Szarek and Ting 1977; Szarek 1979; Ziegler et al. 1981; Batanouny et al. 1991; Andres 1993; Kalapos et al. 1997; Sayed 2001). C3 plants fix carbon using a photosynthetic pathway that produces $\delta^{13}C$ values between −31 and −23‰ (O'Leary 1988). C3 plants from different ecosystems can have different $\delta^{13}C$ values (Cerling et al. 2003), but such variation should be relatively minor on Gibraltar because of its small areal extent. The species listed in Table 2 are almost entirely C3 plants. Only the *Poaceae* family (grasses) are likely to contain C4 species (with a photosynthetic pathway producing $\delta^{13}C$ values between −15 and −7‰), but the two grasses consumed in 2006 are both cool season grasses and are therefore likely to be C3 (Ehleringer and Osmond 1994). None of the plants consumed by these macaques are likely to be CAM plants that use metabolic pathways that produce $\delta^{13}C$ values intermediate between those of C3 and C4 plants (Sayed 2001). Thus, while most foods provided to the macaques or obtained from the local environment are from C3 plants, macaques in contact with tourists are much more likely to obtain C4 carbon from human snack foods containing sucrose or corn sweeteners and are therefore likely to consume a diet slightly enriched in ^{13}C. The consumption of significant amounts of wild C4 or CAM plants could also produce higher $\delta^{13}C$ values, but that seems unlikely based on the feeding observations and the low frequency of C4 plants in the macaques' habitat. C4 plants are relatively rare in Europe, comprising between 0.5 and 2.5% of the flora, with many being invasive or naturalized species (Andres 1993; Kalapos et al. 1997).

The slightly higher $\delta^{15}N$ values seen in the Middle Hill samples are consistent with a diet containing a larger amount of wild plant foods and lower amounts of agricultural products such as snack foods.

The provisioned foods listed in Table 1 are obtained from commercial markets and can therefore be expected to have lower $\delta^{15}N$ values than wild foods. Synthetic agricultural fertilizers are produced from atmospheric nitrogen and have $\delta^{15}N$ averaging near 0 ± 3‰, whereas unfertilized soils have $\delta^{15}N$ values ranging from about 4 to 12‰ (Hebert and Wassenaar 2001; Annable et al. 2007). Consumption of a greater amount of wild plant foods obtained by foraging instead of human provisioned foods is a likely explanation for the slightly higher $\delta^{15}N$ values of the Middle Hill macaques. Lower legume consumption is another possible explanation for the observed pattern, as legumes generally are depleted in ^{15}N compared to other plant foods. One legume (green beans) was provisioned in 2000 (Table 1), but it was a very minor portion of the provisioned foods. The same is true for the single wild legume consumed (Table 2). The uncontrolled contribution of peanuts (also a legume) by tourists and their guides is difficult to estimate, but could contribute significantly to reduced $\delta^{15}N$ values at the sites most frequented by tourists.

One previous study of lemurs showed that high ^{15}N levels could be the result of suboptimal health (Loudon et al. 2007). Unpublished data from GONHS and the Veterinary office of Gibraltar indicate that the macaques of Middle Hill are not malnourished. Weight and measurement data collected in 1999, 2006, and 2009 indicate that Middle Hill body size fully overlaps with the other sites, and morbidity and mortality data are similar to the other groups in the reserve. Females of Middle Hill have healthy reproductive cycles and short birth intervals (Heistermann et al. 2006) providing further support that the macaques are not malnourished. The higher $\delta^{15}N$ values for the Middle Hill macaques are also unlikely to be due to other factors such as water stress (Ambrose 1991) or trophic level effects (DeNiro and Epstein 1981) because all groups have access to water on a daily basis and have not been observed to eat other animals, except occasional consumption of ants.

Differences in mean $\delta^{13}C$ and $\delta^{15}N$ values between groups are likely related to varied levels of exposure to tourists, an important and variable component of the anthropogenic ecology of the Gibraltar macaques. The increased reliance on naturally occurring forage is probably responsible for the reduced $\delta^{13}C$ and increased $\delta^{15}N$ values of the Middle Hill group when compared to the other four groups. This hypothesis should be tested with additional feeding observations and isotopic analysis of food items consumed. The isotopic patterns suggest that quantifying consumption of non-provisioned, non-naturally occurring foods provided by tourists and their guides should be an important focus of future field studies in order to most effectively characterize the socioecological pressures in the Gibraltar macaques.

Human tourists and nonhuman primates interact frequently in many different parts of the world (Fuentes

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2006b; Fuentes et al. 2007a; Fuentes and Hockings 2010) and thus anthropogenic ecologies are core to our models of the ecosystems. Field observations can be used to determine the intensity of human/nonhuman primate interactions at such sites, but it is a very time-consuming approach. This study indicates that isotopic analysis of hair can be used to quickly and economically assess one aspect of the intensity of impact of such anthropogenic ecologies given a basic prior understanding of the species ecology. The isotopic data can be used to define hypotheses in order to create more effective and economical field observation programs, likely to give a better understanding of the ecological context and characteristics at sites where humans and nonhuman primates interact in substantive ways. These isotopic measurements are especially useful because they could be used to quickly assess the likely spots for intensive, food-based, interactions between humans and nonhuman primates, and thus identify the most probable locations for testing micro-level variations in feeding behavior, habitat quality, food choice, and their relation to broader socioecological themes.

Acknowledgments We wish to thank the GONHS staff, especially Dale Lageua and Damien Holmes, the participants of the University of Notre Dame Gibraltar research teams, Drs. Lisa Jones-Engel and Gregory Engel, and Dr. Mark Pizarro. The University of Notre Dame Institute for Scholarship in the Liberal Arts and the Office of the Dean of the College of Arts and Letters partially supported this research. We also thank Dr. Julia Lee-Thorp and an anonymous reviewer for insightful commentary on earlier versions of the manuscript.

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