Obligate herbivory in an ancestrally carnivorous lineage: the giant panda and bamboo from the perspective of nutritional geometry

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Running Headline: Nutritional geometry of the giant panda

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Summary

1. Herbivores face various nutritional challenges in their life cycles, challenges that may become increasingly acute under ongoing environmental changes.

2. Here, focusing on calcium, phosphorus, and nitrogen, we used nutritional geometry to analyze individual-based data on foraging and extraction efficiencies, and combined these with data on reproduction and migratory behavior to understand how a large herbivorous carnivore can complete its life cycle on a narrow and seemingly low quality bamboo diet.

3. Behavioral results showed that pandas during the year switched between four main food categories involving the leaves and shoots of two bamboo species available. Nutritional analysis suggests that these diet shifts are related to the concentrations and balances of calcium, phosphorus and nitrogen. Notably, successive shifts in range use and food type corresponded with a transition to higher concentrations and/or a more balanced intake of these multiple key constituents.

4. Our study suggests that pandas obligatorily synchronize their seasonal migration and reproduction with the disjunct nutritional phenologies of two bamboo species. This finding has potentially important implications for habitat conservation for this species and, more generally, draws attention to the need for understanding the nutritional basis of food selection in devising management plans for endangered species.

Key words: feeding strategy, giant panda, nutritional geometry, life cycle, reproductive timing, Right-angled mixture triangles, seasonal migration

Introduction

It is widely accepted that herbivores face nutritional challenges, including low digestive efficiency of food due to high fiber (Milton 1979), plant-produced toxins (Rosenthal & Berenbaum 1991), and nutritionally imbalanced foods (Ritchie 2000; Elser *et al.* 2000, 2007). Such nutritional challenges may grow worse under ongoing climate change that shifts the range, timing, and physiological conditions of forage plants (Tuanmu *et al.* 2012). In response to such challenges, animals have evolved behavioral, developmental and physiological adaptations that interact across timescales to facilitate homeostasis and maintain performance (Mayntz *et al.* 2005; Rothman *et al.* 2011). The science of nutritional ecology aims to understand the ways that these interactions mediate the relationships between nutrient needs and ecological constraints (Raubenheimer *et al.* 2009). This helps to inform our understanding of the ecological and evolutionary processes that have shaped the diversity of animal foraging modes and to devise management strategies for endangered species and their habitats (Moore & Foley 2005; Raubenheimer *et al.* 2012).

An important requirement for maintaining fitness in the face of changes in nutrient supply or demand (e.g. at different stages in the life cycle) is compensatory homeostatic adjustment whereby foraging behavior and physiological processing of nutrients counter-act the changes (Raubenheimer *et al.* 2012). Such changes typically involve balancing the gain of several nutrients (e.g. usable energy, protein and amino acids, minerals), and are highly significant for individual fitness and also population dynamics (Pyke 1984; Lewis & Kappeler 2005; Taillon *et al.* 2006). In particular, females have specific nutritional requirements to support the demands of reproduction, and animals that live in seasonal environments must often adjust the

72 timing of reproduction and the pattern of foraging behavior to meet these nutritional demands

73 (Goldizen *et al.*1988; Rubenstein & Wikelski 2003).

74   The giant panda (*Ailuropoda melanoleuca*) is an endangered, obligate herbivore that

75 diverged early within an otherwise carnivorous clade (Qiu & Qi 1989; Wei *et al.* 2012; Zhao

76 *et al.* 2013). Uniquely within the order Carnivora, pandas specialize (~99%) on various

77 species of bamboo, resulting in a diet that is generally believed to be of poor quality due to

78 low protein and high fiber and lignin contents, contributing to its low dry matter digestibility

79 (Schaller *et al.* 1985; Hu *et al.* 1990; Wei *et al.* 1999; Zhu *et al.* 2011). Despite being

80 exclusively herbivorous, the giant panda retains the simple stomach and short gastrointestinal

81 tract typical of carnivores (Dierenfeld *et al.* 1982), and consequently needs to eat large

82 amounts of poorly digestible foods (Hu *et al.* 1990). This high degree of specialization on

83 large quantities of low quality food taken from a small number of plant species renders the

84 giant panda vulnerable to extinction in the face of environmental change (Colles *et al.* 2009).

85 Further, its recently acquired herbivorous lifestyle provides an especially interesting

86 opportunity for evaluating how shifts in herbivore behavior allow a species to cope with food

87 quality challenges involving the levels and balance of essential nutrients.

88   Previous studies have highlighted a number of interesting ecological and life history

89 characteristics that might be associated with the ability of panda populations to survive as

90 bamboo specialists (Schaller *et al.* 1985; Pan *et al.* 2001). Nevertheless, these relationships

91 remain poorly understood, largely because of the difficulty of obtaining intensive behavioral

92 data for these secretive animals. Other bears give birth to unusually small, altricial young, a

93 trait that has been associated with hibernation (Garshelis 2004). However, pandas do not

94   hibernate and yet they have the shortest gestation period (3 – 5.5 months) and give birth to

95   offspring that are the smallest of any bear species (Garshelis 2004; merely 0.1% of the

96   mother's weight). As with other bears, pandas have an embryonic diapause, known as

97   seasonal delayed implantation, in which the embryo remains suspended in the uterus in a state

98   of arrested development until it attaches and resumes growth, sometimes months later

99   (Schaller *et al*. 1985). While the adaptive significance of this remains uncertain (Thom *et al*.

100   2004), delayed implantation is believed to be an ecological adaptation to adjust the timing of

101   mating and the rearing of offspring to different seasonal environments (Sandell 1990).

102   In this study we used a combination of direct behavioral observations and

103   individual-based characterization of food intake and egestion to assess the nutritional

104   consequences of the seasonal food choices of pandas. We relate these choices to the timing of

105   altitudinal migration and other major life history events including seasonal mortality, mating,

106   gestation, parturition and lactation. We focused our analysis on the mineral nutrients calcium

107   and phosphorus, as well as nitrogen as a proxy for protein, because of the critical roles that

108   these nutrients play in growth and reproduction of animals, including mammals (White 1993;

109   McDowell 1996; Moen *et al*. 1999; Sterner & Elser 2002). Importantly, mammalian

110   requirements for calcium and phosphorus, principally for bone growth, are critically

111   inter-dependent (Van Soest 1994; Underwood & Suttie 1999) and thus of special interest in

112   the study of nutritional ecology of vertebrate herbivores. To examine the roles of these

113   multiple dimensions of nutritional quality and their inter-dependencies, we organize our

114   analysis using nutritional geometry, an approach for modeling the inter-active effects of

115   nutrients on animals (Raubenheimer 2011). Our objectives were: i. to elucidate the

116 relationships between the distinctive adaptations of these newly obligate herbivores, seasonal

117 habitat choice, food selection and nutrient gain in the extreme nutritional environment to

118 which they have become specialized, and ii. to learn whether the respective foods and habitats

119 of the giant pandas are inter-changeable (alternative sources of the same resources), or

120 complementary (provide different combinations of essential nutrients). The study thus

121 provides fundamental insight into the nutritional ecology of a highly unusual and ecologically

122 threatened herbivore, as well as critical information for the management and conservation of

123 panda habitat.

Methods

Study site and animals

126   This study was conducted in Foping Reserve, a key panda reserve, in the Qinling

127 Mountains, China. The Qinling Mountains contain a high density of wild giant pandas with a

128 population of 273 individuals (State Forestry Administration- China 2006). Two bamboo

129 species are the main diet resource of the pandas there, wood bamboo (*Bashania fargesii*) and

130 arrow bamboo (*Fargesia qinlingensis*), which grow at mean elevations of 1600 m and 2400 m,

131 respectively. These two bamboo species have different life histories. Wood bamboo (*WB*)

132 produces shoots in May and the shoots begin to sprout abundant new leaves in August. In

133 contrast, arrow bamboo (*AB*) produces shoots in early June and its shoots sprout a limited

134 number of new leaves in the following spring and considerably more new leaves in summer.

135 The leaves of *WB* persist year-round while the *AB* leaves drop off in winter.

136   With approval from the State Forestry Administration in China (2009-261), a total of six

137 pandas, three adult females and males, respectively, were fitted with GPS/VHF collars (Lotek

138   Wireless Inc., Ontario, Canada; Nie *et al*. 2012a.b, Zhang *et al*. 2014). This made it possible

139   to conduct intensive behavioral observations and collect food samples, enabling us to

140   determine individual-level seasonal food intake and obtain paired food- fecal samples for

141   chemical analysis and assessment of relative digestive extraction efficiencies.

142   **Observations of foraging behaviour and sample collection**

143   Using the GPS collars, over six years we tracked pandas from short distances (usually

144   10-20 m) to examine their seasonal pattern of food selection. Food and dung samples were

145   collected in the four foraging seasons of 2009 and 2010. During this period we also tracked

146   pandas to conduct behavioral observations at intervals of 3-5 days for each individual, except

147   when inclement weather prevented this. These observations enabled us to determine which

148   bamboo species, tissues, and ages were chosen by pandas. Paired food and fresh feces

149   samples were collected at each observed feeding patch during different foraging periods

150   year-round. We defined a feeding patch as an area with a size of ~300 x 300 m within which a

151   panda was observed feeding for at least 24 hours (because the gut passage time is usually

152   around 10-12 hours; Schaller *et al*. 1985). Bamboo leaf and shoot samples were collected

153   according to the age of plants; that is, one- and multi-year old leaves, and new and old shoots,

154   respectively. All food and fecal samples were coded by the feeding patch, dried in the field

155   station, and the plant samples were sorted by different bamboo species and tissues. The dried

156   plant and fecal samples were stored in zip-lock bags in the field for transport to laboratory.

157   **Life cycle and mortality data collection**

158   To examine the possible relationship between nutrition and reproduction strategy, we

conducted a study of the reproductive ecology of giant pandas by tracking collared animals

over six years from 2007 to 2012 (Nie *et al*. 2012a.b). We also analyzed long-term (37 years)

historical data of panda death and illness events in the wild from Foping Reserve records.

These data were used to explore the potential effect of food resource quality on the individual

lifespan and population dynamics of this endangered species.

Laboratory analyses

A total of 263 plant and fecal samples were collected in the field, including 66 shoots

and 47 fecal samples during shoot foraging season, and 100 leaf and 50 fecal samples in leaf

foraging season. All samples were ground to powder with a common multi-functional

laboratory mill and oven-dried at 70℃ and then weighed before laboratory analyses. We used

the micro-Kjeldahl method (Bremner 1996) to analyze N concentrations (% of dry mass). P

contents (% of dry mass) were measured by the ammonium molybdate method after

persulfate oxidation (Kuo 1996), standardized against known reference materials. Ca contents

(% of dry mass) were determined using an atomic absorption spectrometer after hydrofluoric

acid oxidation (Langmyhr & Thomassen 1973).

Data Analysis

We used Right-angled Mixture Triangles (RMTs, Raubenheimer 2011) to explore the

relationships among the proportional contents of nutrients in the foods and published

estimates of nutrient requirements. To estimate digestive extraction efficiencies of Ca, P and

N, we compared the proportional compositions of food samples with the associated feces

using RMTs. This method does not yield the absolute digestive efficiencies of separate

180   nutrients, for which measures are needed of the absolute intake and excretion of each nutrient.

181   Rather, by comparing the concentrations of nutrients in the food and matched feces we are

182   able to establish the *relative* extraction efficiencies of the focal nutrients (Raubenheimer 2011).

183   For example, if the concentration of P in the feces was half that in the matched foods, we

184   could not conclude that P was extracted with 50% efficiency, because we would not know the

185   extent to which the change in P concentration from food to feces was due to the extraction of

186   other nutrients (i.e., changes in the denominator rather than numerator in the concentration

187   ratio). However, if the Ca:P ratio in the food was twice the Ca:P ratio in the feces, then we

188   could conclude that Ca was extracted with higher efficiency (by a factor of 2) than P. Since in

189   this analysis we were interested in the relative extraction efficiencies of Ca, P and N, we

190   chose to use an RMT model in which each nutrient was expressed as a percentage of the sum

191   of the three nutrients [e.g., $\%Ca = Ca/(Ca+P+N) \times 100$] rather than as a percentage of the total

192   sample mass (i.e., grams Ca/100g sample). This enables the relative digestive efficiencies of

193   all three nutrients to be compared in a single model, and also excludes from the denominator

194   unaccounted components that might otherwise confound the comparison of relative extraction

195   efficiencies (Raubenheimer 2011). We could then use as a baseline the null model in which all

196   three nutrients are extracted from the food with equal efficiency, indicated in RMTs as the

197   situation where the Ca-P-N ratio of feces and the associated food is the same (i.e., the

198   composition points for food and feces are superimposed). Alternative outcomes would be

199   indicated by the vectors of displacement of feces composition relative to food; for example, if

200   the Ca:P ratio in feces is lower than in food, this indicates that Ca was extracted with higher

201   efficiency than P.

202   We used t-tests to compare the concentrations and ratios of nutrients in the foods of

203   pandas, and to compare the ratios of nutrients in the foods with matched fecal samples to

204   establish relative digestive efficiencies (as explained above). Levene's Test was used to test

205   for equality of variances, and where the null hypothesis of equal variances was rejected we

206   applied a modified t-test that does not assume equal variances. A Kolmogorov-Smirnov Test

207   was used to compare the observed monthly frequencies of mortality with the random null

208   model. One-sample t-tests were used to compare dietary calcium: phosphorus ratios with the

209   required ratios from the literature. All tests were performed using IBM SPSS v. 20.

210   **Results**

211   **Seasonal migration, feeding, reproduction and mortality**

212   **Seasonal movement and foraging patterns**

213   A mean of 52.5 ± 6.5 observation days were collected year-round for each animal with a

214   range of 43 to 61 days. Over an annual cycle, pandas fed on two bamboo species located at

215   different elevations, which we refer to as winter habitat and summer habitat (Fig. 1, 2). All six

216   collared pandas in this study showed a similar pattern of foraging transition, switching to *WB*

217   shoots in early May (range: 30 April to 4 May) when the shoots reached a height $(31.0 \pm 5.75$

218   cm) sufficient that pandas could eat them. In early June (3 June to 11 June), pandas moved to

219   higher elevation and switched to *AB* shoots, within a short period from 3 June to 11 June at

220   the time that low-elevation *WB* shoots had grown tall $(247 \pm 23.6$ cm) and become lignified.

221   Similarly, pandas switched to *AB* leaves when the shoots of *AB* had grown tall $(233 \pm 12.1$

222   cm). The mean time of the migrations to low elevation was at the end of August, within the

223   period of 12 August - 10 September. Finally, pandas preferred the younger leaves during the

224   two leaf periods. Thus, based on the seasonal migration and the specific tissues eaten from

225   these two bamboo species, there were four distinct foraging periods, two in which leaves and

226   two in which shoots were eaten: wood bamboo leaf period (*WBl*), wood bamboo shoot period

227   (*WBs*), arrow bamboo shoot period (*ABs*) and arrow bamboo leaf period (*ABl*, Fig. 2). During

228   the *ABl* period, pandas sometimes ingested a small portion of the stems when eating leaves

229   but we did not include these infrequent occurrences in the data analysis.

230   **Reproductive timing in giant pandas**

231   Over several consecutive years, March (15 mating events; 68%) and April (7 mating

232   events; 32%) were the main months for mating for the Qinling Mountain pandas. Pregnancy

233   lasted 4-5 months (collared females: 137-143d; 146-151d; 132-138d), of which 1.5-2 months

234   comprised post-implantation gestation (Schaller *et al*. 1985). The females gave birth in a short

235   period between mid-August and early September: there were 4 births in mid-August (57%); 2

236   births in late August (29%) and one in early September (14%) during our study (Fig. 2).

237   **Seasonal pattern of mortality of giant pandas**

238   A total of 25 dead or ill pandas were observed in the wild over the past 37 years in

239   Foping Reserve. More than half (52%) of these occurrences were in March and April, a

240   frequency that was statistically greater than expected by chance ($Z = 1.61$; $P = 0.01$). This

241   period corresponds to the end of the longest time on any of the four diets—the *WB* leaf period

242   (Fig. 2).

243   **Nutritional composition of foods**

244   **Nitrogen-phosphorus relationships**

245   Figure 3a shows the composition of the seasonal diets in terms of N and P and a range of

246   N to P ratios that are likely to encompass biomass requirements of mammals based on

247   proximate chemical composition and investment in muscle and bone for an animal of this

248   body size (Elser *et al*. 1996). The nutritional implications in relation to N and P for pandas for

249   the spring switch from old *WB* leaves to *WB* shoots are denoted by the solid arrow labeled 1

250   in Fig. 3a. Young shoots (early May) had substantially higher concentrations of N ($P < 0.001$)

251   and P ($P < 0.001$) than the leaves, with disproportionately more P and hence a lower N:P ratio

252   ($P < 0.001$). As the shoots matured through May and early June the concentrations of both N

253   ($P < 0.0001$) and P ($P < 0.0001$) dropped, but the N:P ratio remained unchanged ($P = 0.097$).

254   The arrow marked 2 in Fig. 3a shows nutritional changes associated with the switch from

255   old *WB* shoots to young *AB* shoots in June (see Fig. 2). The N:P ratio did not differ between

256   the foods ($P = 0.98$), but the concentrations of both N ($P < 0.0001$) and P ($P < 0.0001$) were

257   higher in *AB* shoots, indicating a shift to foods that allow greater intake of these nutrients.

258   By mid-July the concentrations of both N and P in the *AB* shoots had significantly

259   declined ($P < 0.001$), although the N:P ratio barely changed ($P = 0.057$). This corresponded

260   with a switch in July from the older shoots to young leaves of *AB* (solid arrow marked 3 in

261   Fig. 3a), which were higher both in N ($P < 0.0001$) and P ($P < 0.0001$), with an increased N:P

262   ratio ($P < 0.0001$).

263   Between mid-July and August, the concentrations of N ($P < 0.0001$) and P ($P < 0.0001$)

264   had decreased in *AB* leaves, and the N:P ratio had increased from 11.9 ± 0.35 to 13.9 ± 0.57 (*P*

265    $= 0.005$), whereupon female pandas migrated to the lower altitude foraging site and fed on

266   young leaves of *WB*. These leaves had a significantly higher P content ($P = 0.016$) with

267   similar N content ($P = 0.997$), and consequently a lower N:P ratio ($P = 0.012$) than the older

268   *AB* leaves.

269   From August to April, when the pandas once again switched to young shoots of *WB*

270   (arrow 1 in Fig. 3a), the concentrations of both N ($P < 0.0001$) and P ($P < 0.0001$) in *WB*

271   leaves decreased, and the N:P ratio increased ($P = 0.002$).

272   **Calcium-phosphorus relationships**

273   The relationships between Ca and P in the dietary transitions by pandas through the

274   annual cycle are shown in Fig. 3b. The significant increase in dietary P concentration (see

275   above) corresponding with the spring switch from leaves to shoots of *WB* (Fig. 2) was

276   accompanied by a reduction in Ca ($P < 0.0001$), and consequently a strong decrease in the

277   Ca:P ratio ($P < 0.0001$). Thereafter, as the shoots aged, the concentration of Ca dropped ($P <$

278   0.0001) together with the concentration of P (see above). However, Ca dropped more steeply

279   than P, resulting in a significant reduction in the Ca:P ratio ($P < 0.0001$). The Ca:P ratios of

280   shoots in the early, mid and late season were 0.26 ± 0.02, 0.19 ± 0.02 and 0.09 ± 0.03,

281   respectively; all of these are substantially lower than the Ca:P ratio of 1-2 recommended in

282   the diets of mammals (van Soest 1994, Underwood & Suttie 1999; Buchman & Moukarzel

283   2000) ($P < 0.0001$). In contrast, the mean Ca:P ratio of mature *WB* leaves was 3.4 ± 0.24,

284   which is significantly higher than the maximum of 2 recommended for mammals ($P <$

285   0.0001).

286   The switch from the old shoots of *WB* to young shoots of *AB* (arrow 2 in Fig. 3b) was

287   associated not only with a significant increase in P (see above), but also with a proportionately

288   larger increase in Ca ($P < 0.0001$). Consequently, the Ca:P ratio was higher (closer to the

289   recommended range) in young *AB* (0.25±0.02) than the older *WB* shoots (0.09±0.03, *P* <

290   0.0001). As *AB* shoots aged, there was a reduction in P (see above) and Ca (*P* < 0.0001), and

291   a decrease in the Ca:P ratio from 0.25 to 0.10 (*P* < 0.0001).

292   In mid-July, when the pandas switched from shoots to young leaves of *AB* (arrow 3 in Fig.

293   3b), the leaves were significantly higher in both P (above) and Ca (*P* < 0.0001), with a

294   substantially higher Ca:P ratio (*P* < 0.0001). At a value of 0.10±0.02, the Ca:P ratio of shoots

295   was an order of magnitude lower than the recommended minimum for mammals of 1 (*P* <

296   0.0001), while the value for leaves (2.49±0.08) was marginally but significantly greater than

297   the recommended maximum ratio of 2 (*P* < 0.0001).

298   By mid-August the leaves of *AB* had reduced in both P (see above) and Ca (*P* < 0.0001)

299   concentrations, but the Ca:P ratio remained unchanged (*P* = 0.84). At this point the female

300   pandas moved to a lower elevation and switched to the younger leaves of *WB*, which had a

301   significantly higher P content (above) and marginally higher Ca (0.51±0.04% vs.0.43±

302   0.02%; *P* = 0.08). The Ca:P ratio did not differ between the leaves of the two species (*P* =

303   0.97).

304   From mid-August, when female pandas started eating young *WB* leaves, to April when

305   they switched from the now older leaves to young *WB* shoots (arrow 1 in Fig. 3b), P

306   concentration in the leaves dropped (above) but there was no change in Ca (*P* = 0.63).

307   Consequently, the dietary Ca:P ratio of the *WB* leaves increased from 2.5±0.13 in

308   mid-August to 3.4±0.24 in April (*P* = 0.002).

309   **Relative digestive extraction efficiencies**

310   Our analysis (Table 1) showed that, relative to N, both Ca and P were enriched in feces

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compared with shoots, whether the food species was *WB* (Fig. 4a) or *AB* (Fig. 4b). This

demonstrates that N was extracted from shoots with higher relative efficiency than either P or

Ca. Additionally, the Ca:P ratio in feces was higher than in shoots, indicating that Ca was

extracted with lower relative efficiency than P, thus exacerbating the effective deficit of Ca

relative to P in shoots. In both species of bamboo, the feces associated with leaves were

relatively enriched in Ca, whereas the proportional concentration of P was statistically

unchanged in leaves and feces (Table 1, Figs 4a and 4b).

Discussion

This is the first field study to use nutritional geometry to explore the relationship

between the balance of essential nutrients, selection of foraging habitat, and the life cycle of a

highly endangered herbivore species. Our data showed pandas experience marked seasonal

foraging changes with four primary foraging periods corresponding to the annual phenology

of the two bamboo species. The nutritional quality of the diet was heterogeneous through the

year, both in terms of the absolute concentrations of N, P, and Ca and the proportional

balance of these nutrients. Seasonal diet switches corresponded with shifts in quantities of

these key nutrients, as did the life cycle, reproduction and pattern of altitudinal migration. As

we will discuss, the close correspondence between animal life history events, shifting range,

and forage quality suggest that nutritional balancing is a contributing component that

maintains the population of this endangered species, an insight that may be crucial in its

conservation as well as that of other endangered species that have narrow dietary ranges.

Our analysis showed that the N-P-Ca composition of diets varied with bamboo species,

plant part, and the age of the plant part. Age influenced primarily the concentration of

333 nutrients, which declined as both leaves and shoots matured (Figs 3a and 3b), most likely due

334 to an increase in plant structural components such as cellulose, hemicellulose, and lignin (Hu

335 *et al*. 1990). In the absence of reliable measures of daily food intake (Rothman *et al*. 2011),

336 which cannot readily be obtained for wild giant pandas in the field, it is difficult to interpret

337 the significance of the concentration of a nutrient in foods in relation to the animal's

338 requirement for that nutrient. Invariably, however, diet switches by pandas corresponded with

339 a change to younger, more nutrient-rich alternatives, whether this involved a different plant

340 part (e.g., arrow 1 in Figures 3a and 3b) or species (e.g. arrow 2 in Figures 3a and 3b). This

341 preference for younger tissues could relate to their higher nutrient concentrations compared

342 with older tissues in which nutrients are diluted by greater concentrations of structural

343 components. Additionally, the biomechanical properties associated with plant structural

344 components can also reduce the nutritional quality of foods (Clissold *et al*. 2009). Although

345 we did not measure plant-produced allelochemicals, these might play a similar role

346 (Launchbaugh *et al*. 2001).

347   Nutrient concentrations also differed between species of bamboo, but these differences

348 were contingent on the plant part. Specifically, the young shoots of wood bamboo had

349 considerably higher N, P, and Ca concentrations than the young arrow bamboo shoots,

350 whereas the species difference was less marked and reversed for leaves (Figs 3a and 3b). The

351 greatest difference between plant parts, however, was in the balance of nutrients. Leaves had a

352 higher N:P ratio (Fig. 3a) and a substantially higher Ca:P ratio (Fig. 3b) than did shoots, and

353 this contrast applied for both bamboo species. Such differences in nutrient balance can be a

354 significant parameter of food quality, because nutrient balance determines the ways that

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355　nutrients interact in their effects on consumers (Sterner & Elser 2002; Simpson &

356　Raubenheimer 2012).

357　　　An important consideration in inferring the functional significance of seasonal diet

358　switches in giant panda is therefore their implications for nutrient balance. Thus, the spring

359　switch by the giant pandas in our study from leaves to shoots of wood bamboo corresponded

360　with increased dietary N content, which could well be an important functional driver of the

361　switch (White 1993). Significantly, dietary P content increased to a proportionally even

362　greater extent than N, and consequently the dietary N:P ratio decreased. Since the N:P ratio of

363　wood bamboo leaves was higher than the optimal range, the decrease corresponding to the

364　switch to shoots brings the N:P ratio in the diet more in line with estimated requirements (the

365　shaded area in Fig. 3a). The higher P concentrations in the shoots of wood bamboo do,

366　however, have important consequences for giant pandas in relation to the dietary Ca:P ratio.

367　　　Dietary Ca:P ratios of 1:1 to 2:1 are recommended for mammals (Fig. 3b), with excesses

368　of either nutrient interfering with the absorption and metabolism of the other (Robbins 2001).

369　When the Ca:P ratio drops much below 1, P impedes absorption of the already limiting Ca,

370　resulting in Ca resorption from bones and ultimately osteomalacia (softening of the bones)

371　and associated diseases. In both human and animal studies, Ca:P ratios less than 0.5 have been

372　associated with reduced bone mass density and compromised bone strength (Calvo & Tucker

373　2013). These effects can be particularly acute in relation to reproduction because of its

374　increased calcium requirements for lactation and bone growth (Schulkin 2001). It is therefore

375　noteworthy that the high levels of P in young wood bamboo shoots in our study resulted in

376　Ca:P ratios of considerably less than 1 (0.2) . In contrast, Ca-P ratios in leaves were closer to

377 the recommended range for mammals, being marginally above 2 (Fig. 3b). In general, Ca:P

378 ratios higher than 2 (surplus Ca) are tolerated by herbivores to a greater extent than ratios less

379 than 1 (surplus P) (Robbins 2001).

380   It is important to note that measures of Ca:P ratios in plant foods might not accurately

381 represent the biologically effective Ca:P ratios, because a proportion of these elements might

382 be bound in molecular complexes that render them unavailable (Suttle 2010). For example

383 phytic acid is an important storage form of P in many plant tissues (especially seeds), and is

384 poorly digested by non-ruminant herbivores. However, if the relative excess of P in the panda

385 diet was significantly influenced by unavailable P in this way, then we would expect the feces

386 would be more highly P-enriched relative to Ca, but this was not the case. Rather, the feces

387 produced from bamboo shoots were enriched in Ca relative to P (i.e. the Ca:P ratio of feces

388 was greater than the Ca:P ratio of the shoots), and therefore the post-absorptive Ca:P ratio

389 associated with shoots was even lower than 0.2 (Fig. 4). The relatively high Ca levels in the

390 feces are consistent with the interfering effect of surplus P on Ca absorption discussed above.

391 By contrast with shoots, the feces associated with leaves were enriched in Ca but had

392 statistically similar P contents to the leaves (Fig. 4). This selective egestion of Ca would bring

393 the Ca:P ratio of leaves, which was marginally higher than 2, more closely in line with the

394 recommended range.

395   Our nutritional analysis therefore implies that, when pandas switch from a diet of old

396 leaves to shoots of wood bamboo at around the time of mating, they shift from a diet that is

397 low in both N and P with a N:P ratio that exceeds the maximum recommended for mammals

398 to a diet that is higher in both nutrients and has an N:P ratio within the recommended range

399 (Fig. 3a). With the subsequent switch to arrow bamboo shoots, the dietary concentrations of

400 both N and P were reduced but the N:P ratio was very close to the center of the expected N:P

401 range (~6.75, Fig. 3a). These high shoot N and P contents likely help support construction of

402 the placenta and the growing embryo during fetal development. During this period, however,

403 the dietary Ca:P ratio was considerably lower than considered necessary to support

404 reproduction in mammals, and was only restored with the subsequent switch to arrow bamboo

405 leaves (Figs 2 and 3b).

406   These dynamics lead us to suggest that perhaps delayed implantation provides a means

407 for pandas to postpone the Ca investment in lactation and bone growth, synchronizing these

408 more closely with a leaf-based diet that can support them. On the other hand, the relatively

409 low levels of both N and P, and the high N:P ratios in the autumn and winter diet, present

410 additional challenges for panda reproduction. Specifically, both N and P are required for

411 tissue growth, and there would be obvious fitness penalties for pandas that could not acquire

412 these in sufficient quantities for reproduction. An interesting possibility is that this could be

413 related to the evolutionary maintenance and enhancement of the short gestation period of

414 pandas, and the extremely small size of the offspring at birth (Garshelis 2004). Giving birth to

415 altricial young would ease the burden on the mother for acquiring limiting nutrients, by

416 enabling the offspring to start independent feeding (i.e. weaning) earlier. Having both mother

417 and offspring eating to meet their own respective nutrient needs would allow the pair to

418 process bamboo and acquire limiting nutrients at a greater rate than if the burden fell on the

419 mother alone. Barclay (1994) used similar reasoning to argue that the long development time

420 for flight, which delays independent foraging in flying vertebrates (bats and birds), might

421   impose constraints on Ca acquisition for bone growth and explain why these animals

422   generally have small litters. The peak in panda mortality in March and April is also consistent

423   with an interpretation that the extended low quality of the winter diet of leaves is nutritionally

424   stressful, highlighting the need for both mother and offspring to forage for limiting nutrients.

425   In summary, our analysis has shown that young shoots of wood bamboo were high in P

426   and N but had a Ca:P ratio markedly lower than is considered necessary to support bone

427   growth in mammals. In June, the levels of P, Ca and N dropped in the maturing shoots of

428   wood bamboo, whereupon the pandas migrated to higher elevation. This allowed them to

429   switch to arrow bamboo shoots, which had higher levels of P, N and Ca but again a

430   sub-optimally low Ca:P ratio. By early August, during the late stages of gestation, nutrient

431   levels in arrow bamboo shoots had dropped and the pandas switched to feeding on the leaves

432   of the same species. These had higher nutrient levels, in particular Ca, a more favorable Ca:P

433   ratio, and a better Ca:P absorption profile than bamboo shoots. In August, females returned to

434   the lower elevation feeding sites where birthing coincided with the availability of young wood

435   bamboo leaves, with high nutrient content and a high Ca:P ratio. However, as wood bamboo

436   leaves aged through the winter, their P content decreased, reaching their lowest level in the

437   period that coincides with the highest historical mortality rate. At that point, the pandas again

438   switched to young wood bamboo shoots once they became available.

439   Overall, our results also suggest that the two bamboo species are nutritionally

440   interchangeable but that different plant parts (shoots and leaves) are not. Rather, the shoots

441   and leaves are nutritionally complementary resources, with shoots providing primarily N and

442   P, but deficient in Ca, which is provided by the leaves. Both species of bamboo are,

443 nonetheless, critical for the pandas, because their asynchronous phenology, coupled with

444 seasonal altitudinal migration, enables the pandas to complete their life cycle on this low

445 diversity and highly specialized diet. Our insights into the phenological dynamics of panda

446 nutrition have important implications for managing the conservation of this charismatic

447 species in the face of climate change. Tuanmu *et al*. (2012) recently modelled likely future

448 distributions of wood and arrow bamboo in light of several IPCC climate projections for the

449 Qinling Mountain region (our study area). They noted strong potential for range contraction

450 and elevation shifts in these species, changes that are likely to lead to phenological

451 mismatches between the timing of panda life cycle events and the nutritional suitability of

452 bamboo. Such possibilities highlight the need for a systems-approach to panda conservation,

453 in which pandas, both species of their food plants, and their respective habitats, both current

454 and projected, are all afforded protection. More broadly, this work gives us a new insight into

455 animal nutritional ecology of potential benefit to further research in the field of animal

456 ecology and conservation biology, especially for species that face serious nutritional

457 challenges due to accelerating environmental change.

458

459 **Acknowledgements**

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603

604 **Table 1**. Comparison of the N, P and Ca concentrations and Ca/P ratio in foods (shoots and

605 leaves) from two species of bamboo and the feces produced by giant pandas from eating the

606 respective foods. All concentrations are calculated as a proportion of the sum of the three

607 components [e.g. %Ca = (Ca/N+P+Ca) x 100]. Statistics are from independent samples t-tests

608 comparing the N, P, Ca composition of each food with the composition of the relevant feces.

609 Positive t-values indicate decreased concentration in feces relative to food, while negative

610 t-values indicate increased concentration in feces relative to food.

611

	Bashania fargesii (wood bamboo)		*Fargesia qinlingensis* (arrow bamboo)	
Component	Leaves	Shoots	Leaves	Shoots
N	$t_{(38.270)} = 8.150$, $P < 0.0001$	$t_{(30.016)} = 11.731$, $P < 0.0001$	$t_{(58)} = 4.197$, $P < 0.0001$	$t_{(23.083)} = 15.464$, $P < 0.0001$
P	$t_{(38.810)} = 1.375$, $P = 0.177$	$t_{(37.258)} = -9.095$, $P < 0.0001$	$t_{(58)} = 0.640$, $P = 0.524$	$t_{(23.782)} = -10.914$, $P < 0.0001$
Ca	$t_{(39.741)} = -8.791$, $P < 0.0001$	$t_{(26.320)} = -9.974$, $P < 0.0001$	$t_{(58)} = -4.141$, $P < 0.0001$	$t_{(22.970)} = -11.942$, $P < 0.0001$
Ca/P	$t_{(36.798)} = -6.936$, $P < 0.0001$	$t_{(33.803)} = -6.873$, $P < 0.0001$	$t_{(25.590)} = -3.373$, $P = 0.001$	$t_{(26.928)} = -8.529$, $P < 0.0001$

612

613

Figure legends

Figure 1. Annual pattern of migration of giant pandas corresponding with a diet transition

between two bamboo species in our study area. We used the GPS location data of two of the

GPS collared pandas to exemplify the seasonal foraging migration pattern of giant pandas in

this area. All the six collared pandas in our study lived in the same winter (September-May)

habitat area at low elevation where wood bamboo (*Bashania fargesii*) is located. In summer,

two of them moved to summer habitat 1 while four moved to summer habitat 2, at high

elevation where arrow bamboo (*Fargesia qinlingensis*) is located.

Figure 2. Scheme relating the annual pattern of diet selection and habitat elevation to the

reproductive cycle of giant pandas. Foods: *WBl* = wood bamboo leaves; *WBs* = wood bamboo

shoots; *ABs* = arrow bamboo shoots; *ABl* = arrow bamboo leaves. Life history events: MS =

mating season, DII = delayed implantation interval, BS = birthing season.

Figure 3. Implications in terms of N:P (a), Ca:P (b) of seasonal diet shifts in giant pandas.

Green = wood bamboo (*WB*), orange = arrow bamboo (*AB*); circles = shoots, squares = leaves;

no border on symbols = young tissue; thin border = intermediate, thick border = old. Solid red

arrows show active switches by pandas between foods, dashed blue arrows = seasonal

changes in composition of foods being eaten. The shaded area denotes the recommended

range for N-P, Ca-P ratios in the diets of mammals.

Figure 4. Comparison of the Ca-N-P composition of foods (shoots and leaves) and the

associated feces in (a) wood bamboo (*WB*) and (b) arrow bamboo (*AB*). Circle-orange =

shoots; Circle-empty = shoot-associated feces; Oval-green = leaves; Oval-empty =

635   leaf-associated feces. Radials show mean Ca-P ratios, and negatively-sloped diagonals (%N

636   isolines) show the mean percentage of N relative to the sum of N, P and Ca in each sample.

637   Values for the % N isolines are shown in square brackets. Solid lines represent foods (leaves =

638   green, shoots = orange) and dashed lines represent feces associated with leaves (green) or

639   shoots (orange). The shaded area shows the range of Ca-P ratios recommended in the diets of

640   mammals.