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1 **Molecular analysis of the diets of snakes: changes in prey exploitation**
2 **during development of the rare smooth snake *Coronella austriaca*.**

3
4 DAVID S. BROWN, KATIE L. EBENEZER and WILLIAM O. C. SYMONDSON

5
6 Cardiff School of Biosciences, Sir Martin Evans Building, Cardiff University, Museum
7 Avenue, Cardiff CF10 3AX, UK

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9
10
11
12
13 Correspondence: D.S. Brown: Fax:+44 (0)29 208 74116; E-mail: brownds@cardiff.ac.uk

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17 Running title: Molecular analysis of diet in snakes

18
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20 diagnostics, *Natrix natrix*, smooth snake

21 **Abstract**

22 Reptiles are declining in many parts of the world, mainly due to habitat loss and
23 environmental change. A major factor in this is availability of suitable food. For many
24 animals, dietary requirements shift during developmental stages and a habitat will only be
25 suitable for conserving a species if it supports all stages. Conventional methods for
26 establishing diet often rely on visual recognition of morphologically identifiable features of
27 prey in faeces, regurgitates or stomach contents, which suffer from biases and poor
28 resolution of taxa. DNA-based techniques facilitate non-invasive analysis of diet from faeces
29 without these constraints. We tested the hypothesis that diet changes during growth stages of
30 smooth snakes (*Coronella austriaca*), which have a highly restricted distribution in the UK
31 but are widespread in continental Europe. Small numbers of the sympatric grass snake
32 (*Natrix natrix*) were analysed for comparison. Faecal samples were collected from snakes
33 and prey DNA analysed using PCR, targeting amphibians, reptiles, mammals and
34 invertebrates. Over 85% of smooth snakes were found to have eaten reptiles and 28% had
35 eaten mammals. Predation on mammals increased with age and was entirely absent among
36 juveniles and sub-adults. Predation on reptiles did not change ontogenetically. Smooth
37 snakes may, therefore, be restricted to areas of sufficiently high reptile densities to support
38 young snakes.

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44 **Introduction**

45 The distributions of snakes in temperate regions may be strongly influenced by the presence
46 of winter hibernation sites (Prior & Weatherhead 1996; Harvey & Weatherhead 2006) and
47 by temperature and the ability to thermoregulate (Huey 1991; Reinert 1993; Row & Blouin-
48 Demers 2006). However, the “ideal free distribution theory” (Fretwell & Lucas 1970;
49 Fretwell 1972) predicts that the distribution of any predator will reflect that of its prey, and
50 that this is most often the driving factor. The home ranges of black pine snakes (*Pituophis*
51 *melanoleucus lodingi*) (Baxley & Qualls 2009), water pythons (*Liasis fuscus*) (Madsen &
52 Shine 1996) and carpet pythons (*Morelia spilota metcalfei*) (Heard *et al.* 2004), for example,
53 have all been found to be associated with the abundance of their prey. While the distribution
54 of predators may be restricted to areas of sufficiently high prey density, ontogenetic shifts in
55 diet, a common phenomenon among vertebrates, can mean that a predator’s distribution may
56 be dependent upon the spectrum of different prey available at particular stages of its life.
57 Differences between juveniles and adults in their prey species selection, and the size of prey,
58 have been observed in fish (McCormick 1998; Reñones *et al.* 2002), birds (Price & Grant
59 1984), mammals (Dickman 1988; Page *et al.* 2005) and reptiles (Herrel & O’Reilly 2006),
60 and is commonly seen in snakes (Lind & Welsh 1994; Pizzatto *et al.* 2009; reviewed in
61 Shine & Wall 2007). Frequently, juveniles eat smaller prey and a narrower range of species
62 than adults. This may simply be a function of differences in relative body sizes of predators
63 and prey, but can also be attributed to inexperienced foraging ability (Rutz *et al.* 2006),
64 differential habitat use due to changes in predator avoidance / territory defense with age, or
65 in order to reduce intraspecific competition (Angelici *et al.* 1997).

66 Reptiles in Britain, as elsewhere, are in decline (Wilkinson & Arnell 2011) as
67 habitats are continually destroyed, fragmented or unsympathetically managed. Their ranges
68 are increasingly becoming narrower, leading to extinctions in many regions (Prestit 1971;
69 Howes 1973). In the UK, the smooth snake (*Coronella austriaca*) is considered endangered
70 due to its severely restricted distribution to a few strongholds on heathlands in Dorset and
71 Hampshire, southern England, the reasons for which are not clear. Britain is home to two
72 other sympatric snakes, the adder (*Vipera berus*) and the grass snake (*Natrix natrix*), both of
73 which are much more widely distributed. The grass snake is found up to, and occasionally
74 beyond, 56°N, approximately the border of England and Scotland. Smooth snakes range
75 almost as far north as grass snakes throughout mainland Europe, up as far as 60°N in
76 Sweden, which corresponds to a vegetational and climatological boundary (Gasc *et al.*
77 1997). Thus, a distribution in the UK that is restricted by temperature is unlikely. While
78 smooth snakes are only found on sandy lowland heath in Britain, throughout continental
79 Europe they are found in a variety of different habitats (pine forests, mixed riverside forests,
80 bogs, vegetation bordering fields, bramble patches, orchards and open grassland (Beebee &
81 Griffiths 2000)), and so habitat structure does not appear able to explain their UK
82 distribution. Alternatively, distribution may be more ecological, a function of diet, prey
83 availability, prey diversity and competition with sympatric snakes for food (Phelps 1978;
84 Goddard 1984; Drobenkov 1995).

85 Smooth snakes are generally considered to be reptile specialists throughout
86 continental Europe (Duguay 1961; Andrén & Nilson 1976, 1979; Street 1979; Drobenkov
87 1995; Rugiero *et al.* 1995). However, their diet in the UK has been subject to debate, and
88 while there is agreement over the main range of prey taken (amphibians, reptiles and small

89 mammals) the importance of each is unclear. Goddard (1981, 1984), using morphological
90 analyses of faeces and regurgitates, found the proportion of smooth snakes which had
91 consumed small mammals was more than twice that of smooth snakes that had consumed
92 reptiles. Goddard (1984) speculated that smooth snakes were not reptile specialists, but
93 rather generalists consuming prey in relation to its availability, and that the higher reptile
94 component of their diet in continental Europe simply reflected the higher relative densities of
95 reptiles there. This was supported by Rugeiro *et al.* (1995) whose faecal and regurgitate
96 analyses of smooth snakes in Italy revealed they were consuming lizards, snakes and mice in
97 proportion to their ratios in the wild. However, juvenile smooth snakes have showed an
98 innate feeding preference for lizards (Goddard 1984), suggesting that smooth snakes may
99 initially be restricted to a reptile diet, which broadens with increasing age, size and
100 experience. At an even younger age, smooth snakes might be restricted to a diet of
101 invertebrates, with a number of reports of invertebrates in their diet (Spellerberg & Phelps
102 1977; Nature Conservancy Council 1983; Rugiero *et al.* 1995).

103 The diets of Britain's other native snakes are more firmly established, both in the UK
104 and throughout Europe, with adders found to have a very broad diet which includes
105 amphibians, reptiles and birds, but predominantly small mammals (Prestt 1971; Drobenkov
106 1995), while grass snakes are thought to be amphibian specialists that take little other prey
107 (Drobenkov 1995). Although there is overlap in the diet of adders with both grass snakes and
108 smooth snakes (Drobenkov 1995), the home ranges of adders seldom overlap those of the
109 others snake species (Spellerberg & Phelps 1977), whereas grass snakes and smooth snakes
110 are frequently found together. As a result, there is greater potential for competition between
111 these two species. Grass snakes occasionally include reptiles in their diet (Luiselli & Rugiero

112 1991; Capula *et al.* 1994; Drobenkov 1995; Filippi *et al.* 1996; Luiselli & Capula 1997) and
113 small mammals (Luiselli & Rugiero 1991; Luiselli & Capula 1997; Gregory & Isaac 2004)
114 and smooth snakes have been found to eat amphibians (Nature Conservancy Council 1983),
115 although these are considered to be a small components of their diets. However, snake size
116 and age are seldom accounted for in these studies, which have usually been conducted on
117 adults only and may be missing critical information if there are ontogenetic shifts in diets. If
118 smooth snakes are dependent on a narrow range of specific prey as juveniles, then the
119 abundance and distribution of those prey may place restrictions on their population density
120 and may drive them into competition with grass snakes, adders and other predators.

121 Conventional analyses of faeces or regurgitates for morphologically identifiable
122 features of prey are constrained by the presence of undigested remains and the ability to
123 accurately identify them. Snakes are known to be able to digest prey thoroughly, digesting
124 even bones and other hard parts (Secor 2008). Certainly, if soft-bodied invertebrate prey,
125 such as slugs or earthworms, were included in their diet then traditional methods would not
126 be able to identify them. Molecular techniques, in particular the detection of prey DNA in
127 faeces (Symondson 2002), has enabled detailed analyses of prey consumed by vertebrates
128 including fish (Saitoh *et al.* 2003; Jarman & Wilson 2004), birds (Jarman *et al.* 2004; Deagle
129 *et al.* 2007), and mammals (Jarman *et al.* 2002, 2004; Marshall *et al.* 2010; Clare *et al.* 2009,
130 2011; Razgour *et al.* 2011). Next generation sequencing (NGS) has been successfully
131 applied to analyse the diet of the legless lizard (*Anguis fragilis*) (Brown *et al.* 2012) and the
132 effects of season and sex on the diet of the Turtle-headed sea snake (*Emydocephalus*
133 *annulatus*) were also identified using a DNA sequencing approach (Goiran *et al.* 2013).
134 Species-specific PCR primers, which are a less costly alternative to NGS, have not

135 previously been applied to analyses of reptile diet. Such molecular approaches allow
136 standardized non-invasive screening of reptile faeces for target prey.

137 Here we used molecular tools to investigate predation by smooth snakes and address
138 the hypothesis that there are ontogenetic changes in the diet of smooth snakes which may be
139 responsible for their severely restricted distribution. In addition, a preliminary study was
140 made on predation by sympatric grass snakes to investigate the potential for the approach to
141 identify resource partitioning between these sympatric snakes.

142

143 **Methods**

144

145 *Field sites and faecal collection*

146 A total of 53 faecal samples were collected from smooth snakes during monthly visits to two
147 English sites (Ringwood and Creech) from April–September in 2007 and 2008, the active
148 period for British reptiles (Beebee & Griffiths 2000). The Ringwood site (50°52'N, 1°51'W)
149 consists of just under a hectare of unimproved grassland adjacent to ericaceous heathland
150 and coniferous woodland. The Creech site (50°39'N, 2°06'W) is an area of ericaceous
151 heathland comprising common heather (*Calluna vulgaris*), bell heather (*Erica cinerea*) and
152 gorse (*Ulex* spp.). Both sites are managed by The Herpetological Conservation Trust and are
153 typical of habitats in Southern England where smooth snakes are found. The opportunity was
154 also taken to collect further faecal samples from a small number of grass snakes (n=14),
155 collected at the same time and from the same sites, to test the ability of the molecular
156 detection methods on another species and to provide limited comparative information on
157 their diets.

158 Faecal samples were collected into 2 mm microcentrifuge tubes by gentle palpation
159 of the animals. Snout-vent length (SVL), used as a proxy for age, and total weight were
160 measured. All snakes were photographed, allowing individual identification based on unique
161 banding patterns and colouration. To avoid pseudoreplication, snakes previously caught
162 were excluded from analysis. The appropriate license was obtained from Natural England.

163

164 *DNA extraction, PCR and sequencing*

165 All animal material used for DNA extractions were donated by small mammal and
166 herpetological groups, having been found dead during animal surveys. Animals collected
167 included common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), bank vole (*Myodes*
168 *glareolus*), common shrew (*Sorex araneus*), pygmy shrew (*S. minutus*), water shrew
169 (*Neomys fodiens*), brown rat (*Rattus norvegicus*), yellow necked mouse (*Apodemus*
170 *flavicollis*), house mouse (*Mus musculus*), palmate newt (*Lissotriton helveticus*), smooth
171 newt (*L. vulgaris*), common lizard (*Lacerta vivipara*), sand lizard (*L. agilis*), slow worm (the
172 legless lizard *Anguis fragilis*), common frog (*Rana temporaria*), adder (*V. berus*), grass
173 snake (*N. natrix*) and smooth snake (*C. austriaca*). The DNeasy® Tissue Kit (Qiagen) was
174 used for extraction of DNA from tissue. All DNA was amplified by PCR with the universal
175 forward primer LCO1498 (Folmer *et al.* 1994) and the reverse primer HCO1777 (5'-
176 ACTTATATTGTTTATACGAGGGAA-3') (Brown 2010) with the following conditions:
177 1X buffer, 2 mM MgCl₂, 0.5 mM dNTP (Invitrogen), 0.5 μM of each primer, 0.38 U *Taq*
178 polymerase (Invitrogen) and 2 μL of DNA in/ 25 μL PCR reaction with an initial
179 denaturation at 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 45
180 s, and a final extension at 72 °C for 5 min. Amplification was visualized by gel

181 electrophoresis stained with ethidium bromide. Double-distilled water was included as a
182 negative control to test for contamination.

183 PCR products were sequenced for species for which sequences were not readily
184 available on Genbank (slow worm, common lizard and adder). They were cleaned using
185 ExoSAP in the following reaction: 10 µL of each PCR product, 0.25 µL Exonuclease I, 0.5
186 µL SAP (shrimp alkaline phosphatase) and incubated for 45 min at 37°C and 15 min at 80°C.
187 Cleaned product was then used in sequencing PCR using a Big Dye™ terminator sequencing
188 kit (Promega, Madison, WI, USA). Sequences were checked for errors using Sequencher
189 3.1.2.

190 DNA from faecal samples were extracted using the QIAamp® DNA Stool Mini Kit
191 (Qiagen) in accordance with the manufacturer's instructions.

192

193 *Species- and group-specific primer design*

194 Cytochrome b sequences were downloaded from Genbank for the following species: smooth
195 snake (Accession no. EU022673), water vole (*Arvicola amphibius*, AF159400), bank vole
196 (EU035710), field vole (DQ663658), common shrew (GU827395), pygmy shrew
197 (GU827394), yellow-necked mouse (AF159392), wood mouse (*Apodemus sylvaticus*,
198 HQ158102), house mouse (AB125774), common frog (FJ030872), palmate newt (U55948),
199 smooth newt (DQ821238) and red-spotted toad (*Bufo punctatus*, DQ085775, used as a proxy
200 for *B. bufo*). Primers for common frog, smooth newt and small mammals were designed by
201 eye using BioEdit (version 7.0.4.1) to align homologous sequences and NetPrimer (Premier
202 Biosoft International) to check for self-dimers, cross-dimers, hairpin structures and melting
203 temperatures. Cytochrome oxidase I sequences were downloaded from Genbank for smooth

204 snake (AY122752) and grass snake (AY122664) and aligned with sequences for slow worm,
205 common lizard and adder. Primers were designed for slow worm and common lizard.

206 Other primers used included those for bank vole (BV-CG95 and BV-CG266),
207 common shrew (SA520 and SA628) and pygmy shrew (SM421 and SM544), targeting
208 cytochrome b (Moran *et al.* 2008), plus group-specific primers for earthworms (185F and
209 14233R) (Harper *et al.* 2005) and arionid slugs (Harper *et al.* 2005), which target the 12S
210 rRNA region. Species-specific primers were designed or selected for prey species known to
211 be common components of smooth snake and grass snake diet (Drobenkov 1995).

212

213 *Primer optimization and screening*

214 A temperature gradient PCR was performed for each primer set to determine the highest
215 temperature at which the target DNA would amplify. Each primer pair was tested for target-
216 specificity against DNA from all other potential prey species. PCR was performed using a
217 Peltier Thermal Cycler (Bio-Rad Laboratories, CA, USA). PCR concentrations used were
218 the same as those described above, but with a PCR cycle of 94 °C for 3 min, 35 cycles of 94
219 °C for 30 s, the highest working annealing temperature for that primer pair for 45 s and 68
220 °C for 45 s, and a final extension at 68 °C for 10 min.

221 Specificity was achieved for common shrew, common frog, smooth newt, common
222 lizard, slow worm and the small mammals (Table 1). The bank vole primers CG95/CG266
223 (Moran *et al.* 2008) cross-amplified with field vole at all temperatures, but with no other taxa
224 at 58 °C. The pygmy shrew primers SM421/SM544 (Moran *et al.* 2008) cross-amplified with
225 common shrew and water shrew at all temperatures, but were group-specific to all shrews at
226 53 °C. Between 52 °C and 64 °C the common shrew primers SA520/SA628 (Moran *et al.*

227 2008) resulted serendipitously in bands that were species-specific in pygmy shrews (with a
228 *ca.*150 base pair fragment) and water shrew (with a *ca.*250 base pair fragment), both
229 distinguishable from the *ca.*200 bp fragment for common shrew. These may be the result of
230 amplification of pseudogenes, but they proved to be reliable species-specific markers that
231 could separate the three species of shrew in snake faeces. The common lizard primers
232 LCO1498/LV1714R cross-amplified with sand lizard between 53-62 °C and were used as
233 general lacertid primers at 53 °C.

234 All faecal samples were screened with each primer pair twice. Target DNA was
235 included as a positive control, to ensure PCR success, and water was included as a negative
236 control to check for contamination.

237

238 *Statistics*

239 The effects of smooth snake SVL, weight and sex, along with site, month, year, temperature,
240 rainfall and sunshine on predation of various prey were explored within a Generalised Linear
241 Model (GLM). Weight, SVL, temperature, rainfall and sunshine were treated as covariates
242 and all other predictors as factors. Weather information was obtained from the Met Office.
243 The effects of grass snake SVL, only, were considered within GLMs investigating their
244 predation on prey, due to the small sample size. A binomial error distribution was used with
245 a logit link function. All analyses were conducted in the R version 2.8.2. Patterns of
246 predation by the two snake species on each prey species were analysed. However,
247 comparisons between prey were not made due to possible differences between primers in the
248 ability of their amplicons to survive digestion (King *et al.* 2008).

249

250 **Results**

251

252 *Predation by smooth snakes*

253 The primary prey of smooth snakes was reptiles (Fig. 1), with no significant effect of
254 predator age/SVL on their consumption. However, there was a significant effect of both
255 snake SVL and site on predation of shrews, with the probability of predation increasing with
256 snake size ($\chi^2 = 10.4$, $df = 1$, $P=0.003$, Fig. 2a) and a much higher probability of predation at
257 Ringwood ($n=24$) than at Wareham ($n=29$) ($\chi^2 = 8.8$, $df = 1$, $P=0.001$, Fig. 2a). Similar
258 effects of SVL and site were also seen when predation on all small mammals combined was
259 analysed (SVL: $\chi^2 = 5.5$, $df = 1$, $P=0.020$; site: $\chi^2 = 5.0$, $df = 1$, $P=0.026$, Fig. 2b).

260 There was a significant effect of month on smooth snake predation on slow worms
261 ($\chi^2 = 18.3$, $df = 4$, $P=0.001$), lacertids ($\chi^2 = 10.2$, $df = 4$, $P=0.038$) and on all lizards
262 combined ($\chi^2 = 11.1$, $df = 4$, $P=0.025$). Predation on reptiles fluctuated between months but
263 was high throughout the entire season. Even in August, when predation on reptiles was at its
264 lowest, it was still above 50%. Predation on reptiles between the two sites did not
265 significantly differ, with 85.7% of smooth snakes at Ringwood and 83.3% at Wareham
266 having consumed them.

267 Predation on earthworms (18%) and slugs (0%) was minimal or absent and there was
268 no significant effect of any of the variables considered. Predation on smooth newts (3%) and
269 common frogs (9%) was too low to explore statistically.

270

271 *Predation by grass snakes*

272 Prey detection in grass snakes was also successful, although results should be treated with
273 caution given the small sample size (N=14). Snake SVL had a highly significant negative
274 effect on predation on reptiles (SVL: $\chi^2 = 10.4$, $df = 1$, $P=0.001$), with all grass snakes below
275 550mm in SVL ($n=10$) testing positive for reptile DNA but all those above 600mm ($n=4$)
276 testing negative.

277 There was no effect of grass snake SVL on newt predation. All other prey (small
278 mammals, common frog and earthworm) were preyed on too infrequently for statistical
279 analysis.

280

281 *Comparison of smooth snake and grass snake diet*

282 Predation on small mammals by smooth snakes was 28%, twice that of grass snakes. The
283 range of small mammals eaten by smooth snakes was wider and non-overlapping with those
284 eaten by grass snakes; smooth snakes consumed common shrews, pygmy shrews and voles,
285 whereas grass snakes were only found to have eaten water shrew (Fig. 1). There was no
286 significant difference in predation by the two snake species on common lizards or lacertids
287 (common lizards and sand lizards combined), but predation on slow worms was significantly
288 higher in smooth snakes ($\chi^2 = 5.98$, $df = 1$, $P=0.014$). Predation on amphibians (in particular
289 smooth newts) was over ten times higher in grass snakes than in smooth snakes (Fisher's
290 exact test, $P<0.001$).

291

292 **Discussion**

293

294

295 *Smooth snakes*

296 The focus of this study was on the diet of smooth snakes, reflecting interest in the
297 conservation of this species and its unusual and restricted distribution patterns. The main
298 prey of these snakes (N=53) was found to be other reptiles (84.5% tested positive) followed
299 by small mammals (28.0%).

300 Predation on reptiles was similar at each of the sites, with 85.7% of smooth snakes at
301 Ringwood and 83.3% at Wareham having consumed them. However, predation on small
302 mammals differed between the two sites, with twice as many testing positive at Ringwood
303 (38.3%) as at Wareham (16.7%), probably reflecting differences in prey availability at the
304 two locations. The Ringwood site has a variety of different habitats in close proximity to the
305 heathland, including grassland and forest, which are likely to support more small mammals
306 than the open heathland of Wareham. These results indicate that small mammals may not be
307 an essential part of smooth snake diet, but are taken in accordance with their availability, as
308 suggested by Goddard (1984) and Rugiero *et al.* (1995). Reptiles, however, appear
309 predominant in their diet, regardless of the availability of alternative prey.

310 Smooth snakes showed increased predation on shrews ($P=0.003$) and small
311 mammals generally ($P=0.020$) as they grew larger. Taking SVL as a proxy for age
312 (Bronikowski & Arnold 1999; Gignac & Gregory 2005), this indicates an ontogenetic shift
313 in smooth snake diet, with very few small mammals taken when the snakes are young but
314 increasing predation as they grow. This may be explained either by a greater initial
315 preference for reptile prey or by an inability to find, handle or consume small mammals
316 when young (Shine & Wall 2007). No smooth snakes below 300 mm in SVL, equating
317 approximately to a three year old snake (Goddard 1984), were found to have consumed any

318 small mammals, so in these first few years their diet was likely to have been almost
319 exclusively reptile. There was no change in predation on reptiles (common lizard, lacertids
320 generally or slow worm) with snake size, with predation on them starting when smooth
321 snakes were as small as 190 mm in SVL, within their first year. Most probably the youngest
322 smooth snakes are eating juvenile lizards. Thus they continue eating lizards throughout their
323 life, while incorporating small mammals as they grow larger / older.

324 If the geographical distribution of smooth snakes in the UK is restricted by prey
325 availability then it is most likely that this restriction is at the juvenile stage, when their diet is
326 at its narrowest and they are almost entirely dependent on juvenile lizards. While smooth
327 snakes are clearly capable of eating invertebrate prey, only 17% were found to have
328 consumed earthworms, and juveniles were no more likely to consume them than adults. No
329 snakes were found to have consumed any *Arion* slugs despite their abundance at the field
330 sites. It is quite possible that positives recorded for earthworm consumption by smooth
331 snakes were in fact the result of secondary predation (Harwood *et al.* 2001; Sheppard *et al.*
332 2005). Slow worms were shown to be major consumers of earthworms in a separate study
333 (Brown *et al.* 2012) and therefore earthworm DNA may have ended up in the guts of smooth
334 snakes following slow worm consumption. Based on tongue-flick experiments, Pernetta *et*
335 *al.* (2009) found that smooth snakes showed a preference for the scent of lizard and mammal
336 prey over invertebrates, even as juveniles. Van de Bund (1964) and Spellerberg (1977) both
337 suggested that the narrow food preference of young smooth snakes make them particularly
338 vulnerable, more so than grass snakes and adders which have more diverse diets (Drobenkov
339 1995). Slow worms and common lizards are ubiquitous throughout the UK, and so the
340 distribution of smooth snakes would be expected to be more widespread if it were primarily

341 determined by the distribution of lizard prey. However, it may be that smooth snakes are
342 restricted not just to areas where lizards are present, but to areas with a sufficiently high
343 density of juvenile lizards. The heaths of southern England have higher densities of common
344 lizards, sand lizards and slow worms than anywhere else in the country (Braithwaite *et al.*
345 1989).

346

347 *Grass snakes*

348 Grass snakes are usually associated with damp and aquatic environments, hunting the prey
349 found in these habitats, particularly amphibians (Drobenkov 1995; Gregory & Isaac 2004).
350 Although sample size was limited, it was also apparent that amphibians were a major dietary
351 component, with 64.3% testing positive (mainly for smooth newts) compared with a rate of
352 just 5.2% in smooth snakes. Predation by grass snakes on small mammals was exclusively
353 on water shrews, again an aquatic prey. Interestingly, however, a larger proportion of grass
354 snakes were found to be consuming reptile prey (68.2%, Fig. 1) than previous studies have
355 found (Drobenkov 1995; Gregory & Isaac 2004). There was no significant difference
356 between consumption of common lizards by grass snakes and smooth snakes, indicating the
357 potential for competition between these species.

358

359 *Analysis by PCR*

360 Molecular diagnostics revealed detailed and clear information on reptile diets and the effects
361 of developmental stage on prey choice. This approach allows for standardized non-invasive
362 analyses and monitoring of diets, particularly cost- and time-effective where prey-specific
363 primers are already developed. There are potential limitations to these approaches: prey

364 species may be digested at different rates which may affect detectability (e.g. Deagle &
365 Tollit 2007), and primers may differ in sensitivity (Symondson 2002), but these potential
366 biases can be reduced by targeting DNA amplicons of a similar size and on the same gene or
367 by evaluating sensitivity by serial dilution tests (e.g. Chen *et al.* 2000). Unlike some
368 traditional methods, such as forced regurgitation, it is not possible to determine the size of
369 prey or the number of prey individuals consumed by a predator and where this information is
370 desired a combination of approaches is the best possible practice.

371 In this study, with a sample of just 14 grass snakes taken opportunistically, it is too
372 early to project any conclusions onto the wider population, although these findings
373 corroborated many previous studies of grass snake diet (Drobenkov 1995; Gregory & Isaac
374 2004) while also hinting that predation on slow worms may be higher than thought at sites
375 such as these where they are abundant.

376 UK smooth snakes were shown to be almost entirely dependent on lizard prey as
377 juveniles, restricting them to areas of high lizard density. Management plans to maintain
378 smooth snake populations, relocate endangered colonies or attempts to restore their
379 distribution to historical ranges, should focus on creating optimum lizard habitats. This
380 should include lizard surveys to identify hotspots where smooth snake reintroductions might
381 be viable, with maintenance of lizard-friendly habitat. This study offers both insight into the
382 limited distribution of smooth snakes and presents a new tool to aid reptile conservation.

383

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389

390 **References**

391

392 Andrén, C., and G. Nilson (1976) Hasselsnoken (*Coronella austriaca*) - en utrotningshotad
393 ormat! *Fauna Flora, Stockholm*, **71**, 61-76.

394 Andrén, C., and G. Nilson (1979) Hasselsnoken (*Coronella austriaca*) - I Norden-en isolerad
395 och ekologiskt sarstalld ras? *Fauna Flora, Stockholm*, **74**, 89-96.

396 Angelici, F.M., L. Luiselli, and L. Rugiero (1997) Food habits of the green lizard, *Lacerta*
397 *bilineata*, in central Italy and a reliability test of faecal pellet analysis. *Italian Journal*
398 *of Zoology*, **64**, 267-272.

399 Baxley, D.L., C.P. Qualls. 2009. Black pine snake (*Pituophis melanoleucus lodingi*): Spatial
400 ecology and associations between habitat use and prey dynamics. *Journal of*
401 *Herpetology* **43**: 284-293.

402 Beebee, T., and R. Griffiths. 2000. *Amphibians and Reptiles*. HarperCollins, London.

403 Braithwaite, A.C., J. Buckley, K.F. Corbett, P.W. Edgar, E.S. Haslewood, G.A.D.

404 Haslewood, T.E.S. Langton, and W.J. Whitaker. 1989. The distribution in England of
405 the smooth snake (*Coronella austriaca* Laurenti). *Herpetological Journal* **1**: 370-376.

406 Bronikowski, A.M., and S.J. Arnold. 1999. *The evolutionary ecology of life-history variation*
407 *in the garter snake *Thamnophis elegans**. *Ecology* **80**: 2314-2325.

408 Brown DS. 2010. Molecular analysis of the trophic interactions of British Reptiles. PhD
409 thesis.

- 410 Brown, D.S., S.N. Jarman, and W.O.C. Symonson. 2012. Pyrosequencing of prey DNA in
411 reptile faeces: analysis of earthworm consumption by slow worms. *Molecular Ecology*
412 *Resources* **12**: 259-266.
- 413 Capula, M., L. Rugiero, and L. Luiselli. 1994. Ecological observations on the Sardinian
414 grass snake, *Natrix natrix cetti*. *Amphibia-Reptilia* **15**: 221-227.
- 415 Chen Y, Giles KL, Payton ME, Greenstone MH. 2000. Identifying key cereal aphid
416 predators by molecular gut analysis. *Molecular Ecology* **9**: 1887–1898.
- 417 Clare, E.L., E.E. Fraser, H.E. Braid, M.B. Fenton, and P.D.N. Hebert. 2009. Species on the
418 menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a
419 molecular approach to detect arthropod prey. *Molecular Ecology* **18**: 2532-2542.
- 420 Clare EL, Barber BR, Sweeney BW, Hebert PDN, Fenton MB. 2011. Eating local:
421 influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular*
422 *Ecology* **20**: 1772-1780.
- 423 Deagle, B.E., N.J. Gales, K. Evans, S.N. Jarman, S. Robinson, R. Trebilco, and M.A.
424 Hindell. 2007. Studying seabird diet through genetic analysis of faeces: A case study
425 on macaroni penguins (*Eudyptes chrysolophus*). *PLoS ONE* **2**: e831.
- 426 Deagle BE, Tollit DJ. 2007. Quantitative analysis of prey DNA in pinniped faeces: potential
427 to estimate diet composition? *Conservation Genetics* **8**: 743–747
- 428 Dickman, C.R. 1988. Age-related dietary change in the European hedgehog, *Erinaceus*
429 *europaeus*. *Journal of Zoology* **215**: 1-14.
- 430 Drobenkov, S.M. 1995. Comparative analysis of the feeding of sympatric snakes *Vipera*
431 *berus* (L.), *Natrix natrix* (L.) and *Coronella austriaca* (L.). *Russian Journal of Ecology*
432 **26**: 197-201.

- 433 Duguy, R. 1961. Le cycle annuel d'activite de *Coronella austriaca* Laur.; d'apex les
434 observations manuscrites inedites de Ramond Rollinat. *Terre Vie* **15**: 401-435.
- 435 Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for
436 amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan
437 invertebrates. *Molecular Marine Biology & Biotechnology* **3**: 294-299.
- 438 Filippi, E.M., L. Capula, L. Luiselli, U. Agrimi. 1996. The prey spectrum of *Natrix natrix*
439 (Linnaeus, 1758) and *Natrix tessellata* (Laurenti, 1768) in sympatric populations.
440 *Herpetozoa* **8**: 155-164.
- 441 Fretwell, S.D. 1972. Populations in a seasonal environment. Princeton University Press.
442 Princeton, NJ.
- 443 Fretwell, S.D., H.J.Jr. Lucas. 1970. On territorial behaviour and other factors influencing
444 habitat distribution in birds. *Acta Biotheoretica* **19**: 16-36.
- 445 Gasc, J-P., *et al.* 1997. Atlas of Amphibians and Reptiles in Europe. Societas Europaea
446 Herpetologica and Muséum National d'Histoire Naturelle, Paris.
- 447 Gignac, A., and P.T. Gregory. 2005. The effects of body size, age, and food intake during
448 pregnancy on reproductive traits of a viviparous snake, *Thamnophis ordinoides*.
449 *Ecoscience* **12**: 236-243.
- 450 Goddard, P. 1981. Ecology of the smooth snake *Coronella austriaca* Laurenti in Britain.
451 PhD thesis, University of Southampton.
- 452 Goddard, P. 1984. Morphology, growth, food habits and population characteristics of the
453 smooth snake *Coronella austriaca* in southern Britain. *Journal of Zoology (London)*
454 **204**: 241-257.

- 455 Goiran C, Dubey S, Shine R. 2013. Effects of season, sex and body size on the feeding
456 ecology of turtle-headed sea snakes (*Emydocephalus annulatus*) on IndoPacific
457 inshore coral reefs. *Coral Reefs* **32**: 527-538.
- 458 Gregory, P.T., and L.A. Isaac. 2004. Food habits of the grass snake in Southeastern England:
459 Is *Natrix natrix* a generalist predator? *Journal of Herpetology* **38**: 88-95.
- 460 Harper, G.L., R.A. King, C.S. Dodd, J.D. Harwood, D.M. Glen, M.W. Bruford, and W.O.C.
461 Symondson. 2005. Rapid screening of invertebrate predators for multiple prey DNA
462 targets. *Molecular Ecology* **14**: 819-827.
- 463 Harvey, D.S., and P.J. Weatherhead. 2006. Hibernation site selection by eastern Massasuga
464 rattlesnakes (*Sistrurus catenatus catenatus*) near their northern range limit. *Journal of*
465 *Herpetology* **40**: 66-73.
- 466 Harwood, J.D., S.W. Phillips, K.D. Sunderland, and W.O.C. Symondson. 2001. Secondary
467 predation: quantification of food chain errors in an aphid-spider-carabid system using
468 monoclonal antibodies. *Molecular Ecology* **10**: 2049-2057.
- 469 Heard, G.W., D. Black, and P. Robertson. 2004. Habitat use by the inland carpet python
470 (*Morelia spilota metcalfei*: Pythonidae): seasonal relationships with habitat structure
471 and prey distribution in a rural landscape. *Austral Ecology* **29**: 446-460.
- 472 Herrel, A., and J.C. O'Reilly. 2006. Ontogenetic scaling of bite force in lizards and turtles.
473 *Physiological and Biochemical Zoology* **79**: 31-42.
- 474 Howes, C.A. 1973. The history and distribution of reptiles and amphibians in southeast
475 Yorkshire and the Doncaster district. *Naturalist, Hull*: 121-32.
- 476 Huey, R.B. 1991. Physiological consequences of habitat selection. *American Naturalist* **137**:
477 S91-S115.

- 478 Jarman, S.N., B.E. Deagle, and N.J. Gales. 2004. Group-specific polymerase chain reaction
479 for DNA-based analysis of species diversity and identity in dietary samples. *Molecular*
480 *Ecology* **13**: 313-1322.
- 481 Jarman, S.N., N.J. Gales, M. Tierney, P.C. Gill, and N.G. Elliott. 2002. A DNA-based
482 method for identification of krill species and its application to analysing the diet of
483 marine vertebrate predators. *Molecular Ecology* **11**: 2679-2690.
- 484 Jarman, S.N., and S.G. Wilson. 2004. DNA-based species identification of krill consumed
485 by whale sharks. *Journal of Fish Biology* **65**: 586-591.
- 486 King, R.A., D.S. Read, M. Traugott, W.O.C. Symondson. 2008. Molecular analysis of
487 predation: a review of best practice for DNA-based approaches. *Molecular Ecology*
488 **17**: 947-963.
- 489 Lind, A.J., and H.H.Jnr. Welsh. 1994. Ontogenetic changes in foraging behavior and habitat
490 use by the Oregon garter snake, *Thamnophis atratus hydrophilus*. *Animal Behaviour*
491 **48**: 1261-1273.
- 492 Luiselli, L., and M. Capula. 1997. Food habits, growth rates, and reproductive biology of
493 grass snakes, *Natrix natrix* (Colubridae) in the Italian Alps. *Journal of Zoology*
494 (London) **241**: 371-380.
- 495 Luiselli, L., and L. Rugiero. 1991. Food niche partitioning by water snakes (genus *Natrix*) at
496 a freshwater environment in central Italy. *Journal of Freshwater Ecology* **6**: 439-444.
- 497 Madsen, T., and R. Shine. 1996. Seasonal migration of predators and prey – a study of
498 pythons and rats in tropical Australia. *Ecology* **77**: 149-156.
- 499 Marshall, H.D., K.A. Hart, E.S. Yaskowiak, G.B. Stenson, D. McKinnon, and E.A. Perry.
500 2010. Molecular identificaion of prey in the stomach contents of harp seals

501 (*Pagophilus groenlandicus*) using species-specific oligonucleotides. *Molecular*
502 *Ecology Resources* **10**: 181-189.

503 McCormick, M.I. 1998. Ontogeny of diet shifts by a microcarnivorous fish, *Cheilodactylus*
504 *spectabilis*: Relationship between feeding mechanics, microhabitat selection and
505 growth. *Marine Biology* **132**: 9-20.

506 Moran, S., P.D. Turner, and C. O'Reilly. 2008. Non-invasive genetic identification of small
507 mammal species using real-time polymerase chain reaction. *Molecular Ecology*
508 *Resources* **8**: 1267-1269.

509 Nature Conservancy Council. 1983. The ecology and conservation of amphibian and reptile
510 species endangered in Britain. *English Nature Status (Wildlife)*, Peterborough.

511 Page, B., J. McKenzie, and S.D. Goldsworthy. 2005. Dietary resource partitioning among
512 sympatric New Zealand and Australian fur seals. *Marine Ecology Progress Series* **293**:
513 283-302.

514 Pernetta, A.P., C.J. Reading, and J.A. Allen. 2009. Chemoreception and kin discrimination
515 by neonate smooth snakes, *Coronella austriaca*. *Animal Behaviour* **77**: 363-368.

516 Phelps, T.E. 1978. Seasonal movement of the snakes *Coronella austriaca*, *Vipera berus* and
517 *Natrix natrix* in southern England. *British Journal of Herpetology* **5**: 755-761.

518 Pizzatto, L., O.A.V. Marques, and K. Facure. 2009. Food habits of Brazilian boid snakes:
519 Overview and new data, with special reference to *Corallus hortulanus*. *Amphibia-*
520 *Reptilia* **30**: 533-544.

521 Prestt, I. 1971. An ecological study of the viper *Vipera berus* in Britain. *Journal of Zoology*
522 (London) **164**: 373-418.

- 523 Price, T.D., and P.R. Grant. 1984. Life history traits and natural selection for small body size
524 in a population of Darwin's finches. *Evolution* **38**: 483-494.
- 525 Prior, K.A., and P.J. Weatherhead. 1996. Habitat features of black rat snake hibernacula in
526 Ontario. *Journal of Herpetology* **30**: 211-218.
- 527 Razgour O, Clare EL, Zeale MRK, Hanmer J, Schnell IB, Rasmussen M, Gilbert TP, Jones
528 G (2011) High-throughput sequencing offers insight into mechanisms of resource
529 partitioning in cryptic bat species. *Ecology and Evolution* 1, DOI: 10.1002/ece3.49.
- 530 Reinert, H.K. 1993. Habitat selection in snakes. Pages 201-240 in R.A. Seigel and J.T.
531 Collins, editors. *Snakes: Ecology and Behavior*. McGraw Hill, New York.
- 532 Reñones, O., N.V.C. Polunin, and R. Goni. 2002. Size related dietary shifts of *Epinephelus*
533 *marginatus* in a western Mediterranean littoral ecosystem: an isotope and stomach
534 content analysis. *Journal of Fish Biology* **61**: 122-137.
- 535 Row, J.R., and G. Blouin-Demers. 2006. Thermal quality influences habitat selection at
536 multiple spatial scales in milksnakes. *Ecoscience* **13**: 443-450.
- 537 Rugiero, L., M. Capula, E. Filippi, and L. Luiselli. 1995. Food habits of Mediterranean
538 populations of the smooth snake (*Coronella austriaca*). *Herpetological Journal* **5**: 316-
539 318.
- 540 Rutz, C., M.J. Whittingham, and I. Newton. 2006. Age-dependent diet choice in an avian top
541 predator. *Proceedings of the Royal Society B: Biological Sciences* **273**: 579-586.
- 542 Saitoh, K., M. Takagaki, and Y. Yamashita. 2003. Detection of Japanese flounder-specific
543 DNA from the gut contents of potential predators in the field. *Fisheries Science* **69**:
544 473-477.

545 Secor, S.M. 2008. Digestive physiology of the Burmese python: broad regulation of
546 integrated performance. *Journal of Experimental Biology* **211**: 3767-3774.

547 Sheppard, S.K., J.R. Bell, K.D. Sunderland, J. Fenlon, D.J. Skirvin, and W.O.C. Symondson.
548 2005. Detection of secondary predation by PCR analyses of the gut contents of
549 invertebrate generalist predators. *Molecular Ecology* **14**: 4461-4468.

550 Shine R, Wall M. 2007. Why is intraspecific variation in foraging biology more common in
551 snakes than in lizards? In: Reilly SM, McBrayer LB, Miles DB (eds). *Lizard ecology*.
552 Cambridge University Press, Cambridge, pp173-208.

553 Spellerberg, I.F. 1977. Behaviour of a young smooth snake, *Coronella austriaca* Laurenti.
554 *Biological Journal of the Linnean Society* **9**: 323-330.

555 Spellerberg, I.F., and T.E. Phelps. 1977. Biology, general ecology and behaviour of the
556 snake *Coronella austriaca*. *Biological Journal of the Linnean Society* **9**: 133-164.

557 Street, D. 1979. *The Reptiles of Northern and Central Europe*. Batsford BT, London.

558 Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. *Molecular*
559 *Ecology* **11**: 627-641.

560 van de Bund, C.F. 1964. De verspreiding van de reptielen en amfibieën in Nederland.
561 *Lacerta* **22**: 1-72.

562 Wilkinson, J.W., and A.P. Arnell. 2011. NARRS Report 2007-2009: Interim results of the
563 UK National Amphibian and Reptile Recording Scheme Widespread Species Surveys.
564 ARC Research Report **11/01**.

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Table 1. Species- and group-specific primers, with target mitochondrial gene, optimised annealing temperature and amplified product size.

| TARGET SPECIES/GROUP | PRIMERS | | GENE | ANNEALING TEMP. (°C) | PRODUCT SIZE |
|----------------------|--------------------------------------|---|------|----------------------|--------------|
| | FORWARD | REVERSE | | | |
| Common frog | RTF (TACAGCCGATACCTCCCTC) | RTR (TTCATGTCTCTTTGTAGAGG) | cytb | 62 | 176 |
| Smooth newt | LHF (GACTCGTACGAAACATCCA) | LHR (CGCCTATATATGGAATAGCGG) | cytb | 55.5 | 243 |
| Common lizard | LCO1498 (Folmer <i>et al.</i> 1994) | LV1714R (CCCGAACCCACCAATTATTAC) | COI | 62 | 216 |
| Lacertid spp. | LCO1498 (Folmer <i>et al.</i> 1994) | LV1714R (CCCGAACCCACCAATTATTAC) | COI | 53 | 216 |
| Slow worm | LCO1498 (Folmer <i>et al.</i> 1994) | AF1608R GGCTGGCTTAACTCTGCG | COI | 54 | 110 |
| Small mammal spp. | MM14701 (TGACAAACATACGAAAACACACCCAT) | MM14905 (ATGTGTGTTACTGATGAAAAGGCTGTTAT) | cytb | 55.5 | 206 |
| Bank / field vole | CG95 (Moran <i>et al.</i> 2008) | CG266 (Moran <i>et al.</i> 2008) | cytb | 58 | 171 |
| Common shrew | SA520 (Moran <i>et al.</i> 2008) | SA628 (Moran <i>et al.</i> 2008) | cytb | 64 | 108 |
| Pygmy shrew | SA520 (Moran <i>et al.</i> 2008) | SA628 (Moran <i>et al.</i> 2008) | cytb | 52 | ca.150 |
| Water shrew | SA520 (Moran <i>et al.</i> 2008) | SA628 (Moran <i>et al.</i> 2008) | cytb | 52 | ca.250 |
| General shrew spp. | SM421 (Moran <i>et al.</i> 2008) | SM544 (Moran <i>et al.</i> 2008) | cytb | 53 | 108 |
| Earthworm spp. | 185F (Harper <i>et al.</i> 2005) | 14233R (Harper <i>et al.</i> 2005) | 12S | 65 | 225-236 |
| <i>Arion</i> spp. | Ai1F (Harper <i>et al.</i> 2005) | AR2R (Harper <i>et al.</i> 2005) | 12S | 57 | 208-221 |

Figure legends

Figure 1. Proportion of smooth snakes ($n=58$) and grass snakes ($n=14$) testing positive for different mammal, reptiles, amphibian and invertebrate prey using specific primers in PCR.

Figure 2. Predicted probability of predation by smooth snakes (with SE, dotted line) on **a**) shrews (common and pygmy) and **b**) all small mammals, showing significant difference between sites and a significant effect of snake length (determined by GLM).

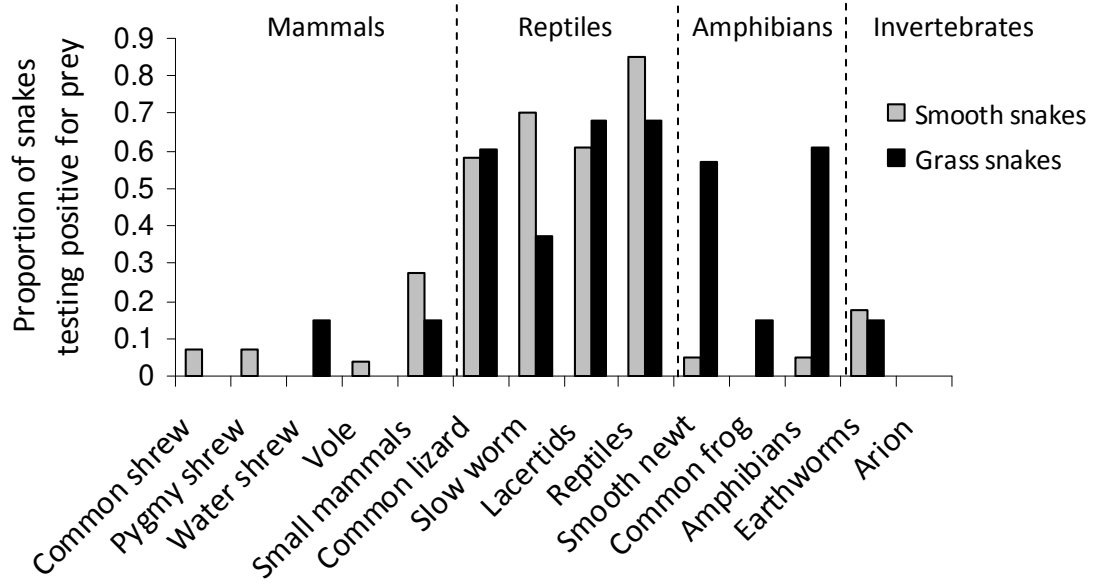


Figure 1.

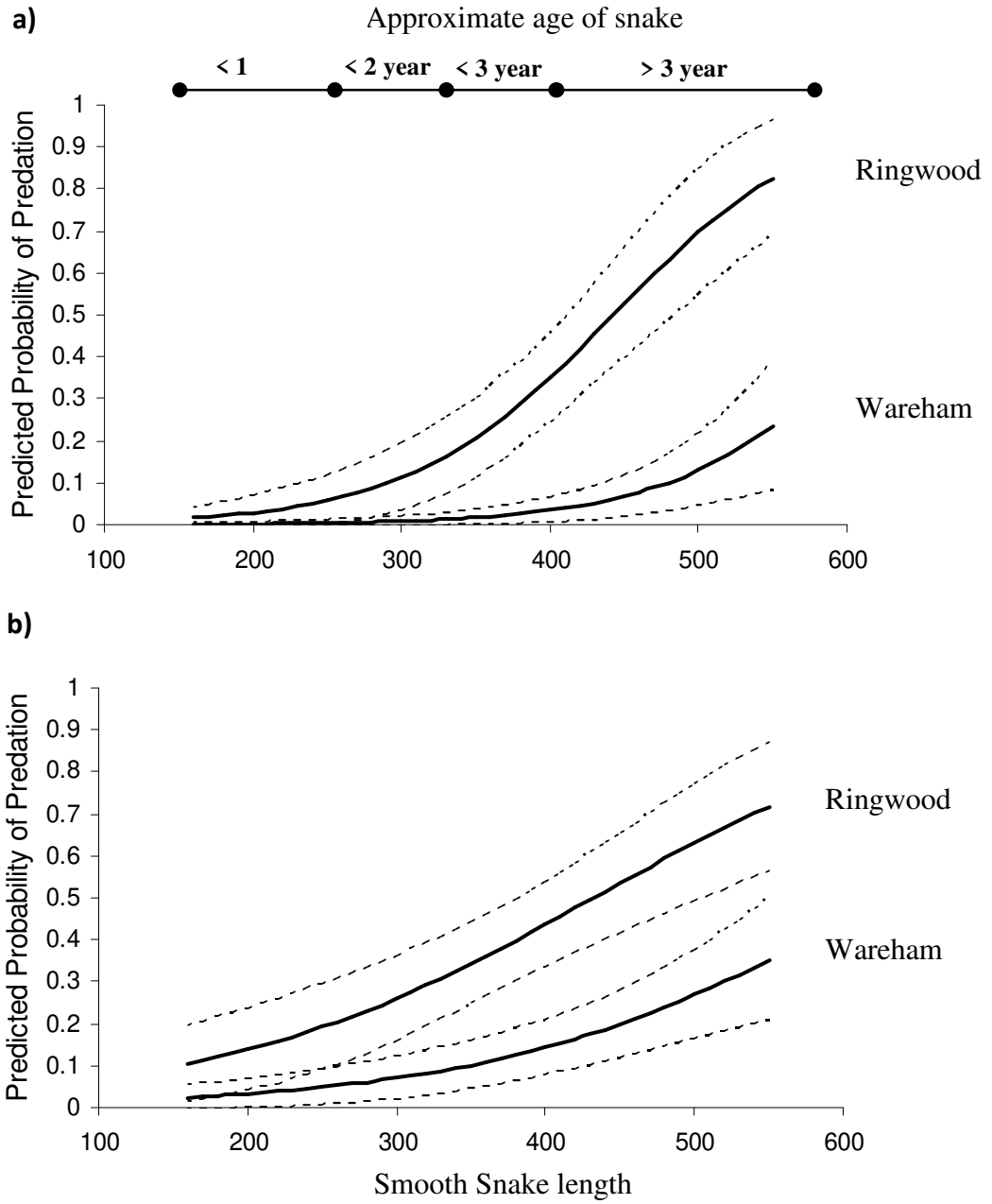


Figure 2.

| Year | Month | Sex | Site | SVL.cm | VTL.cm | Total.Length | Weight | Mean.Tem | Rainfall.mn |
|------|-----------|--------|----------|--------|--------|--------------|--------|----------|-------------|
| 2007 | August | Female | Wareham | 190 | 40 | 230 | 9 | 19.4 | 77 |
| 2007 | August | Male | Ringwood | 145 | 130 | 275 | 20 | 19.4 | 77 |
| 2007 | August | Female | Wareham | 250 | 50 | 300 | 20 | 19.4 | 77 |
| 2007 | August | Male | Wareham | 250 | 60 | 310 | 20 | 19.4 | 77 |
| 2007 | August | Female | Ringwood | 355 | 75 | 430 | 30 | 19.4 | 77 |
| 2008 | August | Female | Ringwood | 280 | 85 | 365 | 11.5 | 18.6 | 35.1 |
| 2008 | August | Male | Wareham | 360 | 100 | 460 | 36.7 | 20.2 | 92.8 |
| 2008 | August | Male | Wareham | 360 | 100 | 460 | 25 | 20.2 | 92.8 |
| 2008 | August | Male | Wareham | 420 | 130 | 550 | 39.5 | 20.2 | 92.8 |
| 2007 | July | Female | Ringwood | 180 | 40 | 220 | 10 | 19.8 | 121.7 |
| 2007 | July | Female | Wareham | 310 | 70 | 380 | 21 | 19.8 | 121.7 |
| 2007 | July | Female | Ringwood | 370 | 80 | 450 | 34 | 19.8 | 121.7 |
| 2007 | July | Male | Wareham | 350 | 115 | 465 | 35 | 19.8 | 121.7 |
| 2007 | July | Male | Wareham | 365 | 115 | 480 | 42 | 19.8 | 121.7 |
| 2007 | July | Male | Wareham | 365 | 115 | 480 | 42 | 19.8 | 121.7 |
| 2008 | July | Male | Ringwood | 160 | 30 | 190 | 4 | 20.2 | 92.8 |
| 2008 | July | Female | Wareham | 330 | 60 | 390 | 26 | 19.3 | 158.3 |
| 2008 | July | Male | Ringwood | 390 | 80 | 470 | 29 | 19.3 | 158.3 |
| 2008 | July | Male | Ringwood | 440 | 100 | 540 | 33 | 19.3 | 158.3 |
| 2007 | June | Female | Ringwood | | | | | 19.6 | 123.8 |
| 2007 | June | Female | Ringwood | | | | | 19.6 | 123.8 |
| 2007 | June | Female | Wareham | | | | | 19.6 | 123.8 |
| 2007 | June | Female | Wareham | 340 | 60 | 400 | 33 | 19.6 | 123.8 |
| 2007 | June | Male | Ringwood | 345 | 100 | 445 | 42 | 19.6 | 123.8 |
| 2007 | June | Male | Ringwood | 345 | 110 | 455 | 37 | 19.6 | 123.8 |
| 2008 | June | Male | Wareham | 250 | 60 | 310 | 10 | 19.1 | 44.4 |
| 2008 | June | Female | Ringwood | 260 | 60 | 320 | 15 | 19.1 | 44.4 |
| 2008 | June | Male | Wareham | 310 | 80 | 390 | 16.8 | 19.1 | 44.4 |
| 2008 | June | Male | Wareham | 330 | 70 | 400 | | 19.1 | 44.4 |
| 2008 | June | Male | Ringwood | 340 | 110 | 450 | 36 | 19.1 | 44.4 |
| 2008 | June | Male | Wareham | 380 | 100 | 480 | 37.7 | 19.1 | 44.4 |
| 2008 | June | Male | Wareham | 380 | 115 | 495 | 32 | 19.1 | 44.4 |
| 2008 | June | Male | Wareham | 400 | 120 | 520 | 48.9 | 19.1 | 44.4 |
| 2007 | May | Female | Wareham | | | | | 19.6 | 123.8 |
| 2007 | May | Male | Ringwood | 210 | 50 | 260 | 10 | 19.6 | 123.8 |
| 2007 | May | Male | Wareham | 255 | 65 | 320 | 18 | 16.6 | 119.4 |
| 2007 | May | Female | Wareham | 340 | 60 | 400 | 33 | 16.6 | 119.4 |
| 2007 | May | Female | Ringwood | 340 | 75 | 415 | 23 | 16.6 | 119.4 |
| 2007 | May | Male | Ringwood | 320 | 100 | 420 | 55 | 16.6 | 119.4 |
| 2007 | May | Female | Ringwood | 360 | 60 | 420 | 40 | 16.6 | 119.4 |
| 2008 | May | Male | Ringwood | 250 | 50 | 300 | 11.4 | 18.3 | 79.8 |
| 2008 | May | Male | Ringwood | 250 | 60 | 310 | 14.2 | 18.3 | 79.8 |
| 2008 | May | Male | Ringwood | 290 | 90 | 380 | 16.7 | 18.3 | 79.8 |
| 2008 | May | Female | Ringwood | 380 | 60 | 440 | 30 | 18.3 | 79.8 |
| 2008 | May | Male | Ringwood | 370 | 90 | 460 | 33 | 18.3 | 79.8 |
| 2007 | September | Female | Ringwood | | | | | 18.6 | 35.1 |
| 2007 | September | Female | Wareham | | | | | 18.6 | 35.1 |
| 2007 | September | Male | Ringwood | 260 | 80 | 340 | 12.4 | 18.6 | 35.1 |
| 2007 | September | Male | Ringwood | 350 | 80 | 430 | 24.3 | 18.6 | 35.1 |
| 2007 | September | Male | Ringwood | 370 | 100 | 470 | 31.1 | 18.6 | 35.1 |
| 2008 | September | Male | Ringwood | 240 | 60 | 300 | 6.4 | 17.7 | 82 |
| 2008 | September | Female | Wareham | 350 | 80 | 430 | 14 | 17.7 | 82 |
| 2008 | September | Male | Wareham | 420 | 130 | 550 | 27.7 | 17.7 | 82 |

Supplementary Material S2. Forward and reverse *cytochrome b* primers designed for a) common frog, b) smooth newt and c) small mammals showing alignments with other British amphibian, reptile and small mammal species. Reverse COI primers designed for d) common lizard and e) slow worm showing alignments with other British reptile species. LCO1498 (Folmer *et al.* 1994) was used as the forward primer with each COI reverse. (~) given where no sequence data was available.

| | <i>Prey species</i> | <i>Forward primer</i> | <i>Reverse primer</i> |
|----|---------------------|------------------------------------|-------------------------------------|
| a) | Common frog | 5' -CCTCTACAAAGAGACATGAA-3' | 5' -TACAGCCGATACCTCCCTC-3' |
| | Smooth newt | CATATTTAAAGAGACCTGAA | TACAGCAGACACACAATCA |
| | Palmate newt | CATATTTAAAGAGACATGAA | CACAGCAGACACACAATCA |
| | Red-spotted toad | TCTCTTTAAAGAGACCTGAA | CACAGCTGATACATCCATA |
| | Smooth snake | CCTAAATAAAAACGTCTGAC | CACAGCTAACATTAACCTT |
| | Water vole | CACCTTCATAGAAACATGAA | TACATCAGACACAATAACA |
| | Bank vole | CAATATAATTGAAACCTGAA | TACATCAGACACATCAACA |
| | Field vole | CAACATAATCGAAACATGAA | TACATCAGACACAGCAACA |
| | Common shrew | CATATACTTAGAAACATGAA | CACATCAGACACAATAACT |
| | Pygmy shrew | TATATACTTAGAAACATGAA | CACATCAGACACAATAACT |
| | Yellow-necked mouse | CAACATAATTGAAACCTGAA | CACATCAGATACATCAACA |
| | Wood mouse | TATTTTTATAGAAACATGAA | CACATCAGACACAATAACA |
| | House mouse | TACATTTATAGAAACCTGAA | CACATCAGATACAATAACA |
| | <i>Prey species</i> | <i>Forward primer</i> | <i>Reverse primer</i> |
| b) | Smooth newt | 5' -GATTAGTGCGAAACATTCA-3' | 5' -CGCCTATATATGGGATCGCTG-3' |

| | | |
|---------------------|----------------------|------------------------|
| Common frog | GACTCCTTCGTAATCTTCA | AGCCAATGTAGGGGGCGGCTG |
| Palmate newt | GACTCGTACGAAACATCCA | CGCCTATATATGGAATAGCGG |
| Red-spotted toad | GACTCCTACGCAACCTCCA | TTCCAATATATGGAGCAGCGG |
| Smooth snake | GAATAATACAAAACCTACA | ~~~~~ |
| Water vole | GATTAATTCGATATTTACA | TTCCGATGTATGGAATTGCTG |
| Bank vole | GACTTATTCGCTATATAACA | TGCCGATGTAAGGGATAGCTG |
| Field vole | GACTTATCCGATATATAACA | TGCCCTACGTAGGGGATGGCTG |
| Common shrew | GACTAATCCGATACCTTCA | AGCCGATATAAGGGATTGCTG |
| Pygmy shrew | GACTAATCCGCTATCTCCA | AGCCGATGTAAGGGATTGCTG |
| Yellow-necked mouse | GGCTGATCCGCTATACCCA | TGCCGATGTAGGGGATGGCTG |
| Wood mouse | GACTAATTCGATATATAACA | TTCCGATGTATGGAATTGCTG |
| House mouse | GACTAATCCGATATATAACA | TTCCAATATATGGGATGGCTG |

Prey species *Forward primer* *Reverse primer*

| | | | |
|-----------|----------------------|---|---|
| c) | Small mammals | 5' -TGACAAACATACGAAAAACACACCCAT-3' | 5' -ATATGGGCGATAGATGAGAATGCGAGGGA-3' |
| | Common frog | ~~~~~ | ATGTGAGCAACTGACGAGAATGCTGATTG |
| | Smooth newt | CCCACACTTTACGAAAGACCCATCCCT | ATGTGGGCTACTGATGAGAATGCTGATTG |
| | Palmate newt | CCCACCCTATACGAAAAACCCATCCGC | ATGTGGGCTACAGATGAGAAAGCTATGGA |
| | Red-spotted toad | ~~~~~ | ATATGAACAACGGATGAGAAGGCAAGGTT |
| | Smooth snake | ~~~~~ | ATATGAGTTACTGAAGAGAATGCTGTTAT |
| | Water vole | TGACAAACATTCGAAAAACACACCCCC | ATGTGGGCAACTGATGAGAATGCTGTTGA |
| | Bank vole | ~~~~~ | ATGTGGGCTACTGATGAGAATGCTGTTGC |
| | Field vole | ~~~~~ | ATGTGTGTGACTGATGAGAAAGCAGTTAT |
| | Common shrew | ~~~~~ | ATGTGCGTGACTGATGAGAAGGCAGTTAT |
| | Pygmy shrew | ~~~~~ | ATATGGGCGACTGATGAAAATGCTGTTGA |
| | Yellow-necked mouse | TGACAATTATTCGAAAAAACATCCAT | ATATGGGTCACTGAAGAAAATGCTGTTAT |

| | | |
|-------------|-----------------------------|-------------------------------|
| Wood mouse | ~~~~~ | ATGTGTGTTACTGATGAAAAGGCTGTTAT |
| House mouse | TGACAAACATACGAAAAACACACCCAT | ~~~~~GAGGGA |

Prey species

Reverse primer

d) Common lizard

5' -CCCGAACCCACCAATTATTAC-3'

| | |
|--------------|-----------------------|
| Slow worm | ~CCGAATCCGCCGATCATAAT |
| Smooth snake | ATGTATCAACATAAAACCTAA |
| Grass snake | GTGTATTAATATAAAACCTAA |
| Adder | ~CCAAAGCCCCGATTATAAT |

Prey species

Reverse primer

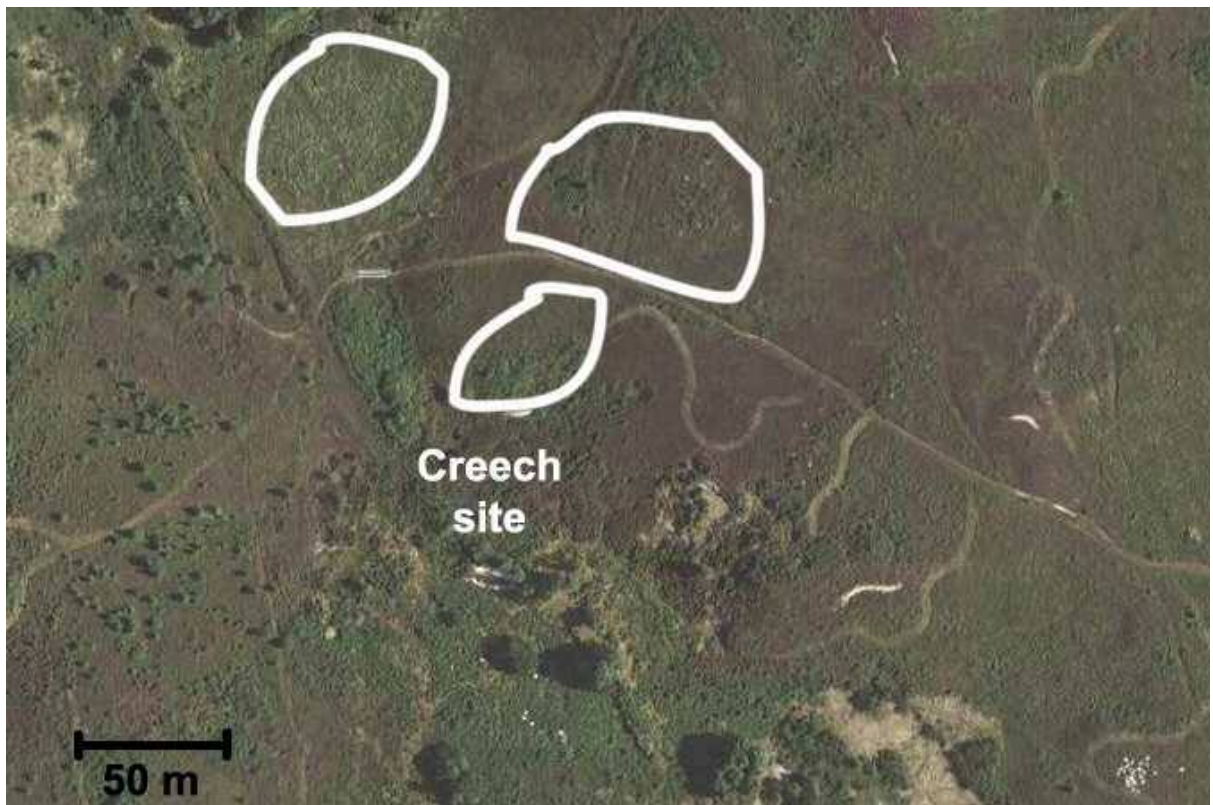
e) Slow worm

5' -GGCTGGCTTAACTCTGCG-3'

| | |
|---------------|--------------------|
| Common lizard | GGTTGGCTTAGTTCGGTT |
| Smooth snake | GCAGCAGCAATTACCATA |
| Grass snake | GCGGCAGCGATTACTATA |
| Adder | GGCTGAGTGAGTTCTATT |

Table S3

| | Number of predators testing positive for prey | | | |
|----------------------------|--|------|------------------------------|------|
| | Smooth snakes (<i>N</i> =53) | | Grass snakes (<i>N</i> =14) | |
| | <i>n</i> | % | <i>n</i> | % |
| Pygmy shrew | 3 | 5.7 | 0 | 0.0 |
| Water shrew | 0 | 0.0 | 2 | 14.3 |
| Bank vole | 2 | 3.8 | 0 | 0.0 |
| Small mammals | 15 | 28.3 | 2 | 14.3 |
| Common lizard | 31 | 58.5 | 9 | 64.3 |
| Slow worm | 38 | 71.7 | 5 | 35.7 |
| Lacertids | 33 | 62.3 | 5 | 35.7 |
| Reptiles | 45 | 84.9 | 10 | 71.4 |
| Smooth newt | 2 | 3.8 | 8 | 57.1 |
| Common frog | 0 | 0.0 | 2 | 14.3 |
| Amphibians | 2 | 3.8 | 9 | 64.3 |
| Earthworms | 9 | 17.0 | 2 | 14.3 |
| Slugs (<i>Arion</i> spp.) | 0 | 0.0 | 0 | 0.0 |



S2.

a)



b)

