

1 Heterochrony of puberty in the European Badger (*Meles meles*) can
2 be explained by growth rate and group-size: Evidence for two
3 endocrinological phenotypes

4
5 Nadine Adrianna Sugianto¹, Chris Newman¹, David Whyte Macdonald¹, Christina Dagmar
6 Buesching^{1*}

7
8 ¹ Wildlife Conservation Research Unit, Department of Zoology, University of Oxford,
9 Oxford, UK.

10
11 *Corresponding author

12 E-mail: christina.buesching@zoo.ox.ac.uk

13

14

15

16

17

18

19

20

21

22

23

24

25 **Abstract**

26 Puberty is a key stage in mammalian ontogeny, involving endocrinological,
27 physiological and behavioural changes, moderated by intrinsic and extrinsic factors. Thus,
28 not all individuals within one population achieve sexual maturity simultaneously. Here, using
29 the European badger (*Meles meles*) as a model, we describe male testosterone and female
30 oestrone profiles (using Enzyme-immunoassays) from first capture (3 months, post-weaning)
31 until 28 months (attaining sexual maturity and final body size), along with metrics of somatic
32 growth, scent gland development and maturation of external reproductive organs as well as
33 intra-specific competition. In both sexes, endocrinological puberty commenced at ca. 11
34 months. Thereafter, cub hormone levels followed adult seasonal hormone patterns but at
35 lower levels, with the majority of cubs reaching sexual maturity during their second mating
36 season (22-28 months). Interestingly, there was evidence for two endocrinological
37 phenotypes among male cubs (less evident in females), with early developers reaching sexual
38 maturity at 11 months (first mating season) and late developers reaching sexual maturity at
39 22-26 months (second mating season). Early developers also attained a greater proportion of
40 their ultimate adult size by 11 months, exhibiting faster growth rates than late developers
41 (despite having similar adult size). Male cubs born into larger social groups tended to follow
42 the late developer phenotype. Our results support the hypothesis that a minimum body size is
43 required to reach sexual maturity, which may be achieved at different ages, even within a
44 single population, where early maturity can confer individual fitness advantages and enhance
45 population growth rate.

46

47 **Key words:** *developmental heterochrony, European badger, oestrone, puberty, sexual*
48 *maturity, testosterone.*

49 **Introduction**

50 Puberty represents a key stage in mammalian ontogeny during which a variety of
51 endocrinological, physiological, and behavioural changes occur [1]. It is marked by the
52 development of secondary sexual characteristics [2], the first occurrence of ovulation/oestrus
53 in females and the onset of spermatogenesis in males [3]. During puberty, typically both
54 sexual and somatic maturation are completed [4], but some species may continue to grow
55 even after reaching sexual maturity [5]. Although the age at puberty depends predominantly
56 on intrinsic genetic factors, its timing can be moderated by a variety of additional extrinsic
57 factors, such as food availability, seasonal variation, environmental conditions [6-8] and/ or
58 the presence of conspecifics [3], as well as dynamic interactions between these factors [4].
59 Thus, typically not all members of a species [9], or even all individuals within one population
60 [1, 10-13] mature simultaneously or at the same rate, leading to heterochrony [14-15]. In
61 mammals, the onset of puberty typically depends on attaining a minimum body size (often a
62 certain functional proportion of the final adult body size: [10,16], and conspecifics may reach
63 this minimum required body size at different ages (e.g., dairy calves require 56-60% of adult
64 body weight which they may reach between 49.8 and 58.2 weeks of age: [10]). Individuals
65 that experience restricted resources during development therefore tend to undergo puberty at
66 an older age than do individuals that experienced abundant resources and are thus in better
67 nutritional condition [13], consequently there can be a trade-off between somatic growth and
68 puberty/ reproductive activity [17].

69 A distinctive endocrinological feature of puberty is the full activation of the
70 hypothalamic-pituitary-gonadal (HPG) axis. This involves the episodic release of
71 gonadotropin releasing hormones (GnRH) by the hypothalamus, which in turn activates the
72 anterior pituitary gland to secrete luteinizing hormone (LH) and follicle stimulating hormone
73 (FSH) that instigate the generation of gametes and release of sex steroids [18]: in males, LH

74 stimulates testosterone production from the interstitial cells of the testes (Leydig cells), and
75 FSH stimulates testicular growth and enhances the production of an androgen-binding protein
76 by the Sertoli cells, which are a component of the testicular tubules necessary for sustaining
77 maturing sperm cells [2]. In females, FSH stimulates the ovarian follicle(s), causing one/
78 several ovum/ ova to grow, and triggers the production of follicular oestrogen. This rise in
79 oestrogen then causes the pituitary gland to cease production of FSH and to increase LH
80 production instead. This rise in LH levels in turn causes the ovum/ ova to be released from
81 the ovary, resulting in ovulation [2]. Therefore, oestrogen and testosterone levels are low
82 throughout the prepubertal period, but increase immediately prior, during and after puberty,
83 until they reach adult concentrations [19-20].

84 Nevertheless, although the physiological processes of puberty have been the subject
85 of numerous studies [6, 8, 21], for many mammals, especially in wild-living populations,
86 knowledge regarding the factors driving sexual development and potential heterochrony in
87 puberty onset is still lacking [7, 20-21]. Particularly in seasonal breeders, it is often difficult
88 to determine sexual maturity unambiguously, because even adults exhibit periods of
89 reproductive quiescence when males cease spermatogenesis and females do not undergo
90 oestrus cycles [22-23]. Most carnivores (for exceptions see [24-26]), and all mustelids [27],
91 undergo such periods of seasonal reproductive quiescence, and in many the timing of their
92 sexual maturity is still being evaluated [26]. Here, we use the European badger (*Meles meles*;
93 henceforth “badger”) as a model seasonally breeding carnivore to investigate the
94 endocrinological changes and concomitant ontological development of male and female
95 genitalia during puberty, and examine how onset of puberty can be affected by body size and
96 intra-specific competition resulting in developmental heterochrony.

97

98 **Badger reproduction and development**

99 The badgers' mating season is restricted mainly to January-March, although further
100 matings can occur throughout the summer months [28] with local population density
101 determining the number of additional oestrous cycles ranging from nil to monthly [29-30].
102 During the mating season, scent marking activity increases [31], where particularly the
103 subcaudal gland secretion plays an important role in group-cohesion and olfactory mate
104 guarding [32-33], as well as resource defense and reproductive advertisement [34-35]. This is
105 reflected by significant elevation in the production of subcaudal secretion [32] as well as
106 changes to the secretion's chemical composition [34]. During the mating season, all mature
107 males have large scrotal testes, and females exhibit a distinctly swollen, pink and everted
108 vulva [36]. In contrast, during autumnal reproductive quiescence, males have smaller testes
109 that ascend into the body cavity, while females cease to exhibit vulval swelling [36]. Sex
110 steroid levels also exhibit distinct seasonal patterns in sexually mature badgers [37, 30, 36]:
111 In males, testosterone levels are high in spring and summer, low in autumn and peak during
112 the winter mating season. In females, oestrone levels are high in spring, low in summer, peak
113 in autumn and remain elevated for pregnant females in winter but decline in non-pregnant
114 females. As in all carnivores, male badgers have a baculum (os penis; [38]) that provides
115 mechanical support during copulation, thus enabling prolonged intromission and mate-
116 guarding through copulatory tying [39-40], facilitates sperm transport [41], and helps trigger
117 ovulation in species with induced ovulation such as badgers [28].

118 Badgers produce one litter annually (with mean litter size at our study site = 1.4 ± 0.06 ,
119 range of 1-4 cubs, where 93% litters comprise less than 3 cubs: [42]), born between mid
120 January – mid March (76% of cubs in the UK are born in mid-February: [42]). Newborn cubs
121 are highly altricial, and their eyes and ear canals do not open until they reach 5 weeks of age
122 [43]. Cubs are weaned at 6-8 weeks of age [44], during which time they first emerge from

123 their underground den, termed a sett [45], and are fully integrated into the social group at 14-
124 16 weeks of age [45]. Growth rate has been shown to vary among cubs depending on
125 resource availability, which is affected by prevailing weather conditions, linked to food
126 (mainly earthworm) availability [46] and natal sett quality [47-48], as well as infection with a
127 highly pathogenic intestinal coccidian (*Eimeria melis*), where surviving infected male and
128 female cubs exhibit, respectively, 5 cm and 3.5 cm shorter mature body-length [49].

129 In high density areas (such as our study population in Wytham Woods: 44.55 ± 5.37
130 (SE) badgers/km²; [42, 50]), cubs take longer to reach adult size than in lower density areas
131 [51, 52] and remain smaller than those living at lower density [51, 53]). Cubs start producing
132 subcaudal gland secretion when they are approximately 4 months old [32] but anoint
133 themselves with secretion from adults (a behaviour termed ‘scent-theft’) at a much younger
134 age [45], signifying the importance of this secretion in badger sociality [33]. Reports of
135 sexual maturity vary considerably, ranging from 9-12 months [54-55] to 18 months [32]; but
136 most studies evade the issue of exact age at puberty and simply state that female badgers
137 would not be able to breed until reaching the age of 2 years due to delayed embryonic
138 implantation (reviewed in [43]). No studies to-date have investigated potential developmental
139 heterochrony between individuals within the same population or cub cohort.

140 Here, we describe for the first time male testosterone and female oestrone profiles for
141 cubs, commencing from the time of first capture (i.e., at the end of the closed season when
142 cubs are 3 months of age and are fully weaned) through to the age of 28 months (i.e., when
143 all badgers have reached sexual maturity: [54]; as well as full adult size: [52]), and report on
144 the ontological development of male and female external genitalia morphology (EGM; i.e.,
145 degree of testicular descent and vulval swelling), baculum length, and testes volume.
146 Because in other mammals, somatic growth as well as sexual maturity have been shown to
147 vary among individuals within the same population [1,10], we then investigate if all cubs in

148 our sample mature at the same rate, or if there is evidence for different ontological strategies
149 in terms of hormone profiles, skeletal growth and the production of subcaudal gland secretion
150 (as reported in other species: [16]).

151

152 **Materials and methods**

153 **Badger Trapping and Sampling**

154 Data were collected from a high-density badger population in Wytham Woods,
155 Oxfordshire, UK (51°46:26 N, 1°19:19 W; for details see [42]) between 1995-2016, as part of
156 an ongoing long-term research project. Following the methodology described in [56], all
157 badgers received a permanent unique tattoo at first capture (typically as cubs: [42, 57],
158 allowing individual identification (ID) and reliable aging.

159 Badgers were trapped in every month except during the closed season under the
160 Protection of Badgers Act, 1992 (December-April), although, in some years, additional
161 trapping was conducted under special license in December and early January before the end
162 of the first pregnancy trimester [42]. The developmet of immature badgers could therefore be
163 followed in: (1) *spring* (May/June) at end of the main mating period when cubs are fully
164 weaned but spring weather can impact cub growth [58]; (2) *summer* (July/August/September)
165 during additional mating activity previously reported in other high-density badger
166 populations [29] and the period of lowest food abundance [46, 57]; (3) *autumn*
167 (October/November) during reproductive quiescence and highest food abundance; and (4)
168 *winter* (December/January) during the main mating season when cold weather may affect
169 thermal energy balance in badgers [59].

170

171 **Somatic measurements and classification of external genitalia**

172 **morphology**

173 Head-body length (to the nearest 5mm), zygomatic arch width (to the nearest 1mm),
174 and body weight (to the nearest 100g) were measured for all captured individuals, and a Body
175 Condition Index (BCI) was calculated as $\log_{10}(\text{weight})/\log_{10}(\text{body length})$. Subcaudal gland
176 secretion was scooped out of the subcaudal pouch using a rounded stainless-steel spatula
177 [32], and the volume estimated by eye to the nearest 0.05 ml. The spatula was disinfected
178 between individuals using absolute ethanol [60]. External Genitalia Morphology (EGM) was
179 recorded in both sexes and categorised according to Sugianto et al. [36] in females as normal,
180 intermediate or swollen vulva, and in males as ascended, intermediate or descended testes.
181 Male baculum length, testes length, width and scrotal thickness were measured (in mm), and
182 the testicular volume was calculated (in mm^3) as $(L \times W \times H) \times 0.71$, where L= testicle length
183 – scrotal pinch, W= testicle width – scrotal pinch, and H = testicle width – scrotal pinch [61].

184

185 **Blood Sampling and Hormone Measurements**

186 Blood samples ($n_{\text{males}}= 119$; $n_{\text{females}}= 63$; chosen from the available data set to
187 represent each month – except the closed season, see above) were collected for
188 endocrinological analyses via jugular venepuncture, using vacutainer tubes (Becton-
189 Dickinson) with K2-EDTA (ethylene diamine tetraacetic acid) anticoagulant. Sampling times
190 were standardized to account for circadian variation in hormonal profiles [36-37], and blood
191 samples were centrifuged within 30 minutes of sampling at 10°C for 10 min under 2,500
192 rpm/ 1470G. Plasma was transferred into Eppendorf tubes and frozen at -20°C immediately.

193

194 All sex steroid titres were analysed using Enzyme-immunoassays (EIA) and analysed
195 at the Chester Zoo Endocrinology Laboratory, UK. Oestrone was measured in
196 microtitreplates coated with polyclonal antiserum raised against oestrone EC R522 [62].
197 Plasma samples were un-extracted and used for measurement after dilution with assay buffer
198 at the ratio of 1:10. Duplicate 20µl aliquots of oestrone standard (0.195-200 pg/well), diluted
199 plasma, and quality controls were combined with 50µl oestrone glucuronide coupled to
200 horseradish peroxidase (oestrone-glucuronide-HRP) as label, and incubated at room
201 temperature for 2 hours. Plates were washed five times and blotted dry after incubation,
202 followed by an addition of 100 µL peroxidase substrate solution (ABTS) to each well. Plates
203 were covered and incubated at room temperature until the '0' wells reached approximately
204 1.0 optical density and read at 405 nm using a Spectrophotometer Opsys MR (Dynex). Assay
205 sensitivity at 90% binding was 3.1 pg. Intra-assay coefficients of variation (CV, calculated as
206 the average value from the individual CVs for all of sample duplicates), were 8.21 % (high)
207 and 6.05 % (low); inter-assay variation (repeated measurements of high and low-value
208 quality controls across plates) was 13.96 % (high) and 13.62 % (low) respectively.

209 Testosterone was measured in microtitre plates coated with anti-testosterone R156/7
210 (OEM-Concepts, UK). Samples (un-extracted) were analysed by dilution in 1:4 assay buffer.
211 Duplicate 50µl aliquots of testosterone standards (2.3-600 pg/well), samples and quality
212 controls were then combined with 50µl horseradish peroxidase (testosterone-HRP) as label.
213 After incubation in the dark at room temperature for 2 hours, plates were washed 5 times and
214 blotted dry, followed by addition of HRP-substrate (100 µL) to each well. Plates were
215 covered and incubated at room temperature until the '0' wells reached 1.0 optical density and
216 were then read at 405 nm, using a Spectrophotometer (Opsys MR; Dynex). Assay sensitivity
217 at 90% binding was 1.6 pg. The testosterone intra-assay coefficients of variation were 14.69

218 % (high) and 6.18 % (low), and inter-assay variation of high and low-value quality controls
219 was 9.15 % (high) and 5.23 % (low).

220

221 **Statistical analysis**

222 All statistical analyses were performed using RStudio (0.99.896) and R (R-3.2.4).
223 Patterns of residuals, normality, and mean variance for each model were checked using R
224 diagnostic plots. Generalized Additive Models (GAM) were used to generate trend lines for
225 sex steroid levels (males: testosterone; females: oestrone) against age (3-28 months) using a
226 smoothing function. A non-linear mixed model (random effect: badger identification/ tattoo
227 number, ID) using the *nlme* and *sslogic* function was used to form a growth curve (providing
228 an asymptote value as output) for bacculum length against age (3-28 months, n=773). To
229 determine the age at which the bacculum ceased to grow, the percentage of the predicted
230 bacculum length towards the asymptote (in the adult population) was calculated. Testes
231 volume (n=597) and subcaudal secretion volume (n_{male}=1233; n_{female}=1284) trend lines were
232 generated against age (3-28 months) by fitting a GAM model. Interactions between
233 proportions of EGM (males: descended, intermediate, ascended testes, n=1136; females:
234 normal, intermediate, swollen vulva, n=1174) with age (3-28 months) were analysed using a
235 Chi-square test.

236

237 **Developmental heterochrony**

238 **Endocrinology and EGM**

239 Our GAM average trends (above) provided a legitimate basis of hormonal
240 heterochrony in both sexes, where some cubs appeared to reach puberty earlier than others
241 (i.e., the existence of two discrete groups that differ in their sex-steroid hormone levels at a

242 certain age: high levels above and low levels below the GAM line benchmark, providing two
243 developmental categories, or phenotypes). However, because endocrinological sample sizes
244 were limited, we also repeated all analyses described below on EGM-based groups at the age
245 of 11 months during their first mating season, which enhanced sample sizes. That is, in
246 addition to comparing cubs with high vs low sex-steroid levels we also compared male cubs
247 with ascended vs descended testes and female cubs with a normal vs swollen vulva;
248 excluding intermediate conditions in both sexes to avoid ambiguity.

249

250 **Somatic growth**

251 We subsequently compared head-body length, zygomatic arch width, BCI, and
252 subcaudal secretion volume between these two groups using a linear model (including year as
253 a factor to account for established inter-annual variation in growth patterns: [49, 52] to
254 determine potential concurrent differences in physical development at the point of hormonal
255 divergence. If significant differences were found in any of the skeletal size measures, the
256 differences in head-body length and zygomatic arch width between adult size (above 28
257 months) to the size at the age at which divergence occurred were calculated in all individuals
258 and compared between the respective groups, to determine heterochronous residual growth
259 between the two groups. We then constructed growth curves for repeatedly captured
260 individuals in each group based on the rates of increase in head-body length (which provide a
261 reliable indicator for overall skeletal growth and development of badger cubs: [52]),
262 employing a non-linear mixed model (random effect: ID) using the *nlme* and *sslogic* function.
263 Where individuals were not recaptured at this precise target-age (within the 28 months
264 period), we used the closest recapture point available for these analyses.

265

266 **Social factors affecting the timing of puberty**

267 In addition, we investigated if social factors/ intra-specific competition affected the
268 timing of sexual maturity by comparing hormone levels at the age when these phenotypic
269 groups diverged with the total number of adults and cubs in that cub's natal group and natal
270 sett (as some groups utilize several setts: [63]), with year included as a factor. The total
271 number of resident adults and cubs was determined annually by assigning residency
272 according to the rules in Annavi et al. [50]; see also Sugianto et al. [52]).

273

274 **Results**

275 **Endocrinological changes during the first 28 months**

276 As predicted, throughout their first summer, all cubs had significantly lower sex-
277 steroid levels than did adults. However, at the age of 11-12 months, i.e. during their first
278 mating season, male cubs showed a small peak in testosterone (GAM: Edf= 8.631, R-
279 sq.(adj)= 0.566, GCV= 2.230, Deviance explained= 59.9%, $p < 0.001$, Fig 1), which was then
280 followed by the seasonal pattern of testosterone levels typical for adult males (high in spring
281 and summer, and low in autumn: [37]), with a pronounced peak that reached levels typical for
282 adults during their second mating season (22-28 months).

283

284 **Figure 1. Testosterone levels (ng/ml) in males aged 3-28 months.**

285

286 In females, oestrone levels increased gradually from the age of 3 up to 11 months, at
287 which point they almost reached adult levels (GAM: Edf= 5.218, R-sq.(adj)= 0.216, GCV=
288 733.1, Deviance explained= 28.2%, $p = 0.009$, Fig 2). Patterns then followed the seasonal
289 oestrone pattern typical for adults, with levels being relatively high in spring (13-16 months)

290 and decreasing in summer (17-19 months), remaining low during autumn (20-21 months,
291 reproductive quiescence) and winter (22 months, December: implantation) after which time
292 they increased again towards spring (27-28 months), this time reaching adult levels
293 (73.28 ± 28.06 pg/ml; [30]). Nevertheless, inter-individual variation among females was
294 considerable during the second summer (months 15-20), i.e., from the end of their first
295 mating season until autumnal reproductive quiescence.

296

297 **Figure 2. Oestrone levels (pg/ml) in females aged 3-28 months.**

298

299 **Changes in external genitalia during the first 28 months**

300 In both sexes, there was a significant interaction between EGM and age (in months)
301 (males_{n=1136}: $X^2 = 937.04$, $df = 36$, $p < 0.001$; females_{n=1174}: $X^2 = 418.47$, $df = 34$, $p < 0.001$; Fig
302 3a, b). The majority of male cubs had scrotal (i.e., fully descended) testes for the first time at
303 the age of 5-6 months (83.3% and 70.4% respectively), while during their first autumn (8-9
304 months) the majority had ascended testes (63.6% and 77.1% respectively). During their first
305 mating season in January (11 months), the largest proportion (41.5%) of male cubs had
306 descended testes, while both ascended and intermediate proportions were each 29.3%. During
307 the following spring (15 months) and summer (19 months) the majority of males (94.8% and
308 82.9%, respectively), had descended testes and followed the adult seasonal pattern thereafter.

309

310 **Figure 3. EGM changes in males (a) and females (b) aged 3-28 months.**

311

312 In females, the earliest vulval swellings (4.4% swollen; 20% intermediate vulva) were
313 recorded at the age of 11 months during their first mating season (Jan). The proportions of
314 females with intermediate and swollen vulva increased during the next spring-summer (15-19

315 months; intermediate: spring= 41.4%, summer= 30.5%; swollen: spring= 10.3%, summer=
316 6.2%) and decreased in autumn (20-21 months; intermediate: 7.8%, swollen: 1.5%). During
317 their third spring, the highest percentage of female cubs had either intermediate (50.4%) or
318 fully developed vulval swelling (19.2 %), congruent with adult states [36].

319 In males, testicular volume started to increase markedly at the age of 11 months (from
320 $848.69 \pm 475.20 \text{ mm}^3$ at the age of 4-6 months_{n=12}; $1331.27 \pm 1289.97 \text{ mm}^3$ at the age of 7-9
321 month_{n=32}; to an average of $3449.73 \pm 1572.04 \text{ mm}^3$ at 11 months_{n=18}) and peaked during the
322 first mating season ($5326.45 \pm 2674.88 \text{ mm}^3$, n=60; winter-early spring; GAM: Edf= 6.921, R-
323 sq.(adj) = 0.242, Deviance explained = 25.1%, p>0.001, Fig 4). Average testicular volume
324 then decreased towards an autumnal minimum at 20-21 months ($3209.42 \pm 2283.41 \text{ mm}^3$,
325 n=20) and followed the adult seasonal pattern thereafter, reaching a slightly higher peak
326 ($5698.26 \pm 2409.85 \text{ mm}^3$, n=187) in the second mating season (22-28 months), with sizes
327 comparable to adult values (winter: 6650.82 mm^3 , spring: 5776.31 mm^3 : [36]).

328

329 **Figure 4. Testes volume (mm³) in males aged 3-28 months.**

330

331 Male baculum length increased consistently month by month for the first year, when
332 baculum growth rates slowed and reached 99% towards the asymptote of 86.03 mm
333 predicted by our model at the age of 23-24 months (Fig 5).

334

335 **Figure 5. Baculum length growth curve at age of 3-28 months in males.**

336

337 **Subcaudal gland activity during the first 28 months**

338 Both sexes started producing subcaudal gland secretion at a similar age (during first
339 capture at 3 months; Fig 6). Nevertheless, secretion volume was very low (unmeasurable

340 traces in males and 0.04 ± 0.09 ml in females $(n=72)$ and increased only slowly towards their first
341 mating season at age 11 months (0.38 ± 0.36 ml in males $n=41$ and 0.19 ± 0.13 ml in females $n=47$).
342 Thereafter, secretion volume increased substantially in both sexes (GAM: male: Edf= 8.45,
343 R-sq.(adj)= 0.56, GCV= 0.18, Deviance explained= 56.2%, $p < 0.001$, Fig 6a; female: Edf=
344 8.83, R-sq.(adj)= 0.37, GCV= 0.04, Deviance explained= 38.2%, $p < 0.001$, Fig 6b). In male
345 yearlings, secretion volume peaked in spring-summer (13-18 months, 1.04 ± 0.61 ml, $n=292$),
346 decreased towards an autumn-minimum (20-21 months; 0.56 ± 0.39 ml, $n=97$) and peaked
347 again (with higher secretion volume) in their second winter-spring (23-28 months; 1.17 ± 0.58
348 ml, $n=210$), following the typical seasonal pattern and secretion volume of adults (average
349 values for spring= 1.06 ± 0.67 ml, $n=1004$; summer= 0.92 ± 0.66 ml, $n=1059$; autumn=
350 0.60 ± 0.50 ml, $n=790$; winter= 0.90 ± 0.60 ml, $n=347$ ml). Female yearlings showed a first
351 slight peak in secretion their second spring (13-16 months; 0.35 ± 0.24 ml, $n=150$), after which
352 volume decreased slightly during summer-autumn (17-19 months, 0.32 ± 0.35 ml, $n=189$ and
353 20-21 months, 0.27 ± 0.17 ml, $n=110$), but then started to increase again at the end of winter
354 (24 months), peaking at an average of 0.52 ± 0.41 ml in spring (27-28 months), and following
355 the adult pattern thereafter (average values for spring = 0.41 ± 0.34 ml, $n = 1287$; summer =
356 0.33 ± 0.32 ml, $n = 1286$; autumn = 0.25 ± 0.24 ml, $n = 933$ ml; winter = 0.24 ± 0.26 ml, $n = 207$).

357

358 **Figure 6. Subcaudal secretion volume (ml) changes in males (a) and females (b) aged 3-**
359 **28 months.**

360

361 **Evidence for two categories of cubs: Early- and Late-Developers**

362 **Endocrinological evidence for early and late developers**

363 In both sexes, some individuals appeared to reach puberty earlier than others
364 evidencing the existence of two endocrinological phenotypes: early- and late developers (Fig

365 1, 2), clustering into two distinct trait types according to the GAM line benchmark which
 366 exposed significantly different sex-steroid levels (for detailed results see Table 1 and 2).
 367 However, the age at which these early- and late development categories became apparent,
 368 differed between male and female cubs. For males, some (3/7= 42.9%) cubs reached puberty
 369 during their first year (HT, testosterone levels above the GAM line at 11 months of age),
 370 while the remainder reached puberty during their second year (LT, testosterone levels below
 371 the GAM line at 11 months, reaching pubescent levels at 22-28 months of age; Fig 1). In
 372 contrast, in females, the two possible endocrinological phenotypes diverged at age 15 -18
 373 months (younger cubs either had more unified levels or sample sizes were too small to
 374 signify a difference; Fig 2), where some females had above-average oestrone levels (HO;
 375 oestrone above the GAM line) and some below-average levels (LO; oestrone below GAM
 376 line). In both sexes, these endocrinological phenotypes manifested independent of calendar
 377 year.

378

379 **Table 1. Differences in somatic development between early and late developers from**
 380 **endocrinological and EGM grouping in male cubs.**

Grouping based on hormone levels in male cubs					
Endocrinological and somatic difference (at 11 months) between groups					
Endocrinological and somatic parameter	Groups based on hormone level				Linear model statistics (accounting for year as covariate)
	HT		LT		
	Avg + std	N	Avg + std	N	
Testosterone level (ng/ml)	5.58±2.19	3	1.15±1.14	4	F _{1,4} =9.534, p=0.037
Zygomatic arch (mm)	83.67±2.52	3	76.00±4.32	4	F _{1,4} =6.333, p=0.066
Head body length (mm)	656.67±23.09	3	610±23.45	4	F _{1,4} =8.448, p=0.044
Body Condition Index	0.31±0.01	3	0.29±0.02	4	F _{1,4} =5.831, p=0.073

Subcaudal secretion volume (ml)	0.33±0.32	3	0.14±0.08	4	$F_{1,4}=1.164, p=0.341$
Residual growth (11 – 28 months) difference between groups					
Somatic parameter	Groups based on hormone level				Linear model statistics (accounting for year as covariate)
	HT		LT		
	Avg + std	N	Avg + std	N	
Zygomatic arch (mm)	5.67±0.58	3	12.25±0.96	4	$F_{1,4}=180.437, p<0.001$
Head body length (mm)	28±26.96	3	77.5±19.36	4	$F_{1,4}=102.892, p<0.001$
Grouping based on EGM in male cubs					
Somatic difference (at 11 months) between groups					
Somatic parameter	Groups based on hormone level				Linear model statistics (accounting for year as covariate)
	DT		AT		
	Avg + std	N	Avg + std	N	
Zygomatic arch (mm)	83.11±3.82	9	79.40±4.33	10	$F_{1,16}=1.969, p=0.180$
Head body length (mm)	668.57±24.21	14	632.08±51.28	12	$F_{1,22}=9.389, p=0.006$
Body Condition Index	0.32±0.01	13	0.30±0.03	12	$F_{1,22}=10.611, p=0.004$
Subcaudal secretion volume (ml)	0.54±0.44	17	0.13±0.11	11	$F_{1,25}=10.730, p=0.003$
Residual growth (11 – 28 months) difference between groups					
Somatic parameter	Groups based on hormone level				Linear model statistics (accounting for year as covariate)
	DT		AT		
	Avg + std	N	Avg + std	N	
Zygomatic arch (mm)	6.50±3.25	8	11.60±4.12	10	$(F_{1,15}=7.805, p=0.014$
Head body length (mm)	33.21±15.14	14	70±38.79	12	$F_{1,23}=12.620, p=0.002$

381

382

383 **Table 2. Differences in somatic development between assumed phenotypes from endocrinological and**
 384 **EGM grouping in female cubs.**

Grouping based on hormone levels in female cubs					
Endocrinological and somatic difference (at 15 - 18 months) between groups					
Endocrinological and somatic parameter	Groups based on hormone level				Linear model statistics (accounting for year as covariate)
	HO		LO		
	Avg + std	N	Avg + std	N	
Oestrone level (ng/ml)	86.06±12.72	9	38.31±15.32	8	$F_{1,14} = 48.283, p < 0.001$
Zygomatic arch (mm)	80.44±3.81	9	81.29±3.15	7	$F_{1,13} = 0.219, p = 0.647$
Head body length (mm)	672.22±23.6	9	662.5±35.15	8	$F_{1,14} = 0.700, p = 0.417$
Body Condition Index	0.3±0.02	9	0.3±0.04	8	$F_{1,14} = 0.014, p = 0.909$
Subcaudal secretion volume (ml)	0.48±0.42	8	0.9±1.46	5	$F_{1,10} = 0.273, p = 0.613$
Grouping based on EGM in female cubs					
Somatic difference (at 15 - 18 months) between groups					
Somatic parameter	Groups based on hormone level				Linear model statistics (accounting for year as covariate)
	SV		NV		
	Avg + std	N	Avg + std	N	
Zygomatic arch (mm)	83.41±2.22	22	82.85±3.96	124	$F_{1,143} = 0.446, p = 0.505$
Head body length (mm)	678.1±18.54	21	675.53±26.59	148	$F_{1,166} = 0.188, p = 0.665$
Body Condition Index	0.3±0.01	21	0.3±0.02	147	$F_{1,165} = 0.051, p = 0.822$
Subcaudal secretion volume (ml)	0.36±0.22	21	0.32±0.21?	142	$F_{1,160} = 0.574, p = 0.450$
Somatic difference (at 11 months) between groups					
Somatic parameter	Groups based on hormone level				Linear model statistics (accounting for year as covariate)
	SV		NV		
	Avg + std	N	Avg + std	N	

Zygomatic arch (mm)	83.5±7.78	2	78.2±3.79	25	$F_{1,24} = 3.105, p=0.091$
Head body length (mm)	675±21.21	2	645.88±37.43	34	$F_{1,33} = 1.247, p= 0.272$
Body Condition Index	0.32±0.02	2	0.31±0.03	34	$F_{1,33} = 0.764, p=0.388$
Subcaudal secretion volume (ml)	0.2±0.0	2	0.18±0.14	33	$F_{1,33} = 0.221, p=0.641$

385

386

387 **Differences in somatic development between early and late developers**

388 **Males**

389 Comparing the extent of somatic development between the two endocrinological
390 phenotypes (at age 11 months), we found that HT males (n=3) had significantly larger head-
391 body length than LT cubs (n=4), were larger overall, and showed a near significant difference
392 in zygomatic arch width and BCI; but did not differ in the volume of subcaudal gland
393 secretion they produced.

394 To test whether the early (endocrinological) development of individuals assigned to
395 the HT group was simply the product of a more rapid development to adult size and were not
396 just larger cubs but indeed early-developers (and were thus closer to being fully developed,
397 sexually mature adults), we compared the differences in head-body length and zygomatic
398 arch width at the age above 28 months (i.e., fully developed adults) with those of cubs
399 assigned to HT- and LT endocrinological categories at the age of 11 months. The difference
400 in these measurements between HT-cubs and adults was significantly smaller than in LT-cubs
401 and adults, confirming that HT-cubs were closer to being fully developed adults (see Table 1).

402 For males, comparing the growth curves of HT (8 repeat-measures over 33 months
403 from 3 individuals) and LT types (32 repeat-measures over 33 months from 4 individuals)
404 revealed a trend (albeit non-significant, likely due to limited sample sizes) for LT-cubs to
405 grow more slowly than HT-cubs, despite ultimately reaching similar adult head-body lengths

406 ($X^2=1.873$, $df=7$, $p=0.392$; non-linear mixed model), where HT had already reached 95% of
407 the maximum head body length at the age of 11 months, whilst LT reached this percentage
408 later at the age of 14 months. This difference in body size disappeared at the age of 19-20
409 months, when growth rates of HT- and LT-males equalised (Fig 7).

410

411 **Figure 7. Body length growth curve of HT (open circles) and LT (solid black circles)**

412 **groups, age: 4-33 months**

413

414 We repeated these analyses based on the degree of testicular descent at the age of 11
415 months, comparing cubs that had fully descended testes (DT; assumed to have reached
416 puberty; $n=18$) with those that had ascended testes (AT; assumed to have not reached
417 puberty; $n=13$). Overall, these showed similar differences in somatic development to
418 categories arising according to endocrinological groups (for detailed results see Table 1). DT
419 cubs were considerably longer (head-body length), were in significantly better body
420 condition/BCI, and had significantly more subcaudal secretion at 11 months of age than AT
421 males; but no difference was found in zygomatic arch width. DT cubs also had significantly
422 smaller adult-cub differences in head-body length and zygomatic arch width than AT cubs,
423 which corroborated that (like HT-males) they were closer to adulthood.

424 Mirroring the trend found in the endocrinological phenotypes, there was a significant
425 difference in the growth curve of these two groups (Fig 8; $X^2=10.087$, $df=8$, $p=0.018$), with
426 DT cubs (117 repeat-measures taken over 35 months from 18 cubs) growing faster, and
427 reaching adult size earlier than AT cubs (99 repeat-measures taken over 35 months from 13
428 cubs). At the age of 19-20 months this difference disappeared and growth rates of DT and AT
429 cubs equalised.

430

431 **Figure 8. Body length growth curve of descended testes (DT) cubs and ascended testes**
432 **(AT) cubs, age: 3-35 months**

433

434 **Females**

435 In contrast, for females, we detected no (significant) differences between any of the
436 somatic parameters, nor for subcaudal gland volume, when comparing between the early
437 ($n_{HO}=9$) and late ($n_{LO}=8$) developing endocrinological phenotypes (see Table 2). To ensure
438 that the somatic similarity found between the two endocrinological phenotypes was not an
439 artefact of the smaller sample size for our endocrinological dataset, we used vulva category at
440 the ages between 15-18 months as the criteria defining stage of development. Again, on this
441 basis, we also found no significant differences (see Table 2) in any somatic parameters nor
442 subcaudal gland volume between SV cubs ($n=24$) and NV ($n=152$), evidencing that both
443 vulva condition types exhibited similar body size by age.

444 However, because some female cubs first exhibit vulval swelling at the age of 11
445 months ($n_{SV}=2$, $n_{NV}=34$), we repeated these analyses (see Table 2) at this younger age, but
446 again found no significant difference in BCI, head-body length, nor subcaudal gland volume,
447 although we did detect a slight difference in zygomatic arch between these groups.

448

449 **Social factors influencing the timing of puberty**

450 At the age of 11 months (see Table 3), testosterone levels in male cubs tended to be
451 lower in larger natal social groups and setts, albeit without statistical significance, although
452 the high R-value (0.53 and 0.63 for resident adults in natal social group and sett respectively)
453 evidences a strong correlation, where non-significance is likely due to low sample sizes; with
454 a similar interaction evidenced by an even higher R-value with number of other cubs present
455 in the natal social group and sett (0.76 and 0.85 for other resident cubs in natal social group

456 and sett respectively). That is, cubs born/ growing up in smaller social groups and/ or setts
 457 may be more likely to be early developers than those born/ growing up in larger groups/ setts.
 458 Using degree of testicular descent at the age of 11 months instead of the endocrinological
 459 phenotype, however, suggested no trend (see Table 3).

460

461 **Table 3. Social factors affecting the timing of puberty in male and female cubs**

Social factors affecting timing of puberty in male cubs (11 months)		
Social factor	Testosterone	EGM
Number of adults in natal social group	R= -0.53; $F_{1,4}= 1.407$, p=0.301	R= -0.39; $F_{1,26}= 1.052$, p=0.314
Number of adults in natal sett	R= -0.631; $F_{1,3}= 1.446$, p=0.316	R= 0.31; $F_{1,23}= 1.994$, p=0.171
Number of cubs in natal social group	R= -0.763; $F_{1,3}= 3.527$, p=0.157	R= 0.36; $F_{1,22}= 2.543$, p=0.125
Number of cubs in natal sett	R= -0.851; $F_{1,4}= 5.359$, p=0.082	R= 0.46; $F_{1,17}= 2.555$, p=0.128
Social factors affecting timing of puberty in female cubs (15 – 18 months)		
Social factor	Oestrone	EGM
Number of adults in natal social group	R= -0.157; $F_{1,14}=0.0393$, p=0.846	R= -0.1; $F_{1,172}=0.272$, p=0.603
Number of adults in natal sett	R= -0.116; $F_{1,14}=0.016$, p=0.901	R= -0.23; $F_{1,165}=2.281$, p=0.133
Number of cubs in natal social group	R= -0.267; $F_{1,14}=0.4299$, p=0.523	R= 0.02; $F_{1,144}=0.002$, p=0.963
Number of cubs in natal sett	R= -0.092; $F_{1,13}=0.052$, p=0.823	R= -0.02; $F_{1,102}=0.005$, p=0.944

462

463 Female cubs showed only very weak negative relationships between oestrone levels at

464 the age of 15-18 months with the number of adults resident in their natal social group and sett,
465 as well as the number of other cubs in their natal social group and sett. When cubs were
466 categorised on the basis of their EGM at the age of 15-18 months, again no effects were
467 found (see Table 3).

468

469 **Discussion**

470 We demonstrate that, in badgers, puberty begins in both sexes at ca. 11 months of age,
471 when cubs develop similar seasonal sex-steroid patterns to adults. Furthermore, in both sexes,
472 all parameters that support reproductive activity and mating-associated behaviours, such as
473 external genitalia morphology (and in males also testes volume), and subcaudal gland
474 secretion volume, show similar developmental patterns to sex steroid levels, further
475 corroborating the onset of puberty [1]. The increase of sex-steroid levels likely triggers
476 changes in EGM [36], as well as in the activity of species-specific subcaudal glands
477 important in the context of reproduction and sexual advertisement [64]. Nevertheless, cub
478 titres typically remained lower than those reported for adults (males: [37]; females: [30]) until
479 their second mating season (22-26 months).

480 In male cubs, testosterone levels remained low and exhibited no seasonal variation
481 until the first winter mating season, when they started to increase, reaching a smaller peak
482 than in adults [37]. Levels then remained elevated until the end of the mating season, and
483 followed the seasonal pattern typifying adults thereafter. By the time male cubs reached the
484 second population breeding season, their testosterone titres had reached higher levels
485 (compared to the first mating season) in accord with adults [37]. Baculum growth also
486 reached the population-based asymptote by the cubs' second mating season, indicating the
487 completion of sexual development. These findings support the hypothesis by Whelton and
488 Power [38], who measured baculum length post-mortem in road kill and culled badgers,

489 positing that the observed abrupt decrease in baculum growth rate coincides with sexual
490 maturity; although their post-mortem study was unable to verify this conclusion through
491 endocrinological measurements.

492 In contrast, in females, we observed a gradual increase in oestrone from the age of 3-
493 11 months (May-January) without any noticeable seasonal variation. After age 11 months,
494 however, cub oestrone levels started to follow the same seasonal pattern as adults [30], with
495 high levels in spring and low levels in summer. Nevertheless, although adult females oestrone
496 levels typically increase in autumn and remain elevated until blastocyst implantation in
497 December [30], cub oestrone levels remained low until the next mating season (January),
498 implying that – counter to observations from other, low-density studies [54] - no female cubs
499 in our dataset were capable of mating successfully during their first year, corroborating
500 genetic results from our study population reported previously [65].

501 Inter-individual variation in plasma sex-steroid levels, however, was considerable
502 among same-age cubs of either sex, and we observed two distinct categories: early and late
503 developers. We infer these to qualify as distinct phenotypic response types, given the
504 potential fitness advantage of early maturity [66], but set against the reality of resource
505 limitation and social stress in wild populations typically precluding all individuals from
506 engaging in the maximal developmental response [51]. In male cubs, endocrinological
507 profiles and EGM indicated that some had reached sexual maturity at the age of 11 months,
508 while the remaining cubs likely achieved this only during their second winter. At this time all
509 male cubs showed similarly large testosterone peaks comparable to adult levels and puberty
510 had concluded. Similarly, there was substantial variation in the proportion of the final body
511 length males had achieved by age 11 months, mirroring the differences in testosterone levels
512 observed during this period. This increase in head body length ceased by age ca. 18 months
513 (99 % towards asymptote; [52]) which is also the age at which body lengths equalised when

514 dividing males according to both testes descent categories (DT and AT as well as
515 endocrinological categories HT and LT). Nevertheless, when we cross-referenced against
516 assigned paternity data, none of the individuals exhibiting high hormone levels at 11 months
517 (HT=3 individuals) were assigned cubs the spring after. For female cubs, variation in
518 oestrogen levels was high during their second summer (May-Sept/ Oct), indicating that not all
519 females reached sexual maturity at the same age, but that puberty onset varied between 15-18
520 months of age.

521 The existence of different ontological phenotypes (i.e., early and late developers) has
522 been described in other members of the Mustelidae, and has been linked to body size and
523 species-specific life history traits [27] For example, in captive sables (*Martes zibellina*), a
524 small proportion of all individuals are reported to reproduce at 15 months of age, whereas the
525 majority (80%) of males and females starts breeding at the age of 27 months [27]. Our results
526 support observations from badgers in Sweden, where spermatozoa were first recorded in male
527 cubs at the age of 12 months [54]. Nevertheless, in this Swedish low-density population, the
528 majority of males reached puberty in their first year, and only a minority of males did not
529 produce spermatozoa until their second summer, or even winter (24 months: [54]). In our
530 high-density population, in contrast, most males reached puberty in their second year.

531 Overall, our results support the hypothesis that mammals typically need to reach a
532 threshold body size for sexual maturation [10]. Thus, the age at which puberty occurs is
533 likely not only influenced by the gene load of the individual but also by ecological factors
534 such as access to food (affected by weather/ climate), competition, and differences in
535 demographic factors [1]. Resource availability tends to vary across time and space [67], and
536 access to resources is further constrained by the number of competing conspecifics present
537 leading to social stress [63]. Consequently, energy budgets can differ substantially between
538 individuals even within a single population/ year, with the potential to drive considerable

539 variability in the timing of sexual maturity [1]: Individuals that develop under poor
540 nutritional conditions, or subject to more social stress resulting from competition, usually
541 reach sexual maturity at slower rates [11-13] as implied by our observation that male cubs
542 born into bigger setts and larger social groups tended to be biased toward the late developer
543 phenotype.

544 Generally, in mammals (especially those with polygynous mating systems), males
545 tend to grow more quickly than females [68], and ultimately attain a larger body-size (i.e.,
546 dimorphism; see Badyaev [69]). Our data show that this is also the case in badgers (see also
547 Sugianto et al. [52]; NB, our measurements were made after weaning, and thus obviating
548 differential maternal investment effects; [70]). Thus, males are likely also more vulnerable to
549 resource limitation and social competition, with the potential to impact their degree of
550 development by the end of their first year, explaining the observed delay in puberty in larger
551 social groups [71- 72]. Our findings, are congruent with those for female brown bears (*Ursus*
552 *arctos*), where adult body size shows a negative relationship with population density [73]:
553 female bears are larger and grow faster in areas with better environmental conditions, while
554 with higher resource competition, females are smaller and grow more slowly. As in our
555 badger study, bears have been shown to compensate for slower growth rates by delaying
556 reproductive activity [73] at the potential cost of lower lifetime reproductive success [74].
557 Similar negative correlations between population density and individual growth rate has been
558 reported in the northern fur seal (*Callorhinus ursinus*; [75]), polar bears (*Ursus maritimus*; in
559 terms of smaller juvenile body length [76], and adult body size [77]), and in American black
560 bears (*Ursus americanus*; with lower yearling weight; [78]). Similarly, in farmed red deer
561 (*Cervus elaphus*), [79] found that the growth rate of subordinate females was 2.5 times
562 slower, and average daily weight gain of all juvenile hinds was significantly impaired, under
563 high stocking density. Demographic effects have also proven to affect the onset of maturation

564 in female baboons (*Papio cynocephalus*), where first menstruation was earlier in smaller
565 groups where individuals experience less social stress and competition [80].

566 We thus conclude that the asynchronous timing of puberty, leading to two
567 heterochronous phenotypes, can occur even within a single population, and is likely caused
568 by individuals attaining the required minimum body size according to different time scales.
569 Ultimately, capacity to breed at a young(er) age can have profound effects on life-history
570 trade-offs (see [81] with early-life success often being critical to an individual's fitness [66]
571 and can substantially enhance population growth rate [82].

572

573 **Acknowledgements**

574 We acknowledge the long-term support of the People's Trust for Endangered Species
575 (PTES) for the Wytham Badger Project. NAS was supported by a DPhil scholarship from
576 Indonesia Endowment for Education 2014-2018, and CDB was supported by a Research
577 Fellowship from the Poleberry Foundation. Dr. Sue Walker's help for hormone analyses in
578 Chester Zoo Laboratory, Chester is warmly acknowledged. All protocols and procedures
579 employed were approved by the Animal Welfare and Ethical Review board of Oxford
580 University's Zoology Department and procedures were conducted under the Animals
581 (Scientific Procedures) Act, 1986 and Natural England licenses (PPL: 30/3379)

582

583 **References**

- 584 1. Onyango PO, Gesquiere LR, Altmann J, Alberts SC. Puberty and dispersal in a wild
585 primate population. *Horm Behav.* 2013; 64(2): 240-249.
- 586 2. Adkins-Regan E. *Hormones and Animal Social Behavior*. Princeton University Press.
587 Princeton, NJ and Oxford, UK. 2005. pp 415.
- 588 3. Evans ACO, O'Doherty JV. Endocrine changes and management factors affecting puberty
589 in gilts. *Livest Prod Sci.* 2001; 68(1): 1-12.
- 590 4. Tena-Sempere M. Keeping puberty on time: novel signals and mechanisms involved. *Curr*
591 *Top Dev Biol.* 2013; 105: 299-329.

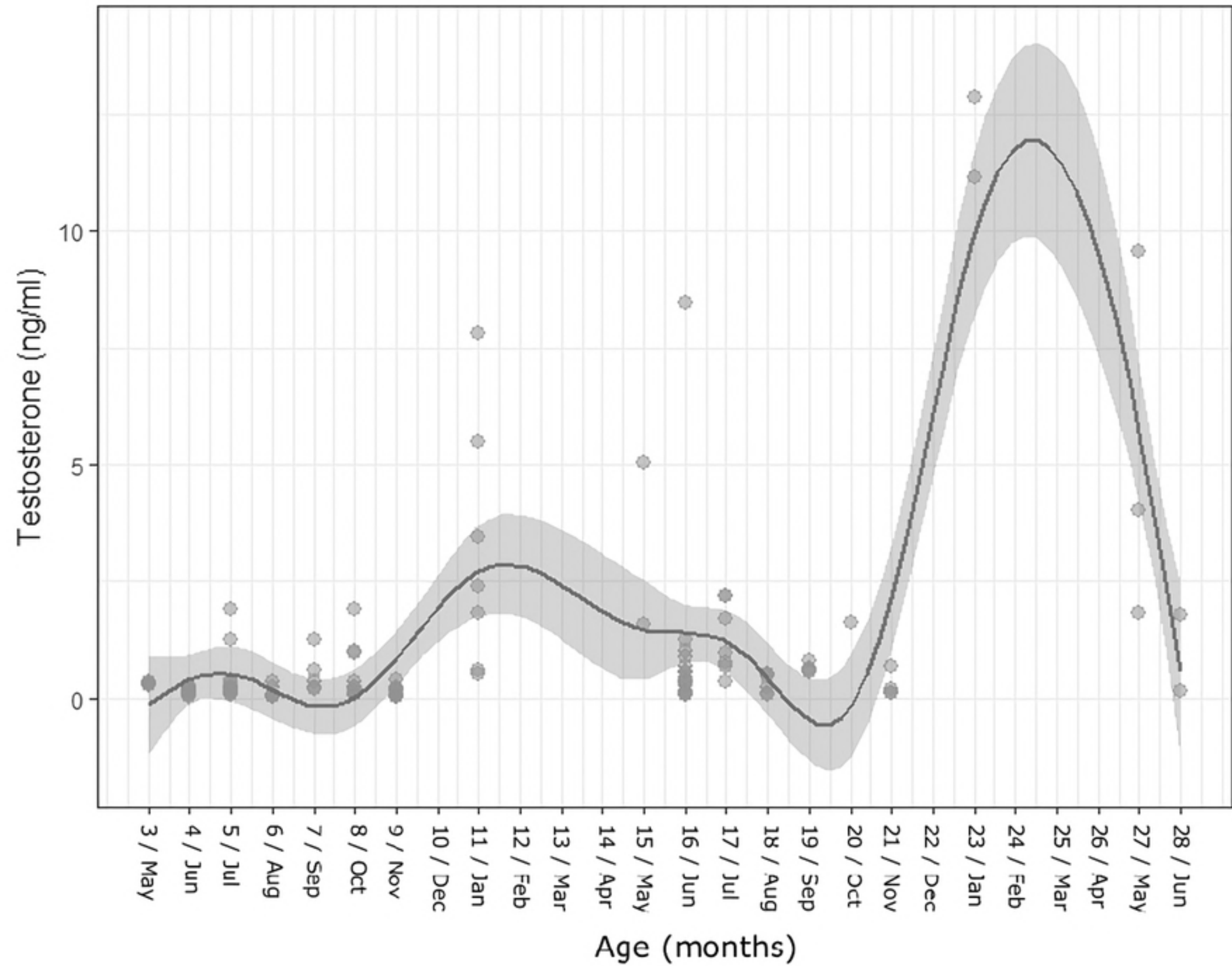
- 592 5. Stamps J, Krishnan VV. Sexual bimaturation and sexual size dimorphism in animals with
593 asymptotic growth after maturity. *Evol Ecol.* 1997; 11(1): 21-39.
- 594 6. De Jonge FH, Bokkers EAM, Schouten WGP, Helmond FA. Rearing piglets in a poor
595 environment: developmental aspects of social stress in pigs. *Physiol Behav.* 1996; 60(2):
596 389-396.
- 597 7. Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP. The timing
598 of normal puberty and the age limits of sexual precocity: variations around the world,
599 secular trends, and changes after migration. *Endocr Rev.* 2003; 24(5): 668-693.
- 600 8. Abeygunawardena H, Dematawewa CMB. Pre-pubertal and postpartum anestrus in
601 tropical Zebu cattle. *Anim Reprod Sci.* 2004; 82: 373-387.
- 602 9. Delemarre-van de Waal HA, van Coeverden SC, Engelbregt MJ. Factors affecting onset of
603 puberty. *Hormone Research in Paediatrics.* 2002; 57(Suppl. 2): 15-18.
- 604 10. Freetly HC, Kuehn LA, Cundiff LV. Growth curves of crossbred cows sired by Hereford,
605 Angus, Belgian Blue, Brahman, Boran, and Tuli bulls, and the fraction of mature body
606 weight and height at puberty. *J Anim Sci.* 2011; 89(8): 2373-2379.
- 607 11. Plaistow SJ, Lapsley CT, Beckerman AP, Benton TG. Age and size at maturity: sex,
608 environmental variability and developmental thresholds. *Proceedings of the Royal
609 Society B: Biological Sciences.* 2004; 271(1542): 919.
- 610 12. Alberts SC. Magnitude and sources of variation in male reproductive performance. In:
611 Mitani JC, Call J, Kappeler PM, Palombit RA, Silk JB, editors. *The Evolution of Primate
612 Societies.* University of Chicago Press. 2012. p 412-431.
- 613 13. Pusey A. Magnitude and sources of variation in female reproductive performance. In:
614 Mitani J C, Call J, Kappeler P M, Palombit R A, Silk J B, editors. *The Evolution of
615 Primate Societies.* University of Chicago Press. 2012; 343-366.
- 616 14. Klingenberg CP. Heterochrony and allometry: the analysis of evolutionary change in
617 ontogeny. *Biol Rev.* 1998; 73(1): 79-123.
- 618 15. Smith KK. Sequence heterochrony and the evolution of development. *J Morphol.* 2002;
619 2002; 252(1): 82-97.
- 620 16. Day T, Rowe L. Developmental thresholds and the evolution of reaction norms for age
621 and size at life-history transitions. *Am Nat.* 2002;. 159(4): 338-350.
- 622 17. Green WC, Rothstein A. Trade-offs between growth and reproduction in female bison.
623 *Oecologia.* 1991;.86(4): 521-527.
- 624 18. Plant TM. The male monkey as a model for the study of the neurobiology of puberty
625 onset in man. *Mol Cell Endocrinol.* 2006; 254: 97-102.
- 626 19. Fitzgerald J, Butler WR. Seasonal effects and hormonal patterns related to puberty in ewe
627 lambs. *Biol Reprod.* 1982; .27(4): 853-863.
- 628 20. Beehner JC, Gesquiere L, Seyfarth RM, Cheney DL, Alberts SC, Altmann J. Testosterone
629 related to age and life-history stages in male baboons and geladas. *Horm Behav.* 2009;
630 56(4): 472-480.
- 631 21. Sisk CL, Foster DL. The neural basis of puberty and adolescence. *Nat Neurosci.* 2004;
632 7(10): 1040-1047.
- 633 22. Curlew JD. Seasonal prolactin secretion and its role in seasonal reproduction: a review.
634 *Reprod Fertil Dev.* 1992; 4(1): 1-23.
- 635 23. Revel FG, Ansel L, Klosen P, Saboureau M, Pévet P, Mikkelsen JD, Simonneaux V.
636 Kisspeptin: a key link to seasonal breeding. *Rev Endocr Metab Disord.* 2007; 8(1): 57-65.
- 637 24. Goeritz F, Neubauer K, Naidenko SV, Fickel J, Jewgenow K., Investigations on
638 reproductive physiology in the male Eurasian lynx (*Lynx lynx*). *Theriogenology.* 2006;
639 66:1751-1754.

- 640 25. Jewgenow K, Naidenko SV, Goeritz F, Vargas A, Dehnhard M. Monitoring testicular
641 activity of male Eurasian (*Lynx lynx*) and Iberian (*Lynx pardinus*) lynx by fecal
642 testosterone metabolite measurement. *Gen Comp Endocrinol.* 2006; 149:151–8.
- 643 26. Jewgenow K, Songsasen N. Reproduction and advances in reproductive studies in
644 carnivores. In: Holt WV, Brown JL, Comizzoli P, editors. *Reproductive sciences in*
645 *animal conservation.* Springer, New York, NY. 2014. pp. 205-239.
- 646 27. Amstislavsky S, Ternovskaya Y. Reproduction in mustelids. *Anim Reprod Sci.* 2000; 60:
647 571-581.
- 648 28. Yamaguchi N, Dugdale HL, Macdonald DW. Female Receptivity, Embryonic Diapause,
649 and Superfetation in the European Badger (*Meles meles*): Implications for the
650 Reproductive Tactics of Males and Females. *Q Rev Biol.* 2006; 81(1): 33-48.
- 651 29. Corner LA, Stuart LJ, Kelly DJ, Marples NM. Reproductive Biology Including Evidence
652 for Superfetation in the European Badger *Meles meles* (Carnivora: Mustelidae). *PLoS*
653 *ONE.* 2015; 10(10): e0138093.
- 654 30. Sugianto NA, Heistermann M, Newman C, Macdonald DW, Buesching CD. Delayed
655 implantation combined with superfoetation provides a flexible mechanism for assuring
656 mating success in the European badger (*Meles meles*). *J Comp Physiol A.* Subm.a
- 657 31. Buesching CD, Macdonald DW. Scent-marking behaviour of the European badger (*Meles*
658 *meles*): resource defence or individual advertisement?. In: *Chemical signals in Vertebrates*
659 9. Springer, Boston, MA. 2001. p 321-327.
- 660 32. Buesching CD, Newman C, Macdonald DW. Variations in colour and volume of the
661 subcaudal gland secretion of badgers (*Meles meles*) in relation to sex, season and
662 individual-specific parameters. *Mamm Biol - Zeitschrift für Säugetierkunde.* 2002a;
663 67(3): 147-156.
- 664 33. Buesching CD, Stopka P, Macdonald DW. The social function of allo-marking in the
665 European badger (*Meles meles*). *Behaviour.* 2003; 140(8): 965-980.
- 666 34. Buesching CD, Waterhouse JS, Macdonald DW. Gas-chromatographic analyses of the
667 subcaudal gland secretion of the European badger (*Meles meles*) part I: chemical
668 differences related to individual parameters. *J Chem Ecol.* 2002b; 28(1): 41-56.
- 669 35. Buesching CD, Macdonald DW. Variations in scent-marking behaviour of European
670 badgers *Meles meles* in the vicinity of their setts. *Acta Theriol.* 2004; 49(2): 235-246.
- 671 36. Sugianto NA, Buesching CD, Heistermann M, Newman C, Macdonald DW. Linking
672 plasma sex-steroid levels to the condition of external genitalia in European badgers
673 (*Meles meles*): A critical evaluation of traditional field methodology. *Mamm Biol. In*
674 *press*
- 675 37. Buesching CD, Heistermann M, Macdonald DW., Seasonal and inter-individual variation
676 in testosterone levels in badgers *Meles meles*: evidence for the existence of two
677 endocrinological phenotypes. *J Comp Physiol A.* 2009; 195(9): 865-871.
- 678 38. Whelton HJ, Power SB. The use of badger bacula as a method of age determination in a
679 badger population infected with tuberculosis. In: *Biology and Environment: Proceedings*
680 *of the Royal Irish Academy.* 1993; Vol. 93B No. 1: 45-47.
- 681 39. Verrell, P. A. Primate penile morphologies and social systems: further evidence for an
682 association. *Folia Primatol.* 1992; 59(2): 114-120.
- 683 40. Dixson AF. Baculum length and copulatory behaviour in carnivores and pinnipeds (Grand
684 Order Ferae). *J Zool.* 1995; 235(1): 67-76.
- 685 41. Stockley P. The baculum. *Curr Biol.* 2012; 22(24): R1032-R1033.
- 686 42. Macdonald DW, Newman C, Buesching CD. Badgers in the rural landscape—conservation
687 paragon or farmland pariah: Lessons from the Wytham badger project. In: Macdonald
688 DW, Feber RE, editors. *Wildlife conservation on farmland Volume 2: Conflict in the*
689 *Countryside.* Oxford University Press. 2015. pp 65-95.

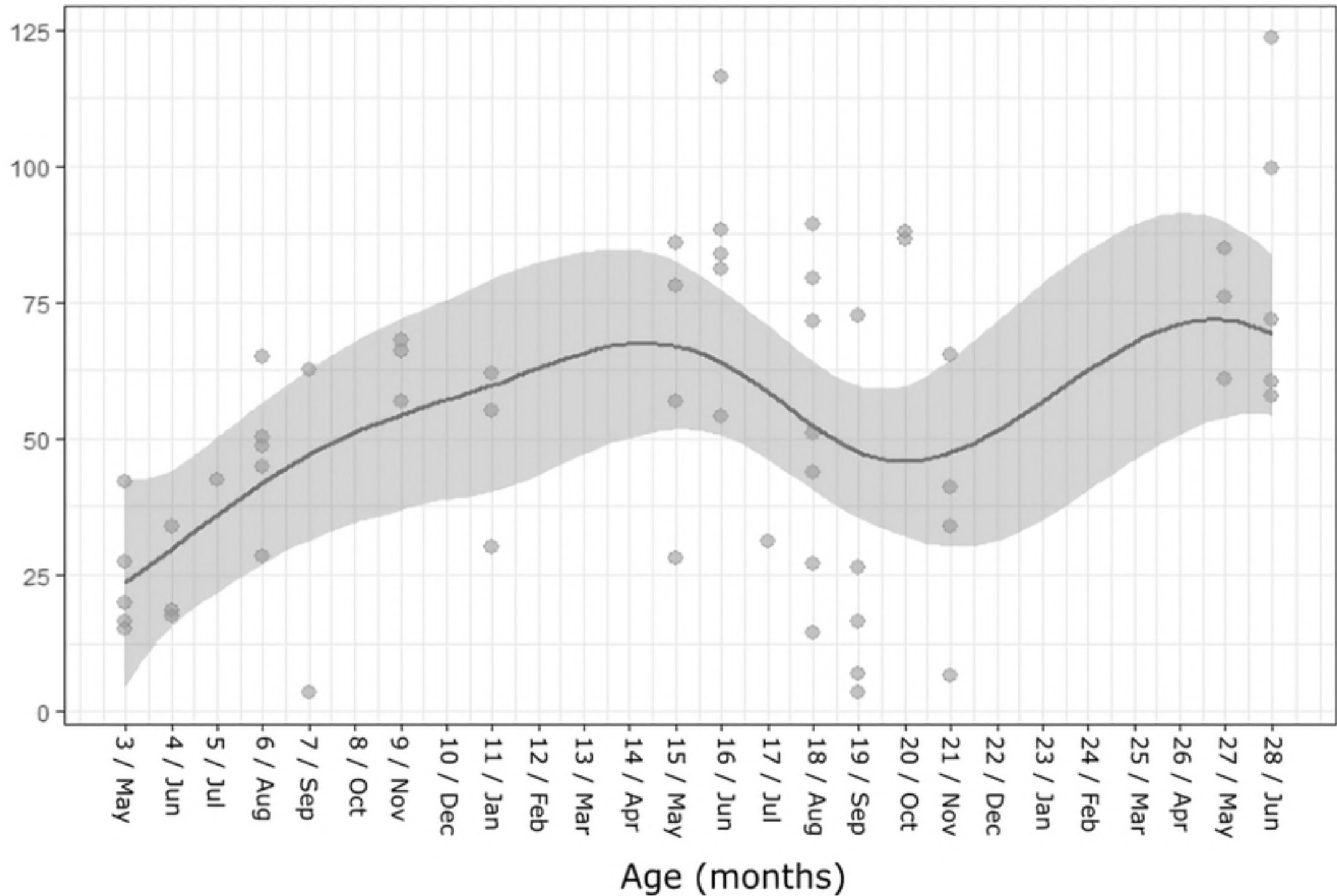
- 690 43. Roper TJ, Badger. Volume 114. Harper Collins, United Kingdom. 2010. pp 416.
- 691 44. Woodroffe R, Macdonald DW. Helpers provide no detectable benefits in the European
692 badger (*Meles meles*). J Zool. 2000; 250(1): 113-119.
- 693 45. Fell RJ, Buesching CD, Macdonald DW. The social integration of European badger
694 (*Meles meles*) cubs into their natal group. Behaviour. 2006; 143(6): 683-700.
- 695 46. Nouvellet P, Newman C, Buesching CD, Macdonald DW. A multi-metric approach to
696 investigate the effects of weather conditions on the demographic of a terrestrial mammal,
697 the European badger (*Meles meles*). PloS ONE. 2013; 8(7): e68116.
- 698 47. Kaneko Y, Newman C, Buesching CD, and Macdonald DW. Variations in Badger (*Meles*
699 *meles*) Setts Microclimate: Differential Cub Survival between Main and Subsidiary Setts,
700 with Implications for Artificial Setts Construction. Int J Ecol. 2010; Article ID 859586, 1-
701 10. <https://doi.org/10.1155/2010/859586>.
- 702 48. Tsunoda M, Newman C, Buesching CD, Macdonald DW, Kaneko Y. Badger setts
703 provide thermal refugia, buffering changeable surface weather conditions. J Therm Biol.
704 2018; 74: 226-233.
- 705 49. Newman C, Macdonald DW, Anwar MA. Coccidiosis in the European badger, *Meles*
706 *meles* in Wytham Woods: infection and consequences for growth and survival.
707 Parasitology. 2001; 123(2):133-142.
- 708 50. Annavi G, Newman C, Dugdale HL, Buesching CD, Sin YW, Burke T, Macdonald DW.
709 Neighbouring-group composition and within-group relatedness drive extra-group
710 paternity rate in the European badger (*Meles meles*). J Evol Biol. 2014; 27(10): 2191-
711 2203.
- 712 51. Macdonald DW, Newman C, Stewart PD, Domingo-Roura X, Johnson PJ. Density -
713 dependent regulation of body mass and condition in badgers (*Meles meles*) from Wytham
714 Woods. Ecology. 2002; 83(7): 2056-2061.
- 715 52. Sugianto NA, Newman C, Johnson P, Macdonald DW, Buesching CD. Extrinsic factors
716 affecting cub development attributing to sexual dimorphism in the European badger
717 (*Meles meles*). Subm.b
- 718 53. Delahay RJ, Carter SP, Forrester GJ, Mitchell A, Cheeseman CL. Habitat correlates of
719 group size, bodyweight and reproductive performance in a high-density Eurasian badger
720 (*Meles meles*) population. J Zool. 2006; 270(3): 437-447.
- 721 54. Ahnlund H. Sexual maturity and breeding season of the badger *Meles meles* in Sweden. J
722 Zool. 1980; 190(1): 77-95.
- 723 55. Neal E, Cheeseman C. Badgers. Poyser Natural History. London, UK. 1996. pp 271.
- 724 56. Sun Q, Stevens C, Newman C, Buesching CD, Macdonald DW. Cumulative experience,
725 age-class, sex and season affect the behavioural responses of European badgers (*Meles*
726 *meles*) to handling and sedation. Animal Welfare. 2015; 24(4): 373-385.
- 727 57. Macdonald DW, Newman C, Nouvellet PM, Buesching CD. An analysis of Eurasian
728 badger (*Meles meles*) population dynamics: implications for regulatory mechanisms. J
729 Mammal. 2009; 90(6): 1392-1403.
- 730 58. Macdonald DW, Newman C, Buesching CD, Nouvellet P. Are badgers ‘Under the
731 Weather’? Direct and indirect impacts of climate variation on European badger (*Meles*
732 *meles*) population dynamics. Glob Change Biol. 2010; 16(11): 2913-2922.
- 733 59. Byrne AW, Fogarty U, O’Keeffe J, Newman C. In situ adaptive response to climate and
734 habitat quality variation: spatial and temporal variation in European badger (*Meles meles*)
735 body weight. Glob Change Biol. 2015; 21(9): 3336-3346.
- 736 60. Sin YW, Buesching CD, Burke T, Macdonald DW. Molecular characterization of the
737 microbial communities in the subcaudal gland secretion of the European badger (*Meles*
738 *meles*). FEMS Microbiol Ecol. 2012; 81(3): 648-659.

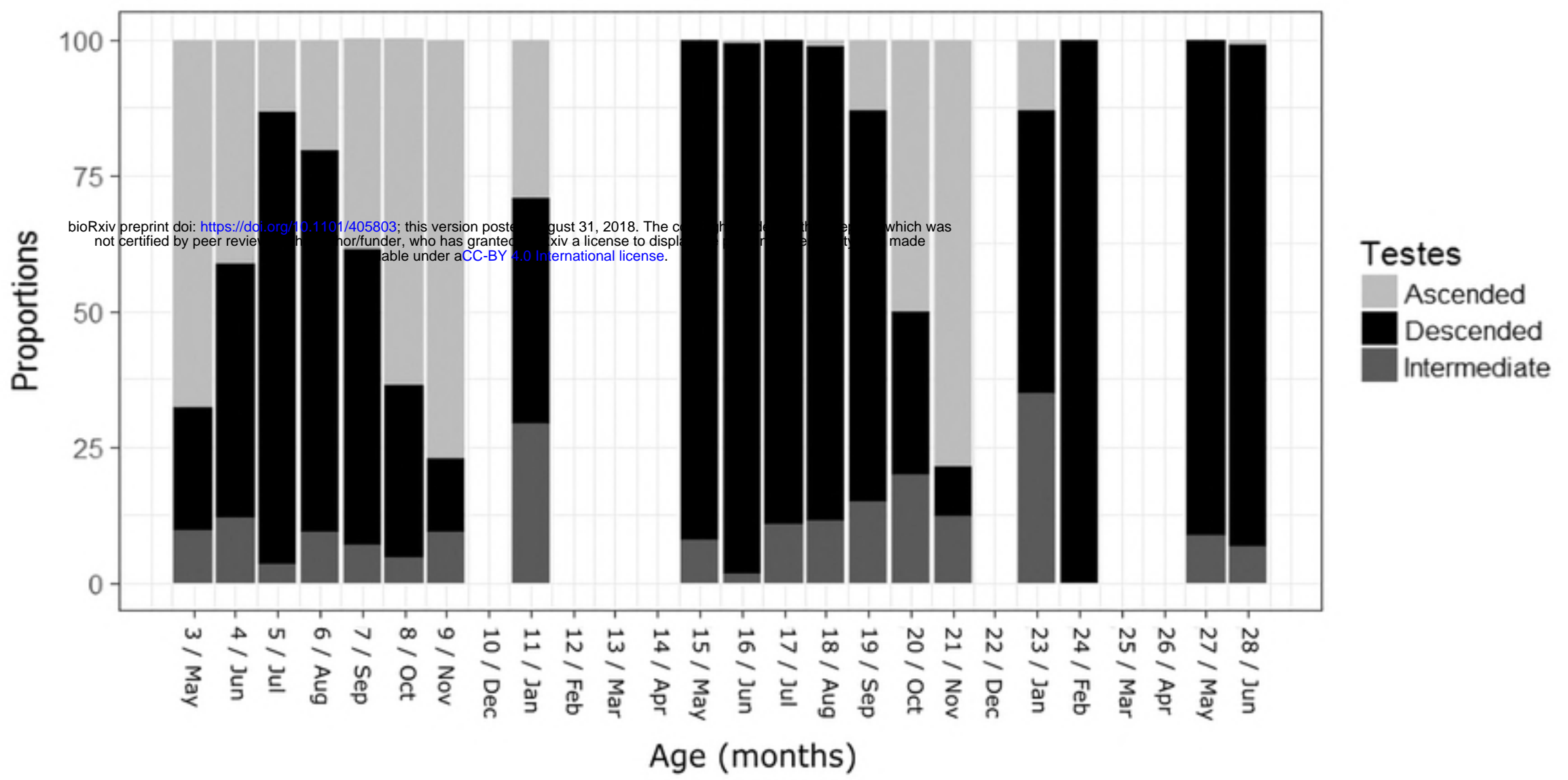
- 739 61. Paltiel HJ, Diamond DA, Di Canzio J, Zurakowski D, Borer JG, Atala A. Testicular
740 volume: comparison of orchidometer and US measurements in dogs. *Radiology*. 2002;
741 222(1): 114-119.
- 742 62. Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL.
743 Relationship of serum estradiol and progesterone concentrations to the excretion profiles
744 of their major urinary metabolites as measured by enzyme immunoassay and
745 radioimmunoassay. *Clin Chem*. 1991; 37(6): 838-844.
- 746 63. Macdonald DW, Newman C, Dean J, Buesching C D, Johnson P J. The distribution of
747 Eurasian badger, *Meles meles*, setts in a high-density area: field observations contradict
748 the sett dispersion hypothesis. *Oikos*. 2004; 106(2): 295-307
- 749 64. Asa CS. Reproductive biology and endocrine studies. *In*: Boitani L, Powell RA, editors.
750 Carnivore ecology and Conservation: A handbook of techniques. Oxford University
751 Press. 2012. pp 506
- 752 65. Dugdale HL, Pope LC, Newman C, Macdonald DW, Burke T. Age- - specific breeding
753 success in a wild mammalian population: selection, constraint, restraint and senescence.
754 *Mol Ecol*. 2011; .20(15): 3261-3274.
- 755 66. Abrams PA. The fitness costs of senescence: the evolutionary importance of events in
756 early adult life. *Evol Ecol*. 1991; 5(4): 343-360.
- 757 67. Macdonald DW, Johnson DDP. Patchwork planet: the resource dispersion hypothesis,
758 society, and the ecology of life. *J Zool*. 2015; 295(2): 75-107.
- 759 68. Birgersson B, Ekvall K. Early growth in male and female fallow deer fawns. *Behav Ecol*.
760 1997; 8(5): 493-499.
- 761 69. Badyaev AV. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual
762 size dimorphism. *Trends Ecol Evol*. 2002; 17(8): 369-378.
- 763 70. Clutton-Brock TH, Albon SD, Guinness FE. Parental investment and sex differences in
764 juvenile mortality in birds and mammals. *Nature*. 1985; 313(5998): 131-133.
- 765 71. LeBlanc PJ, Obbard M, Battersby BJ, Felskie AK, Brown L, Wright PA, Ballantyne JS.
766 Correlations of plasma lipid metabolites with hibernation and lactation in wild black bears
767 *Ursus americanus*. *J Comp Physiol B*. 2001; 171(4): 327-334.
- 768 72. Festa-Bianchet M, Jorgenson JT, Wuhart WD. Early weaning in bighorn sheep, *Ovis*
769 *canadensis* affects growth of males but not of females. *Behav Ecol*. 1994; 5(1): 21-27.
- 770 73. Zedrosser A, Dahle B, Swenson JE. Population density and food conditions determine
771 adult female body size in brown bears. *J Mammal*. 2006; 87(3): 510-518.
- 772 74. Stearns SC. The evolution of life histories. Oxford University Press, New York. 1992. pp
773 249.
- 774 75. Fowler CW. Density dependence in northern fur seals (*Callorhinus ursinus*). *Mar Mam*
775 *Sci*. 1990; .6(3): 171-195.
- 776 76. Derocher AE, Stirling I. Geographic variation in growth of polar bears (*Ursus maritimus*).
777 *J Zool*. 1998; 245(1): 65-72.
- 778 77. Derocher AE, Wiig Ø. Postnatal growth in body length and mass of polar bears (*Ursus*
779 *maritimus*) at Svalbard. *J Zool*. 2002; 256(3): 343-349.
- 780 78. Garshelis DL, Hellgren EC. Variation in reproductive biology of male black bears. *J*
781 *Mammal*. 1994; 75(1): 175-188.
- 782 79. Blanc F, Th eriez M. Effects of stocking density on the behaviour and growth of farmed
783 red deer hinds. *Appl Anim Behav Sci*. 1998; 56(2-4): 297-307.
- 784 80. Altmann J, Alberts SC. Intraspecific variability in fertility and offspring survival in a
785 nonhuman primate: behavioral control of ecological and social sources. *In*: Wachter KW,
786 Bulatao RA, editors. Offspring: The Biodemography of Fertility and Family behavior.
787 National Academy Press, Washington DC. 2003. p 140-169.

- 788 81. Campbell RD, Rosell F, Newman C, Macdonald DW. Age-related changes in somatic
789 condition and reproduction in the Eurasian beaver: Resource history influences onset of
790 reproductive senescence. *PloS ONE*. 2017; 12(12): e0187484.
- 791 82. Promislow DE, Harvey PH. Living fast and dying young: a comparative analysis of
792 life-history variation among mammals. *J Zool*. 1990; 220(3): 417-437.
793

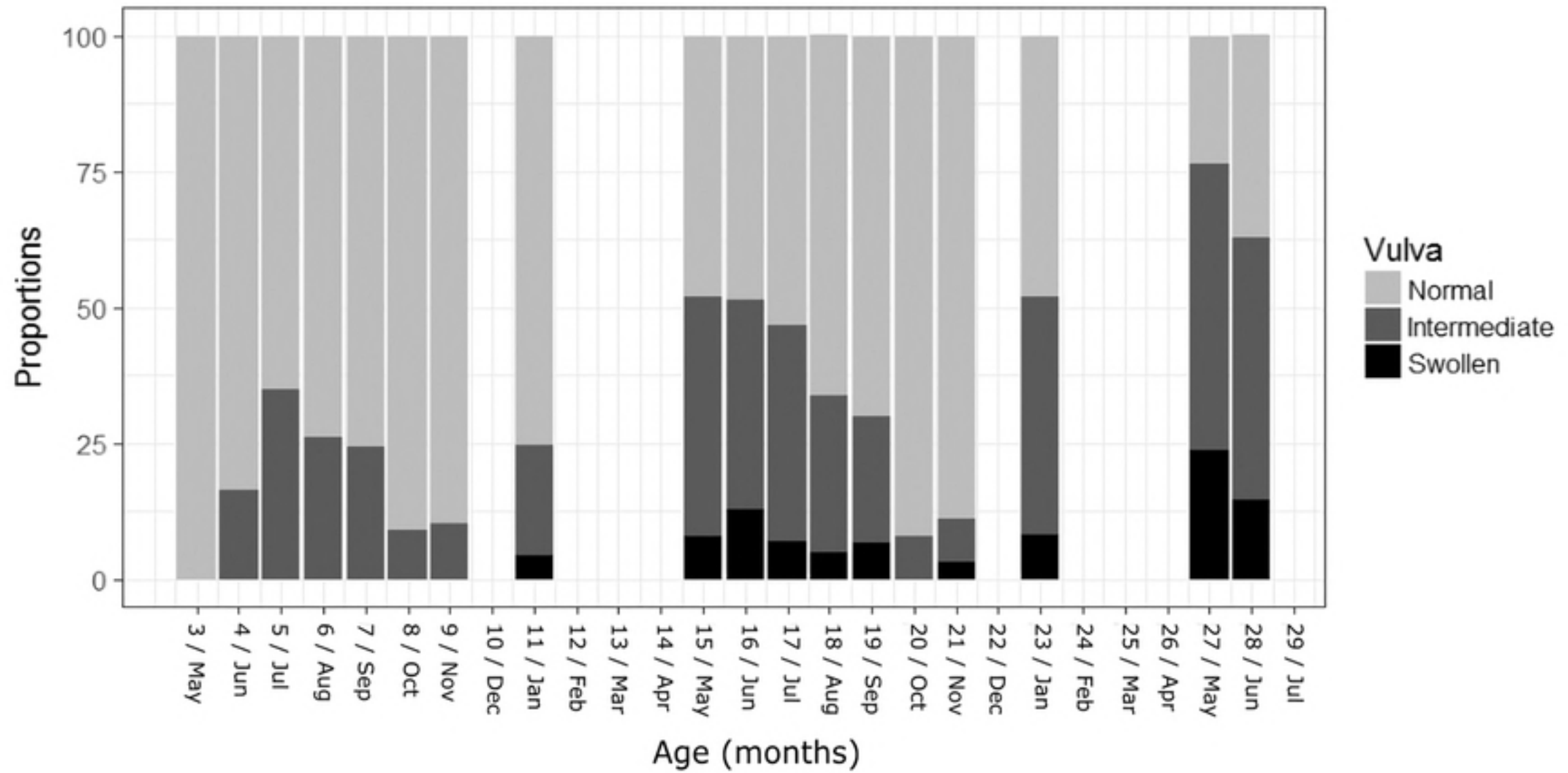


Oestrone (pg/ml)





(a)



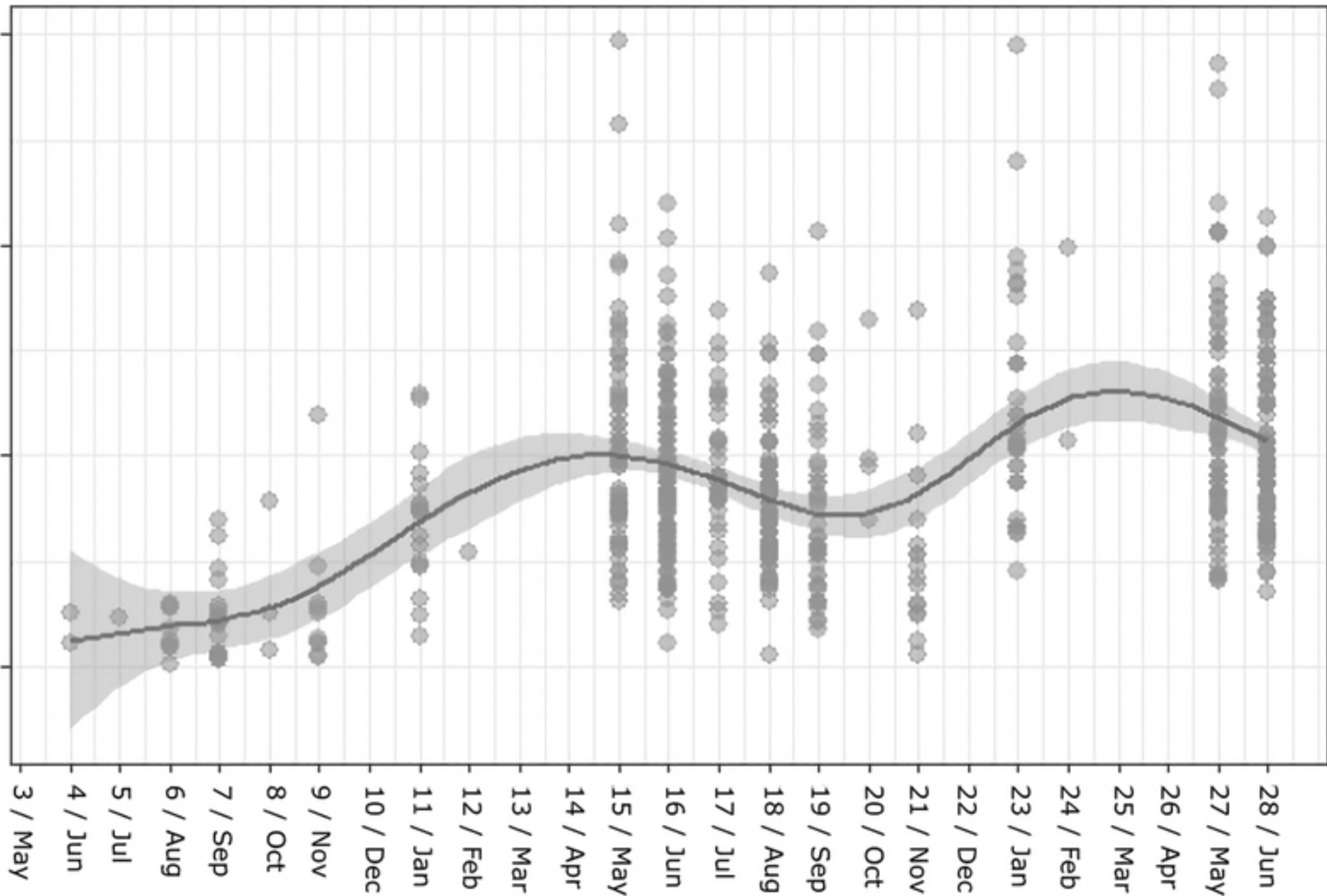
(b)

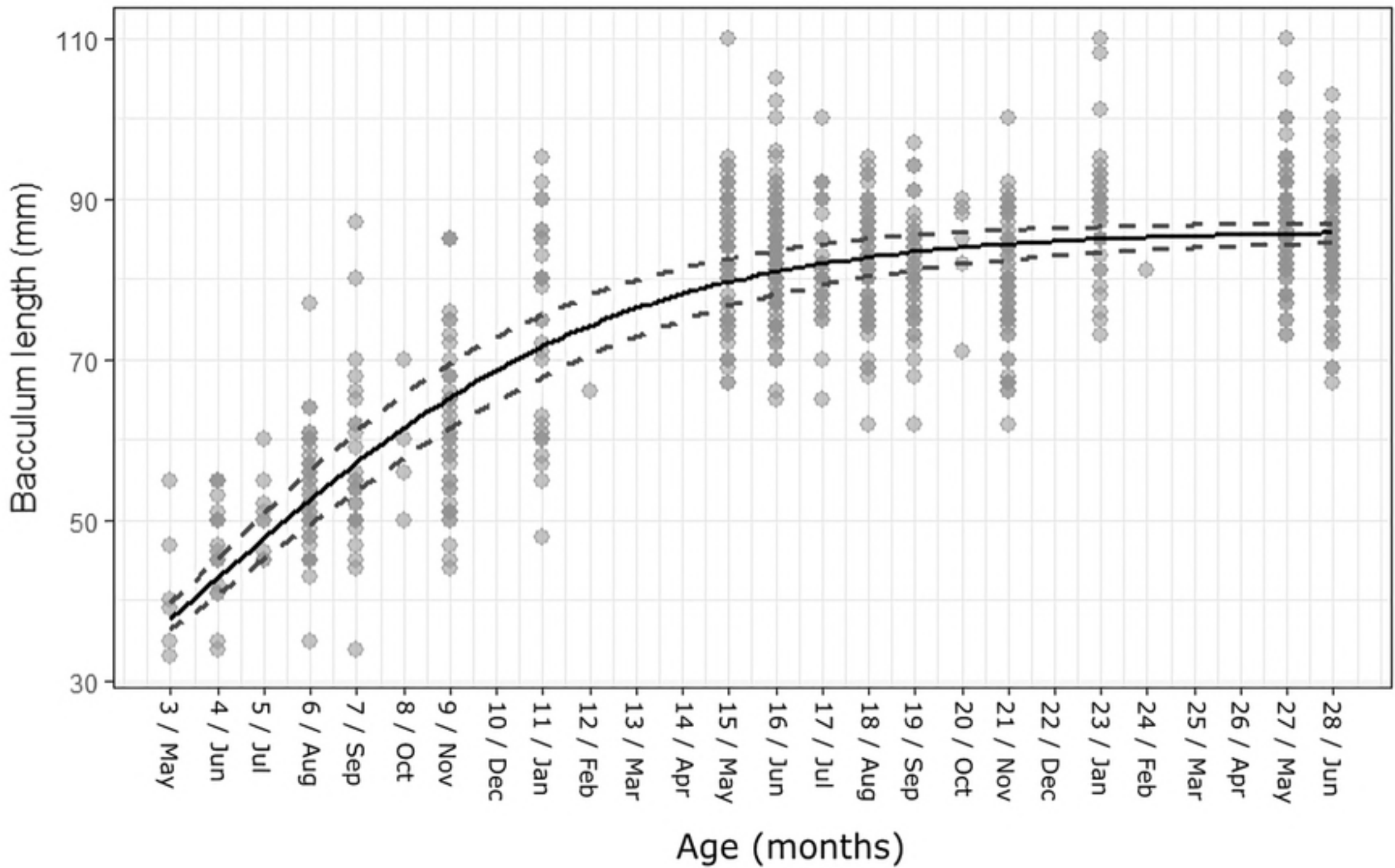
Testes volume (mm³)

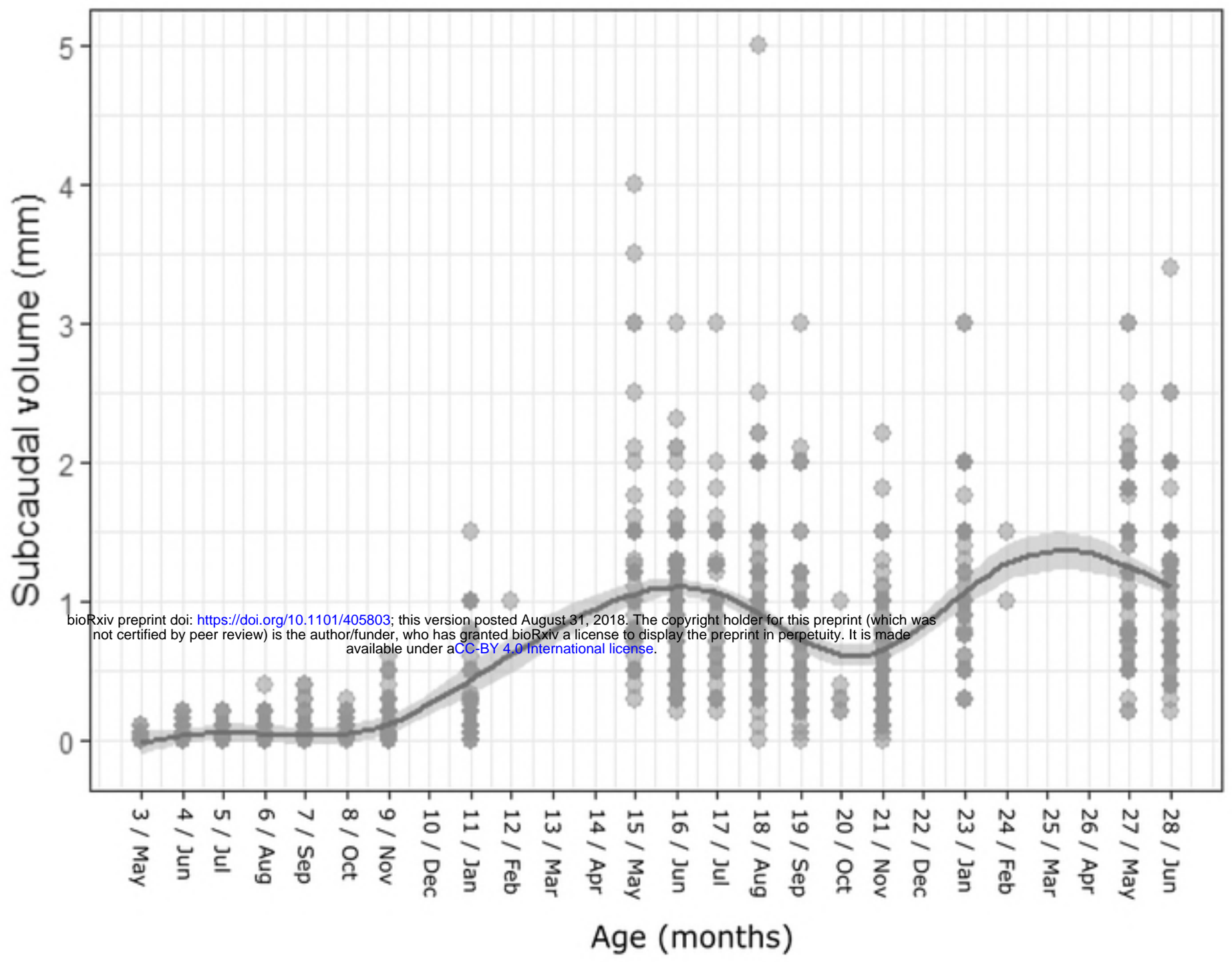
15000
10000
5000
0

3 / May
4 / Jun
5 / Jul
6 / Aug
7 / Sep
8 / Oct
9 / Nov
10 / Dec
11 / Jan
12 / Feb
13 / Mar
14 / Apr
15 / May
16 / Jun
17 / Jul
18 / Aug
19 / Sep
20 / Oct
21 / Nov
22 / Dec
23 / Jan
24 / Feb
25 / Mar
26 / Apr
27 / May
28 / Jun

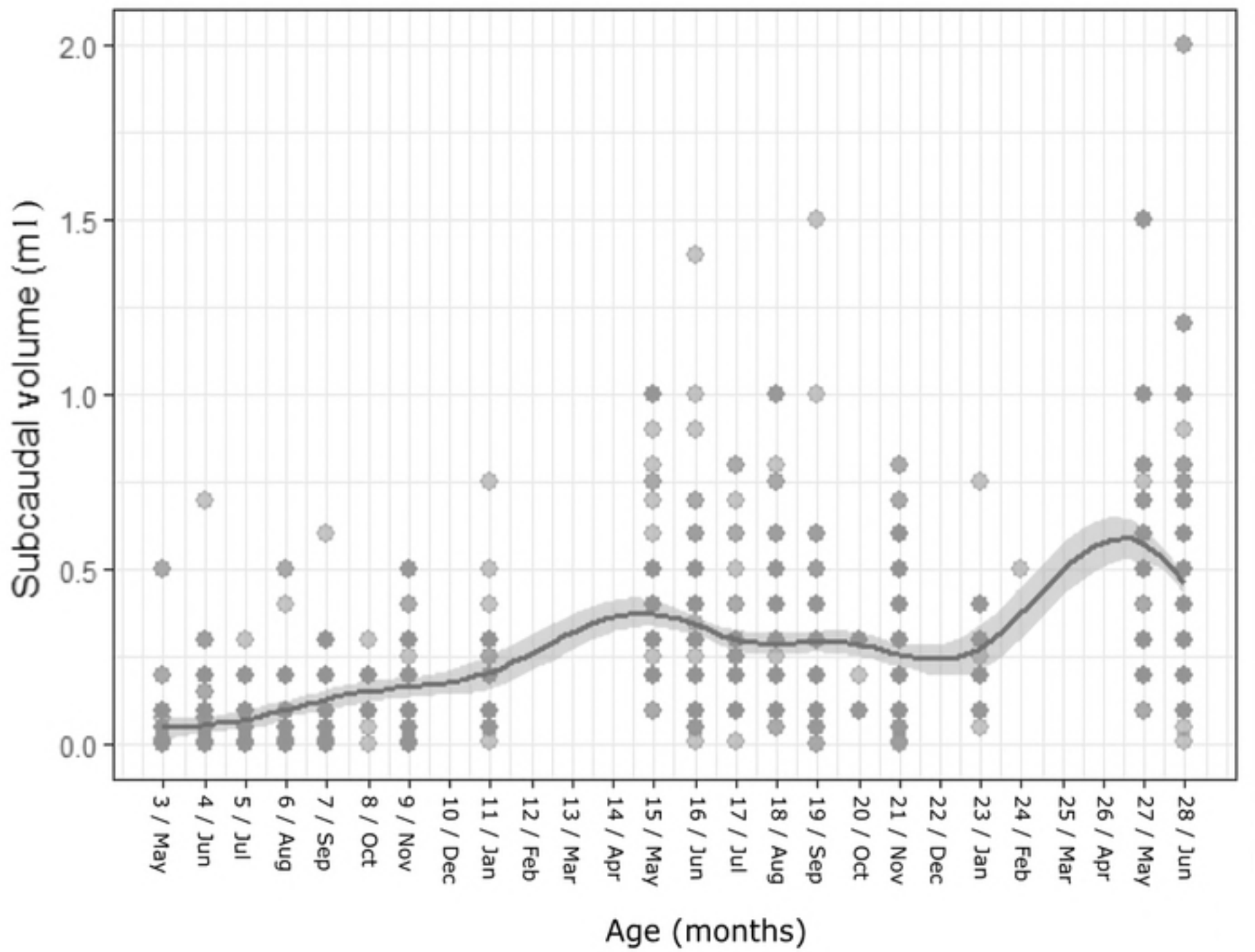
Age (months)







(a)



(b)

