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Growth dynamics and size-based age assignment in a wild population of *Elaphe quatuorlineata* from Central Italy

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2 **Abstract.**

3 Information on age and growth in snake populations is still limited because these animals are difficult
4 to observe and monitor in the wild. Here, we present a longitudinal study conducted between 2010
5 and 2025, characterizing the ontogenetic trajectories of the four-lined snake (*Elaphe quatuorlineata*)
6 in a Mediterranean ecosystem. This study included morphometric data from 649 temporarily captured
7 individuals (416 males, 233 females) assessed during a traditional cultural festival in Cocullo,
8 Abruzzo. Using capture–mark–recapture data from PIT-tagged individuals, we modelled sex-specific
9 growth dynamics using a nonlinear mixed-effects formulation of the Fabens equation, which requires
10 no assumption about absolute age. The model revealed clear female-biased SSD, driven by sex-
11 specific growth trajectories: males grew faster ($K = 0.242 \text{ year}^{-1}$) but reached a smaller asymptotic
12 snout–vent length ($SVL_{\text{inf}} = 141.57 \text{ cm}$), whereas females grew more slowly ($K = 0.176 \text{ year}^{-1}$) but
13 attained a larger asymptotic size ($SVL_{\text{inf}} = 153.71 \text{ cm}$). We also documented a record for the species
14 in Italy, with up to 195 cm of total body length for a female. The maximum size-derived age
15 assignments were approximately 28 years (female) and 19 years (male). Because these values are
16 inferred from body size rather than measured directly, they should be regarded as approximate age
17 assignments rather than demographic estimates of longevity. Our results provide the first long-term
18 description of growth patterns in a free-ranging population of *E. quatuorlineata* and may contribute
19 to future studies on the species.

20
21 **Keywords.** *Elaphe quatuorlineata*, Capture-mark-recapture, Sexual size dimorphism, Citizen
22 science, Growth dynamics, Colubridae.

INTRODUCTION

Age and growth rate are important life-history variables. In snakes, growth and body size are closely related to reproductive investment, survival and lifespan (Shine and Charnov, 1992). These traits are also useful for assessing individual condition and population variability, and informing appropriate management policies (Shine, 1978a, 1978b, 1980; Madsen, 1983). These morphological and biological features can be sensitive to environmental and anthropogenic threats requiring specific conservation strategies (Scott and Seigel, 1992; Dodd, 1993; Filippi and Luiselli, 2000; Pellegrini et al., 2017).

However, these traits are often difficult to measure in wild snake populations due to their elusive nature, low detectability, particularly of juveniles, and the logistical constraints associated with field studies (Bonnet et al., 2011). As a result, most of the available information derives from studies conducted on captive individuals (Lah et al., 2025), from indirect approaches, such as skeletochronology (Castanet and Naulleau, 1974; Fornasiero et al., 2016) or shedding rate assessments (Aldridge and Brown, 1995). Importantly all the methods listed above carry uncertainty and are rarely validated against individuals of known age.

The four-lined snake, *Elaphe quatuorlineata* (Lacépède, 1789), is the largest European colubrid and a key predator in Mediterranean ecosystems (Filippi et al., 2005; Capula and Filippi, 2011). Despite its ecological importance (Filippi and Luiselli, 2001, 2003, 2006; Luiselli and Filippi, 2006), quantitative growth models based on long-term mark-recapture data are lacking and largely restricted to some captive observations on subadults (Bruno, 1984; Schulz, 1996; Esser, 2009; Lah et al., 2025).

In this study we report data collected during a long-term citizen science approach (see Marini et al., 2023; Mendoza-Roldan et al., 2024; Ugochukwu et al., 2024; Alves et al., 2025; Fagundes-Moreira et al., 2025; Montinaro et al., 2025; Miglianti et al., 2026) associated with the traditional rite of San Domenico in Cocullo (l'Aquila, Abruzzo Region, Italy), in which *E. quatuorlineata* individuals are temporarily collected, exhibited, and released in the wild by *Serpari* (local snake-hunters). Until the early 1900s it is reported that some individuals were sometimes killed at the end

of the rite or sold (Harrison, 1907). Since that time, the release of all snakes into the wild has been taking place for at least a hundred years being made compulsory by the Regional Law of Abruzzo 50/1993.

Hence, this century-long, human mediated capture/exhibition/release cycle offers a rare opportunity to examine a large sample of wild snakes over an extended period of time as described in Montinaro et al. 2025.

In order to provide a preliminary baseline characterization of growth patterns and sex differences in *E. quatuorlineata*, we tested three predictions. First, if female-biased SSD in this species arises from divergent ontogenetic trajectories rather than from differences in adult size alone, males and females should differ in asymptotic length, in the growth coefficient, or in both. Second, if fecundity selection acts primarily on trunk length, the dimorphism should be expressed in snout–vent length rather than in total body length. Third, if SSD originates at least partly through sex-specific growth dynamics, males and females should differ in their growth trajectories rather than only in the sizes attained by the largest adults.

Age classes, however, could only be estimated indirectly from body size, and the resulting estimates should be treated as provisional (see Material and Methods).

MATERIAL AND METHODS

Captured snakes were monitored, annually from 2010 to 2025 during the 2–3 days preceding the date of the event (May 1st), with the exception of 2020 and 2021 due to the Covid-19 Pandemic. Sampling relies on the traditional capture of snakes by *Serpari*, which is limited to the period preceding the San Domenico rite (i.e., from 19th March to 30th of April). This timing potentially biases observations toward active adults, specifically males in search of mates (Montinaro et al., 2025), and toward individuals that are more active and detectable and it does not represent a random sample of the population.

For each individual, we recorded species, sex (examining the morphology of the cloacal region or inserting a metal probe into the cloaca; Vetere et al., 2025), age class (i.e., subadult or adult), biometric measurements (weight, WT, using a portable electronic balance, precision to ± 1 g; snout–vent length, SVL; tail length, TL; total body length, TBL to the nearest ± 1 cm). All individuals were implanted subcutaneously with a Passive Integrated Transponder (PIT tag) (Montinaro et al., 2025).

After the event, or within a few days, each snake was released at the original capture site.

Subadult and adult *E. quatuorlineata* were distinguished based on different dorsal coloration and pattern and in relation to size (Capula and Filippi, 2011; Danelis and Bjelica, 2024). Subadults display a type of pattern with dark dorsal blotches or partially developed longitudinal markings on a lighter background, whereas adults show a uniform dorsal coloration with four well-defined dark longitudinal stripes on an olive to brown ground color (Fig. 1).

Our primary inference on individual growth relies on the mixed-effects Fabens model (1965) method, a modification of the Von Bertalanffy (1938) growth model adapted for mark-recapture data. The Fabens method estimates growth parameters directly from the observed increase in length between successive captures over a known time interval (for our study one year) and does not require any assumption about the individual's absolute age. The model estimates asymptotic length (SVL_{inf}) and growth coefficient (K) from the difference in length between first capture (SVL_1) and recapture (SVL_2) over a known time interval (Δt , years):

$$SVL_2 = SVL_{inf} - (SVL_{inf} - SVL_1) e^{(-K \Delta t)}$$

Growth intervals were derived from the individual capture histories reconstructed from the complete 2010–2025 PIT-tag database. Relative to an earlier compilation of the same dataset, capture histories were re-examined and corrected prior to analysis: capture events that had previously been paired incorrectly were re-paired as consecutive captures, previously omitted recaptures were recovered, and a small number of transcription errors in dates and measurements were amended. From the reconstructed capture histories, all consecutive-capture pairs were then screened.

102 Since decreases in SVL are not biologically plausible within the observed intervals, negative
103 growth increments were interpreted as likely measurement or recording errors and were excluded
104 from the primary growth analysis. We have, however, retained other biologically plausible positive
105 intervals relating to the same individuals. Measurement error models that explicitly incorporate
106 uncertain length observations, such as the approach proposed by Eaton and Link (2011), could
107 potentially make full use of these observations and represent a useful direction for future analyses.
108 This interpretation was further supported by the observation that, among individuals showing at least
109 one apparent negative interval, a large proportion (36 of 68 males, 53%; 12 of 23 females, 52%)
110 subsequently showed a positive growth increment in a later recapture interval. Under this procedure,
111 the final growth dataset comprised 215 growth intervals from 126 males and 98 growth intervals from
112 61 females. Overall, of all growth intervals that could in principle be derived from the raw capture
113 histories (i.e., all consecutive-capture pairs, before screening), 32.4% (103 of 318) of male intervals
114 and 22.2% (28 of 126) of female intervals were excluded under this criterion.

115 Growth parameters were estimated using the Fabens (1965) equation fitted as a nonlinear
116 mixed-effects model (function nlme, package nlme v.3 in R; Pinheiro and Bates, 2000), with
117 individual identity (ID) included as a random-effects grouping factor on SVL_{inf} . This structure
118 explicitly accounts for the non-independence of repeated measurements from the same individual,
119 correcting for pseudoreplication inherent in mark-recapture growth datasets with unequal numbers of
120 recaptures per individual (Wang and Milton, 2000).

121 Because the Fabens equation is conditionally linear in a random effect on SVL_{inf} , the marginal
122 likelihood of this model has a closed form. Model fitting was therefore verified by refitting from
123 multiple randomised starting values and by relaxing the default iteration limits of nlme, to confirm
124 that the reported solution corresponds to the global maximum of the likelihood rather than to a
125 premature stopping point of the optimisation algorithm.

126 As a sensitivity analysis, we also tested a random-effects structure allowing individual variation
127 in both SVL_{inf} and K. Because a large proportion of individuals in the combined dataset (111 of 187)

128 contributed only a single growth interval and therefore could not inform individual-level estimates of
129 K, this two-random-effects model was refitted on the subset of individuals with two or more growth
130 intervals ($n = 76$; 202 growth intervals). A likelihood-ratio test confirmed that allowing K to vary
131 among individuals significantly improved model fit even within this balanced subset (L. Ratio =
132 20.40, $df = 1$, $P < 0.0001$), supporting the existence of genuine individual variation in growth rate.
133 However, because parameter estimates from this more complex model were less stable when applied
134 to the full dataset (including single-interval individuals), the simpler model with a random intercept
135 on SVL_{inf} alone was retained as the primary model reported throughout this study, ensuring
136 comparability and stability across the full sample.

137 To test whether growth parameters differed between sexes, sex was included as a fixed-effect
138 predictor in a sequence of nested models: (i) a null model with SVL_{inf} and K shared between sexes;
139 (ii) an intermediate model with sex-specific SVL_{inf} only; and (iii) a full model with sex-specific
140 SVL_{inf} and K. Models were fitted by maximum likelihood and compared via sequential likelihood-
141 ratio tests (LRT), together with AIC and BIC. Parameter estimates and standard errors for each sex
142 were obtained by refitting the selected model with each sex, in turn, set as the reference category.

143 Since these animals were taken from the wild, their exact age could not be determined. We
144 nevertheless assigned approximate age classes in order to visualise the growth trajectory on an age
145 axis. As these data were derived in part from body measurements, in this text we refer to them as 'age
146 estimates based on size' rather than 'chronological ages'. They should therefore be regarded as
147 approximate and exploratory in nature, and not as independently measured ages. Age classes were
148 assigned as follows: (i) subadults were assigned an initial age class from the relative sizes reported in
149 the literature (newborn: up to 41 cm; 1 year: up to 60 cm; 2 years: up to 80 cm; 2.5 years: up to 97
150 cm; Schulz, 1996; Cattaneo, 1999; Esser, 2009; Lah et al., 2025); (ii) accounting for parturition
151 occurring in late August and early September (Capizzi et al., 1996; Capula and Filippi, 2011) and for
152 animals being captured after hibernation; (iii) drawing on direct experience with the species; and (iv)
153 using measurements from 8 females (subadults and adults) and two males held in captivity (Lah and

154 Žagar, unpublished data). Subsequent age classes for these same individuals were assigned from the
155 intervals between annual or pluriannual recaptures. For the remaining individuals, an initial age was
156 assigned from the SVL/age relationship established above, and subsequent age classes were assigned
157 from recaptures.

158 Because the absolute age was unknown, the Fabens model was used as the sole model for
159 parameter estimation. The resulting sex-specific estimates of the standardised body length (SVL) and
160 the K value were subsequently plotted on an illustrative age axis, with the growth trajectory anchored
161 to the mean body length (SVL) of newborn individuals measured in captivity ($L_0 = 32.9$ cm, $n = 8$,
162 range 29–36 cm). The corresponding t_0 was calculated as

163
$$t_0 = \ln(1 - L_0/SVL_{inf}) / K.$$

164 This transformation was used solely to plot the growth trajectories derived from the Fabens
165 model on an age axis and did not constitute an independent age-based growth analysis. Age estimates
166 derived from body size were not used to estimate SVL_{inf} or K.

167 Sexual size dimorphism is negligible at birth in snakes, so the same L_0 was applied to both
168 sexes. Fig. 3 represents an age-axis visualisation of the growth trajectories derived from the Fabens
169 model and anchored to a measured neonate size. The curves are shown for illustrative purposes only
170 and should not be interpreted as an independently fitted von Bertalanffy growth model. The size-
171 derived age classes are used only to position the observed points along the horizontal axis and were
172 not used for parameter estimation. The curves were plotted in R (v4.6.0; R Core Team 2024) for
173 graphical representation only. For cross-sectional morphometric comparisons (Tables 1 and 2), only
174 the first capture of each individual was used to avoid pseudoreplication arising from repeated
175 measurements of recaptured individuals. The morphometric variables were analysed by t-test,
176 ordinary least square regression (after log-transformation of variables), Pearson's correlation
177 coefficient (r). Statistical analysis was carried out using PAST software ver. 5.3.(Hammer et al., 2001)
178 and R Statistical Software (v4.6.0; R Core Team 2024).

179

RESULTS

Out of the 358 adult males, 211 adult females, 58 subadult males, and 22 subadult females captured throughout the observation period, 28.8% of the females ($n = 67$) and 34.6% of the males ($n = 144$) were recaptured at least once (maximum number of recaptures per individual $n = 6$ females, $n = 8$ males).

We recorded the maximum total body length currently documented for the species in Italy: a female with a TBL of 195 cm, whereas the longest male measured 191 cm of TBL.

The shortest adult male measured $SVL = 62.0$ cm, the longest subadult male measured $SVL = 114.5$ cm and the shortest adult female measured $SVL = 67$ cm, the longest subadult female measured $SVL = 125$ cm consistent with the expected size overlap between subadult and adult individuals, a life-stage distinction based primarily on dorsal pattern rather than SVL ; this is distinct from the separate, SVL -based age-class estimation used only to position observations on the age axis of Fig. 3. These figures should be interpreted as a graphical representation of the Fabens estimates on an approximate age axis rather than an independent age-based growth model. Descriptive statistics and comparative analyses for morphometric traits are summarized in Tables 1 and 2.

The mixed-effects Fabens model provided a good fit to the mark-recapture data ($n = 313$ growth intervals from 187 individuals). Sequential likelihood-ratio tests comparing nested models (Tab. 3) showed that including sex as a predictor of SVL_{inf} improved model fit relative to the null model ($LRT = 13.56$, $df = 1$, $P = 0.0002$), and that further allowing K to differ between sexes provided a further improvement in fit, although with weaker statistical support ($LRT = 4.65$, $df = 1$, $P = 0.031$). Both the asymptotic length and the growth-rate coefficient thus differ between males and females (Tab. 4), although the evidence for a sex difference in K is considerably weaker than that for a difference in SVL_{inf} .

The random-effects standard deviation of SVL_{inf} among individuals was 6.96 cm, and the residual standard deviation was 3.33 cm. An initial attempt to fit an independent von Bertalanffy model to the size-derived age data was unsuccessful: approximately 25% of observations exceeded

206 asymptotic length obtained from the fit in both sexes, and the resulting parameters contradicted those
207 obtained from the age-independent Fabens model (see ‘Methods’ for details). This result is presented
208 here because it directly motivated our decision to derive the age-axis growth curves in Fig. 3 from
209 the Fabens parameters rather than from an independent fit. Individual growth trajectories estimated
210 from the mixed-effects Fabens model are shown in Fig. 2.

211 Expressing the same parameters on an age axis, with t_0 anchored to the measured neonate size
212 (see Methods), gives $t_0 = -1.37$ years for females and -1.09 years for males. The corresponding curves
213 are shown superimposed on the size-at-age observations in Fig. 3. Under these curves, 95% of the
214 asymptotic length is attained at approximately 15.6 years in females and 11.3 years in males.

215 The maximum age assigned by our size-based procedure was approximately 28 years in a
216 female (SVL 165 cm) and approximately 19 years in a male (SVL 152 cm).

217

218 DISCUSSION

219 Data from the present study represent, to our knowledge, the first study to estimate sex-specific
220 growth parameters in a free-ranging population of *E. quatuorlineata* using a nonlinear mixed-effects
221 Fabens model. By combining morphometric data with capture–recapture records over 14 field seasons
222 between 2010 and 2025, we identified patterns of sexual size dimorphism, sex-specific growth
223 trajectories, and differences in estimated longevity indicators. Both sexes exhibited rapid juvenile
224 growth followed by progressively slower growth as they approached their asymptotic size.

225 Sequential likelihood-ratio tests indicated that males and females differ in asymptotic length
226 and, with weaker support, in growth rate (Tab. 3). This suggests a growth trade-off: males grow faster
227 but attain a smaller asymptotic size, whereas females grow more slowly but reach a larger asymptotic
228 size (Tab. 4). Notably, a similar pattern has been observed in some other snake species (Rose et al.,
229 2022). Given the limited evidence supporting a difference in K , we consider the difference in
230 asymptotic length to be the better-supported component of this trade-off, and the difference in growth
231 rate as requiring confirmation with a larger number of recapture intervals. This pattern is consistent

232 with theoretical expectations that sexual size dimorphism may arise through sex-specific ontogenetic
233 trajectories rather than through differences in adult size alone (Shine, 1993; Fairbairn et al., 2007). In
234 species with female-biased sexual size dimorphism (SSD), prolonged growth and increased trunk size
235 are commonly interpreted as consequences of fecundity selection, whereby larger females may gain
236 reproductive advantages through increased abdominal capacity and reproductive output (Shine,
237 1993). As already noted in the 'Methods', the appreciable proportion of excluded growth intervals,
238 despite the close to ideal measurement conditions of this study, several trained researchers working
239 together during a concentrated annual event, with a logistical support unavailable in most field
240 contexts, suggests that comparable or higher error rates should be expected, and explicitly checked
241 for, in mark-recapture studies conducted under more typical field conditions, where snakes may be
242 measured by a single researcher, under greater time pressure, and sometimes in unfavorable
243 environmental conditions.

244 A methodological point deserves emphasis, because it bears directly on how growth and
245 longevity are inferred in snake populations. Growth models based on recapture such as the Fabens
246 equation are generally preferred for reptiles precisely because they do not require any assumption
247 about absolute age (Wang and Milton, 2000). An alternative, less robust approach, which involves
248 assigning ages based on body size and then fitting an age-based growth model to the resulting size-
249 at-age data, is nonetheless sometimes attempted when only cross-sectional size data are available.
250 Our dataset illustrates why this alternative can fail. When we fitted a von Bertalanffy model to our
251 size-derived age assignments, the estimated asymptotic length was exceeded by approximately a
252 quarter of the observations in both sexes, residuals increased systematically with age, and the sex-
253 specific parameters contradicted those obtained from the same animals by the age-independent
254 Fabens analysis. Because the age variable was itself constructed from SVL, the size-at-age
255 relationship could not reproduce the deceleration that the von Bertalanffy function requires.
256 Increment-based estimation, which uses only measured changes in length between successive

captures of identified individuals, avoids this problem entirely, and we would encourage its use in preference to size-derived age reconstruction wherever mark–recapture data are available.

The higher proportion of excluded intervals in males relative to females (32.4% vs. 22.2%) most plausibly reflects the larger number of recapture opportunities per male (up to 8 recaptures vs. 6 in females), providing more occasions for isolated measurement error to occur, not a true sex-specific difference in measurement reliability.

Beyond growth rate and asymptotic SVL, the morphometric comparisons summarized in Tab. 1 reveal a more nuanced pattern of sexual dimorphism. While adult females had significantly greater SVL than males, adult males had significantly longer tails, and the two sexes did not differ significantly in total body length or body weight (Tab. 1). This pattern is consistent with a compensatory scaling relationship between SVL and tail length, whereby the proportionally longer tails of males, likely related to the accommodation of paired hemipenes, a common feature of male snakes (King, 1989), offset their shorter trunk length, resulting in overall similar total body length and mass between the sexes. This suggests that the female-biased SSD emerging from sex-specific growth trajectories operates primarily on trunk length (SVL), not on overall body size, and may reflect sex-specific selective pressures acting differentially on trunk versus tail length (e.g., fecundity selection favouring longer trunks, and hence greater abdominal volume, in females, versus reproductive or locomotor functions favouring longer tails in males), rather than a generalized difference in growth investment between sexes.

The biologically relevant differences correspond to approximately 12 cm in asymptotic SVL and 0.065 year^{-1} in K between sexes. In addition, the comparatively small number of subadults captured, particularly subadult females ($n = 22$, versus $n = 58$ subadult males), limits the precision of growth and morphometric comparisons for younger age classes and should be considered when interpreting any sex-specific patterns involving subadults. A sensitivity analysis confirmed genuine individual variation in growth rate (K), in addition to asymptotic length, though this could only be reliably quantified in the subset of individuals with multiple recaptures; future studies with more

283 extensive recapture histories per individual would allow this source of variation to be modelled
284 directly across the full population.

285 In general, the mechanisms driving sexual size dimorphism in snakes are multifactorial (Liang
286 et al., 2025). Because the sexes differ not only in asymptotic length but also, albeit weakly, in the
287 growth coefficient K , divergence is not confined to the largest, oldest individuals but is already
288 present in the rate of approach to asymptotic size, consistent with our third prediction that sex
289 differences should be detectable in the growth trajectory itself. Shine (1990) proposed that SSD may
290 arise through differences in the timing and duration of growth between the sexes, with divergence
291 occurring either before or after sexual maturity, and the relative importance of each pathway appears
292 to vary depending on the snake species: pre-maturational divergence is well documented in some taxa
293 (see, for example, Meter et al., 2022), whereas in others, such as garter snakes (*Thamnophis* spp.)
294 (Rose et al., 2022), the sexes exhibit comparable growth rates and size divergence appears to result
295 from other mechanisms. Our finding of a difference in asymptotic length and, more tentatively, in
296 growth rate indicates sex-specific growth trajectories in *E. quatuorlineata*. However, because neither
297 chronological age nor age at sexual maturity was independently known, the present data do not allow
298 us to determine whether this divergence originates mainly before or after sexual maturation.

299 The highest size-derived age assignments in our sample were approximately 28 years (female)
300 and 19 years (male), and the growth curves indicate that 95% of asymptotic length is reached at
301 roughly 16 and 11 years respectively. Both figures suggest a species in which growth continues over
302 many seasons, but neither constitutes an estimate of maximum longevity: the first is bounded by our
303 assignment procedure and by the ages present in the sample, the second describes the approach to
304 asymptotic size rather than survival.

305 These age assignments implicitly assume that each individual follows the population's average
306 growth trajectory. However, the mixed-effects Fabens model quantifies considerable individual
307 variation in asymptotic size (SD of $SVL_{inf} = 6.96$ cm), and this variation has a non-negligible effect
308 on inferred age, consistent with the substantial individual variability in growth documented in other

ectotherms (King et al., 2016). For example, one would expect an individual with an asymptote equal to one standard deviation above the population mean (160.7 cm rather than 153.7 cm) to reach a given size (for example, 150 cm) at around 14.0 years of age, compared with 19.8 years for an individual following the population's average trajectory, representing a difference of almost six years for the same observed length. The case of the record-holding female (SVL = 165 cm) directly illustrates this concept: her observed length exceeds the population's average asymptotic length (153.71 cm) entirely, implying a personal asymptote well above the mean and, consequently, an age that is likely younger than that would be inferred by applying the population's average parameters. Size derived age estimates for exceptionally large individuals should therefore be interpreted with particular caution, as they may reflect above-average individual growth potential rather than advanced age. This same source of individual variation may also help explain the substantial size overlap between subadult and adult individuals: rather than reflecting measurement error or misclassification, it may indicate genuine individual variation in the size, and possibly the age, at which the dorsal pattern transition associated with maturity occurs. Distinguishing the sources of this variation linked to age from those linked to size, as well as their implications for life history, would require independent data on age and represents a promising direction for future research on this species.

In principle, it would be possible to estimate the age at sexual maturity by anchoring the growth curve derived from Fabens to a known size at maturity, following the same approach used for t_0 (Fig. 3). However, as noted previously, the size classes of subadults and adults overlap substantially in our dataset (see 'Results'), indicating that SVL alone does not define a discrete maturity threshold in this species. Any estimate of age at maturity derived from a single size threshold would therefore be equally subject to the biases associated with individual variation discussed earlier for other size-derived age estimates. Robust estimates of age at maturity in snakes are typically based on individuals of known age and on direct assessment of reproduction (e.g. cloacal smears or palpation to verify gravidity; King, 2026), an approach not available for the present population, which was sampled opportunistically.

335 Comparative data on longevity in ecologically similar species remain scarce. Fornasiero et al.
336 (2016) reported maximum estimated ages of approximately 14–18 years in *Hierophis viridiflavus* and
337 *H. carbonarius*, two sympatric Mediterranean colubrids, and *Pantherophis guttatus* and *Lampropeltis*
338 *getula californiae* are known to reach 25+ years in captivity (Robert et al., 2007). We deliberately
339 refrain from ranking *E. quatuorlineata* against these figures: the *Hierophis* estimates rest on
340 skeletochronological age markers and the captive records on animals of known age, whereas ours rest
341 on size-derived assignments, and the three are not methodologically comparable. Establishing
342 whether *E. quatuorlineata* is in fact longer-lived than these species, as its larger body size might lead
343 one to expect (Shine and Charnov, 1992), will require independent ageing of a subsample.

344 Independently of longevity, the growth parameters themselves carry a conservation implication:
345 a growth coefficient of 0.18–0.24 year⁻¹ means that individuals of both sexes require the better part
346 of a decade to approach adult size. Populations with slow individual growth recover slowly from adult
347 mortality, which reinforces concern about the anthropogenic pressures documented for this species
348 (Filippi and Luiselli, 2000; Filippi and Luiselli, 2006; Filippi and Politi, 2010). If longer age estimates
349 for females are subsequently confirmed, it would be important to understand whether it is linked to
350 factors intrinsic to the species or extrinsic factors. Among the latter, one might consider, for example,
351 possible greater exposure to various sources of risk factors (predation, roadkill, intentional killing) of
352 males during the mating season, given that snake males are known to range widely during the
353 breeding season or spend more time in open areas (because doing so somehow increases their success
354 in locating females), increasing their exposure to various sources of mortality (Weatherhead and
355 Hoysak, 1989; Bonnet et al., 1999). *E. quatuorlineata* is exposed to several environmental pressures
356 in Mediterranean ecosystems (Filippi, 2003; Filippi and Luiselli, 2002, 2006; Luiselli and Filippi,
357 2006), understanding its growth dynamics and age structure is essential for informed management
358 strategies. Our sampling design was restricted to a short annual period and relied on opportunistic
359 captures by Serpari. Consequently, the dataset is likely biased toward active, reproductively engaged
360 adults, and caution is therefore required when extrapolating these results to the entire population.

Extending PIT-tag monitoring in future years, paired with skeletochronological validation or other independent ageing methods on a subsample, would resolve the age-related uncertainties flagged above.

In conclusion, this long-term capture–mark–recapture study provides the first quantitative description of sex-specific growth dynamics in a free-ranging population of *E. quatuorlineata*. Results from the present study indicate that females attain a larger asymptotic SVL, whereas males approach a smaller asymptotic size more rapidly, although the evidence for sex differences in growth rate is weaker than that for asymptotic length. These findings highlight the importance of longitudinal, age-independent approaches for studying growth in wild snakes and show the limitations of inferring chronological age from body size alone. Hence, continued monitoring, ideally combined with independent ageing methods are advocated to better refine estimates of growth, maturity and longevity and to improve our understanding of the life-history traits relevant to the conservation of this species.

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523 **Tab. 1.** Means of different morphometric characters and relative statistical analysis (SVL = snout-
524 vent length; TL = tail length; TBL = total length; WT = weight. (NB. in adult females' sample size
525 varies because of missing measurements).

Adult males	n	Mean	Adult females	n	Mean	t-test	P
SVL (cm)	358	123.91 ± 11.73	SVL (cm)	208	129.19 ± 12.86	4.98	< 0.001
TL (cm)	358	29.56 ± 3.56	TL (cm)	207	25.52 ± 4.08	12.29	< 0.001
TBL	358	153.47 ± 13.69	TBL	208	154.58 ± 14.75	0.91	= 0.36
WT (g)	356	695.75 ± 201.40	WT (g)	207	691 ± 210.83	0.23	= 0.82
Subadult males	n	Mean	Subadult females	n	Mean	t-test	P
SVL (cm)	58	88.70 ± 16.81	SVL (cm)	22	97.16 ± 19.61	1.92	= 0.06
TL (cm)	58	21.78 ± 4.53	TL (cm)	22	20.77 ± 4.13	0.91	= 0.36
TBL	58	110.48 ± 21.01	TBL	22	117.93 ± 23.33	1.37	= 0.17
WT (g)	58	248.29 ± 111.94	WT (g)	22	329.64 ± 158.75	2.48	< 0.05

531 **Tab. 2.** Summary of the intercepts (a) and slopes (b) of the log-log (allometric) regression
 532 equations $\log(Y) = \log(a) + b \cdot \log(X)$ fitted to log-transformed morphometric variables (see
 533 Methods), together with Pearson's correlation coefficients (r). Confidence limits (2SE) are given for
 534 the slope and intercept.

Adult females	log (a) (2SE)	b (2 SE)	r	P
TBL to WT	-3.25 (-3.85, - 2.65)	2.77 (2.50, 3.05)	0.82	< 0.001
SVL to TL	0.34 (-0.18, 0.86)	0.50 (0.25, 0.75)	0.27	< 0.001
Adult males	log (a) (2SE)	b (2SE)	r	P
TBL to WT	-3.80 (- 4.26, -3.34)	3.03 (2.82, 3.24)	0.84	< 0.001
SVL to TL	0.21 (-0.07, 0.49)	0.60 (0.47, 0.73)	0.43	< 0.001
Subadult females	log (a) (2SE)	b (2 SE)	r	P
TBL to WT	-3.16 (-4.18, - 2.13)	2.71 (2.21, 3.21)	0.93	< 0.001
SVL to TL	-0.35 (-0.65, -0.05)	0.84 (0.69, 0.99)	0.93	< 0.001
Subadult males	log (a) (2SE)	b (2 SE)	r	P
TBL to WT	-3.45 (-3.73, -3.12)	2.84 (2.70, 2.98)	0.98	< 0.001
SVL to TL	-0.58 (-0.79, -0.37)	0.98 (0.88, 1.09)	0.93	< 0.001

539 **Tab. 3.** Comparison of nested nonlinear mixed-effects Fabens models. Likelihood-ratio tests (LRT)
 540 compare each model with the next simpler model (1 vs 2; 2 vs 3).

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Model	Sex specific parameters	df	AIC	BIC	logLik	L.Ratio	P
1 (null)	- (shared)	4	1782.52	1797.51	-887.26	-	-
2	SVL _{inf} only	5	1770.97	1789.70	-880.48	13.56	0.0002
3 (full)	SVL _{inf} and K	6	1768.32	1790.80	-878.16	4.65	0.031

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546 **Tab. 4.** Age-axis representation of growth parameters derived from the Fabens model. Growth
 547 parameters expressed on an age axis. SVL_{asyp} and K are the age independent estimates obtained
 548 from the mixed-effects Fabens model (see Methods). The parameter t_0 was derived by constraining
 549 the curve to pass through the mean measured neonate SVL (32.9 cm) and was not estimated
 550 independently. Age at 95% of asymptotic length was derived from these parameters

551

Sex	SVL _{asyp} (cm)	K(year ⁻¹)	Derived to (years)	Age at 95% SVL asyp
Males	141.57 ± 1.42	0.242 ± 0.018	-1.09	11.3 years
Females	153.71 ± 3.04	0.176 ± 0.022	-1.37	15.6 years

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556 **Fig. 1.** Subadult (left) and adult (right) of *E. quatuorlineata*.

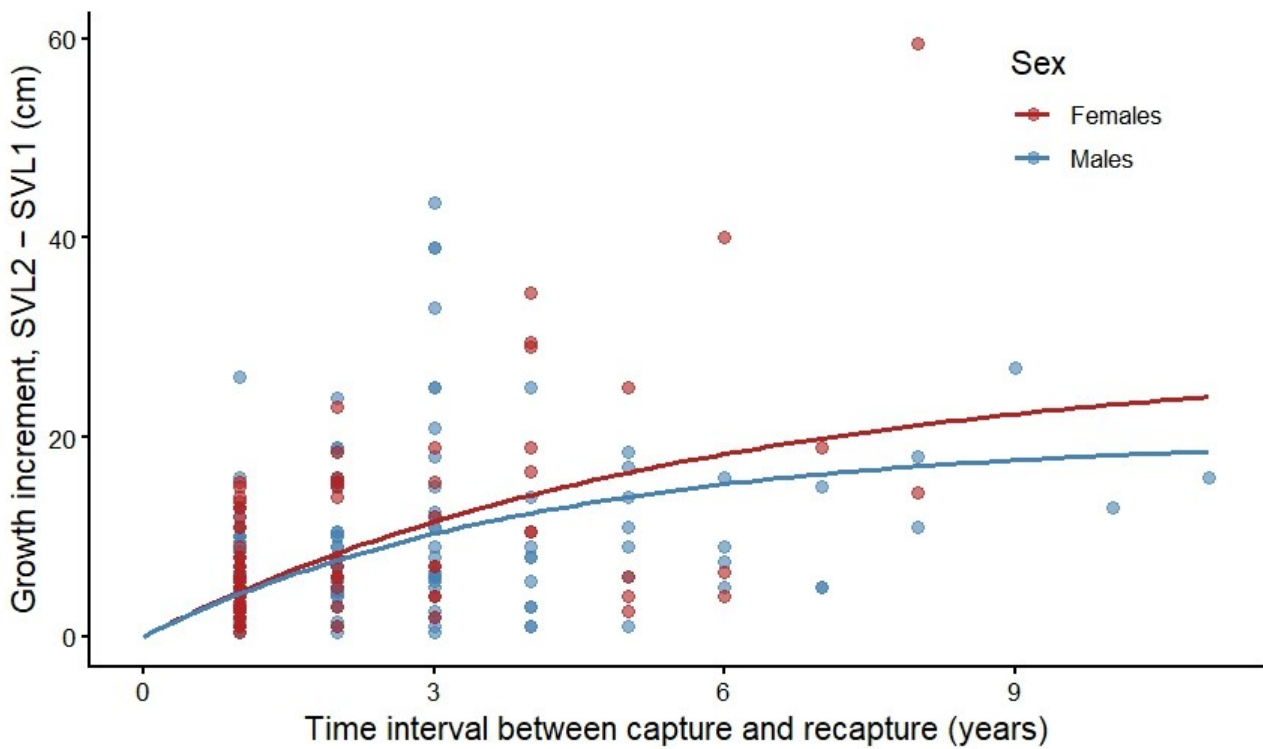


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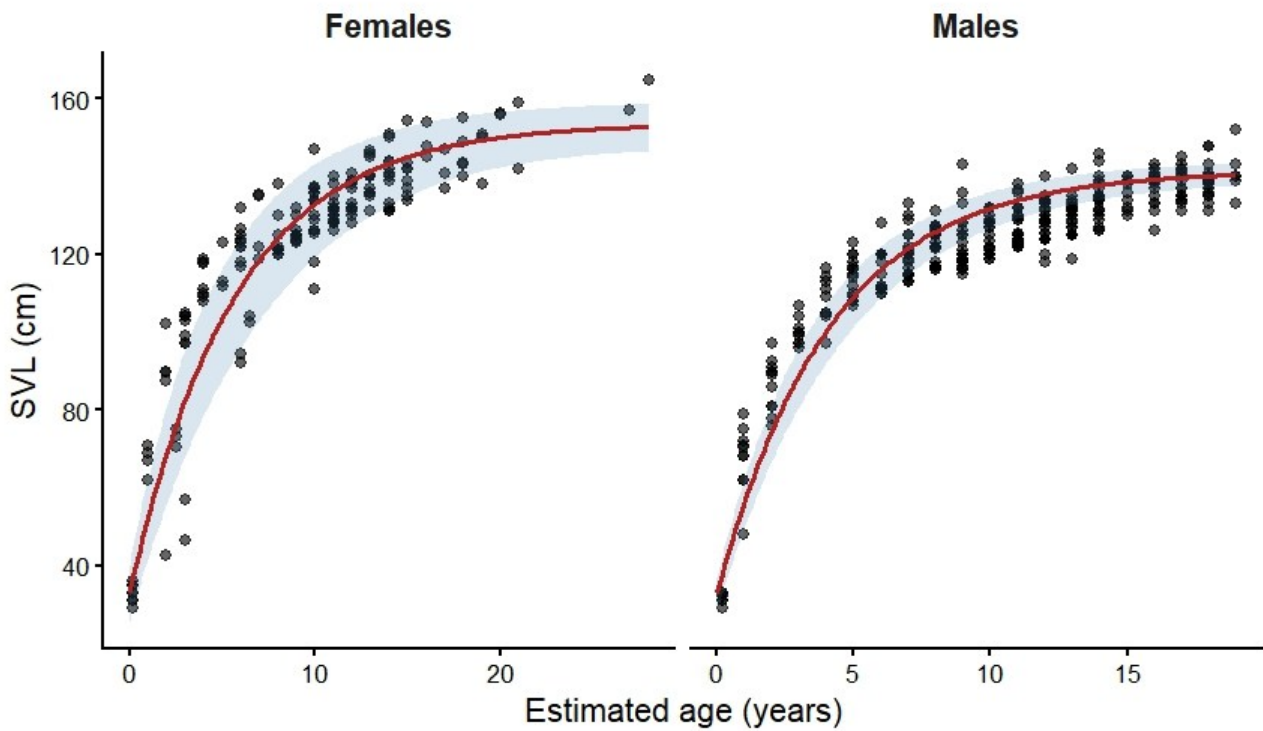
560 **Fig. 2.** Individual growth increments between successive captures of *E. quatuorlineata* estimated
561 using a nonlinear mixed-effects Fabens model for males and females. Points represent the observed
562 growth increment (SVL2 - SVL1) as a function of the time interval between capture and recapture
563 (years). Solid lines represent prediction from the fitted mixed-effects Fabens model, evaluated at the
564 sex specific mean initial SVL (SVL1).



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566 **Fig. 3.** Size-at-age observations for *E. quatuorlineata* (black dots), separated by sex (females, left
567 panel; males, right panel), with the age-axis representation of the growth trajectory derived from the
568 mixed-effects Fabens estimates (red line) and its 95% confidence interval (shaded band), obtained
569 by propagating the standard errors of SVLin_f and K (Tab. 4) through 2,000 simulated parameter
570 draws. Ages on the horizontal axis are size-derived estimates and are shown for illustration only; the
571 curve is not fitted to these data.

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