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Age-related sex differences in body condition and telomere dynamics of red-sided garter snakes

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Abstract

Life-history strategies vary dramatically between the sexes, which may drive divergence in sex-specific senescence and mortality rates. Telomeres are tandem nucleotide repeats that protect the ends of chromosomes from erosion during cell division. Telomeres have been implicated in senescence and mortality because they tend to shorten with stress, growth and age.We investigated age-specific telomere length in female and male red-sided garter snakes, Thamnophis sirtalis parietalis. We hypothesized that age-specific telomere length would differ between males and females given their divergent reproductive strategies. Male garter snakes emerge from hibernation with high levels of corticosterone, which facilitates energy mobilization to fuel mate-searching, courtship and mating behaviours during a two to four week aphagous breeding period at the den site. Conversely, females remain at the dens for only about 4 days and seem to invest more energy in growth and cellular maintenance, as they usually reproduce biennially. As male investment in reproduction involves a yearly bout of physiologically stressful activities, while females prioritize self-maintenance, we predicted male snakes would experience more age-specific telomere loss than females. We investigated this prediction using skeletochronology to determine the ages of individuals and qPCR to determine telomere length in a cross-sectional study. For both sexes, telomere length was positively related to body condition. Telomere length decreased with age in male garter snakes, but remained stable in female snakes. There was no correlation between telomere length and growth in either sex, suggesting that our results are a consequence of divergent selection on life histories of males and females. Different selection on the sexes may be the physiological consequence of the sexual dimorphism and mating system dynamics displayed by this species.

Authors

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2   **Age-related sex differences in body condition and telomere dynamics of red-sided garter**

3   **snakes**

4   <u>Short running title: Sex differences in telomeres</u>

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42 determine telomere length in a cross-sectional study. For both sexes, telomere length was

43 positively related to body condition. Telomere length decreased with age in male garter

44 snakes, but remained stable in female snakes. There was no correlation between telomere

45 length and growth in either sex, suggesting that our results are a consequence of divergent

46 selection on life histories of males and females. Different selection on the sexes may be the

47 physiological consequence of the sexual dimorphism and mating system dynamics displayed

48 by this species.

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50 Keywords: telomeres, condition, life history strategies, sex-differences, reptile

51 Introduction

52 Life history strategies vary widely both between and within species. Such strategies

53 describe how limited resources are used and prioritised [1, 2], generating trade-offs between

54 different physiological processes that mediate growth, reproduction and survival [3-5]. For

2

55 example, organisms that "live fast" are characterised by rapid growth and maturation, and

56 high reproductive output, but age more quickly and have short lifespans [6, 7]. Conversely,

57 organisms that "live slow" grow and mature more gradually and have lower reproductive

58 output, but age more slowly and have longer life spans [6, 7]. Reproduction-longevity trade-

59 offs are often difficult to detect within a population due to condition-mediated positive

60 correlations between natural history traits [8, 9]. However, there should be a link between

61 condition, cellular maintenance and aging. Body condition reflects the efficient collection,

62 assimilation and deployment of resources and depends on the individual's capacity to cope

63 with handicaps like infection, injury, parasitism and environmental stress throughout

64 ontogeny [10-15].

65   As long-lived organisms age, they tend to experience reduced survival and

66 reproductive output that may be mediated by condition [16, but see, 17]. One mechanism

67 linking differences in life histories, lifespans and aging appears to be variation in telomere

68 dynamics [18-20]. Telomeres are hexameric tandem repeat sequences of 5'-TTAGGG-3' at

69 the ends of chromosomes that typically shorten over the life of an organism due to repeated

70 cellular divisions and damage caused by reactive oxygen species [ROS; 21, 22]. Among

71 species, telomere dynamics may covary with life history strategies [23, 24], and the rate of

72 telomere attrition correlates with lifespan [18, 25]. However, it is unclear whether short

73 telomeres cause death or whether they are correlated with some other mechanism of

74 senescence [19, 26, 27]. Body condition indices (BCI: body mass controlled for structural

75 length) may be a useful measurement of somatic maintenance that is associated with longer

76 telomeres [e.g., 28].

77   Interspecific differences in telomere attrition are likely due to prioritising cellular

78 maintenance (e.g., DNA-repair) over other cellular functions [29, 30], as autosomal mutations

79 are negatively correlated with lifespan [mammals, 31]. DNA damage can lead to mutations,

80   telomere loss, and cellular senescence; thus, the maintenance of the genome likely explains

81   telomere length stability in longer lived organisms [32, 33]. To date, most studies of

82   telomere dynamics and life history strategies have focused on interspecific comparisons [18,

83   20, 23, 26]. While these studies have yielded insight into telomere dynamics, elucidating the

84   mechanisms underlying the observed trends is complicated by genetic variation between

85   species. Studying organisms that exhibit intraspecific differences in reproductive tactics

86   and/or life history strategies provides a natural experimental scenario to study telomere

87   dynamics while minimising the noise of interspecific genetic variation. For example, females

88   and males often exhibit sex-related differences in reproductive strategies and sexual selection

89   [34-38], which may result in sex-specific telomere dynamics [25, 39-41]. Thus, we sought to

90   investigate telomere dynamics in a highly dimorphic species with well-characterised life

91   history and reproductive strategies: the red-sided garter snake, *Thamnophis sirtalis parietalis*

92   (a non-venomous colubrid).

93   Red-sided garter snakes are sexually dimorphic with respect to body size, with

94   females growing approximately 30% longer, on average, than males. [42]. In the Interlake

95   populations of Manitoba, Canada, red-sided garter snakes hibernate for eight months in

96   communal dens and emerge *en masse* in spring, to form large aggregations where males

97   scramble to locate and mate with females [43, 44]. Mating activity at the dens lasts ~6 weeks

98   from late April through May [45] with some males mate-searching and courting for two to

99   four weeks [43, 46, 47].

100   During the spring breeding season, male garter snakes are aphagous and have

101   relatively high levels of corticosterone [48-52]. Courtship and copulatory plug production are

102   energetically expensive [44, 53, 54], and males may lose 10% of body mass during two

103   weeks of mate-searching, courtship and mating [46, 52]. In other species, physiological stress

104   and fasting lead to increased ROS production, the depletion of endogenous antioxidants, and

increased cellular damage and senescence [55-63]. One of the hallmarks of male aging is poor sperm performance, which is strongly influenced by oxidative and other physiological stressors [reviewed in 64]. Indeed, larger [and therefore older, 65] male red-sided garter snakes have poorer sperm performance than smaller males [66], suggesting that these males undergo senescence in the wild.

In contrast to males, female garter snakes seem to prioritise growth and maintenance over short-term reproductive success. Females reach sexual maturity at three years of age, while males are sexually mature at one or two years [67]. Most females mate every year before migrating to feeding grounds [68], but they reproduce only when they have acquired sufficient body mass or "capital", which is typically every other year [69, 70]. Like most snakes, female garter snakes do not provide post-natal parental care [71]. Furthermore, female fecundity increases with body length [72-74] and, presumably, also with age because snakes exhibit indeterminate growth [65]. Biennial reproduction and increasing reproductive fitness with age may generate selection on increased cellular maintenance, body condition, and growth in females. In this species, body condition is positively correlated with fat mass (Uhrig et al. unpublished data). With such life history variation between the sexes, the red-sided garter snake is an exceptional model for investigating how different reproductive strategies and telomere dynamics interact, while minimising the genetic variance that makes interspecific studies difficult to interpret.

We hypothesized that the sex-specific reproductive strategies of red-sided garter snakes would be associated with differences in age-related declines in telomere lengths. This study aims to determine: i) the relationship between body condition, telomere length and age in garter snakes, and ii) whether this relationship differs with sex. We predict that male garter snakes will experience greater telomeric attrition with age than females, due to the much

129   more intense reproductive investment in males. Furthermore, if females are investing more in

130   somatic maintenance than males, we expect females will maintain better body condition.

131

132   Materials and methods
133

134   At the peak of breeding season (May 10, 2015), we collected an excess of snakes by hand

135   from mating aggregations with the aim to collect the full range of body lengths found at our

136   Inwood, Manitoba study site (males: N = 100; females: N = 50). We transported snakes to

137   Chatfield research station, 16 km away, where they were weighed (±0.01g) and measured for

138   snout-vent length (SVL: ±1mm) where we culled our sample to ensure an equal distribution

139   of sizes for each sex. We selected the 4 longest and 4 shortest animals of each sex and an

140   even distribution of intermediate sizes, obtaining a final sample of 42 males and 30 females

141   (see FIGURE 1a), the remaining 78 animals were returned to the point of capture the next

142   day. All animals were adults; juveniles are only rarely found at den sites [RTM > 25 years of

143   pers. obs.; 43, 72]. Blood (<0.1mL) for telomere analysis was taken from the caudal vein,

144   added to 300 µL of RNAlater and frozen (-30˚C) until DNA extraction. Approximately 1cm

145   of Tails tissue was collected for skeletochronological aging; see expanded methods in

146   supplemental document 1 for more details.

147   Skeletochronology/Histology

148   Individual age was estimated by a modified version of the technique described by Waye and

149   Gregory [76, 1999] and Clesson, Bautista [77]. Vertebrae were examined microscopically

150   and the number of growth rings was identified for each animal; see supplement for more

151   details.

6

Quantifying telomere length

Telomere length was measured using real-time quantitative PCR (qPCR) as we have done

previously [78] using the 18S ribosomal RNA (18S) gene as the non-variable in copy number

reference gene [78-80]; see supplement more details.

Statistical analyses

We calculated two measures of body condition indices (BCI). In both cases BCI is the

standardized residuals (mean = 0; standard deviation =1) from linear regressions of ln(body

mass) as a function of ln(SVL) [81]. We ran this linear regression model once with males

and females pooled, and it was clear that females had much higher BCI than males.

Therefore, it was more biologically relevant to generate BCI for each sex separately using a

separate regression model for each sex, thus creating BCI specific for each sex (ssBCI) to

account for differences in allometry [81]. Growth was calculated as size (SVL)/age. Visual

inspection of regression plots for male telomere length given age suggested a curvilinear

relationship as has been described in many taxa, including squamate reptiles [26, 40, 82-85],

and F-tests we used to formally test the goodness of fit for first-order versus quadratic

regressions. We used ANCOVA to test for age-specific sex differences in telomere length

and body condition. When we found a significant sex by age interaction we used the Johnson-

Neyman (J-N) procedure to determine ages where the sexes differed in condition [86]. All

analyses were conducted in SigmaPlot 13.0, except the J-N procedure which was conducted

in MS Excel on the spreadsheet provided as a supplement in White [86]. See supplement for

more details.

Results

Skeletochronology, size and body condition

Age and sex predicted body size (SVL): older animals were longer and females were

significantly longer than males of the same age (ANCOVA: Sex x Age P = 0.487 (dropped

179   from model): $R^2 = 0.366$; Age: $F_{1,69} = 14.636$, $P < 0.001$; Sex: $F_{1,69} = 16.569$, $P < 0.001$;

180   FIGURE 1a). The shape of the age distributions was not different between the sexes

181   (Kolmogorov-Smirnov test: $D = 0.205$, $P = 0.412$) and females in our sample were

182   significantly older than males ($F_{1,70} = 6.384$, $P = 0.014$; mean (range), Females: 4.3 y (2-9 y);

183   Males: 3.5 y (2-6 y)). There was a significant Sex x Age interaction on BCI (ANCOVA: $R^2 =$

184   0.542; Age: $F_{1,69} = 5.403$, $P = 0.023$; Sex: $F_{1,69} = 0.003$, $P = 0.953$; Sex x Age $F_{1,69} = 8.695$, P

185    $= 0.004$), suggesting that females and males differentially maintain body condition as they

186   age. Because of the significant Sex x Age interaction, we computed the region of non-

187   significance for the age-effect on BCI between the sexes (-8.454 to 2.029 yrs) using the

188   Johnson-Neyman procedure [86]. This approach demonstrates that BCI differed between the

189   sexes at ages greater than 2.03 years, which included most of the snakes in this sample

190   (FIGURE 1b; note age values < 0 are meaningless and omitted from the figure). Given the

191   profound sex-differences in body condition, we recalculated BCI for each sex with separate

192   regressions (i.e., "sex-specific" BCI) and reran the analysis. We still found a significant Sex x

193   Age interaction ($P = 0.023$), which revealed that sex-specific body condition tends to increase

194   with age in females, but decreases with age in males (Supplemental Figure 2). We used this

195   sex-specific BCI (ssBCI) to explore the relationship between body condition and telomere

196   length in further analyses.

197   Telomere length and age
198   Telomere length was shorter in males than females ($F_{1,70} = 7.288$, $P = 0.009$) . The

199   relationship between telomere length and age was different for males and females. Age did

200   not predict telomere length in females (Females: simple linear regression $R^2 = 0.000$, $F_{1,29} =$

201   0.005, $P = 0.945$: quadratic regression; $R^2 = 0.000$, $F_{2,28} = 0.050$, $P = 0.951$; FIGURE2a).

202   However, in males, telomeres shorten with age, a relationship better fit by quadratic

203   regression than linear regression (test of first order = null hypothesis vs quadratic: $F_{2,41} =$

204   5.538, P = 0.024: simple linear regression: $R^2 = 0.108$, $F_{1,41} = 4.856$, P =0.033; quadratic

205   regression: $R^2 = 0.219$, $F_{2,39} = 5.472$, P =0.008; FIGURE 2b).

206

207   ## Telomere length, body size and growth
208   Although age and SVL were directly related in both sexes (see above), SVL and telomere

209   length were not related (ANCOVA Sex x SVL P = 0.538 (dropped interaction): $R^2 = 0.095$;

210   Sex: $F_{1,69} = 5.900$, P = 0.018; SVL: $F_{1,69} = 0.057$, P = 0.813). Separate analyses to test for a

211   quadratic relationship, as was found in males for age and telomere length, showed no

212   evidence for a relationship between SVL and telomere length in either sex (Females P =

213   0.200; Males P = 0.229). Finally, growth (size/age) was not significantly associated with

214   telomere length (either SVL/age: $R^2 = 0.052$, P = 0.085; residual SVL given age: $R^2 = 0.031$,

215   P = 0.137; or sex-specific residual SVL given age: $R^2 = 0.001$, P = 0.766).

216

217   ## Telomere length and body condition
218   Sex-specific body condition (ssBCI) and blood telomere length were positively correlated

219   ($R^2 = 0.131$, $F_{1,70} = 10.564$, P = 0.002), and, although females had higher ssBCI than males,

220   the relationship between ssBCI and telomere length was the same for both sexes (ANCOVA

221   Sex x ssBCI: P = 0.510 (dropped interaction): $R^2 = 0.145$; Sex: $F_{1,69} = 7.601$, P = 0.007;

222   ssBCI: $F_{1,69} = 4.005$, P = 0.049, FIGURE 3).

223   ## Discussion
224   Sex differences in aging may result from sex-specific optimization of investment to

225   reproduction and somatic maintenance in response to the challenges of different life history

226   strategies between the sexes. We have shown that body condition positively correlates with

227   telomere length in both sexes of red-sided garter snakes, which supports our assertion that

228   body condition is an intuitive measure of somatic investment. However, the relationship

229   between body condition and age differed strikingly between sexes, with females maintaining

their body condition with age, while condition decreased with age in males. Likewise,

telomeres were exponentially shorter in older male garter snakes, while the telomere lengths

of females are independent of age. Non-linear relationships between telomere length and age

have been shown in several taxa [e.g., 82, 85], and is consistent with an exacerbating cycle of

cellular damage and increased dysfunction seen in aging humans [87]. Females had the

longest telomeres and were the oldest individuals in our sample, suggesting they live longer

than males in this population. These results support our prediction that males experience

greater telomere loss with age due to prioritisation of current reproduction over cellular

maintenance and longevity. Overall, the decrease in both body condition and telomere length

in males with age suggests that they senesce at an earlier age than females.

Telomere shortening has been implicated as a cost of reproduction in several species.

For example, in blue tits (*Cyanistes caeruleus*), when brood size was experimentally

increased, parents experienced a decrease in blood telomere length, with males suffering from

greater telomere loss than females [88]. Relative reproductive success seems to result in

greater telomere attrition in common terns (*Sterna hirundo*) [89]. For both male and female

Atlantic silversides (*Menidia menidia*), gonadal somatic index (GSI: gonad mass relative to

total body mass) was negatively correlated with telomere length and lifespan [90]. These

studies suggest increased reproductive investment comes at a cost of telomere attrition.

Studies of telomere dynamics are rare in reptiles and only two reports on snakes.

Bronikowski [91] reported telomere lengths for male wandering garter snakes (*Thamnophis*

elegans). Wandering garter snakes are an interesting species for studying telomere dynamics

because, in the mountains of Northern California there are two eco-types with very different

life-histories: one short lived "meadow" eco-type and a long-lived "lakeshore" eco-type [92-

94]. As in our study, Bronikowski [91] showed declining telomere length with male age (up

to 12 years of age, based on skeletochronology), but was unable to find among eco-type

255 differences, and did not report telomere lengths for females. In water pythons (*Liasis fuscus*)

256 of Northern Territory, Australia, females have longer telomeres than males [83], similar to

257 our study. Furthermore, telomere length increased from hatching to four years of age, but

258 declined very slightly with age in both sexes up to 18 years of age [83].

259 Why might selection on telomere dynamics differ between male and female garter snakes?
260   Our study is observational and cross-sectional, so our causal interpretation of the sex-

261 specific differences in the relationship between age and telomere length is necessarily

262 tentative. In Manitoba's Interlake region, winter temperatures often hover around -40˚C for

263 weeks and, since snow provides insulation from the cold, there are likely cryptic mass

264 fatalities deep within dens during years of light snowfall [72]. The snakes' brief three to four

265 month active season begins and ends with chance freezes and floods that lead to mass

266 mortality events that are likely to generate selection on rapid growth and early maturity in

267 both sexes [72, 95]. Mortality due to these stochastic events is usually not consistently biased

268 toward either sex and adult sex-ratio is 1:1 [43, 72, 95]. Predation and road kills are not sex

269 biased either [95]. However, a mass mortality event could differentially affect size classes

270 among sexes. For example, a winterkill event in 1998-1999 shifted the size distribution

271 toward smaller animals in subsequent years in both sexes, but the largest females were most

272 strongly affected [95]. Small males, and to a lesser extent large females, are more likely to be

273 trapped and suffocate in large mating aggregations (> 500 animals) [96]. Such events could

274 cull a size class or spare only old females with the longest telomeres, generating results

275 similar to ours. Nevertheless, we have not witnessed similar events in our yearly visits since

276 1999, thus other explanations may better fit our results.

277   Males engage in energetically expensive reproductive behaviour annually, while most

278 females generally reproduce biennially. Although male size affects mating success when a

279 single pair of males competes for copulation, the effect is small to non-existent in the largest

280 aggregations at the den sites, reducing selection for increased male size [97, 98]. The largest

281   females, however, are able to reproduce annually, leading to greater fecundity and generating

282   higher selection on female growth and longevity [72-74, 99]. Females in Interlake

283   populations seem to have higher reproductive output given female size than populations

284   farther south in less harsh climates with longer feeding/growth seasons [72, 100]. Therefore,

285   selection on cellular maintenance and longevity are likely to be stronger in females than

286   males because the costly mechanisms that prevent telomere loss are balanced by increasing

287   fecundity with age and size in females, but have fewer benefits for males.

288   What physiological mechanisms might explain sex-specific telomere attrition?
289   We do not know the specific mechanisms that lead to sex-differences in telomere

290   length, but there are several non-mutually exclusive hypotheses to explain our results. For

291   these ectotherms, body temperature and metabolic rate are very low during winter brumation

292   [~1°C 101] and only rise in late April when the ground warms. Both sexes enter winter

293   hibernacula at the same time [72], but males, on average, emerge earlier than most females.

294   Therefore, body temperature and metabolic rate will be lower, for slightly longer, in females

295   than males. Lower body temperature associated with torpor is correlated with positive effects

296   on telomere length and somatic maintenance in some mammals [e.g., 102].

297   High levels of corticosterone experienced by males during the mating season [51]

298   may increase metabolism, but also may increase mitochondrial ROS production, DNA

299   damage, and telomere erosion [33, 58, 103, 104]. The high energetic demands of courtship

300   and mating of aphagous males [53] likely limits the resources that can be allocated to DNA

301   repair mechanisms, limiting the chance for telomere repair [105]. For example, as the

302   increased male-male competition among male rhesus macaques (*Macaca mulatta*), is

303   correlated with DNA oxidative damage (8-OHdG) and shorter lifespan [59]. In red-sided

304   garter snakes, the energy for antioxidant synthesis, DNA repair and telomere maintenance is

305   limited by male fasting [29, 56, 57, 61, 106]. Fasting itself may increase oxidative stress [55,

306   60, 62, 63]. Fasting increases the generation of mitochondrial ROS and lipid peroxidation in

307 rats (*Rattus norvegicus*) [63]. Fasting male northern elephant seals (*Mirounga angustirostris*)

308 exhibit increased oxidative damage to DNA and lipids [60]. Given the stochastic mortality,

309 weak sexual selection on male size, and oxidative stress induced by during energetically

310 costly courtship and mating while fasting, selection to mitigate damage by ROS via

311 investment in cellular maintenance and growth may be weak in male red-sided garter snakes.

312 Weak selection for enhanced cellular maintenance might explain both the reduction of body

313 condition and telomere length with age. This may be the consequence of selection for a live

314 fast, die young strategy in males.

315 Females were in better body condition than males in our study, which generally

316 indicates they have larger energy stores than males [107]. In brown tree snakes, *Boiga*

317 *irregularis*, this additional energy reserve correlates with lower levels of corticosterone [108],

318 potentially leading to lower stress overall and more stable telomere length [30, 109].

319 Furthermore, having greater energy reserves may allow for greater expenditure on

320 antioxidants and cellular repair. Species of snakes that live longer are capable of producing a

321 stronger response to DNA damage by activating repair mechanisms and experience lower

322 levels of mitochondrial reactive oxygen species, which presumably generates less oxidative

323 damage to DNA [91, 110]. We show that female *T. sirtalis parietalis* have a greater lifespan

324 than males and may potentially use mechanisms similar to those of other snakes to maintain

325 genome stability and telomere length. The underlying mechanisms causing the sexual

326 dimorphism may provide explanations for sex-specific differences in telomere length.

327 Sexual size dimorphism varies greatly across taxa, and trends associated with

328 dimorphism, lifespan, and telomere attrition are not consistent [25, 111]. For garter snakes,

329 the difference in size between males and females seems to be controlled by testicular

330 androgens suppressing growth in males [112]. Testosterone can reduce cellular resistance to

331 free radicals [113], leading to increased DNA damage and telomeric attrition [20, 114]. In the

closely related red-spotted garter snake, *Thamnophis sirtalis concinnus*, females treated with

an estrogen receptor antagonist, tamoxifen, experienced a decrease in growth rate [115],

suggesting that estrogen plays a role in the sexual size dimorphism observed in *T. sirtalis*

parietalis. Estrogens act as antioxidants and/or stimulate endogenous antioxidant and cellular

repair mechanisms [116-118] potentially reducing ROS and leading to the telomeric stability

observed in the present study and in females across other taxa [25, 40]. The most

energetically demanding component of reproduction for female garter snakes is the

production of yolk proteins (i.e., vitellogenesis) [119]. There is evidence that the yolk protein,

vitellogenin, may act as an antioxidant, [120-124] reducing DNA damage, telomere attrition,

and cellular senescence at a time when cellular respiration and ROS production are highest.

Thus, selection acting on the mechanisms that increase female growth and provisioning of

offspring seem to also favour antioxidant production, a reduction in oxidative stress, and

cellular repair involved in slowing the aging process.

In the current cross-sectional study, we investigated differences in telomeres within a

single species. We found that telomere dynamics are strongly linked with sex and therefore

life history strategies. Sex-specific telomere dynamics may be tightly linked to selection on

males for early reproduction and costs associated with yearly energetic investment in

courtship and mating while fasting. In contrast, females have biennial reproduction and

investment in somatic maintenance has a fitness payoff of greater fecundity with increasing

size later in life. Future studies should include longitudinal data, increased sampling of the

largest size classes, the measurement of telomerase activity, general DNA damage, and

antioxidant production throughout the entire active season, to assess our hypothesis that

females live longer by investing more in cellular maintenance and repair than males.

364   **Ethics Statement**
365   Procedures performed on animals were approved by Oregon State University [IACUC
366   ACUP-4317 and the research was conducted under permit from Manitoba Conservation
367   [WB16264].

368   **Data accessibility**
369   Has been uploaded to Dryad

370   **Competing interests statement**
371   The authors declare they have no competing interests

372   **Authors' contributions statement**
373   All authors made significant intellectual and material contributions to this paper.

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Figures with legends

697

698 **Figure 1**: **a)** Age (years) and sex predicted body size (ln (snout to vent length): ln(svl)): older

699 animals were longer and females were significantly longer than males of the same age. Open

700 circles indicate males and solid triangles indicate females (note: for clarity with overlapping

701 data points, male data are offset slightly to the right). The least-squares regression lines were

702 calculated separately for females (solid line, r = 0.400) and males (dashed line, r = 0.445). **b)**

703 Body condition (BCI) differed with age and sex and there was a significant sex x age

704 interaction (P = 0.004). Females had higher BCI than males and BCI decreased with male age

705 but not females. Open circles indicate males and solid triangles indicate females. The least-

706 squares regression lines were calculated separately for females (solid line, r = 0.015) and

707 males (dashed line, r = -0.479). The diagonal hatched box (Age = 0.00 to 2.03), is the age-

708 range through which BCI did not differ between females and males as determined by the

709 Johnson-Neyman procedure [86].

710

711
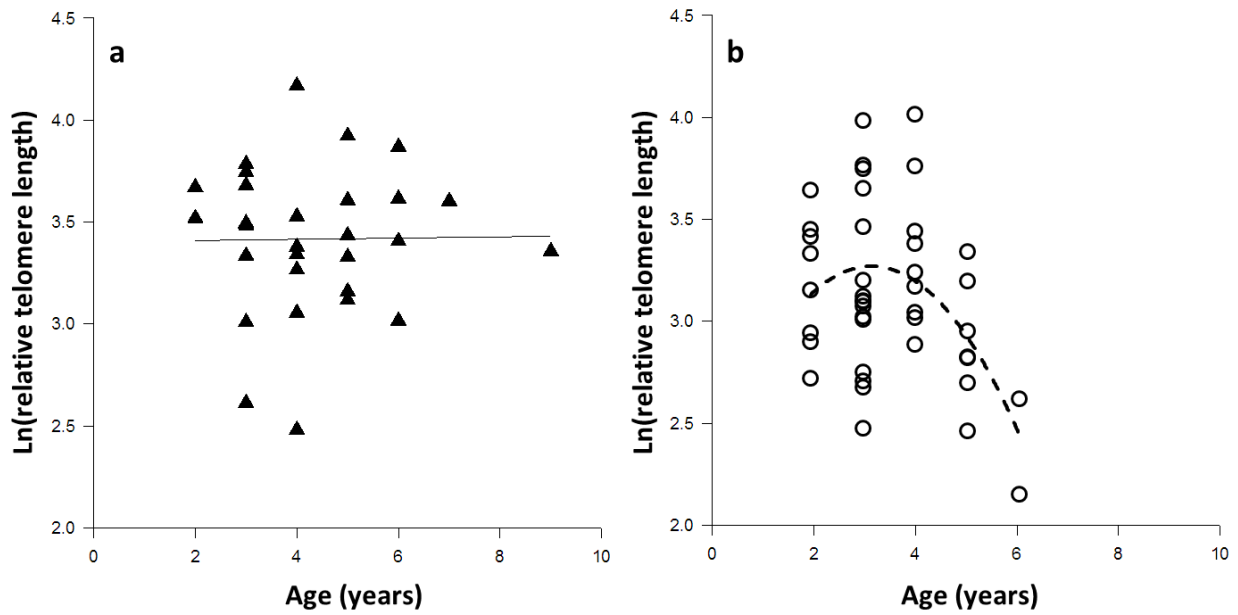
Figure 2: The relationship between natural log of blood telomere length and age in years was

different for females (a) and males (b). (a) Age did not predict telomere length in females

(Females: simple linear regression; $r = 0.013$, $F_{1,29} = 0.005$, $P = 0.945$: quadratic regression; r

$= 0.067$, $F_{2,29} = 0.050$, $P = 0.951$). (b) However, in males, telomeres shorten with age, which

is better fit by quadratic regression than a linear regression (test of first order = null

hypothesis vs quadratic: $F_{2,41} = 5.538$, $P = 0.024$: quadratic regression: $r = 0.468$, $F_{2,41} =$

5.472, $P = 0.008$).

723
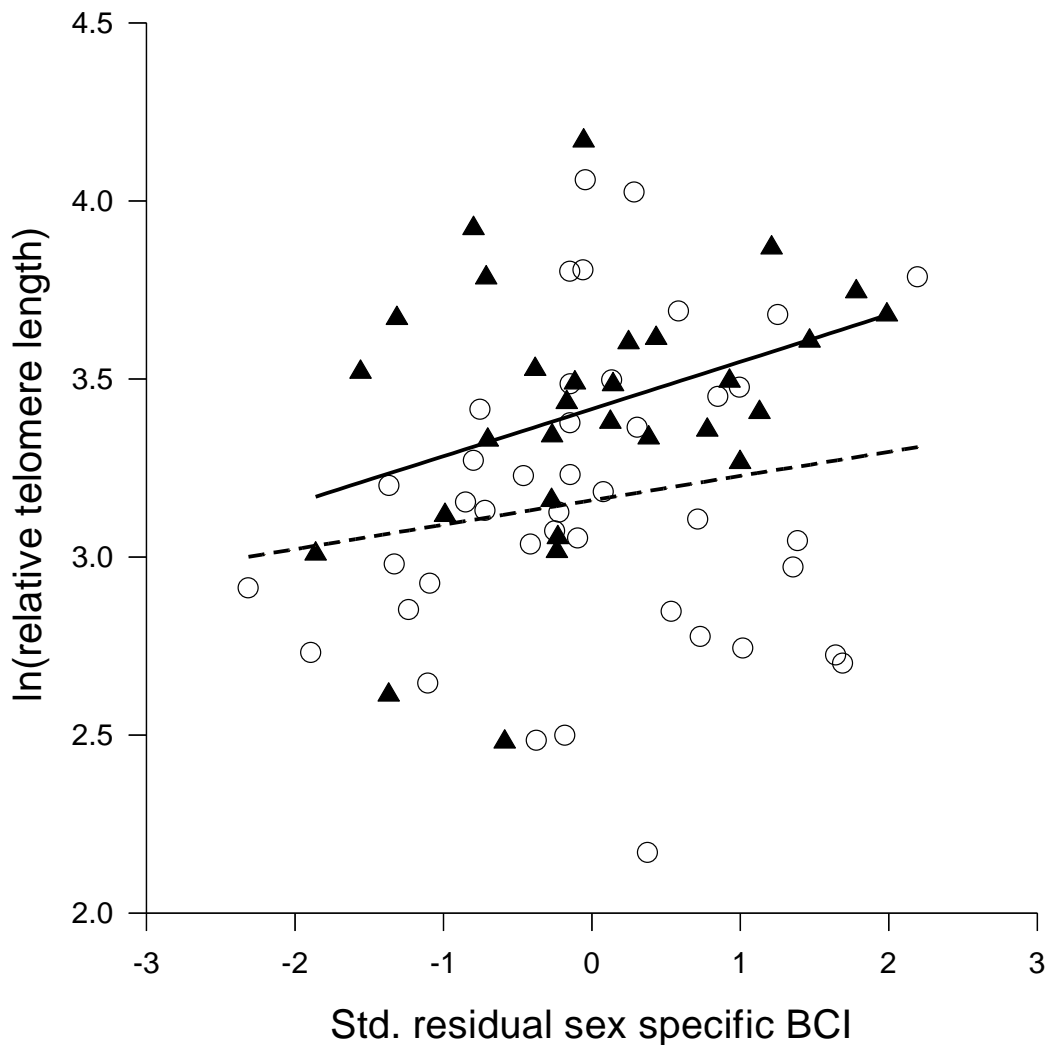
Figure 3: Combined sex-specific body condition (standardized residuals from separate

regressions of body mass given snout-to-vent length for each sex) and natural log of blood

telomere length were positively correlated (r = 0.602). Females had higher BCI than males,

but the relationship between BCI and telomere length was the same. Open circles indicate

males, and solid triangles indicate females. The least-squares regression lines were calculated

separately for females (solid line, r = 0.362) and males (dashed line, r = 0.506).

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