

HIGHLIGHTS

- **Sex-steroid levels declined gradually with age, starting at age 6 in males and 5.5 years in females.**
- **Decline in somatic parameters commenced at the age of 3 years in both sexes.**
- **Reproductive senescence in females tends to involve “restraint”, coinciding with hormonal decline.**
- **Reproductive senescence in males tends to involve “constraint”, coinciding with somatic decline.**
- **There was evidence of over 2 years of Post Reproductive Life Span in both sexes.**

Reproductive and Somatic Senescence in the European Badger (*Meles meles*): Evidence from Lifetime Sex-Steroid Profiles

Nadine Adrianna Sugianto^a, Chris Newman^a, David W. Macdonald^a, Christina D. Buesching^{a*}

^a Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Recanati-Kaplan Centre, Abingdon Road, Tubney House, Tubney, Oxfordshire OX13 5QL, UK.

*Corresponding Author: christina.buesching@zoo.ox.ac.uk

Abstract

Among the Carnivora, there is sparse evidence for any substantive fitness benefits of post reproductive lifespan (PRLS, survival after reproductive cessation, RC). Using the European badger (*Meles meles*) as a model species, we analyzed sex-specific cross-sectional endocrinological and morphological data to investigate: 1) age-dependent reproductive decline in sex-steroid levels versus prime reproductive age; 2) age-dependent declines in somatic condition and reproductive advertisement (from subcaudal scent gland secretion); 3) changes in reproductive success with age due to somatic and endocrinological decline; 4) occurrence of RC, PRLS, and post reproductive representation (PrR) in the population with reference to pre-pubescent hormone levels and evidenced by fewer cub assignments from pedigree. We provide strong evidence for a gradual, not abrupt, decline in sex-steroid levels with age, with both sexes following a concave (down) quadratic trend. For both sexes, the onset of decline in somatic condition commenced at the age of 3 years. In contrast, decline in reproductive hormones started at age ca. 5.5 years in females and 6 years in males, with similar rates of decline thereafter. Subcaudal gland secretion volume also decreased in both sexes, especially after age 5, suggesting less investment in reproductive advertisement. After age 3, fewer (surviving) females were assigned cubs. This coincided with the onset of somatic decline but came earlier than hormonal decline (5.5 years onwards). The decrease in offspring assignments commenced later in males at age 5-6 years; concomitant with onset of testosterone decline at 6 years. This suggests that, contrary to females, in males declining body condition does not preclude reproductive success (no 'restraint') in advance of hormonal senescence ('constraint'). There was evidence of female PRLS, with very old adults living up to 2.59 ± 1.29 years after RC; although in males this evidence was weaker. We discuss the implications of these findings for RC and PRLS in the context of adaptive and non-adaptive hypotheses.

Keywords: European badger, post-reproductive lifespan (PRLS), reproductive strategies, reproductive cessation (RC), post-reproductive representation (PrR), somatic condition, testosterone, oestrone

1. Introduction

A crucial question for understanding the life-histories of wild-living mammals, and related population ecology, is whether individuals experience a post reproductive life-span (PRLS: Medawar 1952; Cohen 2004; or post-reproductive representation, PrR, as a population equivalent: Levitis et al., 2011), and what proportion of individuals live long enough to reach post-reproductive age. This leads to a second question: even if older individuals do remain endocrinologically capable of reproducing, what proportion actually produce young successfully? Thirdly, are there constraints, such as the somatic condition of the individual, or the age structure among competing individuals in that population, that influence later-life reproductive capacity (Croft et al., 2015).

The evolutionary origins of PRLS have been much debated (vom Saal et al., 1994; Austad 1997; Peccei 2001; Shanley and Kirkwood 2001), where natural selection should disfavour the maintenance of any proportion of the population not maximizing reproductive potential. A possible exception to this among social species may arise if older individuals provide genuine benefits to the reproductive success of kin – termed the grandmother hypothesis in primatology (generally involving a slowing of somatic senescence without favouring extension of the fertile span; Hawkes et al., 1998; Alvarez et al., 2000). To-date there has been little evidence to support PRLS among the Carnivora, or corroboration or substantive fitness benefits, although a few studies on social species indicate it can occur (see Malcolm and Marten 1982; Cohen 2004).

Nevertheless, counter to this, in species/ populations where starvation, disease and predation take a systematic toll on cohorts, there are always fewer older individuals in a population than there are younger ones. This implies an age beyond which selection becomes too weak to prevent the onset of reproductive senescence (Croft et al., 2015), resulting in the eventual evolution of physiological mechanisms leading to reproductive and somatic senescence (Gavrilov and Gavrilova 2001). Nevertheless, the strength of selection on alleles with age-specific fitness effects is expected to weaken with increasing age, and detrimental genetic traits (such as PRLS) can persist (or even be favoured) provided their deleterious effects are delayed until an advanced age is reached, relative to the pervading schedule of mortality. In long-lived mammals, reproductive potential typically follows a bell shaped curve; increasing after puberty to a prime age peak, then decreasing until death (Jorgenson et al., 1997), where reports of menopause in non-human mammals are restricted to toothed whales (Ellis et al.,

2018). There is, however, no evolutionary benefit of maintaining fertility if somatic senescence progresses to the stage that successful reproduction is precluded, and although onset of somatic senescence generally occurs earlier than reproductive senescence, reproductive cessation (RC; Cohen et al., 2004) typically occurs prior to somatic condition becoming inadequate to sustain reproductive activity (Berube et al., 1999; Mysterud et al., 2002; Schwartz et al., 2003; Nussey et al., 2008; Descamps et al., 2008).

This raises the important question of evaluating the extent to which further reproduction is precluded by inadequate hormone production (failure to ovulate / reduced spermatogenesis – but see below), termed ‘constraint’, versus the failure of reproductively capable older individuals to realize successful offspring production because of inadequate somatic condition and/or competition from younger conspecifics, termed ‘restraint’ (see McNamara et al., 2009). Additionally, once individuals approach the conclusion of their reproductive lifespan, they may also make a ‘terminal investment’ (Clutton-Brock 1984), involving a disproportionate allocation to reproduction and sacrificing subsequent survival (see Campbell et al., 2017).

The selective basis of senescence differs between the sexes in relation to their reproductive physiology, investment in offspring and associated resource allocation schedules (Bonduriansky et al., 2008), where females typically experience constraint (and in rare cases menopause) while males experience restraint due to reduced competitive ability (Bribiescas et al., 2012). Consequently, reproductive senescence (Finch 1990) and somatic senescence (Reed et al., 2008) are expected to follow different sex-specific trajectories, which can occur asynchronously (Cohen 2004) leading to heterochrony across populations (Campbell et al., 2017).

1.1 Senescence in male reproductive strategies

Male reproductive strategies generally involve weaker selection for longevity, typically following a ‘live fast, die young’ schedule (Carranza and Pérez-Barbería 2007). In most mammals, particularly highly polygynous species, males invest predominantly in maximizing the number of females they mate with, often resulting in high intra-sexual competition for mate access. This is an energetically costly, high-risk strategy, but one that can yield higher fitness over short time periods (Clutton-Brock and Isvaran 2007; Preston et al., 2011). This strategy can, however, incur substantial cumulative somatic damage, which is detrimental to

survival (Carranza and Perez-Barberia 2007) and likely to result in accelerated physiological decline with age (Kotiaho 2001; Bonduriansky et al., 2008).

Aside from promoting aggressive competition (Mougeot et al., 2004; Blas et al., 2006), testosterone also causes reduced immunocompetence, increased susceptibility to oxidative stress, and increased basal metabolic rate and energy expenditure (Bribiescas 2006; Alonso-Alvarez et al., 2007; Cox and John-Alder 2007). In most mammals, males can sustain spermatogenesis until the end of their lifespan (Cohen 2004), although often of lower quality (Møller et al., 2009). Testosterone is produced by leydig cells and not by the sperm itself, and thus sperm production can continue even with low circulating testosterone levels (vom Saal and Finch 1988). Nevertheless, older males tend to undergo physical decline, invest less in sexual traits (e.g. reproductive advertisement, controlled by testosterone), and have poorer mating performance, and thus reduced reproductive fitness (Cohen 2004).

1.2 Senescence in female reproductive strategies

Aside from some female social mammals investing in reproductive suppression (Creel and Creel 1991), a female's primary route to greater fitness is via allocating more available energy into offspring, leading to a more conservative lifetime reproductive strategy than in males (Bonduriansky et al., 2008). Gestation and lactation, however, incur unavoidable costs in pursuit of female fitness (see Clutton-Brock et al., 1989), and therefore physiological decline among older females can lead to less energy allocated to reproduction (Kirkwood and Austad 2000; Myrsetrud et al., 2001). While males are able to increase reproductive fitness by enhancing mating efforts (sacrificing longevity), females are limited by the frequency of oestrus cycles/ number of oocytes, pregnancy duration, and inter-birth interval (largely driven by hormonal homeostasis; Adkins-Regan 2005; Nussey et al., 2009). This can lead to greater variance in relative fitness between males (depending on social system) than between females (Nussey et al., 2009), due to females having less strategic flexibility and more fixed investments in attaining only a single litter per reproductive cycle (Clutton-Brock et al., 1981; Vinogradov 1998). Ultimately, oocyte depletion (affecting oestrogen levels, as they are produced by mature follicles: Adkins-Regan 2005) leads to RC prior to death in menopausal mammal species (although not all undergo RC; Nozaki et al., 1995; Cohen 2004), which has the potential to be more impactful on population dynamics (depending on relative age / sex pyramids) than RC among elderly males (Rankin and Kokko 2007).

1.3 Study Species: The European badger

Here we investigate endocrinological changes with age in a generalist carnivore, the European badger (*Meles meles*; hereafter ‘badger’). These data, and associated inventory of blood samples, allowed us to select candidate individuals with longer realized lifespan (up to the age of 13 years), from the broader population. We also benefitted from a genetic pedigree for this population (Annavi et al., 2011) and from previous genetic analyses examining changes in breeding success and assigned litter size with age, which started at different ages for males and females (Dugdale et al., 2011a). This allowed us to test for reproductive restraint among older individuals that were apparently still capable of reproduction from their hormone levels.

Badgers provide an informative model for examining patterns of reproductive senescence and RC. In the UK, they live in groups of up to 29 mixed sex individuals (da Silva et al., 1994; study population average group size = 11.3) in which several females can breed (Dugdale et al., 2011b). This provides the potential opportunity for allo-maternal care, as seen in other group-living Carnivora (Macdonald and Moehlman 1982); a type of society where the ‘mother’ (Williams 1957) and ‘grandmother’ (Hawkes et al., 1998) adaptive hypotheses of senescence would be plausible. Groups involve high rates of natal philopatry (Macdonald et al., 2008), which enables us to evaluate the ‘inter-generational reproductive conflict’ or ‘adaptive kinship’ hypotheses (Cant and Johnstone 2008) and the ‘altriciality lifespan’ hypothesis (Peccei 1995). Badgers are also polygynandrous and promiscuous (Dugdale et al., 2007; Annavi et al., 2014) giving plausibility to the ‘patriarch’ hypothesis (Marlowe 2000), where older males may accrue a mating advantage.

Badgers are seasonal breeders (Amstislavsky and Ternovskaya 2000) that produce one litter annually (mean litter size = 1.4 ± 0.06 , range of 1 - 4 cubs, where 93% litters comprise less than 3 cubs: Macdonald et al., 2015). Cubs are typically born during mid-February in the UK (76% of births; although parturition can occur from mid January – mid March; Neal and Cheeseman 1996; Yamaguchi et al., 2006) and first emerge from underground burrow systems (termed setts) around mid-April, being weaned by mid-May (Fell et al., 2006). The main mating season occurs during the post-partum oestrus in January-March (extending until May/ June; Yamaguchi et al., 2006; Sugianto et al., subm.). Scent marking activity using subcaudal gland secretion increases markedly during the mating season (Buesching and Macdonald 2001). This functions in olfactory mate guarding (Buesching et al., 2002a,

Buesching et al., 2003), resource defense and reproductive advertisement (Buesching et al., 2002b, Buesching and Macdonald 2004). During autumn, badgers go through reproductive quiescence and typically reach their maximum weight and body condition (Woodroffe and Macdonald 1995a; Macdonald et al., 2010; Sugianto et al., 2018).

Female badgers exhibit delayed implantation, where blastocysts remain suspended in the uterus lumen for up to 11 months (Corner et al., 2015), linked to an endocrinological physiology where more oestrone than oestradiol is produced to sustain pre-implanted blastocysts (see Sugianto et al., *subm.*). Badgers reach sexual maturity around 11 months old in both sexes (Sugianto et al., 2019), but, in our population, typically do not start breeding until the age of 2 years (Macdonald et al., 2009; Dugdale et al., 2011a). Once badgers reach sexual maturity, their circulating sex-steroid levels show a distinct seasonal pattern (Sugianto et al., 2019): In males, testosterone levels are high in spring, slightly lower in summer, lowest in autumn and peak during the winter mating season (Buesching et al., 2009). In females, oestrone levels are high in spring, low in summer, peak in autumn and remain high for pregnant females in winter but decline in non-pregnant females (Sugianto et al., *subm.*).

Confirmed maximum lifespan in badgers in this focal Wytham study population ranges up to 14 years (Macdonald et al., 2015), although only ca. 21.4% (26% females; 16.6% of males; Bright Ross et al., *pers. comm.*) live to age 7 years. Successful breeding in female badgers requires sufficient fat reserves (Woodroffe and Macdonald 1995a) and, in both sexes, involves conspicuous reproductive advertisement (predominantly by scent marking with energetically costly subcaudal gland secretions: Buesching et al., 2003, Buesching and Macdonald 2004, Allen et al., *subm.*). Therefore, breeding has been linked to a reduction in somatic condition (due to pregnancy and lactation in females; mating effort in males), higher bite wounding rates, higher parasite infestation, and anaemia (especially in males; Woodroffe and Macdonald 1995a; Macdonald and Newman 2002; Buesching et al., 2009).

Using this model species to understand the mechanisms underlying reproductive senescence, we examine cross-sectional endocrinological and morphological data to investigate, for each sex, if and when individuals exhibit:

- 1) age-dependent reproductive declines in sex-steroid levels (during their extended mating season) relative to their prime age of reproduction;

- 2) age-dependent declines in somatic condition and reproductive advertisement (subcaudal scent secretion volume), relative to the onset of reproductive senescence.
- 3) changes in reproductive success with age due to somatic and endocrinological decline
- 4) occurrence of RC and PRLS in the badger population with reference to pre-pubescent hormone levels and evidenced by fewer cub assignments, from pedigree (Dugdale et al., 2011a; Annavi et al., 2014 and pers comm).

We discuss the implications of our results for RC and PRLS in the context of the above outlined adaptive and non-adaptive hypotheses.

2. Materials and Methods

2.1 Badger Trapping and Sampling

Samples were drawn from a 29 year data set (1987-2016) detailing the life-histories of 1692 individual badgers from Wytham Woods, Oxford, UK (51°46:26 N, 1°19:19 W; for details see Savill et al., 2010; Macdonald et al., 2015). The extant population averaged 183 ± 43 adults and 50 ± 19 cubs per year (Bright Ross et al., pers. comm.) over this period, utilizing a 6 km² range. These data, and an associated inventory of blood samples, allowed us to select candidate individuals with longer realized lifespan (up to the age of 13 years) from the broader population. We also benefitted from a genetic pedigree for this population (Annavi et al., 2014; unpubl. data).

Following the methodology described in Macdonald and Newman (2002; 2009; see also Sun et al., 2015), badgers were trapped three times annually, routinely over two weeks, in cage traps baited with peanuts: (1) in spring (May/June) during the post-weaning and late-mating period, (2) in summer (August) during the second mating peak reported previously in other badger populations, and (3) in autumn (November) during reproductive quiescence when badgers reach their maximum body weight, prior to a legal closed season during pregnancy and cub rearing through until April 30th (Protection of Badgers Act, 1992).

Captured animals were transferred to holding cages and transported to a central field station between 6.30 - 8.00 am, and then sedated by intramuscular injection of 0.2 ml ketamine hydrochloride/ kg body weight (McLaren et al., 2005; Thorton et al., 2005). All badgers received a permanent unique tattoo, allowing individual identification (ID) and subsequent aging. All protocols and procedures employed were approved by the Animal Welfare and

Ethical Review board of the University of Oxford's Zoology Department, and procedures were conducted under the Animals (Scientific Procedures) Act, 1986 (currently PPL: 30/3379).

2.2 Somatic measurements and subcaudal gland secretion volume

Following sedation, we undertook somatic measurements for all individuals. Body-length was measured from the tip of the snout to the base of the sacrum (in mm) and individuals weighed to the nearest 100g. From this we calculated a Body-Condition Index (BCI), as $\log(\text{weight})/\log(\text{body length})$, supplemented by a direct measure of body-condition assessed by scoring body-fat along the spine and pelvis from 1 = emaciated to 5 = fat (see Buesching et al., 2009). We also collected subcaudal gland secretion from the subcaudal pouch using a rounded stainless-steel spatula, and volume was estimated to the nearest 0.05 ml. (Buesching et al., 2002a, b).

2.3 Blood Sampling and Hormone Measurements

Blood samples were collected by jugular venepuncture into K2-EDTA vacutainer tubes (Becton-Dickinson) for endocrinological analyses. Following collection (within 30 minutes of sampling), samples were centrifuged at 10°C for 10 min at 2,500 rpm/ 1470G. Plasma was transferred into Eppendorf tubes and frozen immediately at -20°C. Sampling times were standardised to account for circadian variation in hormonal profiles (Buesching et al., 2009). To accommodate gaps in individual life-history recapture records (Cam et al., 2002; Van de Pol and Verhulst 2006), we analyzed a cross-section of hormone samples across years from available population archives. Therefore, from 1692 individuals (11,360 captures) record, we selected a single sample from 152 females and 203 males, to include badgers of all ages 0-13. Had we focused only on longitudinal hormone profiles of longer-lived individuals, this could have failed to detect different hormone profile characteristics by excluding shorter-lived individuals and caused bias due to selective mortality (i.e., the residual surviving proportion of the population tends to be comprised of higher quality individuals; e.g. Nussey et al., 2011).

All sex steroid titres were analysed using microtitreplate Enzyme-immunoassays (EIA). Testosterone and oestrone were measured following the methodology described in Sugianto et al. (2018). The testosterone intra-assay coefficient of variation was 14.69% (high) and 6.18% (low), and inter-assay variation of high and low-value quality controls were 9.15%

(high) and 5.23% (low). The oestrone intra-assay coefficient of variation were 8.21% (high) and 6.05% (low); inter-assay variation of high and low-value quality controls were 13.96% (high) and 13.62% (low) respectively.

2.4 Statistical analysis

All data processing and statistical analyses were performed using RStudio (0.99.896) and R (R-3.2.4). Patterns of residuals, normality, and mean variance relationship for each model were checked using diagnostic plots in R to ensure that the models chosen were the best fit for the data.

2.4.1 Age-dependent reproductive declines in sex-steroid levels

In long lived mammals, age-dependent reproductive potential typically follows a bell-shaped curve (Jorgenson et al., 1997; Dugdale et al., 2011a). Sex-steroid hormones are likely to be related to this reproductive potential, and therefore we fitted a quadratic model (age^2) for testosterone and oestrone levels against age (males: 0-13 years, females: 0-12 years) during spring ($n_{\text{males}} = 67$; $n_{\text{females}} = 49$), summer ($n_{\text{males}} = 84$; $n_{\text{females}} = 60$), and autumn ($n_{\text{males}} = 52$; $n_{\text{females}} = 43$) to analyse trends in endocrinological senescence. To confirm that this relationship was indeed quadratic, as opposed to linear, we analyzed the significance of age compared to age^2 in relation to the hormone levels using the ANOVA function.

We then used the quadratic curves of sex-steroid levels during spring ($n_{\text{males}} = 67$; $n_{\text{females}} = 49$) to analyse differences in schedules of endocrinological senescence between males and females, referenced against standardised (towards peak-value/concentration) hormone levels (with oestrone in females and testosterone in males on different scales).

2.4.2 Age-dependent changes in seasonal sex-steroid patterns

Badgers are known to exhibit a distinct seasonal hormonal pattern relating to their reproductive behavior (Sugianto et al., 2018; Buesching et al., 2009). Therefore, we examined here for seasonal effects on age-group specific senescence patterns. Age groups for both males and females were defined based on quadratic models fitted to spring hormone data (May/June: i.e., peak mating season). Using the age where the curve reached its peak, we then defined the age groups against this reference as: Cubs < 1 year, Yearlings (Y): $1 \leq Y < 2$ years (which tended to have hormone levels $< 80\%$ of the adult peak), Young adults (YA): $2 \leq YA < 6$ years (hormone levels between 80-100% of the adult peak), Old adults (OA): $6 \leq$

OA < 9 years (hormone levels declining from 100-80% of adult peak), and Very old adults (VOA) (hormone levels < 80% of adult peak): ≥ 9 years (see Figure 1 and Table 1). The seasonal mean and standard deviation of hormone levels (for both sexes respectively) of each age class were then plotted to inspect for any apparent changes in reproductive strategies. The significance of the seasonal patterns for each age group was then analyzed using a linear model with hormone levels as response and season, age group and their interaction as parameters. As our focus was on differences between VOA hormone levels compared to other age groups, a-priori contrast tests were conducted between hormone levels of the VOA group against the other age groups for each season.

Table 1. Sample size of age classes (cub, yearling, young adult, old adult, and very old adult) during spring, summer, and autumn for males and females.

Season	Age Class	Sample size	
		Female	Male
Autumn	Cub	5	9
	Yearling	5	6
	Young adult	13	23
	Old adult	9	11
	Very old adult	11	3
Spring	Cub	7	10
	Yearling	5	9
	Young adult	16	20
	Old adult	10	17
	Very old adult	11	11
Summer	Cub	6	18
	Yearling	8	14
	Young adult	18	23
	Old adult	18	16
	Very old adult	10	13

Figure 1

2.4.3 Age-dependent declines in somatic condition and reproductive advertisement

To investigate trends in somatic senescence, we analyzed metrics of BCI, body condition, and subcaudal gland secretion volume against age (years) separately in spring, summer, and autumn (Table 2) using GAM (Generalized Additive Model) models to fit smoothing

functions (quadratic models were not used because there was no indication, unlike in hormones, that these parameters showed a quadratic response. Here samples sizes were greater, taken directly from the long-term population database, without the constraint of laboratory analysis of samples.

Table 2. Sample sizes of BCI, subcaudal gland volume, and body condition in both sexes during spring summer and autumn.

Somatic parameter	Season	Sample Size	
		Males	Females
BCI	spring	703	869
	summer	687	744
	autumn	422	556
Subcaudal gland secretion volume	spring	535	723
	summer	475	559
	autumn	294	451
Body condition	spring	652	813
	summer	559	513
	autumn	360	513

3. Results

3.1 Endocrinological senescence

3.1.1 Males

For males, we found a significant concave down quadratic (age^2) relation between testosterone levels and age in spring (age term : $F_{1,64} = 4.89$, $p = 0.031$; age^2 term : $F_{1,64} = 12.16$, $p < 0.001$; Fig. 2a) and summer (age term : $F_{1,81} = 1.89$, $p = 0.172$; age^2 term : $F_{1,81} = 13.85$, $p < 0.001$; Fig. 2b). Levels initially increasing from cubs to adulthood, then decreased beyond hormone peak. In autumn no significant relationship between testosterone levels and age or age^2 was found (age term : $F_{1,49} = 0.15$, $p = 0.705$; age^2 term : $F_{1,49} = 1.82$, $p = 0.183$; Fig. 2c), most likely due to a reproductive quiescence in the autumn.

Figure 2

Figure 3 shows the seasonal testosterone patterns of each age group. A significant relationship between testosterone and season was found for all age groups (season term: $F_{2,188}$

= 9.92, $p < 0.001$). Testosterone levels were highest in spring, declining through summer and autumn (see Buesching et al., 2009) for all age groups except for pre-pubescent cubs (for which testosterone levels increased from spring to adult level by autumn: Sugianto et al., 2019). Although the VOA age group had lower mean testosterone levels, a-priori contrast tests in spring and summer (autumn not tested due to no apparent age relationship) indicated this difference between VOA testosterone was not significant versus other age groups (cubs, Y, YA, OA), due to high inter-individual variation (especially in spring, Fig.3).

Figure 3

3.1.2 Females

In females, we also found a significant concave down quadratic (age^2) relationship between oestrone levels and age in spring (age term : $F_{1,46} = 0.37$, $p = 0.544$; age^2 term : $F_{1,46} = 16.02$, $p < 0.001$; Fig. 4a) and autumn (age term : $F_{1,40} = 2.23$, $p = 0.144$; age^2 term : $F_{1,40} = 37.45$, $p < 0.001$; Fig. 4c). Levels increased from cubs to adulthood and then decreased beyond their hormonal peak. In summer no significant relationship was found between oestrone levels and age or age^2 (age term : $F_{1,57} = 0.002$, $p = 0.963$; age^2 term : $F_{1,57} = 0.22$, $p = 0.642$; Fig. 4b), as oestrone levels are known to decline during this period (Sugianto et al., 2018; Sugianto et al., *subm.*).

Figure 4

Figure 5 shows the seasonal oestrone patterns across age groups. A significant relationship between oestrone and season was apparent (season term : $F_{2,188} = 9.92$, $p < 0.001$) where a typical seasonal pattern (high in spring, low in summer, high again in autumn; Sugianto et al., 2018; Sugianto et al., *subm.*) was evident for all age groups except for pre-pubescent cubs (hormone levels increased from pre-pubescent in spring to adult levels in autumn; Sugianto et al., 2019). A-priori tests in spring and autumn (summer not tested due to no age relationship) indicated a significant difference between VOA oestrone levels versus YA and OA age groups in autumn (YA : estimate = 9.74, std. error = 4.76, $t_{\text{value}} = 2.04$, $p = 0.048$; OA : estimate = 13.40, std. error = 5.35, $t_{\text{value}} = 2.51$, $p = 0.017$); no significant difference was seen in spring due to large inter-individual variation of hormone levels (similar to males, Fig.3).

Figure 5

3.1.3 Age-dependent male and female hormonal decline after reproductive prime age

Both males and females exhibited similar standardized hormone profiles (quadratic concave down) in spring, with sex-steroid levels highest during the main mating season. Females reached their reproductive peak/ prime-age at 5.5 years, slightly earlier than males, which peaked at 6 years (Fig. 6). Hereafter, both sexes showed similar schedules of hormonal decline indicating reproductive senescence.

Figure 6

3.2 Age-dependent somatic decline

We also identified a gradual post-maturity reduction in BCI and body condition as badgers became older, for both sexes in all seasons. The GAM model, testing BCI values as a smoothed function of age, revealed that BCI (Fig. 7) started to decline after age 3 years, but more markedly after age 5 years. Decline for both sexes was most evident in autumn, less so in summer and weakest in spring (Fig. 7).

Figure 7

This gradual reduction in somatic condition for both sexes with age in all seasons was further corroborated by our simpler metric of body condition, with onset starting at age 3 years, but with a steeper rate of decline beyond age 5 years (Fig. 8).

Figure 8

3.3 Age-dependent reduction in subcaudal gland volume as proxy of reproductive advertisement

Subcaudal gland volume underwent a gradual decline with age, especially after 5 years of age, for both sexes in all seasons, but still followed broad typical changes in seasonal volume patterns (Fig. 9). The negative trend was more abrupt in males, while females underwent a more gradual transition, as shown in the GAM analyses (Fig. 9).

Figure 9

4. Discussion

4.1 Age-dependent declines in sex-steroid levels

From our endocrinological analyses, we provide strong evidence for a gradual, but not abrupt, decline in sex-steroid levels with age. We found a significant concave (down) quadratic relationship between age and sex-steroid levels in both sexes, indicating a decline in hormone levels as individuals aged. This decline started at 6 and 5.5 years respectively for males and females. In both sexes, the VOA age group tended to have lower mean hormone levels; however this was only significant for oestrone levels in autumn compared to the prime reproductive age group (YA and OA).

The function of oestrone in badgers is to sustain pre-implanted blastocysts during delayed implantation (Thom et al., 2004; Yamaguchi et al., 2006; Sugianto et al., *subm.*). Therefore lower autumnal oesterone levels among VOA females would likely be insufficient to sustain blastocysts until winter implantation (in which case blastocysts are reabsorbed) (Sugianto et al., *subm.*).

During the main spring mating season VOA badgers of both sexes had higher inter-individual hormone level variation when compared to all other age groups (ranging from adult to cub levels), causing non-significant apriori results. A closer inspection of this variability among VOA males (11 individuals, ≥ 9 years of age) revealed that 45.5% (5/11 sampled) maintained testosterone levels within 80% of peak production (2.2 ng/ml peak), while levels dropped below 80% to pre-pubescent levels (i.e., < 1.76 ng/ml) in the remaining 54.5% (6/11 sampled). Similarly, among VOA females (11 individuals, ≥ 9 years of age) a proportion (36.4%: 4/11 sampled) maintained adult hormone levels (>70 pg/ml), while oestrone levels had dropped to pre-pubescent levels (<70 pg/ml) in the other 63.6% (7/11 sampled). The quadratic sex steroid curves (Fig 2. and 4) illustrate that normal adult oestrone levels of >70 pg/ml only occur in the youngest individuals of the VOA age group and are no longer found in older VOA individuals (aged > 10), explaining the significant quadratic decline with age. This implies divergent reproductive strategies among individuals surviving until old age (Buesching et al., 2009).

4.2 Age-dependent declines in somatic condition and reproductive advertisement relative to reproductive senescence.

For both sexes, the onset of decline in somatic condition commenced earlier, starting at age 3 years, than any decline in reproductive hormones, starting at age 5-6 years; congruent with general trends in other mammal species (Berube et al., 1999; Mysterud et al., 2002). We thus observed that both sexes exemplify heterochrony between rates of somatic and hormonal senescence (Berube et al., 1999; Mysterud et al., 2002; Schwartz et al., 2003; Cohen 2004; Nussey et al., 2008).

We also observed a decrease in subcaudal volume with age, suggesting that investment in reproductive advertisement (Buesching and Macdonald 2001; Buesching et al., 2003) also decreases. This was most evident among badgers older than 5 years, and aligned with the decline in hormone levels for both sexes in all seasons. In general, this decrease was more abrupt in males, and more gradual in females, especially during the spring (i.e., the peak in female production of subcaudal gland secretion) and summer when both sexes decrease production in preparation for autumn reproductive quiescence (Buesching et al., 2002a). Similar declines in sexual advertisement with age can be seen in the curvilinear decline of antler growth linked to dominance status and reproductive success in male red deer (*Cervus elaphus*; Kruuk et al., 2002) and white-tailed deer (*Odocoileus virginianus*; Scribner et al., 1989). Similarly, older male house mice (*Mus musculus domesticus*) produce lower concentrations of involatile signaling proteins in their urine (MUPs), which serve in mate attraction (Garratt et al., 2011).

These age-related declines in hormonal levels (5-6 years) and body-condition (3 years) were highly synchronous between males and females (although with different offspring assignment implications, below). This is contrary to most studies in polygynous mammals, where males typically senesce earlier than females, and thus realize shorter lifespans (Metcalf and Monaghan 2003), leading to more rapid male mortality rates (Clutton-brock and Isvaran 2007; Nussey et al., 2009), as seen in studies of black tailed prairie dogs *Cynomys ludovicianus*, red deer *Cervus elaphus*, and African lions *Panthera leo* (see Clutton-Brock and Isvaran 2007)

In our study population, however, there is no significant sex-bias in badger mortality rate (Macdonald and Newman 2002; Macdonald et al., 2009), according with the similar

senescence rates we report between sexes. Due to the badger's polygynandrous mating system, combined with the fact that females undergo delayed implantation and superfoetation (potentially giving a longer mating window; see Yamaguchi et al., 2006; Corner et al., 2015; Sugianto et al., *subm.*), and low rates of inter-group dispersal (19.1%; see Macdonald et al., 2008), we propose that sexual selection may not be as severe as in polygynous mating systems, leading to similar (somatic and hormonal) senescence schedules between the sexes.

4.3 Changes in reproductive success with age

Analysis of maternal assignments, through genetic pedigree for this same population established that fewer older females were assigned cubs, starting at age 3 years (Dugdale et al., 2011a). This is earlier than the hormonal decline we identified, evident from age 5.5 years onwards in females (age attained by 31.8% of individual females born, or 55.8% of those attaining sexual maturity), implying some decoupling between potential and actual reproduction. Reduced offspring assignment with age was, however, more coincident with the onset of somatic decline in females, also occurring around age 3 years. This was supported by the a-priori analysis of seasonal oestrone patterns (Fig. 5), where levels were significantly lower in autumn among VOA females, indicating a reduced capacity to retain and implant blastocysts compared to younger age groups (YA and OA). This was likely due to insufficient body condition to maintain blastocysts after summer until implantation in winter (Sugianto et al., *subm.*).

A similar decline in the number of offspring assignments was evident among males, but commenced later than in females, at age 5 - 6 years (Dugdale et al., 2011a); concomitant with the onset of testosterone decline at age 6 years (age attained by 20.9% of individual males born, or 37.8% of those attaining sexual maturity; Bright Ross pers. comm.). Males exhibited a similar age of onset of somatic decline to females (3 years), showing that declining body condition does not preclude reproductive success in males (no 'restraint') in advance of hormonal senescence ('constraint'; see Dugdale et al., 2011a).

4.4 Occurrence of RC and PRLS in badger population

For males, increasing testosterone levels among cubs (see Fig. 5) initiate sperm production during puberty (Adkins-Regan 2005). Thereafter, it appears that some sperm production can persist throughout the lifetime of individuals (Vom Saal and Finch 1988; Cohen 2004) even though testosterone levels decrease with age – evidenced by the fact that some male badgers

have been assigned parentage up to the age of 13 years (Dugdale et al., 2011a). Importantly, we observed a much greater inter-individual variation in testosterone levels as male badgers advanced into very old age (≥ 9 years; Figure 3). This is consistent with Buesching et al. (2009), who report the existence of two endocrinological phenotypes among older males in this same population.

Cross-referencing testosterone and pedigree data, among the 5 high testosterone VOA individuals we sampled, 2 (= 40%) sired cubs, while only 16.7% (1 of 6) low testosterone VOA male sired a cub. This indicates that, contrary to previous interpretations (Woodroffe et al., 1997; Buesching et al., 2009), a decline in testosterone levels with age does not necessarily exclude males from siring cubs (as sperm is still produced; see Vom Saal and Finch 1988; Adkins-Regan 2005). This flexibility may permit a small proportion of males to make a terminal investment (Clutton-Brock 1984) in late life reproduction. Simultaneously, 3 of these 5 VOA high testosterone males were not assigned offspring, indicating that high testosterone does not necessarily lead to breeding success in older males (indicating constraint), or that they mated with inferior females that did not produce cubs that year. In combination, these observations suggest that complex reproductive performance factors are involved in achieving breeding success for males, such as how testosterone influences mating effort, reproductive advertisement, roaming, sperm quantity and quality, and not simply absolute fertility (Cohen 2004; Møller et al., 2009; Clutton-Brock and Isvaran 2007).

While low testosterone may impair spermatogenesis, it is not extinguished (Adkins-Regan 2005). In contrast, below a certain threshold low oestrone will cause an absolute failure to ovulate in females, and thus total loss of potential fitness (Adkins-Regan 2005; Packer et al., 1998; Cohen 2004). Figures 4a and 4c illustrate that in female cubs oestrone levels develop through ca. 40 pg/ml in spring and 60 pg/ml in autumn, until puberty at ca. 11 months (Sugianto et al., 2019). This infers that oestrus and ovulation are not possible until levels reach ca. 70 pg/ml; this being the minimum age at which conception is apparent in very few individuals according to pedigree (subject to delayed implantation). Applying this 70 pg/ml cut off on the older side of quadratic curve, suggests that the majority (7 of 11 individuals aged 9-12) of older female badgers undergo a decline in oestrone consistent with functional menopause at around age 9 years. However, older females also exhibited more heterogeneity in their hormone levels than younger females, and we identified that 4 of 11 individuals aged 9-12 maintained oestrone levels above the 70 pg/ml threshold during spring. This indicates

that some very old females may not experience breeding constraint and, as per males (Buesching et al., 2009), suggests two reproductive phenotypes. This supports previous findings that very old female badgers can still be assigned parentage, although much more rarely than younger females (with 1 female badger having been assigned offspring at up to 11 years old; Dugdale et al., 2011a). From our data set, only 1 of 4 very old females with high oestrone levels, gave birth to a single cub, while for the remaining 7 very old females with low oestrone levels, none gave birth to cubs, evidencing RC, with an average PRLS of ca. 2.59 ± 1.29 years (range 1.25-4.75 years). Potentially, a proportion of even those high-oesterone phenotype very old females may be constrained by senescence in body-condition, which is known to exert a major restraint on embryonic implantation, delayed implantation and pregnancy in badgers (Woodroffe and Macdonald 1995a; see also Kirkwood and Austad 2000; Myrsterud et al., 2001). Similar somatic, rather than hormonal, limitations on reproduction affect older bighorn ewes, where low body-mass individuals that are less able to ovulate and conceive (Berube et al., 1999); and reproductive fecundity also correlates significantly with body fat in older female Eurasian beavers (*Castor fiber*; Parker et al., 2017).

To understand the proportion of the population that potentially experiences PRLS, we used cub assignment data from Dugdale et. al. (2011a) and survival data from Macdonald et al. (2009) of the same population to calculate the post-reproductive representation (PrR, using the method by Levitis et al., 2011). We found the the age of first reproduction to be 2 years (54.0% cohort survival), the age of last reproduction as 13 years (0.8% cohort survival) in males and 12 years (6.7% cohort survival) in females, with a maximum life expectancy of 14 years in both sexes. The obtained PrR for males was 0.032 ($p = 0.750$) and 0.058 ($p = 0.708$) for females. This indicates that only a small and insignificant portion of any cohort will experience PRLS. The higher PrR for females also supports that females have a definite limit of RC (minimum hormone level) while males do not.

4.5 Support for hypotheses explaining reproductive senescence

The difficulties involved in collecting data from wild populations, combined with the ever-diminishing proportion of cohorts surviving to older age (Nussey et al., 2008), have resulted in reproductive senescence once being considered a rare phenomenon in nature (Comfort 1979). Gradually, however, studies are revealing that a (small) proportion of various mammal

species experience RC and PRLS (see Cohen 2004), although data are particularly lacking for the Carnivora.

Nevertheless, there are difficulties in arguing for PRLS under natural selection. Non-adaptive hypotheses dismiss PRLS as simply a proportion of a population merely being lucky enough to live beyond RC (Peccei 2001), arising as an epi-phenomenon of antagonistic pleiotropy favouring early life fertility (Wood et al., 2001), where PRLS provides an insurance against the risk of dying by chance prior to RC (Tully and Lambert 2011). This would fit badger biology because early-life mortality rates can be high and vary substantially inter-annually (Macdonald and Newman 2002; Macdonald et al. 2009), and thus the contribution to population fertility made by older (and thus likely more experienced) individuals can be important.

Alternatively, under selection where older males secure more matings and offspring (the Patriarch hypothesis: Marlowe 2000), the inseparability of polygenic factors would also result in the extended female lifespan, subsequently experiencing PRLS (Tuljapurkar et al., 2007). This is less plausible for badgers, given that male mating success (but not mounting success) is linked positively with body condition (Dugdale et al., 2011b), where older males show a lower BCI beyond age 3, and older males are assigned fewer, rather than more offspring (Dugdale et al., 2011a).

Cant and Johnston's (2008) adaptive kinship dynamics hypothesis proposes that older females should stop breeding when females of the next generation start to breed. This also does not fit badger senescence patterns well because (i) prime-age female badgers are able to suppress the reproductive capacity of younger females (Woodroffe and Macdonald 1995b), (ii) all females mate but only ca. 45.2% are assigned offspring, per year (Annavi et al., 2014), and (iii) younger females allo-mark prime-age (but not old) females with subcaudal secretion (Buesching et al., 2003). Furthermore, high rates of inter-group visits and extra-group paternity obscure the linearity of intra-sex breeding competition (Macdonald et al., 2008).

Unlike in other group-living carnivores (Macdonald and Moehlman 1982), allo-maternal care is also not a prominent feature of badger society (Fell et al., 2006); no food is provisioned to weaned cubs, and thus helpers provide no detectable benefits (Woodroffe and Macdonald 2000; but see Dugdale et al., 2010). This offers no support for either the 'mother' hypothesis'

(Williams 1957), which proposes that females that terminate reproduction in mid-life gain fitness advantages by investing in previous offspring; or the ‘grandmother hypothesis’ (Hawkes et al., 1998), which proposes that post-reproductive females can increase their inclusive fitness by supporting weaned grand-offspring. Furthermore, despite inter-generational overlap (generation length for this population = 3.09 years; Macdonald and Newman 2002), Cant and Johnstone’s (2008) inter-generational Reproductive Conflict Hypothesis (proposing RC through the segregation of reproductive generations) receives little support due to high natal philopatry and low levels of dispersal leading to high inbreeding coefficients in badgers ($f = 0.010$; Annavi et al., 2014) and high inter-generational relatedness. In classical terms, neither does Peccei’s (1995) altriciality-lifespan hypothesis fit badger biology; but noting that due to induced ovulation and delayed implantation (Macdonald et al., 2017) reproduction represents a substantial component of a female’s time and energy budgets, where early life investments may be linked to later-life RC.

Therefore, perhaps the most parsimonious explanation for RC in badgers – congruent with a general thesis that sociality in badgers is facultative and linked to resource dispersion, not altruism (Macdonald et al., 2015) – is that by ceasing to reproduce, females escape the increased risk of mortality associated with late-life pregnancy, and both sexes escape the demands that maintaining reproductive condition places on somatic condition (Penn and Smith 2007).

5. Conclusion

We demonstrate for the first time an age-related decline in reproductive hormone profiles in a wild-living population of European badgers, and a heterochronic decline in somatic condition, leading to RC, PRLS and PrR, especially in females. With regard to differential rates of reproductive senescence between the sexes, it is important to consider that germ cell production is less tightly coupled to hormonal variations in males than it is in females (Adkins-Regan 2005), causing differences in the potential influence of an age-related decline in sex-steroid levels on reproductive fitness (see Penn and Smith 2007).

Ability to reproduce into old age can enhance individual lifetime reproductive success significantly (Merilä and Sheldon 2000). In capital breeders (Festa-Bianchet et al., 1998), this can relate to the ability to accrue, or continue to be able to accrue resources necessary for late-life reproductive effort (e.g., Campbell et al., 2017). The extent to which late-life breeding contributes to population demographics depends, however, on age-specific survival.

In this badger population c. 90.2% badgers die before reaching the age of 9 years (Bright Ross pers. comm.), thus only 9.8% of badgers born will ever be in a position to experience RC (or continue to breed). Consequently, while we evidence that PRLS can occur in badgers, it is still subject to substantial negative selection pressure (Cohen 2004; Baudisch 2005), where the low post-reproductive representation (PrR; Levitis et al., 2011) of senescent individuals frees resources for more productive younger badgers. Conversely, juvenile mortality is often very high in badgers (Macdonald and Newman 2002; Macdonald et al., 2009, peaking at 48.5% in 2014; cohort size = 66; Bright Ross pers comm.), linked to parasitoses and slow immune development (see Macdonald et al. 2015), and thus there may be heterochronic advantages for a proportion of older individuals to retain breeding capacity to ensure population continuity if substantial failures occur in younger cohorts under adverse inter-annual conditions (Promislow and Harvey 1990); that is, to provide insurance against indeterminacy (Tully and Lambert 2011).

Declarations of interest: none

Acknowledgements

We gratefully acknowledge the long-term support of the People's Trust for Endangered Species (PTES) for the Wytham Badger Project. NAS was supported by a DPhil scholarship from LPDP-Indonesia (Indonesia Endowment for Education) 2014-2018, and CDB was supported by a Research Fellowship from the Poleberry Foundation. Hormone levels were measured by Chester Zoo Laboratory, Chester, UK. All trapping and sampling for this study was carried out under the appropriate UK Home Office (PPL: 30/3379) and Natural England licenses (2018-34017-SCI-SCI) and approved by the University of Oxford's Ethical Committee.

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Figure Captions

Figure 1. Classification of age classes based on the hormone quadratic curve.

Figure 2. Quadratic curves of testosterone (ng/ml) against age in males during spring (a), summer (b), and autumn (c). Blue line represents quadratic model mean while grey area represents 95% confidence interval.

Figure 3. Seasonal mean testosterone levels (ng/ml) and standard deviation of each age class.

Figure 4. Quadratic curves of oestrone (pg/ml) against age in females during spring (a), summer (b), and autumn (c). Blue line represents quadratic model mean while grey area represents 95% confidence interval.

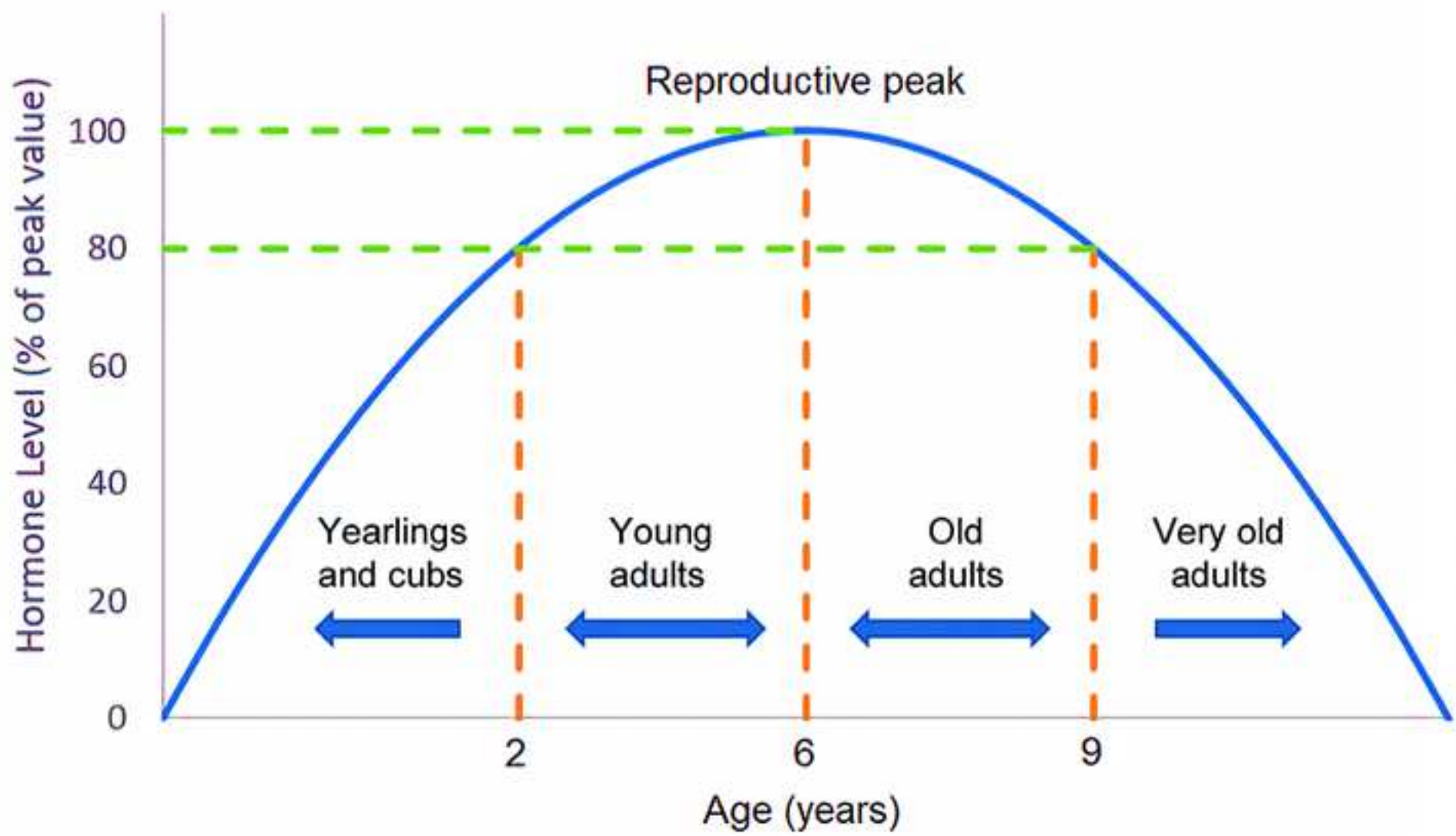
Figure 5. Seasonal mean oestrone levels (pg/ml) and standard deviation of each age class.

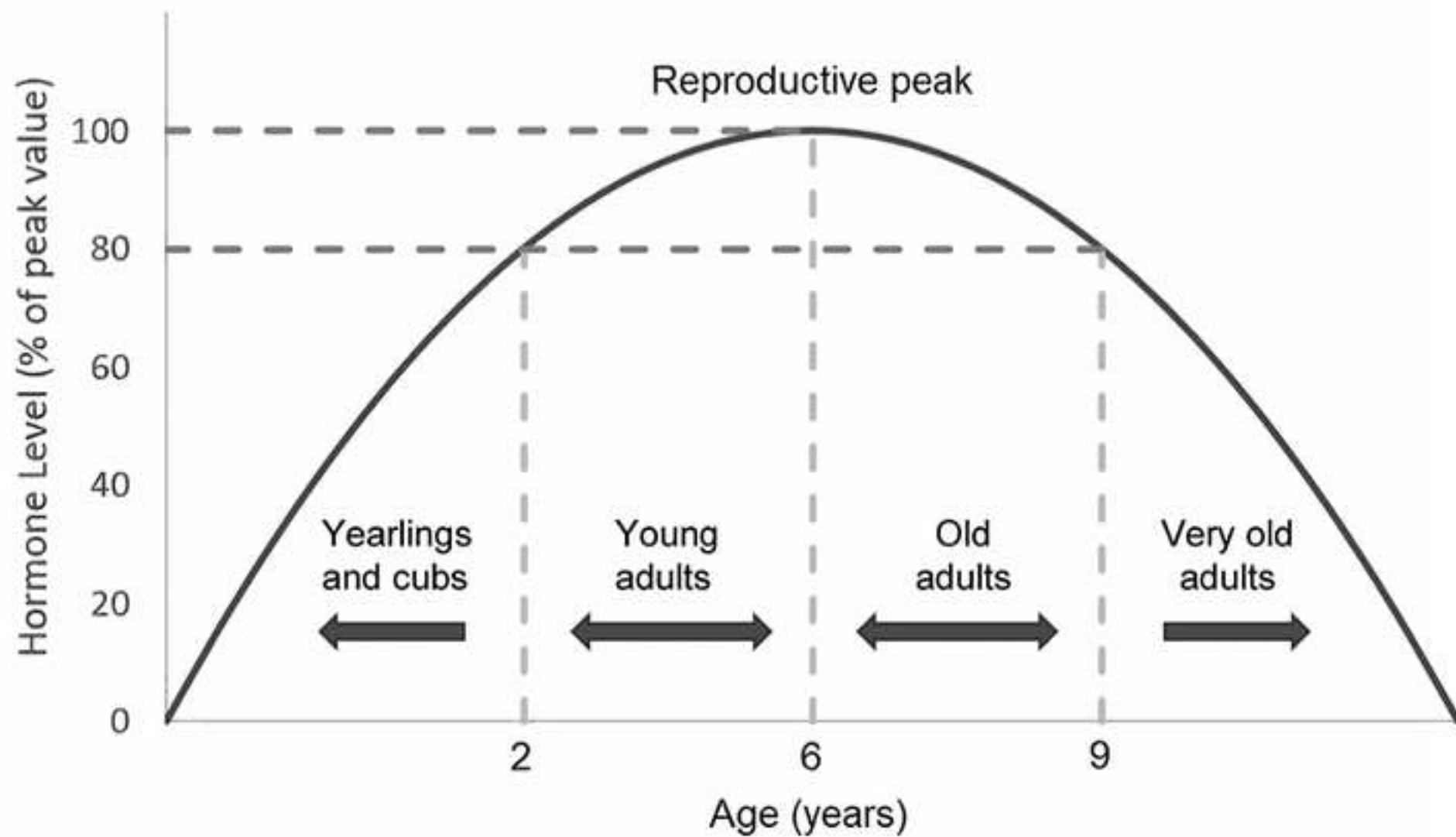
Figure 6. Comparison of standardised male and female quadratic hormone curves during spring. Solid line represents mean hormone level and dotted line represents 95% confidence interval. Standardisation conducted towards peak mean hormone level of each sex.

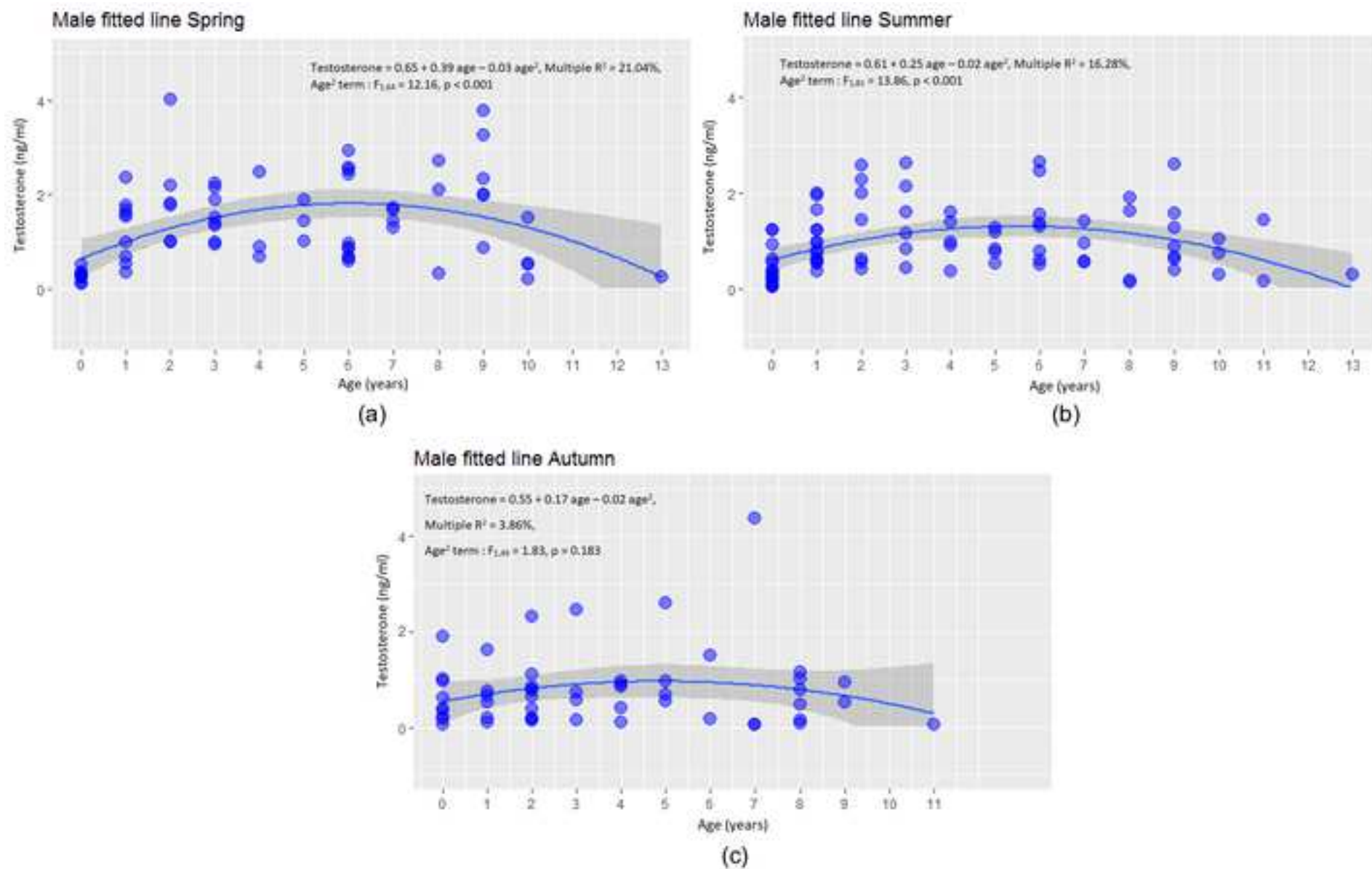
Figure 7. Body condition index (BCI) against age in males and females during spring (a), summer (b), and autumn (c). GAM model average shows the trend line for BCI against age (solid line) and the confidence interval (grey area) using a smoothing function.

Figure 8. Body condition score against age in males and females during spring (a), summer (b), and autumn (c). GAM model average depicts trend line for body condition score against age (solid line) and the confidence interval (grey area) using a smoothing function.

Figure 9. Subcaudal gland volume (ml) against age in males and females during spring (a), summer (b), and autumn (c). GAM model average depicts trend line for subcaudal volume against age (solid line) and the confidence interval (grey area) using a smoothing function.







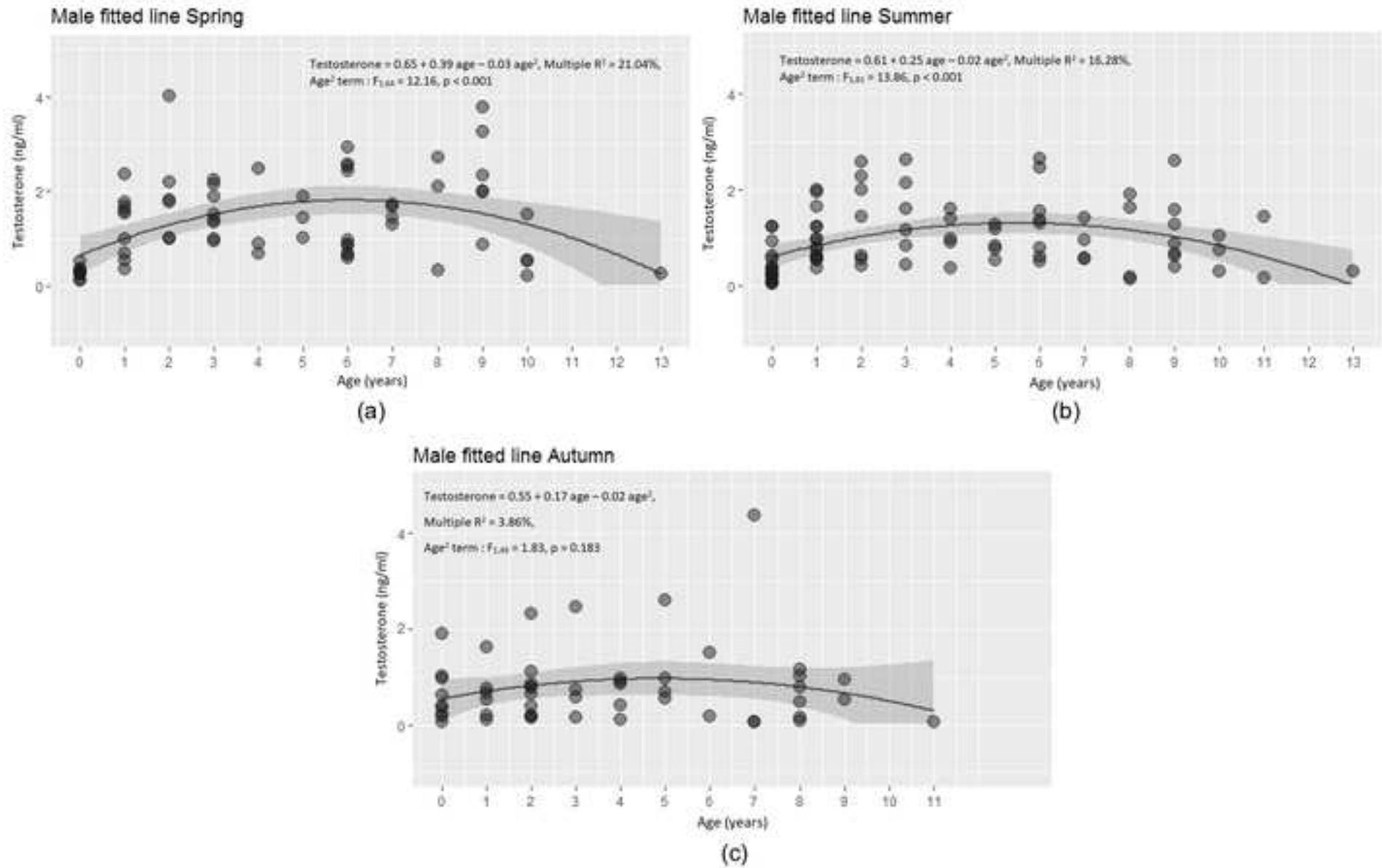
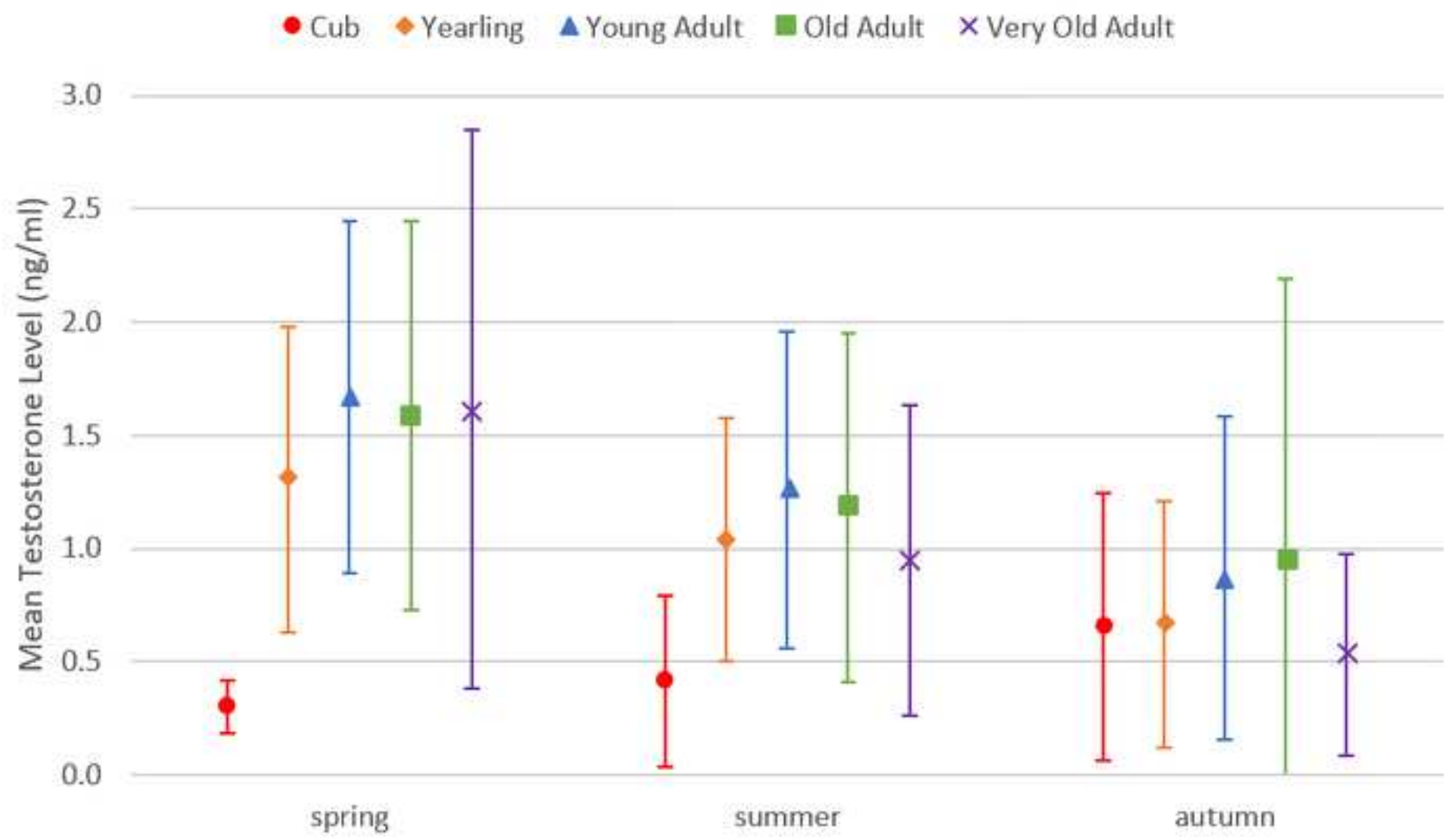
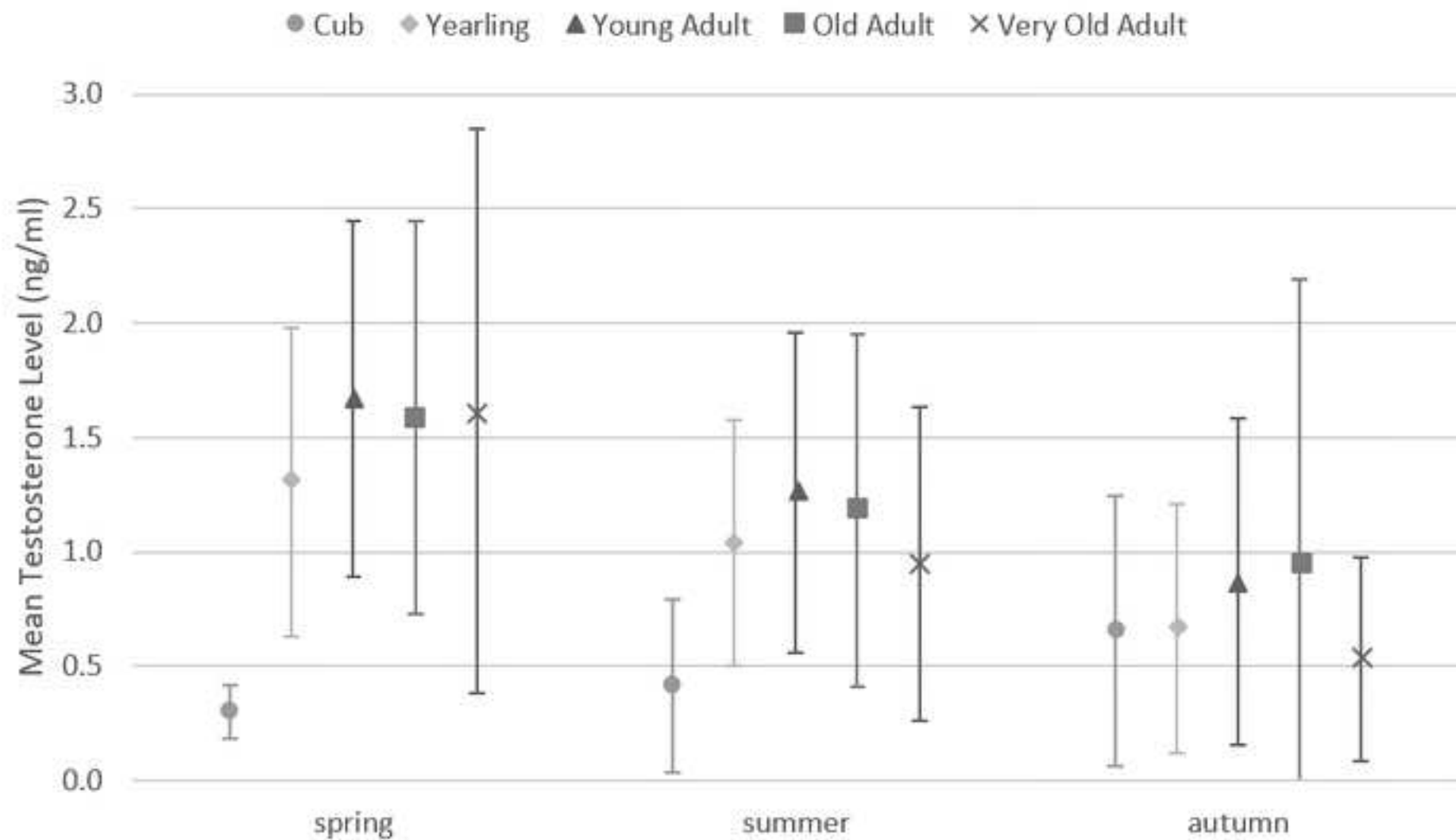
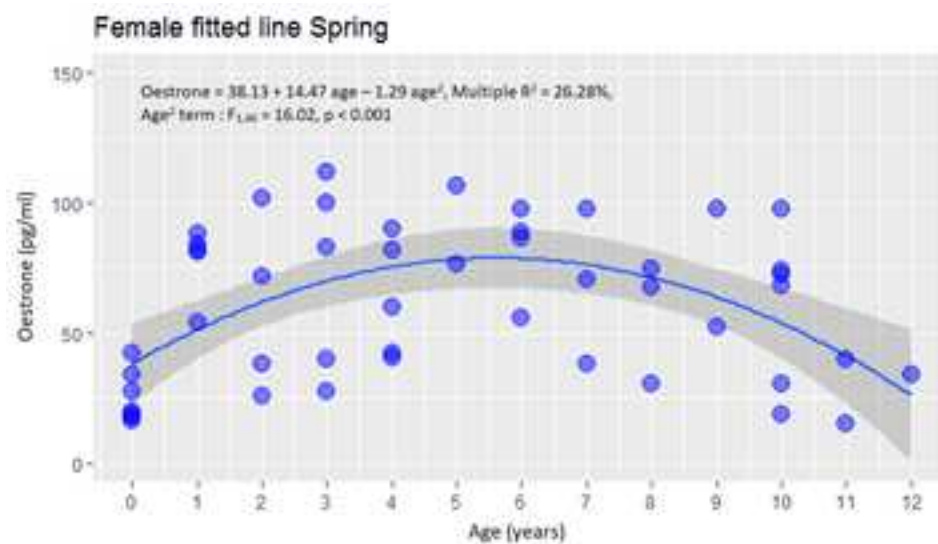


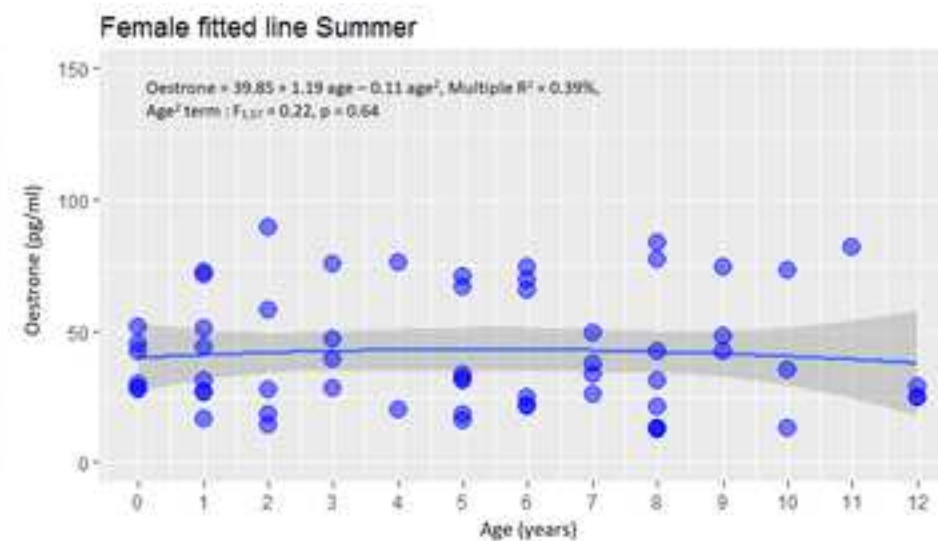
Figure 3 (colour)



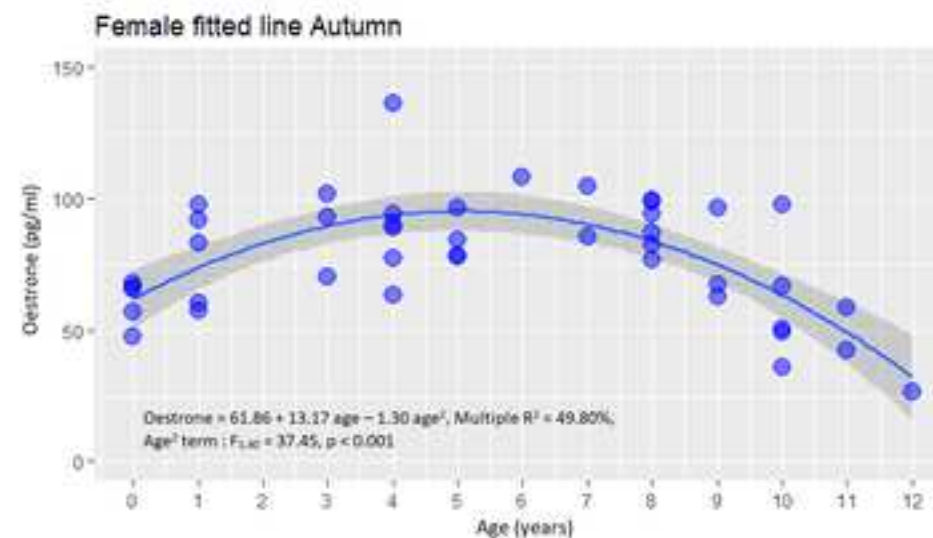




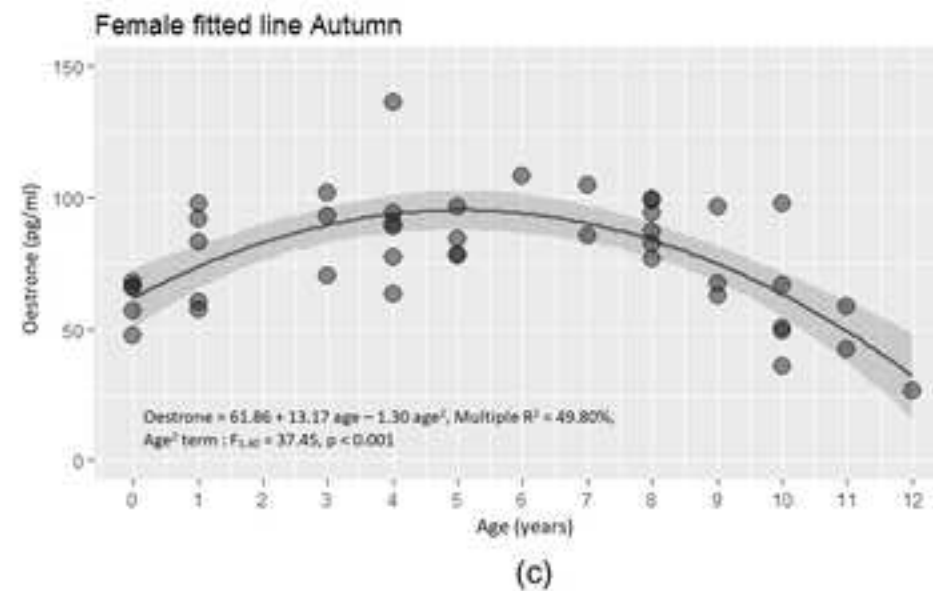
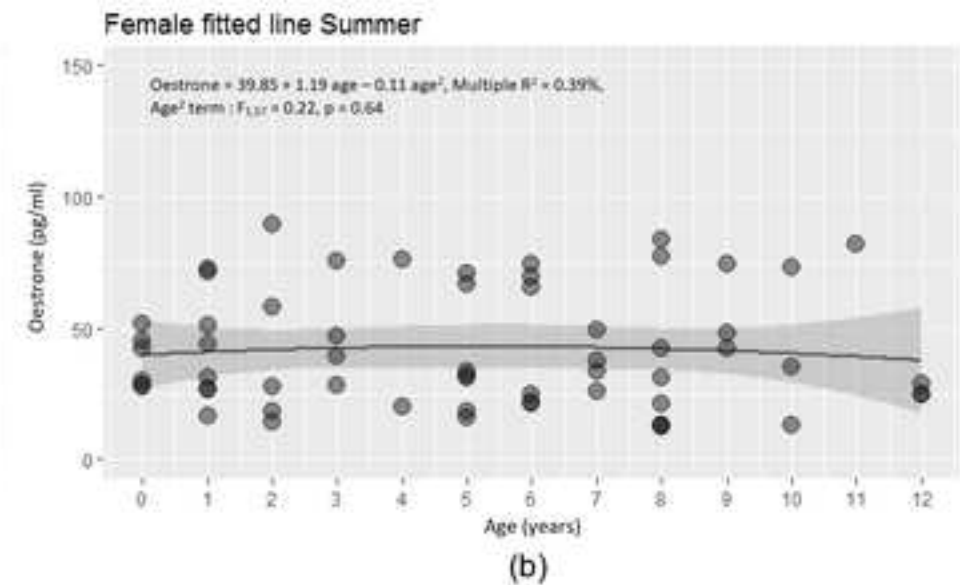
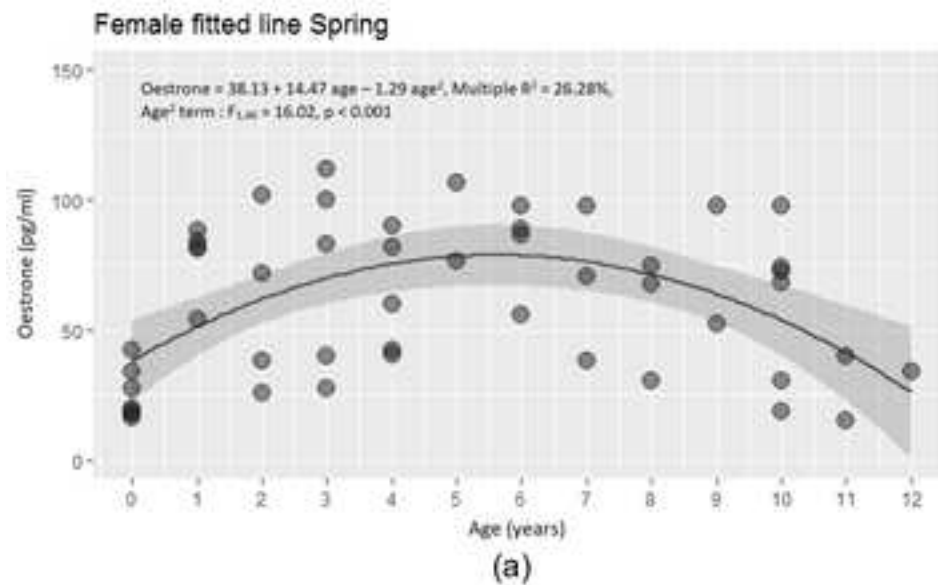
(a)

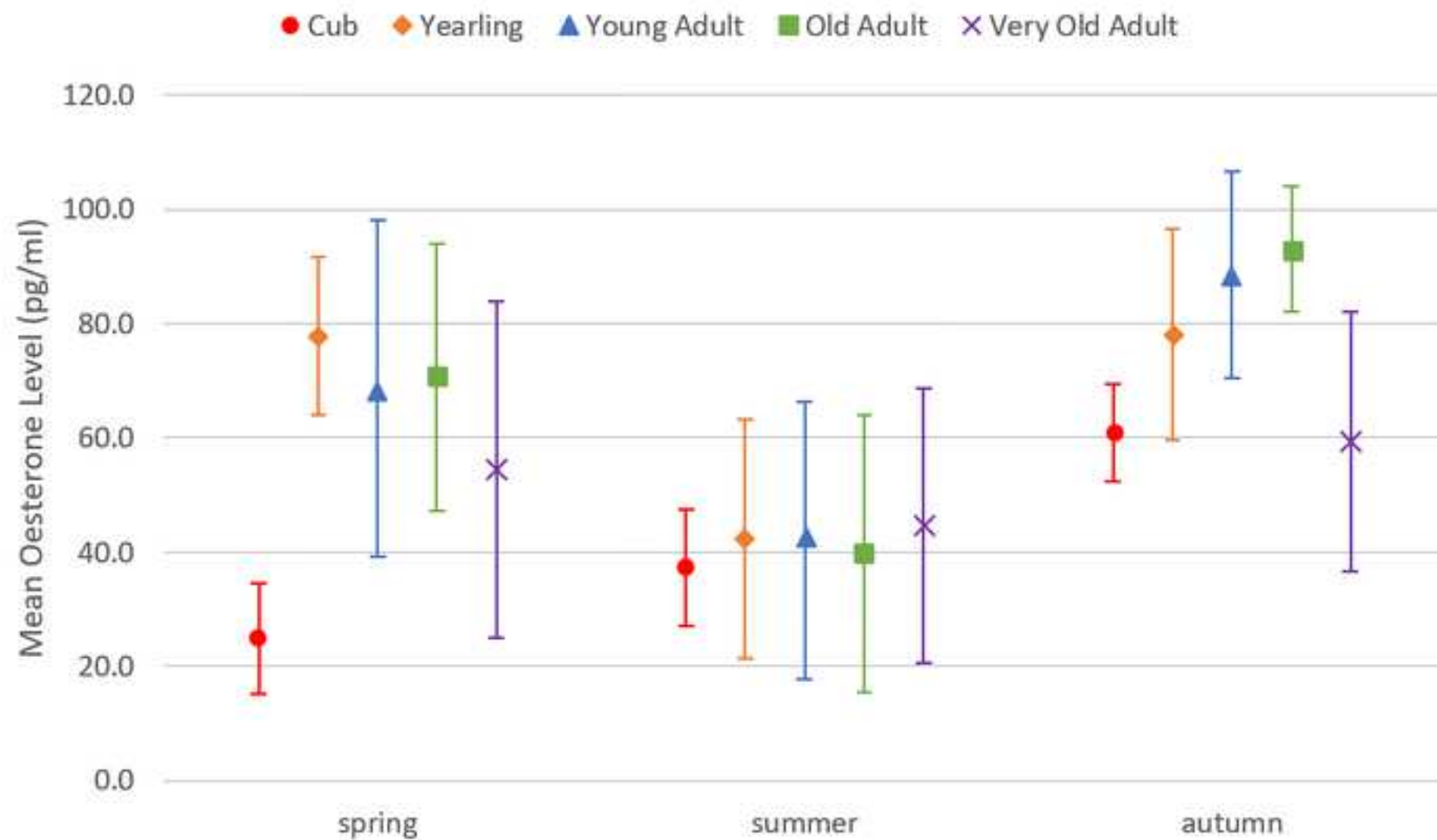


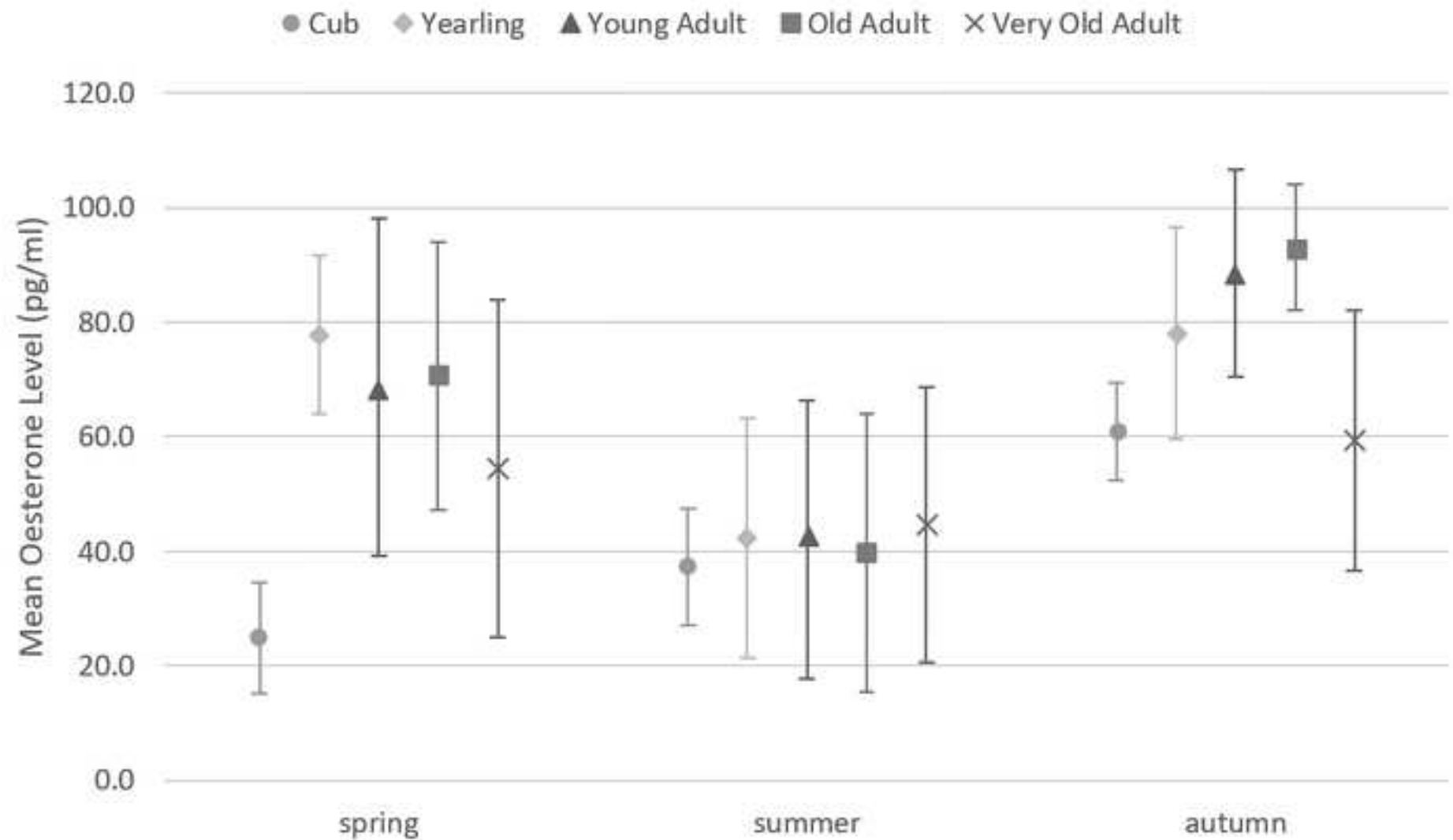
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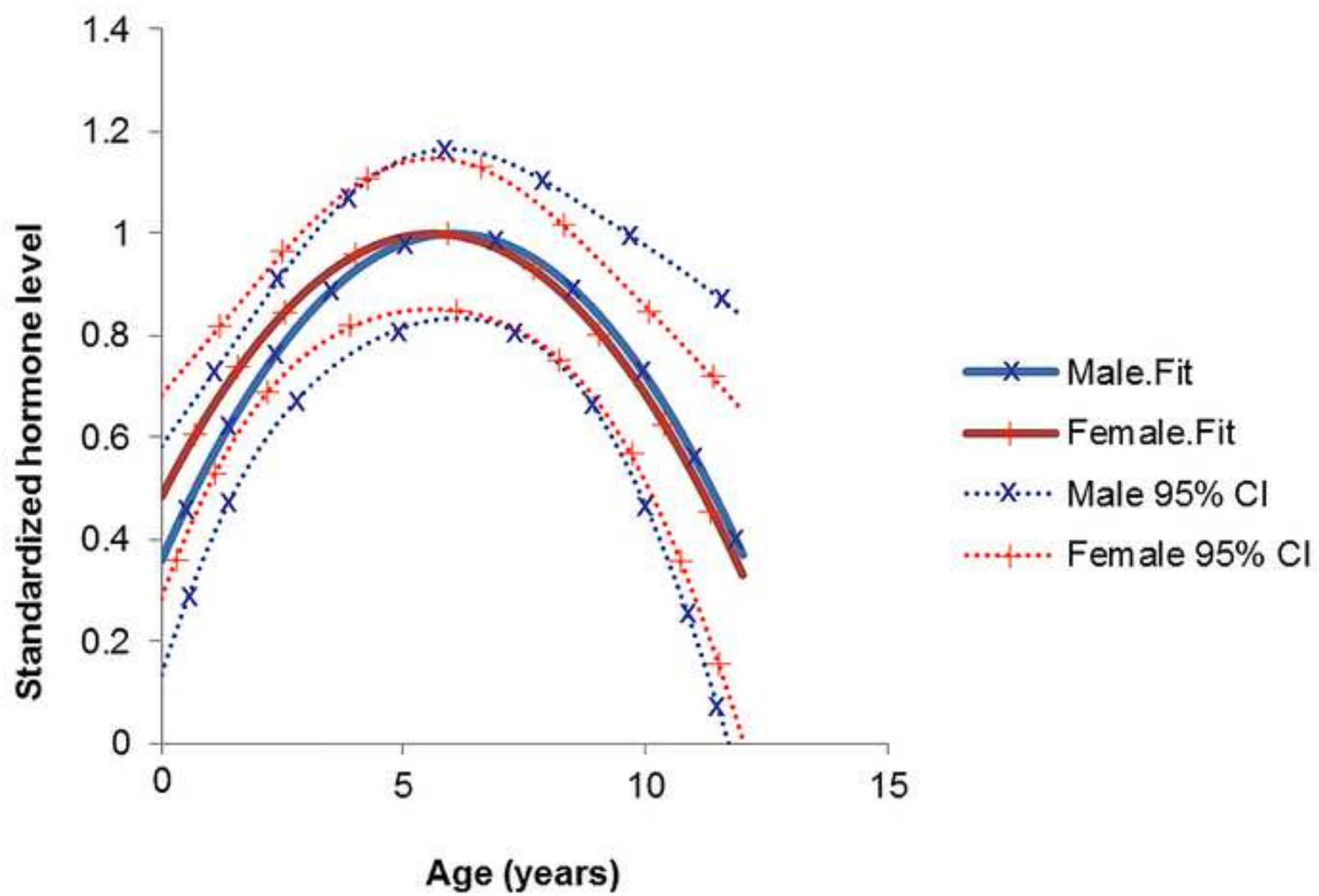


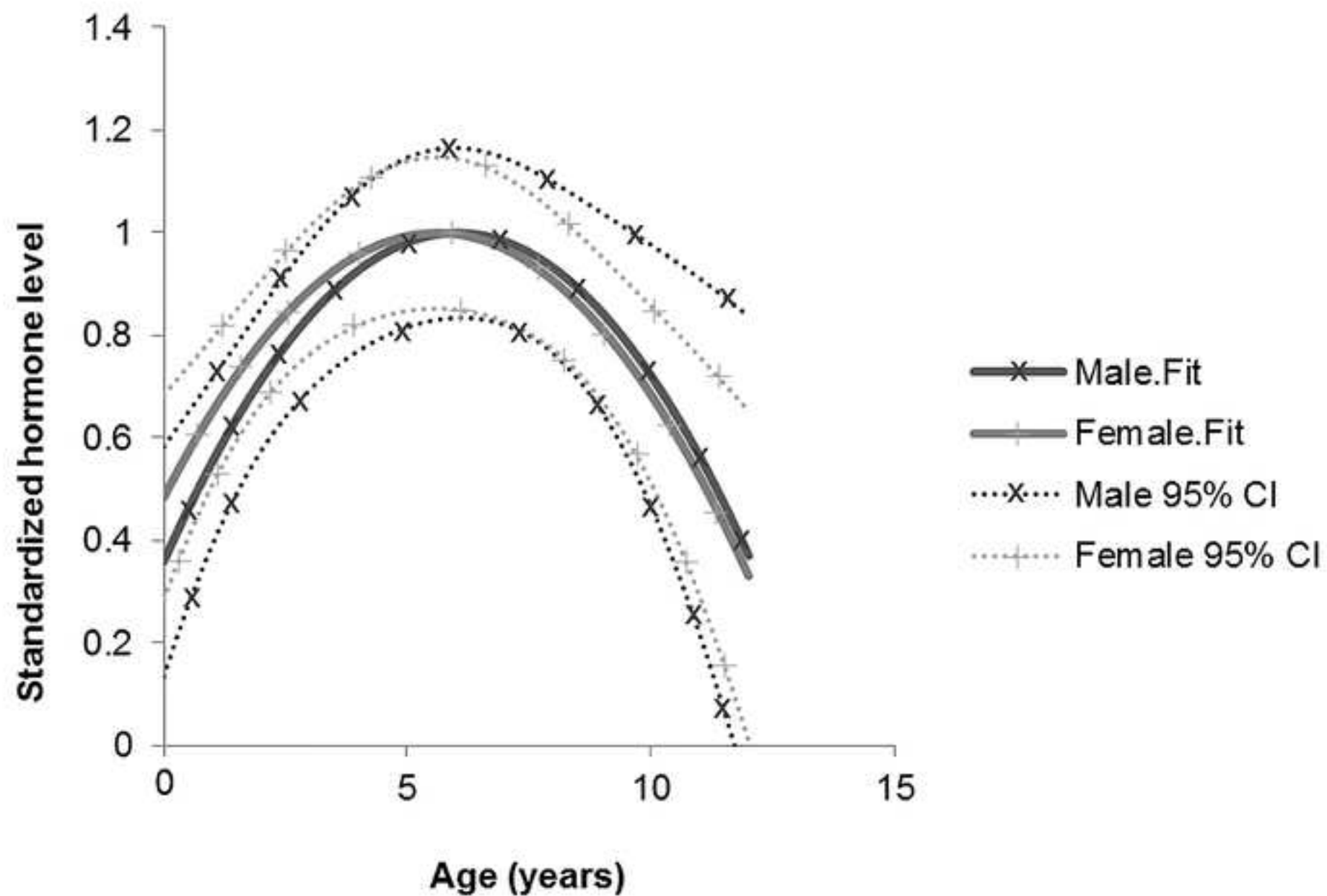
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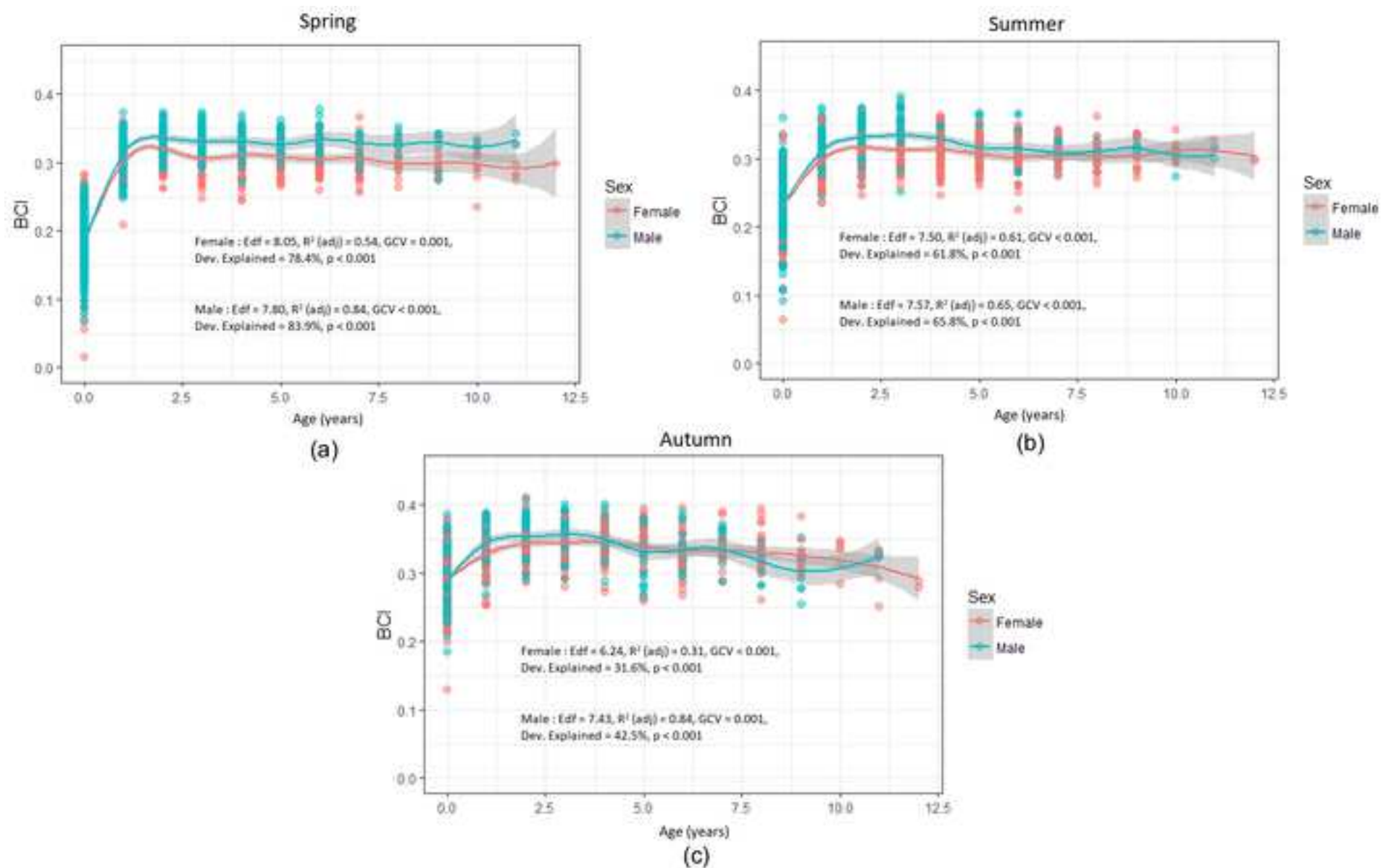


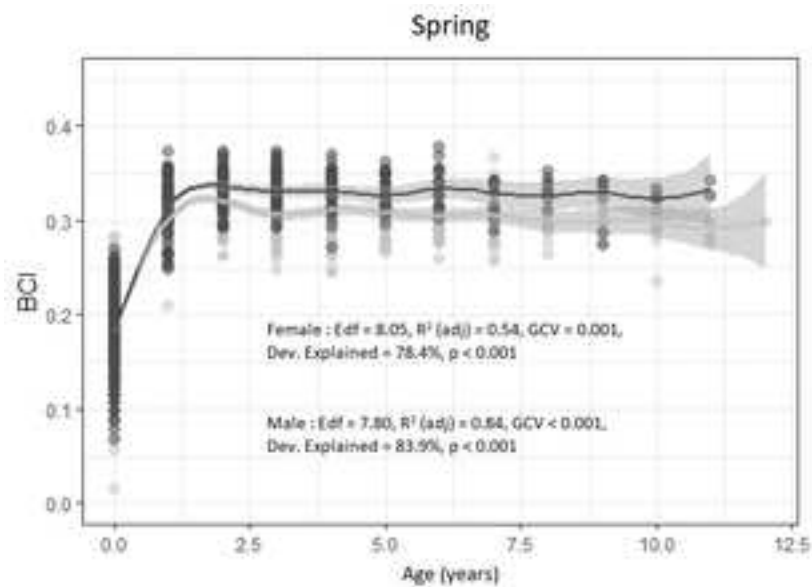




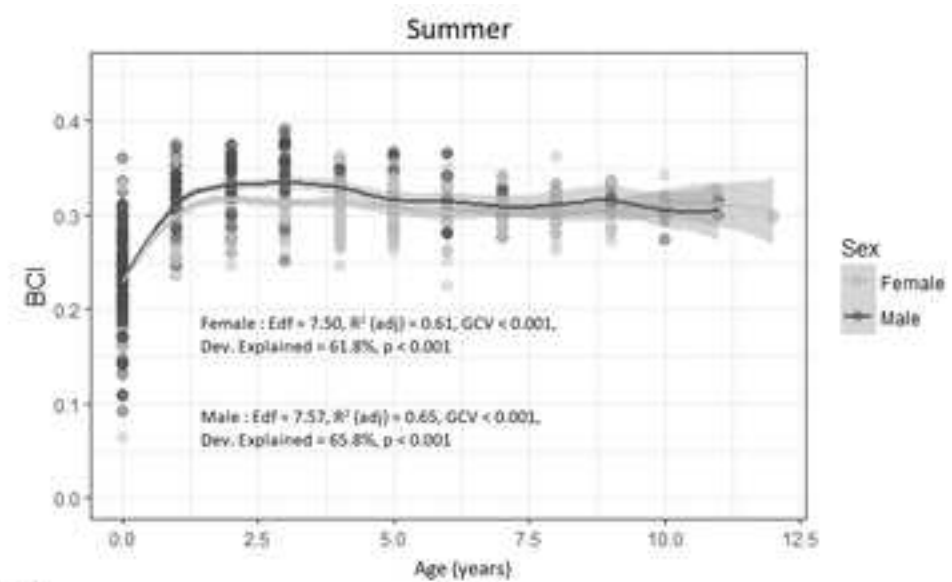




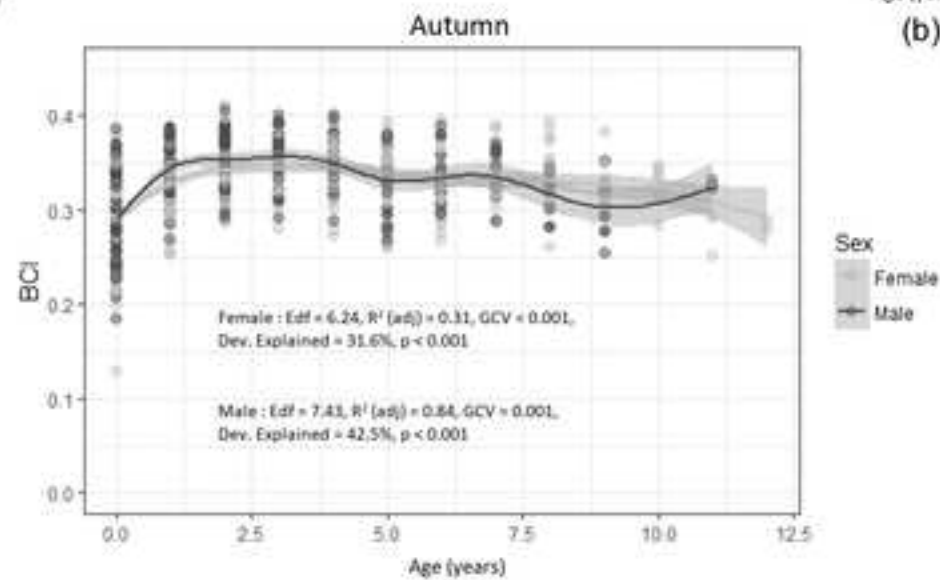




(a)



(b)



(c)

