Environmental Research 289 (2026) 123383

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Seasonal testicular morphometry of European brown bears in relation to pollutants

Maja Lazarus [a,*,1], Agnieszka Sergiel [b,1], Ankica Sekovanić [a], Maja Ferenčaković [c], Marija Dvoršćak [a], Darija Klinčić [a], Ena Oster [d], Slaven Reljić [d], Đuro Huber [d]

[a] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[b] Institute of Nature Conservation of Polish Academy of Sciences, Kraków, Poland
[c] University of Zagreb Faculty of Agriculture, Zagreb, Croatia
[d] University of Zagreb Faculty of Veterinary Medicine, Zagreb, Croatia

ARTICLE INFO

Keywords:
Testis size
Epididymis
Reproductive effect
Wildlife
PCBs
PBDEs
Metal(loid)

ABSTRACT

Inorganic and organic pollutants are capable of affecting the reproductive health of both animals and humans. The resulting pollutant-induced phenotype disorders have been rarely studied in wildlife males that commonly associate with diminished sperm quality leading to subfertility. We investigated testicular morphometry in Dinara-Pindos population of protected European brown bears (*Ursus arctos*) in relation to metal(loid)s (N = 44), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (N = 27), while controlling for age, body condition, and season. The principal approximating generalized linear models included following predictors of testicular mass and size: age (greater mass and larger size in adults than in subadults), toxic As (↓), Pb (↑), and Hg (↓), and essential Mn (↓), Se (↑), and Cu (↓) levels in the testes and/or epididymides. When organic pollutants were included in models explaining testicular mass and size variation instead of metal(loid)s, subcutaneous fat levels of PCB-118 (↑) and PCB-180 (↓), age (greater mass and larger size in adults) and season (higher in breeding than in non-breeding season) showed significant contribution. Higher mass and size of testes were found in breeding season (Mar–Apr) compared to non-breeding season (Oct). Metal(loid) concentrations, reported for the first time in ursid testicular and epididymal tissue, add to very scarce body of data from other wild terrestrial mammals. Associations between morphometric indices of male reproductive organs and certain endocrine-disrupting pollutants highlight potential toxicological relevance for wildlife chronically exposed to diffuse, global pollution sources.

1. Introduction

Successful reproduction is essential for conservation and population sustainability, particularly for strictly protected European carnivores like the brown bear (*Ursus arctos*), which face increasing challenges in landscapes dominated by human activity (Chapron et al., 2014). Widespread anthropogenic pollution by endocrine-disrupting organic and inorganic contaminants endangers reproductive health and viability in exposed wildlife and humans (Rodríguez-Estival and Mateo, 2019; Rodríguez-Jorquera et al., 2017). Pollutant-driven disturbance in reproductive organs, endocrine and immune system all affect reproductive functionality (Sonne, 2010 and references cited therein). Toxic elements like cadmium (Cd), lead (Pb), mercury (Hg) and arsenic (As) negatively affect aforementioned systems in males by several mechanisms: a) accumulation in reproductive organs, b) induction of oxidative stress, c) cytotoxicity on Leydig and Sertoli cells, d) structural damage to testis vasculature and blood-testis barrier, e) epigenetic modification, f) disturbance of the hypothalamus-pituitary-gonadal axis, g) inflammation, and h) substitution of essential elements in enzymes by ion mimicry (Anyanwu and Orisakwe, 2020; Arteaga-Silva et al., 2021; de Angelis et al., 2017; Tan et al., 2009). Health outcomes include, among others, a dose-dependent decrease in the mass and size of the

* Corresponding author.
E-mail addresses: mlazarus@imi.hr (M. Lazarus), sergiel@iop.krakow.pl (A. Sergiel), asekovanic@imi.hr (A. Sekovanić), mferencakovic@agr.hr (M. Ferenčaković), mdvorscak@imi.hr (M. Dvoršćak), dklincic@imi.hr (D. Klinčić), eoster@vef.unizg.hr (E. Oster), slaven.reljic@gmail.com (S. Reljić), djuro.huber@gmail.com (Đ. Huber).
[1] These authors contributed equally as first authors.

https://doi.org/10.1016/j.envres.2025.123383
Received 19 August 2025; Received in revised form 27 October 2025; Accepted 19 November 2025
Available online 21 November 2025

testes due to atrophy of the seminiferous epithelium in experimental rodents, followed by a reduction in testosterone levels, sperm count, and motility (Apostoli et al., 1998; Arteaga-Silva et al., 2021; Bhardwaj et al., 2021; de Angelis et al., 2017; Machado-Neves, 2022; Renu et al., 2018; Satarug et al., 2022; Wirth and Mijal, 2010; Zhu et al., 2020). However, inconsistencies in the reported changes have been attributed to notable strain-related variation in the sensitivity and accumulation of toxic metal(loid)s in the testes and epididymides (Apostoli et al., 1998). Larger relative testis size has evolved as an adaptation for greater sperm production (Dixson and Anderson, 2004). Testis size is therefore widely used as an indicator of reproductive health in wild mammals (Sonne et al., 2006, 2007; Williams et al., 2021). In the relatively few wildlife studies conducted, species-specific differences, seasonal factors, and fluctuations in essential metal(loid) levels have all been observed to influence the accumulation of toxic metal(loid)s in tissues. Metal(loid)-related changes in testicular morphology have been studied in environmentally exposed wild yellow-necked mice (*Apodemus flavicollis*; Damek-Poprawa and Sawicka-Kapusta, 2003), red deer (*Cervus elaphus*; Castellanos et al., 2015; Reglero et al., 2009), roe deer (*Capreolus capreolus*; Blottner et al., 1999; Vuarin et al., 2025), sheep (*Ovis aries*; Heidari et al., 2021), and camels (*Camelus dromedarius*; Ullah et al., 2023), though the findings revealed inconsistent trend. More frequently, studies have focused on abnormalities in sperm parameters in wild animals (Castellanos et al., 2015; Chen et al., 2018), domestic animals (Meligy et al., 2019; Ribeiro et al., 2022), and humans (Apostoli et al., 1998; de Angelis et al., 2017; Tariba Lovaković, 2020; Zečević et al., 2025), in relation to metal(loid) concentrations in blood or seminal plasma. In ursids, blood lead (Pb) levels in captive giant pandas (*Ailuropoda melanoleuca*) were inversely related to live sperm count (Chen et al., 2018).

Persistent organic halogenated pollutants negatively affect testes and epididymides size, sperm parameters and testosterone level through disruption of receptor binding activity of endocrine hormones in human and animal studies (Ahmad et al., 2003; Rodprasert et al., 2021; Vested et al., 2014; Williams et al., 2021), and free-ranging polar bears (*Ursus maritimus*; Ciesielski et al., 2023; Oskam et al., 2003; Sonne et al., 2007, 2006)). The prominent seasonality in ursid reproduction highlights the need to carefully consider biological and ecological factors in pollutant-related studies of reproductive and endocrine toxicity. Generally, the breeding season in ursids is characterized by higher testosterone levels, larger testis size and mass, and increased spermatogenesis in spring and early summer compared to the non-breeding season (i.e., fall) (Howell-Skalla et al., 2002; S. S. Palmer et al., 1988; Spörndly-Nees et al., 2019; Tsubota et al., 1997; White et al., 2005a).

Recent findings of toxicologically relevant cadmium (Cd) and lead (Pb) levels (Lazarus et al., 2017, 2024b), potential metal-related impairment of oxidative balance (Lazarus et al., 2020, 2024b), and endocrine effects (Lazarus et al., 2023, 2024a) in the European brown bears have raised questions regarding their impact on testicular tissue and related functions. Due to the high content of polyunsaturated fatty acids and high cell division rate, testes are known to be particularly sensitive to oxidative stress, one of the main mechanisms of metal(loid) toxicity (Arteaga-Silva et al., 2021). The aim of this study was, therefore, to report for the first time the levels of metal(loid)s in the testes of European brown bears, the primary male reproductive organ responsible for spermatogenesis and related hormone synthesis. In addition, we hypothesized that toxic metal(loid)s in the testes and epididymides, along with organic pollutants in adipose tissue (polychlorinated biphenyls, PCBs and polybrominated diphenyl ethers, PBDEs) of brown bears negatively associate with testicular size and mass. These hypotheses were tested while accounting for potential variation due to age, body condition index, breeding season, and the presence of essential metal(loid)s.

2. Materials & methods

2.1. Ethical statement

In Croatia, the national Brown Bear Management Plan, together with its annual Action Plans, requires the collection of biological samples from all recorded mortality events. For individuals not killed explicitly for scientific purposes, sample collection is exempt from approval by local ethics committees. At the European level, the brown bear is strictly protected under the Habitats Directive, which regulates the legal acquisition and use of specimens. In Croatia, however, 10–16 % of the Croatian part of Dinara-Pindos population (Huber et al., 2008, 2019), that counts approx. 1,000 individuals (range 858–1063; Skrbinšek et al., 2019), is annually allocated to trophy hunting, permitted under the derogation provisions of the Directive (Council Directive 92/43/EEC, article 16). Hunting is allowed during two designated seasons, from February to May and from September to December. For the present study, we relied on the sample collection framework established in the Management Plan and annual Action Plans, as well as on official exemptions issued by the Ministry of Economy and Sustainable Development for actions otherwise prohibited with strictly protected species. This framework enabled the collection of material from human-induced mortality events, including traffic-related fatalities.

2.2. Sample collection

Sixty-five testicles were sampled from 63 free-ranging and two captive Dinara-Pindos European brown bears in the Lika and Gorski kotar regions of Croatia (Fig. 1) between 2015 and 2024. Although these regions lack point sources of pollution, the reported presence of metal(loid)s and organic pollutants in bears (Jagić et al., 2024; Lazarus et al., 2017, 2023, 2024a) indicates the importance of long-range atmospheric transport and deposition, as well as geogenic sources in the studied area (Lazarus et al., 2024b). Majority of the samples ($N = 54$) were collected while conducting mortality sampling during two regular hunting seasons enabled under the Habitat Directives derogation provisions and in accordance with the Brown Bear Management Plan. Additionally, nine free-ranging bears were sampled following road or railroad collisions, while two captive bears were sampled during castration. Based on recorded total body mass (M) and length (L), the body condition index (BCI) was calculated using the formula: $BCI = (\ln M - 3.21 \times \ln L + 11.64)/(0.29 - 0.017 \times \ln L)$, as established by Cattet et al. (2002) for North American brown bears. The mass, length, and width of the encapsulated left and right testes were measured for only 44 bears sampled between 2021 and 2024. Average testicular mass (TM) and size ($TL \times TW$) was used in the following analyses. Testicles were stored at −80 °C in plastic tubes until metal(loid) analyses. Subcutaneous adipose tissue was collected from 24 bears sampled between 2021 and 2022, and stored at −80 °C in plastic zip-lock bag for further analyses of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) as previously reported (Jagić et al., 2024). Age was determined by counting cementum annuli of the first premolar (Matsons Lab, Milltown, Montana, USA; Matson et al., 1993).

2.3. Metal(loid) analyses

Partially thawed testicular parenchyma (4 g) and epididymal head and body (3 g) were excised using a ceramic knife and freeze-dried for 72 h (HyperCOOL HC3055, LabTech Srl, Korea). The average moisture content was 84 %. Portions (0.2 g) of testis and epididymis were then acid digested (2 mL of purified nitric acid and 2 mL of ultrapure water) in respective quartz tubes using a microwave digestion system (UltraCLAVE IV, Milestone, Italy). Metal(loid)s (As, Ca, Cd, Cu, Hg, Mn, Pb, Se, Tl, and Zn) were quantified by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 8900, Agilent Technologies, Santa Clara, CA, USA) using the previously detailed method (Vihnanek Lazarus et al.,

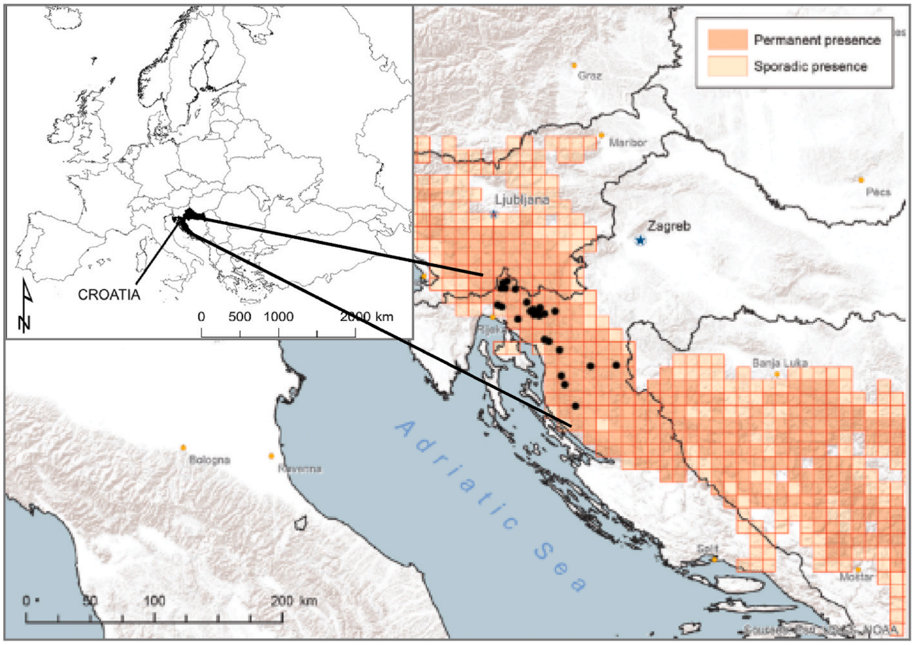

Fig. 1. Map of Croatia with displayed locations (black dots) of sampling testes from Dinara-Pindos population of European brown bears (*Ursus arctos*) in 2015–2024 period. Map adjusted from source (Kaczensky et al., 2024).

2013). A review of the literature on the reproductive impacts of specific toxic metal(loid)s, as well as their interactions with essential metal(loid)s in testicular tissue, informed the selection of metal(loid)s included in this study. Quality control was carried out by digesting and measuring duplicate samples of certified reference material BCR-185R Bovine liver, and ERM – BB184 Bovine muscle (European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Belgium) together with testicular samples.

2.4. Statistical analyses

All statistical analyses were conducted using the JMP® Student Edition 18.2.0 (SAS Institute Inc., Cary, NC, USA). Graphical representations were created using R version 4.5.0 (R Core Team, 2025) within the RStudio environment (version 2024.12.1, Posit Software, PBC) and TIBCO Statistica® software, version 14.0.0.15 (TIBCO Software Inc., Palo Alto, CA, USA).

Multiple linear regression models (JMP® Student Edition 18.2.0) were constructed using the Generalized Regression framework to identify predictors of testicular mass and size parameters in 44 bears. No excessive multicollinearity was detected. Predictors included age group, season, body condition index, selected metal(loid)s level (As, Ca, Cd, Cu, Hg, Mn, Pb, Se, Tl, and Zn), seven indicator PCBs (CB-28, -52, −101, −118, −138, −153, −180), and PBDEs (BDE-28, -47, −99, −100, −153, −154, −183) concentrations. Bears in their second year of life were categorized as yearlings, subadults if ≥ 2 and <4 years old, and adults if aged ≥ 4 years (Knott et al., 2014; Lazarus et al., 2023). Due to the low number of yearlings ($N = 2$), data for this age group were analyzed together with data from the subadult group, although they are visualized separately in Fig. 2A. Based on the observation of high testosterone levels in the earlier study (Fig. 2B; Lazarus et al., 2024a) and the fact that spermatogenesis and steroidogenesis begin before the breeding season (White et al., 2005a), we classified samples collected from late March to July as testes sampled during the breeding period, whereas samples

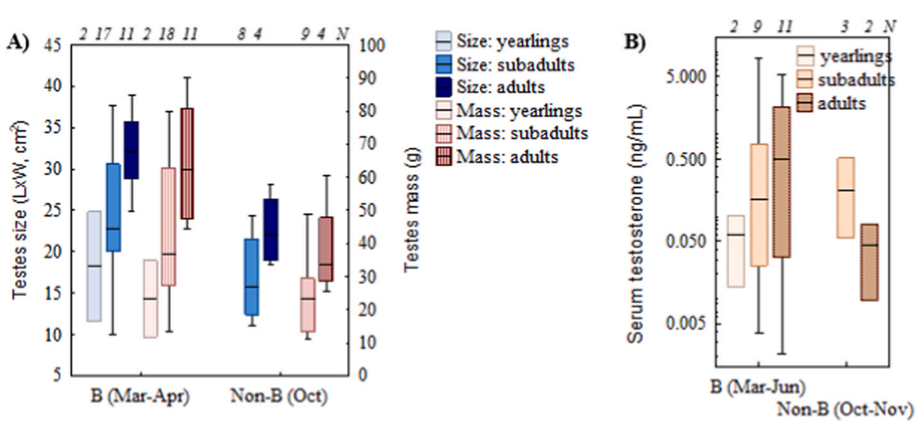

Fig. 2. Size (cm^2) and mass (g) of testes of 44 brown bears from Dinara-Pindos population sampled in Croatia in 2015–2024 categorized by breeding season (B, breeding; Non-B, non-breeding) and age group (A). Statistically significant differences in size and mass of testes were noted between yearling and subadult bears compared to adults, and between two seasons. Serum testosterone (ng/mL) on a log scale in brown bears from Carpathian and Dinara-Pindos population sampled in 2014–2019 (Lazarus et al., 2024a) categorized by breeding season and age group (B). Boxes denote first and third quartile with central line as median, whiskers denote range. Number of individual bears per group is presented in italic (N).

collected from August to December were classified as representing the non-breeding period. Metal(loid)s and organic pollutants were modeled separately due to the limited number of sampled bears, and the distinct toxicological properties of these two pollutant groups, although we are aware their effects on testicular cell morphology may be similar. The number of predictor effects allowed in the final models varied depending on the type of analysis. More details are available in the Supplementary material. Model selection and evaluation were based on the Akaike Information Criterion corrected for small sample sizes (AICc), ΔAIC values, Akaike weights, and adjusted R-squared (R^2adj) values. Models with a ΔAIC ≤2 were considered to have substantial support and were statistically indistinguishable from the best model, in line with Burnham and Anderson (2002).

Data preparation prior to the analyses included winsorization (Cu and Cd in testes, and Ca in epididymis), substitution of data below limit of detection (LOD) with LOD/2 values (Hg, 4/65 in testes, and 9/61 in epididymides) and certain variable transformations to meet normality and homogeneity of the variance assumptions (more details in the Supplementary material**)**. Of the 27 adipose tissue PCB and PBDE concentrations reported previously (Jagić et al., 2024), data for 24 individuals with matching testis morphometry data were used in this study for modelling possible variations of testes mass and size by these compounds. Differences between the age groups (subadults *vs*. adults) and seasons (breeding *vs*. non-breeding) were tested with Student's t-test or Welch's *t*-test (N = 65). Metal(loids) levels for paired measurements (testis *vs*. epididymis level) were compared using the Wilcoxon signed-rank test (N = 61). The presence of linear associations between toxic and essential metal(loid)s were assessed using Spearman's rank correlation coefficient, while associations between BCI and other continuous variables by employing Pearson's correlation coefficient.

3. Results

3.1. Variation drivers of testes mass and size – association with metal (loid)s

The set of principal approximating generalized linear models used to assess variations in bear testicular mass and size based on age, BCI, season, toxic and essential metal(loid)s (testicular and epididymal, respectively) is listed in Table S1 in the Supplementary material. Only the top model was further characterized (Table 1) and discussed in the text.

Taking into account testicular metal(loid)s, the top-ranked model selected to explain the variation in testicular mass (74 %; Table S1) included age (higher in adults), a negative effect of Mn and As in testes, and a positive effect of Se and Pb in testes (Table 1). The variation in the testicular size (69 %; Table S1) was best explained by age (larger size in adults), a negative effect of Mn, Mn and Tl interaction (Mn x Tl), and Hg x Tl in testes, and a positive effect of Se in testes (Table 1). The addition of Hg, Tl (positive effect) and Mn x Tl contributed significantly to the best model, but these variables separately had no effect on testicular size (p = 0.513, p = 0.155, and p = 0.057, respectively).

On the other hand, modeling variations in testicular mass (70 %; Table S1) by taking into account epididymal metal(loid)s revealed a positive effect of Se and a negative effect of Mn, Hg and Mn x Se interaction (Table 1). Inclusion of Mn and Mn x Se in epididymides showed to be important for the model, but none of these two variables exhibited a significant independent association with testicular mass (p = 0.106 and p = 0.054, respectively). For testicular size, age (larger size in adults), Cu and Hg (negative effect), Se and As (positive effect) in epididymides contributed to the top model, explaining 65 % of variation.

Table 1
Coefficient estimates (with 85 % confidence intervals, CI) and significance (p-value) for predictors (testis/epididymis/fat) from the top ranked models describing mass and size of testes in 44 free-ranging brown bears (Dinara-Pindos population) sampled in Croatia in 2021–2024[a].

Response variable	Model rank	Explanatory variable (predictor)	Estimate (85 % CI)	p-value
Metal(loid)s in testis (T)				
Testes mass (TM)	1	Age (↓)	−7.11 (−9.93, −4.30)	**<0.0001**
		Mn_T (↓)	−37.2 (−45.5, −28.8)	**<0.0001**
		As_T (↓)	−6.03 (−10.6, −1.40)	0.063
		Se_T (↑)	0.049 (0.041, 0.057)	**<0.0001**
		Pb_T (↑)	6.93 (2.02, 11.8)	**0.045**
Testes size	1	Age (↓)	−2.30 (−3.53, −1.07)	**0.009**
(TLxW)		Mn_T (↓)	−11.2 (−14.7, −7.62)	**<0.0001**
		Se_T (↑)	0.016 (0.013, 0.020)	**<0.0001**
		Hg_T	0.479 (−0.590, 1.55)	0.513
		Tl_T	1.71 (−0.023, 3.44)	0.155
		Mn_T x Tl_T (↓)	−5.21 (−9.10, −1.32)	0.057
		Hg_T x Tl_T (↓)	−2.99 (−4.40, −1.59)	**0.003**
Metal(loid)s in epididymis (E)				
Testes mass (TM)	1	Mn_E (↓)	−9.58 (−18.1, −1.07)	0.106
		Se_E (↑)	34.7 (26.8, 42.5)	**<0.0001**
		Hg_E (↓)	−4.99 (−7.07, −2.92)	**0.001**
		Mn_E x Se_E (↓)	−22.4 (−39.0, −5.84)	0.054
Testes size	1	Age (↓)	−2.44 (−3.85, −1.02)	**0.016**
(TLxW)		Se_E (↑)	10.4 (7.47, 13.4)	**<0.0001**
		Cu_E (↓)	−14.5 (−21.6, −7.36)	**0.005**
		As_E (↑)	4.23 (1.87, 6.59)	**0.012**
		Hg_E (↓)	−1.63 (−2.48, −0.773)	**0.008**
PCBs and PBDEs in fat				
Testes mass (TM)	1	Age (↓)	−13.3 (−16.6, −10.1)	**<0.0001**
		Season (↑)	10.4 (6.86, 14.0)	**0.0001**
Testes size	1	Age (↓)	−4.73 (−6.22, −3.23)	**0.0001**
(TLxW)		PCB-118 (↑)	4.33 (2.60, 6.06)	**0.001**
		PCB-180 (↓)	−4.33 (−6.42, −2.25)	**0.006**

[a] Models were selected according to sample-size-corrected Akaike's Information Criterion (AIC_c) scores (ΔAIC ≤2). Predictors coloured blue denote variables that do not substantially improve the model, as indicated by 85 % confidence intervals overlap zero (Arnold, 2010). Effect direction (arrow) is given only for variables without 85 % confidence intervals overlap zero. (↑) sign denotes positive effect on response variable, while (↓) sign denotes negative effect on response variable; negative effect for age variable denotes age2 (adults) > age1 (subadults); positive effect for season variable denotes breeding season1 (spring, Mar–May) > non-breeding season2 (fall, Oct). Significant p-values are bolded. Some predictors were ln transformed (As_T, Pb_T, Tl_T, Hg_T, Se_E, Hg_E, As_E, Cu_E, PCB-118, PCB-180), but this was not indicated in the table to increase clarity of presentation.

3.2. Variation drivers of testes mass and size – association with PCBs and PBDEs

The top-ranked models evaluating testicular mass by taking into account organic pollutants quantified in brown bear adipose tissue, included age (higher mass in adults) and season (higher mass in breeding season) as principal contributors, which together explained 45 % of the variation (Table 1, Table S1). The testicular size variation (60 %) was best explained by age (higher mass in adults), a positive effect of PCB-118, and a negative effect of PCB-180.

3.3. Body and testis morphometry

This study included male brown bears aged from 1 to 13 years. Body mass differed ($p < 0.001$) between subadult (1–3 y; N = 39, mean ± SEM, 115 ± 8 kg) and adult (≥4 y; N = 21, 209 ± 10 kg) bears, as well as BCI ($p < 0.001$). Total body length differed ($p < 0.001$) between subadults (N = 39, 163 ± 3 cm) and adults (N = 21, 188 ± 2 cm). Season (breeding: March–July *vs.* non-breeding: Aug–December) did not influence body mass, body length and BCI. Fig. 2A presents the average size and mass of bear testes, categorized by sampling month and age group. While yearlings are graphically distinguished from subadults in the figure, they are combined with subadults for statistical comparisons against the adult group. Testicular mass ($p < 0.001$) and size ($p = 0.002$) were significantly lower in subadult (N = 29, 35.6 ± 3.6 g and 21.5 ± 1.6 cm^2, respectively) compared to adult bears (N = 15, 57.3 ± 4.9 g and 29.6 ± 1.6 cm^2, respectively). Higher testicular mass ($p = 0.004$) and size ($p = 0.004$) were found in March/April (N = 31, 48.9 ± 3.9 g and 27.0 ± 1.5 cm^2, respectively) compared to October (N = 13, 29.1 ± 4.2 g and 18.8 ± 1.6 cm^2, respectively) in 44 bears. Testicular size was highly positively associated with testicular mass (N = 42, r = 0.88, $p < 0.001$).

3.4. Metal(loid)s in testis and epididymis

All metal(loid)s were detectable, except Hg in four testicular and nine epididymal samples. Metal(loid)s in testes strongly correlated with respective metal(loid)s in epididymal tissue (As r = 0.80, $p < 0.001$; Cd r = 0.83, $p < 0.001$; Hg r = 0.74, $p < 0.001$; Se r = 0.84, $p < 0.001$; Tl r = 0.86, $p < 0.001$; Zn r = 0.71, $p < 0.001$), while moderate correlations were noted for Mn (r = 0.50, $p < 0.001$) and Pb (r = 0.42, $p < 0.001$), and weak correlations for Cu (r = 0.26, $p = 0.047$). Levels of Ca ($p < 0.001$), Cu ($p = 0.022$), Mn ($p < 0.001$), Se ($p < 0.001$), Tl ($p < 0.001$), and Zn ($p < 0.001$) were higher in testes than in studied epididymides, while opposite was true for As ($p < 0.001$), Cd ($p < 0.001$), and Pb ($p < 0.001$), which were higher in epididymides. Significant correlations of toxic with essential metal(loid)s in testis and epididymis are presented in Fig. 3.

Results of testing differences in metal(loid) levels between subadults and adults are presented in Table 2. Selenium and Zn levels in both testicular ($p < 0.001$) and epididymal tissue ($p < 0.001$ and $p = 0.0006$, respectively) were higher in adult bears compared to subadults. On the contrary, subadults had higher testicular levels of Hg than adults ($p = 0.012$).

Significant seasonal differences in metal(loid) levels are shown in Fig. 4. Testes and epididymides had higher Mn ($p = 0.006$ and $p = 0.002$, respectively), Se levels ($p < 0.001$), and Zn ($p < 0.001$ and $p = 0.002$, respectively), Tl in testes ($p = 0.022$) and Pb in epididymides ($p = 0.022$) in the breeding season (spring, Mar–May) than in the non-breeding season (Jul–Dec). On the contrary, testicular Cd ($p = 0.003$), Hg ($p = 0.044$) and Pb ($p = 0.010$) were lower in the breeding season compared to non-breeding season.

4. Discussion

4.1. Variation drivers of testes mass and size – association with metal (loid)s

Our modeling results indicated that age, along with levels of certain essential (Mn, Se, and Cu) and toxic metal(loid)s (As, Pb, and Hg) in the testes and/or epididymides, explained large variation (65–74 %) in testes mass and size in European brown bears.

Among these, age, Mn, and Se levels in both the testes and epididymides were consistently associated with variation in both testicular mass and size. Adult European brown bears had larger and heavier testes compared to subadults, as reported by White et al. (2005b). Testicular development patterns observed in our study align with findings in polar bears, whose testes continue to grow until sexual maturity at around 6–7 years of age (Spörndly-Nees et al., 2019). However, brown bears in southern Europe are generally reported to reach sexual maturity earlier, around 4 years of age, compared to their northern counterparts (Frković et al., 2001; Knott et al., 2014).

Season significantly predicted variation in testicular mass in the validated model (Table 1), with testes of Dinara-Pindos brown bears being larger during the breeding than the non-breeding season after controlling for age, as reported in other ursid species (Howell-Skalla et al., 2002, 2000; Spörndly-Nees et al., 2019; White et al., 2005b, and references therein).Testicular size reached only about 45 % of their peak size during the non-breeding season (Howell-Skalla et al., 2002). This variation mirrors seasonal fluctuations in serum testosterone levels, which are regulated by photoperiod (Palmer et al., 1988; Tsubota et al., 1997; White et al., 2005a, and references therein). Elevated serum

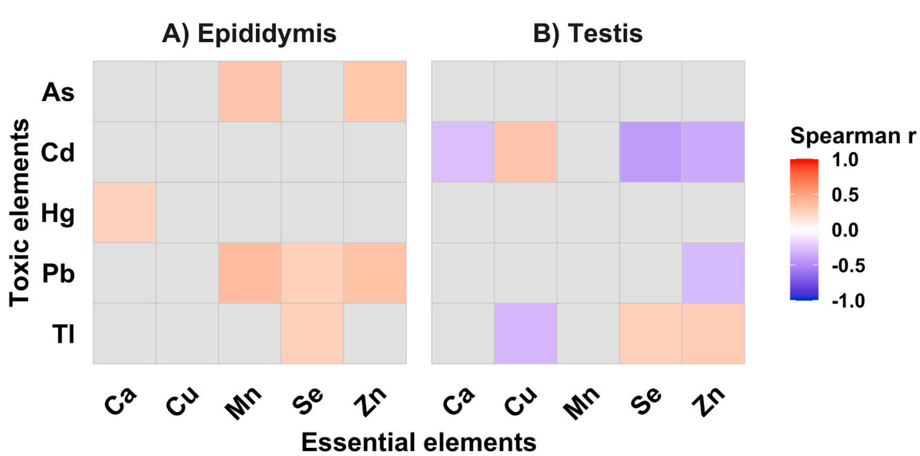

Fig. 3. Heatmap of Spearman correlation coefficients (r) between toxic and essential metal(loid)s in 61 epididymal (A) and 65 testicular (B) tissues of brown bears from Dinara Pindos population sampled in Croatia in 2015–2024.

Table 2
Age-related differences in levels of metal(loid)s (on dry mass of tissue) in testicular and epididymal tissue of 65 brown bears from Dinara-Pindos population sampled in Croatia in 2015–2024 (mean ± SEM, median (range))[a].

	Testis			Epididymis		
	Subadults (<4 y), N = 42	Adults (≥4 y), N = 23	Group differences	Subadults (<4 y), N = 39	Adults (≥4 y), N = 22	Group differences
As (µg/kg)	5.39 ± 0.40, 4.68 (1.54–11.6)	5.36 ± 0.96, 3.95 (1.47–19.4)	NS	6.12 ± 0.51, 5.95 (1.49–12.9)	6.50 ± 0.90, 5.48 (1.15–18.2)	NS
Ca (mg/kg)	435 ± 9, 439 (322–549)	466 ± 16, 440 (387–717)	NS	367 ± 8, 362 (302–486)	355 ± 21, 337 (212–696)	NS
Cd (µg/kg)	164 ± 14, 159 (48.1–330)	176 ± 19, 163 (26.5–515)	NS	181 ± 17, 139 (70.2–343)	214 ± 24, 172 (26.7–618)	NS
Cu (mg/kg)	5.10 ± 0.19, 4.96 (3.40–11.5)	5.26 ± 0.14, 5.34 (3.20–6.54)	NS	4.60 ± 0.17, 4.21 (3.28–7.52)	5.03 ± 0.19, 4.68 (3.13–6.68)	NS
Hg (µg/kg)	7.74 ± 2.18, 2.74 (<0.497–77.9)	2.14 ± 0.27, 1.75 (<0.497–4.59)	t(63) = -2.58 p = 0.012	5.85 ± 1.44, 3.00 (<0.497–43.2)	3.14 ± 0.64, 2.20 (<0.497–12.6)	NS
Mn (mg/kg)	2.41 ± 0.07, 2.41 (1.64–3.31)	2.60 ± 0.06, 2.63 (1.59–3.01)	NS	1.29 ± 0.08, 1.19 (0.586–2.55)	1.44 ± 0.08, 1.43 (0.602–2.25)	NS
Pb (µg/kg)	29.0 ± 4.2, 19.2 (7.02–162)	32.1 ± 5.8, 17.4 (5.74–120)	NS	61.2 ± 9.3, 40.1 (12.5–242)	66.4 ± 10.1, 47.7 (11.1–187)	NS
Se (mg/kg)	1.38 ± 0.06, 1.22 (0.847–2.01)	1.81 ± 0.08, 1.87 (0.928–2.44)	t(63) = 4.31 p < 0.0001	0.840 ± 0.049, 0.712 (0.447–1.65)	1.52 ± 0.16, 1.61 (0.493–3.17)	t(59) = 3.99 p = 0.0004
Tl (µg/kg)	11.8 ± 1.29, 9.85 (2.16–43.0)	12.0 ± 1.2, 11.7 (3.95–25.9)	NS	5.59 ± 0.70, 4.45 (0.896–22.0)	5.80 ± 0.74, 4.38 (1.38–14.7)	NS
Zn (mg/kg)	78.8 ± 2.63, 74.9 (51.6–116)	95.9 ± 2.5, 98.5 (51.1–113)	t(63) = 4.77 p < 0.0001	65.8 ± 2.8, 63.7 (32.7–113)	86.3 ± 5.6, 84.2 (48.5–151)	t(59) = 3.64 p = 0.0006

[a] In the calculation of descriptive statistics, Hg levels below the detection limit of the method (DL) were assigned the value of the DL/2 and presented with <DL sign. Group differences were tested with Student or Welch *t*-test and presented as t(df) and p-value or NS – not significant.

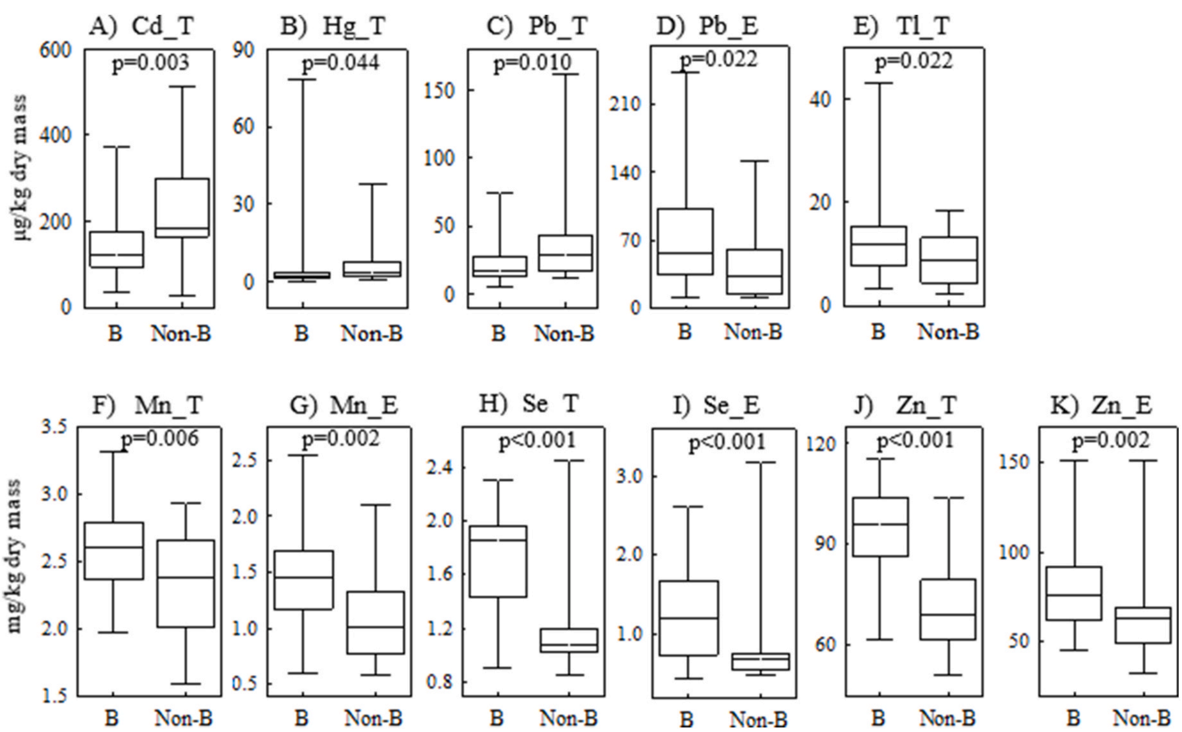

Fig. 4. Levels of testicular (T) and epididymal (E) metal(loid)s in relation to breeding (B; Mar–Jul) and non-breeding season (Non-B; Aug–Dec) of 65 brown bears from Dinara-Pindos population sampled in Croatia in 2015–2024. Boxes denote first and third quartile with central line as median, whiskers denote range. Level of significance (p) for tested differences between two seasons is denoted above box-whisker plots.

testosterone levels typically observed in May were already detectable in March in European populations (Fig. 2B; Lazarus et al., 2024a). Testicular size increased from January until April and May in American black bears (*Ursus americanus*; Howell-Skalla et al., 2000) and in North American brown bears, with a peak in June in adults (White et al., 2005a), but not subadults (White et al., 2005b). In contrast, the trends in testicular size and mass observed in brown bears in the present study only partially align with these earlier findings, as we observed a clear decline from March–April through to October in both subadult and adult individuals (Fig. 2A).

Seasonal differences in metal(loid) levels in the Dinara-Pindos population of brown bears were previously established in kidney and liver tissues, and were proposed to result predominantly from variations in food items consumed between spring and fall, including excessive food intake during fall hyperphagia, which contrasts with lower intake in spring (Lazarus et al., 2017, 2024b). Interestingly, higher fall (non-breeding season) testicular levels of Cd, Hg, and Pb, but lower levels of Mn, Pb, Se, Tl, and Zn (Fig. 4), aligned well with seasonal variations observed in renal tissue (higher Cd and Cu, but lower Hg, Mn, Pb, Tl, and Zn in fall; Lazarus et al., 2024b) and hepatic tissue (higher Cu and Hg, but lower Mn, Tl, and Zn in fall; Lazarus et al., 2017). Indeed, correlation analysis between metal(loid) levels in the testes or epididymides and the corresponding kidney tissues (Lazarus et al., 2024b) of individuals included in this study revealed moderate to high correlations ($N = 49$, $r = 0.30–0.79$, $p < 0.05$; data not shown), with the strongest correlations observed for Cd and Tl. These findings suggest that testicular and epididymal tissues can moderately to very accurately reflect Ca, Cd, Cu, Hg, Mn, Pb, and Tl levels in renal tissue, and *vice versa*. This alignment in metal(loid) accumulation across different tissues, including the testes, is reported here for the first time in bears. A similar pattern was observed in wild yellow-necked mice, where site-specific changes in kidney and liver Pb levels were accompanied by corresponding changes in testicular Pb and Zn levels, though not in Cd and Fe (Damek-Poprawa and Sawicka-Kapusta, 2003).

Arsenic accumulated to a lesser extent in the testes than in the epididymides of brown bears from this study, which may have contributed to the contrasting effects observed between these organs. Furthermore, testicular As levels were negatively associated with testis mass, supporting similar adverse effects previously demonstrated in adult and subadult laboratory rats (*Rattus norvegicus*) and mice (*Mus musculus*) (Guimarães-Ervilha et al., 2025; Renu et al., 2018; Souza et al., 2021). However, in environmentally exposed red deer, no significant relationship was observed between As levels and testicular size or mass (Reglero et al., 2009). Interestingly, red deer from both control and mining areas accumulated up to ten times more As in their testes compared to the bears in this study (range: LOD–100 *vs*. 1.15–19.4 μg/kg dry mass (Reglero et al., 2009)). Correlation analysis in this study revealed positive associations of As with Mn and Zn (Fig. 3). The interaction of As with these essential elements may be attributed to its reported effects on the expression of metallothionein (MT) and the Cu-, Zn- and Mn-containing superoxide dismutase (SOD) enzyme in rats (Rachamalla et al., 2022; Souza et al., 2021, and references therein). Arsenic was shown to reduce the activity of the SOD by displacing essential metal cofactors from its active site. The resulting release of Mn or Zn ions, along with the accumulation of superoxide radicals, may stimulate the synthesis of metallothionein (MT), a well-known antioxidant protein with a high binding affinity for As (Rachamalla et al., 2022; Peraza et al., 1998).

In brown bears, testicular Pb levels showed a positive association with testis mass. Experimental studies in rodents have reported conflicting results regarding the effects of Pb on testis mass and size, with findings ranging from negative to no impact (Apostoli et al., 1998; Massányi et al., 2003; Wirth and Mijal, 2010). Therefore, the observed increase in testicular mass in bears with higher Pb levels may be a result of adaptive response of cells trying to compensate for the Pb-related adverse effects, similar to those reported in red deer from mining areas in Spain (Reglero et al., 2009) and roe deer from polluted regions in Germany (Blottner et al., 1999). In contrast, histopathological damage with notably elevated testicular Pb levels were observed in yellow-necked mice inhabiting zinc smelter compared to mice from other industrial and control sites (Damek-Poprawa and Sawicka-Kapusta, 2003). Elevated levels of Pb and Cd in the testes of sheep have been linked to reduced testicular size and increased sperm abnormalities (Heidari et al., 2021). Similarly, camels from industrial regions of Pakistan exhibited decreased testis mass and size, corresponding with higher testicular concentrations of Cd and Pb compared to other regions (Ullah et al., 2023). In roe deer from central eastern France, testicular mass showed a negative association with hepatic Cd levels and no significant relationship with Pb levels (Vuarin et al., 2025).

The highest Pb concentration measured in this study (242 μg/kg dry mass) was two orders of magnitude lower than the levels associated with impaired spermatogenesis in rats (2000 μg/kg wet mass (Apostoli et al., 1998)). However, interpretation should be taken with caution due to the interspecific differences. A few bears with the highest testicular Pb levels had their renal Pb concentrations around the population median (3.97 mg/kg dry mass; Lazarus et al., 2024b), suggesting no toxicological relevance. The average Pb levels in bear testes and epididymides (mean 30.1 and 63.1 μg/kg dry mass, respectively) were consistent with values reported in both control and environmentally exposed red deer (mean 28–81 μg/kg dry mass (Castellanos et al., 2015; Reglero et al., 2009)), but substantially lower than levels observed in wild yellow-necked mice (mean 140–6960 μg/kg dry mass; Damek-Poprawa and Sawicka-Kapusta, 2003), sheep (approx. 500–800 μg/kg dry mass; Heidari et al., 2021) or camels (Ullah et al., 2023). The observed interspecific differences may arise from physiological variations influencing the absorption, distribution, metabolism, and excretion of metal(loid)s, as well as from differing exposure levels associated with habitat and dietary characteristics.

Mercury ranged lowest among toxic metal(loid)s in brown bear from this study and was negatively associated with testes mass, which may result from testicular degeneration and atrophy, and impaired steroidogenesis established in Hg-exposed experimental animals (Anyanwu and Orisakwe, 2020; Massányi et al., 2003; Zhu et al., 2000). Reproductive effect studies of Hg exposure in wild animals largely involve birds, while threshold values of Hg and other metal(loid)s in mammalian wildlife are mostly unknown (Vuarin et al., 2025). However, several indicators of altered testicular dimensions, histological characteristics, and functional impairment have been reported in Kermani rams chronically exposed to the polluted environment of a copper smelter (Heidari et al., 2021).

Although identified as a pollutant of toxicological concern in a small portion of the brown bear population (Lazarus et al., 2017, 2024b), Cd was not a significant predictor of testicular mass or size as shown in this study. Renal Cd concentrations in a few bears with the highest testicular levels were around the population median (60.2 mg/kg dry mass; Lazarus et al., 2024b) or up to twice as high, but still below the renal toxicity threshold for mammals (435 mg/kg dry mass, recalculated from wet mass assuming 77 % water content; Scheuhammer, 1991). Compared to the limited data available for other species, testicular Cd levels in bears (range 26.5–515 μg/kg dry mass) were higher than those reported for red deer (<LOD–38 μg/kg dry mass; Reglero et al., 2009), but lower than levels observed in wild yellow-necked mice (mean 340–700 μg/kg dry mass; Damek-Poprawa and Sawicka-Kapusta, 2003).

Our results suggest a negative association between testicular/epididymal Mn levels, and organ mass and size. A similar trend, attributed to reproductive tissue degeneration and reduced sperm quality, has been reported in orally exposed experimental animals and occupationally exposed workers (Adedara et al., 2017; ATSDR, 2012; Gomes-Silva et al., 2023). However, we propose that the bears in this study were neither exposed to elevated Mn levels through their diet nor exhibited significant Mn accumulation in the testes that could lead to such adverse effects. The observed trend may instead be attributed to species-specific differences in metal(loid) accumulation and susceptibility in testicular tissue (Apostoli et al., 1998). The testicular Mn levels measured in both control and fly ash-exposed raccoons (*Procyon lotor*; med 2–2.4 mg/kg dry mass, recalculated from wet mass according to 84 % of water and a factor of 6.25; Souza et al., 2013) were within the same range as those found in the brown bears analyzed in this study (med 2.51 mg/kg dry mass).

Adequate Se levels, much like Mn, play an important role in testicular development, spermatogenesis, testosterone metabolism, and protection against oxidative stress. Both deficient and excessive Se levels can lead to morphological and functional defects (Behne et al., 1996; Boitani and Puglisi, 2008). In this study, Se levels were a significant factor influencing variation in testicular size and mass in bears, showing an opposite trend compared to Mn. Positive association of bear testis size and mass in relation to Se contrasted negative correlation reported in red deer (Reglero et al., 2009). Indeed, Se deficiency in rats has been shown to impair testicular morphology by reducing testosterone biosynthesis and the biological activity of key selenoenzymes (Behne et al., 1996). Due to the essential role of Se in reproductive health, its concentration in mammalian testes is tightly regulated and predominantly found in the enzyme phospholipid hydroperoxide glutathione peroxidase (GPx4 (Boitani and Puglisi, 2008)). The Se levels measured in Croatian bears (med 1.53, range 0.447–3.17 mg/kg dry mass) were somewhat lower than those reported in raccoons (med 2.75–3.3 mg/kg dry mass, recalculated from wet mass using a factor of 6.25; Souza et al., 2013) and of higher range than in red deer from control and mining area in Spain (0.07–1.42 mg/kg dry mass; Reglero et al., 2009), taking into account species-specific differences. Selenium and Zn were the only essential metal(loid)s that differed between subadult and adult bears. Zinc, but not Se was also found higher in renal tissue of adult brown bears (Lazarus et al., 2024b). The higher testicular Se levels in adult bears are consistent with findings showing increased Se during puberty, regulated by gonadotropic hormones, to meet the growing demand for the Se-containing enzyme GPx4 during sperm maturation (Behne et al., 1996; Ursini et al., 1999).

The underlying cause of higher epididymal Cu levels associated with smaller testis size observed in bears from this study is not apparent. Both Cu deficiency and excess can lead to cellular atrophy due to the inability of Cu to maintain oxidative balance (Bhardwaj et al., 2021). Assessing the adequacy of Cu levels in bears is challenging without established reference values for this species. However, we can confirm that the renal Cu values in majority of bears from this study fall within the range previously reported for this population (Lazarus et al., 2017). Additionally, the negative relationship between testicular size and Cu observed in brown bears may, to some extent, reflect an adaptive response to the accumulation of toxic metal(loid)s. Such accumulation could enhance the production of oxygen radicals, thereby triggering increased expression and activity of Cu-containing superoxide dismutase (SOD) enzymes or metallothioneins (MT), leading to greater Cu influx into epididymal tissue (Chen et al., 2020; Reglero et al., 2009; Valko et al., 2005). The Cu levels in the testes of bears from this study ranged from 3.20 to 11.5 mg/kg dry mass, which is comparable to levels reported in red deer (3.79–10.5 mg/kg dry mass; Reglero et al., 2009) and raccoons (median 6.4–7.5 mg/kg dry mass, recalculated from wet mass using a conversion factor of 6.25; Souza et al., 2013).

4.2. Variation drivers of testes mass and size – association with PCBs and PBDEs

None of the quantified PCBs and PBDEs in adipose tissue (Jagić et al., 2024) showed a significant impact on testicular mass in Croatian brown bears when modeled alongside biological and ecological factors. A considerable proportion (45 %) of the variation in testicular mass was attributed to age and season, whose impact was discussed earlier in this paper (section 4.1.). Body condition, previously reported to negatively affect PCB levels and positively influence testis mass (Williams et al., 2021), showed no effect on testis size or mass in the brown bears studied here. Interestingly, we found two indicator PCBs (−118 and −180) with opposing associations to testis size. A decrease in testis size related to organic pollutants has been associated with reduced diameters of seminiferous and epididymal tubules in ursids (Sonne et al., 2006), which are known to directly influence spermatogenic activity (Ahmad et al., 2003; Dixson and Anderson, 2004). The negative association of PCB-180 with testis size in this study aligns with reduced testosterone levels previously observed in polar bears (Oskam et al., 2003). In that same study, PCB-118 was not found to be associated with testosterone levels, although a general decline in testosterone was reported in relation to the sum of PCBs. Conversely, Sonne et al. (2006) reported negative associations between the sum of PBDEs, but not the sum of PCBs, and testicular length in polar bears. Organohalogenated compounds have been proposed to influence the size of reproductive organs by disrupting the hypothalamic-pituitary-gonadal axis, resulting in altered reproductive hormone levels (Oskam et al., 2003; Sonne, 2010).

5. Conclusions

Testicular mass and size of brown bears were used here for the first time to assess reproductive health in relation to chronic environmental exposure to metal(loid)s, PCBs, and PBDEs, while accounting for age, body condition, and season. We identified pollutant metal(loid)s (As, Hg, and Pb) and PCBs (PCB-118 and PCB-180) as important contributors to morphological variation in testes of bears, affected also by age and season. Greater mass and larger size of testes were noted when higher Se, but lower Mn and Cu levels were present in testicular and epididymal tissue. The confirmed accumulation of toxic metal(loid)s in testes, subject to species-specific differences, adds to the list of organs and systems affected by environmental pollutant exposure in free-ranging brown bears. Testicular morphometry is thus confirmed as a useful tool for postmortem assessment of reproductive health in wildlife, when adjusted for key biological and ecological factors. This research area would benefit from future studies incorporating histopathological examinations of male and female reproductive organs, effect biomarkers aligned with the most probable mechanisms of pollutant toxicity, reproductive hormone profiling, and assessments of sperm function in brown bears.

CRediT authorship contribution statement

Maja Lazarus: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Agnieszka Sergiel:** Writing – review & editing, Project administration, Funding acquisition, Data curation. **Ankica Sekovanić:** Validation, Methodology, Investigation, Data curation. **Maja Ferenčaković:** Writing – original draft, Visualization, Software, Methodology, Data curation. **Marija Dvoršćak:** Validation, Methodology, Investigation, Data curation. **Darija Klinčić:** Validation, Methodology, Investigation, Data curation. **Ena Oster:** Methodology, Investigation. **Slaven Reljić:** Writing – review & editing, Funding acquisition, Data curation. **Đuro Huber:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation.

Funding sources

Sample collection was conducted along field research benefitting from the following funding: “HUNT” project of the 7th Framework Programme for Research and Technological Development (212160), LIFE DINALP BEAR (LIFE13 NAT/SI/000550) and CARNIVORA DINARICA (INTERREG SI-HR 410), project no. 4230/17 funded by Plitvice Lakes National Park, project no. 2198-1-90-01/01-19-2 funded by Paklenica National Park, funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 665778 through National Science Centre in Poland, within the frames of project no. 2016/23/P/NZ9/03951 (BearHealth), EURONATUR, Bernd Thies Foundation, project no. 2020/04/X/NZ4/01327 (MINIATURA 4 call; year 2021) funded by the National Science Centre in Poland, project no. PAN.BFB.S.BDN.617.022.2021 (AnthroBear) supported by funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 847639, from the Ministry of Science and Higher Education, within the frames of PASIFIC call 1, and internal research funds of the Institute of Nature Conservation of Polish Academy of Sciences in Krakow, Poland. Analyses were partly supported by the Ministry of Science and Education of the Republic of Croatia through Institutional Funding, within the projects HumEnHealth, and EnvironPollutHealth funded by the European Union – NextGenerationEU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006), and performed using the facilities and equipment funded within the European Regional

Development Fund project KK.01.1.1.02.0007 "Research and Education Centre of Environmental Health and Radiation Protection – Reconstruction and Expansion of the Institute for Medical Research and Occupational Health". Funding source(s) had no involvement in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank the local hunters, experts, and all project members who assisted in the field during bear handling and sample collection. We also acknowledge the invaluable work of Dr. Tatjana Orct in element quantification.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.123383.

Data availability

Data will be made available on request.

References

Adedara, I.A., Abolaji, A.O., Awogbindin, I.O., Farombi, E.O., 2017. Suppression of the brain-pituitary-testicular axis function following acute arsenic and manganese co-exposure and withdrawal in rats. J. Trace Elem. Med. Biol. 39, 21–29. https://doi.org/10.1016/j.jtemb.2016.07.001.

Agency for Toxic Substances and Disease Registry (ATSDR), 2012. Toxicological Profile for Manganese. Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services, Atlanta, Georgia, USA.

Ahmad, S.U., Tariq, S., Jalali, S., Ahmad, M.M., 2003. Environmental pollutant Aroclor 1242 (PCB) disrupts reproduction in adult male rhesus monkeys (*Macaca mulatta*). Environ. Res. 93, 272–278. https://doi.org/10.1016/S0013-9351(03)00110-5.

Anyanwu, B.O., Orisakwe, O.E., 2020. Current mechanistic perspectives on male reproductive toxicity induced by heavy metals. J. Environ. Sci. Heal. Part C Toxicol. Carcinog. 38, 204–244. https://doi.org/10.1080/26896583.2020.1782116.

Apostoli, P., Kiss, P., Porru, S., Bonde, J.P., Vanhoorne, M., ASCLEPIOS study Group, 1998. Male reproductive toxicity of lead in animals and humans. Occup. Environ. Med. 55, 364–374. https://doi.org/10.1136/oem.55.6.364.

Arnold, T.W., 2010. Uninformative parameters and model selection using akaike's information criterion. J. Wildl. Manage. 74, 1175–1178. https://doi.org/10.2193/2009-367.

Arteaga-Silva, M., Arenas-Rios, E., Bonilla-Jaime, H., Damian- Matzumura, P., Limon-Morales, O., Hernandez-Rodriguez, J., Marquez-Aguiluz, D., 2021. Neuroendocrine effects of cadmium exposure on male reproductive functions. Front. Biosci. 26, 4895. https://doi.org/10.2741/4895.

Behne, D., Weiler, H., Kyriakopoulos, A., 1996. Effects of selenium deficiency on testicular morphology and function in rats. J. Reprod. Fertil. 106, 291–297. https://doi.org/10.1530/jrf.0.1060291.

Bhardwaj, J.K., Paliwal, A., Saraf, P., 2021. Effects of heavy metals on reproduction owing to infertility. J. Biochem. Mol. Toxicol. 35, e22823. https://doi.org/10.1002/jbt.22823.

Blottner, S., Frölich, K., Roelants, H., Streich, J., Tataruch, F., 1999. Influence of environmental cadmium on testicular proliferation in roe deer. Reprod. Toxicol. 13, 261–267. https://doi.org/10.1016/S0890-6238(99)00014-3.

Boitani, C., Puglisi, R., 2008. Selenium, a key element in spermatogenesis and male fertility. In: Cheng, C.Y. (Ed.), Molecular Mechanisms in Spermatogenesis. Landes Bioscience and Springer Science+Business Media, New York, NY, USA, pp. 65–73. https://doi.org/10.1007/978-0-387-09597-4.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Inference: a Practical Information-Theoretic Approach, second ed. Springer-Verlag, New York, NY, USA. https://doi.org/10.1007/b97636.

Castellanos, P., del Olmo, E., Fernández-Santos, M.R., Rodríguez-Estival, J., Garde, J.J., Mateo, R., 2015. Increased chromatin fragmentation and reduced acrosome integrity in spermatozoa of red deer from lead polluted sites. Sci. Total Environ. 505, 32–38. https://doi.org/10.1016/j.scitotenv.2014.09.087.

Cattet, M.R.L., Caulkett, N.A., Obbard, M.E., Stenhouse, G.B., 2002. A body-condition index for Ursids. Can. J. Zool. 80, 1156–1161. https://doi.org/10.1139/z02-103.

Chapron, G., Kaczensky, P., Linnell, J.D.C., von Arx, M., Huber, D., Andrén, H., López-Bao, J.V., Adamec, M., Álvares, F., Anders, O., Balčiauskas, L., Balys, V., Bedő, P., Bego, F., Blanco, J.C., Breitenmoser, U., Brøseth, H., Bufka, L., Bunikyte, R., Ciucci, P., Dutsov, A., Engleder, T., Fuxjäger, C., Groff, C., Holmala, K., Hoxha, B., Iliopoulos, Y., Ionescu, O., Jeremić, J., Jerina, K., Kluth, G., Knauer, F., Kojola, I., Kos, I., Krofel, M., Kubala, J., Kunovac, S., Kusak, J., Kutal, M., Liberg, O., Majić, A., Männil, P., Manz, R., Marboutin, E., Marucco, F., Melovski, D., Mersini, K., Mertzanis, Y., Mysłajek, R.W., Nowak, S., Odden, J., Ozolins, J., Palomero, G., Paunović, M., Persson, J., Potočnik, H., Quenette, P.-Y., Rauer, G., Reinhardt, I., Rigg, R., Ryser, A., Salvatori, V., Skrbinšek, T., Stojanov, A., Swenson, J.E., Szemethy, L., Trajçe, A., Tsingarska-Sedefcheva, E., Váňa, M., Veeroja, R., Wabakken, P., Wölfl, M., Wölfl, S., Zimmermann, F., Zlatanova, D., Boitani, L., 2014. Recovery of large carnivores in Europe's modern human-dominated landscapes. Science 346, 1517–1519. https://doi.org/10.1126/science.1257553.

Chen, J., Jiang, Y., Shi, H., Peng, Y., Fan, X., Li, C., 2020. The molecular mechanisms of copper metabolism and its roles in human diseases. Pflugers Arch. Eur. J. Physiol. 472, 1415–1429. https://doi.org/10.1007/s00424-020-02412-2.

Chen, Y., Liu, Q., Ma, Q., Maltby, L., Ellison, A.M., Zhao, Y., 2018. Environmental toxicants impair liver and kidney function and sperm quality of captive pandas. Ecotoxicol. Environ. Saf. 162, 218–224. https://doi.org/10.1016/j.ecoenv.2018.07.008.

Ciesielski, T.M., Sonne, C., Smette, E.I., Villanger, G.D., Styrishave, B., Letcher, R.J., Hitchcock, D.J., Dietz, R., Jenssen, B.M., 2023. Testosterone and persistent organic pollutants in East Greenland male polar bears (*Ursus maritimus*). Heliyon 9, e13263. https://doi.org/10.1016/j.heliyon.2023.e13263.

Damek-Poprawa, M., Sawicka-Kapusta, K., 2003. Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. Toxicology 186, 1–10. https://doi.org/10.1016/S0300-483X(02)00595-4.

de Angelis, C., Galdiero, M., Pivonello, C., Salzano, C., Gianfrilli, D., Piscitelli, P., Lenzi, A., Colao, A., Pivonello, R., 2017. The environment and male reproduction: the effect of cadmium exposure on reproductive functions and its implication in fertility. Reprod. Toxicol. 73, 105–127. https://doi.org/10.1016/j.reprotox.2017.07.021.

Dixson, A.F., Anderson, M.J., 2004. Sexual behavior, reproductive physiology and sperm competition in male mammals. Physiol. Behav. 83, 361–371. https://doi.org/10.1016/j.physbeh.2004.08.022.

Frković, A., Huber, D., Kusak, J., 2001. Brown bear litter sizes in Croatia. Ursus 12, 103–105.

Gomes-Silva, A.P., de Medeiros, P. da C., Silva, L.N., Araújo Santiago, M. da S., Perobelli, J.E., 2023. Exposure to manganese during sertoli cell formation and proliferation disturbs early testicular development in rats. Reprod. Toxicol. 120, 108447. https://doi.org/10.1016/j.reprotox.2023.108447.

Guimarães-Ervilha, L.O., Assis, M.Q., Iasbik-Lima, T., da Silva Bento, I.P., Machado-Neves, M., 2025. Could the effect of arsenic on the testis be reversed after removing the insult? A meta-analysis study. Biol. Trace Elem. Res. https://doi.org/10.1007/s12011-025-04513-4. Epub ahead of print.

Heidari, A.H., Zamiri, M.J., Nazem, M.N., Jafarzadeh Shirazi, M.R., Akhlaghi, A., Ansari Pirsaraei, Z., 2021. Detrimental effects of long-term exposure to heavy metals on histology, size and trace elements of testes and sperm parameters in Kermani Sheep. Ecotoxicol. Environ. Saf. 207, 111563. https://doi.org/10.1016/j.ecoenv.2020.111563.

Howell-Skalla, L.A., Bunick, D., Nelson, R.A., Bahr, J.M., 2000. Testicular recrudescence in the male black bear (*Ursus americanus*): changes in testicular luteinizing hormone-, follicle-stimulating hormone-, and prolactin-receptor ribonucleic acid abundance and dependency on prolactin. Biol. Reprod. 63, 440–447. https://doi.org/10.1095/biolreprod63.2.440.

Howell-Skalla, L.A., Cattet, M.R.L., Ramsay, M.A., Bahr, J.M., 2002. Seasonal changes in testicular size and serum LH, prolactin and testosterone concentrations in male polar bears (*Ursus maritimus*). Reproduction 123, 729–733. https://doi.org/10.1530/rep.0.1230729.

Huber, Đ., Bišćan, A., Reljić, S., Domazetović, Z., Frković, A., Majnarić, D., Majić-Skrbinšek, A., Sindičić, M., Šprem, N., Modrić, M., Lipošćak, M., Žuglić, T., 2019. Brown bear management plan for the Republic of Croatia [Plan gospodarenja smeđim medvjedom (*Ursus arctos* L.) u Republici Hrvatskoj (in Croatian)]. Ministry of Agriculture – Directorate of Forestry, Hunting and Wood Industry and Ministry of Environmental Protection and Energy, Directorate for Nature Protection, Zagreb, Croatia, pp. 1–104. https://poljoprivreda.gov.hr/UserDocsImages/dokumenti/sume/gospodarenje_divljaci/Plan%20gospodarenja%20medvjedom%202019_final.pdf.

Huber, Đ., Jakšić, Z., Frković, A., Štahan, Ž., Kusak, J., Majnarić, D., Grubešić, M., Kulić, B., Sindičić, M., Skrbinšek, A.M., Lay, V., Ljuština, M., Zec, D., Laginja, R., Francetić, I., 2008. Brown bear management plan for the Republic of Croatia. Ministry of Regional Development, Forestry and Water Management, Directorate for Hunting and Ministry of Culture, Directorate for the Protection of Nature. Zagreb, Croatia, pp. 1–89. https://poljoprivreda.gov.hr/UserDocsImages/dokumenti/sume/smedji_medvjed/Plan_gospodarenja_smedim_medvjedom_engleski.pdf.

Jagić, K., Dvoršćak, M., Sergiel, A., Oster, E., Lazarus, M., Klinčić, D., 2024. First data on polybrominated diphenyl ethers and temporal trend of polychlorinated biphenyls in European brown bear as a bioindicator species. Chemosphere 362, 142637. https://doi.org/10.1016/j.chemosphere.2024.142637.

Kaczensky, P., Ranc, N., Hatlauf, J., et al., 2024. Large carnivore distribution maps for Europe 2017 – 2022/23. Dryad. https://doi.org/10.5061/dryad.3xsj3txrc.

Knott, E.J., Bunnefeld, N., Huber, D., Reljić, S., Kereži, V., Milner-Gulland, E.J., 2014. The potential impacts of changes in bear hunting policy for hunting organisations in Croatia. Eur. J. Wildl. Res. 60, 85–97. https://doi.org/10.1007/s10344-013-0754-3.
Lazarus, M., Sergiel, A., Ferenčaković, M., Sekovanić, A., Reljić, S., Pađen, L., Janz, D.M., Oster, E., Zwijacz-Kozica, T., Zięba, F., Selva, N., Huber, Đ., 2024a. Trace element contaminants and endocrine status of European brown bears assessed using blood as a matrix. Sci. Total Environ. 957. https://doi.org/10.1016/j.scitotenv.2024.177486.
Lazarus, M., Sergiel, A., Orct, T., Ferenčaković, M., Tariba Lovaković, B., Žunec, S., Rašić, D., Oster, E., Reljić, S., Macur, J., Huber, Đ., 2024b. Apex carnivores coping with metal(loid) pollution and oxidative stress: biological and environmental drivers of variation in kidney of European brown bear. Environ. Pollut. 363, 125285. https://doi.org/10.1016/j.envpol.2024.125285.
Lazarus, M., Sergiel, A., Ferenčaković, M., Orct, T., Kapronczai, L., Pađen, L., Janz, D.M., Reljić, S., Zwijacz-Kozica, T., Zięba, F., Selva, N., Huber, Đ., 2023. Stress and reproductive hormones in hair associated with contaminant metal(loid)s of European brown bear (*Ursus arctos*). Chemosphere 325, 138354. https://doi.org/10.1016/j.chemosphere.2023.138354.
Lazarus, M., Orct, T., Sergiel, A., Vranković, L., Marijić, V.F., Rašić, D., Reljić, S., Aladrović, J., Zwijacz-Kozica, T., Zięba, F., Jurasović, J., Erk, M., Maślak, R., Selva, N., Huber, Đ., 2020. Metal(loid) exposure assessment and biomarker responses in captive and free-ranging European brown bear (*Ursus arctos*). Environ. Res. 183, 109166. https://doi.org/10.1016/j.envres.2020.109166.
Lazarus, M., Sekovanić, A., Orct, T., Reljić, S., Kusak, J., Jurasović, J., Huber, Đ., 2017. Apex predatory mammals as bioindicator species in environmental monitoring of elements in Dinaric Alps (Croatia). Environ. Sci. Pollut. Res. 24, 23977–23991. https://doi.org/10.1007/s11356-017-0008-0.
Machado-Neves, M., 2022. Effect of heavy metals on epididymal morphology and function: an integrative review. Chemosphere 291, 133020. https://doi.org/10.1016/j.chemosphere.2021.133020.
Massányi, P., Trandžík, J., Nad, P., Koréneková, B., Skalická, M., Toman, R., Lukáč, N., Strapák, P., Halo, M., Turčan, J., 2003. Concentration of copper, iron, zinc, cadmium, lead, and nickel in boar semen and relation to the spermatozoa quality. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 38, 2643–2651. https://doi.org/10.1081/ESE-120024453.
Matson, G., Van Daele, L., Goodwin, E., Aumiller, L., Reynolds, H., Hristienko, H., 1993. A laboratory manual for cementum age determination of Alaska Brown Bear first premolar teeth. Alaska Department of Fish and Game, Division of Wildlife Conservation, Anchorage, AK, U.S. State of Alaska. https://matsonslab.com/wp-content/uploads/2016/02/Alaska_Brown_Bear_First_Premolar_Teeth.pdf.
Meligy, A.M.A., Waheed, M.M., El-bahr, S.M., 2019. Effect of heavy metals arsenic, cadmium, and lead on the semen variables of dromedary camels (*Camelus dromedarius*). Anim. Reprod. Sci. 208, 106115. https://doi.org/10.1016/j.anireprosci.2019.106115.
Oskam, I.C., Ropstad, E., Dahl, E., Lie, E., Derocher, A.E., Wiig, Ø., Larsen, S., Wiger, R., Skaare, J.U., 2003. Organochlorines affect the major androgenic hormone, testosterone, in male polar bears (*Ursus maritimus*) at Svalbard. J. Toxicol. Environ. Heal. - Part A 66, 2119–2139. https://doi.org/10.1080/15287390390211342.
Palmer, S.S., Nelson, R.A., Ramsay, M.A., Stirling, I., Bahr, J.M., 1988. Annual changes in serum sex steroids in male and female black (*Ursus americanus*) and polar (*Ursus maritimus*) bears. Biol. Reprod. 38, 1044–1050. https://doi.org/10.1095/biolreprod38.5.1044.
Peraza, M.A., Ayala-Fierro, F., Barber, D.S., Casarez, E., Rael, L.T., 1998. Effects of micronutrients on metal toxicity. Environ. Health Perspect. 106 (Suppl. 1), 203–216. https://doi.org/10.1289/ehp.98106s1203. Suppl 1.
Rachamalla, M., Chinthada, J., Kushwaha, S., Putnala, S.K., Sahu, C., Jena, G., Niyogi, S., 2022. Contemporary comprehensive review on arsenic-induced male reproductive toxicity and mechanisms of phytonutrient intervention. Toxics 10, 744. https://doi.org/10.3390/toxics10120744.
Reglero, M.M., Taggart, M.A., Castellanos, P., Mateo, R., 2009. Reduced sperm quality in relation to oxidative stress in red deer from a lead mining area. Environ. Pollut. 157, 2209–2215. https://doi.org/10.1016/j.envpol.2009.04.017.
Renu, K., Madhyastha, H., Madhyastha, R., Maruyama, M., Vinayagam, S., Valsala Gopalakrishnan, A., 2018. Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity. Life Sci. 212, 37–58. https://doi.org/10.1016/j.lfs.2018.09.045.
Ribeiro, I.M., Viana, A.G. de A., Carvalho, R.P.R., Waddington, B., Machado-Neves, M., 2022. Could metal exposure affect sperm parameters of domestic ruminants? A meta-analysis. Anim. Reprod. Sci. 244, 107050. https://doi.org/10.1016/j.anireprosci.2022.107050.
Rodprasert, W., Toppari, J., Virtanen, H.E., 2021. Endocrine disrupting chemicals and reproductive health in boys and men. Front. Endocrinol. 12, 1–45. https://doi.org/10.3389/fendo.2021.706532.
Rodríguez-Estival, J., Mateo, R., 2019. Exposure to anthropogenic chemicals in wild carnivores: a silent conservation threat demanding long-term surveillance. Curr. Opin. Environ. Sci. Heal. 11, 21–25. https://doi.org/10.1016/j.coesh.2019.06.002.
Rodríguez-Jorquera, I.A., Vitale, N., Garner, L., Perez-Venegas, D.J., Galbán-Malagón, C.J., Duque-Wilckens, N., Toor, G.S., 2017. Contamination of the upper class: occurrence and effects of chemical pollutants in terrestrial top predators. Curr. Pollut. Reports 3, 206–219. https://doi.org/10.1007/s40726-017-0061-9.
Satarug, S., Gobe, G.C., Vesey, D.A., 2022. Multiple targets of toxicity in environmental exposure to low-dose cadmium. Toxics 10, 1–20. https://doi.org/10.3390/toxics10080472.
Scheuhammer, A.M., 1991. Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. Environ. Pollut. 71, 329–375. https://doi.org/10.1016/0269-7491(91)90036-V.
Skrbinšek, T., Konec, M., Bragalanti, N., Calderola, S., Groff, C., Huber, Đ., Majić Skrbinšek, A., Molinari-Jobin, A., Molinari, P., Rauer, G., Reljić, S., Pagon, N., Černe, R., Jelenčič, M., 2019. 2019 annual population status report for brown bears in northern Dinaric Mountains and south-eastern Alps. https://dinalpbear.eu/wp-content/uploads/2023/11/Population-Status-Report-2019_V1.1.FINAL_.pdf.
Sonne, C., 2010. Health effects from long-range transported contaminants in Arctic top predators: an integrated review based on studies of polar bears and relevant model species. Environ. Int. 36, 461–491. https://doi.org/10.1016/j.envint.2010.03.002.
Sonne, C., Dietz, R., Born, E.W., Riget, F.F., Leifsson, P.S., Bechshøft, T., Kirkegaard, M., 2007. Spatial and temporal variation in size of polar bear (*Ursus maritimus*) sexual organs and its use in pollution and climate change studies. Sci. Total Environ. 387, 237–246. https://doi.org/10.1016/j.scitotenv.2007.07.020.
Sonne, C., Leifsson, P.S., Dietz, R., Born, E.W., Letcher, R.J., Hyldstrup, L., Riget, F.F., Kirkegaard, M., Muir, D.C.G., 2006. Xenoendocrine pollutants may reduce size of sexual organs in East Greenland polar bears (*Ursus maritimus*). Environ. Sci. Technol. 40, 5668–5674. https://doi.org/10.1021/es060836n.
Souza, A.C.F., Machado-Neves, M., Bastos, D.S.S., Couto Santos, F., Guimarães Ervilha, L. O., Coimbra, J.L., de, P., Araújo, L. de S., Oliveira, L.L. de, Guimarães, S.E.F., 2021. Impact of prenatal arsenic exposure on the testes and epididymides of prepubertal rats. Chem. Biol. Interact. 333, 109314. https://doi.org/10.1016/j.cbi.2020.109314.
Souza, M.J., Ramsay, E.C., Donnell, R.L., 2013. Metal accumulation and health effects in raccoons (*Procyon lotor*) associated with coal fly ash exposure. Arch. Environ. Contam. Toxicol. 64, 529–536. https://doi.org/10.1007/s00244-012-9865-1.
Spörndly-Nees, E., Holm, L., van Beest, F.M., Fakhrzadeh, A., Ekstedt, E., Letcher, R., Magnusson, U., Desforges, J.P., Dietz, R., Sonne, C., 2019. Age and seasonal variation in testis and baculum morphology in East Greenland polar bears (*Ursus maritimus*) in relation to high concentrations of persistent organic pollutants. Environ. Res. 173, 246–254. https://doi.org/10.1016/j.envres.2019.03.036.
Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in humans and wildlife. Crit. Rev. Toxicol. 39, 228–269. https://doi.org/10.1080/10408440802233259.
Tariba Lovaković, B., 2020. Cadmium, arsenic, and lead: elements affecting male reproductive health. Curr. Opin. Toxicol. 19, 7–14. https://doi.org/10.1016/j.cotox.2019.09.005.
Tsubota, T., Howell-Skalla, L., Nitta, H., Osawa, Y., Mason, J.I., Meiers, P.G., Nelson, R. A., Bahr, J.M., 1997. Seasonal changes in spermatogenesis and testicular steroidogenesis in the male black bear *Ursus americanus*. J. Reprod. Fertil. 109, 21–27. https://doi.org/10.1530/jrf.0.1090021.
Ullah, S., Ennab, W., Wei, Q., Wang, C., Quddus, A., Mustafa, S., Hadi, T., Mao, D., Shi, F., 2023. Impact of cadmium and lead exposure on camel testicular function: environmental contamination and reproductive health. Animals 13. https://doi.org/10.3390/ani13142302.
Ursini, F., Heim, S., Kiess, M., Maiorino, M., Roveri, A., Wissing, J., Flohé, L., 1999. Dual function of the selenoprotein PHGPx during sperm maturation. Science 285, 1393–1396. https://doi.org/10.1126/science.285.5432.1393.
Valko, M., Morris, H., Cronin, M., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12, 1161–1208. https://doi.org/10.2174/0929867053764635.
Vested, A., Giwercman, A., Bonde, J.P., Toft, G., 2014. Persistent organic pollutants and male reproductive health. Asian J. Androl. 16, 71–80. https://doi.org/10.4103/1008-682X.122345.
Vihnanek Lazarus, M., Sekovanić, A., Kljaković-Gašpić, Z., Orct, T., Jurasović, J., Kusak, J., Reljić, S., Huber, Đ., 2013. Cadmium and lead in grey wolf liver samples: optimisation of a microwave-assisted digestion method. Arh. Hig. Rada Toksikol. 64, 395–403. https://doi.org/10.2478/10004-1254-64-2013-2323.
Vuarin, P., Rey, B., Herrada, A., François, D., Crini, N., Scheifler, R., Fritsch, C., Lemaître, J., Saïd, S., 2025. Exposure to toxic trace metals is negatively associated with testis mass in wild male roe deer. Environ. Pollut. 383, 126825. https://doi.org/10.1016/j.envpol.2025.126825.
White, D., Berardinelli, J.G., Aune, K.E., 2005a. Seasonal differences in spermatogenesis, testicular mass and serum testosterone concentrations in the grizzly bear. Ursus 16, 198–207. https://doi.org/10.2192/1537-6176(2005)016[0198:SDISTM]2.0.CO;2.
White, D., Berardinelli, J.G., Aune, K.E., 2005b. Age variation in gross and histological characteristics of the testis and epididymis in grizzly bears. Ursus 16, 190–197. https://doi.org/10.2192/1537-6176(2005)016[0190:AVIGAH]2.0.CO;2.
Williams, R.S., Curnick, D.J., Brownlow, A., Barber, J.L., Barnett, J., Davison, N.J., Deaville, R., ten Doeschate, M., Perkins, M., Jepson, P.D., Jobling, S., 2021. Polychlorinated biphenyls are associated with reduced testes weights in harbour porpoises (*Phocoena phocoena*). Environ. Int. 150, 106303. https://doi.org/10.1016/j.envint.2020.106303.
Wirth, J.J., Mijal, R.S., 2010. Adverse effects of low level heavy metal exposure on male reproductive function. Syst. Biol. Reprod. Med. 56, 147–167. https://doi.org/10.3109/19396360903582216.
Zečević, N., Kocić, J., Perović, M., Stojsavljević, A., 2025. Detrimental effects of cadmium on male infertility: a review. Ecotoxicol. Environ. Saf. 290. https://doi.org/10.1016/j.ecoenv.2024.117623.
Zhu, Q., Li, X., Ge, R.S., 2020. Toxicological effects of cadmium on mammalian testis. Front. Genet. 11, 1–9. https://doi.org/10.3389/fgene.2020.00527.
Zhu, X., Kusaka, Y., Sato, K., Zhang, Q., 2000. The endocrine disruptive effects of mercury. Environ. Health Prev. Med. 4, 174–183. https://doi.org/10.1007/BF02931255.