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# Telomere length in relation to persistent organic pollutant exposure in white-tailed eagle (*Haliaeetus albicilla*) nestlings from Sweden sampled in 1995–2013

Elisabeth Hansen<sup>a,\*</sup>, Tove Skotnes<sup>a,b,1</sup>, Jan Ove Bustnes<sup>b</sup>, Björn Helander<sup>c</sup>, Igor Eulaers<sup>d</sup>, Jiachen Sun<sup>e</sup>, Adrian Covaci<sup>f</sup>, Bård-Jørgen Bårdsen<sup>b</sup>, Sandrine Zahn<sup>g</sup>, Francois Criscuolo<sup>g</sup>, Sophie Bourgeon<sup>a</sup>

<sup>a</sup> UiT – the Arctic University of Norway, Department of Arctic and Marine Biology, Hansine Hansens Veg 18, NO-9019 Tromsø, Norway

<sup>b</sup> Norwegian Institute for Nature Research (NINA), Framcenteret, Hjalmar Johansens Gate 14, NO-9296 Tromsø, Norway

<sup>c</sup> Swedish Museum of Natural History, Department of Environmental Research and Monitoring, Box 50007, SE-10405 Stockholm, Sweden

<sup>d</sup> Department of Bioscience, Aarhus University, Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark

<sup>e</sup> School of Environment, Jinan University, West Huangpu Avenue 601, 510632 Guangzhou, Guangdong, China

<sup>f</sup> Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, BE-2610 Wilrijk, Belgium

<sup>g</sup> Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

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## ABSTRACT

Telomeres are used as biomarkers of vertebrate health because of the link between their length, lifespan, and survival. Exposure to environmental stressors appears to alter telomere dynamics, but little is known about telomere length and persistent organic pollutant (POP) exposure in wildlife. The white-tailed eagle (WTE; *Haliaeetus albicilla*) is an avian top predator that accumulates high levels of POPs and may subsequently suffer adverse health effects. Here we study the Baltic WTE population that is well documented to have been exposed to large contaminant burdens, thereby making it a promising candidate species for analyzing pollutant-mediated effects on telomeres. We investigated telomere lengths in WTE nestlings ( $n = 168$ ) over 19 years and examined legacy POP concentrations (organochlorines and polybrominated diphenyl ethers) in whole blood and serum as potential drivers of differences in telomere length. Although we detected significant year-to-year variations in telomere lengths among the WTE nestlings, telomere lengths did not correlate with any of the investigated POP concentrations of several classes. Given that telomere lengths did not associate with POP contamination in the Baltic WTE nestlings, we propose that other environmental and biological factors, which likely fluctuate on a year-to-year basis, could be more important drivers of telomere lengths in this population.

## 1. Introduction

Telomeres are repeated sequences of non-coding DNA that cap the ends of chromosomes and play an important role in stabilizing and protecting the coding sequences in eukaryote genomes (Blackburn, 1991). Telomeres shorten with every cell division, with the length eventually shortening to such an extent that the process triggers cell senescence (Angelier et al., 2018; Blackburn, 2000; Louzon et al., 2019). There are relationships between increased rates of telomere shortening and sub-optimal environmental conditions such as dramatic changes in

climate (Mizutani et al., 2013), warmer and wetter springs (van Lieshout et al., 2021), unfavorable natal or wintering conditions (Angelier et al., 2013; Watson et al., 2015), and low adult habitat quality (Apfelbeck et al., 2019; Wilbourn et al., 2017), possibly mediated through increased oxidative stress (the imbalance within an organism between free radicals' production and antioxidants availability or activity) (Haussmann and Marchetto, 2010; von Zglinicki, 2002). Contrasting environmental conditions can therefore translate into inter-individual variability in telomeres (Ibanez-Alamo et al., 2018; Kärkkäinen et al., 2019) further influencing lifespan (Giraudeau et al., 2019; Monaghan and Haussmann,

\* Corresponding author.

E-mail address: [elisabeth.hansen@uit.no](mailto:elisabeth.hansen@uit.no) (E. Hansen).

<sup>1</sup> Joint first authorship.

2006). Finally, as telomeres are robust, they allow a reliable retrospective analysis (Blackburn, 1991) to assess the impact of environmental stressors on wildlife.

The white-tailed eagle (hereafter referred to as WTE; *Haliaeetus albicilla*) is an avian top predator associated with aquatic habitats in many Eurasian countries (Cramp and Simmons, 1983). Due to its feeding habits and apex trophic position, the WTE accumulates high levels of persistent organic pollutants (POPs) and may therefore suffer detrimental health effects (Helander, 1985; Helander et al., 1982, 2002; Letcher et al., 2010; Sonne et al., 2020). For example, the Swedish WTE population on the Baltic coastline has suffered reproductive failure and population decline during the 1950s until the 1980s as a consequence of contamination by legacy POPs (Helander, 1985; Helander et al., 1982, 2002). Poor breeding success has also been reported for other WTE populations in the Baltic Sea region (Koivusaari et al., 1980; Scharenberg and Struwe-Juhl, 2006). Thus the WTE represents a species that is useful for examination of possible detrimental impacts of exposure to environmental POPs (Helander et al., 2008).

Although the production and use of many POPs (particularly organochlorine compounds) were banned or restricted decades ago, they remain highly present in the environment and biota (AMAP, 2016). Even low levels of POPs may induce sub-lethal health effects, such as endocrine disruption (Monclus et al., 2018; Nordstad et al., 2012; Verboven et al., 2010), immune system impairment (Bustnes et al., 2004; Hansen et al., 2020; Sagerup et al., 2009), and oxidative stress (Costantini et al., 2014; Fenstad et al., 2016; Sletten et al., 2016). While the relationship between POP exposure and telomere lengths has been investigated in some avian species (Blevin et al., 2016, 2017; Sebastiano et al., 2020; Sletten et al., 2016), findings are often inconsistent or inconclusive (Louzon et al., 2019), and wildlife ecotoxicology lacks long-term studies on how telomere lengths may fluctuate both over time and in relation to POP exposure.

In the present study, we examined telomere lengths and POP concentrations in WTE nestlings from the Swedish Baltic coast sampled across 19 years (1995–2013). In nestlings, telomere dynamics are less confounded by biological age and life-long exposure to other environmental factors, and are thus better suited than adults to examine the associations between telomere lengths and POP exposure (Boonekamp et al., 2014; Eastwood et al., 2019; Heidinger et al., 2012; Wood and Young, 2019). The current study aimed (1) to examine year-to-year variations in telomere lengths among the WTE nestling cohorts, and (2) to investigate the relationship between POP concentrations and telomere lengths.

## 2. Materials and methods

### 2.1. Field sampling

Sampling of WTE nestlings was carried out on the Baltic coast of Sweden at nine different locations (58°–61°N, 16°–19°E, Fig. 1) in the years 1995–2013 as part of the Swedish Environmental Monitoring Programme (Helander et al., 2008). During March–April, nests were inspected for breeding activity and from mid-May to mid-June, when the nestlings were 4–8 weeks post-hatching, all occupied nests were visited (Helander et al., 2008). There were 1–3 chicks per nest and all nestlings in a brood were ringed and measured for body mass [kg] and wing length [mm]. One specimen per nest was blood sampled (<10 mL) from the brachial vein using a 10 mL syringe with a 25 × 0.6 mm needle. From all samples, a small amount of whole blood (0.5–1.0 mL) was transferred directly to an EDTA-coated syringe and used in the present study for telomere length analysis and sexing. For samples from 1995 to 2002 the remaining blood sample was stored as whole blood, while for samples from 2003 to 2013 the remaining blood sample was centrifuged (2000 rpm for 10 min), separating serum from red blood cells. All blood

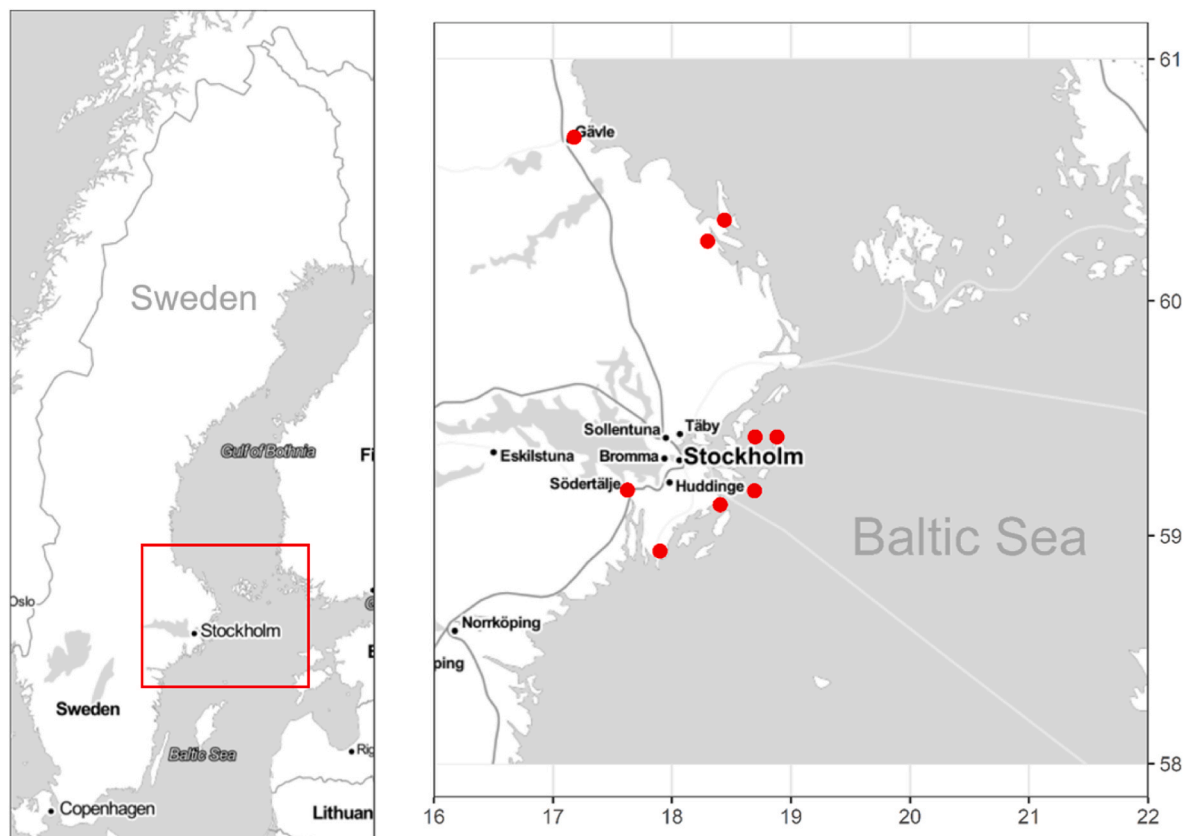


Fig. 1. Baltic coastline of Sweden indicating locations (red circles) where white-tailed eagle nestlings were sampled in 1995–2013. Labelled place names are included for regional context. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(fraction) samples were kept cool in an icebox for short transport in the field and transferred to a freezer ( $-20\text{ }^{\circ}\text{C}$ ) or liquid nitrogen the same day, and subsequently stored at  $-80\text{ }^{\circ}\text{C}$  in the national specimen bank until laboratory analysis. In total, 184 WTE nestlings were blood sampled and morphometrically measured during the study period. Due to differing amounts of individual blood samples, 168 were analyzed for telomere lengths and molecular sexing. Of these, 152 were also analyzed for POPs (whole blood 1995–2002,  $n = 71$ ; serum 2003–2013,  $n = 81$ ).

## 2.2. Molecular sexing analysis

Sex determination of nestlings was performed at IPHC-DEPE (CNRS, France) using the PCR and gel electrophoresis protocols adapted from Helander et al. (2007). In brief, DNA was extracted from whole blood using a commercial kit (NucleoSpin® Blood QuickPure, Macherey Nagel, Germany) and the sections of the sex-linked chromo-helicase-DNA-binding gene (CHD-Z and CHD-W) were amplified by polymerase chain reaction using primers 2550 F and 2718R (Fridolfsson and Ellegren, 1999).

## 2.3. Telomere length analysis

Analysis of telomere length was performed at IPHC-DEPE (CNRS, France) using the qPCR procedure described by Criscuolo et al. (2009) adapted from a human-telomere qPCR methodology published by Cawthon (2002). The description of blood extraction and qPCR amplification conditions of WTE telomeres was previously described in further detail in Sletten et al. (2016). Briefly, genomic DNA was extracted from 5  $\mu\text{L}$  of frozen red blood cells, using an adapted protocol of the commercial kit (NucleoSpin® Blood Quickpure, Macherey Nagel, Germany). Due to the age of the samples (over 19 years of storing) an extreme care was required to assess DNA quantity and quality (using Nanodrop, Thermo Fisher Scientific, USA; and fluorescence, QuantiFluor® ONE dsDNA System, Promega, USA) and no DNA degradation (using gel-migration). Relative telomere length (T/S ratio) were obtained using a ratio of the numbers of amplification cycles between a genomic control gene (S), and the telomeric sequences (T).

Because of the duration of this longitudinal study (nearly 20 years), we have, prior to the measurement of relative telomere length, checked if there is no efficiency variation during the sampling years for the telomeric and the control genes ( $E_{\text{Control}} = 0,992 \pm 0,003$  and  $E_{\text{TEL}} = 0,997 \pm 0,004$  (mean  $\pm$  SE)). All samples were measured in one 384-well thermocycler (CFX-384 Touch Real-Time PCR Detection System, Biorad, USA) using the same amplification conditions for both the control gene and telomere sequences. All samples were used in duplicates, and the qPCR run contained a no template control (distilled water used for DNA dilution) and a calibrator sample dilution curve (issue from a pool of one sample, randomly chosen, per sampling years) to calculate the amplification efficiencies (0.998 for control and 0.996 for telomere amplifications). The qPCR run ended with a melting curve to control for unexpected non-specific amplification artefacts. Relative telomere lengths were calculated following Pfaffl (2001). Intra-run variation of T/S ratio was 0.792, as evaluated using intraclass correlation coefficient (Allyssa R. et al., 2021; Cicchetti, 1994).

## 2.4. POP analysis

POPs were analyzed at the Toxicological Centre, University of Antwerp (Belgium) using whole blood ( $n = 71$ ; 1995–2002) or serum ( $n = 81$ ; 2003–2013). The methods used to analyze organochlorines (polychlorinated biphenyls [PCB] and pesticides) or polybrominated diphenyl ethers (PBDEs) are described in further detail by Eulaers et al. (2011) and Covaci and Voorspoels (2005), respectively. Targeted compounds were 28 PCB congeners (28, 49, 52, 66, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 177, 180, 183, 187, 194, 196/203, 199, 206, and 209), dichlorodiphenyltrichloroethane (*p*,

*p'*-DDT) and its metabolites *p,p'*-DDE and *p,p'*-DDD, five chlordanes (*cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs;  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH), seven PBDE congeners (28, 47, 99, 100, 153, 154, and 183) and two PBDE metabolites (6-MeO-BDE-47 and 2-MeO-BDE-68). POP concentrations are expressed as  $\text{ng mL}^{-1}$  wet weight (*ww*) for whole blood and serum.

Analytical quality assessment and control was supported by the analysis of procedural blanks and addition of internal standard solutions (CB 143,  $\epsilon$ -HCH, BDE 77 and 13C-HCB) to each sample. Every 21 samples, two procedural blanks and one standard reference material were analyzed. Regular analysis of plasma reference material (AM-S-W1705) under the inter-laboratory comparison exercises organized by the Arctic Monitoring and Assessment Programme reveal that the obtained precision is typically less than 20%. All contaminant concentrations were corrected for blank values, and the limit of quantification (LOQ) was set at 3 times the standard deviation of the blank concentrations or determined to be at a 10:1 signal to noise ratio for compounds not detectable in the blanks. The LOQs for the analyzed compounds ranged from 0.02 to 0.10  $\text{ng mL}^{-1}$  *ww*. Mean internal standard recoveries of the internal standards PCB 143,  $\epsilon$ -HCH, and BDE 77 were  $86 \pm 6\%$ ,  $98 \pm 8\%$ , and  $94 \pm 8\%$ , respectively.

## 2.5. Data analysis

Statistical analyses were performed using R version 3.6.1 (R Development Core Team, 2020). To determine whether the statistical models fulfilled their underlying assumptions, we conducted visual inspection of residual plots and normal QQ-plots (Zuur et al., 2010). All tests were two-tailed and the null hypothesis was rejected at an  $\alpha$ -level of 0.05.

Since the same WTE nests were sampled multiple times across the study period, we first chose linear mixed-effect (LME) models in our analyses with Nest ID as the random factor (a random intercepts only) to estimate and account for among-nest variability using the nlme-package (Pinheiro et al., 2017; Pinheiro and Bates, 2000). However, since the random factor in the LME revealed low among-nest variability (i.e., the random intercept variance; results not shown), we also fitted generalized least square (GLS; function 'gls') models (i.e., without the random effect). We compared the model fit of the LME against the GLS based on the second order Akaike Information Criterion (AICc) (Anderson, 2008; Burnham et al., 2002). Namely, the final and selected LME models in our analyses were compared with their GLS equivalent, and the model with the lowest AICc (i.e. the most parsimonious model) was chosen for inference (Zuur et al., 2009).

First, we estimated differences in telomere length among WTE cohorts using a GLS with year (19-level factor and 1995 as baseline year) as a fixed effect. Next, we assessed POP concentrations as drivers of individual telomere length. To do so, we first divided the dataset into whole blood and serum POP concentration samples since these measures are not directly comparable (Batterman et al., 2016). Our statistical analyses only included the responses where POPs were present in >75% of the samples (Table S1), and levels below LOQ were arbitrarily replaced by half the compound-specific LOQ value (Hansen et al., 2020). To reduce the number of compounds used in the statistical analyses, their linear relationships were investigated using Pearson's product moment correlation tests ( $r$ ; Figs. S1 and S2). Since many compounds were significantly correlated, we grouped them based on their commonness (i.e., belonging to the same chemical class) and physiochemical properties (Sletten et al., 2016) and summed the structurally similar compounds such as the PCBs, PBDEs, DDTs (*p,p'*-DDE and *p,p'*-DDD) and Nonachlors (CN and TN). Only HCB,  $\beta$ -HCH, and OxC were treated individually because of their particular physiochemical properties (Sletten et al., 2016). In total, seven groups of POPs were retained in the final analyses: the sum of 24 PCB congeners ( $\sum_{24}\text{PCBs}$ ), the sum of five PBDE congeners ( $\sum_{5}\text{PBDEs}$ ), the sum of two DDT metabolites ( $\sum\text{DDT}$ ), the sum of two Nonachlors ( $\sum\text{Nonachlor}$ ), HCB,  $\beta$ -HCH, and OxC. To attain the

assumption of normality, POP concentrations were ln-transformed prior to analyses (Zuur et al., 2007).

Thereafter, we created a set of *a priori* candidate LME models to investigate POPs as potential drivers of variation in telomere lengths among individual nestlings (Tables S2a and b) by using telomere length as the response variable and POP concentration (each group tested separately for whole blood and serum, respectively) as the explanatory variable. Wing length, sex (factor with female and male as levels) and brood size (factor with one, two and three chicks as levels) were included as additional predictors in the candidate models (Tables S2a and b). The latter three variables were included to control for important state variables expected to affect individual stress levels directly and telomere length indirectly. To avoid problems with multicollinearity, correlated variables were not placed in the same candidate models (Figs. S3a and b) (Zuur et al., 2009). A model selection of the set of candidate LME models was then performed using the *aictab* function in the AICcmodavg package (Mazzerolle, 2019) and the most parsimonious model explaining variation in telomere length was selected based on the lowest AICc ( $\Delta_i \leq 2$ ; Tables S2a and b) (Anderson, 2008; Burnham et al., 2002). Finally, we compared the selected LME with a GLS (Tables S3a–c); in all cases, the GLS models had the lowest AICc and were therefore chosen for inference.

### 3. Results

#### 3.1. Telomere lengths

The overall mean telomere length for all WTE nestling cohorts was  $1.03 \pm 0.03$  (mean  $\pm$  SE) and the individual values ranged from 0.16 to 2.30 (Fig. 2). There were differences in telomere lengths among annual cohorts with significantly longer telomeres being found in 1998–2002, 2004 and 2006 compared to the first year of sampling, 1995 (Fig. 2, Table S4,  $p < 0.05$  in all cases). The shortest telomeres were recorded in 1996 (Fig. 2), but they did not differ significantly from 1995 (Fig. 2, Table S4,  $p = 0.50$ ).

#### 3.2. POP concentrations in whole blood and serum

Concentrations of POPs included in the statistical analyses are presented in Table 1.  $\Sigma_{24}$ PCBs made up the majority of the investigated

POPs and represented 82% of the total concentration in both whole blood and serum (Table 1). Among the organochlorine pesticides (*p,p'*-DDD, *p,p'*-DDE, HCB, OxC, CN, TN, and  $\beta$ -HCH), *p,p'*-DDE contributed with the highest levels and represented 13.5% and 15% of the total whole blood and serum concentrations, respectively (Table 1).  $\Sigma_5$ PBDEs represented less than 1% of the total POP concentration in both matrices (Table 1).

#### 3.3. Relationship between telomere lengths and POP concentrations

Overall, we detected no significant relationships between telomere length and POP concentrations in either whole blood or serum in the WTE nestlings (Fig. 3, Tables S5a and b:  $p > 0.20$  in all cases). While wing length was the only additional predictor variable that was retained in some models (whole blood POP concentrations), it was never significantly correlated with telomere length (Table S5a:  $p > 0.08$  in all cases). Sex and brood size were never retained in any of the models, which indicates that neither sex nor brood size were important predictor variables explaining variation in telomere lengths in the WTE nestlings.

## 4. Discussion

#### 4.1. Telomere length in relation to POP concentrations

Based on the reported links between telomere lengths, fitness, longevity, and survival, telomeres could represent useful biomarkers of environmental stressors in wildlife research (Monaghan, 2014). While telomere erosion has been related to pollution exposure in humans (Barbullushi et al., 2009; De Felice et al., 2012; Hoxha et al., 2009; Zhang et al., 2013), only four studies have investigated the relationship between telomere length and POP concentrations in avian wildlife (Blevin et al., 2016, 2017; Sebastiano et al., 2020; Sletten et al., 2016). In the current study, we found no relationships between telomere lengths and POP concentrations in nestling WTEs, despite examining 152 individuals from a highly polluted population over a period of 19 years (1995–2013). Similar to our study, in a Norwegian population of WTE nestlings showing telomere lengths within the same range as ours but overall lower POP concentrations (on average twice lower for the dominating compounds), Sletten et al. (2016) found no association between telomere length and plasma POPs. In adult black-legged

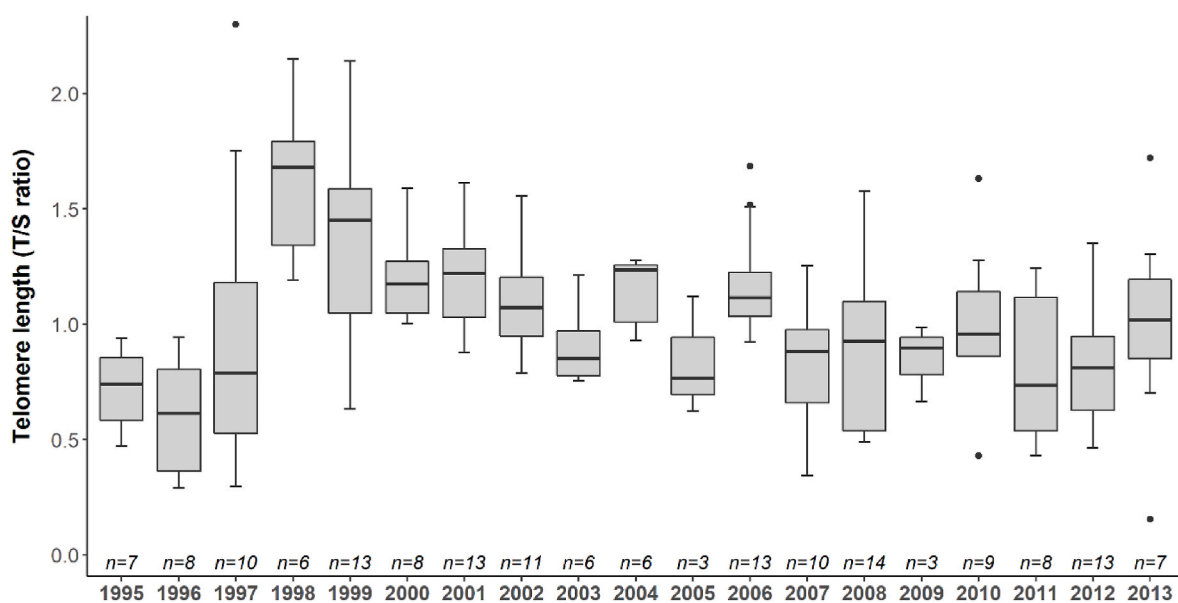


Fig. 2. Boxplots showing the median, 25th–50th quartiles (boxes) and range (whiskers) in the observed telomere lengths among white-tailed eagle nestling cohorts sampled at the Swedish Baltic coast during 1995–2013. Black dots represent extreme values and are plotted individually. Annual sample size ( $n$ ) is indicated above the respective year on the graph.



**Table 1**

Descriptive statistics (mean  $\pm$  SE, median, range [min-max]) for legacy persistent organic pollutant concentrations (ng mL<sup>-1</sup> ww) in white-tailed eagle (*Haliaeetus albicilla*) nestlings sampled at the Swedish Baltic coast during 1995–2002 (whole blood;  $n = 71$ ) and 2003–2013 (serum;  $n = 81$ ).

	Whole blood			Serum		
	Mean	Median	Min-max	Mean	Median	Min-max
$p,p'$ -DDD	0.33 $\pm$ 0.02	0.27	0.02–1.04	0.39 $\pm$ 0.04	0.27	0.05–3.10
$p,p'$ -DDE	7.55 $\pm$ 0.48	6.56	1.02–20.5	14.2 $\pm$ 0.86	12.4	3.16–38.5
$\Sigma$ DDT <sup>a</sup>	7.90 $\pm$ 0.48	6.96	1.07–20.9	14.6 $\pm$ 0.87	12.6	3.33–39.1
HCB	0.55 $\pm$ 0.05	0.49	0.09–3.10	0.64 $\pm$ 0.05	0.51	0.11–2.35
OxC	0.16 $\pm$ 0.01	0.12	0.03–0.44	0.23 $\pm$ 0.01	0.20	0.03–0.58
TN	0.23 $\pm$ 0.01	0.22	0.03–0.49	0.35 $\pm$ 0.02	0.30	0.12–1.19
CN	0.10 $\pm$ 0.01	0.10	0.03–0.22	0.16 $\pm$ 0.01	0.14	0.03–0.53
$\Sigma$ Nonachlor <sup>b</sup>	0.33 $\pm$ 0.02	0.32	0.05–0.69	0.51 $\pm$ 0.03	0.45	0.05–1.71
$\beta$ -HCH	0.41 $\pm$ 0.03	0.38	0.03–1.01	0.53 $\pm$ 0.05	0.48	0.03–2.96
$\Sigma_{24}$ PCBs <sup>c</sup>	45.7 $\pm$ 2.42	39.9	9.54–116	76.0 $\pm$ 4.96	66.5	21.4–241
$\Sigma_5$ PBDEs <sup>d</sup>	0.48 $\pm$ 0.03	0.45	0.07–1.19	0.68 $\pm$ 0.11	0.49	0.19–4.94

<sup>a</sup> Sum of  $p,p'$ -DDD and  $p,p'$ -DDE (see Table S1 for full names and abbreviations).

<sup>b</sup> Sum of TN and CN (see Table S1 for full names and abbreviations).

<sup>c</sup> Sum of 24 congeners (see Table S1 for full names and abbreviations).

<sup>d</sup> Sum of 5 congeners (see Table S1 for full names and abbreviations).

kittiwakes (*Rissa tridactyla*), however, shorter telomeres were associated with higher whole blood OxC levels, but in females only (Blevin et al., 2016). Contrary to OxC, plasma concentrations of perfluoroalkyl substances (PFAS) were positively correlated to telomere lengths in both adult black-legged kittiwakes and glaucous gulls (*Larus hyperboreus*), which indicates that individuals with the highest PFAS concentrations also had longer telomeres (Blevin et al., 2017; Sebastiano et al., 2020). The seemingly disparate findings might be a result of measuring different POP classes (lipophilic organochlorines vs. proteinophilic PFAS) in various study species sampled at different life-history stages. The discrepancies between studies could also stem from different magnitude of POP concentrations detected in the birds (e.g., lower in this study compared to Blevin et al. (2016)).

In the studied population, levels of legacy POPs measured in eggs and feathers from adult WTEs have been steadily decreasing over the 20 years prior to our study period (Helander et al., 2008; Sun et al., 2020), leading to improved breeding success since the 1980s (Helander et al., 2002). In the current study, sampling started in 1995 when DDE and PCB levels were below the thresholds for effects on reproduction (Bignert and Helander, 2015), which could explain the lack of relationships between telomere length and POP concentrations in our study.

Despite not highlighting clear links between telomere length and different POP classes, our study has two major strengths. First, by investigating nestlings whose telomere attrition is greater compared to

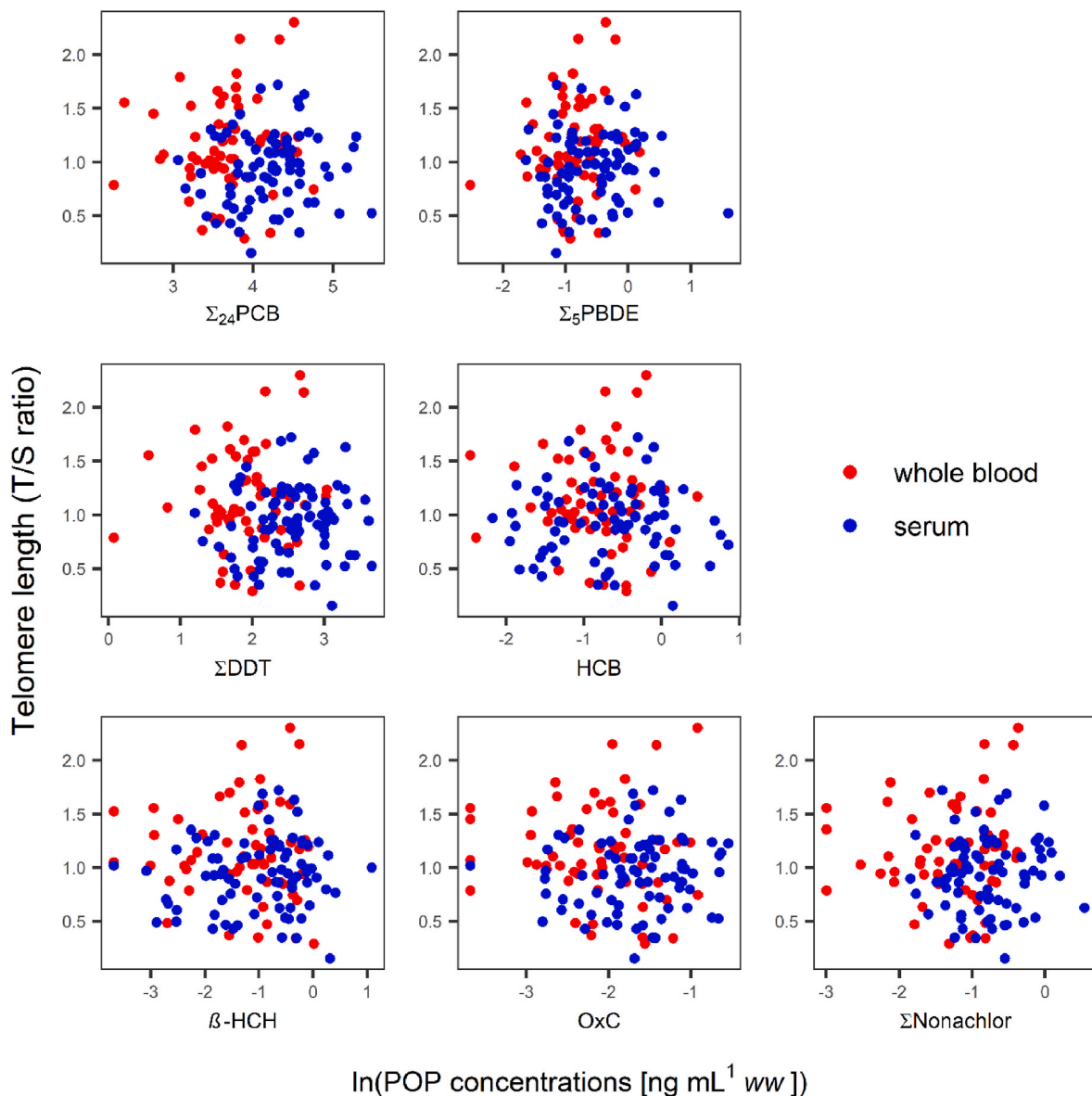
adults (Angelier et al., 2018; Boonekamp et al., 2014; Eastwood et al., 2019; Heidinger et al., 2012; Wood and Young, 2019), our study maximizes the potential of highlighting relationships while reducing the confounding effect of biological age and past exposure towards multiple environmental factors on telomere lengths (Dugdale and Richardson, 2018). Secondly, the long timeseries we analyze decreases the probability of reporting an apparent correlation with telomere length that is really due to coincidental inter annual variation between cohorts.

#### 4.2. Year-to-year variation in telomere lengths

While telomere lengths did not vary with POP concentrations, they did differ on a year-to-year basis among the WTE nestling cohorts. Telomere length at a given time is a result of the initial telomere length (i.e., inherited from the parental gametes) combined with variable shortening from both cell divisions and exposure to stressors (Angelier et al., 2018; Blackburn, 2000; Houben et al., 2008). For example, environmental conditions (e.g. food availability and habitat quality) are suggested as important factors that drive telomere dynamics in wildlife (Foote et al., 2011; Hall et al., 2004; Heidinger et al., 2012; Spurgin et al., 2018). In a longitudinal study on Seychelles warblers (*Acrocephalus sechellensis*), Spurgin et al. (2018) found positive associations between telomere length and food availability (insect abundance) but not body mass. Conditions experienced during early life such as parental care (Viblanco et al., 2020) and sibling competition (Dupont et al., 2018; Nettle et al., 2013, 2015; Watson et al., 2015) also appear to determine telomere dynamics (Monaghan and Ozanne, 2018). We could not measure food availability in the current study, but we did not observe significant relationships between telomere lengths and brood size that could serve as a proxy of food provisioning and/or environmental conditions. Environmental and early life conditions likely fluctuate among years and may therefore give rise to year-to-year variations in telomere lengths in WTE nestlings. Among factors influencing within-species variations in telomere length, Monaghan and Ozanne (2018) pointed out early life conditions as having long-term consequences for health and longevity. Although telomere lengths ranged from 0.16 to 2.30 (T/S ratio) in WTE nestlings of the current study, it remains to be established whether these inter-individual differences can be biologically and ecologically meaningful.

#### 4.3. Telomere length as a biomarker of health in a multiple stressor context

Conducting wildlife ecotoxicological studies in a multiple stressor context has recently gained significant momentum (Bardsen et al., 2018; Bustnes et al., 2015), and allows for a holistic examination of factors that could drive biomarkers of health in wildlife. Nestlings undergo energy-demanding periods of rapid growth and development and may be particularly vulnerable to multiple stress exposure that could have a profound influence on telomere length (Powolny et al., 2020). However, disentangling potential drivers of telomere length appear challenging (Angelier et al., 2018; Dugdale and Richardson, 2018; Monaghan and Ozanne, 2018). Despite studying free-ranging raptor nestlings from a highly polluted population over 19 years, we did not detect a relationship between telomere length and legacy POP contamination in Baltic WTE nestlings. Instead, year-to-year variability in telomere lengths among nestling cohorts suggests that environmental conditions and/or biological drivers as discussed in section 4.2, which likely vary between years, were more important drivers during the study period (Chatelain et al., 2020). Studies show potential indirect effects of environmental stressors on telomere dynamics, which are mediated through physiological mechanisms that are important in maintaining homeostasis (Angelier et al., 2018; Monaghan and Ozanne, 2018; Powolny et al., 2020). Namely, increased oxidative stress and glucocorticoid levels (the major stress hormone in vertebrates) are linked to telomere dynamics (Angelier et al., 2018; Kawanishi and Oikawa, 2004; Lemaitre et al.,



**Fig. 3.** Relationships between telomere lengths (T/S ratio) and persistent organic pollutant (POP) concentrations in white-tailed eagle (*Haliaeetus albicilla*) nestlings sampled at the Swedish Baltic coast (all P-values > 0.05). The investigated POP compounds were the sum of 24 polychlorinated biphenyl congeners ( $\Sigma_{24}$ PCBs), the sum of five polybrominated diphenyl ether congeners ( $\Sigma_5$ PBDEs), the sum of two dichlorodiphenyltrichloroethane metabolites (*p,p'*-DDE and *p,p'*-DDD:  $\Sigma$ DDT), hexachlorobenzene (HCB),  $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH), oxychlorane (OxC), and the sum of *trans*- and *cis*-Nonachlor ( $\Sigma$ Nonachlor). Red dots represent whole blood POP concentrations and blue dots are serum POP concentrations. Estimates from linear-mixed effect models are given in Tables S5a and b. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2021), which are both also associated with POP and metal contamination (Dietz et al., 2019).

In conclusion, we did not detect clear associations between telomere length and concentrations of POPs of different classes in Swedish WTE nestlings, but telomere lengths did vary on a year-to-year basis. Analyzing additional physiological variables, such as oxidative stress parameters and glucocorticoid levels, could further explain variation in individual telomere length, and in combination with multiple environmental stressors, give more insight into the relationship between telomeres and environmental pollutants in avian wildlife.

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#### 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2022.112712>.

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