Exposure to oxychlordane is associated with shorter telomeres in arctic breeding kittiwakes

Pierre Blévin[a*], Frédéric Angelier[a], Sabrina Tartu[a], Stéphanie Ruault[a], Paco Bustamante[b], Dorte Herzke[c], Børge Moe[d], Claus Bech[e], Geir Wing Gabrielsen[f], Jan Ove Bustnes[g], Olivier Chastel[a]

[a] Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 – CNRS & Université de la Rochelle, 79360 Villiers-en-Bois, France

[b] Littoral Environnement et Sociétés (LIENSs), UMR 7266 – CNRS & Université de la Rochelle, 17000 La Rochelle, France

[c] Norwegian Institute for Air Research, NILU, Fram centre, NO-9296 Tromsø, Norway

[d] Norwegian Institute for Nature Research, NINA, Høgskoleringen 9, NO-7034 Trondheim, Norway

[e] Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

[f] Norwegian Polar Research Institute, Fram centre, NO-9296 Tromsø, Norway

[g] Norwegian Institute for Nature Research, NINA, Fram centre, NO-9296 Tromsø, Norway

[*] Corresponding author

Pierre Blévin

Centre d'Etudes Biologiques de Chizé

UMR 7372 – CNRS & Université de la Rochelle

79360 Villiers-en-Bois, France

blevin.pierre@gmail.com

Abstract

Telomeres are DNA-protein complexes located at the end of chromosomes, which play an important role in maintaining the genomic integrity. Telomeres shorten at each cell division and previous studies have shown that telomere length is related to health and lifespan and can be affected by a wide range of environmental factors. Among them, some persistent organic pollutants (POPs) have the potential to damage DNA. However, the effect of POPs on telomeres is poorly known for wildlife. Here, we investigated the relationships between some legacy POPs (organochlorine pesticides and polychlorobiphenyls) and telomere length in breeding adult black-legged kittiwakes (*Rissa tridactyla*), an arctic seabird species. Our results show that among legacy POPs, only blood concentration of oxychlordane, the major metabolite of chlordane mixture, is associated with shorter telomere length in females but not in males. This suggests that female kittiwakes could be more sensitive to oxychlordane, potentially explaining the previously reported lower survival rate in most oxychlordane-contaminated kittiwakes from the same population. This study is the first to report a significant and negative relationship between POPs and telomere length in a free-living bird and highlights sex-related susceptibility to banned pesticides.

Keywords: Seabirds, Svalbard, Contaminants, Organochlorines, PCBs, DNA

Highlights:

- Potential impacts of POPs on telomeres were studied in an arctic seabird.
- No relationship was found between PCBs and telomere length.
- Oxychlordane concentration was associated with shorter telomeres in females.
- This study highlights sex-related sensitivity to banned organochlorine pesticides.

Blévin, Pierre; Angelier, Frédéric; Tartu, Sabrina; Ruault, Stéphanie; Bustamante, Paco; Herzke, Dorte; Moe, Børge; Bech, Claus; Gabrielsen, Geir W.; Bustnes, Jan Ove; Chastel, Olivier.
Exposure to oxychlordane is associated with shorter telomeres in arctic breeding kittiwakes. Science of the Total Environment 2016 ;Volum 563-564. s. 125-130
DOI 10.1016/j.scitotenv.2016.04.096

1. Introduction

Telomeres are DNA-protein complexes located at the end of linear chromosomes which play a critical role in maintaining the genomic integrity (Blackburn, 1991; Monaghan and Haussmann, 2006). The DNA polymerase protein complex cannot replicate the very ends of chromosomes during mitosis, and, consequently telomeres shorten at each cell division (Olovnikov, 1996). When telomeres reach a critical lower threshold, cell division can damage coding DNA leading to apoptosis or replicative senescence (Olovnikov, 1996; Campisi et al., 2001). It was originally thought that telomere loss occurred at a constant rate in individuals through their life, and telomere length could therefore act as an internal 'mitotic clock' to measure the chronological age of organisms into the wild (Haussmann and Vleck, 2002). However, recent studies have shown that telomere length predicts survival (Haussmann et al., 2005; Bize et al., 2009; Salomons et al., 2009; Heidinger et al., 2012; Angelier et al., 2013; Barrett et al., 2013) and is related to a wide range of environmental stressors (Mizutani et al., 2013; Meillère et al., 2015). Consequently, telomere length is considered as more related to biological age than chronological age *per se* (Monaghan and Haussmann, 2006; Barrett et al., 2013).

In humans, telomere erosion can be accelerated by different environmental factors such as exposure to pollutants (Zhang et al., 2013). For instance, it has been reported that outdoor workers exposed to traffic pollution have shorter telomeres than indoor office workers (Hoxha et al., 2009). Similarly, women telomere length decreases as exposure to pollution caused by hazardous wastes increases (De Felice et al., 2012). One underlying mechanism that could potentially explain accelerated telomere shortening is oxidative stress (Von Zglinicki et al., 2000; Zhang et al., 2013). This corresponds to the imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of an organism (Finkel and Holbrook, 2000). When metabolic by-products, such as ROS, are not fully

neutralized by anti-oxidant defenses, they may oxidize cellular macromolecules such as DNA (Houben et al., 2008). Hence, telomere length may partly reflect oxidative stress history of an individual (Houben et al., 2008). Among contaminants, some persistent organic pollutants (POPs) have the potential to damage DNA by triggering oxidative stress and even decrease survival (Fernie et al., 2005; Isaksson, 2010; Letcher et al., 2010; Erikstad et al., 2013; Costantini et al., 2014; Sletten et al., 2016). However, the effect of POPs on telomere length is poorly known for wildlife. To the best of our knowledge, only one study has addressed this topic in a free-living animal with low contamination levels but failed to find any significant relationships (Sletten et al., 2016).

Due to their high volatility and persistence in time, POPs reach remote areas such as the Arctic (Gabrielsen and Henriksen, 2001). Once deposited in marine ecosystems, living organisms assimilate the POPs *via* food intake. The POP concentrations then increase from the marine environment into the organisms and throughout food webs due to bioaccumulation and biomagnification (Letcher et al., 2010). Seabirds are top predators; consequently, they are particularly exposed to POPs contamination. They therefore appear as highly relevant biological models to investigate the influence of POPs on telomere length. A previous study in a Svalbard population of black-legged kittiwakes *Rissa tridactyla* ("kittiwakes" hereafter) has reported high oxidative stress levels in most POPs contaminated individuals (POPs included polychlorobiphenyls: PCBs and one organochlorine pesticide: OCP; Lindsøe, 2012). Additionally, adult survival rate in the same population of kittiwakes was negatively linked to some OCPs (Goutte et al., 2015). In this study, we investigated the relationships between some legacy POPs (OCPs and PCBs) and telomere length in Svalbard kittiwakes. Because telomere length is classically reduced in response to oxidative stress (Von Zglinicki et al., 2000; Zhang et al., 2013) and often tightly linked to survival (Haussmann et al., 2005; Bize et

al., 2009; Salomons et al., 2009; Heidinger et al., 2012; Angelier et al., 2013; Barrett et al., 2013), we predicted that POP levels would be negatively related to telomere length.

2. Materials and Methods

2.1 Study area and sampling collection

Fieldwork was carried out in 2012 from July 12th to July 27th in Krykkjefjellet colony of Kongsfjorden, Svalbard (78°54'N, 12°13'E). A total of 38 individuals (22 males and 16 females) were caught on their nest with a noose at the end of a 5m fishing rod during the chick rearing period. At capture, a 2mL blood sample was collected from the alar vein using a heparinized syringe and a 25G needle to determine legacy POP levels, telomere length and the sex of individuals. Blood samples were stored at -20°C until subsequent analyses. The sex of individuals was determined from red blood cells by polymerase chain reaction (PCR) at the Centre d'Etudes Biologiques de Chizé (CEBC) as previously described (Weimerskirch et al., 2005).

2.2 Telomere assay

Telomere length was determined at the CEBC by Southern blot using the TeloTAGG Telomere Length Assay (Roche, Mannheim, Germany) as previously described and with minor modifications (Foote et al., 2010; Kimura et al., 2010). Telomere length analysis has already been successfully achieved on the same population of Svalbard kittiwakes (Schultner et al., 2014a). Briefly, samples were digested with proteinase K, and DNA was extracted from red blood cells by using the DNeasy blood and tissue kit (Qiagen). DNA quality was checked

by gel electrophoresis and optical density spectrophotometry. Preliminary tests have been conducted to determine the optimal amount of DNA to be used and, for each sample, 0.7 µg of DNA was digested with the restriction enzymes *HinfI* and *RsaI* for 16 h at 37°C. Digested DNA samples were then separated using a pulse-field gel electrophoresis (Bio-Rad) on a 0.8% agarose gel. All samples were run in four gels. Samples were randomly assigned to a gel. Internal controls were run on each gel to measure inter-gel variations. The gels were run at 3.0V/cm with an initial switch time of 0.5 sec to a final switch time of 7 sec for 14 hours. Following that step, the gel was depurinated and denaturated in an alkaline solution. The gel was then neutralized and DNA was transferred onto a nitrocellulose membrane by Southern blot (Hybond N+, Amersham Life Science, Amersham, UK). The membrane was incubated at 120°C for 20 minutes in order to fix the DNA. The DNA was then hybridized with a digoxigenin-labeled probe specific for telomeric sequences and incubated with antidigoxigenin-specific antibody before visualization with a Chemidoc (Bio Rad). Telomere length was then analyzed using ImageJ to extract telomere smear densities. Lane-specific background was subtracted from each density value telomere length (mean value) was then calculated using a window of 5-30 kb that includes the whole smear (Nussey et al., 2014). Inter-gel CV was 1.40%.

2.3 POPs analyses

POPs were analyzed from whole blood at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway. Only compounds that potentially affect survival of kittiwakes were considered in this study (Goutte et al., 2015). Thus, we selected the ∑PCBs (CB-99, -118, -138, -153, -180, -183 and -187), and the OCPs (HCB, *p,p'*-DDE, oxychlordane, *trans*- and *cis*-nonachlor). To a blood total sample of 0.5 to 1.5 ml, a 100 µL internal standard

150 solution was added (^{13}C-labelled compounds from Cambridge Isotope Laboratories: Woburn,
151 MA, USA). The sample was extracted twice with 6ml of n-hexane, after denaturation with
152 ethanol and a saturated solution of ammonium sulphate in water. Matrix removal on florisil
153 columns, separation on an Agilent Technology 7890 GC and detection on an Agilent
154 Technology 5975C MSD were performed as previously decribed (Herzke et al., 2009). For
155 validation of the results, blanks (clean and empty glass tubes treated like a sample, 3 in total)
156 were run for every 10 samples, while standard reference material (3 in total, 1589a human
157 serum from NIST) was run for every 10 samples. The accuracy of the method was within the
158 70 and 108% range.
159
160 *2.4 Statistical analyses*
161
162 Statistical tests were performed using R 2.13.1 (R Development Core Team, 2011).
163 We first checked if telomere length and POP levels differed between sexes using the
164 parametrical test of Welch. The influence of POPs' contamination on telomere length was
165 investigated with Linear Models. Thus, ∑POPs, ∑PCBs, HCB, *p,p'*-DDE, oxychlordane, *cis*-
166 and *trans*-nonachlor were defined as independent variables and telomere length as the
167 dependent variable. Because blood contaminant concentrations differed between males and
168 females (see results), including the factor "sex" and the variable "∑POPs" in the same model
169 could lead to multicollinearity problems and biased results (Graham, 2003). Additionally, it is
170 now well established that males and females can react in different way to environmental
171 stressors such as POPs contamination. Specifically, previous studies conducted on kittiwakes
172 from Krykkjefjellet colony have reported sex differences regarding body condition, hormones
173 levels, endocrine disruptions, phenology, breeding decision and even survival rate (Goutte et
174 al., 2010, 2015; Schultner et al., 2014b; Tartu et al., 2013, 2014, 2015a). Therefore, male and

female kittiwakes were separated in statistical analyses. Diagnostic plots were assessed and Shapiro normality tests were performed on residuals to test whether data sufficiently met the assumption of the linear model. Multiple testing can potentially lead to misleading results, indicating statistical significance in situations where there is none. Consequently, we performed bootstrapping (i.e. resampling method) from the data sets of significant relationship and then assessed diagnostic plot to corroborate the results (Supplementary materials; Chernick and Labudde, 2014). A significance level of $\alpha < 0.05$ was used for all tests.

3. Results

Telomere length was not related to sex (t = -1.438, P-value = 0.160) and ∑POPs tended to be higher in male than in female kittiwakes (t = -1.976, P-value = 0.056). ∑POPs, ∑PCBs, HCB, *p,p'*-DDE, *cis*- and *trans*-nonachlor were not related to telomere length in male nor in female kittiwakes (All P-values ≥ 0.259; Table 1). In females, we found a significant and negative association between oxychlordane and telomere length (Table 1; Fig.1a). However, we found no relationship between oxychlordane and telomere length in males (Table 1; Fig.1b).

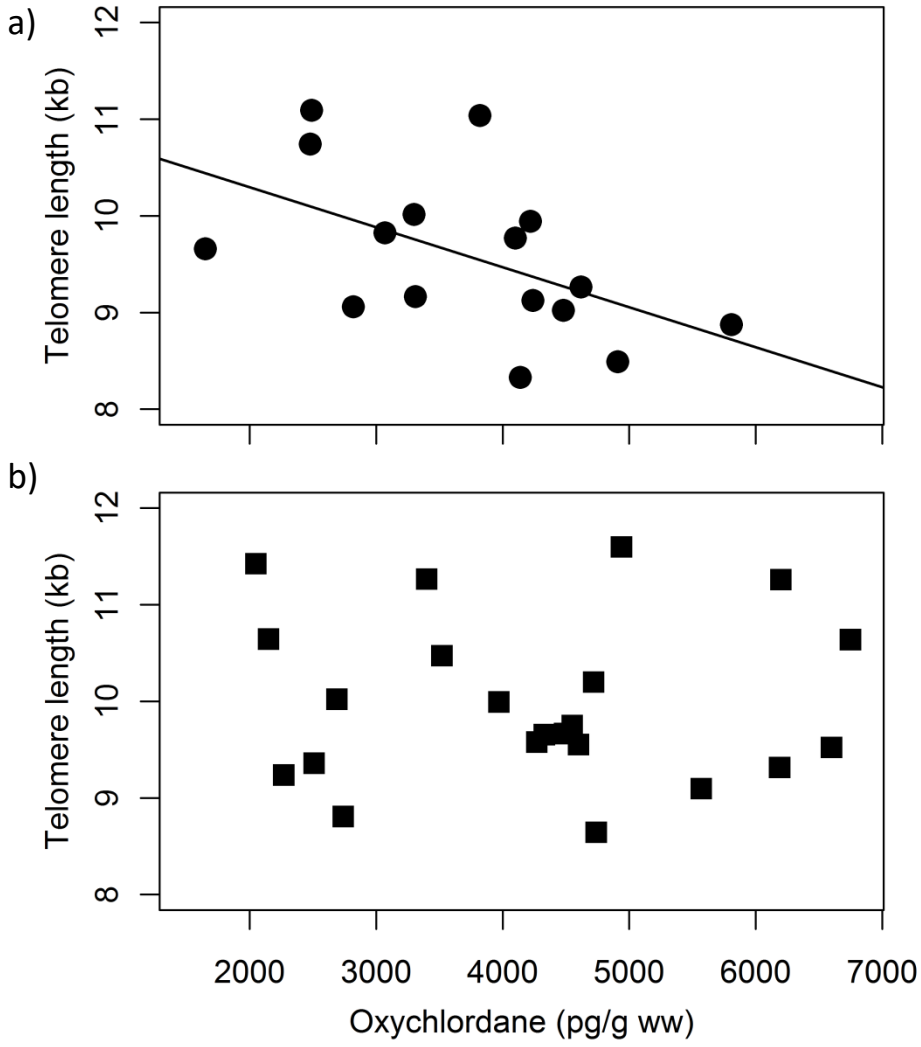
Table 1

Relationships between whole blood ∑POPs, ∑PCBs, OCPs and telomere length in female and male chick-rearing black-legged kittiwakes, *Rissa tridactyla* from Kongsfjorden, Svalbard.

Dependent variables		Independent variables	*df*	*F*	P-value
Females	Telomere length	∑POPs*	1,14	0.802	0.386
		∑PCBs**	1,14	0.833	0.377
		HCB	1,14	0.534	0.477
		p,p'-DDE	1,14	0.066	0.801
		Oxychlordane	1,14	5.343	**0.037**
		cis-nonachlor	1,14	1.246	0.283
		trans-nonachlor	1,14	1.301	0.273
Males	Telomere length	∑POPs*	1,20	0.394	0.537
		∑PCBs**	1,20	0.114	0.740
		HCB	1,20	0.002	0.967
		p,p'-DDE	1,20	1.351	0.259
		Oxychlordane	1,20	0.025	0.876
		cis-nonachlor	1,20	0.077	0.785
		trans-nonachlor	1,20	0.922	0.348

Significant variables are in bold.
*∑POPs: CB-99, -118, -138, -153, -180, -183, -187, HCB, *p,p'*-DDE, oxychlordane, *trans*- and *cis*-nonachlor
**∑PCBs: CB-99, -118, -138, -153, -180, -183, -187

Fig. 1. Relationships between telomere length and whole blood oxychlordane concentrations in female (a) and male (b) chick-rearing black-legged kittiwakes, *Rissa tridactyla* from Kongsfjorden, Svalbard.



4. Discussion

Our results on adult kittiwakes showed that among legacy POPs, only blood concentrations of oxychlordane were negatively associated with telomere length in females but not in males. Oxychlordane and chlordane mixture have already been associated with lower survival in both males and females from the same kittiwake population (Goutte et al., 2015). Our study only reports a negative relationship between telomere length only in females, thus providing a possible mechanism linking oxychlordane exposure and female mortality. Regarding males, it is thus likely that oxychlordane exposure may lead to a lower survival rate in another way, independent of telomere attrition. Effects of contaminants on telomere length are still poorly known; most of the studies have focused on humans (Zhang et al., 2013) and to the best of our knowledge, only one study has investigated the relationships between POPs and telomere length in wildlife (Sletten et al., 2016).

So far, 11 of the 14 studies investigating the relationship between telomere length and environmental and occupational chemical exposure in humans, have reported significant negative associations (Rigolin et al., 2004; Hoxha et al., 2009; Bin et al., 2010; McCracken et al., 2010; Pavanello et al., 2010; Li et al., 2011; Rollison et al., 2011; De Felice et al., 2012; Eshkoor et al., 2012; Hou et al., 2012; Wu et al., 2012). For example, shorter telomeres were associated with pesticide exposure in patients with myelodyplastic syndrome (Rigolin et al., 2004; Rollison et al., 2011). Similarly, a study conducted in an apparent healthy Korean population reported a negative association between telomere length and exposure to high levels of POPs, including OCPs (*p,p'*-DDE, *trans*-nonachlor and oxychlordane), PCBs and polybrominated diphenylethers (Shin et al., 2010). The only study investigating relationships between telomere length and contamination in wildlife has been performed on white-tailed eagle (*Haliaeetus albicilla*) chicks in northern Norway (Sletten et al., 2016). In this study, the

254 authors did not find any relationship between telomere length and POPs, including OCPs,
255 PCBs and perfluorinated compounds. However, the relatively low levels of contaminants in
256 white-tailed eagle chicks compared to those measured in adult kittiwakes may explain the
257 discrepancy in the results. Oxychlordane concentrations in kittiwakes measured in the present
258 study (4019 ± 1315 pg/g ww) were on average, around 3 times higher than those reported for
259 eagle chicks (1483 ± 197 pg/g ww). With this exception, our results are thus consistent with
260 previous works and suggest a negative effect of oxychlordane on telomere length in
261 kittiwakes.

262 Surprisingly, the relationships between oxychlordane and telomere length were sex
263 dependent and a significant relationship was found in females, but not in males. How to
264 explain such a difference? There are some indications that female seabirds may be more
265 sensitive to high levels of OCPs than males. In glaucous gulls (*Larus hyperboreus*) and in
266 kittiwakes, OCPs exposure mostly affected survival rate in females (Erikstad et al., 2013;
267 Goutte et al., 2015). Furthermore, in wandering albatrosses (*Diomedea exulans*), another
268 long-lived seabird species, the relationships between POP levels and oxidative stress were
269 dependent of reproductive effort and breeding females had the highest levels of haptoglobin, a
270 well-known acute phase protein that indicates an ongoing inflammatory response which can
271 limit the spread of oxidative damages, compared to breeding males (Costantini et al., 2014)
272 Thus, the influence of contaminants on oxidative stress may be exacerbated by reproductive
273 effort. Although kittiwakes are considered to share equally incubation and chick rearing duties
274 (Coulson, 2011), egg production represents a significant and important cost for females
275 (Monaghan and Nager, 1997). It is thus possible that the cost of egg production increases the
276 sensitivity of female kittiwakes to oxychlordane. This may therefore explain the sex-
277 dependent relationship between telomere length and oxychlordane burden. Furthermore,
278 although female birds transfer a significant part of their PCB and DDT burden to their eggs,

279 oxychlordane on the other hand, seemed to be more selectively retained by the female, at least
280 in female glaucous gulls (Verboven et al., 2009). In female kittiwakes, it is thus possible that
281 the energetic cost of clutch production added to the maintenance of significant oxychlordane
282 levels would exacerbate the toxic effects of this chlorinated pesticide.

283 Our study was conducted on breeding adults (i.e. at least 3-4 years old; Coulson,
284 2011). However, the birds' age was unknown in our study, thus a possible confounding factor
285 could be that birds with high levels of OCPs are the oldest birds with the shortest telomeres. If
286 so, the negative relationship between oxychlordane and telomere length would be induced by
287 the age of individuals rather by a direct effect of oxychlordane on telomere attrition. Although
288 several studies suggested a negative effect of age on telomere length (Haussmann and Vleck,
289 2002; Haussmann et al., 2003), other reported that telomere loss mainly occurs early in life of
290 long-lived seabird (i.e. between chick and adult stage) rather than during adulthood (Hall et
291 al., 2004; Foote et al., 2010), as is the case in other vertebrates (Frenck et al., 1998; Rufer et
292 al., 1998; Zeichner et al., 1999; Friedrich et al., 2001). Additionally, Haussmann et al. (2003)
293 showed a significant and positive relationship between telomere length and age in another
294 long-lived seabird species, the Letch's storm petrel (*Oceanodroma leucorhoa*). Consequently,
295 effect of age on telomere length appears to be more complex rather than a simple negative and
296 constant decrease of telomere length with age. Furthermore, it has been reported that in
297 several seabird species, blood level of POPs is unrelated to age in adult birds (Bustnes et al.,
298 2003; Carravieri et al., 2014; Tartu et al., 2015b), and rather reaches a steady state of
299 equilibrium once adult (Newton et al., 1981; Henriksen, 1995; Drouillard, 2001; Bustnes et
300 al., 2003). Therefore, it is unlikely that the negative relationship between oxychlordane and
301 telomere length originates from birds with high levels of oxychlordane being the oldest birds
302 with the shortest telomeres.

Previous studies have shown decreased antioxidant enzyme activity in relation to contaminants, such as in herring gulls (*Larus argentatus*), where chicks exposed to PCBs combined with dietary restrictions showed negative relationships between catalase, glutathione peroxidase and contaminant levels (Hegseth et al., 2011). Similarly, significant negative associations between oxychlordane, *p,p'*-DDE, PCB-153 and superoxide dismutase enzyme in white-tailed eagle chicks have been reported (Sletten et al., 2016). Finally, a significant positive relationship between oxychlordane, PCBs and oxidative stress was found in a Svalbard population of adult kittiwakes (Lindsøe, 2012). Consequently, oxychlordane could be possibly involved in the generation of oxidative stress through an increase of ROS which are known to reduce telomere length (Von Zglinicki, 2002). As the nucleobase guanine is a major oxidation target for ROS, the (TTAGGG)n repeats, that constitute vertebrate telomeres, are particularly vulnerable to oxidative attacks (Wang et al., 2010). In our study, *in vivo* biomarkers of oxidative stress were not measured. Thus, further studies measuring at the same time POP levels, proxies of oxidative stress and telomere length are thus needed to test if oxidative stress induced by oxychlordane exposure could be linked to telomere attrition. Among other potential mechanisms, oxychlordane could induce a down-regulation of telomerase activity. Indeed, telomere integrity is largely maintained by a telomerase-based mechanism, in which the enzyme telomerase plays a key role by adding hexameric (TTAGGG) repeats to chromosome ends, partially compensating telomere shortenning (Greider and Blackburn, 1989; Xin et al., 2008). However, this hypothesis seems unlikely since telomerase is generally inactivated in adult somatic cells of most studied species so far (Monaghan and Haussmann, 2006; Vleck et al., 2007; but see Hatakeyama et al., 2008). Consequently, telomerase down-regulation seems to be more a common feature of large and/ or long-lived species (Gomes et al., 2011) rather than being specifically related to contamination levels.

328 Extensively used during more than 35 years as a pesticide, usage of chlordane, of
329 which oxychlordane is a major metabolite, tended to decrease in the 80s (U.S. Department of
330 Health and Human Services, 1994). Banned from use and listed as a legacy POP by the
331 Stockholm convention since 2004, oxychlordane provided a clear diagnostic criterion related
332 to the lethal poisoning in several bird species (Blus et al., 1983, 1985; Stickel et al., 1983;
333 Okoniewski and Novesky, 1993; Stansley and Roscoe, 1999; Wiemeyer, 1996). Furthermore,
334 in an experimental study where female rats were gavage with oxychlordane, high
335 administrated doses (10 $mg.kg^{-1}$) revealed acute toxicity, characterized by feed refusal, rapid
336 weight loss and thymic atrophy. At lower doses (2.5 $mg.kg^{-1}$), female rats showed signs of
337 hepatic changes indicative of microsomal enzyme induction (Bondy et al., 2003). Finally, as
338 previously mentioned, in glaucous gulls and in kittiwakes from Svalbard, oxychlordane has
339 been associated with lower survival rates, especially in females (Erikstad et al., 2013; Goutte
340 et al., 2015). In the present study, among legacy POPs, only oxychlordane was negatively
341 associated with telomere length in females. Our results are thus consistent with the idea that
342 oxychlordane is one of the most toxic POPs (Erikstad et al., 2013). Consequently, female
343 kittiwakes' sensitivity to oxychlordane could affect telomere length, explaining the previously
344 reported lower survival rate in highly contaminated female kittiwakes. On the other side, the
345 lack of relationships between oxychlordane and telomere length in males suggest that the
346 lowest survival rate of most oxychlordane contaminated birds is thus probably not mediated
347 by telomere shortening in male kittiwakes.

348 This study is the first to report a significant and negative relationship between POPs
349 and telomere length in wildlife and therefore partly fills the gap of knowledge about
350 contaminants effect on telomere attrition. However, the present work has some limitations.
351 First, we did not measure oxidative stress or other potential mechanisms. Thus, further studies
352 measuring at the same time POP levels, proxies of oxidative stress and telomere length are

thus needed to test if oxidative stress induced by oxychlordane exposure could be linked to telomere attrition. Secondly, among all tested contaminants only blood oxychlordane concentration was negatively associated with telomere length and even though several studies have reported that oxychlordane is highly toxic for birds, an experimental approach would enable to confirm the acute toxicity of this compound on telomere length through oxidative stress. Finally, our study was conducted on a limited number of individuals and to fully validate our finding, future studies investigating effects of POPs on telomere length should be conducted on a larger sample size and other species.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

This project was supported by Institut Polaire Français (IPEV project 330 to O.Chastel) and by Agence National de la Recherche (ANR project PolarTop to O.Chastel) and by the Research Council of Norway (AVITOX project to J.O. Bustnes). P.Blévin was funded by a PhD grant from University of La Rochelle. This study was approved by the French and Norwegian Ethic committees and by the Governor of Svalbard. The authors thank Linda Hanssen, the staff of the NILU laboratory in Tromsø for their assistance during the POPs analysis, David Pinaud for statistical advices on bootstrap procedures and multiple testing and the two anonymous referees for their constructive comments that definitely improved the earlier version of the manuscript.

References

Angelier, F., Vleck, C.M., Holberton, R.L., Marra, P.P., 2013. Telomere length, non-breeding habitat and return rate in male American redstarts. Funct. Ecol. 27, 342–350.

Barrett, E.L.B., Burke, T.A., Hammers, M., Komdeur, J., Richardson, D.S., 2013. Telomere length and dynamics predict mortality in a wild longitudinal study. Mol. Ecol. 22, 249–259. doi:10.1111/mec.12110

Bin, P., Leng, S.G., Cheng, J., Pan, Z.F., Duan, H.W., Dai, Y.F., Li, H.S., Niu, Y., Liu, Q.J., Liu, Q., 2010. Association between telomere length and occupational polycyclic aromatic hydrocarbons exposure. Zhonghua Yu Fang Yi Xue Za Zhi 44, 535–538.

Bize, P., Criscuolo, F., Metcalfe, N.B., Nasir, L., Monaghan, P., 2009. Telomere dynamics rather than age predict life expectancy in the wild. Proc. R. Soc. B Biol. Sci. 276, 1679–1683. doi:10.1098/rspb.2008.1817

Blackburn, E.H., 1991. Structure and function of telomeres. Nature 350, 569–573.

Blus, L.J., Henny, C.J., Krynitsky, A.J., 1985. Organochlorine-induced mortality and residues in Long-billed Curlews from Oregon. The Condor 87, 563–565.

Blus, L.J., Pattee, O.H., Henny, C.J., Prouty, R.M., 1983. First records of chlordane-related mortality in wild birds. J. Wildl. Manag. 47, 196–198.

Bondy, G., Armstrong, C., Coady, L., Doucet, J., Robertson, P., Feeley, M., Barker, M., 2003. Toxicity of the chlordane metabolite oxychlordane in female rats: clinical and histopathological changes. Food Chem. Toxicol. 41, 291–301.

Bustnes, J.O., Bakken, V., Skaare, J.U., Erikstad, K.E., 2003. Age and accumulation of persistent organochlorines: A study of arctic-breeding glaucous gulls (Larus hyperboreus). Environ. Toxicol. Chem. 22, 2173–2179.

Campisi, J., Kim, S.H., Lim, C.S., Rubio, M., 2001. Cellular senescence, cancer and aging: the telomere connection. Exp. Gerontol. 36, 1619–1637.

Carravieri, A., Bustamante, P., Tartu, S., Meillère, A., Labadie, P., Budzinski, H., Peluhet, L., Barbraud, C., Weimerskirch, H., Chastel, O., Cherel, Y., 2014. Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern ocean predators. Environ. Sci. Technol. 48, 14746–14755. doi:10.1021/es504601m

Chernick, M.R., LaBudde, R.A., 2014. An introduction to bootstrap methods with applications to R. John Wiley & Sons.

Costantini, D., Meillère, A., Carravieri, A., Lecomte, V., Sorci, G., Faivre, B., Weimerskirch, H., Bustamante, P., Labadie, P., Budzinski, H., Chastel, O., 2014. Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. Oecologia. 175, 1107–1116. doi:10.1007/s00442-014-2975-x

Coulson, J.C., 2011. The kittiwake. T & AD Poyser.

De Felice, B., Nappi, C., Zizolfi, B., Guida, M., Sardo, A.D.S., Bifulco, G., Guida, M., 2012. Telomere shortening in women resident close to waste landfill sites. Gene 500, 101–106. doi:10.1016/j.gene.2012.03.040

Drouillard, K.G., 2001. Modeling the toxicokinetics and biomagnification of polychlorinated biphenyls (PCBs) in birds. Thesis, Univ Tromsö, Norway.

Erikstad, K.E., Sandvik, H., Reiertsen, T.K., Bustnes, J.O., Strom, H., 2013. Persistent organic pollution in a high-Arctic top predator: sex-dependent thresholds in adult survival. Proc. R. Soc. B Biol. Sci. 280, 20131483. doi:10.1098/rspb.2013.1483

Eshkoor, S.A., Jahanshiri, F., Ismail, P., Rahman, S.A., Moin, S., Adon, M.Y., 2012. Association between telomere shortening and ageing during occupational exposure. J. Med. Biochem. 31, 211–216.

Fernie, K.J., Shutt, J.L., Mayne, G., Hoffman, D., Letcher, R.J., Drouillard, K.G., Ritchie, I.J., 2005. Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (Falco sparverius). Toxicol. Sci. 88, 375–383. doi:10.1093/toxsci/kfi295

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Foote, C.G., Daunt, F., González-Solís, J., Nasir, L., Phillips, R.A., Monaghan, P., 2010. Individual state and survival prospects: age, sex, and telomere length in a long-lived seabird. Behav. Ecol. 22, 156–161.

Frenck, R.W., Blackburn, E.H., Shannon, K.M., 1998. The rate of telomere sequence loss in human leukocytes varies with age. Proc. Natl. Acad. Sci. 95, 5607–5610.

Friedrich, U., Schwab, M., Griese, E.U., Fritz, P., Klotz, U., 2001. Telomeres in neonates: new insights in fetal hematopoiesis. Pediatr. Res. 49, 252–256.

Gabrielsen, G.W., Henriksen, E.O., 2001. Persistent organic pollutants in Arctic animals in the Barents Sea area and at Svalbard; levels and effects (review). Mem. Natl. Inst. Polar Res. Spec. Issue 54, 349–364.

Gomes, N., Ryder, O.A., Houck, M.L., Charter, S.J., Walker, W., Forsyth, N.R., Austad, S.N., Venditti, C., Pagel, M., Shay, J.W., 2011. Comparative biology of mammalian telomeres: hypotheses on ancestral states and the roles of telomeres in longevity determination. Aging Cell 10, 761–768.

Goutte, A., Angelier, F., Chastel, C.C., Trouvé, C., Moe, B., Bech, C., Gabrielsen, G.W., Chastel, O., 2010. Stress and the timing of breeding: glucocorticoid-luteinizing hormones relationships in an arctic seabird. Gen. Comp. Endocrinol. 169, 108–116.

Goutte, A., Barbraud, C., Herzke, D., Bustamante, P., Angelier, F., Tartu, S., Clément-Chastel, C., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2015a. Survival rate and breeding outputs in a high Arctic seabird exposed to legacy persistent organic pollutants and mercury. Environ. Pollut. 200, 1–9. doi:10.1016/j.envpol.2015.01.033

Graham, M.H., 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84, 2809–2815.

Greider, C.W., Blackburn, E.H., 1989. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 337, 331–337.

Hall, M.E., Nasir, L., Daunt, F., Gault, E.A., Croxall, J.P., Wanless, S., Monaghan, P., 2004. Telomere loss in relation to age and early environment in long-lived birds. Proc. R. Soc. Lond. B Biol. Sci. 271, 1571–1576.

Hatakeyama, H., Nakamura, K.I., Izumiyama-Shimomura, N., Ishii, A., Tsuchida, S., Takubo, K., Ishikawa, N., 2008. The teleost Oryzias latipes shows telomere shortening with age despite considerable telomerase activity throughout life. Mechanisms of Ageing and Development 129, 550–557.

Haussmann, M.F., Winkler, D.W., O'Reilly, K.M., Huntington, C.E., Nisbet, I.C., Vleck, C.M., 2003. Telomeres shorten more slowly in long-lived birds and mammals than in short–lived ones. Proc. R. Soc. Lond. B Biol. Sci. 270, 1387–1392.

Haussmann, M.F., Winkler, D.W., Vleck, C.M., 2005. Longer telomeres associated with higher survival in birds. Biol. Lett. 1, 212–214. doi:10.1098/rsbl.2005.0301

Haussmann, M.F., Vleck, C., 2002. Telomere length provides a new technique for aging animals. Oecologia 130, 325–328. doi:10.1007/s00442-001-0827-y

Hegseth, M.N., Camus, L., Gorbi, S., Regoli, F., Gabrielsen, G.W., 2011. Effects of exposure to halogenated organic compounds combined with dietary restrictions on the antioxidant defense system in herring gull chicks. Sci. Total Environ. 409, 2717–2724. doi:10.1016/j.scitotenv.2011.04.004

Heidinger, B.J., Blount, J.D., Boner, W., Griffiths, K., Metcalfe, N.B., Monaghan, P., 2012. Telomere length in early life predicts lifespan. Proc. Natl. Acad. Sci. 109, 1743–1748. doi:10.1073/pnas.1113306109

Henriksen, E.O., 1995. Levels and congener pattern of PCBs in kittiwake, Rissa tridactyla, in relation to mobilization of bodylipids associated with reproduction. Thesis, Univ Tromsö, Norway.

Herzke, D., Nygård, T., Berger, U., Huber, S., Røv, N., 2009. Perfluorinated and other persistent halogenated organic compounds in European shag (Phalacrocorax aristotelis) and common eider (Somateria mollissima) from Norway: a suburban to remote pollutant gradient. Sci. Total Environ. 408, 340–348.

Houben, J.M.J., Moonen, H.J.J., van Schooten, F.J., Hageman, G.J., 2008. Telomere length assessment: Biomarker of chronic oxidative stress? Free Radic. Biol. Med. 44, 235–246. doi:10.1016/j.freeradbiomed.2007.10.001

Hou, L., Wang, S., Dou, C., Zhang, X., Yu, Y., Zheng, Y., Avula, U., Hoxha, M., Díaz, A., McCracken, J., Barretta, F., Marinelli, B., Bertazzi, P.A., Schwartz, J., Baccarelli, A.A., 2012. Air pollution exposure and telomere length in highly exposed subjects in Beijing, China: a repeated-measure study. Environ. Int. 48, 71–77.

Hoxha, M., Dioni, L., Bonzini, M., Pesatori, A., Fustinoni, S., Cavallo, D., Carugno, M., Albetti, B., Marinelli, B., Schwartz, J., Bertazzi, P., Baccarelli, A., 2009. Association between leukocyte telomere shortening and exposure to traffic pollution: a cross-sectional study on traffic officers and indoor office workers. Environ. Health 8, 41. doi:10.1186/1476-069X-8-41

Isaksson, C., 2010. Pollution and its impact on wild animals: A meta-analysis on oxidative stress. EcoHealth 7, 342–350. doi:10.1007/s10393-010-0345-7

Kimura, M., Stone, R.C., Hunt, S.C., Skurnick, J., Lu, X., Cao, X., Harley, C.B., Aviv, A., 2010. Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. Nat. Protoc. 5, 1596–1607.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995–3043. doi:10.1016/j.scitotenv.2009.10.038

Li, H., Jönsson, B.A.G., Lindh, C.H., Albin, M., Broberg, K., 2011. N-nitrosamines are associated with shorter telomere length. Scand. J. Work. Environ. Health 316–324.

Lindsøe, L.M., 2012. Humoral immunity and oxidative stress in black-legged kittiwake (Rissa tridactyla) and common eider (Sommateria mollissima) from Svalbard - related to bioaccumulated organochlorine contaminants. Master thesis, Univ Oslo, Norway.

McCracken, J., Baccarelli, A., Hoxha, M., Dioni, L., Melly, S., Coull, B., Suh, E., Vokonas, P., Schwartz, J., 2010. Annual ambient black carbon associated with shorter telomeres in elderly men: veterans affairs normative aging study. Environ. Health. Persp. 118, 1564–1570.

Meillère, A., Brischoux, F., Ribout, C., Angelier, F., 2015. Traffic noise exposure affects telomere length in nestling house sparrows. Biol. Lett. 11, 20150559.

Mizutani, Y., Tomita, N., Niizuma, Y., Yoda, K., 2013. Environmental perturbations influence telomere dynamics in long-lived birds in their natural habitat. Biol. Lett. 9, 20130511.

522 Monaghan, P., Haussmann, M.F., 2006. Do telomere dynamics link lifestyle and lifespan?
523 Trends Ecol. Evol. 21, 47–53. doi:10.1016/j.tree.2005.11.007
524 Monaghan, P., Nager, R.G., 1997. Why don't birds lay more eggs? Trends Ecol. Evol. 12,
525 270–274.
526 Newton, I., Bogan, J., Marquiss, M., 1981. Organochlorine contamination and age in
527 sparrowhawks. Environ. Pollut. Ser. Ecol. Biol. 25, 155–160.
528 Nussey, D.H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N., Hartmann, N., Horn,
529 T., Haussmann, M., Olsson, M., Turbill, C., Verhulst, S., Zahn, S., Monaghan, P.,
530 2014. Measuring telomere length and telomere dynamics in evolutionary biology and
531 ecology. Methods Ecol. Evol. 5, 299–310.
532 Okoniewski, J.C., Novesky, E., 1993. Bird poisonings with cyclodienes in suburbia: links to
533 historic use on turf. J. Wildl. Manag. 57, 630–639.
534 Olovnikov, A.M., 1996. Telomeres, telomerase, and aging: Origin of the theory. Exp.
535 Gerontol. 31, 443–448. doi:10.1016/0531-5565(96)00005-8
536 Pavanello, S., Pesatori, A.C., Dioni, L., Hoxha, M., Bollati, V., Siwinska, E., Mielzyńska, D.,
537 Bolognesi, C., Bertazzi, P.A., Baccarelli, A., 2010. Shorter telomere length in
538 peripheral blood lymphocytes of workers exposed to polycyclic aromatic
539 hydrocarbons. Carcinogenesis 31, 216–221.
540 R Development Core Team R: A language and environment for statistical computing., R
541 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.
542 http://www.R-project.org/, 2011
543 Rigolin, G.M., Porta, M.D., Bugli, A.M., Castagnari, B., Mauro, E., Bragotti, L.Z., Ciccone,
544 M., Cuneo, A., Castoldi, G., 2004. Flow cytometric detection of accelerated telomere
545 shortening in myelodysplastic syndromes: correlations with aetiological and clinical–
546 biological findings. Eur. J. Haematol. 73, 351–358.
547 Rollison, D.E., Epling-Burnette, P.K., Park, J.Y., Lee, J.H., Park, H., Jonathan, K., Cole,
548 A.L., Painter, J.S., Guerrier, M., Meléndez-Santiago, J., Fulp, W., Komrokji, R.,
549 Lancet, J., List, A.F., 2011. Telomere length in myelodysplastic syndromes. Leuk.
550 Lymphoma 52, 1528–1536. doi:10.3109/10428194.2011.568648
551 Rufer, N., Dragowska, W., Thornbury, G., Roosnek, E., Lansdorp, P.M., 1998. Telomere
552 length dynamics in human lymphocyte subpopulations measured by flow cytometry.
553 Nat. Biotechnol. 16, 743–747.
554 Salomons, H.M., Mulder, G.A., van de Zande, L., Haussmann, M.F., Linskens, M.H.K.,
555 Verhulst, S., 2009. Telomere shortening and survival in free-living corvids. Proc. R.
556 Soc. B Biol. Sci. 276, 3157–3165. doi:10.1098/rspb.2009.0517
557 Schultner, J., Moe, B., Chastel, O., Bech, C., Kitaysky, A.S., 2014a. Migration and stress
558 during reproduction govern telomere dynamics in a seabird. Biol. Lett. 10, 20130889.
559 Schultner, J., Moe, B., Chastel, O., Tartu, S., Bech, C., Kitaysky, A.S., 2014b. Corticosterone
560 mediates carry-over effects between breeding and migration in the kittiwake Rissa
561 tridactyla. Mar Ecol Prog Ser 496, 125–33.
562 U.S. Department of Health and Human Services., 1994. Toxicological profile for chlordane
563 (update). Agency for Toxic Substances and Disease Registry. TP-93/03.
564 Shin, J.Y., Choi, Y.Y., Jeon, H.-S., Hwang, J.H., Kim, S.A., Kang, J.H., Chang, Y.S., Jacobs,
565 D.R., Park, J.Y., Lee, D.H., 2010. Low-dose persistent organic pollutants increased
566 telomere length in peripheral leukocytes of healthy Koreans. Mutagenesis 25, 511–
567 516. doi:10.1093/mutage/geq035
568 Sletten, S., Bourgeon, S., Bårdsen, B.J., Herzke, D., Criscuolo, F., Massemin, S., Zahn, S.,
569 Johnsen, T.V., Bustnes, J.O., 2016. Organohalogenated contaminants in white-tailed
570 eagle (Haliaeetus albicilla) nestlings: An assessment of relationships to

immunoglobulin levels, telomeres and oxidative stress. Sci. Total Environ. 539, 337–349. doi:10.1016/j.scitotenv.2015.08.123

Stansley, W., Roscoe, D.E., 1999. Chlordane poisoning of birds in New Jersey, USA. Environ. Toxicol. Chem. 18, 2095–2099.

Stickel, L.F., Stickel, W.H., Dyrland, R.A., Hughes, D.L., 1983. Oxychlordane, HCS-3260, and nonachlor in birds: Lethal residues and loss rates. J. Toxicol. Environ. Health Part Curr. Issues 12, 611–622.

Tartu, S., Angelier, F., Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Gabrielsen, G.W., Verboven, N., Verreault, J., Labadie, P., 2015a. Polychlorinated biphenyl exposure and corticosterone levels in seven polar seabird species. Environ. Pollut. 197, 173–180.

Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015b. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ. 505, 180–188. doi:10.1016/j.scitotenv.2014.10.008

Tartu, S., Gabrielsen, G.W., Blévin, P., Ellis, H., Bustnes, J.O., Herzke, D., Chastel, O., 2014. Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in Arctic breeding black-legged kittiwakes. Environ. Sci. Technol. 48, 13504–13510.

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2013. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol. Lett. 9, 20130317.

Verboven, N., Verreault, J., Letcher, R.J., Gabrielsen, G.W., Evans, N.P., 2009. Differential investment in eggs by Arctic-breeding glaucous gulls (*Larus hyperboreus*) exposed to persistent organic pollutants. The Auk 126, 123–133. doi:10.1525/auk.2009.08039

Vleck, C.M., Haussmann, M.F., Vleck, D., 2007. Avian senescence: underlying mechanisms. Journal of Ornithology 148, 611–624.

Von Zglinicki, T., 2002. Oxidative stress shortens telomeres. Trends Biochem. Sci. 27, 339–344.

Von Zglinicki, T., Pilger, R., Sitte, N., 2000. Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. Free Radic. Biol. Med. 28, 64–74.

Wang, Z., Rhee, D.B., Lu, J., Bohr, C.T., Zhou, F., Vallabhaneni, H., de Souza-Pinto, N.C., Liu, Y., 2010. Characterization of oxidative guanine damage and repair in mammalian telomeres. PLoS Genet 6, e1000951.

Weimerskirch, H., Lallemand, J., Martin, J., 2005. Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. J. Anim. Ecol. 74, 285–291.

Wiemeyer, S.N., 1996. Other organochlorine pesticides in birds. Environ. Contam. Wildl. Interpret. Tissue Conc. 99–115.

Wu, Y., Liu, Y., Ni, N., Bao, B., Zhang, C., Lu, L., 2012. High lead exposure is associated with telomere length shortening in Chinese battery manufacturing plant workers. Occup. Environ. Med. 69, 557–563.

Xin, H., Liu, D., Songyang, Z., 2008. The telosome/shelterin complexand its functions. Genome Biology 9, 232.

Zeichner, S.L., Palumbo, P., Feng, Y., Xiao, X., Gee, D., Sleasman, J., Goodenow, M., Biggar, R., Dimitrov, D., 1999. Rapid telomere shortening in children. Blood 93, 2824–2830.

Zhang, X., Lin, S., Funk, W.E., Hou, L., 2013. Republished: Environmental and occupational exposure to chemicals and telomere length in human studies. Postgrad. Med. J. 89, 722–728.

Supplementary materials

Fig. 1. Bootstrapping from the dataset of the significant relationship between oxychlordane and telomere length for female kittiwakes (10 000 iterations). The green axes represent the range limit of hazardous correlations with an interval confidence (IC) of 95%. The red axis corresponds to the Pearson correlation coefficient (r = -0.53) between oxychlordane and telomere length in female kittiwakes. The correlation between oxychlordane and telomere length is outside the range limit of hazardous correlations (IC=95%) testifying that the significant and negative relationship between oxychlordane and telomere length in female kittiwakes is consistent and corroborates our result.