# Perfluorinated substances and telomeres in an Arctic seabird: Cross-sectional and longitudinal approaches

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**Abstract**

Telomeres are non-coding DNA repeats located at the termini of eukaryotic chromosomes, regulated by dynamic processes balancing shortening and maintenance. Despite a mechanism to slow-down telomere shortening, cell division leads to progressive attrition of chromosomes, leading to the onset of cellular senescence or apoptosis. However, telomere restoration based on telomerase activity is the primary mechanism for telomere maintenance. Telomere length is associated to health and survival and can be impacted by a broad panel of environmental factors. However, the effect of contaminants on telomeres is poorly known for living organisms. The aim of this study was to investigate relationships between some poly- and perfluoroalkyl substances (PFASs), body condition and telomere length by using both a cross-sectional and longitudinal approach in adult breeding Black-legged kittiwakes (*Rissa tridactyla*) from Svalbard. First, we examined the associations between absolute telomere length and PFASs contamination in a given year (cross-sectional approach). Second, we investigated the relationships between telomere dynamics and PFASs contamination within a two years' time frame (longitudinal approach). Our results did not show any significant relationships of PFASs and body condition with absolute telomere length in a given year. Surprisingly, we found a positive and significant relationship between PFASs and telomere dynamics in both sexes with elongated telomere in birds bearing the highest concentrations of PFASs. Our study underlines (i) the need to investigate PFAS effects on telomere dynamics with a longitudinal approach and (ii) a potential positive effect of these contaminants on telomere length, with the most contaminated birds showing the slowest rate of telomere shortening or even displaying elongated ones. Our study is the first to report a relationship between PFASs and telomere length in free-living vertebrates. A possible underlying mechanism and other potential confounding factors are discussed.

**Capsule**

Absolute telomere length was unrelated to perfluoroalkyl substances (PFASs) concentration in a given year whereas telomere attrition over 2 years was inversely related to PFASs contamination in adult breeding Black-legged kittiwakes (*Rissa tridactyla*) from Svalbard.

**Keywords:**

- Organic contaminants
- PFASs
- Black-legged kittiwake
- Svalbard
- DNA

**Highlights:**

- Relationships between PFASs and telomeres were studied in Arctic kittiwakes
- Absolute telomere length was unrelated to PFAS concentrations in a given year
- However, telomere attrition over 2 years was inversely related to PFASs burden
- Telomere elongation was observed in 4 birds (23.5%) with high levels of PFASs
- Longitudinal studies seem to be relevant to assess the effect of PFASs on telomeres

## 1. Introduction

Halogenated contaminants such as the poly- and perfluoroalkyl substances (PFASs) are synthetically manufactured chemicals produced since the 1950s. They are mainly used as surfactants and water repellents in numerous industrial and commercial applications because of their unique hydrophobic and oleophobic properties (e.g. fire-fighting foam, waterproof clothing, non-stick coating and impregnation agent for carpets, papers and textiles; Kissa, 2001). PFASs are either released in the environment by direct discharge ("direct emissions") or result from the degradation of precursor compounds ("indirect emissions"; Butt et al., 2010). PFASs are carbon chains varying in length, where hydrogen is replaced by fluorine atoms. Chemical bonds between carbon and fluorine atoms are very strong which make the PFASs thermally and chemically stable, resistant to degradation, and thus extremely persistent in the environment (Key et al., 1997; Muir and de Wit, 2010). Because of atmospheric long-range transport and oceanic currents, some PFASs reach remote areas such as the Arctic marine ecosystem, where they are preferentially deposited because of cold climate (AMAP, 2004; reviewed in Butt et al., 2010; Ellis et al., 2004; Giesy and Kannan, 2001; Prevedouros et al., 2006). The Arctic is therefore considered as a sink for environmental contaminants such as the PFASs. Specifically the perfluoroalkyl carboxylic acids (PFCAs), seem to increase in Arctic marine biota, contrary to the PFOS, a compound that belongs to sulfonic acids (PFSAs) which appears to decline since mid-2000s, after the phase-out by the US company 3M (reviewed in AMAP, 2016; Braune and Letcher, 2013; reviewed in Butt et al., 2010; Rotander et al., 2012; Wania, 2003).

Once deposited in the marine ecosystem, PFASs enter in the food chain with phytoplankton uptake, bioaccumulate in living organisms *via* food intake and increase with the trophic position due to biomagnification (Fang et al. 2014; Haukås et al., 2007; Kannan et

al., 2005; Kelly et al., 2009; Tommy et al., 2004). There is now strong evidence that (i) PFASs accumulate and persist in protein-rich compartments (e.g. blood, liver, kidneys) and (ii) PFASs biomagnification is enhanced as the carbon chain length increases (Aas et al., 2014; reviewed in Butt et al., 2010; Conder et al., 2008; Kelly et al., 2009; Verreault et al., 2005). Indeed, PFAS profiles in liver and/or plasma of four Arctic seabird species, the Thick-billed murres (*Uria lomvia*), the Northern fulmar (*Fulmarus glacialis*), the Glaucous gull (*Larus hyperboreus*) and the Black-legged kittiwake (*Rissa tridactyla*), were dominated by long chained PFCAs (Butt et al., 2007; Tartu et al., 2014; Verreault et al., 2005). As top predators, Arctic seabirds are exposed to relatively high concentrations of environmental contaminants; they are thus considered as extremely pertinent biological indicators to investigate the potential hazardous effects of PFASs on wildlife. To date, our knowledge about effects of PFASs exposure is limited (DeWitt, 2015; Jensen and Leffers, 2008; Lau et al., 2007), especially for free-living animals, although few studies have reported interactions between PFASs and physiology. For instance, several studies conducted on fishes and birds reported high concentrations of thyroid hormones and low levels of stress hormones in most PFASs contaminated individuals (Braune et al., 2011; Liu et al., 2011; Nøst et al., 2012; Tartu et al., 2014). More importantly, it has been suggested that PFASs could decrease the hatching success in two avian species, the Black-Legged kittiwake and the Tree swallow (*Tachycineta bicolor*; Custer et al., 2012; Tartu et al., 2014; but see also Bustnes et al., 2008). Further investigations focusing on wildlife and including more physiological and fitness traits are needed to better assess the impact of contaminants on animals living in natural ecosystems (Kannan, 2011; Lau et al., 2007).

Among potential physiological investigations to be conducted for a better assessment of the toxicological consequences of PFASs exposure, are the telomeres. Telomeres are non-coding DNA repeats located at the termini of eukaryotic chromosomes and play a key role in

ensuring the genomic stability (Blackburn, 1991; Monaghan and Haussmann, 2006). Because the DNA polymerase protein complex is unable to fully achieve the chromosomes replication during mitosis (i.e. end-replication problem), telomere length progressively shortens through life as a consequence of repeated cell divisions (Blackburn, 1991; Olovnikov, 1996; Sedivy, 1998). When telomere length is too short, cell division can damage coding DNA inducing cellular senescence or apoptosis (Blasco, 2007; Campisi et al., 2001; Harley et al., 1990; Olovnikov, 1996). Importantly, telomere length and telomere dynamics have been shown to be reliable predictors of longevity and survival in captive and wild vertebrates (Ashgar et al., 2015; Barrett et al., 2013; Bauch et al., 2014; Bize et al., 2009; Boonekamp et al., 2014; Haussmann et al., 2005; Heidinger et al., 2012; Fairlie et al., 2016; Foote et al., 2010; Salomons et al., 2009). Moreover, recent studies have demonstrated that the rate of telomere shortening varies to a great extent between individuals. Indeed, telomere shortenning has been shown to be accelerated by the occurrence of a wide range of environmental stressors (Angelier et al., 2013; Epel et al., 2004; Hau et al., 2015; Meillère et al., 2015; Mizutani et al., 2013; Salmón et al., 2016; Young et al., 2013) including heavy metals and persistent organic contaminants (Blévin et al., 2016, Stauffer et al., 2017). However, there is still very few information regarding the effects of contaminants on absolute telomere length in free-living animals and no studies have been conducted so far on telomere dynamics, with a longitudinal approach. To the best of our knowledge, a single study has investigated the influence of PFASs on absolute telomere length (with a cross-sectional approach) in free-living birds but did not report any significant relationships (Sletten et al., 2016). Because of this link with survival and environmental stressors, measuring the effect of specific compounds on telomere length and telomere dynamics appear promising to better assess their impact on wildlife (Bateson, 2015).

In Svalbard, Black-legged kittiwakes (*Rissa tridactyla*, hereafter "kittiwakes"), are exposed to a complex cocktail of organic contaminants and heavy metals which are known to correlate with impaired individual fitness and population dynamics (Goutte et al., 2015; Tartu et al., 2013, 2014, 2015, 2016). Kittiwakes are thus potentially sensitive to a broad mixture of contaminants with many possible additive, synergistic, as well as antagonistic effects. The aim of the present study is to investigate the relationships between several measured PFASs (11 PFCAs and 3 PFSAs), body condition and telomere length by using both a cross-sectional and longitudinal approach in adult breeding kittiwakes from Svalbard. First, we examined the relationships between PFASs contamination and absolute telomere length within a given year (cross-sectional approach in 2012). Second, we investigated the associations between PFASs contamination in 2012 and telomere dynamics by sampling the same kittiwakes twice, over a time frame of two years (longitudinal approach, between 2012 and 2014). Predictions are challenging since the impact of PFASs on the survival rate of free-ranging vertebrates remains undocumented with the exception of a study conducted on the glaucous gull where no relationships between PFASs and adult returning rate were found (Bustnes et al., 2008). However, since PFASs are expected to be detrimental for living organisms and appear to disrupt several physiological processes (e.g. endocrine disruption) in wildlife, as well as in laboratory animals (Austin et al., 2003; reviewed in De Witt 2015; reviewed in Lau et al. 2007; Liu et al., 2011), we predict that a high PFASs contamination will be associated with a rapid rate of telomere shortening (longitudinal approach), and thus, with short telomeres (cross-sectional approach).

## 2. Material and methods

175 Fieldwork was conducted in 2012, from 12th to 27th July and in 2014, from 26th June to
176 20th July, within a colony of kittiwakes at Kongsfjorden (78°54'N; 12°13'E), Svalbard. In
177 2012, 44 breeding adults (22 males and 22 females) were trapped while sitting on their nest
178 with a loop at the end of long pole during the chick rearing period. All birds were assigned
179 with a unique three-letter code fixed to the bird's tarsus. We collected a 2 mL blood sample
180 from the alar vein using a heparinized syringe and a 25-gauge needle to assess PFAS
181 concentrations, measure telomere length and determine gender. Then, skull length (head +
182 bill) was measured with an accuracy of 0.1 mm using a calliper and birds were finally
183 weighted to the nearest 2 g with a Pesola spring balance. In 2014, 17 birds (12 males and 5
184 females) out of the 44 kittiwakes caught in 2012 were recaptured after identification at a
185 distance using a telescope. Indeed, in that colony the adult annual survival rate is 85% and the
186 percentage of birds successfully reaching the chick rearing is about 75% (Goutte et al., 2015).
187 Moreover, some birds were not possible to catch. After capture, these birds were blood
188 sampled to assess PFAS concentrations (only 6 birds) and measure telomere length. Blood
189 samples were stored on ice in the field. Plasma and red blood cells, obtained after
190 centrifugation were kept frozen at -20°C before subsequent lab work.

191 Telomere analysis was performed from red blood cells collected in 2012 (n = 38; 22
192 males and 16 females) and in 2014 (n = 17; 12 males and 5 females) at the Centre d'Etudes
193 Biologiques de Chizé in France (CEBC). Indeed, over the 44 individuals caught in total in
194 2012, telomeres analysis was conducted on 38 individuals since not enough blood was left for
195 4 females. Telomere length was measured with the telomere restriction fragment method
196 (TRF) by Southern blot and using the TeloTAGG Telomere Length Assay (Roche,
197 Mannheim, Germany) as previously described and with minor modifications (Foote et al.,
198 2010; Kimura et al., 2010a). Specifically, we have adjusted the quantity of DNA to allow a
199 correct visualisation of the DNA signal on the gels. Briefly, samples were digested with

proteinase K and DNA was extracted from red blood cells using the DNeasy blood and tissue kit (Qiagen). Gel electrophoresis and optical density spectrophotometry were used to check for DNA quality. 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 with a pulse-field gel electrophoresis (Bio-Rad) on a 0.8% agarose gel. Samples were randomly assigned to a gel except those used to assess telomere length dynamics which were treated in the same gel. At total, all samples were run in 4 gels. Internal controls were run on each gel to measure inter-gel variations and each gel was 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 placed in an incubator and dried 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 software and measured from telomere smear densities. Lane-specific background was subtracted from each density and telomere length (mean value) was then calculated within a window of 5-30 kb that includes the whole smear (Nussey et al., 2014). Inter-gel CV was 1.40. Telomere dynamics relates to the difference of telomere length between 2014 and 2012. Molecular sexing was conducted at the CEBC, from red blood cells of samples collected in 2012 (22 males and 22 females) by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) present on sexual chromosomes following Fridolfsson and Ellegren, (1999).

PFAS concentrations were determined from plasma samples collected in 2012 (n = 44; 22 males and 22 females) and 2014 (n = 6; 4 males and 2 females) at the Norwegian Institute for Air Research (NILU) in Tromsø, Norway. We searched for 14 PFASs: perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), linear perfluorooctanesulfonate (PFOSlin), perfluorobutanoate (PFBA), perfluoropentanoate (PFPA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDcA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTrA), and perfluorotetradecanoate (PFTeA). Compounds not detected in 100% of the samples were not included in statistical analyses. Thereby, those remaining for further investigations were PFOSlin, PFNA, PFDcA, PFUnA, PFDoA, and PFTrA. Briefly, a sample (0.5 mL) spiked with internal standards was extracted in acetonitrile (1 mL) by repeated sonication and vortexing. The supernatant was cleaned-up using ENVI-Carb graphitized carbon absorbent and glacial acetic acid. Extracts were analyzed by UPLC/MS/MS. Recovery of the internal standards ranged between 50% and 120% and the deviation of the target concentrations in the SRMs (NIST Human serum 1958) were within the laboratory's accepted range (76-105%; n = 3). All blanks concentrations were below the instrument detection limits. Limit of detection of each compound is given in Table 1.

Statistical analyses were performed using R 3.3.1 (R Core Team, 2016). We first performed a principal component analysis (PCA; "Ade4 package") with individual PFASs in order to reduce the number of explanatory variables. We preferred this method instead of examining each contaminant separately because, (i) PFAS compounds are highly correlated with each other and (ii) it considerably decreases the number of statistical models since testing many models can potentially increase the type I error. The appropriate use of PCA was tested and confirmed through the Kaiser-Mayer-Olkin measure of sampling adequacy (K-M-

249 O = 0.74) and the Bartlett's test of sphericity ($p < 0.001$). The number of significant principal
250 components was selected according to the Kaiser criterion (i.e. eigenvalue higher than 1;
251 Kaiser, 1960). The PCA resulted in one component (PC1), explaining 71% of the total
252 variance and mainly influenced by high concentrations of PFDcA (factor loading: 0.45),
253 PFUnA (0.45), PFOSlin (0.44), PFDoA (0.44) and to a minor extent PFTrA (0.33) and PFNA
254 (0.32). Body condition was calculated with the residuals of the regression of body mass
255 against skull length. The influence of contaminants and body condition in 2012 on absolute
256 telomere length in 2012 and telomere length dynamics were investigated using linear models.
257 Thus, PC1, body condition and sex were considered as explanatory variables while telomere
258 length in 2012 and telomere dynamics were defined as response variables. Because PFAS
259 concentrations in 2012 were different between sexes (Table 1), including the factor “sex” with
260 the PFASs variable in the same model could induce multicollinearity problems and lead to
261 biased results (Graham, 2003). However, it has been proposed to use the variance inflation
262 factor (VIF) as a statistical tool to assess the extent of dependence between explanatory
263 variables. Several studies suggested that below a value of 10, dependence is no longer a major
264 issue (Chatterjee and Price, 1991; Neter et al., 1990), but a more stringent approach is to
265 consider VIF < 3 (Zuur et al., 2009). Because males were more contaminated than females,
266 VIF was then calculated between PC1 and the factor “sex” to ensure that these explanatory
267 variables met independence (VIF = 1.16; calculated with “AED package” developed by Zuur
268 et al., 2009). Biologically relevant models were constructed with PC1, body condition, sex
269 and interactions of PC1 and body condition with sex as predictor variables. The best models
270 were then selected with the bias-adjusted Akaïke’s Information Criterion (AICc), defined as a
271 bias adjustment for small-sample size (Burnham and Anderson, 2002). If AICc values differ
272 by more than 2, the lowest AICc is the more accurate, whereas if AICc differ by less than two,
273 models are considered as fairly similar in their ability to describe the data. Additionally, the

Akaike weight (*Wi*) was estimated and can be interpreted as approximate probabilities that the model *i* is the best one to predict the data, given the candidate set of models (Burnham and Anderson, 2002; Johnson and Omland, 2004). We finally performed diagnostic plots and Shapiro normality tests on residuals to check if the data sufficiently met the linear model assumptions (Zuur et al., 2009). Data were log-transformed when testing for sex differences of PFAS concentrations and when investigating correlations between each PFAS compounds. A significance level of $\alpha < 0.05$ was used for all tests.

## 3. Results

### 3.1. PFAS concentrations

Plasma PFAS mean concentrations ± standard errors for chick-rearing adult kittiwakes in 2012 are listed in Table 1. Linear models to test gender-related differences indicated that all PFASs except PFNA and PFTrA significantly differed between sexes, with males having higher concentrations than females. Such sex-related differences of PFAS concentrations could be attributed either to the ability of females to transfer elevated amounts of contaminants into their eggs (Gebbink and Letcher., 2012) and/ or to sexual differences regarding foraging ecology, with males feeding at higher trophic levels or in more contaminated areas than females. All PFASs (log-transformed) were highly and positively correlated with each other (Pearson correlations: $0.49 \leq r \leq 0.93$, all p-values $< 0.001$; $n = 44$), indicating similar exposure routes. Finally, PFAS concentrations seem to be repeatable (from 2012 to 2014) within the same individuals ($r = 0.59$, $n = 6$; calculated from the repeatability equation developed by Lessels and Boag, 1987). In other words, an individual with relatively high levels of PFASs in 2012 will also show relatively high levels of PFASs in 2014.

However, the sample size is low (n = 6) and further studies conducted on a larger sample size would enable to confirm this statement.

### 3.2. Relationships between PFASs, body condition and telomere length

The model selection to explain absolute telomere length based on PFAS concentrations (PC1) and body condition in 2012 for male and female adult kittiwakes is presented in Table 2. Among the set of candidate models, the null model (parameterized with an intercept only) showed the best fit to the data. None of the other candidate models including sex, PC1 or body condition (as well as the interaction terms with sex) was better than the null model. These variables were therefore not good predictors of absolute telomere length, and PFAS concentrations in 2012 do not appear as good explanatory variables of absolute telomere length in 2012 (PC1, slope: a = 0.06; p = 0.443; Fig. 1).

The model selection to explain telomere dynamics between 2012 and 2014 based on PFAS concentrations (PC1) and body condition in 2012 for male and female adult kittiwakes is presented in Table 3. Among the set of candidate models, the model including PC1 best fitted the data (**Δ**AICc = 2.8). PC1 was significantly and positively related to telomere dynamics (Fig. 2; slope: a = 0.17, p = 0.026). In other words, the most PFASs contaminated individuals in 2012 were those showing the slowest rate of telomere shortening from 2012 to 2014. Body condition and the gender were not were not considered as good predictors of telomere dynamics (Table 3).

## 4. Discussion

We observed no relationships between PFASs, body condition and absolute telomere length when analyzing only one year (cross-sectional approach in 2012). However, the results from the longitudinal approach indicated PFASs in 2012 as the best predictor of telomere dynamics. There was a significant and positive relationship between PFAS plasma concentrations in 2012 and telomere dynamics with the most PFASs-contaminated individuals showing the slowest rate of telomere shortening from 2012 to 2014. Additionally, among the most PFAS contaminated birds, 4 individuals displayed elongated telomeres from 2012 to 2014. This suggests some potential positive effects of PFASs contamination on telomeres. Considering the discrepancy in the findings between the two approaches, our study highlights the need to investigate the effects of PFASs on telomere dynamics with a longitudinal approach, rather than simply measuring absolute telomere length in a single snapshot. In vertebrates, most of telomere shortening occurs early in life, during growth and developmental stages and this rate of early-life shortening varies to a great extent between individuals (Boonekamp et al., 2014; Hall et al., 2004; Foote et al., 2010; Frenck et al., 1998; Friedrich et al., 2001; Rattiste et al., 2015; Salomons et al., 2009; Zeichner et al., 1999). In addition, telomere length can also be affected later in life, in adults, by variation in stressful experiences (Angelier et al., 2013; Epel et al., 2004; Hau et al., 2015; Mizutani et al., 2013; Young et al., 2013). As a result, there is probably a large inter-individual variability in telomere length in adult kittiwakes and this variability may result from several factors that were not taken into account in our analyses (e.g. age, environmental stressors, etc.). This large inter-individual variability can certainly blur the potential effect of PFASs contamination on telomere length when using a cross-sectional approach, possibly explaining why we were not able to detect any correlations between PFASs contamination and absolute telomere length in 2012. Because PFASs contamination appears quite repeatable over two years within the same

individual, the longitudinal approach allows us to relate such PFASs contamination in 2012 with telomere dynamics.

Only three studies have studied the associations between contaminants and telomere length in free-ranging vertebrates (Blévin et al., 2016; Sletten et al., 2016; Stauffer et al., 2017). Thus, this work contributes at filling the gap of knowledge about the potential effects of environmental contaminants on telomere length in wildlife. Contrary to our results from the longitudinal approach, PFASs did not predict telomere length in white-tailed eagle (*Haliaeetus albicilla)* chicks (Sletten et al., 2016). However, this study did not investigate the relationships between contaminants and telomere dynamics, but rather used a cross-sectional approach (i.e. a single measure of telomere length). This could potentially explain the discrepancy between the results of the two studies. Another potential explanation would rely on the difference of concentrations of contaminants between eagle chicks and kittiwake adults but this statement does not seem relevant here. While PFOSlin concentration in kittiwakes (9 884 ± 462 pg/g ww) were on average 4 times lower than those in eagle chicks (40 914 ± 5 746 pg/g ww), PFUnA concentration in kittiwakes (10 746 ± 509 pg/g ww) were on average 2 times higher than those in eagle chicks (5 609 ± 525 pg/g ww). Finally, a recent study conducted on the same kittiwake population showed a negative relationship between telomere length and oxychlordane (Blévin et al., 2016), a metabolite of an organochlorine pesticide considered as very toxic for wildlife (Bustnes et al., 2006; Erikstad et al., 2013; Goutte et al., 2015). Organochlorines and PFASs are structurally opposed, with organochlorines being lipophilic (Frindlay and Defretas, 1971) and PFASs having a high affinity with proteins (Heuvel et al., 1992). Moreover, kittiwakes are exposed to an additional mixture of chemicals, which are not included in this study and which could act on telomere length (Stauffer et al., 2017). Consequently, further investigations focusing on various chemicals, structurally different, may enable to clarify such contrasted results.

Telomere length adjustment is dynamic with both shortening and maintenance events. Despite a mechanism to slow-down telomere shortening, the end-replication problem leads to progressive attrition of chromosomes, leading to the onset of cellular senescence or apoptosis (Blasco, 2007; Campisi et al., 2001; Harley et al., 1990; Olovnikov, 1996). However, telomere restoration based on telomerase activity, an enzyme adding new telomeric sequences onto the ends of chromosomes at each DNA replication, has been shown to be the primary mechanism for telomere maintenance and genomic integrity (Blackburn, 1991, 2005; Greider and Blackburn, 1985). Telomerase is variably active in several somatic and post-somatic tissues throughout the lifespan of long-lived seabirds (Haussmann et al., 2007). This latest study highlighted the very high activity of telomerase in bone marrow during the whole lifespan of two seabird species, the Common tern (*Sterna hirundo*) and the Leach's storm petrel (*Oceanodroma leucorhoa;* Haussmann et al., 2007). The authors stated that "*telomerase activity in bone marrow may be associated with the rate of erythrocyte telomere shortening; birds with lower rates of telomere shortening and longer lifespans have higher bone marrow telomerase activity throughout life*". Indeed, all circulating erythrocytes in birds are produced by the hematopoietic stem cells of the bone marrow (Sturkie and Griminger, 1976), and telomere length measured in erythrocytes appear to mirror the telomere length of stem cells in bone marrow (Kimura et al., 2010a; Vaziri et al., 1994; but see Reichert et al., 2013). Thus, which underlying mechanisms could induce a disruption of telomerase activity and how can it be related to PFASs contamination? Indeed, several correlational and experimental studies have highlighted a potential role of glucocorticoids in determining telomere dynamics: increased glucocorticoids concentration (i.e. corticosterone and cortisol) were associated with a down-regulation of telomerase activity or/and an accelerated rate of telomere shortening (Bauch et al., 2016; Choi et al., 2008; Haussmann et al., 2012; Quirici et al., 2016; Schultner et al., 2014; Young et al., 2016; but see Epel et al., 2010; Young et al.,

2016). Importantly, another investigation conducted in the same kittiwake population reported a negative relationship between baseline corticosterone levels and PFAS concentrations (Tartu et al., 2014). Even if underlying mechanisms are currently unclear, PFASs-induced lower circulating corticosterone levels might potentially result in relatively high telomerase activity in bone-marrow, and therefore in decreased rate of telomere shortening in highly contaminated kittiwakes.

Our study reported some telomere elongation between 2012 and 2014 in 4 kittiwakes. Interestingly, telomere elongation has already been associated with nutritional and climatic factors. Recently, Hoelzl et al. (2016) showed that food supplementation reduces telomere attrition and is even associated with telomere elongation in a wild mammal species, the dormouse (*Glis glis*). Similarly, Bebbington et al. (2016) reported an increased telomere length with high food availability in a small passerine, the Seychelles warbler (*Acrocephalus sechellensis*). Finally, a study conducted on the Black-tailed gull (*Larus crassirostris*) highlighted a potential positive effect of El Niño on telomere dynamics (Mizutani et al., 2013). Therefore, the lower rate of telomere shortenning in most PFASs contaminated kittiwakes highlighted in our study, in combination with good environmental conditions, could potentially explain why we observed telomere elongation in some kittiwakes.

We proposed here one possible underlying physiological mechanism, based on endocrine disruption, potentially explaining the reduced rate of telomere shortening in most PFAS-exposed kittiwakes. Although causality is difficult to assess in correlational studies, the relationships with telomere dynamics reported here may rely on ecological factors, rather than PFASs contamination. Besides, a study conducted in the same kittiwake colony reported a positive relationship between PFASs contamination and body condition in males (Tartu et al., 2014). This could suggest that the apparent positive effect of PFASs on telomere length maybe related to individual quality rather than to PFASs contamination. That is the reason

why we included body condition in our analyses as a potential predictor of telomere length. However, body condition in 2012 was not related to absolute telomere length in 2012 and telomere dynamics. Indeed, telomere length does not fluctuate as fast as the body condition does, which is probably too labile compared to the slower rate of change of telomeres. Therefore, further ecological variables directly linked to feeding ecology (e.g. stable isotopes, protein amounts) of kittiwakes should be included as predictors of telomere length. Indeed, since food ingestion is the main route for PFASs exposure, the most contaminated kittiwakes could be the birds feeding at the highest trophic levels and are possibly the individuals of the highest quality.

Another important point that deserves to be discussed is a potential confounding effect of age which is suggested to negatively affect telomere length (Haussmann and Vleck, 2002; Haussmann et al., 2003). However, this is particularly true for species with shorter lifespans which lose more telomeric repeats with age than species with longer lifespans (Haussmann et al., 2003). Indeed, in long-lived species, telomere loss appears to occur mainly early in life (i.e. between chick and adult stage) rather than during adulthood (Hall et al., 2004; Foote et al., 2010), as is the case in other vertebrates (Frenck et al., 1998; Rufer et al., 1998; Zeichner et al., 1999; Friedrich et al., 2001). Since our study was conducted on breeding adults (i.e. at least 3–4 years old; Coulson, 2011) of a long-lived seabird and because we investigated telomere dynamics, with a longitudinal approach, we have some good reasons to think that age in our study is not a major factor influencing telomere length. However, relationships between age and PFASs in seabirds remains undocumented so far and thus, a potential confounding effect of age on PFAS concentrations here cannot be completely ruled out.

Despite some limitations and a moderate sample size, the positive relationship between PFASs contamination and telomere dynamics reported here could suggest a positive effect of PFASs exposure on telomeres and *in fine*, on survival rate of adult kittiwakes. This

seems to be corroborated by findings from a recent study about PFASs and self-maintenance metabolism (Basal Metabolic Rate) conducted also on kittiwakes which supports the hypothesis that PFASs may stimulate self-maintenance mechanisms (Blévin et al., 2017). However, only capture-mark-recapture (CMR) investigations would enable to confirm this statement and to fully validate our findings, future experimental investigations focusing on the effects of PFASs on telomere length should be carried out with a laboratory avian model.

## Conflict of interest

The authors declare to have no conflicts of interest.


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**Table 1**

Plasma PFAS mean concentrations ± standard errors (ng/ mL ww) in 2012 and limits of detection (LODs) of female and male chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. PFAS gender-related differences have been tested with linear models.

| | | **Males (n = 22)** | **Females (n = 22)** | | |
|---|---|---|---|---|---|
| | **LODs** | **Mean ± SE** | **Mean ± SE** | $F_{1,42}$ | **P-value** |
| PFOSlin[a]* | 704 $10^{-3}$ | 10.85 ± 0.58 | 8.92 ± 0.68 | 5.81 | **0.02** |
| PFNA[b]* | 40.9 $10^{-3}$ | 1.21 ± 0.1 | 1.08 ± 0.14 | 1.92 | 0.173 |
| PFDcA[c] | 61.9 $10^{-3}$ | 2.2 ± 0.12 | 1.63 ± 0.12 | 11 | **0.002** |
| PFUnA[d] | 83 $10^{-3}$ | 12.11 ± 0.64 | 9.38 ± 0.69 | 8.40 | **0.006** |
| PFDoA[e]* | 109 $10^{-3}$ | 2.54 ± 0.14 | 1.99 ± 0.17 | 8.75 | **0.005** |
| PFTrA[f]* | 360 $10^{-3}$ | 11.62 ± 1.41 | 9.68 ± 1.52 | 1.57 | 0.217 |

Significant p-values are in bold.
*Data were log-transformed to meet the assumption of the linear model
[a] PFOSlin: Perfluorooctane sulfonate
[b] PFNA: Perfluorononanoate
[c] PFDcA: Perfluorodecanoate
[d] PFUnA: Perfluoroundecanoate
[e] PFDoA: Perfluorododecanoate
[f] PFTrA: Perfluorotridecanoate

783 **Table 2**

784 AICc model ranking for absolute telomere length in 2012 based on PFAS concentrations
785 (PC1) and body condition in 2012 in chick-rearing adult kittiwakes *Rissa tridactyla* from
786 Kongsfjorden, Svalbard. (n =38, 22 males and 16 females). PFASs were measured in plasma.

| **Models** | **AICc** | **ΔAICc** | ***Wi*** 787 |
|---|---|---|---|
| Null | 99.6 | 0 | 0.35 |
| Sex | 99.8 | 0.3 | 0.30 |
| PC1 | 101.3 | 1.7 | 0,15 |
| Body condition | 101.8 | 2.2 | 0,11 |
| PC1 * Sex | 102.9 | 3.3 | 0,07 |
| Body condition * Sex | 104.8 | 5.3 | 0,02 |

788 AICc, bias-adjusted Akaike's Information Criteria values; *Wi,* AICc weights.

**Table 3**

AICc model ranking for telomere dynamics between 2012 and 2014 based on PFAS concentrations (PC1) and body condition in 2012 in chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard (n =17, 12 males and 5 females). PFASs were measured in plasma.

| **Models** | **AICc** | **ΔAICc** | ***Wi*** |
|---|---|---|---|
| PC1 | 28.8 | 0 | 0.7 |
| Null | 31.7 | 2.8 | 0.17 |
| PC1 * Sex | 34 | 5.2 | 0.05 |
| Sex | 34.4 | 5.6 | 0.04 |
| Body condition | 34.6 | 5.8 | 0.04 |
| Body condition * Sex | 41.8 | 13 | 0 |

AICc, bias-adjusted Akaike's Information Criteria values; *Wi*, AICc weights.

**Fig. 1.** Relationship between PC1 and absolute telomere length in 2012 in chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. The effect of PFAS concentrations in 2012 on telomere length in 2012 was tested with a linear model (slope: a = 0.06, p = 0.443). PC1 is mainly influenced by high concentrations of PFOSlin, PFDcA, PFUnA, PFDoA and to a minor extent PFNA and PFTrA. Males (n = 22) are represented with empty circles and females (n = 16) with filled circles.

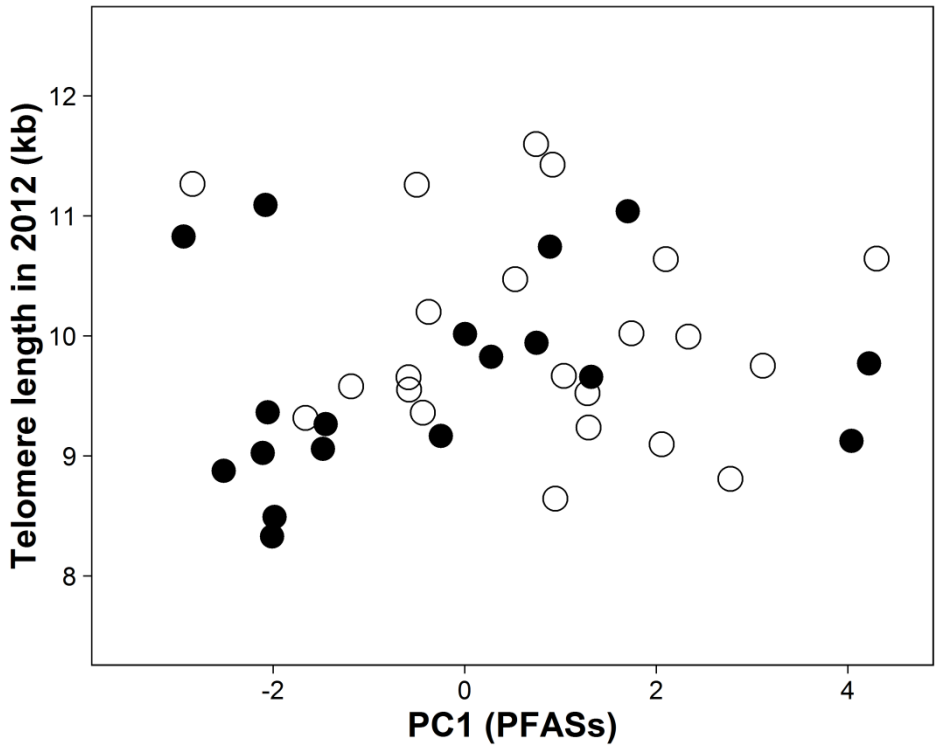

**Fig. 2.** Relationship between PC1 and telomere dynamics (the difference of telomere length between 2012 and 2014) in chick-rearing adult kittiwakes *Rissa tridactyla* from Kongsfjorden, Svalbard. The effect of PFASs in 2012 on telomere dynamics was tested with a linear model (slope: a = 0.17, p = 0.026). PC1 is mainly influenced by high concentrations of PFOSlin, PFDcA, PFUnA, PFDoA and to a minor extent PFNA and PFTrA. Males (n = 12) are represented with empty circles and females (n = 5) with filled circles. Individuals above red dashed line have increased telomere length whereas the ones bellow showed decreased telomere length between 2012 and 2014.