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1 Sex- and age-specific mercury accumulation in a long-lived seabird

2

3 Justine Bertram^{a,*}, Coraline Bichet^b, Maria Moiron^{a,c}, Peter J. Schupp^{d,e}, Sandra Bouwhuis^a

4 ^a Institute of Avian Research, An der Vogelwarte 21, Wilhelmshaven, Niedersachsen, DE 26386

5 ^b Centre d'Etudes Biologiques de Chizé (CEBC), CNRS-La Rochelle Université, Villiers-en-Bois,

6 France

7 ^c Department of Evolutionary Biology, Bielefeld University, 33615, Bielefeld, Germany

8 ^d Carl von Ossietzky Universität Oldenburg, Department for Chemistry and Biology of the Marine

9 Environment, Terramare, Wilhelmshaven, Niedersachsen, DE 26382

10 ^e Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg

11 Oldenburg, DE 26129

12

13 *Author for correspondence: justine.bertram@ifv-vogelwarte.de

14

15 **Abstract**

16 Mercury levels in the environment are increasing, such that they are also expected to accumulate in top-
17 predators, but individual-based longitudinal studies required to investigate this are rare. Between 2017
18 and 2023, we therefore collected 1314 blood samples from 588 individual common terns (*Sterna*
19 *hirundo*) to examine how total blood mercury concentration changed with age, and whether this differed
20 between the sexes. Blood mercury concentrations were highly variable, but all exceeded toxicity
21 thresholds above which adverse health effects were previously observed. A global model showed blood
22 mercury to be higher in older birds of both sexes. Subsequent models partitioning the age effect into
23 within- and among-individual components revealed a linear within-individual accumulation with age in
24 females, and a decelerating within-individual accumulation with age in males. Time spent at the
25 (particularly contaminated) breeding grounds prior to sampling and laying in females were also found
26 to affect mercury concentrations. As such, our study provides evidence that male and female common
27 terns differentially accumulate mercury in their blood as they grow older and calls for further studies of
28 the underlying mechanisms as well as its consequences for fitness components, such as reproductive
29 performance and survival.

30

31 **Keywords**

32 THg, pollution, plasticity, ageing, phenology, longitudinal study

33

34 **Introduction**

35 Industrial activities and other drivers of global change, such as ongoing climate warming, increase
36 environmental mercury levels (Fitzgerald et al., 2007; Eagles-Smith et al., 2018; UN Environment,
37 2019). As mercury enters aquatic ecosystems, microorganisms transform it into its organic and
38 bioavailable form, methylmercury (MeHg). When biota ingest methylmercury, it can accumulate along
39 the food chain (Atwell et al., 1998), exposing long-lived top predators, such as some seabird species, to
40 particularly high levels. As such, seabirds have sometimes been used as bioindicators to monitor
41 environmental mercury contamination and to study its consequences for physiology, behaviour and
42 fitness components (Burger and Gochfeld, 2004). Blood is a tissue regularly used for this purpose, as it
43 enables the measurement of an individual bird's mercury contamination without sacrificing it (Kahle
44 and Becker, 1999). It additionally provides an opportunity for repeated sampling of the same individuals.

45 Repeated blood sampling of seabirds is most feasible during the breeding season, when
46 individuals are usually highly site faithful and can be caught and/or sampled during nest attendance on
47 land. Blood mercury levels during the breeding season largely reflect a combination of recent mercury
48 uptake, dependent on trophic position and contamination of prey items in the main foraging areas
49 (Monteiro and Furness, 1995), and levels of deposition in internal organs (Eagles-Smith et al., 2008;
50 Fromant et al., 2016). For migratory seabirds, however, there may also be carry-over effects from
51 mercury uptake in the wintering areas or during migration (Lavoie et al., 2014; Quillfeldt et al., 2022;
52 Carravieri et al., 2023), the influence of which could depend on the timing of sampling relative to the
53 timing of these other events along the annual cycle (Eagles-Smith et al., 2009a).

54 Besides by timing, local uptake and deposition in internal tissues, blood mercury contamination
55 may be affected by sex. Female birds have often been found to exhibit lower mercury levels than males,
56 potentially suggesting a role for egg laying in the ability of females to excrete part of their mercury
57 (Bond and Diamond, 2009; Ackerman et al., 2020; but see Robinson et al., 2012; Hitchcock et al., 2019).
58 To test whether egg laying could explain (part of) the observed sex difference, one would ideally sample
59 birds of both sexes both prior to, and after, female egg laying, or artificially manipulate the number of
60 eggs females produce, for example by conducting an egg removal experiment, inducing females to

61 produce additional eggs. Alternatively, however, one could test whether variation in clutch size explains
62 variation in post-laying levels of mercury contamination in both sexes, whereby one would expect a
63 negative correlation in females only in case of eggs being a pathway for detoxification.

64 Despite detoxification, potentially via eggs in females (Bond and Diamond, 2009; Ackerman et
65 al., 2020), and via feathers in birds of both sexes (>90%; Braune and Gaskin, 1987a; Whitney and
66 Cristol, 2017), if incomplete, the relative longevity of seabirds might predispose them to
67 bioaccumulation of mercury as they age (Rowe, 2008; Celik et al., 2021). Studies examining the age-
68 specificity of mercury contamination in birds have, however, not yet yielded conclusive evidence for
69 this to occur. Insightful first studies reported the expected positive correlation between age and mercury
70 concentration when comparing birds of different age classes, i.e. chicks vs. adults (Souza et al., 2020;
71 Bighetti et al., 2021). Given that chicks and adults do not only differ in age, but may also differ in diet
72 - with chicks feeding on smaller, potentially less contaminated prey items - and moult pattern - given
73 that chicks are in the process of growing their feathers, potentially depositing mercury - these results,
74 however, do not provide conclusive proof that mercury accumulates as birds grow older. Indeed, among
75 adult seabirds, further studies found either no (Thompson et al., 1993; Becker et al., 2002; Carravieri et
76 al., 2014, 2022; Tartu et al., 2015) or a negative correlation (Agusa et al., 2005; Tavares et al., 2013;
77 Tartu et al., 2014) between age and mercury concentration. Interpretation of the differences in the
78 perhaps surprising results among these latter studies is currently hampered by the fact that all were based
79 on cross-sectional data, which do not facilitate distinguishing between within-individual accumulation
80 of mercury with age and among-individual variation in survival in relation to mercury pollution level
81 (selective disappearance, *sensu* van de Pol and Wright, 2009). Mercury levels could, for example, be
82 positively associated with survival due to better foragers both acquiring more resources to aid survival
83 (as a function of individual quality), but also becoming more contaminated via increased dietary mercury
84 uptake (Binkowski et al., 2021). Alternatively, mercury levels could be negatively associated with
85 survival (Ma et al., 2018a) due to detrimental effects on physiology (Hawley et al., 2009; Lewis et al.,
86 2013; Ma et al., 2018a, 2018b; Adams et al., 2020; Grunst et al., 2023a). Such selective disappearance
87 would result in patterns of age-specific mercury contamination at the population level (here: an in- or
88 decrease, respectively) that could either mask or inflate within-individual patterns of accumulation.

89 When data have been collected longitudinally, methods are available to assess the relative importance
90 of within- and among-individual sources of age-related patterns of mercury contamination (van de Pol
91 and Wright, 2009). These methods have been routinely applied in studies of ageing in birds (see
92 Bouwhuis and Vedder, 2017 for a review), and could be useful when adopted in studies of avian
93 physiology as well.

94 In this study, we assess factors underlying variation in blood mercury concentrations in a long-
95 lived migratory seabird, the common tern (*Sterna hirundo*). Using 1209 blood samples obtained from
96 588 common terns of known age and sex, and collected across seven years, we investigate whether
97 variation in total mercury levels (THg) can be explained by the birds' (i) timing, assessed as the intervals
98 between arrival at the breeding colony, egg laying and blood sampling, (ii) sex, (iii) clutch size and (iv)
99 age. Importantly, after first running a global linear mixed effect model assessing the effect of age, we
100 adopt a longitudinal analysis approach and run a second linear mixed effect model to test whether age-
101 specificity of mercury concentration is best explained by within-individual processes and/or selective
102 (dis)appearance of birds in relation to, or based on, their mercury levels. Third, we add a within-season
103 perspective to our analyses, enabled by the fact that 88 birds were sampled repeatedly either within a
104 single breeding attempt or among two breeding attempts when birds re-nested after having failed.

105

106 **Methods**

107 **Study species and site**

108 Common terns are long-lived seabirds, mainly feeding on fish of a length up to 15 cm (Becker and
109 Ludwigs, 2004). After migration to their wintering areas along the African coast (Kürten et al., 2022),
110 they return to their breeding colony each spring (Dittmann and Becker, 2003; Dittmann et al., 2005).
111 Starting their first breeding attempt on average at an age of three years, common terns usually produce
112 clutches of one to three eggs, which are incubated by both pair members (Becker and Ludwigs, 2004).
113 Their colonial breeding, longevity and high level of site-fidelity (Szostek and Becker, 2012) make
114 common terns particularly suitable for extensive longitudinal studies.

115 We studied the mercury concentration in blood of common terns breeding at a monospecific
116 colony located at the Banter See in Wilhelmshaven, at the German North Sea coast (53°30'40" N,
117 08°06'20" E). Since 1992, this colony is monitored three times weekly to, among others, record laying
118 date and clutch size for each nest, and all locally hatched birds are marked with rings as well as
119 subcutaneously implanted transponders if they reach fledging age (Becker and Wendeln, 1997). The
120 colony site consists of six artificial islands (10.7 x 4.6 m), covered with gravel and surrounded by 60 cm
121 high walls. The walls sustain 44 resting platforms equipped with antennas that allow the automatic and
122 remote detection of arrival date (Moiron et al., 2020) and presence of transponder-marked birds.
123 Additionally, placing mobile antennas around each nest for at least 24 hours allows the identification of
124 breeders and linking them to their nests. The sex of the studied birds is determined molecularly following
125 Becker and Wink (2003).

126

127 **Blood collection**

128 Between May and August 2017 – 2023, we used the antenna system to identify 588 transponder-marked
129 common terns during incubation to collect 1209 blood samples 3 – 36 days after the first egg of their
130 clutch was laid. We collected 47 further blood samples from 45 birds within the same breeding attempt
131 within 1 – 24 days after their first blood collection in 2021 and 2023. Despite replacement clutches being
132 infrequent (approximately 9%), they significantly contribute to the lifetime reproductive success of
133 common terns (Becker and Zhang, 2011) and we made use of their occurrence to additionally collect 58
134 blood samples from 55 individuals within 19 – 55 days after their first blood collection in 2017, 2021,
135 2022 and 2023 (Table 1). In all cases, blood sampling was conducted prior to hatching of the first chick.
136 In total, we collected 1314 blood samples from 588 individuals (Table 1).

137 For blood sampling, we used starved kissing bugs (*Dipetalogaster maxima*; Becker et al., 2006).
138 We inserted one or two third larval instars into an artificial hollow egg, closely resembling real common
139 tern eggs in size and colour. After the target bird was detected on its nest by the automatic antenna
140 system, we replaced one of its real eggs with the “bug-egg”, storing the real egg in a foam-lined box
141 kept warm in an incubator at 37°C. Birds resumed incubation shortly after nest manipulation. Small

142 holes along the artificial egg allowed the bug(s) to suck blood from the incubating bird. Throughout this
 143 process, birds were meticulously observed from observation huts within the colony to prevent sample
 144 contamination by switches between partners. After 20 – 30 minutes, we visually confirmed whether the
 145 bug had successfully obtained a blood meal; if so, we retrieved the artificial egg and returned the real
 146 egg. Up to 500µl blood of the bird was then extracted from the bug's abdomen using a syringe and stored
 147 at -18°C until analysis. To ensure that mercury concentrations in blood sampled this way are not affected
 148 by bug physiology, in 2017, we additionally blood sampled 18 birds (7 males and 11 females) from the
 149 brachial vein using needles and capillaries. A correlation test, performed in R, showed a highly
 150 significant correlation of 0.93 (Fig. S1).

151

152 **Table 1: Details regarding the 1314 blood samples collected from 588 incubating common terns in the years**
 153 **2017 – 2023 (total (males; females)).**

Year	No of 1st blood samples taken	No of 2nd blood samples taken within the same clutch	No of 2nd blood samples taken from a replacement clutch
2017	157 (78; 79)	-	5 (2; 3)
2018	166 (78; 88)	-	-
2019	216 (106; 110)	-	-
2020	107 (51; 56)	-	-
2021	152 (73; 79)	26 (13; 13)	30 (14; 16)
2022	184 (97; 87)	-	11 (7; 4)
2023	227 (114; 113)	21 (8; 13)	12 (7; 5)
total	1209 (597; 612)	47 (21; 26)	58 (30; 28)

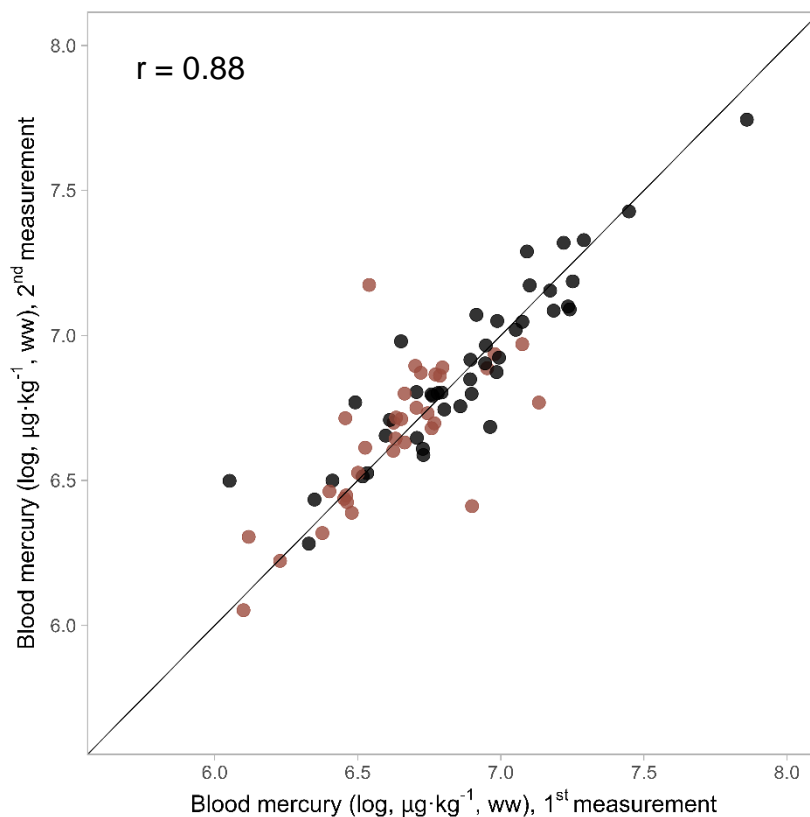
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155 **Mercury analysis**

156 Mercury analysis was performed using a Direct Mercury Analyzer (DMA-80, MLS Mikrowellen-Labor-
 157 Systeme GmbH, Germany) to quantify total mercury concentration (THg), which we report on a wet-
 158 weight (ww) basis, and which provides a reliable proxy for MeHg levels (Renedo et al., 2021). For each
 159 sample, ca. 20 µl of wet whole blood was pipetted into a nickel sample boat and weighted, then dried

160 and thermally decomposed in a stream of oxygen at 750 °C. This process released gaseous mercury that
161 was trapped by gold amalgamation to prevent its combustion along with the remaining compounds of
162 the blood sample. By subsequent heating to 850°C, the mercury was released and determined by atomic
163 absorption spectrometry at 253.7 nm. Each of our 35 measurement batches included an analytical blank
164 to verify the absence of contamination of the device and for 29 out of the 35 od the batches we have the
165 values obtained for a certified reference material (DORM-4; fish protein from National Research
166 Council, Canada, 412 $\mu\text{g}\cdot\text{kg}^{-1}$ dry weight; with a mean percent recovery \pm standard deviation of $109 \pm$
167 7%) to ensure its accuracy as well. The detection limit of THg was $0.0003 \mu\text{g}\cdot\text{g}^{-1}$ and none of the samples
168 showed a value below this limit.

169 As part of the laboratory's quality assurance and quality control (QA/QC) measures, it engages
170 in the QUASIMEME international laboratory performance study program twice a year with satisfactory
171 results. Nevertheless, to evaluate the reliability of our measurement method, we split 77 sufficiently
172 large blood samples to obtain duplicates, which were measured blindly and across batches. The relative
173 difference among duplicates was $10 \pm 11\%$ and a correlation test, using the cor.test function in R, showed
174 a highly significant correlation of 0.88 (Fig. 1), such that all further samples were measured only once.



176

177

178 **Figure 1:** Correlation between two total blood mercury concentration measurements of the same blood sample (n
179 = 43 male samples in black, 34 female samples in red). The line represents the x = y line, the slope of the correlation
180 between both equals 0.82 ± 0.05 SE.

181

182 **Statistical analyses**

183 To assess potential sources of variation in the blood THg levels of common terns, we first used a linear
184 mixed model with ‘blood THg concentration’ (in $\mu\text{g}\cdot\text{kg}^{-1}$ ww) as the dependent variable and ‘year’ and
185 ‘individual identity’ as random effects to account for the non-independence of repeated observations on
186 the same individual and potential environmental fluctuations across years. We \log_e -transformed our
187 dependent variable to meet model assumptions. We included ‘age’ as a linear and a quadratic covariate,
188 and ‘sex’ as 2-level and ‘clutch size’ as 3-level class variables, to not limit ourselves to assuming linear
189 effects. To account for the fact that common terns are migratory, and that the sampled individuals may
190 have taken different amounts of time to transfer from arriving to laying the first egg (Moiron et al.,
191 2023), we added the ‘interval between arrival and laying date’ as a covariate. Moreover, we added the
192 ‘interval between laying and sampling’ as another covariate to correct for any potential methodological
193 variation in the speed with which we could sample birds in the field (which mainly depends on the
194 synchrony of breeding). Finally, we tested for sex-specificity of our candidate variables by including all
195 interactions between ‘sex’ and the terms introduced above.

196 Given our finding of age-specificity of blood mercury levels (see Results and Table S1), we ran a second
197 linear mixed model where used a within-subject age standardization to separate within- and among-
198 individual age effects. Hereto, we partitioned the age of each individual into an ‘average age’ and ‘delta
199 age’ component (following van de Pol and Wright, 2009). An individual’s average age was calculated
200 as the average of all ages at which the bird was sampled, while delta age was calculated as the difference
201 between an individual’s actual age at sampling and its average age (i.e., ‘delta age’ = ‘actual age’ –
202 ‘average age’, see Table S2 for an example). When including both ‘average age’ and ‘delta age’ as

203 covariates in the model, ‘average age’ quantifies the among-individual age effect, whereas ‘delta age’
204 quantifies the within-individual change in THg concentration as birds grow older (van de Pol and
205 Wright, 2009). If the among- and within-individual age effects were to be significantly different from
206 each other, this would indicate that the effect of age among individuals cannot be explained by changes
207 within individuals, meaning that there is age-specific selective (dis)appearance of individuals with
208 certain levels of THg concentrations from the sampled pool of individuals (van de Pol and Wright,
209 2009). In addition to the main effects of ‘average age’ and ‘delta age’, we added the interaction between
210 them to test for non-linearity of any within-individual change in THg concentration with age, such as
211 accelerating or decelerating effects as birds grow older. Finally, as in the first model, we tested for sex-
212 specificity of our candidate variables by including all interactions between ‘sex’ and the introduced
213 terms, as well as the three-way interaction between ‘average age’, ‘delta age’ and ‘sex’. Candidate
214 variables were not highly correlated (Fig. S2), such that we were able to enter them simultaneously.

215 Given that we found a significant interaction between ‘sex’ and ‘interval between arrival and
216 laying date’ (in both the first and second model, see Results and Table S1), we proceeded to run the
217 second model for males and females separately, excluding the interactions with sex as well as the main
218 effect of sex (Table 2b and c). In addition, we repeated these sex-specific models while replacing ‘delta
219 age’ by the ‘actual age’ of an individual, to test whether the ‘average age’ and ‘delta age’ effects were
220 significantly different from one another (van de Pol and Wright, 2009), which would indicate selective
221 (dis)appearance of birds with low or high THg levels (see above, Tables 2b and c).

222 To determine how two THg concentrations obtained from the same bird in the same season
223 correlate, we ran a final linear mixed model with the log_e-transformed ‘second blood THg concentration’
224 as the dependent variable and the ‘first blood THg concentration’ as a covariate. As above, ‘individual
225 identity’ and ‘year’ were included as random effects. ‘Clutch’ of the second sample (same or
226 replacement) and ‘sex’ were included as 2-level class variables. Additionally, we added the interaction
227 between (i) ‘clutch’ and ‘sex’ to test whether the fact that females laid eggs in between the two
228 measurements in cases where the second sample was taken while the birds were incubating their
229 replacement clutch made a difference, as well as (ii) ‘clutch’ and ‘first blood THg concentration’ to
230 account for the fact that the temporal interval between the two measurements was larger in cases where

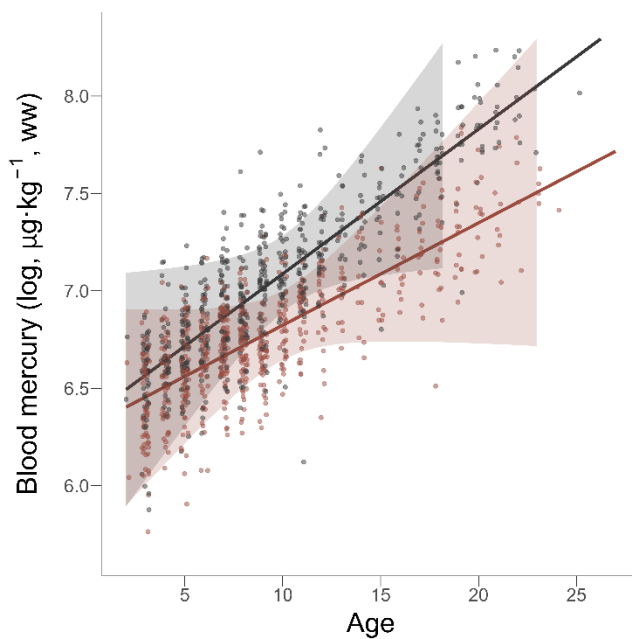
231 the second sample was taken while the birds were incubating a replacement clutch. Further, we included
232 the three-way interaction between ‘clutch’, ‘sex’ and ‘first blood THg concentration’ because we
233 expected the correlation to be stronger for males than females in cases when the clutch was different,
234 but not when the clutch was the same.

235 All analyses were performed and figures created, using R (version 4.3.1, R Core Team 2022).
236 Models were run with the R package “lme4” (Bates et al., 2015). P-values were obtained using the
237 package “lmerTest” (Kuznetsova et al., 2017) with the level of significance set at $p < 0.05$. Data were
238 visualized using the R packages “ggplot2” (Wickham, 2016) or “interactions” (Long, 2022).

239

240 **Results**

241 The total mercury concentration (THg) in 1209 blood samples collected from 588 individual common
242 terns ranged from 0.29 to 3.90 $\mu\text{g}\cdot\text{g}^{-1}$ ww, with a mean \pm SE of 1.00 ± 0.11 . Part of this variation was
243 explained by significant positive linear and negative quadratic age effects, with older birds exhibiting
244 higher THg concentrations in their blood than younger birds (Table S1, Fig. 2).



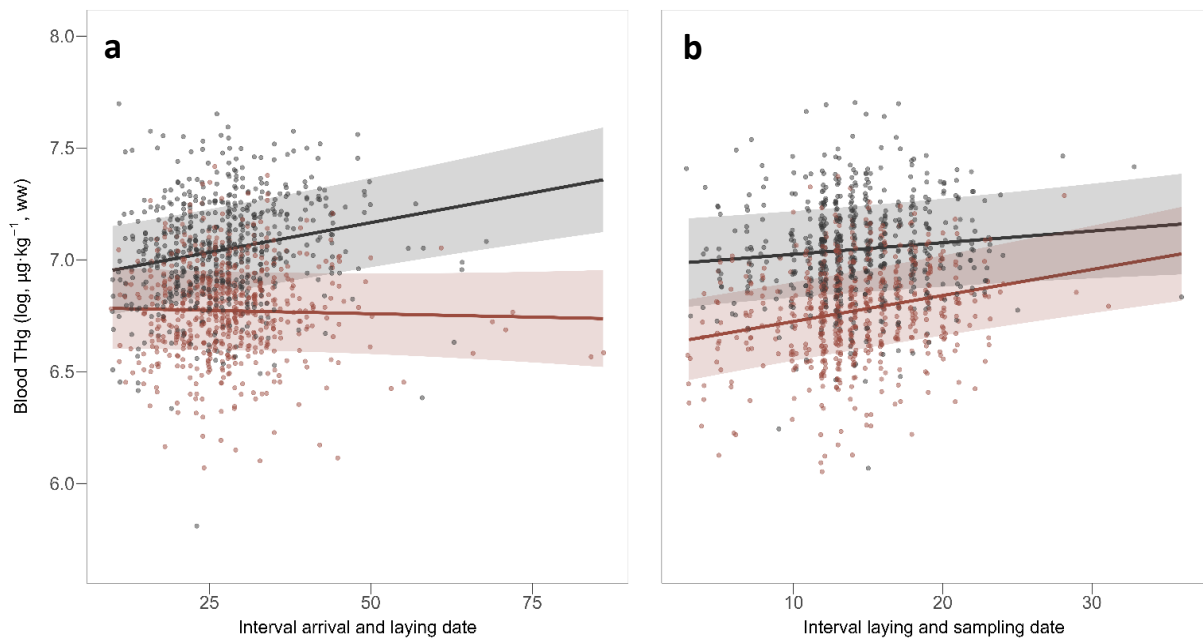
245

246 **Figure 2:** Total blood mercury concentrations of common terns in relation to age. Each point represents
247 the partial residual of a blood sample (black = males, red = females), lines the sex-specific linear
248 regressions and shaded areas the confidence interval.

249 To distinguish between within-individual accumulation of mercury with age and among-individual
250 variation in survival in relation to mercury pollution level driving the observed age effect, we repeated
251 the model splitting age into average and delta age components. This model showed that part of the
252 variation in the terns' blood THg concentrations was explained by a significant interaction between sex
253 and 'interval between arrival and laying date', with the interaction between sex and 'interval between
254 laying and sampling date' and the three-way-interaction between sex, average age and delta age also
255 being close to significance (Table 2a), such that we subsequently ran sex-specific models to assess
256 effects for males and females separately.

257 The sex-specific model for females showed that there was no correlation between the interval
258 between arrival and laying of the first egg and female THg concentration (Fig. 3a, Table 2b), but that
259 THg levels were higher in females with a longer interval between first egg laying and blood sampling
260 (Fig. 3b). In addition, females that laid three eggs had significantly lower THg levels than those that laid
261 one egg, with females laying two eggs showing an intermediate THg level that was not significantly
262 different from either (Fig. S3). Females with a higher average age had higher THg levels than those with
263 a lower average age (Fig. 2), which most likely can be explained by within-individual accumulation of
264 THg with age, given that the within-individual term for delta age showed a trend towards being positive
265 (Fig. 4) and the within- and among-individual estimates not differing from one another, such that
266 selective (dis)appearance in relation to THg should not be underlying variation among females.

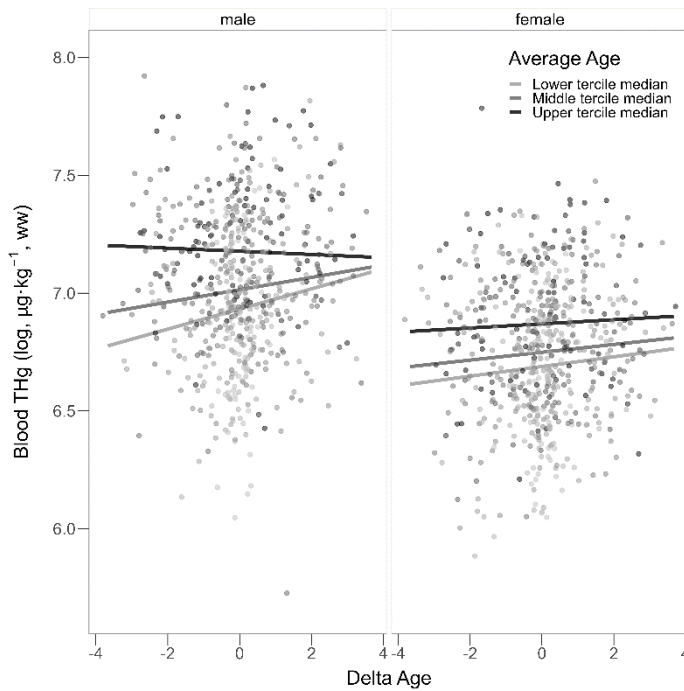
267



268 **Figure 3:** Total blood mercury concentration of male (black) and female (red) common terns in relation to their
 269 (a) interval between arrival and laying date and (b) interval between laying and sampling date, both in days. Each
 270 point represents the partial residual of a blood sample, lines the sex-specific linear regressions, shaded areas the
 271 confidence intervals.

272

273 The sex-specific model for males showed significantly positive correlations between the interval
 274 between arrival and laying (by their partner) and the interval between laying (by their partner) and blood
 275 sampling and male THg levels (Fig. 3a and b, Table 2c). Additionally and interestingly, THg levels were
 276 lower in males whose partner laid a larger clutch, although not significantly so (Fig. S2). Similar to
 277 females, THg levels in the blood of older males were higher than those in younger ones (Fig. 2), which
 278 can be attributed to a significant within-individual accumulation, although one that in males decelerated
 279 with age (Fig. 4), given that the within- and among-individual patterns did not differ from one another,
 280 but did show a significant interaction.

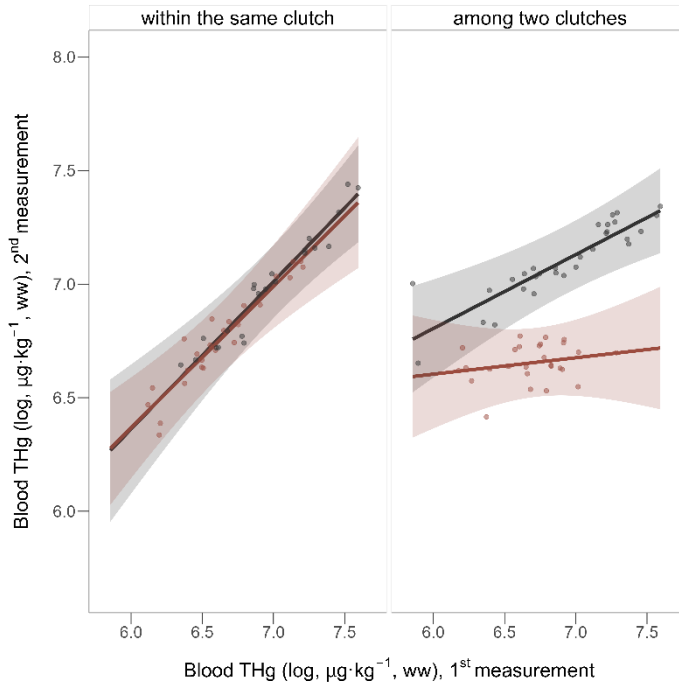


281

282 **Figure 4:** Total blood mercury concentration of male (left) and female (right) common terns in relation to the age,
 283 in years, at which they were sampled relative to the average age at which they were sampled ('delta age'). Each
 284 point represents the partial residual of a blood sample; lines the predicted within-individual change across seven
 285 years for birds sampled at an average age at the lower (5 years), middle (8 years) and upper (14 years) tertile
 286 median (from light grey to black), respectively.

287

288 Our within-season analysis revealed that variation in the THg concentration at the second
 289 measurement within a season can be explained by a significant interaction between clutch and THg
 290 concentration at the first measurement, with there being a trend towards this interaction differing
 291 between the sexes (Table 3). These interactions show that the correlation between the two THg
 292 concentrations is stronger when measured within the same clutch, especially for females (Fig. 5).



293

294 **Figure 5:** Total blood mercury concentration at the second sampling event within a season, either within the same
 295 clutch (n=47, left) or among two clutches (n=58, right). Each point represents the partial residual of a second blood
 296 sample (black = males, red = females), lines the linear regression for each sex and shaded areas the confidence
 297 intervals.

298

299 **Table 2: Results from three models testing whether selected candidate factors explain variation in (log-**
 300 **transformed) blood total blood mercury concentrations ($\mu\text{g}\cdot\text{kg}^{-1}\text{ ww}$) of 1209 samples taken from 588**
 301 **common terns for (a) both sexes combined, (b) females, and (c) males.**

Dependent variable	Estimate \pm SE	t-value	p-value
(a) both sexes (n=1209)			
Intercept	6.577 \pm 0.1097	59.977	< 0.001
Sex (female)	-0.137 \pm 0.105	-1.306	0.192
Clutch size (2)	-0.107 \pm 0.066	-1.613	0.107
Clutch size (3)	-0.095 \pm 0.066	-1.449	0.148
Interval AD – LD	0.005 \pm 0.001	4.611	< 0.001
Interval LD - SD	0.005 \pm 0.002	2.119	0.034
Average age	0.027 \pm 0.003	9.821	< 0.001

Delta age	0.070 ± 0.020	3.523	< 0.001
Sex (female)*clutch size (2)	0.010 ± 0.084	0.119	0.905
Sex (female)*clutch size (3)	-0.015 ± 0.083	-0.186	0.853
Sex (female)*interval AD – LD	-0.006 ± 0.002	-3.661	< 0.001
Sex (female)*interval LD – SD	0.006 ± 0.003	1.877	0.061
Sex (female)*average age	-0.007 ± 0.004	-1.853	0.064
Sex (female)*delta age	-0.043 ± 0.026	-1.654	0.099
Average age*delta age	-0.005 ± 0.002	-3.322	< 0.001
Sex (female)*average age*delta age	0.004 ± 0.002	1.862	0.063

Variance ± SD

Year (r)	0.038 ± 0.196
Identity (r)	0.020 ± 0.144
Residual (r)	0.044 ± 0.211

(b) females (n=612)

Intercept	6.459 ± 0.098	66.211	< 0.001
Clutch size (2)	-0.092 ± 0.050	-1.850	0.065
Clutch size (3)	-0.106 ± 0.050	-2.137	0.033
Interval AD – LD	-0.001 ± 0.001	-0.740	0.460
Interval LD – SD	0.011 ± 0.002	4.589	< 0.001
Average age	0.020 ± 0.003	7.520	< 0.001
Delta age	0.033 ± 0.018	1.799	0.073
Average age*delta age	-0.002 ± 0.002	-1.017	0.310
Average age (sel. d. app.)	0.022 ± 0.011	0.211	0.833
Actual age (sel. d. app.)	0.017 ± 0.010	1.748	0.081

Variance ± SD

Year (r)	0.034 ± 0.183
Identity (r)	0.016 ± 0.125

Residual (r) 0.043 ± 0.207

(c) males (n=597)

Intercept	6.564 ± 0.116	56.629	< 0.001
Clutch size (2)	-0.116 ± 0.068	-1.699	0.090
Clutch size (3)	-0.107 ± 0.068	-1.578	0.115
Interval AD – LD	0.005 ± 0.001	4.507	< 0.001
Interval LD – SD	0.006 ± 0.003	2.232	0.026
Average age	0.028 ± 0.003	9.184	< 0.001
Delta age	0.065 ± 0.021	3.144	0.002
Average age*delta age	-0.005 ± 0.002	-3.131	0.002
Average age (sel. d. app.)	0.018 ± 0.011	1.595	0.111
Actual age (sel. d. app.)	0.009 ± 0.011	0.874	0.382

Variance ± SD

Year (r) 0.044 ± 0.210

Identity (r) 0.027 ± 0.164

Residual (r) 0.045 ± 0.213

302

303 Significant results ($p < 0.05$) are presented in bold. ‘Average age (sel. d. app.)’ and ‘Actual age (sel. d. app.)’, both
304 in years, report the results for the analysis in which we test whether the average and delta age estimates obtained
305 in the main model are significantly different from one another; all other results reported are obtained from the
306 main model in which ‘average age’ and ‘delta age’, also in years, and their interaction are included. ‘Interval AD
307 – LD’ represents the interval between arrival and laying date, ‘Interval LD – SD’ the interval between laying and
308 sampling date, both in days. Clutch size refers to the size of the clutch the bird was incubating while its blood
309 sample was collected.

310

311 **Table 3: Results from a model testing whether clutch (same or replacement), sex and/or log_e-transformed**
 312 **first total blood mercury concentration explain variation in the (log_e-transformed) second total blood**
 313 **mercury concentrations (µg·kg⁻¹ ww) of 88 common terns sampled multiple times within a season.**

Dependent variable	Estimate ± SE	t-value	p-value
Intercept	2.466 ± 0.832	2.964	0.004
Clutch (replacement)	2.387 ± 0.878	2.718	0.009
First Hg concentration	0.649 ± 0.118	5.488	<0.001
Sex (female)	0.171 ± 1.086	0.158	0.875
Clutch (replacement)*first THg concentration	-0.324 ± 0.124	-2.604	0.013
Clutch (replacement)*sex (female)	1.147 ± 1.443	0.795	0.430
Clutch (replacement)* first THg concentration*sex (female)	-0.028 ± 0.159	-0.173	0.863
Clutch (replacement)* first THg concentration*sex (female)	-0.253 ± 0.151	-1.677	0.099
	Variance ± SD		
Year (r)	0.017 ± 0.131		
Identity (r)	0.026 ± 0.161		
Residual (r)	0.013 ± 0.115		

314 Significant results (p < 0.05) are presented in bold.

315

316 **Discussion**

317 In this study, we used seven years of longitudinal data to assess factors underlying variation in total
 318 mercury concentration (THg) in the blood of a long-lived seabird, the common tern. THg concentrations
 319 were highly variable, ranging from 0.29 to 1.80 µg·g⁻¹ ww in females and from 0.35 to 3.90 µg·g⁻¹ ww
 320 in males, showing an overall mean of 1.00 ± 0.11 µg·g⁻¹ ww.

321 Apart from the overall difference in blood THg concentrations between male and female
 322 common terns, we observed differences between the sexes in the correlations between some of our

323 variables of interest and THg concentrations (Table 2a). Consequently, we ran sex-specific analyses.
324 With respect to phenology, for males, the sex-specific analysis showed that males with longer intervals
325 between arrival and the onset of laying by their female, and between laying and sampling had higher
326 blood THg levels (Table 2c). This may suggest that a longer time spent at the breeding area results in
327 males being exposed to a higher dietary uptake of methylmercury (MeHg) compared to the uptake in
328 their foraging areas prior to arrival. This seems supported by a recent study showing an increasing
329 temporal trend in mercury contamination of fish in this area (Kammann et al., 2023). Alternatively or
330 additionally, however, the causality may be reversed, such that males with higher THg levels might have
331 arrived in a poorer condition, for example due to MeHg exacerbating oxidative stress (Hoffman et al.,
332 2011; Costantini et al., 2014; Eckbo et al., 2019; Soldatini et al., 2020) and/or hormone levels (Varian-
333 Ramos et al., 2014), resulting in delayed breeding and/or reduced incubation behaviour, leading to us
334 needing longer to sample them successfully.

335 In females, there was no correlation between the interval between arrival and egg laying and
336 THg concentrations, while the correlation between the interval between laying and sampling and THg
337 concentrations was significantly positive (Table 2b) and showed a trend towards being slightly stronger
338 than in males (Table 2a). This difference between the sexes might be explained by females' ability to
339 reduce mercury concentrations via egg laying (Bond and Diamond, 2009; Ackerman et al., 2020). If a
340 longer time spent at the breeding area indeed results in higher blood THg concentrations due to high
341 environmental contamination, egg laying could initially offset an increase in blood THg concentrations
342 in females, but not males. However, after clutch completion, this additional excretion mechanism would
343 cease, leading to an increase in female THg levels over time as well. This hypothesis is supported by
344 our results obtained from the repeated THg measurements within breeding seasons. Whereas the within-
345 individual correlation between THg concentrations is similarly positive for the sexes if both
346 measurements were obtained from blood samples taken within a single clutch, it is somewhat weaker
347 for females than males if the second blood sample was taken during incubation of a replacement clutch,
348 most likely due to a repeated reduction of THg levels by females through egg laying.

349 A considerable role of egg laying for female THg detoxification is additionally suggested by
350 our finding that the larger the number of eggs in the clutch, the lower the blood THg concentrations.

351 Similar evidence of egg laying being an efficient route of mercury excretion for female terns is provided
352 by studies showing that mercury deposition in eggs decreases across the laying sequence, likely due to
353 a reduced ‘availability’ of mercury in females for excretion into subsequent eggs after the first one is
354 laid (Becker et al., 1992; Akearok et al., 2010; Ackerman et al., 2016). Previous studies, however, also
355 indicated that other factors may additionally contribute to sex-differences in THg contamination
356 (Robinson et al., 2012; Hitchcock et al., 2019). In double-crested cormorants (*Phalacrocorax auritus*),
357 females, for example, were shown to have greater capacity to demethylate mercury in the liver than
358 males (Robinson et al., 2011). Additionally or alternatively, males and females may differ in their levels
359 of mercury excretion (Braune and Gaskin, 1987b). Given that moult does not take place during the early
360 breeding season, excretion through faeces seems the only potential pathway in this respect. We,
361 however, deem this unlikely to be important in our case, because faecal mercury exists only in inorganic
362 form (Morrissey et al., 2005), and the uptake of inorganic mercury is minimal. However, further studies
363 should address the potential impact of excretion mechanisms on sex-specific blood THg levels, as well
364 as the effects of mercury deposited in eggs for the offspring (Ackerman et al., 2020).

365 Older birds of both sexes had significantly higher THg levels in their blood compared to young
366 birds. We found no evidence for selective (dis)appearance of either weakly or heavily contaminated
367 individuals, and our longitudinal data suggest that the higher THg levels in older individuals result from
368 within-individual THg accumulation with age, the shape and extent of which differed somewhat between
369 the sexes. Specifically, within-individual THg concentrations of males exhibited significant decelerating
370 accumulation, whereas in females there was a trend towards a lesser but linear within-individual
371 increase. Again, the ability of females to excrete MeHg through egg laying could explain why we
372 observe a weaker accumulation with age in females than in males. However, this does not explain the
373 trend towards the non-linear component of the within-individual age effect differing between males and
374 females, given that female terns of our population increase their clutch size as they grow older (Zhang
375 et al., 2015) and therefore would, if anything, be expected to show a stronger deceleration with age than
376 males. Sex-specificity of mercury uptake, via the use of differential foraging areas (Carravieri et al.,
377 2014; Militão et al., 2023) or differential prey choice (De Felipe et al., 2019), which additionally might
378 be age-specific, is an alternative explanation that deserves empirical attention.

379 As also alluded to above, birds possess mechanisms that should mitigate the MeHg
380 concentrations in their bodies. It is well-established that a substantial portion of ingested MeHg is
381 deposited in feathers (Braune and Gaskin, 1987a), a phenomenon that may explain why in some bird
382 species, such as wandering albatrosses, adults, undergoing frequent moulting, exhibit lower, rather than
383 higher THg levels in blood compared to immature birds undergoing sporadic moults (Tavares et al.,
384 2013; Bustamante et al., 2016). Moreover, mercury that is not excreted, such as that ingested during
385 non-moulting periods, is typically demethylated in the liver to inorganic mercury by binding with
386 selenium at a high rate (e.g. Eagles-Smith et al., 2009b; Manceau et al., 2021). These highly efficient
387 mechanisms could account for the absence of mercury accumulation in blood observed in previous (but
388 cross-sectional) studies on birds (Thompson et al., 1993; Becker et al., 2002; Carravieri et al., 2014,
389 2022; Tartu et al., 2015). In addition, they could explain the rather slow rate of accumulation found in
390 this study, although the fact that accumulation still occurs suggests that these mechanisms are limited.

391 The difference in the average THg levels between young (first quartile of age, $0.90 \mu\text{g}\cdot\text{g}^{-1}$ ww,
392 $n = 318$) and old (fourth quartile of age, $1.17 \mu\text{g}\cdot\text{g}^{-1}$ ww, $n = 308$) birds equals 30%, which combined
393 with a relatively low individual repeatability of 20% (i.e. total phenotypic variance explained by
394 individual identity as a random effect in the model described in Table 2a), raises concerns about the
395 suitability of blood from birds of unknown age as an accurate bioindicator of environmental mercury
396 levels, at least for common terns. The influence of age on mercury levels might, however, be less
397 pronounced in other study systems, especially when considering additional variables such as isotopic
398 data (Polito et al., 2016; Renedo et al., 2018a, 2018b; Jouanneau et al., 2022), different foraging
399 locations (e.g., Ackerman et al., 2016), or species preying at lower trophic levels (Ma et al., 2021) or
400 having shorter lifespans (Hallinger et al., 2011). Assessing the generality of the age-specificity of blood
401 mercury contamination we found therefore is important. Moreover, further studies are also needed to
402 investigate the age-specificity of (maternal) mercury levels in other commonly used bioindicator tissues,
403 such as feathers (Sun et al., 2020) or eggs (Evers et al., 2003; Goodale et al., 2008).

404 It is noteworthy that the THg values of all of our 1314 blood samples collected from 588
405 individual common terns exceeded the toxicity thresholds at which adverse health effects have been
406 observed, with 57% being at low ($0.2 - 1.0 \mu\text{g}\cdot\text{g}^{-1}$ ww) and 43% being at moderate ($1.0 - 3.0 \mu\text{g}\cdot\text{g}^{-1}$

407 ww) risk levels, following the toxicity benchmarks published by Ackerman *et al.* (2016). As an
408 endocrine disruptor, MeHg has been reported to decrease breeding probability (Tartu *et al.*, 2013; Goutte
409 *et al.*, 2015), to alter risk-taking (Kobiela *et al.*, 2015) or parental behaviour (Tartu *et al.*, 2015) and
410 oxidative stress status (Costantini *et al.*, 2014; Eckbo *et al.*, 2019; Soldatini *et al.*, 2020). Such effects
411 could potentially impair the ability to adapt to environmental changes (Jenssen, 2006; Grunst *et al.*,
412 2023a) or to mount an effective immune response against disease (Hawley *et al.*, 2009; Lewis *et al.*,
413 2013). Moreover, mercury has been associated with a reduced body condition (Amenlineau *et al.*, 2019;
414 Eckbo *et al.*, 2019; but see Carravieri *et al.*, 2022), which can be particularly harmful for migratory birds
415 (Seewagen, 2020) when their flight performance (Ma *et al.*, 2018a) or fat deposition and muscle
416 development (Adams *et al.*, 2020) would be compromised.

417 Overall our study suggests that the risk of suffering from the detrimental effects of mercury
418 pollution differs between the sexes and increases as adult birds age. Although we so far found no
419 evidence of selective (dis)appearance and previous studies on adult seabirds in wild populations mostly
420 also reported no direct correlation between survival and mercury contamination (Thompson *et al.*, 1991;
421 Goutte *et al.*, 2015; Bustamante *et al.*, 2016; Pollet *et al.*, 2017; Amélineau *et al.*, 2019; Chastel *et al.*,
422 2022; but see Calvert *et al.*, 2024), interactions between mercury pollution and other stressors may have
423 synergistic effects (Costantini *et al.*, 2014; Grunst *et al.*, 2023b; Smith *et al.*, 2023) and further studies
424 are needed to assess how mercury pollution interacts with such stressors and whether effects may be
425 sex- and/or age-specific as well.

426

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433

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