Science of the Total Environment 956 (2024) 176935

Contents lists available at ScienceDirect

Science of the Total Environment

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

From mother to egg: Variability in maternal transfer of trace elements and steroid hormones in common eider (*Somateria mollissima*)

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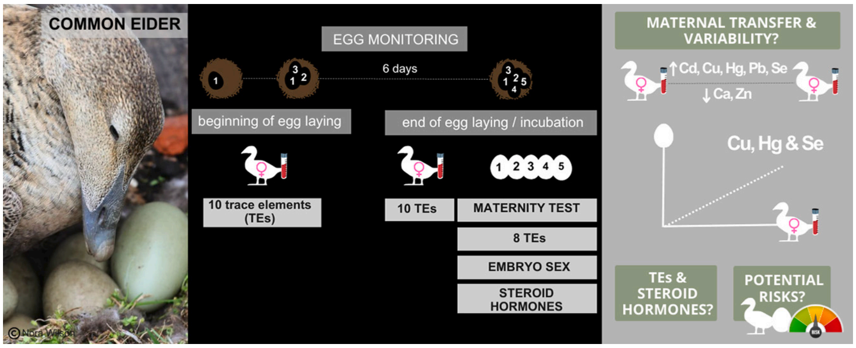
HIGHLIGHTS

- Ten trace elements from laying common eider hens and eggs were studied in Finland.
- Maternal and egg concentrations of Cu, Hg and Se were correlated.
- Except for Pb, concentrations were below toxicity thresholds.
- Egg Pb concentration was the highest reported in this species.
- Egg steroid hormones were not associated with trace elements.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Rafael Mateo

Keywords:
Sea duck
Baltic Sea
Trace elements
Breeding females
Steroid hormones
Lead

ABSTRACT

The Baltic Sea is among the most polluted seas worldwide with elevated concentrations of trace elements (TEs). TEs can induce negative effects on organisms and may be transferred to eggs causing endocrine-disrupting effects on embryos. The Baltic Sea population of common eider (*Somateria mollissima*) has declined over the last thirty years, but the potential contribution of TEs to this decline is understudied. The aim of this study was to assess maternal transfer of TEs during the incubation period. Associations between TEs and steroid hormone concentrations in eggs (androstenedione, testosterone, pregnenolone and progesterone) were also investigated. Ten nests from Bengtskär (Finland) were monitored, for which hens at the beginning and end of the egg-laying were blood-sampled and their clutches were collected. Red blood cells from females ($n = 10$) and homogenized eggs ($n = 44$) were analyzed for 10 TEs (As, Ca, Cd, Cu, Hg, Mg, Ni, Pb, Se and Zn). Maternal and egg concentrations

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https://doi.org/10.1016/j.scitotenv.2024.176935
Received 24 March 2024; Received in revised form 8 October 2024; Accepted 12 October 2024

were correlated for Cu, Hg and Se ($R^2 = 0.51$, $R^2 = 0.51$, $R^2 = 0.52$, respectively and all *p*-values ≤ 0.01). Three eggs had the highest Pb concentrations (1.43–2.24 μg g^{-1} ww) ever reported for this species. Although maternal and egg Pb concentrations were not significantly correlated, those eggs were laid by the same female, also having the highest Pb concentration (3.4 μg g^{-1} ww). Most blood TE concentrations in females were below known toxicity limits, except for Pb where 20 % of 10 females (including one outlier) had concentrations above the toxicity limit reported for subclinical poisoning in Anatini ($>$ 0.2 μg g^{-1} ww). Steroid hormones in eggs were interrelated, but not correlated to TEs. Overall, the results call for more urgent research into the origin and consequences of high Pb concentrations and continued monitoring of the common eider populations in the Baltic Sea.

1. Introduction

During the last century, growing industrialization has accentuated the exploitation of Earth's natural resources (Gautam et al., 2016; Persson et al., 2022), which has intensified global releases of trace elements (TEs) into the environment. TEs are naturally present in the environment, but their availability has been increased by human activities (Persson et al., 2022; Vareda et al., 2019). Some TEs play an essential role in biological functions at low doses but can have sub-lethal and lethal effects at higher doses (Purves, 1985; Strachan, 2010). Others are non-essential metals or metalloids which can be toxic even at very low concentrations (Briffa et al., 2020; Gautam et al., 2016; Purves, 1985). Thus, toxicity of TEs is concentration-dependent, and can be chronic or acute for human health as well as for wildlife (Briffa et al., 2020; Gautam et al., 2016; Izatt, 2016; Vardhan et al., 2019).

In birds, TEs impact life history traits, including gametogenesis, clutch size, hatching and juvenile survival (Beyer and Meador, 2011; Kalisińska, 2019; Scheuhammer, 1987). TEs can also induce various behavioral and developmental abnormalities (*e.g.*, response to stress, movement skills, singing skills, structural malformations), as well as enzymatic, metabolic and hormonal changes (Beyer and Meador, 2011; Kalisińska, 2019; Zala and Penn, 2004).

During egg formation, essential substances such as hormones are transferred for the growth of the embryo, but this also represents a non-negligible route of contaminant off-loading for the female (Nys and Guyot, 2011; Hoffman, 2003). As reserves are mobilized during egg-laying, relationships between concentrations of TEs in eggs and female organs have already been demonstrated (Ackerman et al., 2020). Eggs are a useful biomonitoring matrix to study contaminant levels (Hashmi et al., 2015; Klein et al., 2012) and could represent a relevant less invasive matrix to investigate breeding female contamination (Pacyna-Kuchta, 2023). However, maternal transfer must first be demonstrated and the influence of biological parameters (*e.g.*, egg-laying order, clutch size; Ackerman et al., 2016a,b; Akearok et al., 2010; Sanpera et al., 2000) must be considered by preliminary studies. However, this information is still mostly limited to mercury (Hg) (Ackerman et al., 2020).

During embryogenesis, the embryo is exposed by maternal transfer of contaminants to the egg (Fairbrother et al., 1999; Hamlin and Guillette, 2011; Orłowski et al., 2024) but the incubation period is a critical window of development where even minor changes can induce long-term consequences for individuals and populations (Herring et al., 2010; Ottinger et al., 2008). *Via* embryonic exposure, some TEs such as Hg and lead (Pb) are reported to induce immunotoxicity (Lee et al., 2001) and to modify the endocrine system (Heddle et al., 2020; Herring et al., 2012). Known as endocrine disruptors, Hg and Pb can modify sex steroid hormone excretions (Jayasena et al., 2011; Tartu et al., 2013, 2014), which are involved in sexual differentiation (Adkins-Regan, 2007; Ottinger et al., 2008) and consequently affect the primary and secondary sex ratio (Bouland et al., 2012; Navara, 2013). Ultimately, individual-effects of endocrine disruptors could indirectly induce changes in population dynamics with sex-biased survival and decrease in hatching, fledging, breeding success, and long-term breeding probabilities (Goutte et al., 2014a,b, 2015). While relations between steroid hormones deposited into eggs and persistent organic pollutants (POPs) have already been investigated (French et al., 2001; Jouanneau et al., 2023; Verboven et al., 2008), this is still poorly described for TEs (Ruuskanen et al., 2019).

Several migratory waterbird populations are experiencing global declines, among them the common eider (*Somateria mollissima*, L., 1758, hereafter eider). Eiders are seaducks inhabiting coastal waters of the northern hemisphere (Waltho and Coulson, 2015; Wetlands International, 2012). In Europe, the Baltic/Wadden Sea flyway population of eiders has declined considerably since the 1990s (Desholm et al., 2002; Ekroos et al., 2012; HELCOM, 2023) and is now considered endangered in Europe (BirdLife International, 2021). In addition, in several colonies a male sex bias has also been reported which is currently understudied (Lehikoinen et al., 2008a; Tjørnløv et al., 2019). Several hypotheses are debated to explain these major changes in the population dynamics (*e.g.*, disease outbreaks, parasitism, mussel overexploitation, hunting, aerial and terrestrial predation) (*e.g.*, Christensen and Hounisen, 2014; Desholm et al., 2002; Laursen et al., 2009; Morelli et al., 2021; Tjørnløv et al., 2013). Other causes have been proposed to explain this decline, but they remain poorly studied such as the influence of thiamine deficiency, climate change or pollution (Fox et al., 2015; Lehikoinen et al., 2006; Mörner et al., 2017).

Despite international governance and science-based management in the Baltic Sea, such as the Helsinki Commission (Reusch et al., 2018), eider populations are still declining, requiring more research to determine the causes. Of particular interest in this context is Finland, serving as a major breeding area for >70 % of the flyway population (Desholm et al., 2002; Ekroos et al., 2012). The Finnish population shows a steep decrease of over 50 % compared with the mid-1990s (Hario and Rintala, 2007), particularly in the Gulf of Finland, where the population has decreased by 20 % and is still in decline (Hario and Rintala, 2007; HELCOM, 2023). In addition to the general decrease in eider population size, the proportion of females during the spring migration into the Gulf of Finland dropped from 58 % to 41 % between the 1980s and 2000s (Lehikoinen et al., 2008a). Similarly, the sex ratio of ducklings at hatching was balanced, but the mortality of chicks in their first life stages was female-biased (Lehikoinen et al., 2008b).

Ecotoxicological studies were conducted in the 2000s on eiders in the Gulf of Finland reporting exposure of arsenic (As), Hg, Pb and selenium (Se) (Franson et al., 2000a, 2000b, 2002). Few recent ecotoxicological studies on eiders were again conducted in this area. Breeding female eiders were sampled in 2011 and reported Hg concentrations (mean ± SD: 0.17 ± 0.07 μg g^{-1} ww) correlated with DNA damages in whole blood (Fenstad et al., 2016a, 2016b, 2017). More recently, Hg was analyzed but focusing on muscle and liver in a context of trophic dynamics (Vainio et al., 2022). However, there is a scarcity of knowledge about the potential role of TEs in the eider population decline and biased sex ratio.

In the present study, we aimed to assess maternal transfer of trace elements and its variability during egg laying in the common eider. We sampled blood from laying hen eiders in the beginning and at the end of egg-laying and then collected full clutches of eggs of these females.

The specific objectives of this study were (1) to describe the dynamics of TE concentrations during the egg-laying period in breeding females from a Finnish colony; (2) to assess maternal transfer of TEs to eggs and their variability as a function of maternal condition and embryo sex; (3) to investigate relationships between TEs and steroid hormone concentrations in eggs and (4) to compare TE concentrations with reported levels in eiders and other birds to estimate potential adverse effects. We hypothesized that (i) changes in TE concentrations in the blood occur during the egg-laying period due to redistribution of TEs stored in body reserves and the transfer to eggs of circulating TEs; (ii) TEs are transmitted from the female to eggs as a function of her body condition; (iii) TEs, especially TEs reported as endocrine disruptors, could be associated to hormone concentrations in eggs.

2. Materials and methods

2.1. Study area

Fieldwork was conducted on the Bengtskär island (59°72′32.7"N, 22°49′91.2″E, Finland) located at the interface between the northern Baltic Sea and the Gulf of Finland (Fig. S1). Bengtskär is a small rocky island of around 2 ha with sparse vegetation. Contrary to the overall decline in the region, this breeding population has increased during recent years. In 1990, there were about five nests (Vösa et al., 2017) and in 2021, 522 nests were counted during the breeding season (Arzel & Wilson, unpubl). The island is a tourist attraction for the light house and its eider colony (10,000 to 12,000 visitors per year, Wilson pers. com.). Human activities and active scaring of potential avian predators by the light house staff make it unattractive for avian predators including white-tailed eagles which are otherwise known to cause a top-down effect on the Baltic/Wadden Sea flyway population of eiders (Morelli et al., 2021). Also, there are no terrestrial predators on the island due to its location far from the coast (Vösa et al., 2017). Still, a high number of unhatched eggs and dead ducklings were observed leading to a breeding failure above 40 % in 2019 and 2021 (years during which intensive breeding surveys were carried out, Arzel & Wilson, pers. comm.) which is above the indicator of 5 % reported for seabirds, indicating significant pressures on the population (Cook et al., 2014; OSPAR Commission, 2016).

2.2. Sample collection

All sampling was carried out after approval by the Finnish Regional State Administrative Agency (permit number ESAVI/9500/2021 for collecting blood from the females), the Finnish Hunting Association (permit number 2021-5-000-18962-8 to capture females and collect the clutches), ringing permit of Céline Arzel (to ring the females and take body measurements) and Per and Paula Wilson to operate on the island of Bengtskär.

A comprehensive experimental design was made to obtain an overall insight into maternal transfer of TEs by sampling eider females twice during egg-laying and collecting full clutches from these same females (Fig. 1). However, with the protected status of the common eider, classified as endangered in Europe (BirdLife International, 2021) and the restrictive study design, with two samplings from the same female during egg-laying, only a modest sample size of females to be sampled was approved with the permit obtained.

The study was conducted in 2021, during the incubation period from 10 to 19th of May 2021. At the start of the breeding season, 10 nests that already had one or two eggs were given a nest number and the first egg (s) were marked with a permanent marker. The nests were revisited daily, and each new egg was annotated with a number corresponding to its laying order, adjoined by a letter if two new eggs had been laid (*e.g.*, 3A and 3B). To avoid nest abandonment, female sampling was carried out when they had two to four eggs and appeared to remain on the nests.

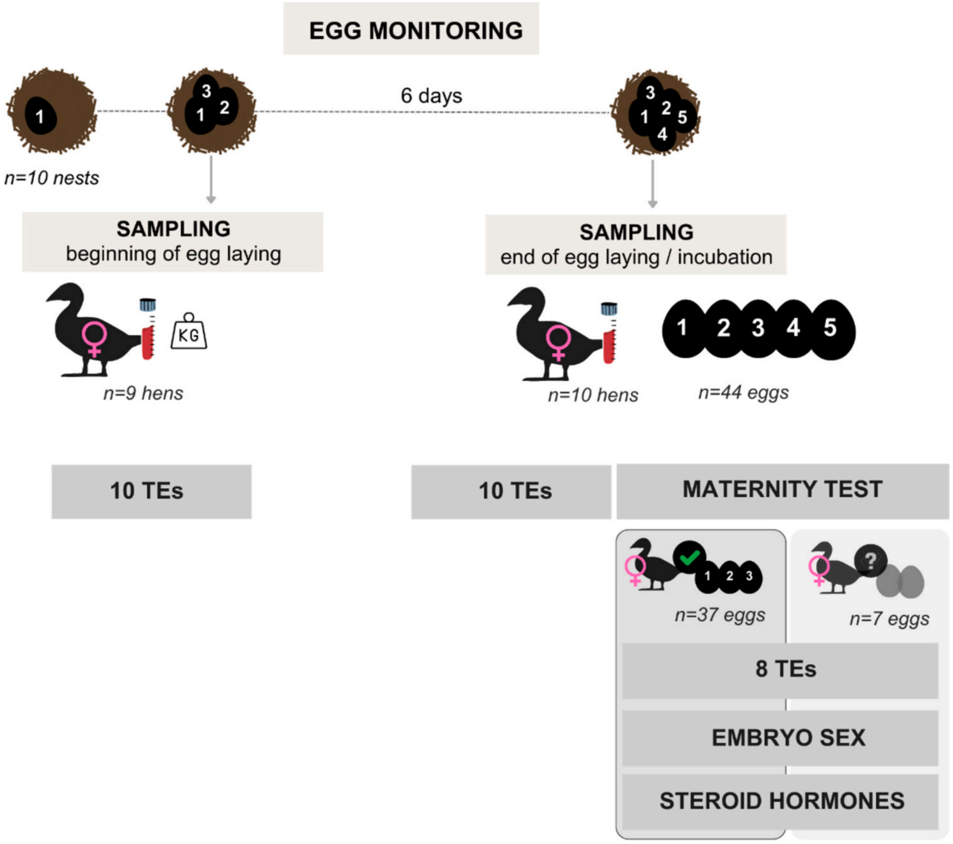

Fig. 1. Experimental design to address the four goals of this study on common eider, *Somateria mollisima*. Abbreviations: TEs: trace elements (As, Ca, Cu, Hg, Ni, Pb, Se and Zn for females and eggs, and Ca and Mg for female eiders only).

Incubating females ($n = 9$) were captured on their nests with dip nets during egg laying, not including one female (10th female, DT077810, Table S1) which had been captured first on a nest 29th of April (sample not used in this study) but was found later on incubating eggs on another nest (second sample). All females ($n = 10$) were resampled a second time at the end of egg-laying/beginning of incubation (*ie.*, six days after the first sampling). In addition, the entire clutches (mean ± SD: 4.4 ± 2.1 eggs per nest) from the ten monitored nests were collected after the second sampling (19th of May 2021). At each capture, the body mass was recorded using a Pesola spring balance (for birds weighing up to 2500 g) or a portable scale for birds weighting >2500 g, with a 20 and 25 g accuracy, respectively (Table S1). In addition, a maximum of 6 mL of blood (< 1 % of an average body mass) was collected from the brachial vein using a syringe with a 23-gauge needle. For each sample, the blood was transferred to lithium heparin tubes and gently inverted 8–10 times before storing in a cold box (approx. 5 °C) for a maximum of 6 h before separation of plasma and red blood cells by centrifugation (2500 rpm, 10 min, EBA 270, Hettich, Germany). Blood samples (plasma and red blood cells) ($n = 19$) and eggs ($n = 44$) were stored at −20 °C in the field. All samples were transported to the University of Turku (UT) in cold boxes with freezer elements and transferred to −20 °C (red blood cells) and − 80 °C (plasma and eggs) freezers. The red blood cells and plasma were shipped on dry ice to NTNU where they were stored at −35 °C until analyses. The eggs were shipped on dry ice to Aarhus University, Denmark, where they were stored at −80 °C until homogenization.

2.3. Measurement and homogenization of the eggs

For the 44 collected eggs, the eggshells were cut at the equator with a Dremel tool (MBM40 Microline from ®Bosch) and frozen egg contents were placed in a 1 L glass jar and weighed. All eggs contained embryos, which were sampled for sex determination by DNA analyses. After defrosting, each egg was homogenized (®Bamix blender, model M200) and transferred to 15 mL Falcon™ tubes. Between each egg, all the equipment was cleaned with soapy water, rinsed with tap water and deionized water, allowed to dry, and finally rinsed with acetone. Moreover, four blanks (“homogenization blank”, hereafter) were made after every tenth egg, by filling a 1 L glass jar with approximately 300 mL of ultra-pure water directly from a Dionex™ IC pure system (Thermo Scientific™). Clean metal spoon and spatula were placed in the water, to simulate the removal of embryonic material for DNA analyses, and each “homogenization blank” was homogenized in the same way as for the eggs. All samples (eggs and homogenization blanks) were stored at −20 °C until transport on dry ice to NTNU where they were stored at −35 °C before analyses.

2.4. Trace elements analyses

The preparation and TEs analyses were conducted at the Department of Chemistry (NTNU, Trondheim, Norway). Subsamples of red blood cells ($n = 19$; 0.58 ± 0.03 g), eggs ($n = 44$; 0.43 ± 0.10 g) and homogenization blanks ($n = 4$; 0.63 ± 0.15 g) were transferred to 15 mL Teflon tubes, previously washed with Milli-Q water and concentrated nitric acid 30 % (HNO_3, 0.6 M).

1.5 mL of concentrated HNO_3 was added to red blood cells samples before digestion in microwave (Milestone, UltraCLAVE). After digestion, red blood cells samples were diluted with Milli-Q water to attain a final weight of ~35 mL (35.56 ± 0.03 g) in 50 mL falcon™ tubes

Following the same procedure, 5 to 10 mL of HNO_3 50 % was added to eggs and homogenization blanks (to account for mass differences between samples) and digested. After digestion, egg samples were also diluted with Milli-Q water to attain a final weight of ~67 mL (73.96 ± 17.14 g) then 15 mL was transferred to 20 mL Falcon™ tubes.

Analyses were performed with High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS). Ten TEs were analyzed: As, cadmium (Cd), copper (Cu), Hg, nickel (Ni), Pb, Se and zinc (Zn) in both red blood cells and egg samples, and calcium (Ca) and magnesium (Mg) in red blood cells samples only. A minimum of two analytical blanks were added in every sample batch to check for possible laboratory contamination. All TEs concentrations were corrected from the analytical blank samples. For the egg samples, the values were also corrected by the mean of the homogenization blanks. Concentrations are converted and expressed to μg g^{-1} wet mass (ww) (Fig. S2). Except Hg and Ni, recovery rates varying between 80 and 99 % of the exact value of the reference material (SI). For Hg, as concentrations were below the limit of detection (LOD) for some samples of the reference material inducing low recovery rates (149 %), Hg concentrations should be taken with caution (SI). Details on quality control and assurances, as well as LODs are provided in Supplementary Information (Table S2).

2.5. Steroid hormone analyses

Hormone extraction and analyses were conducted at the Department of Chemistry (NTNU). Eighteen steroid hormones (Table S3) were extracted from egg samples ($n = 44$) and homogenization blanks (produced during egg homogenization, $n = 4$) using hybrid solid-phase extraction (HybridSPE®) as described earlier (Sait et al., 2023; Vike-Jonas et al., 2021) with minor modifications as detailed in SI.

Analyses of steroid hormones were performed using UPLC-MS/MS with an ACQUITY CSH Fluoro-Phenyl column (100 × 2.1 mm, 1.7 μm, 100 Å, Waters, USA) serially connected to a C18 guard column from Phenomenex (recommended for 2.1 mm ID columns), as previously described (Sait et al., 2023). Briefly, the aquatic phase contained Milli-Q water with 0.1 % volume/volume (*v*/v, hereafter) formic acid and the organic phase contained acetonitrile with 0.1 % (*v*/v) formic acid. In addition to egg samples ($n = 44$) and homogenization blanks ($n = 4$), for quality assurance and quality control, standards ($n = 12$), extraction blanks ($n = 2$), a pooled sample of two eggs and an analytical blank (containing pure methanol) were analyzed.

Hormone concentrations are expressed in ng g^{-1} ww. Details on quality control, as well as calculations and values of LODs are presented in SI (Table S3).

2.6. Maternity test and molecular sexing

The eider is a colonial species known to practice intraspecific nest parasitism also known as conspecific clutch parasitism (Waltho and Coulson, 2015). Thus, a female may lay one or more eggs in another nest or incubate eggs that are not her own. The frequency of this phenomenon is variable and can affect from 4 to 55 % of the nests, with parasitic eggs representing 1 to 14 % of the eggs (Hario et al., 2002; Kilpi et al., 2004; Lusignan et al., 2010; Robertson, 1998; Waldeck et al., 2011; Waldeck and Andersson, 2006). To verify the maternity of the collected eggs, DNA comparisons were made between the collected eggs and the sampled incubating females through microsatellite genotyping. In addition, embryo sex was determined using a molecular approach from embryo samples collected during egg homogenization (Griffiths et al., 1998). Laboratory work was performed at the Center of Evolutionary Applications (UT). The DNA extraction, molecular sexing and microsatellite genotyping methods are described in SI (Table S4). Parentage analyses showed that seven eggs (16 %) were parasitic and thus, the laying order was modified accordingly.

2.7. Statistical analyses

Only TEs and hormones above the LOD for >70 % of the samples (female blood or eggs) were included in the statistical analyses. Respectively, for female blood and egg samples, Ni (detection frequency, df: 44 %) and Cd (df: 23 %) were therefore excluded from the statistical analyses. Values below the LOD (only two eggs for Pb: one egg with maternity known and one parasitic egg) were replaced by half of the LOD of the specific element (Wood et al., 2011).

One female had high Pb concentrations (3.54 and 6.12 μg g^{-1} ww at the beginning and end of egg-laying, respectively) compared to other females (range: 0.10–0.55 and 0.10–0.47 μg g^{-1} ww, respectively). Eggs from this female also presented high concentrations of Pb (0.82–2.25 μg g^{-1} ww) compared to other eggs (< 0.001–0.04 μg g^{-1} ww) which confirmed that these concentrations are not linked to contamination during analyses but consistent with biological outliers. For these outliers, the concentrations of the other TEs were in the same range of values as the other samples. Thus, these samples were retained in the statistical analyses, but statistical analyses without outliers are presented in the SI.

To address our aims, after normality tests, Student *t*-tests or Spearman correlations were performed using R version 4.2.2 (R Core Team, 2020) and a significance level of $\alpha < 0.05$. Statistical linear models were also performed with linear mixed-effects models (LMMs). For each linear model selection, the parsimonious model has been discriminated between all possible models, being the null (without any variable) to complete model according to Akaike's Information Criterion for small sample size (AICc; “AICcmodavg” package, Mazerolle, 2020) (Buckland et al., 1997; Burnham and Anderson, 2004). The model with the lowest AICc was selected except if there was another model with ΔAICc <2. In this case, the model with the least explanatory variables was selected as more parsimonious. The variables included in this best model were considered to have a significant effect. For all models, the homoscedasticity and normal distribution of residuals and random effects were visually assessed on the graphs of residuals *versus* fitted values and Q – Q plot (Zuur et al., 2009). Package ggplot2 were used for graphical illustrations (Wickham, 2011).

2.7.1. Aim 1: dynamics of TE concentrations during egg-laying

For each element, to study its change in blood during the egg-laying period, comparisons of the mean were performed. As normality was not achieved for all TEs, Student's *t*-tests for paired series by permutation were performed (“RVAideMemoire” R package, Hervé, 2022). Here, the sample size was nine hen eiders as the sampling of the 10th female could not be included.

2.7.2. Aim 2: maternal transfer of TEs to eggs and their variability

Preliminarily, relationships between TEs were investigated using Spearman's correlation coefficients (Fig. S3). LMMs were used to study the influence of maternal TE concentration on the concentrations transferred to the eggs and its biological variability (“nmle” package, version 3.1.160, Pinheiro et al., 2023). Separately for each TE (As, Cu, Hg, Pb, Se and Zn), egg TE concentration was used as response variable. Maternal concentrations of this TE at the start of laying, early laying female mass and embryo sex were used as predictor and female identity was added as a random factor to consider for the non-independence of eggs from the same female. Female mass was used as proxy for the health status of the female. Continuous variables (female mass and each maternal TE concentration) were standardized (centered and scaled, mean = 0 and SD = 1) to allow effect size comparisons. For As and Pb, LMMs were used with a variance weight (exponential variance function structure) to deal with the heteroscedasticity structure of the residuals. For these analyses, only eggs for which the maternal identity was known were kept ($n = 32$, excluding parasitic eggs and eggs from the female with no first sampling).

The laying order was not included as factor variable due to the heterogeneity of the number of eggs per clutch (clutch sizes varied from two to six eggs, details in Table S1). For each TE, the difference between the first and second laid eggs was studied using Student's *t*-test for paired series by permutation. For one nest, two eggs were laid by the same female in <24 h and prior to our visit to the nest preventing us to record the laying order. Females lay eggs in an average of 18 to 24 h (Waltho and Coulson, 2015) and DNA analyses confirmed the parentage of these two eggs with the sampled female. Hence, we replicated the analyses to examine whether the results differed based on the designation of which egg was considered laid before the other.

2.7.3. Aim 3: relationships between TEs and steroid hormones in eggs

Preliminarily, relationships between the four steroid hormones detected in >70 % of eggs (androstenedione -AN-, testosterone -TS-, pregnenolone -PREG- and progesterone —P4—) were investigated using Spearman's correlation coefficients (Fig. S4). Similarly, mutual relationships of TEs in eggs were investigated (Fig. S5). All hormones were positively correlated (all *p*-values <0.02), contrary to TEs that presented few significant associations (except, Hg & Pb, Hg & Se, Hg & Cu and Zn & Cu, Fig. S4). Thus, all TEs were investigated separately (As, Cu, Hg, Ni, Pb, Se and Zn) with one steroid hormone (AN, as it was detected in all samples and strongly associated to other hormones, all Spearman correlation coefficients >0.55). Due to statistical differences in steroid hormones between parasitic eggs and other eggs (Wilcoxon-Mann-Whitney tests, *p*-value <0.05 for AN and all *p*-values >0.05 for TS, PREG and P4), only eggs with known maternal identity were kept ($n = 37$). LMs were built with AN concentrations as response variable and egg TE concentrations, embryo sex and their interaction as explanatory variables. Female identity was added as a random factor to consider for the non-independence of eggs.

3. Results

3.1. Dynamics of blood TE concentrations during laying

For the two female blood samplings, the mean (± standard deviation (SD)), median and range of concentrations for each element are presented in Table 1. Of the nine TEs found above the LOD in female blood for >70 % of samples, seven out of nine have significantly different concentrations between the first and second blood sampling. The concentrations increased for Cd, Cu, Hg, Pb and Se while they decreased for Ca and Zn (Table 1 and Fig. S6). For Pb, with or without the outlier, a significant increase is observed between the two samplings (*p*-values <0.05). For As, the mean is not significantly different between the first and second sampling. However, the concentration at the end of egg-laying in one of the females is about five times higher than average. In the absence of this outlier, the As concentrations at the first sampling are significantly higher than the concentrations at the second sampling (paired Student's *t*-test by permutation, *p*-value <0.01).

3.2. Maternal transfer of TEs to eggs and variability

Descriptives statistics for the eggs are presented in Table 1 and detailed descriptive statistics for each clutch are provided in Table S5 and Fig. S7.

Cu, Hg and Se concentrations in eggs were positively correlated with the maternal concentrations (LMMs, Cu: slope estimate [95 % Confidence Interval (CI)]: 0.24 [0.10–0.38], *F*-value$_{1,7} = 11.80$, $R^2 = 0.51$, *p*-value = 0.01; Hg: 0.03 [0.02–0.04], $F_{1,7} = 23.51$, $R^2 = 0.51$, *p*-value <0.01; Se: 0.06 [0.03–0.09], $F_{1,7} = 14.50$, $R^2 = 0.52$, *p*-value <0.01; Fig. 2 and Table S6). For all other TEs, the most parsimonious models were the null models, except for Pb (also without outliers). In this case, Pb appeared to be weakly and negatively associated to female mass (LMM, slope estimate [95 % CI]: 0.15 [−0.15–0.45], $F_{1,7} = 5.30$, Table S6); but 0 was included in the 95 % CI (and *p*-value >0.05) suggesting an uninformative parameter (Arnold, 2010).

TE concentrations in the first two eggs were not significantly different (all *p*-values >0.05, except for Pb in one of the two order laying sequences arrangements; Fig. S8 and Table S7). Except for Pb, the same conclusions were found for the two laying sequence arrangements that were tested (Table S7). For Pb, a significant increase of Pb between the first two eggs (*p*-value = 0.02) was observed for one of the two arrangement, whereas no significant difference were observed for the other arrangement (*p*-value >0.05).

Table 1
Descriptive statistics (mean, standard deviation, median, ranges and *n*: sample size) of TE concentrations (µg g^{-1} ww) in red blood cells of breeding female eiders (*Somateria mollissima*) (beginning and end of egg-laying) and eggs (including parasitic eggs due to similar TE concentrations). Samples of one female were not included because it was sampled with a different interval between the first and second sampling (20 days as compared to one week for all other females). Differences between the first and second samples were tested with Student's *t*-tests for paired series by permutation. Statistical differences (*p*-values $<$0.05) are given in bold. Abbreviation $<$ LOD signifies a value below the limit of detection (LOD$_{\mathrm{Cd}}$: 0.0000003, LOD$_{\mathrm{Ni}}$: 0.00002 and LOD$_{\mathrm{Pb}}$: 0.00005 µg g^{-1} ww).

µg g^{-1} ww	Females			Eggs
	Beginning of laying (*n* = 9)	End of laying (*n* = 9)	*p*-value	3 to 10 days after laying (*n* = 44)
As	0.024 ± 0.005 0.024 [0.017;0.032]	0.030 ± 0.044 0.016 [0.011;0.147]	> 0.05	0.132 ± 0.134 0.095 [0.025;0.861]
Ca	92.235 ± 39.553 80.923 [53.405;172.129]	21.787 ± 4.516 21.995 [15.445;28.893]	**< 0.01**	Not analyzed
Cd	0.0016 ± 0.0003 0.0017 [0.0011;0.0022]	0.0021 ± 0.0005 0.0021 [0.0013;0.0029]	**< 0.01**	Detected in 23 % samples [< LOD;0.0002]
Cu	0.392 ± 0.081 0.381 [0.221;0.521]	0.466 ± 0.072 0.478 [0.293;0.552]	**< 0.01**	0.948 ± 0.316 0.867 [0.481;1.637]
Hg	0.152 ± 0.055 0.146 [0.073;0.249]	0.204 ± 0.075 0.177 [0.085;0.318]	**< 0.01**	0.121 ± 0.047 0.114 [0.042;0.262]
Mg	106.056 ± 5.089 105.673 [99.003;115.852]	102.453 ± 9.073 98.960 [91.103;120.487]	> 0.05	Not analyzed
Ni	Detected in 44 % samples [< LOD;0.003]	Detected in 33 % samples [< LOD;0.077]		0.008 ± 0.002 0.007 [0.004;0.013]
Pb	0.536 ± 1.139 0.092 [0.033;3.544]	0.894 ± 1.964 0.260 [0.096;6.123]	**< 0.05**	0.178 ± 0.523 0.004 [< LOD;2.25]
Se	3.364 ± 1.106 3.532 [1.3141;4.75]	4.378 ± 1.518 4.696 [1.901;6.741]	**< 0.01**	0.473 ± 0.070 0.464 [0.319;0.6433]
Zn	9.066 ± 1.540 8.733 [7.422;12.443]	6.666 ± 0.700 6.365 [5.911;7.879]	**< 0.01**	20.144 ± 2.946 20.651 [9.453;25.786]

3.3. Relationship of TE and steroid hormone concentrations in eggs

Of the 18 steroid hormones analyzed, three were detected in all eggs: AN (range: 2.9–50.6 ng g^{-1} ww), PREG (range: 104.0–1066.6 ng g^{-1} ww) and P4 (range: 45.3–1654.5 ng g^{-1} ww), and TS was quantified in 72 % of the eggs (range: $<$ 0.4–11.2 ng g^{-1} ww). Details on hormone concentrations for each clutch are provided in Table S5. For all TEs, the most parsimonious models to explain hormone concentrations are the null models (none of the variables investigated in this study).

4. Discussion

4.1. Dynamics of blood TE concentrations during laying

Avian egg development requires sufficient concentrations of some TEs such as Ca and Zn which are essential for eggshell production and embryo development (Baumgartner et al., 1978; Orłowski et al., 2016a, b; Perrins, 1996; Hincke, 2012). In this study, we have observed a decrease in Ca and Zn in female red blood cells from early in egg laying to after the last egg is laid. The decrease in these elements may illustrate the cost of breeding, similarly to reported in the Christiansø colony (Denmark) (McPartland et al., 2020b). Eider eggs are produced using endogenous and exogenous sources (Hobson et al., 2015; Jaatinen et al., 2016), inducing the partial uses of reserves made prior to reproduction by the female to meet demands during laying and incubation periods. Pollutants stored in body tissues can be released back into circulation at time of high energy demand such as during reproduction (Fox et al., 2015; Mason et al., 2014; McPartland et al., 2020b). For Cd, Hg and Se, relationships between blood and kidney or liver concentrations in female eiders have already been reported (Wayland et al., 2001). Moreover, Cd and Pb can mimic Ca and thus be stored in the medullary bones (Wilson et al., 2007). The eggshell is known to be a route of excretion of several TEs including metals like Cd and Pb but also Cu, Ni and Zn (Burger, 1994; Orłowski et al., 2016a,b). As a result, these elements are remobilized during eggshell production and incubation (Beyer and Meador, 2011; Kalisińska, 2019; Mora, 2003; Vallverdú-Coll et al., 2015), and could explain the increase in Cd, Cu, Hg and Pb concentrations observed in this study. Contrary to the decrease reported for Mg and Se during incubation in previous studies (Garbus et al., 2020; McPartland et al., 2020b; Wilson et al., 2007), no significant difference for Mg and an increase of Se were observed in this study. However, in our study, sampling was performed at an interval of one week, and during the onset of egg-laying compared to approximately 20 days during the incubation period in other studies. A shorter interval between samplings in our study could explain the differences in TE dynamics compared to previously reported results. Moreover, Se is an essential element for embryonic development, an increase in early laying could be related to a rebalance between the different storage organs and the bloodstream to ensure their maternal transfer (Kalisińska, 2019).

4.2. Maternal transfer of TEs to eggs and variability

In line with maternal transfer of TEs to eggs, we have observed a positive correlation between Cu, Hg and Se in eider female blood and eggs. For eiders, maternal transfer of Hg had already been reported in the first egg while none had been found for Se (Franson et al., 2000b). Several studies have examined maternal transfer of Hg (26 species reviewed by Ackerman et al., 2020) and to a lesser extent for As, Cd, Pb and Se (seabird, waterflow and wader species in Ackerman et al., 2016a; Agusa et al., 2005; Kubota et al., 2002; Vallverdú-Coll et al., 2015). Contrary to a previous study in mallard *Anas platyrhynchos* (Vallverdú-Coll et al., 2015), no correlation was found for Pb in female red blood

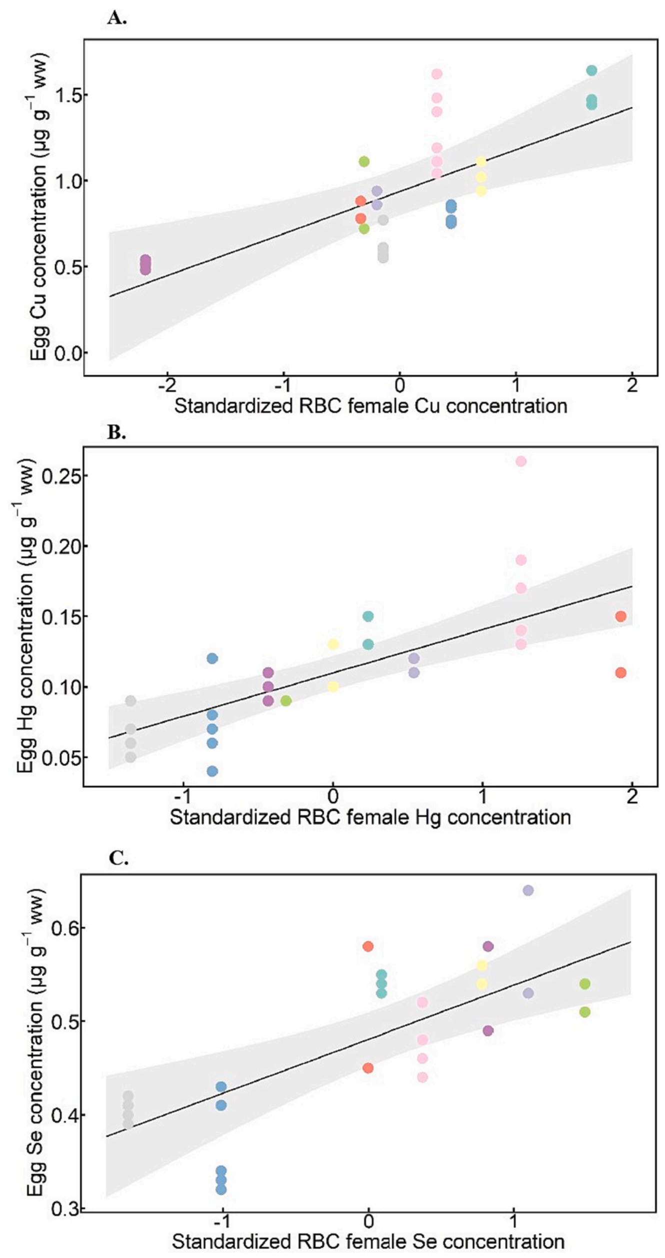

Fig. 2. Results of linear mixed-effects models on female and egg TE concentrations of common eider, *Somateria mollisima.*

A. Relation between egg Cu concentrations and standardized female Cu concentrations ($R^2 = 0.51$, p-value $= 0.01$).

B. Relation between egg Hg concentrations and standardized female Hg concentrations ($R^2 = 0.51$, p-value <0.01).

C. Relation between egg Se concentrations and standardized female Se concentrations ($R^2 = 0.52$, p-value <0.01).

Each circle is an individual egg with colour referring to female identity. For each graph, the solid line represents the fitted model and 95 % confident interval is presented in grey. Abbreviation: RBC: red blood cells.

cells and eggs in the current study. Still, the female with the highest concentrations of Pb in red blood cells produced eggs with the highest Pb concentrations suggesting maternal transfer. Theoretically, a TE decrease in the laying sequence is related to the load of the female, which decreases progressively during laying with the first eggs being more contaminated than the last (Akearok et al., 2010). Thus, it is essential to include this parameter in biomonitoring of contamination (Ackerman et al., 2016b; Akearok et al., 2010; Orłowski et al., 2016a). Our statistical analyses, which focused on the first two eggs only, showed that they are exposed to the same load of TEs (except for Pb for one of the two laying order options). The uneven sampling of clutches with more than two eggs did not allow to statistically explore the differences in TEs along egg laying sequence, which graphically seem to decrease for Hg, Ni and Se (Fig. S8). Other studies showed a decrease in concentrations in the laying sequence, with early eggs being more contaminated with Hg than late eggs (15 species including eider reviewed in Ackerman et al., 2016a, 2016b). Clutch size was also reported to influence maternal transfer in the laying sequence, with intraclutch variations in Hg and Se according to the clutch size for Audouin's gull *Larus audouinii* (Morera et al., 1997; Sanpera et al., 2000).

As conspecific brood parasitism is relatively common and variable between eider colonies (reviewed by Waltho and Coulson, 2015), knowing the exact number laid by the incubating females is difficult, and requires egg maternity tests. Based on microsatellite marker-based genotyping we have shown that 16 % of the eggs among the 10 studied clutches were parasitic eggs (seven parasitic eggs distributed in three nests, with up to three parasitic eggs in a single nest). Development of methods to discriminate parasitic eggs based on molecular methods such as genotyping (Andersson et al., 2019) offers a good alternative to exhaustive frequent monitoring of nest occupancy.

The female condition was also investigated as a factor inducing variability in TE concentrations in eggs but did not seem to have a significant influence on maternal transfer of TEs. Similarly, embryo sex was suggested to be associated with TE concentrations, since TEs such as Hg can affect the endocrine system involved in sex hormone excretion (Tartu et al., 2013, 2014), essential to sex determination during embryogenesis. However, in our study TE concentrations in eggs are not related to embryo sex. To this date, only a few avian studies have examined the associations between TE loads and embryo sex or sex ratio (Bouland et al., 2012).

4.3. *Relationship of TEs and steroid hormone concentrations in eggs*

Of the 18 steroid hormones analyzed, only AN, P4, PREG and TS were detected in >70 % of the individuals and the values were consistent with concentrations reported in other avian species (Kumar et al., 2018; Rubolini et al., 2011; Wang et al., 2023). The associations between TEs and steroid hormones have mostly been studied in breeding adults showing potential endocrine changes (Heath and Frederick, 2005; Jayasena et al., 2011). Disruption of the maternal endocrine system changes hormones deposited into the eggs, as reported with POPs (Jouanneau et al., 2023), and could induce transgenerational endocrine effects. In our study, no significant correlations between steroid hormones and TE concentrations in eggs were observed, suggesting a marginal effect of TEs on steroid hormones concentrations in the eggs. However, female embryos with the highest Pb concentrations appeared to have lower hormone levels than other females (Fig. S9). Eggs were collected precociously, *i.e.* within the first days of incubation, which potentially reflect maternal hormone transfer. We cannot exclude that TE concentrations could affect the future expression pattern of sex steroid hormones after the neuroendocrine system development, requiring further investigations into the later stages of embryonic life.

4.4. *TEs contamination and potential implications*

4.4.1. *Tissue variability and considerations*

Whole blood is a common matrix to study TEs. However, studies report that metals are mainly bound to blood cells, making red blood cells a relevant matrix to study TEs too (Bond and Robertson, 2015; Coeurdassier et al., 2012). In addition, whole blood is commonly centrifuged in order to extract plasma for other analyses as in our case, thus using red blood cells for TEs allows to optimize the sample collection and use. Previous studies have reported a ratio red blood cells – whole blood of 1.8:1 for Cd, Hg and Pb in for birds, including the eider (Coeurdassier et al., 2012; Ma et al., 2020) corresponding to an average

Table 2

Summary of descriptive statistics (mean, standard deviation (SD) and ranges) of TE concentrations (μg g^{-1} ww) on breeding female eiders (*Somateria mollissima*). Abbrevations and symbology are *n*: sample size; WBc: whole blood concentrations obtained by conversion from red blood cells using 1.8 factor; WB: whole blood; xd inc: x days of incubation; ND: no-detected; ?: unspecified; ^{1} Concentrations are converted from nmol g^{-1} ww to ng g^{-1} ww using these conversion values: 1 mol Hg = 200.59 g Hg and 1 mol Se = 78.96 g Se; ^{2} Concentrations are converted from dry mass (dw) to wet mass (ww) using 79.6 % moisture content for blood (Franson et al., 2004) and this formula $ww = dw\ x \left[\frac{(100 - \%moisture)}{100}\right]$ ^{3}SD calculated with Standard Error $*\sqrt{sample\ size}$ ^{4}Average over the four sampling areas of the study. [1] McPartland et al., 2020b [2] Miller et al., 2019 [3] Provencher et al., 2016 [4] Fenstad et al., 2016a, 2016b [5] Wilson et al., 2007 [6] Franson et al., 2004 [7] Franson et al., 2002 [8] Franson et al., 2000b. In Fenstad et al., 2017, blood concentrations of incubating females sampled in 2011 for As, Cu, Pb and Zn were analyzed but no numerical data are provided and thus, these are not included in this table.

Country and colony area	Year	Matrix and *n*	Period	As	Ca	Cd	Cu	Hg	Mg	Pb	Se	Zn	Study
Finland Bengtskär	2021	WBc 9	Start laying	0.014 ± 0.003	51.24 ± 21.97	0.0009 ± 0.0002	0.22 ± 0.05	0.08 ± 0.03	58.92 ± 2.83	0.30 ± 0.63	1.87 ± 0.62	5.04 ± 0.85	This study
Finland Bengtskär	2021	WBc 10	End laying	0.016 ± 0.023	12.15 ± 2.37	0.0012 ± 0.0003	0.26 ± 0.04	0.11 ± 0.03	57.32 ± 4.92	0.45 ± 1.04	2.38 ± 0.81	3.74 ± 0.38	This study
Denmark Christiansø	2018	WB 27	4d inc	0.012 ± 0.003	58.88 ± 14.82	0.0008 ± 0.0004	0.34 ± 0.07	0.21 ± 0.10	72.52 ± 6.97	0.22 ± 0.31	2.95 ± 0.82	5.55 ± 0.54	[1]
Denmark Christiansø	2018	WB 27	24d inc	0.013 ± 0.004	51.02 ± 5.95	0.0010 ± 0.0005	0.37 ± 0.068	0.18 ± 0.09	67.68 ± 7.71	0.26 ± 0.24	2.32 ± 0.69	5.12 ± 0.61	[1]
Denmark Christiansø	2017	WB 23	4d inc	0.014 ± 0.003	95.10 ± 36.1	0.0008 ± 0.0003	0.33 ± 0.06	0.18 ± 0.06	70.29 ± 7.39	0.04 ± 0.06	3.89 ± 1.18	6.98 ± 1.39	[1]
Denmark Christiansø	2017	WB 23	24d inc	0.016 ± 0.005	50.50 ± 4.44	0.0010 ± 0.0004	0.36 ± 0.05	0.17 ± 0.06	64.49 ± 6.15	0.06 ± 0.06	3.11 ± 1.10	5.09 ± 0.85	[1]
Alaska Kaktovik	2014	WB 20	≥ 15d inc (15-25d)			0.008 ± 0.001	0.47 ± 0.01	0.20 ± 0.01		0.069 ± 0.003	10.40 ± 0.85		[2]
Canada Mittivik Island	2014	WB 92	pre-laying			0.003 ± 0.001		0.22 ± 0.06		0.009 ± 0.006	4.15 ± 1.36		[3]
Canada Mittivik Island	2013	WB 98	pre-laying			0.002 ± 0.001		0.19 ± 0.05		0.009 ± 0.006	4.11 ± 1.13		[3]
Finland Tvärminne^{1}	2011	WB 21	26d inc					0.17 ± 0.07			1.37 ± 0.68		[4]
Svalbard Kongsfjorden^{1}	2011	WB 26	26d inc					0.11 ± 0.03			4.46 ± 2.36		[4]
Alaska Tutakoke River	2004	WB 44	inc							0.05 ± 0.01	8.71 ± 3.51		[5]
Alaska Kigigak island	2004	WB 135	inc							0.05 ± 0.01	6.74 ± 0.21		[5]
Alaska Kigigak island	2003	WB 67	inc							0.04 ± 0.01 (detcted in 48 % of samples)	7.11 ± 0.25		[5]
Alaska Kigigak island	2002	WB 45	inc							0.27 ± 0.16	5.77 ± 0.25		[5]
Alaska Tutakoke River	2002	WB 61	inc							0.15 ± 0.11	6.62 ± 0.24		[5]
Alaska - Beaufort Sea^{2,3}	2000	WB 40	?	Detected in 2.5 % of samples [ND;0.08]		0.0061 ± 0.0038	0.40 ± 0.05		63.44 ± 3.73	0.05 ± 0.02	7.36 ± 2.23	5.34 ± 0.70	[6]
Finland Gulf of Finland^{4}	1999 2000	WB 101	inc							0.07 ± 0.01			[7]
Finland Gulf of Finland^{4}	1998	WB 224 in	inc							0.06	1.37		[8]
Finland Gulf of Finland^{4}	1997	total	inc							0.10	1.75		[8]

hematocrit value of 55.56 %. Hematocrit values were not recorded as part of our study, but hematocrit values were collected in breeding females of common eider from the same Baltic-Wadden Sea population in 2015–2018 on three Danish islands (mean ± SD: 53.5 ± 8.1 %, $n = 171$) (Sonne and Garbus, unpubl., Ma et al., 2020). Therefore, for comparisons with other studies, we decided to keep a conservative approach and only present converted TEs concentrations in red blood cells concentrations in whole blood by applying a 1.8 factor. Comparison of concentrations converted in whole blood for As, Ca, Cu, Mg, Ni, Se and Zn with other studies need caution in the interpretation, as the conversion ratio red blood cells - whole blood are not known for these elements.

4.4.2. *Bengtskär concentrations in blood and eggs compared to eider colonies in Finland and elsewhere*

For most studied TEs, concentrations in breeding females are consistent with those reported in the literature over the last 15 years (Table 2). When comparing the Bengtskär colony with another eider colony in the Gulf of Finland sampled approximately 10 years ago, Cd, Cu and Hg have decreased by a factor two (Cd: ~0.002, Cu: ~0.42 and Hg: ~0.17 μg g^{-1} ww in whole blood, graphical approximations, Fenstad et al., 2017). Se concentrations (mean: 1.87 and 2.38 μg g^{-1} ww at the beginning and end of egg-laying, respectively) were in the same order of magnitude as reported from other colonies including the Finnish colonies sampled in 1997, 1998 and 2011 (mean: 1.75, 1.37 and 1.37 μg g^{-1} ww, respectively) (Fenstad et al., 2016a, 2016b; Franson et al., 2000b; Table 2). Moreover, As, Mg and Zn concentrations were in the same order of magnitude than concentrations already reported, especially in Baltic Sea colonies (Finland and Denmark) (Fenstad et al., 2017; McPartland et al., 2020b).

For eggs, concentrations are difficult to compare with other studies because they vary with embryonic stage (Klein et al., 2012; Orłowski et al., 2016a,b). However, concentrations of As, Hg and Se seemed consistent with values found in a Finnish colony in 1997 (Franson et al., 2000b). Similarly, all TE concentrations were also in the same order of magnitude with eggs from other colonies (Table 3).

Finally, for Pb, one female had concentrations (3.4 μg g^{-1} ww) >10 times higher than the maximum mean values reported in the literature for eider ducks (Table 2). Including these concentrations in the averages, the values at Bengtskär were above the values reported in blood and eggs from other colonies (Table 2 and 3). Excluding this female, female concentrations at both samplings (0.09 ± 0.09 and 0.13 ± 0.07 μg g^{-1} ww in whole blood, 1st and 2nd sample, respectively) were still higher than concentrations reported from some other colonies in Alaska (Provencher et al., 2016; Wilson et al., 2007), the Baltic Sea (Christiansø in 2017 and Finland archipelagos in 1990's and 2011; Fenstad et al., 2017; Franson et al., 2000b, 2002; McPartland et al., 2020b). In particular, mean Pb concentrations were twice the concentrations found in a Finnish colony ten years ago (Fenstad et al., 2017). However, it was lower than concentrations reported in a Baltic Sea colony (Christiansø, Denmark) in 2018 (McPartland et al., 2020b). Pb concentrations in the eggs of this eider female (mean ± SD (range): 1.50 ± 0.70 (0.82–2.25) μg g^{-1} ww) were up to 20 times higher than concentrations previously reported (Table 3) and were the highest Pb concentrations ever reported for this species.

4.4.3. *TEs & potential toxicity*

Blood is a matrix which is more frequently used to establish toxicity thresholds. In the female blood, except for Pb, TE concentrations in the current study appeared to be within limits that can be considered as harmless. Indeed, for Cd, Cu and Hg, concentrations were below toxicity thresholds for these TEs. Cd concentrations corresponded to the reported norm (< 0.05 μg g^{-1} ww in whole blood) and were well below the concentrations for which the first signs of toxicity are observed (> 0.26 μg g^{-1} ww in whole blood) (Wayland and Scheuhammer, 2011). Similarly, Cu concentrations were in the range of the reported values in non-exposed birds (0.2–0.6 μg g^{-1} ww in whole blood) (Fenstad et al., 2017; Osofsky et al., 2001). For Hg, Evers et al. (2008) defined a toxicity threshold for common loon *Gavia immer* reproduction, for blood concentrations higher than 3 μg g^{-1} ww in whole blood. Low Hg concentrations at Bengtskär, ranging from 0.04 to 0.17 μg g^{-1} ww were well below this threshold, even lower that the no risk toxicity category (< 0.2 μg g^{-1} ww) reported by Ackerman et al., (2016c) in birds. However, even low concentrations of Hg (range: [0.04;0.43] μg g^{-1} ww) can indirectly affect corticosterone levels, as described for eiders (Provencher et al., 2016).

For other essential elements than Cu (Ca, Mg, Se and Zn), toxicity thresholds are not defined but presented mostly concentrations close to which reported in other colonies (Table 2). At the end of egg-laying, Ca and Zn presented low concentrations, until eight times lower than reported in the literature (Table 2). Deficiency of TEs is known to cause deleterious effects (Baumgartner et al., 1978; Frassinetti et al., 2006; Kalisińska, 2019) but can also reflected the transitory cost of reproduction (McPartland et al., 2020b). For As, few correlative studies have investigated its effects on wild birds, particularly using blood (reviewed in Sánchez-Virosta et al., 2015). However, As concentrations were in the same order of magnitude than values reported in other Baltic colonies (Fenstad et al., 2017; McPartland et al., 2020b, Table 2). Finally, for Ni, few data are available about its toxicity, but <50 % of female eiders had Ni concentrations above the LOD and with very low levels. However, Ni was detected in all eggs, and since Ni appears implicate in enzyme activity and regulate hormone activity (Binkowski, 2019), low Ni concentrations could meet physiological needs. Toxicity in eggs has been little studied and general toxicity thresholds have therefore not been established for avian eggs. Only Hg toxicity thresholds in common loon eggs were defined with a low-risk category for Hg concentrations below 0.60 μg g^{-1} ww (Evers et al., 2003), suggesting that Hg egg concentrations in our study were not harmful. This is consistent with other Hg concentrations reported in egg birds (Scheuhammer et al., 2001). Moreover, for other TEs except Pb, the concentrations in eggs were consistent with the values already reported in the literature on eider eggs (Table 3).

4.4.4. *Special case of Pb: risks and implications*

For Pb, at the first and second sampling, two females (22 % of the nine females at the beginning of egg-laying and 20 % of the 10 females at the end of egg-laying) were above the subclinical concentrations (~0.2–0.5 μg g^{-1} ww in whole blood) reported for Anatini (Franson and Pain, 2011). In particular, one female had very high concentrations (calculated 1.97 and 3.4 μg g^{-1} ww in whole blood at the beginning and end of egg-laying, respectively). Such concentrations are higher than those reported in other studies on common eiders (except, females with concentrations of 6.6 and 7.0 μg g^{-1} ww in whole blood reported in Wilson et al., 2007) and notably above the thresholds for severe poisoning (> 0.5 μg g^{-1} ww in whole blood). Especially, these concentration is comparable with blood concentrations reported in birds of prey exposed to lead residues (Franson and Pain, 2011). Possibly, concentrations of this female could reflect contamination due to the ingestion of Pb pellet or debris either at wintering sites or locally, especially as Bengtskär was the place of a battle and bombardment during the Second World War, debris may still be present. It is worth noting that we did not observe any differences in behavior or appearance when handling this female compared to the other females. Even at sub-lethal concentrations, Pb can induce a delay in the date of arrival on the eider breeding grounds as well as an alteration in enzymatic activity (decreased delta-aminolaevulinic acid dehydratase activity) and body condition (Franson et al., 2002; Provencher et al., 2016). Some Pb concentrations in eggs are also high with the highest concentrations reported so far for this species (2.25 μg g^{-1} ww in this study; 1.34 μg g^{-1} ww reported in Burger et al., 2008). Particularly, concentrations of Pb above 0.1 μg g^{-1} ww in mallard ducklings reduced weight at hatching, induced immunological changes, increased oxidative stress and mortality within seven days of hatching (Vallverdú-Coll et al., 2015).

Table 3

Summary of descriptive statistics (mean and standard deviation) of TE concentrations (μg g^{-1} ww) on eider eggs (*Somateria mollissima*). Abbreviations are *n*: sample size; ND: no-detected; ?: unspecified; < LOD: value below the detection limit (LOD$_{Cd}$: 0.0000003 and LOD$_{Pb}$: 0.00005 μg g^{-1} ww); d. inc.: days of incubation; d. lay.: days of laying; ^{1} Concentrations are converted from dry mass (dw) to wet mass (ww) using 64.7 % moisture content for egg (Burnham et al., 2021; Franson et al., 2004 and the following formula ww $= \text{dw} \times \left[\frac{(100 - \%\text{moisture})}{100}\right]$) ^{2} SD calculated with Standard Error $*\sqrt{\text{sample size}}$ ^{3} Median [1] Smith et al., 2022 [2] Pratte et al., 2015 [3] Burnham et al., 2021 [4] Burger et al., 2008 [5] Akearok et al., 2010 [6] Peck et al., 2016 [7] Franson et al., 2004 [8] Franson et al., 2000b.

Country and colony area	Year	Period	*n*	As	Cd	Cu	Hg	Ni	Pb	Se	Zn	study
Finland Bengtskär	2021	3–9 days of inc	44	0.13 ± 0.13	Detected in 23 % of samples	0.95 ± 0.32	0.12 ± 0.05	0.007 ± 0.002	0.18 ± 0.52	0.47 ± 0.07	20.14 ± 2.95	This study
Greenland East Bay^1	2019	1–13 d. lay. – 1st egg	50				0.36 ± 0.13					[1]
Greenland East Bay^1	2018	1–13 d. lay. – 1st egg	46				0.38 ± 0.14					[1]
Greenland East Bay^1	2017	1–13 d. lay. – 1st egg	12				0.52 ± 0.22					[1]
Greenland East Bay^1	2016	1–13 d. lay. – 1st egg	13				0.45 ± 0.16					[1]
Canada Nova Scotia	2015	Nest <4 eggs	45	0.12 ± 0.03	0.0003 ± 0.0001	0.84 ± 0.20	0.08 ± 0.04		0.005 ± 0.002	0.33 ± 0.09	11.7 ± 3.3	[2]
Greenland Manson Islands^1	2014	?	12				0.08 ± 0.03					[3]
Alaska Amchitka & Kiskaf^1	2007	?	52	0.27 ± 0.02			0.15 ± 0.08		0.11 ± 0.04	0.61 ± 0.05		[4]
Greenland East Bay^{1,2}	2008	“Early eggs”	9				0.18 ± 0.01					[5]
Greenland East Bay^{1,2}	2008	“Late egg”	9				0.12 ± 0.01					[5]
Canadian high Arctic^1	2004, 2005	Early inc	5				0.31 ± 0.08					[6]
Alaska Beaufort Sea^{1,2}	2000	Varying	20	0.09 ± 0.06	Detected in 30 % of samples	1.94 ±0.75	0.20 ± 0.06	Varying with development status	Detected in 35 % of samples	0.80 ± 0.19	17.76 ± 7.40	[7]
Finland Gulf of Finland^3	1997	First eggs	21	< 0.10			0.10			0.55		[8]

Further investigations are required to evaluate the potential association with hatchability and juvenile survival.

Finally, a high annual variability of Pb has been already reported, especially in the western Baltic with a 4.7-fold-increase in Pb concentrations in incubating females was reported between 2017 and 2018 (McPartland et al., 2020b; Wilson et al., 2007). Thus, a longitudinal study should be considered to account for the possible year-to-year variability also in the current colony. Likewise, future studies are still needed to trace the origin of Pb for example by Pb isotopic measurements (see Lam et al., 2020; McPartland et al., 2020a).

5. Conclusion

The aim of this study was to assess maternal transfer of TEs and to examine the dynamics of blood TE concentrations in female eiders during egg-laying. Associations between TEs and steroid hormone concentrations in eggs were also investigated. In addition, knowledge of recent TE concentrations in a colony in the northeastern Baltic Sea has been updated and compared with concentrations previously reported and toxicity thresholds. Except for Pb, TE concentrations in the blood of females as well as in their eggs were consistent with reported values especially in the western Baltic Sea and below known toxicity limits. Pb concentrations exceeded toxicity thresholds, in particular with high concentrations for one female and its eggs including the highest egg concentration ever reported for this species. These high Pb concentrations raise the question of the frequency of these extreme cases in the colony, as well as on the Northern Baltic Eider population, where 70 % of the flyway breeding population is located. Continued monitoring of this colony would allow to consider the annual variability and to discriminate the possible sources of Pb contamination.

CRediT authorship contribution statement

Prescillia Lemesle: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation. **Sunniva H. Frøyland:** Writing – review & editing, Investigation. **Amalie Ask:** Writing – review & editing, Supervision, Investigation. **Junjie Zhang:** Writing – review & editing, Investigation. **Tomasz M. Ciesielski:** Validation, Methodology. **Alexandros G. Asimakopoulos:** Writing – review & editing, Resources. **Kristina Noreikiene:** Writing – review & editing, Methodology, Investigation. **Nora M. Wilson:** Writing – review & editing, Investigation. **Christian Sonne:** Writing – review & editing, Resources. **Svend Erik Garbus:** Resources. **Veerle L.B. Jaspers:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Céline Arzel:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Acknowledgments

This work was funded by the University of Turku Foundation and the Academy of Finland (grant no. 336261 and grant no. 333400 to Céline Arzel) and carried out as a collaboration between the University of Turku and the Department of Biology at NTNU. PL was supported by an ERASMUS internship grant. The authors thank the team of NTNU Chemistry Department: Anica Simic and Kyyas Seyitmuhammedov for the help with the trace element analyses. We are grateful to Juho Jolkkonen for his participation in the field work at Bengtskär. We also express our gratitude to Bengtskär Oy, especially Henrik and Peppi Wilson, for facilitating the fieldwork. Thanks are due to Katja Salminen and Meri Lindqvist (Center of Evolutionary Applications, University of Turku) for performing the maternity analyses of the eggs. Hematocrit data were collected by Sven Erik Garbus and Christian Sonne as part of research projects funded by The Danish Environmental Protection Agency, 15. Juni Fonden, Jægernes Naturfond, SKOV A/S (Glyngøre 7870 Roslev, Denmark) and the BONUS BALTHEALTH project. The authors would like to thank four anonymous reviewers for their constructive feedback to improve the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176935.

Data availability

data will be availaible on Zenodo after approval.

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