Environment International 142 (2020) 105874

Contents lists available at ScienceDirect

Environment International

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

Lead isotopic signatures in blood from incubating common eiders (*Somateria mollissima*) in the central Baltic Sea

Molly McPartland[a], Svend-Erik Garbus[b], Syverin Lierhagen[c], Christian Sonne[b,d], Åse Krøkje[a,*]

[a] Norwegian University of Science and Technology (NTNU), Department of Biology, Høgskoleringen 5, NO-7491 Trondheim, Norway
[b] Aarhus University, Department of Bioscience, Arctic Research Centre (ARC), Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark
[c] Norwegian University of Science and Technology (NTNU), Department of Chemistry, Høgskoleringen 5, NO-7491 Trondheim, Norway
[d] Henan Province Engineering Research Center for Biomass Value-added Products, School of Forestry, Henan Agricultural University, Zhengzhou 450002, China

ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Avian
Baltic Sea
Lead poisoning
Isotope
HR-ICP-MS
Biomonitoring

ABSTRACT

The Christiansø colony of common eiders (*Somateria mollissima)* in the central Baltic Sea were exposed to high levels of Pb during the 2018 breeding season that were not present in 2017. Due to these high Pb blood levels, the present study investigated possible Pb sources and Pb dynamics within this vulnerable colony. We analyzed body mass and lead isotopic ratios (Pb-IRs) in blood taken from the same incubating eiders at the early (day 4) and late (day 24) stages of incubation during the 2018 breeding season (n = 23). Pb-IRs 208/207, 208/206, 206/207, and 207/206 were analyzed using high resolution inductively coupled mass spectrometry. We found largely similar Pb-IRs from the different stages of incubation indicating a predominantly constant endogenous source of Pb exposure. We suggest the increasing Pb levels come from pre-nesting and nesting foraging and from medullary bone release. The similar Pb-IRs also indicate continued metabolization of the medullary bone to meet the nutritional and energy demands of incubation. Comparisons to Pb-IR reports from the Baltic Sea showed multiple sources of pollution distinguished by a difference between Pb-IRs in individuals with Pb blood concentrations > 500 µg/kg ww and < 500 µg/kg ww. The most highly contaminated individuals in the present study had Pb-IRs similar to those of Pb ammunition indicating shot pellet uptake. This study further emphasizes the need for continued biomonitoring of the Christiansø colony, including fecal sampling and environmental field sampling to identify the origin and extent of dietary Pb exposure on Christiansø. As a representative unit of the Baltic Flyway population; the Christiansø colony provides an important opportunity for continued investigation into Pb contamination, population dynamics, and declines.

1. Introduction

Environmental lead (Pb) pollution is a well-documented factor causing mortality and population declines in avian species (Pain et al., 2019a). Waterfowl, in particular, are susceptible to Pb poisoning due to their diverse habitats and foraging behavior (Haig et al., 2014). Waterfowl are predominantly exposed to Pb through ingestion although wounding has also been a documented source (Mallory et al., 2004; Clausen et al., 2017). Pb is absorbed by the gastrointestinal tract with 5–15% efficiency, although dietary deficiencies of iron (Fe), zinc (Zn), magnesium (Mg) and calcium (Ca) can enhance this absorption (Abadin et al., 2007; Skerfving and Bergdahl, 2014). Once absorbed, mobile Pb in the blood has shown to inhibit delta-aminolevulinic acid dehydratase (ALAD) through Zn mimicry (Jaishankar et al., 2014) and become incorporated into bone as a Ca replacement (Elder et al., 2014).

In bone tissue, Pb has a half-life of ca. 30 years, making it a useful material to detect lifelong Pb exposure in avian species (Pain et al., 2019a). Liver and kidney tissue have been used as well (Franzen-Klein et al., 2018; Slabe et al., 2020) although, like bone, they require destructive sampling methods and are therefore not always suitable when studying an endangered or threatened species. Alternatively, blood and feces have been implemented as a minimally-invasive means of analyzing metal contamination without sacrifice (Berglund, 2018; Lam et al., 2020; Slabe et al., 2019). This method has been criticized for compromising scientific rigor as blood and feces both reflect information on Pb intake from dietary exposure within the last two weeks (Craighead and Bedrosian, 2008). However, this is not completely founded as blood also reflects remobilized Pb from stored deposits in the medullary bone, liver, and kidneys during egg production, moulting, and other critical life-history events (Veerle et al., 2004; Mason et al., 2014). The relationships between bone Pb concentrations and that in the blood or excrement have not been extensively studied

* Corresponding author.
E-mail address: ase.krokje@ntnu.no (Å. Krøkje).

https://doi.org/10.1016/j.envint.2020.105874
Received 28 January 2020; Received in revised form 14 May 2020; Accepted 14 May 2020
Available online 22 June 2020

and therefore must be considered cautiously (Wayland et al., 2001; Berglund, 2018).

Within the Baltic Sea, Pb is considered a pollutant of concern (Zalewska et al., 2015; HELCOM, 2018, 2019) and has been documented above toxicity threshold levels in waterfowl leading to adverse health effects and death (Falandysz et al., 2001; Pain et al., 2019a; Lam et al., 2020). Approximately half of all Pb inputs into the Baltic Sea originate from atmospheric deposition however, phasing out efforts have shown a temporal decrease in pollution trends with no recent reports of Pb poisoning in waterfowl (HELCOM, 2010, 2018). Nevertheless, poisoning of avian species through unintentional ingestion of Pb from fishing tackle and ammunition pellets is a persistent problem world-wide and within the Baltic Sea (Helander et al., 2009; Martinez-Haro et al., 2011; Legagneux et al., 2014; Berny et al., 2015; Madry et al., 2015; Pain et al., 2019b). Although total and partial bans of lead ammunition have been implemented in 23 countries across the European Union (EU), certain countries bordering the Baltic Sea, such as Poland and Lithuania, currently have no regulations (Mateo and Kanstrup, 2019). Continued usage of Pb ammunition is estimated to cost the EU €383–€960 million euros, which includes not only wildlife impact expenses but also human hospitalizations and IQ reduction in children (Pain et al., 2019a). While Denmark and the Netherlands' total ban on lead ammunition is intended to be protective of both wildlife and humans (Kanstrup et al., 2016), without a concerted effort from hunters and industry across the EU, the Baltic Sea and its inhabitants still face the risk that Pb exposure poses.

The Common eider (*Somateria mollissima*), hereafter eider, is the largest sea duck in the northern hemisphere and are found along the coastlines of Scandinavia, Iceland, Svalbard, and much of northern Canada. As partially migratory birds, eiders have an expansive habitat making them vulnerable to spatial and temporal patterns of Pb availability (Haig et al., 2014). The Baltic/Wadden Sea Flyway population of eiders consists of ca. 900,000 birds from Norway, Sweden, Denmark, Finland, Estonia, Germany and the Netherlands (Desholm et al., 2002; Christensen et al., 2013; Waltho and Coulson, 2015). The Flyway population has shown drastic population declines in the last two decades with largely unknown explanations (Ekroos et al., 2012). From 2000 to 2009 the total number of breeding pairs decreased by 48%, while the wintering numbers decreased by 36% (Ekroos et al., 2012). Further, the Flyway population has experienced three mass mortality events since 2000, cumulatively killing thousands of birds (Camphuysen et al., 2002, Garbus et al., 2019). Two of these events occurred within the Christiansø colony known to breed on the island of Christiansø, Denmark (Fig. 1) in the central Baltic Sea (Garbus et al., 2018, 2019). The Christiansø colony consists of 10% of the breeding Danish Eiders and overwinters at the western part of the Baltic Sea to the southern Dutch part of the Wadden Sea, although exact locations are unknown (Noer, 1991). Consisting of ca. 1500 females this colony is a representative unit of the Baltic/Wadden Flyway population and has similarly also suffered from population declines (Lyngs, 2014) partly explained by limitations in their preferred food, the blue mussel (*Mytilus edulis*) (Larsson et al., 2014), or high prevalence of acanthocephalan parasites (Garbus et al., 2018, 2019). However, this does not fully explain the observed mass mortality events or mortality of birds in good body condition (Laursen and Møller, 2014; Garbus et al., 2018, 2020).

Recently, Pb exposure has been suggested to contribute to the declines observed in the Christiansø colony (Lam et al., 2020). Incubating eiders have shown remarkably large increases in Pb blood concentrations between the breeding seasons of 2017 and 2018 (Lam et al., 2020). In 2017, Pb blood levels were well below avian toxicological thresholds of concern (Lam et al., 2020). However, Pb blood concentrations increased by 361% from 2017 to 2018 and 470% as compared to eiders sampled in the northern Baltic Sea seven years prior (Fenstad et al., 2017). As eiders feed from the benthic food web they are exposed to shallowly buried lead ammunition and fishing tackle (Mateo and Kanstrup, 2019). Additionally, mussels and sediments sampled close to Christiansø and in neighboring areas in the Baltic Sea have reported high Pb concentrations indicating possible year-round exposure (HELCOM, 2019). These Pb concentrations are of the utmost concern as similar instances of highly elevated Pb blood concentrations have resulted in Pb poisoning and mortality in Baltic Sea eiders (Hollmén et al., 1998; Franson et al., 2000).

Lam et al. (2020) also found an equally concerning increase of Pb blood concentrations from early to late incubation observed in the same females from the Christiansø colony. Incubation is a critical life-history event where the brooding parent remains on her nest for 26 days and loses 23–46% of her body mass (Korschgen, 1977; Bolduc and Guillemette, 2003; Bustnes et al., 2012; Waltho and Coulson, 2015; Garbus et al., 2020). Eiders are considered largely capital breeders and therefore build up energy reserves prior to this incubation period (Waltho and Coulson, 2015). However, it has been shown that gizzard size is built up favorably over body condition at the wintering location to increase food processing capacity upon arrival at the breeding grounds (Laursen et al., 2019a). Additionally, observations of female eiders have reported reprieves from incubation to forage for food with increasing frequency as body condition deteriorates (Garbus, 2016). On Christiansø, incubating eiders have shown to enter the third stage of fasting, as marked by a shift from lipid to protein catabolism (Garbus et al., 2020). This indicates an increased likelihood of stored Pb deposits becoming remobilized along with bone and liver tissue to account for energy demands and egg production (Borch-Iohnsen et al., 1991; Alonso-Alvarez et al., 2002). Compounding upon this, eiders have shown signs of severe immunosuppression and oxidative stress towards the end of incubation (Hanssen et al., 2003, 2005). Taken together this is an indication that possible Pb exposure on Christiansø is posing a real threat to an already energetically stressed parent and, therefore, the viability of her offspring (Lam et al., 2020).

In the present study, we analyzed Pb isotopic ratios (Pb-IRs) (Pb 208/206, Pb 208/207, Pb 207/206 and, Pb 206/207) from incubating eiders on Christiansø, Denmark during the 2018 breeding season, both at early and late stages of this critical incubation period. As Pb isotopes and their ratios are distinctive between different Pb deposits, we are able to use them as a means of source identification (Scheuhammer and Templeton, 1998) which is vital for the preservation and protection of the eiders in the Baltic Sea. We additionally utilized multivariate statistics to determine the usefulness of Pb-IRs to evaluate the toxicokinetics of Pb during nesting. Our study is unique in that it reflects Pb-IRs from both lifelong and recent exposure due to remobilization of Pb from bone for egg production and energy demands during the incubation period (Hargreaves et al., 2011). We hypothesized that as more body mass was lost, we would observe a change in Pb-IR "fingerprints" as the source of exposure transitioned from dietary to stored Pb deposits in bone. In tandem, we expected dissimilar Pb-IRs between the early and late stages of incubation, further reflecting this transition in exposure.

2. Methods

2.1. Study area, design, and permissions

The present study was carried out on Frederiksø, the eastern-most island of Christiansø (Ertholmene), Denmark (55°19′N 15°11′E) in the central Baltic Sea (Fig. 1). The eiders arrive on Christiansø from their wintering location in the Wadden Sea between late-February and mid-April (Lyngs, 2014). Study plots were chosen and checked daily for the appearance of new nests with females pre-incubating 1–2 eggs. Danish Law protects all adults, chicks, eggs, and nests (Wildlife Management and Hunting Act; present LBK nr. 265 of 31/03/2019). Permission was obtained to handle eggs, nest material, and female eiders from the Nature Agency and the Danish Ministry of Environment and Food (NST-304-0008). Permit no. 2017-15-0201-01205 (case no. 2017-15-0201-01205/MABJE) was granted to handle incubating eiders and conduct

Fig. 1. Study area on the island of Christiansø (designated by red pin). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Source: ArcGis.

blood sampling from The National Committee for the Protection of Animals used for Scientific Purposes.

2.2. Field sampling

Replicate samples were obtained from 23 incubating eiders from early and late incubation during the 2018 breeding season. Day 4 was considered early incubation and occurred between April 15 – 20 and day 24 was considered late incubation and occurred between April 30 – May 5. Using a fishing rod ending with a nylon snare, the female eiders were taken from their nests, non-destructively sampled, and returned to their nests for continued incubation.

For more procedural details on blood sampling and body mass measurements refer to Samour (2006). A spring scale (Pesola Spring Balance) with 10 g accuracy was used to measure body mass and ~1 ml of blood was taken per 100 g of the bird's body mass from the brachialis vein under the wing. Whole blood (1 ml) was allocated for High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) analysis. Blood samples were transferred to a sterile 4 ml BD Vacutainer® Lithium Heparin tube, placed in a −20 °C freezer within four hours of sampling, and after field work was completed, transported to Trondheim, Norway where the samples were stored at −80 °C.

2.3. Lead concentration and isotopic analysis

The analysis of Pb and Pb 208/206, Pb 208/207, Pb 207/206 and, Pb 206/207 was performed at the Norwegian University of Science and Technology (NTNU). Prior to HR-ICP-MS analysis 500–1000 mg of whole blood was transferred to 15 ml acid-washed Teflon tubes specifically used in conjunction with the high-pressure microwave system, UltraClave (Milestone). Samples were digested in the UltraClave microwave (temperature up to 240 °C and pressure of 160 bar) for two hours in 2 ml of Scanpure nitric acid 50% (ultrapure grade, HNO_3). The samples were diluted to a volume of 24–27 ml with Milli-Q ion-exchanged water and placed in 15 ml vials for HR-ICP-MS analysis.

Reference materials samples (Seronorm trace elements whole blood L-2, lot 1206264, REF 210105) and three blanks were analyzed in conjunction with ^{208}Pb isotope to ensure quality of the analysis. Pb isotopic ratios were normalized versus certified National Institute of Standards and Technology (NIST) lead isotope standards (standard reference material (SRM) 981; 24.1442 ± 0.0057% for ^{206}Pb, 22.0833 ± 0.0027% for ^{207}Pb, and 52.3470 ± 0.0086% for ^{208}Pb). NIST standards were used to correct for instrumental mass discrimination/fractionation and verification of analytical procedure. All reference material was within the approved range for analyzed elements and results were corrected from blank samples. The lower limit of detection (LOD) was equivalent to the limit of quantification (LOQ) and was set to 3 times the standard deviation of the average blank measurement (Jian-Li et al., 2015) or in a situation where the instrumental detection limit (IDL) was larger, the LOD was set to the latter. Blood concentrations of Pb are presented in Lam et al. (2020) in µg/L and in the supplemental information (Table S1). Isotopic ratios were selected based on what is most utilized in the literature on avian species, inciting the need for both Pb 206/207 and the inverse 207/206, as well as the relative abundance of each isotope and the limitations of what our spectrometer can detect. Pb-IRs were provided by the spectrometer as ratios corrected for mass bias and reported with 95% confidence. Individual isotope quantities of ^{207}Pb, ^{204}Pb, and ^{206}Pb were not provided by the spectrometer.

2.4. Statistical analysis

All statistical analyses were performed with R (R Core Team, 2019), the free statistical computing and graphics software. The package 'ggplot', 'lmer4′, and 'MuMin' were used for graphical representation and linear mixed effect modeling of Pb-IRs, respectively. Correlograms were visually inspected to determine any grouping, patterns, or differences between days of incubation. Statistical differences between Pb-IRs (208/206, 208/207, 207/206, and its inverse 206/207) and body mass on day 4 and day 24 of incubation were tested using paired t-tests. To further test our hypothesis, linear mixed effect models were run with each Pb-IR as a response variable and body mass as a predictor variable.

The eider ID was included in the modeling as a random factor to account for the nested structure within the data. Initially, body mass and day were both included as predictor variables, however, issues of collinearity between body mass and day (vif = 25.249) forced the exclusion of one variable. In conjunction with our hypothesis, body mass was kept in the mixed modeling to address the stress of incubation in relation to source identification. In situations where mass was significant in the mixed modeling, further correlations between residuals from day 4 and day 24 were quantified using Pearson's product-moment coefficients. This enables analysis of additional Pb-IR variation from early to late incubation that was not accounted for by body mass and therefore provides new information concerning the usefulness of Pb-IRs as a means of source identification in a critical life history event. Pb-IRs were log-transformed prior to all statistical testing to improve normalcy of the dataset. Diagnostic plots in R (Residuals vs. Fitted, Normal QQ, Scale-Location, and Residuals vs. Leverage plot) were used to validate that all assumptions for mixed effect modeling were met.

To compare our results to regional Pb-IR values, Pb 208/206 and 206/207 were selected as they are the most extensively researched ratio for Pb source identification in avian species (Scheuhammer and Templeton, 1998; Madry et al., 2015; Binkowski et al., 2016). There were no statistical differences in mean Pb-IRs from day 4 to day 24 of incubation for Pb 208/206 and 206/207 (Table 1) and were therefore combined in the following analysis. To check for possible groupings or patterns amongst Pb blood concentrations, individuals were divided by Pb blood levels of 0–200 µg/kg ww, 200–500 µg/kg ww, and > 500 µg/kg ww. This is representative of toxicological thresholds for subclinical (200–500 µg/kg ww) and clinical (> 500 µg/kg ww) Pb poisoning. As environmental samples on Christiansø were not obtained during the 2018 breeding season, reports of Pb-IR values from proximal countries (Poland, Denmark, Germany, and Sweden) were instead used as a reference of possible sources. However, as available literature only reports singular values for Pb-IRs, statistical testing between Christiansø eider blood and potential sources was not possible. Instead, graphical representation was visually inspected to analyze clustering and trends between the Pb-IRs in the present study and those reported in current literature.

3. Results

3.1. Body mass and Pb-IRs

The mean ± SD, median and range of body mass (g) and Pb 208/206, 208/207, 207/206, 206/207 are summarized in Table 1. Body mass ± SD was significantly higher on day 4 (2181.39 ± 29.87) of the incubation period than on day 24 (1553.60 ± 32.12) ($t = 26.02$, $p < 0.001$). Pb-IRs increased from day 4 to day 24 of incubation in the following order: Pb 207/206 > 206/207 > 208/206 > 208/207. Although Pb 208/207 showed a significant difference between day 4 and day 24 ($t = -4.32$, $p < 0.001$) the difference in means was 0.49% (Table 1) and correlograms of Pb 208/206 and 208/207, Pb 208/207 and 206/207, and Pb 208/206 and 208/207 showed no apparent differences between day 4 and day 24 of the incubation period (Fig. 2).

3.2. Linear mixed-effect modeling

Body mass was a significant and negative predictor variable for Pb 208/207 ($t = 5.65$, $p < 0.001$). Body mass also showed a slightly significant negative effect on Pb 206/207 ($t = 2.09$, $p = 0.29$) and had no effect on Pb 208/206 (Table 2). However, conditional R^2 values were larger than marginal R^2 values by 85.45% and 78.81%, for models Pb 208/206 and 208/207, respectively (Table 2). This indicates the random factor accounts for most of the variation in the dataset. Analysis between residuals from models Pb 208/207 and 206/207 showed a strong correlation between day 4 and day 24 (208/207: $r = -0.956$, $p < 0.001$, 206/207: $r = -0.835$, $p < 0.001$) further indicating that

Table 1
Mass (g) and Pb isotope ratios given as Mean ± SD, median and range in incubating eiders on Christiansø, Denmark during the breeding season of 2018 for day 4 (n = 25) and day 24 (n = 23) of incubation.

	Mass #		Pb 208/207 $		Pb 208/206		Pb 207/206		Pb 206/207	
	Day 4	Day 24	Day 4	Day 24	Day 4	Day 24	Day 4	Day 24	Day 4	Day 24
Mean ± SD	2181.39 ± 29.87	1553.60 ± 32.12	2.4584 ± 0.0228	2.4700 ± 0.0330	2.0768 ± 0.0193	2.0778 ± 0.0228	0.8447 ± 0.0130	0.8412 ± 0.0154	1.1841 ± 0.0184	1.1891 ± 0.0219
Median	2205	1575	2.4522	2.4598	2.0741	2.0744	0.8458	0.8433	1.1821	1.1857
Range	1993–2475	1225–1865	2.4210–2.5184	2.4292–2.5522	2.0318–2.1221	2.0329–2.1628	0.8173–0.8704	0.8122–0.8762	1.1487–1.2232	1.1411–1.2311

#: significantly higher body mass on day 4 compared to day 24 ($df = 22$, $t = 26.02$, $p < 0.001$).
$: significantly different Pb-IR on day 4 compared to day 24 (208/207: $df = 22$, $t = -4.32$, $p < 0.001$).

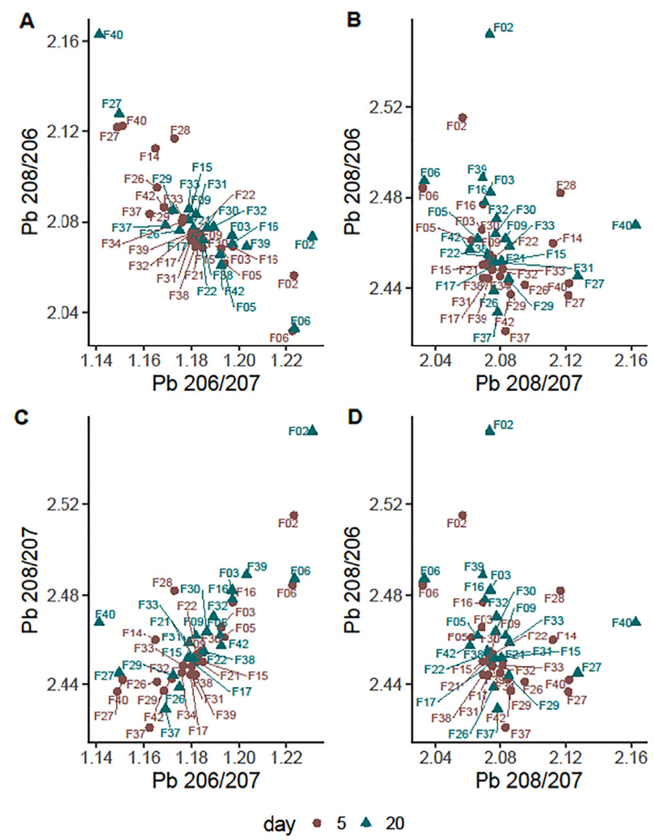

Fig. 2. Correlogram of Pb isotopic ratios: (A) 208/206 and 206/27, (B) 208/206 and 208/207, (C) 208/207 and 206/207, and (D) 208/206 and 208/207 in incubating eider on Christiansø, Denmark during the breeding season of 2018. The dark red circles represent day 4 while the green triangles represent day 24. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the random factor accounts for most of the variation in Pb 208/207 and 206/207 (Fig. 3).

3.3. Regional Pb-IR values

Separating Pb-IRs by Pb blood levels of 0–200 µg/kg ww, 200–500 µg/kg ww, and > 500 µg/kg ww showed distinct clustering of individuals with Pb blood levels > 500 µg/kg ww at approx. 1.15 and approx. 2.12 for 206/207 and 208/206, respectively (Fig. 4). Individuals with Pb blood levels between 0 and 500 µg/kg ww showed a general negative liner trend with no apparent differences. Individuals with Pb blood concentrations > 500 µg/kg ww showed distinct Pb-IR values that were closely grouped with reported Pb-IR values of lead shot ammunition pellets available from Poland manufacturers described by Binkowski et al. (2016) (Fig. 4). However, individuals with Pb blood concentrations < 500 µg/kg ww did not show similarities to this lead ammunition. Furthermore, the Pb-IR values from European gasoline, European coal combustion (Novák et al., 2003), and natural Pb (Komárek et al., 2008) were dissimilarly grouped to the Pb-IRs reported in the Christiansø eider colony in the present study (Fig. 4).

4. Discussion

Pb has shown to be a persistent problem in the Baltic Sea (HELCOM, 2019) with numerous reports of Pb poisoning leading to the mortality of eiders (Hollmén et al., 1998; Franson et al., 2000; Lam et al., 2020). On Christiansø, Denmark during the breeding season of 2018, 48% of the sampled incubating eiders had Pb blood concentrations surpassing thresholds of subclinical poisoning, while 13% had levels surpassing thresholds of clinical poisoning (Lam et al., 2020). Furthermore, increases in Pb blood concentrations from the early to late stages of incubation incited the need for additional investigation into possible pollution sources as well as analysis of the toxicokinetics of Pb during this critical life-history event. As the eiders sampled in this study are representative of the Christiansø colony and therefore the larger population, it is predicted 25% of the eiders in the Baltic/Wadden Flyway population may be facing health risks posed by Pb pollution.

4.1. Comparison of body mass and Pb-IRs from early to late incubation

In the present study the range for all Pb-IRs in blood overlapped between day 4 and day 24 indicating exposure to an unchanged Pb source from the early to late stages of incubation. Although Pb 208/207 was significantly different between days of incubation the difference of means was only 0.47%. Furthermore, there were no significant differences between Pb 208/206, 207/206 and its inverse 206/207. A constant endogenous Pb source due to lack of feeding seems a logical conclusion as eiders are considered extreme capital breeders and remain on their nests for 26-days (Waltho and Coulson, 2015). However, recent reports by Garbus (2016) and Jaatinen et al. (2016) have found that eiders in poor body condition show no seasonal change in energy allocation and utilized local feeding to meet the energy demands of reproduction. Eiders are known to arrive on Christiansø 2–4 weeks prior to egg-laying (Lyngs, 2014) where they build body condition and fat reserves in preparation for reproduction (Waltho and Coulson, 2015; Laursen et al., 2019a, 2019b). Eider Pb exposure on the breeding grounds has previously been accounted to pre-nesting foraging, most commonly on blue mussels (Wilson et al., 2007, Lam et al., 2020). Mussel soft tissue has been documented to have Pb concentrations of 0.18 µg/g ww in locations surrounding Christiansø (HELCOM, 2019). Furthermore, the status of blue mussels, sediment, and fish liver in the same area has been termed “not good” by Helcom Indicators (HELCOM, 2019). While these are possible pollution sources, uptake and release of Pb in blue mussels has shown to be a slow process taking two to three years (Right et al., 1997), indicating that we would expect to see similar levels of Pb in 2017, which was not observed. However, Lam et al., (2020) reported significantly larger eider body mass in 2017 than 2018

Table 2
Mixed effect model for Pb 208/206, Pb 208/207, and Pb 206/207 as a function of body mass in incubating eiders on Christiansø, Denmark. Individual was included as a random factor. The summary table includes the predictor variables and coefficient values, the standard error (SE), t-values and the significance levels (p-values) of each coefficient. The significance level was set to $p = 0.05$. The asterisks, *,**,***, represent a p-value of < 0.05, < 0.01 and < 0.001 respectively. Marg R^2 and Cond. R^2 represent the marginal and conditional R squares.

Pb-IRs	Predictor Variables	Coeff. value	SE	t- value	p-value		Marg. R^2	Cond. R^2
Pb 208/206	Intercept	0.7364	0.0043	169.69	< 0.001	***	0.3%	85.75%
	Body mass	−0.0011	0.0012	0.91	0.352			
Pb 208/207	Intercept	0.8975	0.0018	504.57	< 0.001	***	10.38%	89.19%
	Body mass	−0.0049	0.0008	5.65	< 0.001	***		
Pb 206/207	Intercept	0.1662	0.0030	54.63	< 0.001	***	1.10%	87.07%
	Body mass	−0.0032	0.0015	2.09	0.029	*		

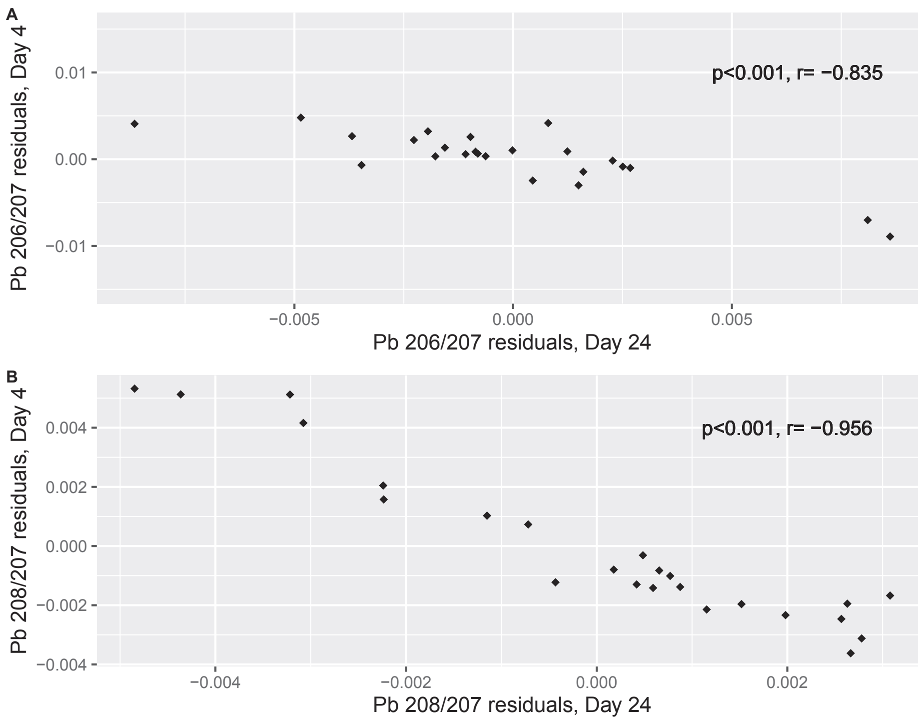

Fig. 3. Correlation plots of day 4 and day 24 residuals from models (A) Pb 206/207 as a function of body mass and (B) Pb 208/207 as a function of body mass. Pearson's product correlation coefficient (r) and p-value are represented in the upper left-hand corner of each graph.

at both stages of incubation. This could be indicative of a transition to income breeding resource allocation during the 2018 breeding season explaining the increase in Pb blood levels from 2017 to 2018 (Jaatinen et al., 2016). Additionally, this would also explain increased exposure to the same Pb source over the incubation period in 2018. However, internal regulation, remobilization, and reproductive physiology play a role in Pb blood concentrations and therefore must be considered as well.

4.2. Mechanisms of uptake

There was a significant relationship between body mass and Pb 208/207 as well as Pb 206/207 in the present study. This is a unique analysis and can therefore not be compared to other studies. However, in the same eiders, Lam et al. (2020) reported no correlation between body mass and Pb blood concentrations which was not to be expected as Pb is not stored in fat but instead in bone which is remobilized during incubation irrespective of body mass loss (Pain et al., 2019a; Lam et al., 2020). Supporting our hypothesis, the relationship between Pb-IRs and

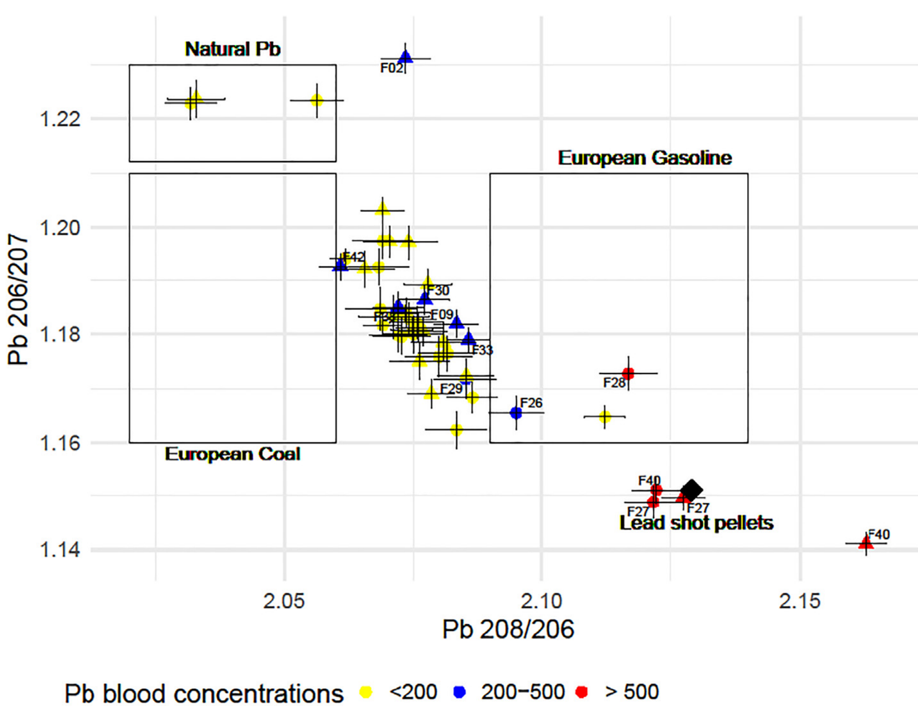

Fig. 4. Correlogram of Pb 208/206 and Pb 206/207 from blood of incubating eiders on Christiansø, Denmark during the 2018 breeding season. Yellow dots indicate eiders with Pb blood concentrations < 200 µg/kg ww, blue indicates 200–500 µg/kg ww, and red indicates > 500 µg/kg ww. Only eiders with blood concentrations > 200 µg/kg ww have been labelled with an ID. European gasoline, Central European coal, and natural Pb isotopic compositions were reported up by Komárek et al. (2008) and Novák et al. (2003). Lead shot ammunition were reported by Binkowski et al. (2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

body mass is instead an indication that as body mass is lost, the source of Pb in the eiders' blood changes. However, there is likely not a causal relationship between body mass and Pb-IRs as reflected by the large difference between conditional and marginal R^2 values (Løseth et al., 2019). The residuals of these models were also strongly correlated between days of incubation further supporting the significance of individual variation (Løseth et al., 2019). Body mass serves as an indication of the stress of breeding and reflects many additional confounding factors that cannot be included in the mixed models. This information taken together indicates other physiological factors, such as bone remobilization for egg production or liver metabolization for energy demands, explain the variation of Pb-IRs observed in the mixed models.

Increases in Pb blood concentrations during eider incubation have previously been accounted for by mobilization of stored Pb deposits from the medullary bone (Franson et al., 2005; Wilson et al., 2007). During egg-shell calcification the avian medullary bone shows rapid decreases in mineral content and size of trabeculae (Kerschnitzki et al., 2014). As Pb chemically mimics Ca it is easily incorporated into medullary bone and released during egg-shell formation (Finley and Dieter, 1978). Further, the blood-rich trabecular (spongy) bone, such as the medullary bone, has shown to have much higher Pb enrichment than compact bone (Cretacci and Parsons, 2010), resulting in a larger dose of Pb during egg-shell calcification.

In the present study, eiders were sampled on day 4 of incubation, during which eggs had already been laid. Therefore, at the beginning of incubation it can be assumed that Pb stored in the medullary bone has already been mobilized into the bloodstream possibly masking Pb-IR fingerprints from dietary exposure on the breeding grounds (Franson et al., 2005; Wilson et al., 2007; Kerschnitzki et al., 2014). If early incubation sampling had occurred prior to medullary bone mobilization it is possible the significant differences observed in Pb 208/207 would be reflected in Pb 208/206 and 207/206 as well as dietary intake was the primary source of Pb. However, given the similarities between Pb-IRs throughout incubation, it follows logically that after egg-laying nutritional demands of fasting caused continued metabolization of the medullary bone, resulting in further Pb release (Wilson et al., 2007). As mass is lost, due to fasting during incubation, Pb deposits are released from the medullary bone becoming the predominate source of Pb in the bloodstream and reflecting the significant differences observed in Pb 208/207 and the relationship between body mass and Pb 208/207 and 206/207. Pb bone accumulation spans a wide range (Ethier et al., 2007) although as the medullary bone has a rapid turnover rate in avian species (Kerschnitzki et al., 2014) it may reflect yearly Pb accumulation as opposed to lifelong Pb exposure as cortical bone would portray (Cretacci and Parsons, 2010). This would explain the large increase of Pb blood concentrations from the breeding seasons of 2017 to 2018 observed in the Christiansø colony (Lam et al., 2020). While these results confirm our hypothesis, it is impossible to determine the extent of Pb exposure from dietary intake versus bone mobilization and therefore the extend of Pb exposure on the breeding grounds versus the wintering grounds. Feces have been used as a non-destructive sampling method (Martinez-Haro et al., 2011; Berglund, 2018) and, if applied to future sampling on Christiansø could elucidate the contribution of dietary exposure during incubation to overall Pb blood concentrations.

4.3. Exposure from lead ammunition and health effects

There were dissimilar Pb-IRs between individuals with Pb blood concentrations $>$ 500 µg/kg ww and $<$ 500 µg/kg ww. This indicates different sources of exposure between individuals that were surpassing clinical thresholds of Pb poisoning and those that were not. Pb blood concentrations between 200 and 500 µg/kg ww are still well above avian toxicological thresholds and have been linked to genotoxicity, reduced reproduction, and liver and brain lesions (Pain et al., 2019a). Despite phasing out efforts from the EU, a large percentage of Pb in the Baltic Sea is a result of atmospheric deposition from mines, metal smelters, coal-fired power plants and the fertilizers from developing countries without the same regulations (HELCOM, 2010, 2019). Pb-IRs produced from European coal combustion and gasoline were both obtained from Novák et al. (2003), though were dissimilar to the Pb-IRs in Christiansø eider blood. This is expected as lead gasoline was phased out in the EU through the 1990s and completely banned in 2005 (EU-Commission et al., 1998). Likewise, natural Pb in the Baltic Sea is dissimilar to the patterns in the present study and therefore not a likely pollution source (Komárek et al., 2008).

However, in the present study, individuals with Pb blood concentrations $>$ 500 µg/kg ww had similar isotopic fingerprints to those in lead shot ammunition pellets available from Poland manufactures and reported by Binkowski et al. (2016). This is an indication that the most highly contaminated individuals are experiencing recent Pb poisoning from ingested Pb ammunition, either at the wintering grounds or the breeding grounds. Although without comparisons within and between blood samples and Pb ammunition, we cannot conclusively determine the cause of poisoning. However, Pb ammunition is a common and widespread cause of poisoning in avian species today (Haig et al., 2014; Pain et al., 2019b). Regardless of regulations limiting or banning Pb ammunition, one million waterfowl across Europe die each year from unintentional ingestion (Andreotti et al., 2018). Denmark has banned the use of Pb ammunition since 1996 (Kanstrup et al., 2016) and designated Christiansø as an EU Habitat Area and nature reserve (Danish Nature Policy, 2014). Despite these efforts, the results of this study show the importance of an EU-wide ban on Pb ammunition in protecting wildlife, especially migratory birds, against Pb poisoning. The United States and Canada have both implanted total bans on lead ammunition in waterfowl hunting leading to a 64% decline in annual Pb poisoning in Mallards (Anderson et al., 2000). Even with a total ban, lead ammunition can remain buried in the sediment for years (Mateo and Kanstrup, 2019) or degrade and enter the food web in smaller particles (Right et al., 1997). Pb has not shown to bioaccumulate in the Baltic Sea biota (Szefer, 1991) meaning that limiting ingestion would substantially lower concentrations found in wildlife (Helander et al., 2009). However, without unanimous legislation banning Pb ammunition, we cannot hope to preserve the health and success of the Baltic/Wadden Flyway population.

4.4. Limitations of Pb-IRs in source identification

Excluding the most highly contaminated individuals, there was a general negative linear relationship between Pb-IRs demonstrating a lack of distinct clustering of individuals. This is likely indicative of composite factors involved in Pb pollution and is common in locations that have no single major pollution source (Nakata et al., 2017). It is also possible there were limited differences in Pb isotope compositions of current pollution sources (Nakata et al., 2017). For example, paint, solder, and batteries have all been shown to have similar Pb-IRs making them difficult to differentiate (Mukal et al., 1993).

Limitations in data availability of Pb-IRs in and around the Baltic Sea also largely constricted the scope of this study. Future sampling of abiotic and biotic factors on Christiansø may broaden the capacity of Pb-IRs to identify pollution deposits if the sources are detectable, such as e.g. mussel supply. However, even with this sampling, as mentioned previously, the Baltic Sea accumulates Pb largely from atmospheric deposition creating additional challenges in source identification. As more than 50% of the world-wide production of Pb becomes atmospheric, reconstructing origin using Pb-IRs can be an impossible task (Weiss et al., 1999). This study highlights the limitations of Pb-IRs for source identification in an area such as the Baltic Sea that is known to accumulate Pb deposits from many different locations (HELCOM, 2019).

While Pb ammunition is a common avenue of exposure and poisoning in avian species, it does not fully explain the Pb levels documented in the breeding season of 2018 (Lam et al., 2020). Fecal sampling is needed to determine the extent of dietary Pb exposure on Christiansø and Pb-IRs from blue mussels and environmental field samples could be informative in determining Pb origin. Along with energetic constraints and disease (Christensen, 2008), Pb exposure is now predicted to contribute to the observed population declines in the Baltic/Wadden Flyway population (Lam et al., 2020). Most concerning is Pb's possible contribution to the mass mortality events observed in 2000 in the Dutch Wadden sea (Camphuysen et al., 2002) and in 2007 and 2015 on Christiansø (Garbus et al., 2018, 2019). While autopsies were performed on the dead eiders in 2015, Pb levels were not analyzed (Garbus et al., 2018, 2019). However, clinical signs of chronic Pb exposure as described by Haig et al. (2014) include lethargy and anorexia both of which were documented in 2015. Furthermore, Pb poisoning has shown to cause immunosuppression (Koivula and Eeva, 2010) which may have contributed to the large number of parasitic infections found in the dead eiders (Camphuysen et al., 2002; Garbus et al., 2019). Sustained or increased Pb exposure from continued hunting practices, fishery activity, and changing annual atmospheric deposition could all result in increased risk to this vulnerable colony. For example, with a warmer and drier climate, as is expected with climate change, Pb atmospheric flux and deposition have shown to be greater (Guo et al., 2019). This could explain some of the differences we see in the two years included this study and also serves as a warning for the possibility of greater Pb exposure in the future.

5. Conclusion

There were limited significant differences between Pb-IRs at the different stages of incubation indicating the likelihood of a largely constant Pb source during nesting. While exposure to Pb on the breeding ground is a probable contributing factor to Pb blood concentrations, medullary bone release of stored Pb deposits also occurs. Pb-IRs from individuals with blood concentrations $<$ 500 μg/kg ww did not show distinct clustering and were dissimilar to Pb-IRs from leaded gasoline and natural Pb from the Baltic Sea. However, individuals with blood concentrations $>$ 500 μg/kg ww showed similar clustering to lead ammunition, indicating the Baltic Sea's eiders are still suffering from Pb poisoning due to hunting practices. It is clear that during this energetically expensive time, eiders are dealing with an additional and possibly lethal stressor that has shown to cause population declines previously and may be contributing to multiple mass mortality instances. We urge additional environmental field sampling, fecal sampling, and continued biomonitoring of the Christiansø colony both for its own preservation and for the information it provides concerning pollution levels and impacts within the Baltic Sea.

CRediT authorship contribution statement

Molly McPartland: Writing - original draft, Writing - review & editing, Methodology, Visualization, Software, Data curation. **Svend-Erik Garbus:** Funding acquisition, Methodology, Writing - review & editing. **Syverin Lierhagen:** Resources, Formal analysis, Validation, Methodology. **Christian Sonne:** Project administration, Methodology, Funding acquisition, Writing - original draft, Writing - review & editing. **Åse Krøkje:** Project administration, Methodology, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

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

The study was approved by the Danish Nature Agency (SVANA). The chemical analysis was funded by the Norwegian University of Science and Technology (project no. 82505106). For funding of the fieldwork, we acknowledge The Danish Environmental Protection Agency, 15. Juni Fonden, Jægernes Naturfond, SKOV A/S (Glyngøre 7870 Roslev, Denmark) and BONUS BALTHEALTH that has received funding from BONUS (Art. 185), funded jointly by the EU, Innovation Fund Denmark (grants 286 6180-00001B and 6180-00002B), Forschungszentrum Jülich GmbH, German Federal Ministry of Education and Research (grant FKZ 03F0767A), Academy of Finland (grant 311966) and Swedish Foundation for Strategic Environmental Research (MISTRA). In addition, we are grateful to the people of Christiansø for their help during sampling and fieldwork.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105874.

References

Abadin, H., Ashizawa, A., Stevens, Y.W., Llados, F., Diamond, G., Sage, G., Citra, M., Quinones, A., Bosch, S., and Swarts, S. (2007). Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry (US), Atlanta, GA, 1st edition. 1–414 pp.

Alonso-Alvarez, C., Velando, A., Ferrer, M., Veira, J.A.R., 2002. Changes in plasma biochemistry and body mass during incubation in the Yellow-legged Gull. Waterbirds 25, 253–258.

Anderson, W.L., Havera, S.P., Zercher, B.W., 2000. Ingestion of lead and nontoxic shotgun pellets by ducks in the Mississippi Flyway. J. Wildl. Manag. 63, 848–857.

Andreotti, A., Guberti, V., Nardelli, R., Pirrello, S., Serra, L., Volponi, S., Green, R.E., 2018. Economic assessment of wild bird mortality induced by the use of lead gunshot in European wetlands. Sci. Total Environ. 610, 1505–1513.

Berglund, Å.M.M., 2018. Evaluating blood and excrement as bioindicators for metal accumulation in birds. Environ. Pollut. 233, 1198–1206.

Berny, P., Vilagines, L., Cugnasse, J.-M., Mastain, O., Chollet, J.-Y., Joncour, G., Razin, M., 2015. Vigilance Poision: Illegal poisoning and lead intoxication are the main factors affecting avian scavenger survival in the Pyrenees (France). Ecotoxicol. Environ. Saf. 118, 71–82.

Binkowski, Ł.J., Meissner, W., Trzeciak, M., Izevbekhai, K., Barker, J., 2016. Lead isotope ratio measurements as indicators for the source of lead poisoning in Mute swans (*Cygnus olor*) wintering in Puck Bay (northern Poland). Chemosphere 164, 436–442.

Bolduc, F., Guillemette, M., 2003. Human disturbance and nesting success of Common Eiders : Interaction between visitors and gulls. Biol. Conserv. 3207, 77–83.

Borch-Iohnsen, B., Holm, H., Jørgensen, A., Norheim, G., 1991. Seasonal siderosis in female eider nesting in Svalbard. J. Comp. Pathol. 104, 7–15.

Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Fenstad, A.A., Nordstad, T., Borgå, K., Gabrielsen, G.W., 2012. Temporal dynamics of circulating persistent organic pollutants in a fasting seabird under different environmental conditions. Environ. Sci. Technol. 46, 10287–10294.

Camphuysen, C.J., Berrevoets, C.M., Cremers, H.J.W.M., Dekinga, A., Dekker, R., Ens, B.J., Piersma, T., 2002. Mass mortality of common eiders (*Somateria mollissima*) in the Dutch Wadden Sea, winter 1999/2000: Starvation in a commercially exploited wetland of international importance. Biol. Conserv. 106, 303–317.

Clausen, K.K., Holm, T.E., Haugaard, L., Madsen, J., 2017. Crippling ratio: A novel approach to assess hunting-induced wounding of wild animals. Ecol. Ind. 80, 242–246.

Craighead, D., Bedrosian, B., 2008. Blood lead levels of common ravens with access to big-game offal. J. Wildl. Manage. 72, 240–245.

Cretacci, Y., Parsons, P.J., 2010. Localized accumulation of lead within and among bones from lead-dosed goats. Environ. Res. 110, 26–32.

Christensen. T.K., 2008. Factors affecting population size of Baltic common eiders Somateria mollissima. PhD thesis. Dept. of Wildlife Ecology and Biodiversity, NERI. National Environmental Research Institute, University of Aarhus, Denmark. 1–204 pp.

Christensen, T.K., Asferg, T., Madsen, A.B., Kahlert, J., Clausen, P., Laursen, K., Sunde, P., Haugaard, L., 2013. Jagttidsrevision 2014. Vurdering af jagtens bæredygtighed i forhold til gældende jagttider. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi. Videnskabelig rapport fra DCE – Nationalt Center for Miljø og Energi nr. 66. 108 pp. http://dce2.ai.dk/pub/SR66.pdf (accessed January 2016, in Danish).

Danish Nature Policy, 2014. Our shared nature. Danish Ministry of the Environment, Danish Nature Agency, 1–64 pp.

Desholm, M., Christensen, T.K., Ens, B., 2002. Status of the Baltic/Wadden Sea population of the Common Eider (*Somateria mollissima*). Wildfowl 53, 167–203.

Ekroos, J., Fox, A.D., Christensen, T.K., Petersen, I.K., Kilpi, M., Jónsson, J.E., Öst, M., 2012. Declines amongst breeding eider (*Somateria mollissima*) numbers in the Baltic / Wadden Sea flyway. Ornis Fennica 89, 81–90.

Elder, A., Nordberg, G., Kleinman, M., 2014. Routes of Exposure, Dose, and Toxicokinetics of Metals. In: Handbook on the Toxicology of Metals, 4th edition. Elsevier, Oxford, UK, pp. 45–70.

Ethier, A.L.M., Braune, B.M., Scheuhammer, A.M., Bond, D.E., 2007. Comparison of lead residues among avian bones. Environ. Pollut. 145, 915–919.

EU-Commission et al., 1998. EC of the European parliament and of the council of 13 October 1998 relating to the quality of petrol and diesel fuels and amending council 93/12/EEC (directive). Official Journal L, 350, 12.
Falandysz, J., Ichihashi, H., Szymczyk, K., Yamasaki, S., Mizera, T., 2001. Metallic elements and metal poisoning among White-tailed Sea Eagles from the Baltic south coast. Mar. Pollut. Bull. 42, 1190–1193.
Fenstad, A.A., Bustnes, J.O., Lierhagen, S., Gabrielsen, K.M., Öst, M., Jaatinen, K., Krøkje, Å., 2017. Blood and feather concentrations of toxic elements in a Baltic and an Arctic seabird population. Mar. Pollut. Bull. 114, 1152–1158.
Finley, M.T., Dieter, M.P., 1978. Influence of laying on lead accumulation in bone of mallard ducks. J. Toxicol. Environ. Health 4, 123–129.
Franson, J.C., Hollmén, T., Poppenga, R.H., Hario, M., Kilpi, M., 2000. Metals and trace elements in tissues of common eiders (*Somateria mollissima*) from the Finnish archipelago. Ornis Fennica 77, 57–63.
Franson, J.C., Hollmén, T., Poppenga, R.H., Hario, M., Kilpi, M., Smith, M.R., 2005. Selected trace elements and organochlorines: some gindings in blood and eggs of nesting common eiders (*Somateria Mollissima*) from Finland. Environ. Toxicol. Chem. 19, 1340–1347.
Franzen-Klein, D., McRuer, D., Slabe, V.A., Katzner, T., 2018. The use of lead isotope analysis to identify potential sources of lead toxicosis in a juvenile bald eagle (*Haliaeetus leucocephalus*) with ventricular foregin bodies. J. Avian Med. Surg. 32, 34–39.
Garbus, S.E., Christensen, J.P., Buchmann, K., Jessen, T.B., Lyngs, P., Jacobsen, M.L., Sonne, C., 2019. Haematology, blood biochemistry, parasites and pathology of common eider *(Somateria mollisima*) males during a mortality event in the Baltic. Sci. Total Environ. 683, 559–567.
Garbus, S.E., Christensen, J.P., Lyngs, P., Eulaers, I., Mosbech, A., Dietz, R., Sonne, C., 2020. Changes in blood biochemistry of incubating Baltic Common Eiders (*Somateria mollisima*). J. Ornithol. 161, 25–33.
Garbus, S.E., Lyngs, P., Christensen, J.P., Buchmann, K., Eulaers, I., Mosbech, A., Sonne, C., 2018. Common eider (*Somateria Mollissima*) body condition and parasitic load during a mortality event in the Baltic proper. Avian Biol. Res. 11, 167–172.
Garbus, S.E., 2016. Health, Behaviour, Egg Failure and Starvation-mortality of Incubating Common Eiders (*Somateria mollissima*) at Christiansø, Central Baltic Sea. Univeristy of Copenhagen. Masters thesis. 1–73 pp.
Guo, B., Wang, J., Lin, C., He, M., Ouyang, W., 2019. Anthropogenic and lithogenic fluxes of atmospheric lead deposition over the past 3600 years from a peat bog, Changbai Mountains, China. Chemosphere 227, 225–236.
Hanssen, S.A., Hasselquist, D., Folstad, I., Erikstad, K.E., 2005. Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. Proc. Roy. Soc. B: Biol. Sci. 272, 1039–1046.
Hanssen, S.A., Folstad, I., Erikstad, K.E., 2003. Reduced immunocompetence and cost of reproduction in common eiders. Oecologia 136, 457–464.
Haig, S.M., D'Elia, J., Eagles-Smith, C., Fair, J.M., Gervais, J., Herring, G., Schulz, J.H., 2014. The persistent problem of lead poisoning in birds from ammunition and fishing tackle. The Condor 116, 408–428.
Hargreaves, A.L., Whiteside, D.P., Gilchrist, G., 2011. Concentrations of 17 elements, including mercury, in the tissues, food and abiotic environment of Arctic shorebirds. Sci. Total Environ. 409, 3757–3770.
Helander, B., Axelsson, J., Borg, H., Holm, K., Bignert, A., 2009. Ingestion of lead from ammunition and lead concentrations in white-tailed sea eagles (*Haliaeetus albicilla*) in Sweden. Sci. Total Environ. 407, 5555–5563.
HELCOM, 2010. Hazardous substances in the Baltic Sea – an integrated thematic assessment of hazardous substances in the Baltic Sea. Balt Sea Environ. Proc. 120B, 1–94.
HELCOM, 2018. Inputs of hazardous substances to the Baltic Sea. Balt Sea Environ. Proc No. PCL6. 1-26 pp.
HELCOM, 2019. HELCOM Indicators. HTML: http://www.helcom.fi/baltic-sea-trends/indicators/metals/results-and-confidence/.
Hollmén, T., Franson, J.C., Poppenga, R.H., Hario, M., Kilpi, M., 1998. Lead poisoning and trace elements in common eiders *Somateria mollissima* from Finland. Wildlife Biol. 4, 193–203.
Jaatinen, K., Öst, M., Hobson, K.A., 2016. State-dependent capital and income breeding: A novel approach to evaluating individual strategies with stable isotopes. Front. Zool. 13, 1–28.
Jian-Li, H.E., Tao, P., Jie, X., Han-Hui, D., Dong-Dong, C., Zhen-Feng, Y., Chun-Lin, F., Cun, L., 2015. Determination of 20 perfluorinated compounds in animal liver by HPLC- MS/MS. Chi J. Analyt. Chem. 43, 40–48.
Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicol. 7, 60–72.
Kanstrup, N., Thomas, V.G., Krone, O., Gremse, C., 2016. The transition to non-lead rifle ammunition in Denmark: National obligations and policy considerations. Ambio 45, 621–628.
Kerschnitzki, M., Zander, T., Zaslansky, P., Fratzl, P., Shahar, R., Wagermaier, W., 2014. Rapid alterations of avian medullary bone material during the daily egg-laying cycle. Bone 69, 109–117.
Koivula, M.J., Eeva, T., 2010. Metal-related oxidative stress in birds. Environ. Pollut. 158, 2359–2370.
Komárek, M., Ettler, V., Chrastný, V., Mihaljevič, M., 2008. Lead isotopes in environmental sciences: A review. Environ. Int. 34, 562–577.
Korschgen, C.E., 1977. Breeding Stress of Female Eiders in Maine. J. Wildl. Manage. 41, 360–373.
Lam, S.S., McPartland, M., Noori, B., Garbus, S.E., Lierhagen, S., Lyngs, P., Dietz, R., Therkildsen, O.R., Christensen, T.K., Tjørnløv, R.S., Kanstrup, N., Fox, A.D., Sørensen, I.H., Arzel, C., Krøkje, Å., Sonne, C., 2020. Lead concentrations in blood from incubating common eiders (*Somateria mollissima*) in the Baltic Sea. Environ. Int. 137, 105582.
Larsson, K., Hajdu, S., Kilpi, M., Larsson, R., Leito, A., Lyngs, P., 2014. Effects of an extensive *Prymnesium polylepis* bloom on breeding eiders in the Baltic Sea. J. Sea Res. 88, 21–28.
Laursen, K., Møller, A.P., 2014. Long-term changes in nutrients and mussel stocks are related to numbers of breeding eiders *Somateria mollissima* at a large baltic colony. PLoS ONE 9, e95851.
Laursen, K., Møller, A.P., Haugaard, L., Öst, M., Vainio, J., 2019a. Allocation of body reserves during winter in eider *Somateria mollissima* as preparation for spring migration and reproduction. J. Sea Res. 144, 49–56.
Laursen, K., Møller, A.P., Öst, M., 2019b. Body condition of Eiders at Danish wintering grounds and at pre – breeding grounds in Åland. J. Ornithol. 160, 239–248.
Legagneux, P., Suffice, P., Messier, J.S., Lelievre, F., Tremblay, J.A., Maisonneuve, C., Bêty, J., 2014. High risk of lead contamination for scavengers in an area with high moose hunting success. PLoS ONE 9, 1–8.
Løseth, M.E., Flo, J., Sonne, C., Krogh, A.K.H., Nygård, T., Bustnes, J.O., Jaspers, V.L.B., 2019. The influence of natural variation and organohalogenated contaminants on physiological parameters in white-tailed eagle (*Haliaeetus albicilla)* nestlings from Norway. Environ. Res. 177, 108586.
Lyngs, P., 2014. Christianso Fieldstation http://www.chnf.dk/aktuelt/edf14/edfugl14.php.
Madry, M.M., Kraemer, T., Kupper, J., Naegeli, H., Jenny, H., Jenni, L., Jenny, D., 2015. Excessive lead burden among golden eagles in the Swiss Alps. Environ. Res. Lett. 10, 1–9.
Mallory, M.L., Braune, B.M., Wayland, M., Gilchrist, H.G., Dickson, D.L., 2004. Contaminants in common eiders (*Somateria mollissima*) of the Canadian Arctic. Environ. Rev. 12, 197–218.
Martinez-Haro, M., Taggart, M.A., Martín-Doimeadiós, R.R.C., Green, A.J., Mateo, R., 2011. Identifying sources of Pb exposure in waterbirds and effects on porphyrin metabolism using noninvasive fecal sampling. Environ. Sci. Technol. 45, 6153–6159.
Mason, L.H., Harp, J.P., Han, D.Y., 2014. Pb neurotoxicity: Neuropsychological effects of lead toxicity. Biomed Res. Int. 2014, 1–8.
Mateo, R., Kanstrup, N., 2019. Regulations on lead ammunition adopted in Europe and evidence of compliance. Ambio 48, 989–998.
Mukal, H., Furuta, N., Fujii, T., Ambe, Y., Sakamoto, K., Hashimoto, Y., 1993. Characterization of sources of lead in the urban air of asia using ratios of stable lead isotopes. Environ. Sci. Technol. 27, 1347–1356.
Nakata, H., Nakayama, S.M.M., Oroszlany, B., Ikenaka, Y., Mizukawa, H., Tanaka, K., Ishizuka, M., 2017. Monitoring lead (Pb) pollution and identifying pb pollution sources in Japan using stable Pb isotope analysis with kidneys of wild rats. Int. J. Environ. Res. Public Health 14, 1–13.
Noer, H., 1991. Distributions and movements of eider (*Somateria mollissima*) populations wintering in Danish waters, analysed from ringing recoveries. Danish Rev. Game Biol. 14, 1–32.
Novák, M., Emmanuel, S., Vile, M.A., Erel, Y., Véron, A., Pačes, T., Hovorka, J., 2003. Origin of lead in eight central European peat bogs determined from isotope ratios, strengths, and operation times of regional pollution sources. Environ. Sci. Technol. 37, 437–445.
Pain, D.J., Dickie, I., Green, R.E., Kanstrup, N., Cromie, R., 2019a. Wildlife, human and environmental costs of using lead ammunition: An economic review and analysis. Ambio 48, 969–988.
Pain, D.J., Mateo, R., Green, R.E., 2019b. Effects of lead from ammunition on birds and other wildlife: A review and update. Ambio 48, 935–953.
R Core Team (v.3.6.1, 2019). R: A Language and Environment for Statistical Computing. R Foun- dation for Statistical Computing, Vienna, Austria.
Right, F., Johansen, P., Asmund, G., 1997. Uptake and release of lead and zinc by blue mussels. Experience from transplantation experiments in Greenland. Mar. Pollut. Bull. 34, 805–815.
Samour, J., 2006. Diagnostic value of hematology. In: Harrison, J. G., and Lightfoot, T. editors. Clinical Avian Medicine Volume 2. Florida Spix Publishing, 587–610 pp.
Scheuhammer, A.M., Templeton, D.M., 1998. Use of stable isotope ratios to distinguish sources of lead exposure in wild birds. Ecotoxicology 7, 37–42.
Skerfving, S., Bergdahl, I., 2014. Lead. In Handbook on the Toxicology of Metals, 4th edition. Elsevier, Oxford, UK, pp. 911–967.
Slabe, V.A., Anderson, J.T., Cooper, J., Brown, B., Ortiz, P., Buchweitz, J., Katzner, T., 2019. Lead in piscivorous raptors during breeding season in the Chesapeake Bay region of Maryland and Virginia, USA. Environ. Toxicol. Chem. 38, 862–871.
Slabe, V.A., Anderson, J.T., Cooper, J., Miller, T.A., Brown, B., Wrona, A., Katzner, T., 2020. Feeding ecology drives lead exposure of facultative and obligate avian scavengers in the eastern United States. Environ. Toxicol. Chem. 39, 882–892.
Szefer, P., 1991. Interphase and trophic relationships of metals in a southern Baltic ecosystem. Sci. Total Environ. 101, 201–215.
Veerle, J., Tom, D., Rianne, P., Lieven, B., Ronny, B., Marcel, E., 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, *Parus major*. J. Environ. Monit. 6, 356–360.
Waltho, C., Coulson, J., 2015. The Common Eider, 1st edition. Bloomsbury Publishing, London, UK, pp. 1–276.
Wayland, M., Garcia-Fernandez, A.J., Neugebauer, E., Gilchrist, H.G., 2001. Concentrations of cadmium, mercury and selenium in blood, liver and kidney of common eider ducks from the Canadian arctic. Environ. Monit. Assess. 71, 255–267.
Weiss, D., Shotyk, W., Appleby, P.G., Kramers, J.D., Cheburkin, A.K., 1999. Atmospheric Pb deposition since the industrial revolution recorded by five Swiss peat profiles: Enrichment factors, fluxes, isotopic composition, and sources. Environ. Sci. Technol. 33, 1340–1352.
Wilson, H.M., Flint, P.L., Powell, A.N., 2007. Coupling contaminants with demography: Effects of lead and selenium in Pacific eiders. Environ. Toxicol. Chem. 26, 1410–1417.
Zalewska, T., Woroń, J., Danowska, B., Suplińska, M., 2015. Temporal changes in Hg, Pb, Cd and Zn environmental concentrations in the southern Baltic Sea sediments dated with 210Pb method. Oceanologia 57, 32–43.