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Lead concentrations in blood from incubating common eiders (*Somateria mollissima*) in the Baltic Sea

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ABSTRACT

Here we investigate if lead may be a contributing factor to the observed population decline in a Baltic colony of incubating eiders (*Somateria mollissima*). Body mass and blood samples were obtained from 50 incubating female eiders at the Baltic breeding colony on Christiansø during spring 2017 ($n = 27$) and 2018 ($n = 23$). All the females were sampled twice during early (day 4) and late (day 24) incubation. The full blood was analysed for lead to investigate if the concentrations exceeded toxic thresholds or changed over the incubation period due to remobilisation from bones and liver tissue. Body mass, hatch date and number of chicks were also analysed with respect to lead concentrations. The body mass (mean ± SD g) increased significantly in the order: day 24 in 2018 (1561 ± 154 g) < day 24 in 2017 (1618 ± 156 g) < day 4 in 2018 (2183 ± 140 g) < day 4 in 2017 (2359 ± 167 g) (all $p < 0.001$). The lead concentrations increased significantly in the opposite order i.e. day 4 in 2017 (41.7 ± 67.1 µg/L) < day 24 in 2017 (55.4 ± 66.8 µg/L) < day 4 in 2018 (177 ± 196 µg/L) < day 24 in 2018 (258 ± 243) (all $p < 0.001$). From day 4 to 24, the eider females had a 1.33-fold increase in blood lead concentrations in 2017 and a 1.46-fold increase in 2018. Three of the birds (13%) sampled in 2018 had lead concentrations that exceeded concentrations of clinical poisoning (500 µg/L) and eleven (48%) had concentrations that exceeded the threshold for subclinical poisoning (200 µg/L). In 2017, none of the birds exceeded the high toxic threshold of clinical poisoning while only one (4%) exceeded the lower threshold for subclinical poisoning. Three of the birds (6%) sampled in 2018 had lead concentrations that exceeded those of clinical poisoning while 12 birds (24%) resampled in both years exceeded the threshold for subclinical poisoning. In addition, lead concentrations and body mass on day 4 affected hatch date positively in 2018 (both $p < 0.03$) but not in 2017. These results show that bioavailable lead in bone and liver tissue pose a threat to the health of about 25% of the incubating eiders sampled. This is particularly critical because eiders are largely capital breeding which means that incubating eiders are in an energetically stressed state. The origin of lead in incubating eiders in the Christiansø colony is unknown and it remains an urgent priority to establish the source, prevalence and mechanism for uptake. The increase in lead from day 4 to day 24 is due to bone and liver remobilization; however, the additional lead source(s) on the breeding grounds needs to be identified. Continued investigations should determine the origin, uptake mechanisms and degree of exposure to lead for individual birds. Such research should include necropsies, x-ray, lead isotope and stable C and N isotope analyses to find the

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lead sources(s) in the course of the annual cycle and how it may affect the population dynamics of the Christiansø colony which reflects the ecology of the Baltic eiders being suitable for biomonitoring the overall flyway.

1. Introduction

Lead is a toxic heavy metal absorbed by the gastro-intestinal tract with an efficiency of ca. 10%, concentrated in soft tissue such as liver and brain (Abadin et al., 2007; Nordberg et al., 2014; Skerfving and Bergdahl, 2014) and accumulated in bone as a calcium replacement (Ethier et al., 2007; Goyer, 1997). Lead also causes lesions in the central nervous system by interfering with the regulatory actions of calcium through oxidative stress, cytotoxicity, as well as liver and renal damage (Hollmén et al., 1998; Sanders et al., 2009). The main lead excretion route is through the kidneys with a half-life of 30–40 days in blood and soft tissue (Nordberg et al., 2014).

In the Baltic Sea, atmospheric lead deposition has declined significantly over the past 30 years due to phasing out efforts. Since year 2000, concentrations have stabilised at a low level as reflected in marine sediments and water (Bartnicki et al., 2017; Leipe et al., 2013; Zalewska et al., 2015). Despite these temporal declines, lead remains a major concern with higher than background concentrations measured in certain areas of the Baltic Sea (HELCOM, 2010; Leipe et al., 2013; Zalewska et al., 2015). Part of this lead originates from hunters using leaded ammunition, resulting in locally elevated environmental lead concentrations (Pain et al., 2019). This has caused poisoning of waterbirds and secondary poisoning of raptors, scavengers and carnivores (Pain et al., 2019). For waterbirds such as the common (*Somateria mollissima*) and spectacled eiders (*S. fischeri*), lead exposure is predominantly through ingestion but also by wounding (Flint et al., 1997; Franson et al., 1995; Mallory et al., 2004). Unexplained mortality events of common eiders during the breeding season have been reported from five different incidents in North America (Madin, 2009), one in the Dutch Wadden Sea (Camphuysen et al., 2002) and three in the Baltic Sea (Garbus et al., 2018a, 2019), but it remains unknown if any of these are caused by lead poisoning.

The common eider is the largest and heaviest sea duck in the Northern Hemisphere. The Danish eiders are part of the biological subunit of the Baltic/Wadden Sea Flyway population, which consists of an estimated 900,000 birds (Christensen et al., 2013; Waltho and Coulson, 2015). The Flyway is comprised of breeding populations from Finland, Sweden, Denmark, southern Norway and Germany. Between 1990 and 2000 the number of Flyway wintering eiders in Danish waters has decreased from 800,000 to 380,000 birds and overall, the Flyway population has experienced decreases from 1.2 mill to around 760,000 individuals (Delaney and Scott, 2002, 2006).

The Christiansø colony is a representative unit of the Baltic Flyway population located in the Southern part of the Baltic Proper and is the second largest colony in Denmark (Christensen and Bregnballe, 2011). The local breeding population numbered 1445 and 1750 nesting

Fig. 1. Study area at Christiansø in the Baltic Sea.

females in 2007 and 2015, respectively. The eiders on Christiansø are migrating between wintering grounds at the western part of the Baltic Sea south to the Dutch part of the Wadden Sea. They return to the summer breeding grounds from late February to early April (Lyngs, 2014). Reflecting the Flyway population, the Christiansø colony has also experienced population declines in the past decades (Lyngs, 2014). Starvation due to fishery activities and food web changes affecting blue mussel (*Mytilus edulis*) stocks are potential causes of the population decline but does not fully explain the mortality of birds in good body condition (Cramp and Simmons, 1977; Laursen and Møller, 2014; Madin, 2009). In addition, high prevalence of acanthocephalan parasites has also been associated with mortality in eiders (Camphuysen et al., 2002; Garbus et al., 2018a, 2019).

As lead is known to be a prevalent toxic element in water birds (De Francisco et al., 2003; Pain et al., 2019), we explored lead concentrations in incubating female eiders in the colony of Christiansø. Due to previous reports of lead poisoning from pellet ingestion in the Baltic (Falandysz et al., 2001; Pain et al., 2019), we investigated if lead may be a contributing factor to the observed population decline in the Christiansø colony though multivariate statistical analyses. We hypothesize that body mass would decrease due to starvation and egg production and that lead exposure would increase due to bone and organ (i.e. liver tissue) remobilisation during the incubation period.

2. Materials and methods

2.1. Study area, design and permissions

The study was performed on Christiansø island, northeast of Bornholm in the Central Baltic Sea (55°19′N; 15°11′E; Fig. 1) during two incubation periods from 5 April 2017 to 15 May 2018. The colony hosts 1500 nesting females and outside of the breeding season, the eiders migrate through Danish waters to the Wadden Sea, returning from late-February to mid-April (Lyngs, 2014). Three study plots were selected on Frederiksø and the north-eastern and southern part of Christiansø, having approximately 1200 incubating eiders. The areas were inspected daily to locate new nests, with 1–2 pre-incubated eggs, for blood sampling of the nesting females. Nests, adults, eggs and chicks of eiders are protected according to Danish law (Wildlife Management and Hunting Act; present LBK nr. 265 of 31/03/2019) and a permission to handle female eiders was granted by the Nature Agency and the Danish Ministry of Environment and Food (NST-304-0008). Blood samples and handling of incubating females was conducted under the permit no. 2017-15-0201-01205 (case no. 2017-15-0201-01205/MABJE) from The National Committee for the Protection of Animals used for Scientific Purposes.

2.2. Blood sampling and body mass

Blood samples and information on body mass were collected from 50 birds representing ca. 3% of the total colony. The 50 birds were all sampled twice i.e. early and late incubation. The sampling took place in April (day 4 = early incubation) and May (day 24 = late incubation) in both 2017 (n = 27) and 2018 (n = 23) (Table 1). In 2017, samples were collected during April 5–10 and again during April 25–30 while in 2018 samples were collected during April 15–20 and again during April 30 to May 5. Blood sampling was performed in the brachial vein using a 23/25G needle. All samples were transferred to a sterile 4 ml BD Vacutainer® Lithium Heparin tube and frozen at − 20 °C until chemical analysis. To handle the birds as briefly as possible, only body mass (i.e. not full biometrics) was recorded using a Pesola Spring balance with 10 g accuracy. For each eider, the number of hatched chicks was recorded.

2.3. Chemical analyses

Whole blood samples were analyzed for concentrations of elements at the Department of Chemistry, NTNU using High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS). Before the beginning of the HR-ICP-MS analyses, between 500 and 1000 mg of whole blood was transferred to acid washed 15 ml Teflon tubes designed for UltraClave and 2 ml of Scanpure nitric acid 50% (HNO3) was added to each vial. Samples were then digested for 2 h in an UltraClave (Milestone), a high pressure microwave system reaching up to temperatures of 240 °C and a pressure of 160 bar. The samples were then diluted with Milli-Q water to a volume of 24–27 ml and transferred to 15 ml vials for HR-ICP-MS analysis. HR-ICP-MS was carried out using a Thermo Finnigen model Element 2 instrument. To ensure the quality of the analysis, three reference material samples (Seronorm trace elements whole blood L-2, lot 1206264, REF 210105) were also analysed including isotope Pb^{208}. Three blanks were added to monitor contamination during each analysis. The reference material was within the approved range for all analyzed elements. The lower limit of detection (LOD) was set to the highest value of either the calculated instrument detection limit (IDL) or three times the standard deviation of the blanks. Calculations of IDL were made by analyzing solutions containing decreasing concentrations of each element. The concentration resulting in a relative standard deviation of 25% (n = 3 scans) was chosen as the IDL with baseline corrections. The concentrations of lead, calcium and nickel are reported as µg/L. These three element were analysed as they may mimic each other.

2.4. Statistical analyses

All multivariate statistical analyses were preformed using the statistical computing program R (R core Team, 2019). R packages ggplot, MuMin, Lmer4, and corrplot were used for visual representation, linear mixed models and correlation analyses, respectively. Mixed model linear regressions were used to test for differences in lead concentrations and mass between sampling days and years and their interaction.

Table 1
Body mass (g) and lead concentrations (µg/L) in blood of incubating eiders collected in 2017 (n = 27) and 2018 (n = 23) at Christiansø in the Baltic proper.

	2017				**2018**			
	Day 4		*Day 24*		*Day 4*		*Day 24*	
	Mass	Lead	Mass	Lead	Mass	Lead	Mass	Lead
Mean	2359[#,¥]	41.7[§,€]	1618[#,¥]	55.4[§,€]	2183[#,¥]	177[§,€]	1561[#,¥]	258[§,€]
SD	167	67.1	156	66.8	140	196	154	243
Range	2050–2665	10.3–355	1445–2105	8.34–366	1960–2410	45.2–875	1225–1865	30.1–1050

[#] : significantly higher body mass at day 4 compared to day 24 (both $p < 0.001$).
[¥] : Significantly higher body mass in 2017 compared to 2018 (both $p < 0.001$).
[§] : significantly higher lead concentrations at day 24 compared to day 4 (both $p < 0.001$).
[€] : Significantly higher lead concentrations in 2018 when compared to 2017 (both $p < 0.001$).

The eider ID was included as a random variable to account for the non-independence of samples within years. Linear regression analysis was employed to test if body mass or body mass loss affected lead concentrations in the blood on day 4 and day 24 of incubation. Sampling year was also included as a predictor variable to further support the results of the t-tests used to determine differences between years. To explore alternative variables in the dataset, linear regression analysis was run to test the effect of lead on lay date and number of hatched ducklings. The two days of incubation were analysed separately for two reasons, namely to isolate the different effects of early and late body mass on Pb blood concentrations and secondly to avoid issues of collinearity between body mass and day (ANOVA 80%). In all linear models, interactions were initially included, though removed in a stepwise manner to achieve the most parsimonious and simplified model. A correlation matrix was also run to visualize the relationships between variables; number of eggs, number of ducklings, numbering of lost ducklings, body mass on day 4, body mass on day 24, body mass loss, Pb blood concentrations on day 4, Pb blood concentrations on day 24, hatch date and lay date as well as the correlations among lead, calcium and nickel. All linear model assumptions were ensured using Residuals vs. Fitted, Normal QQ, Scale-Location, and Residuals vs. Leverage plots. The criteria for statistical significance was set to 0.05. The output from all statistical analyses conduced in R is found in Supplementary Information.

3. Results

3.1. Body mass

Body mass of the females is summarized in Table 1. For 2017, the body mass ± SD was 2359 ± 167 g and 1618 ± 156 g at day 4 and 24, respectively. In 2018, the body mass was 2183 ± 140 at day 4 and 1561 ± 154 g at day 24. Fig. 2 shows that body mass increased in the following order: day 24 in 2018 < day in 2017 < day 4 in 2018 < day 4 in 2017 and that a single high body mass was recorded at day 24 in 2017. The statistically analyses showed that body mass was significantly the lowest at day 24 compared to day 4 while the females in year 2018 had a significantly lower body mass than the females in 2017 (see Supplementary Information) (both $p < 0.001$).

3.2. Lead concentrations

The results from the blood samples analysed for lead are shown in Table 1. Mean lead concentration ± SD at day 4 and 24 in 2017 was 41.7 ± 67.1 μg/L and 55.4 ± 66.8 μg/L, respectively. In 2018, the mean lead concentrations were 177 ± 196 μg/L at day 4 and 258 ± 243 μg/L at day 24. Fig. 3 shows that lead concentrations increased in the following order: day 4 in 2017 < day 24 in 2017 < day 4 in 2018 < day 24 in 2018 and that high values are found for all four samples. Linear mixed effect models showed that Pb was significantly higher at day 24 compared to day 4 and that 2018 was significantly higher compared to 2017 (see Supplementary Information) (both $p < 0.001$). Furthermore, body mass did not significantly predict the blood lead concentrations on day 4 or day 24 (both $p > 0.08$).

From day 4 to 24, eider females showed 1.33 and 1.46-fold increases in blood lead concentrations in 2017 and 2018, respectively (Table 1). Furthermore, there were no significant correlations between any of the three elements lead, calcium and nickel that may mimic each other (all $p > 0.05$; $r = -0.26$ to 0.13).

3.3. Hatch date and number of chicks

Due to egg dumping and loss from gull predation, the number of hatched chicks ranged from one to eight across the years of 2017 and 2018. Linear models showed that the lead concentrations and body mass at day 4 affected hatch date positively in 2018 but not in 2017 (both $p < 0.03$). There were no significant effects from lead on the number of hatched chicks per female (see Supplementary Information) (all $p > 0.05$). Likewise, mass or mass loss did not affect the number of hatched chicks (all $p > 0.05$).

3.4. Toxic thresholds

Fig. 4 shows the paired samples divided into day 4 and day 24 for year 2017 and 2018, respectively, in relation to two establish toxic thresholds (vertical lines). Three of the birds (13%) sampled in 2018 had lead concentrations that exceeded concentrations of clinical poisoning of 500 μg/L and eleven (48%) had concentrations that exceeded the threshold for subclinical poisoning of 200 μg/L. In 2017, none of the birds exceeded the high toxic threshold of clinical poisoning while only one (4%) exceeded the lower for subclinical poisoning.

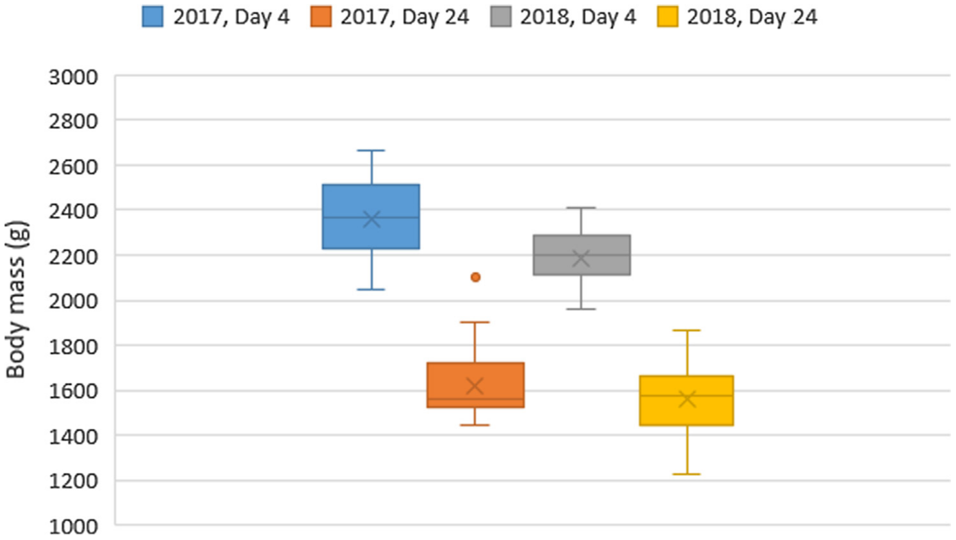

Fig. 2. Box and whisker showing from below minimum, Q1, median (×), mean, Q3 and maximum of paired samples of body mass of incubating eiders collected day 4 and 24 in 2017 ($n = 27$) and 2018 ($n = 23$) at Christiansø in the Baltic proper.

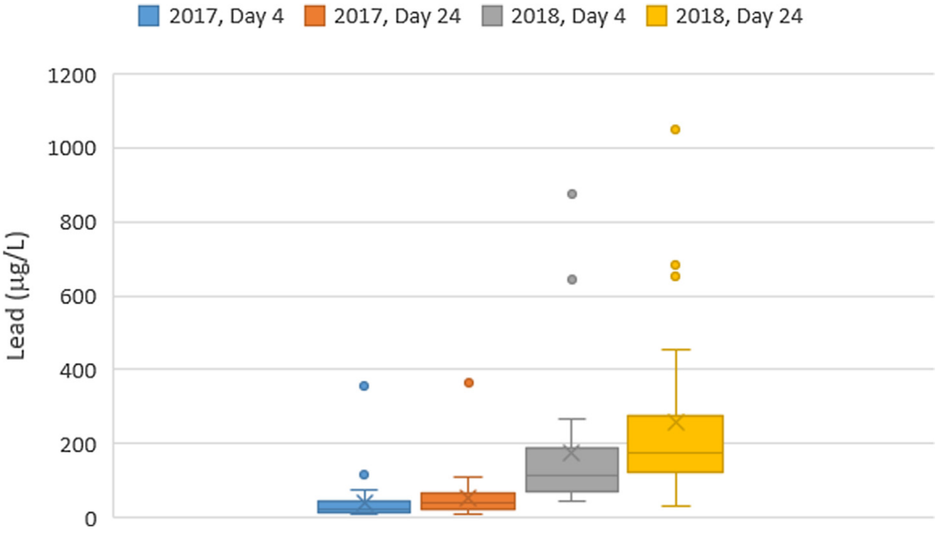

Fig. 3. Box and whisker showing from below minimum, Q1, median (×), mean, Q3 and maximum of lead concentrations in paired blood samples of incubating eiders collected at day 4 and 24 in 2017 (n = 27) and 2018 (n = 23) at Christiansø in the Baltic proper.

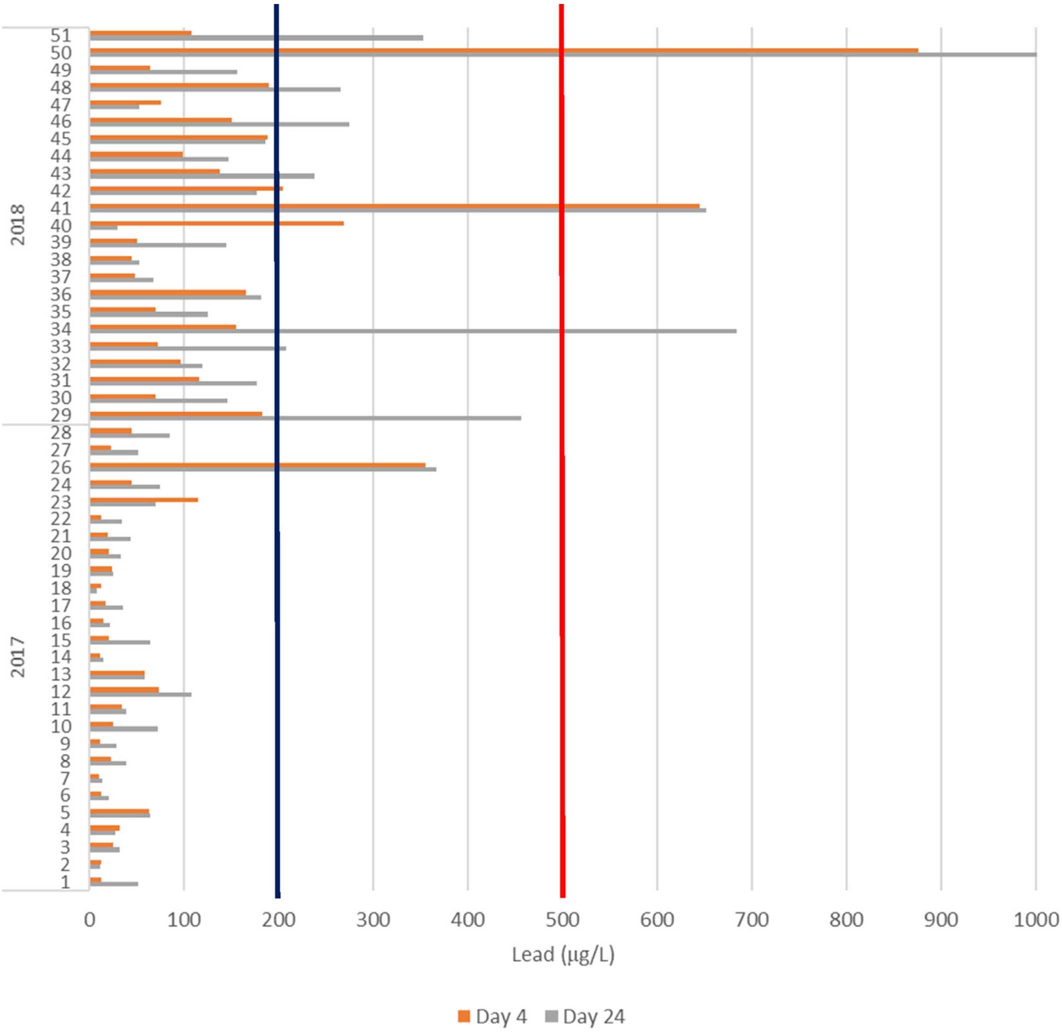

Fig. 4. Lead concentrations in paired blood samples from incubating eiders at day 4 and 24 sampled in 2017 (n = 27) and 2018 (n = 23) at Christiansø in the Baltic Sea. The concentrations are compared with thresholds for subclinical poisoning (dark blue vertical line: 200 µg/L) and clinical poisoning (red vertical line: 500 µg/L) in *anseriformes* according to Pain et al. (2019). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. Body mass

As the sole incubator amongst the sexes and a largely “capital” breeder (Meijer and Drent, 1999), female eiders undergo food deprivation and starvation during the breeding season to protect eggs from predation and to secure embryo development (Waltho and Coulson, 2015). The loss in body mass from early to late incubation reflects starvation which may lead to depleted energy reserves causing increased time away from the nest to feed (Garbus et al., 2018a, 2018b) or nest abandonment (Korschgen, 1977). Overall, the critical threshold of body mass at which death is inevitable for incubating eiders is around 1100 g (Korschgen (1977). In this study none of the eiders at day 24 approached this body mass threshold. This contrasts with the level of emaciation found among female eiders during the earlier mortality events on Christiansø in 2007, 2015 and 2016, where the main cause of death was associated with food deprivation and high parasitic burdens (Garbus et al., 2018a, 2019). Lead was not analysed in these individuals and therefore, the potential links between lead intoxication and mortality cannot be evaluated from these years. However, two clinical signs of lead intoxication are lethargy and poor body condition, both of which were documented in the mass mortality event of 2015 as many of the eiders were found dead on their nests in a state of starvation (Garbus et al., 2018a).

4.2. Lead exposure and health effects

Lead increased 1.33-fold and 1.47-fold over the course of incubation in 2017 and 2018, respectively. Previous studies have shown that eiders in later stages of incubation had higher blood concentrations of lead compared to those in earlier stages, which supports our results on incubating eiders at Christiansø (Fenstad et al., 2017; Franson et al., 1998, 2000; Wilson et al., 2007). During incubation, lead is mobilised from bones and liver because of calcium and energy demands for egg production (Hargreaves et al., 2010). This explains the higher concentrations found at day 24 compared to day 4 i.e. at the end and start of incubation period, respectively (Ethier et al., 2007; Franson and Pain, 2011; Williams et al., 2018). Leaner birds may be at risk of greater lead exposure over the incubation period as they are forced into a more severe state of starvation and therefore re-mobilize more calcium and energy from bone and liver, which releases more lead as well (Hargreaves et al., 2010). Despite the differences in body mass from 2017 and 2018 there was no significant difference between lead increases in the present study. This indicates that the larger blood concentration of lead in 2018 was likely due to a new source of exposure that was not present in 2017. Three of the incubating eiders (13 percent) from 2018 had levels exceeding concentrations of clinical poisoning typically leading to alterations in muscular-driven motor functions while eleven (48 percent) exceeded the threshold for subclinical poisoning leading to genotoxicity, reduced reproduction, and liver and brain lesions (Pain et al., 2019). In 2017, none of the birds exceeded the high toxic threshold of clinical poisoning, while only 1 (4%) exceeded the lower threshold for subclinical poisoning. Elevated blood lead concentrations of 100–600 μg/L have been reported in common eiders from the Gulf of Finland and Bothnia and were associated with liver lesions (Hollmén et al., 1998). The birds from Chrisiansø in 2018 had similar blood concentrations, which suggests that the Christiansø colony experienced similar health effects. This is relevant and points towards lead being a possible co-factor in the Christianø colony decline over the past 20–30 years, a fact which makes the colony suitable for biomonitoring the health and population dynamics of the Baltic flyway. The highest lead level found in the present study was in a female sampled at day 4 in 2018. This individual had a blood lead concentration of 1488 μg/L, which is lethal according to Pain et al. (2019), and probably explains why we were unable to relocate her again at the end of the incubation period. In addition, the birds in the Christiansø colony have shown clinical signs associated with starvation and lead intoxication during previous mortality events (Garbus et al., 2018a, 2019; Hollmén et al., 1998).

Lead is mobilized from bones to blood, although transfer to eggs is low, except in highly contaminated areas where hens typically have continuously elevated lead blood concentrations masking bone release during incubation (Korbecki et al., 2019). Therefore, sampling eggs is not recommended to monitor and assess lead exposure and toxicity in Baltic eiders. However, blood sampling, as used in the present study to represent current exposure, is non-invasive and allows re-sampling of the same individual birds, which provides information of potential critical life-history events (Fenstad et al., 2016; Letcher et al., 2010).

4.3. Sources of lead

Previous studies have shown that elevated lead in eiders may be due to ingested lead pellets or fragments during foraging (Flint et al., 1997; Hollmén et al., 1998; Olney, 1960). In studied avian species, high lead concentrations occurred due to ingestion of lead originating from ammunition or fishing tackle (Haig et al., 2014; Helander et al., 2009; Grade, 2019). In Denmark, although the use of lead shot in hunting ammunition has been made illegal since 1996, accumulations of pellets remain shallowly buried in sediments, which are still accessible to ducks sifting through such material, especially in heavily hunted localities. Other countries surrounding the Baltic Sea still allow the use of lead shot and bullets, which together with historical lead pellets could be the source of lead in the blood of these birds (Kanstrup et al., 2016; Mateo and Kanstrup, 2019; Sonne et al., 2019).

Having similar chemical properties to calcium, high concentrations of lead may be found in species like blue mussels (*Mytilus edulis*) which is an important prey species for the eider and may contribute to their exposure (Eisler, 2009; Phillips, 1976). This may be particularly important for eiders during egg laying (especially calcium for egg formation) and incubation given that they deplete body stores of these essential elements to meet their requirements at these times (Wayland and Scheuhammer, 2011). Pre-nesting foraging by eiders around the colony at Christiansø is likely to be a lead source to breeding females. The lead concentrations in sediments, mussels, and fish liver of the Baltic Sea clearly show that the area that surround Christiansø is “not good” status according to the Helcom Indicators (HELCOM, 2019). Mean concentrations of lead in fish liver in the Gulf of Finland was above 0.2 μg/ww while it was around 0.05–0.07 μg/g ww around Christiansø and only 0.02 μg/g ww in Kattegat (HELCOM, 2019). Similarly, concentrations of lead in mussel soft tissue in the area around Christiansø was above 0.18 μg/g ww. This means that eiders eating mussels and crustaceans are likely to be exposed to high lead concentrations at both their breeding and wintering grounds (HELCOM, 2019). Since the exact wintering ground locations for these individual birds remain unknown, this limits further investigation on the lead concentrations in prey. However, concentrations in blue mussels are known to range from 0.1 to 0.4 μg/g ww elsewhere in the Baltic (Fliedner et al., 2018). A Canadian study has shown that birds bioaccumulate a wide range (3–311 μg/g) of lead concentrations in their bones (Ethier et al., 2007). Altogether, this may help to explain how the Christiansø eiders experience elevated lead blood concentrations, but it does not explain the overall increases from 2017 to 2018 (Ethier et al., 2007; Goyer, 1997).

4.4. More research needed

The origin of lead in incubating eiders in the Christiansø colony is unknown and it remains an urgent priority to establish the source, prevalence and mechanism for uptake. The increase in lead from day 4 to day 24 is due to bone and liver remobilization; however, the additional lead source on the breeding grounds is urgent to identify and continue to monitor. At present, there is little idea of the specific source of the lead. Eiders have muscular gizzards, highly effective at crushing blue mussel shells for their digestion, so it seems less likely that they actively seek out grit and ingest lead shot and pellets this way. Therefore, more research including necropsies and x-ray and lead, C, and N stable isotope analyses are required to investigate the uptake and toxic effects of lead on breeding adults and off-loading to their offspring (Nakano, 2016).

Several factors have been hypothesised to explain the declines in the eider population at Christiansø and the Baltic flyway (Lyngs, 2014), which includes energetic constraints and exposure to contaminants and diseases (Christensen, 2008). Energy constraints have often been related to the nutritional quality of blue mussels that constitute their primary food source (Cramp and Simmons, 1977). In fact, a decline in the use of agricultural fertilisers (which have accumulated in inshore marine areas through runoff) has been proposed as a major cause for the decline in the Baltic Sea eider population over the last decades due to fewer and slower growing blue mussels (Laursen and Møller, 2014). *Prymnesium polylepis* algal blooms have also been suggested as a cause of reduced eider body condition, contributing to increasing numbers of non-breeding eiders (Larsson et al., 2014). High prevalence of acanthocephalan parasites has previously been associated with mortality events in eiders from the Baltic Sea (Camphuysen et al., 2002; Garbus et al., 2018a, 2019). Finally, dietary deficiencies in calcium, iron and zinc may increase lead uptake (Abadin et al., 2007; Skerfving and Bergdahl, 2014). This is particularly important for eiders during incubation where they may experience depletion of these essential elements (Wayland and Scheuhammer, 2011). It is worth mentioning that elevated lead concentrations were positively linked to hatch date in the present study, which may contribute to nest abandonment and population declines. Lead is known to, for example, affect egg size and hatching in pigeons and it is therefore likely that the high Pb levels may have delayed the hatch date in 2018 (Williams et al., 2017). Given the previous mortality events in the Christiansø colony it cannot be ruled out that lead exposure may be a co-factor in mortalities and population declines of the incubating eider females.

5. Conclusions

The body mass of the female eiders decreased from early to late incubation meanwhile lead increased in both 2017 and 2018. The reason for this lead increase is remobilisation from bone and liver tissue because of increased energy and calcium requirements for egg production and nest protection. The higher concentrations in 2018 likely occurred due to a new source of exposure. Six percent of the birds had lead concentrations that exceeded those of clinical poisoning, increasing the risk of neuro-muscular symptoms, while 24% of the birds exceeded the threshold for subclinical poisoning, increasing the risk of tissue damage, anaemia and reproductive impairment. As capital breeders, incubating eiders are already energetically stressed and clearly show that bioavailable lead is posing a threat to their health. Therefore, we urge further investigation of lead toxicological effects on adult female eiders, especially to determine the origin, uptake mechanisms and degree of exposure to lead of different individuals. Such research should include necropsies, x-ray, lead isotope and stable C and N isotope analyses to find the lead sources(s) in the course of the annual cycle and how it may affect the population dynamics of the colony. We urge the use of the Christiansø colony for biomonitoring the ecology and health of the Baltic flyway because of its sensitivity and geographical location. This should include more research on multiple stressors such as lead through the annual cycle including year-to-year fluctuations and population dynamics.

CRediT authorship contribution statement

Su Shiung Lam: Writing - review & editing. **Molly McPartland:** Writing - review & editing, Writing - original draft, Methodology, Visualization, Software, Data curation. **Brenley Noori:** Writing - original draft, Methodology, Software, Data curation. **Svend-Erik Garbus:** Funding acquisition, Methodology, Writing - review & editing. **Syverin Lierhagen:** Resources, Formal analysis, Validation, Methodology. **Peter Lyngs:** Methodology, Supervision, Investigation, Writing - review & editing. **Rune Dietz:** Writing - review & editing, Funding acquisition. **Ole Roland Therkildsen:** Writing - review & editing, Funding acquisition, Investigation. **Thomas Kjær Christensen:** Writing - review & editing, Funding acquisition, Investigation. **Rune Skjold Tjørnløv:** Methodology, Investigation, Writing - review & editing. **Niels Kanstrup:** Writing - review & editing. **Anthony D. Fox:** Writing - review & editing. **Iben Hove Sørensen:** Writing - review & editing. **Céline Arzel:** Writing - review & editing. **Åse Krøkje:** Project administration, Funding acquisition, Supervision, Writing - review & editing. **Christian Sonne:** Project administration, Conceptualization, Methodology, Funding acquisition, Writing - original draft, Writing - review & editing, Data curation.

Declaration of Competing Interest

We report that there are no conflicts of interests, and that the submitted manuscript has been reviewed and approved by all co-authors, and is not under consideration for publication elsewhere.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105582.

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