Variation in skull bone mineral density of ringed seals (Phoca hispida) from the Gulf of Bothnia and West Greenland between 1829 and 2019

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1. Introduction

The continuous renewal of bone (i.e., bone remodelling) is mediated mainly by two different bone cell types. While osteoblasts are responsible for the formation, bone resorption is performed by osteoclasts. The process of bone remodelling is influenced by matrix-embedded osteocytes, which are terminally differentiated osteoblasts (Bonewald, 2002). It not only serves for the renewal of bone in response to skeletal loading, but also for the maintenance of mineral homeostasis (i.e., calcium, phosphate) (Rolvien et al., 2017). While the remodelling process is ideally balanced, it is influenced by multiple environmental factors including hormones, vitamins, nutrients and organochlorines (Hill, 1998; Johansson et al., 2002; Leder et al., 2001; Roos et al., 2010; Sarazin et al., 2000; Sonne et al., 2004). Environmental contaminants in particular may affect this process, resulting in an imbalance that leads to a decrease in bone mass and pathological changes including porosity, alveolar deterioration and decreased overall bone density (Bergman et al., 1992; Lind et al., 2003; Sonne et al., 2004).

Some of the most widely used chemicals over the past 80 years are organochlorines, which include polychlorinated biphenyls (PCB) and chlorinated pesticides such as dichlorodiphenyl trichloroethane (DDT). PCBs and DDTs are found in all fresh, brackish and marine waters around the world (Ogata et al., 2009). These substances were regulated and banned in most parts of the world during the 70′ and 80′s (AMAP, 1998), but due to their persistence and tendency to bioaccumulate, they can still be found in ecosystems worldwide today. For the Baltic Sea the...
Stockholm Convention banned the usage of PCB in 2001 while Sweden already restricted the use of PCB in 1978. Before the ban, a decrease in the seal populations of both ringed (Pusa hispida) and grey seals (Halichoerus grypus) was observed in the Baltic (Helle, 1980; Harding & Härkönen, 1999).

The reduction of the population size was due to intensive hunting during the first part of the century but later the exposure to organochlorine contaminants, affected the seals’ reproductive system and led to infertility (Bergman, 1999; Bergman & Olsson, 1986; Helle, 1980; Reijnders, 1986; Harding & Härkönen, 1999). Between 2003 and 2016, the growth rate of the ringed seal populations in the Baltic Sea was less than the intrinsic capacity (the biotic potential or maximal growth rate for a species) (HELCOM, 2018).

Multiple publications report on organochlorines and their effects on the immune, endocrine, reproduction and skeletal systems of aquatic mammals (Beineke et al., 2005; Bergman et al., 1992; Das et al., 2006; Jepson et al., 2016; Lind et al., 2003; Routti et al., 2008). In Eurasian otters (Lutra lutra) from Sweden, PCBs have been shown to be correlated positively with cortical bone variables including cortical area, cortical mineral content and cortical thickness (Roos et al., 2010). In subadult Eastern Greenland polar bears, a negative correlation between skull bone mineral density (BMD) and PCB and chlordane burden was found (Sonne et al., 2004). Moreover, a study of Routti et al. (2008) showed a relationship between contaminants and endocrine homeostasis and also demonstrated that the bone lesions of Baltic grey seals could be a combination of contaminant-mediated vitamin D and thyroid disruption. The study showed that seals in the Baltic Sea are also exposed to higher concentrations of PCB and DDT than in Canada because the concentrations in the environment are much higher in the former region (Routti et al., 2008).

Furthermore, it was found that the mandible of Baltic male grey seals had the lowest bone density between 1986 and 1997 compared to the time between 1850 and 1955 (Lind et al., 2003). Grey seals, as well as ringed seals are top predators in the marine ecosystem and accumulate high concentrations of environmental contaminants such as PCBs in the internal organs, tissues and blubber. As a top predator, the ringed seal is important for the native ecosystem and is supposed to have a significant effect on structuring its ecosystem (Appollonio, 2002; Ray, 1981; Klotz & Kühn, 2002; Moore, 2008; Sergio et al., 2005).

Therefore, the health status of the ringed seals is used for the evaluation of the overall health and status in a way that this phocid is utilized as a sentinel species (Gulland & Hall, 2005).

As a result of the contaminant influence and the slow recovery of the ringed seal population, it has been listed as vulnerable in the HELCOM Red List and has the IUCN Criteria A3c, which means that these seals are under special protection. Against this background it is important to know how they are affected by pollutants in order to be able to take effective actions (HELCOM, 2013). The present study aims to document the historical event of large-scale environmental pollution by investigating the BMD of ringed seal skulls from the Baltic Sea over the period 1897 to 2018.

2. Materials and methods

2.1. Samples

The samples from Sweden originate from ringed seals that were found dead (N = 11), bycaught (N = 10) or hunted by locals (N = 14), for 68 individuals the cause of death is unknown. The Greenland samples were hunted by locals. The animals were shot according to the existing hunting law in the respective countries. A sample of 103 ringed seal skulls from the Gulf of Bothnia and 200 ringed seal skulls from west Greenland were examined (Fig. 1; Table 1). The 103 ringed seal skulls from the Gulf of Bothnia (scattered along the northern Swedish east coast) were provided by the Swedish Museum of Natural History (SMNH) in Stockholm, while the skulls from Greenland (Qaanaaq and Qeqertarsuaq) were provided by the Aarhus University and Zoological Museum in Copenhagen, Denmark. The skulls were collected in the period between 1897 and 2018 (Gulf of Bothnia) and the skulls from Greenland between 1829 and 2019. At the SMNH, there are only records of exact treatment method for the individual skulls from recent years. Upon ocular inspection, the skulls from 1897 to 1922 were found to be probably macerated and/or boiled carefully. From 1933 until the 1980s the skulls were boiled with or without adding a cleaning agent. From the end of the 1980s it became more common to use dermestid beetles, and from 2008 and onwards all the skulls were treated with this method. Roos et al. (2010) found no significant differences in bone mineral density between the boiling method at SMNH and cleaning by dermestid beetles. However, too harsh treatment with boiling and chemicals can result in markedly rugged surfaces on the bone. Skulls that had been treated this harshly were excluded from the study. The Zoological Museum in Copenhagen exposed the skulls to different temperatures to clean them. Occasionally, soap was also used to clean the bones. After preparation, the skulls were stored dry at room temperature at the museum.

The age of the animals was determined by the growth layers counted in the cementum of the canine teeth collected from the lower jaw. First, a decalcification took place, followed by sectioning and staining with toluidine blue as described in Stirling et al. (1977) and Dietz et al. (1991). 37 ringed seal skulls from the Gulf of Bothnia had already been determined by the SMNH in the same manner. All individuals were separated into three sex and age classes based on age determination: adult males (≥ 5 years old), adult females (≥ 4 years old) and juveniles (remaining individuals) (Atkinson, 1997; Kelly et al., 2010).

Additionally, the skulls were divided into three groups, according to year of collection. The first group included the skulls from 1829 to 1957, the second from 1958 to 1989 and the last group from 1994 to 2019. These periods represent different contaminant levels in the seals. The first period describes a period with an absence up to very low PCB levels (Supplementary Fig. A1) in the environment prior to the industrial production and can be considered as baseline. In this period, 167 skulls were available, where 58 individuals originate from the Gulf of Bothnia and 109 individuals from Greenland (Table 1). The second period covers the most intense production phase of PCBs (Supplementary Fig. A1) and the highest peak of PCBs in the Baltic and includes 40 skulls (Gulf of Bothnia: 26, Greenland: 14, Table 1). In the last period, which represents the decreasing PCB concentrations (AMAP, 1998, Supplementary Fig. A1) in the Baltic, 96 skulls were available (Gulf of Bothnia: 19, Greenland: 77, Table 1).

2.2. Identification of sex

The samples came from 114 individuals of the Gulf of Bothnia, of which 59 were of known sex (24 males, 33 females, 2 juvenile males). In order to determine the sex of the 55 individuals with unknown sex, six skull lengths were measured (Table 2, Fig. 2). Each distance was measured on both sides of the skulls, using a vernier caliper (Covetrus Inc., 7 Custom House St. Portland ME, 04101). The skulls were measured by two persons and the mean value was used in the calculations. The skull length measurements from individuals with known sex were then tested for differences between the sexes. The aim of the analysis was to predict the sex of individuals with unknown sex by identifying significant sexual differences in ringed seal skull lengths.

2.3. Bone mineral density (BMD)

Bone mineral density (BMD) was examined for 103 skulls from the Gulf of Bothnia (25 females, 20 males, 58 unknown) and 200 skulls from Greenland’s west coast (64 females, 82 males, 54 unknown), after all skulls which were damaged or not complete were excluded. Dual-energy x-ray absorptiometry (DXA, Lunar Prodigy iDXA; GE Healthcare;
Madison, WI, USA) was used for this, which is a non-invasive and commonly used method for studying BMD (Dowthwaite et al., 2011). In humans it serves for the detection of osteoporosis. The skulls were scanned with a speed of 5.65 mm/s and a resolution of 0.5 × 0.61 mm (Boudousq et al., 2005). Macerated skulls are difficult to scan, therefore a water tank, which replaced skin and tissue, stands above the samples during the scan process (Fig. 3). After scanning, the results were analysed by the enCORE-software (v15 – GE HealthCare Lunar, Buckinghamshire, UK), which produced an image of the skulls and calculated the BMD in grams per square centimetre (g/cm²). In the analysis the software automatically identifies bone tissue, based on the different X-ray absorption coefficients of bone and other tissue. Nevertheless, this process is not always reliable and the scanner does not necessarily identify the whole skull as bone tissue. This can be manually corrected as the process is supervised.

2.4. Statistical analyses

The statistical analyses were performed with R (version 3.4.3, R Core Team, 2014) using the packages "jtools" (Long, 2019), “sm” (Bowman & Azzalini, 2018) and “mgcv” (Wood, 2011).

A binomial generalized linear model (GLM) was used to test for differences in skull length measurements (OPF, POH, CBL, P4-M2, C-M2, I1-M2) between sexes. The aim of the analysis was to subsequently differentiate the sex of seals with unknown sex. This model was only tested with the age group "adult", as other age groups were under-represented, and both sides of the skull were analysed separately. The most parsimonious model was selected in a step-wise procedure, as recommended by Zuur et al. (2009). Each explanatory variable (i.e. each skull length measurement) was subsequently dropped from the full model and then this reduced model was compared to the full model by applying likelihood ratio tests using the R function anova. Only significant model terms were retained. For both sides, the most parsimonious model included only OPF ($p_{\text{left}} = 0.015$, $p_{\text{right}} = 0.04$).

The BMD was tested for normality by applying the Shapiro-Wilk test. Level of significance was set at $p \leq 0.05$ and a trend was considered between $0.05 < p \leq 0.10$ based on Sonne et al. (2004).

The next step was a linear regression analysis (LM), where the relationship of BMD versus period (before, during, after) and also the relationship of BMD versus sex (female, male, unknown) were tested for the skulls from the Baltic Sea and Greenland Sea. For the comparison of both areas first the relationship of BMD versus both areas were tested. To test for period differences an ANCOVA was used. BMD was the dependent variable and period, as well as both areas were independent variables.

### Table 1

|                | Gulf of Bothnia | West Greenland |
|----------------|-----------------|----------------|
|                | Period 1 1897–1933 | Period 2 1960–1989 | Period 3 2007–2018 |
|                | Period 1 1829–1957 | Period 2 1958–1987 | Period 3 1994–2019 |
| Total          | 58              | 26             | 19             | 109             | 14             | 77             |
| Adult          | 26              | 25             | 15             | 47              | 2              | 17             |
| Juvenile       | 28              | 2              | 2              | 4               | 1              | 18             |
| Unknown (Un.) Age group | 4                | 1              | 2              | 58              | 11             | 42             |
| Known sex      | 1               | 26             | 18             | 66              | 5              | 75             |
| Males          | 1               | 11             | 8              | 44              | 1              | 37             |
| Adult          | 0               | 11             | 6              | 28              | 0              | 12             |
| Juvenile       | 0               | 0              | 1              | 1               | 1              | 7              |
| Un. Age group  | 0               | 0              | 1              | 16              | 0              | 18             |
| Females        | 0               | 15             | 10             | 22              | 4              | 38             |
| Adult          | 0               | 14             | 9              | 10              | 1              | 5              |
| Juvenile       | 0               | 0              | 1              | 3               | 0              | 11             |
| Un. Age group  | 0               | 1              | 0              | 9               | 3              | 22             |

Fig. 1. Study areas in the Baltic Sea and West Greenland. Samples were predominantly collected in the Gulf of Bothnia and in the locations marked with a triangle at the west coast of Greenland.
To investigate the effect of organochlorines, particularly PCB, on BMD, reliable estimates of PCB values in ringed seals were needed. As PCB concentrations could only be measured in 17 of the available skull samples (Tab. A1., Supplementary), we modelled total PCB content in blubber samples of ringed seals from the Gulf of Bothnia (Table 1). The relationship between BMD and PCB levels was then tested with a linear regression model. After that, the relationship between PCB levels and sex was tested in a separate linear regression model.

3. Results

3.1. Gulf of Bothnia

3.1.1. Sex

The GLMs revealed that only OPF on both sides seems to be significantly different between sexes ($p_{\text{left}} = 0.02$, $p_{\text{right}} = 0.04$). Although there is a significant difference, the difference is relatively small and thus biologically not meaningful: mean OPF right females = 8.11 ± 0.36 cm and males = 8.38 ± 0.39 cm, mean OPF left females = 8.16 ± 0.34 cm and males = 8.38 ± 0.39 cm (Fig. 4). Thus, it is not possible to reliably distinguish sexes only on skull morphometric measurements and to identify the sex of individuals of unknown sex in this way.

3.1.2. BMD and sex differences

BMD was analysed in 103 skulls from the Gulf of Bothnia divided into 25 females (including 1 juvenile), 20 males and 58 unknown individuals representing the period 1897–2018. The exact composition of each time period group can be found in Table 1. Both sexes had significantly higher BMD (mean: female = 0.83 ± 0.16 g/cm², male = 0.91 ± 0.30 g/cm², unknown = 0.62 ± 0.10 g/cm²) than the unknown category ($LM_{\text{unknown vs males}} < 0.001$, $LM_{\text{unknown vs females}} < 0.001$) (Fig. 5). No statistically significant difference could be seen between BMD of males and females.

3.1.3. Time trends

Regarding samples from the Bothnian Bay, 58, 26 and 19 skulls were used for the first, second and third periods, respectively (Table 1). The BMD levels of the individuals from the pre-pollution period (1897–1933) were significantly lower than from the pollution period (1960–1989) and from the post-pollution period (2007–2018) ($p_{\text{pollution}} < 0.001$, $p_{\text{post-pollution}} < 0.001$; mean: pre-pollution period = 0.62 ± 0.10 g/cm², pollution period = 0.91 ± 0.14 g/cm², post-pollution period = 0.81 ± 0.32 g/cm²) (Fig. 5).

3.1.4. BMD vs. Contaminants

BMD was analysed against modelled PCB concentrations in ringed seals from the Gulf of Bothnia (Table 1). The relationship between BMD

### Table 2

Definition of the skull lengths of the Baltic ringed seals and Greenland ringed seals (based on Bechshøft et al. 2008).

| Length | Definition |
|--------|------------|
| OPF    | Maximal distance between the opistocranion and the postorbital process of the frontal bone. |
| CBL    | Condylar length. |
| P4-M2  | Length from the anterior margin of the 4th premolar to the posterior margin of the 2nd molar. |
| C-M2   | Length from the anterior of the alveole of the canine, to the posterior of the alveole of the 2nd molar. |
| M2     | Length from the anterior margin of the 4th premolar to the posterior margin of the 2nd molar. |
| POH    | Postorbital height. |

Fig. 2. The six lengths measured on the ringed seal skulls of the Gulf of Bothnia in the Baltic Sea and West Greenland. 1 = OPF, 2 = POH (upper picture), 3 = CBL, 4 = P4-M2, 5 = C-M2, 6 = M2 (bottom picture).

assumed a PCB content of 0 as a baseline for the model. For some field work campaigns, the original measurements were not available but only median total PCB values and the corresponding sample sizes were reported. As those sample sizes were usually high, the median values were considered robust. Therefore, the values were repeated as often as the sample size and as such entered in the analysis to make the GAM more robust to outliers from measurements of single individuals. The coefficient of determination was high ($R^2 = 81.7\%$) indicating a good model fit. The model output was then used to predict total PCB content of ringed seals from 1897 to 2018 (Supplementary Material 1, Fig.A1). The model values were subsequently assigned to the skull samples from the Gulf of Bothnia based on sampling year. The relationship between BMD and PCB levels was then tested with a linear regression model.

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and PCB concentrations was significant (LM, \( p < 0.001 \), Fig. 6). BMD increased with increasing PCB. Males on average have a higher PCB concentration than females between 1897 and 2018 (mean: males = 55672.71 ± 46694.47 ng/g, females = 25094.83 ± 30006.85 ng/g).

3.2. Greenland

3.2.1. BMD and age group/sex differences

BMD was analysed in 225 skulls divided into 64 females, 82 males and 79 unknown individuals. In contrast to the Gulf of Bothnia the Greenland population showed a significantly higher BMD in males than in females (LM, \( p = 0.04 \); mean: males = 0.76 ± 0.35 g/cm²). Furthermore the BMD was higher in adult individuals than in juvenile individuals (LM, \( p = 0.02 \); mean: adult = 0.73 ± 0.39 g/cm², juvenile = 0.57 ± 0.1 g/cm²), as well as the group of individuals of unknown sex (LM, \( p = 0.04 \); mean: unknown = 0.71 ± 0.23 g/cm², juvenile = 0.57 ± 0.1 g/cm²) (Fig. 7).

3.2.2. Time trends

During the whole study period none of the tested variables were significant. The BMD showed no significant variation over the time span from 1829 to 2019 (Fig. 7).

3.3. Greenland vs. Gulf of Bothnia

3.3.1. BMD and country differences

The BMD varies between sexes and sea regions. In the Gulf of Bothnia, the BMD of the females varies between 0.453 g/cm² and 1.12 g/cm² (median: 0.74 g/cm²) and the BMD of the males varies between 0.574 g/cm² and 1.987 g/cm² (median: 0.72 g/cm²). Around Greenland the BMD range of the females varies between 0.252 g/cm² and 1.127 g/cm² (median: 0.64 g/cm²) and of the males between
0.334 g/cm² and 2.774 g/cm² (median: 0.72 g/cm²). No significant difference between the BMD levels of the Greenland population and the Gulf of Bothnia could be shown. The gender composition for the Gulf of Bothnia was for the pre-pollution period one male and 57 individuals with unknown sex. For the pollution period it was 11 males and 15 females and for the post-pollution period eight males, ten females and one unknown. The gender composition for the Greenland individuals were for the pre-pollution period 44 males, 22 females, 43 unknowns; and for the pollution period one male, four females and nine unknowns; and for the post-pollution period 37 males, 38 females and two unknowns.

3.3.2. Time trends
For the first period, prior to PCB production, 167 skulls were available, in the second period 40 skulls were examined and for the third period, after the highest peak of contaminant concentration, 96 skulls were available (Table 1). The Greenland population showed a significantly higher BMD in the pre-pollution period than the population in the Gulf of Bothnia (LM, p < 0.001, Fig. 8). The population of the Gulf of Bothnia showed significantly higher BMD values in the pollution and post-pollution period than the population of Greenland (LM, p_{pollution} < 0.001, p_{post-pollution} = 0.005, Fig. 8).

4. Discussion

4.1. Identification of sex
Analysis of the possibility to identify sex based on skull measurements was performed on adult Baltic ringed seals. The test of both skull sides showed a significant difference between sex and OPF (maximal distance between the opistocranion and the postorbital process of the frontal bone), but the difference was relatively small and thus biologically not meaningful in ringed seals. The sex classification based on skull length was also tested in polar bear studies (Bechshaft et al., 2008), where significant differences between the sexes were detected indicating a marked sexual dimorphism. In contrast to that, female and male ringed seals showed no obvious sexual dimorphism, neither in body length nor in head form. Another study examined the age and sex classification of the skulls of the Mediterranean monk seal (Monachus monachus) (Brombin et al., 2009) using a method which is based on landmarks. With this method it was possible to detect differences between age classes, but there was no apparent sexual dimorphism similar to ringed seals in this study. Thus, it is not possible to distinguish the sex of ringed seals via skull morphometric measurements and genetic analyses are needed to reliably confirm the sex.

4.2. Bone mineral density of skulls from ringed seals
The skulls from Greenlandic ringed seals showed that males had a significantly higher BMD than females, which can be explained by the effect of the reproduction periods on the females’ body. Females lose BMD during their pregnancy and lactation (Kalkwarf et al., 1997). Ringed seals give birth to one pup and the suckling period lasts up to 7 weeks (Atkinson, 1997). In this time, females mobilise much of their own calcium and phosphate for the pup (Cross et al., 1995; Krebs et al., 1997; Ramsay & Stirling, 1988). Due to requirements for calcium during gestation, the bone density is lower compared to males. The same result was found in Greenlandic polar bears and in humans (Cross et al., 1995; Sonne et al., 2004). The variation of BMD in females seen in this study could also be due to the influence of sexual steroids on the skeletal morphology and physiology. The oestrogen level is important for the bone mineral absorption and the skeletal growth in pups, juveniles and adults (Lind et al., 2004; Ding et al., 2008; Saggese et al., 1997; Yilmaz et al., 2005). An inhibition of the oestrogen secretion can lead to a BMD decrease (Saggese et al., 1997).
4.3. Period differences and time trends in skull BMD

Individuals from the Baltic Sea had a significantly higher skull BMD from the period between 1957 and 1990 compared with those from the pre-pollution period. The same can be seen with individuals from the post-pollution period. These results suggest that there could be a linkage between increased BMD and increased contaminant loads. The BMD of males and females increase in the pollution period. The differences between the sampling areas illustrate possible effects from PCB on bones and indirect effects through sterility that cause female BMD not to decrease. The contaminant concentrations in ringed seals around Greenland are almost 100-fold lower than in the Gulf of Bothnia (Skåre, 1996; Letcher et al., 2010, Harding et al. unpublished). The PCB concentrations, during the pollution period, were very high in the tissue from ringed seals of the Gulf of Bothnia (Bjourlid et al., 2018) and reached levels, which can affect immunological parameters. It is known, that environmental contaminants suppress some mechanisms of the immune system (Dean et al., 1982), because the immune system is also influenced by sex steroids, such as oestrogen and progesterone, and stress hormones (Rooney et al., 2003). Such suppressions in turn could be indirectly linked to the present variation in skull BMD (Lind et al., 2004; Routti et al., 2008), as a suppressed immune system cannot protect the organism from harmful impacts (Rooney et al., 2003). Many other studies show a decrease in skull BMD during the period of high levels of pollution in different species, like grey seals, polar bears (Ursus maritimus) and Eurasian otters (Bergman et al., 1992, Roos et al., 2010 or Sonne et al., 2004). In all studies, whether the BMD decreased, increased, the changes were probably caused by hormonal disturbances, which are possibly induced by PCB (Routti et al., 2008). An inhibition of the oestrogen level can affect the BMD (Saggese et al., 1997) and the disruption of the thyroid homeostasis can also affect the bone homeostasis (Routti et al., 2008). The contaminants may suppress the circulating vitamin D levels (Routti et al., 2008), which affects the circulating calcium and phosphate levels.

4.4. Contaminants and effects

Different studies showed that PCB can potentially affect endocrine homeostasis, which can lead to changes in bones (Haave et al., 2003; Letcher et al., 2010; Sonne et al., 2004). These results may suggest endocrine-related effects (Lind et al., 2003; Sonne et al., 2004), because PCB and DDT can bind at sexual steroid receptors and intensify or inhibit the reactions of the normal hormone cascade. This is supported by a study on harbour seals between 1981 and 2014, where healthy harbour seal skulls were compared to skulls with rather pathological changes, and the healthy harbour seal skulls showed an increase in bone density (Pertoldi et al., 2018). However, higher BMD levels do not necessarily indicate better bone quality or stronger bones (Lind et al., 2000, 2004; Pertoldi et al., 2018). Despite higher BMD levels the bones may become brittle (Lind et al., 2000) if the increase in BMD is due to PCB contamination. For instance, PCB impaired bone strength and bone composition in rats (Lind et al., 2000). A study of Lind et al. (2004) demonstrated that the cortical part of the bone of female juvenile American alligators becomes larger, but loses in mass and the trabecular bone becomes smaller, but wins in mass. As a higher amount of the bones consisted of trabecular bone structures, the overall BMD rose (Lind et al., 2004). Nevertheless, these changes lead to a porous bone structure as a result of differences in the estrogenic hormone balance. As similar results were found in this study, the ringed seals could be exposed to estrogenic compounds resulting in the increase of BMD, because such changes are characteristic of oestrogens (Breen et al., 1998). The same effects could be seen in terrestrial vertebrates like mice (Breen et al., 1998). Nonetheless, many publications show a loss of BMD related to an increase in contaminants (Lind et al., 2000, 2003; Roos et al., 2010; Sonne et al., 2004), which could be a result of exposure to anti-estrogenic compounds (Bergman et al., 1992; Lind et al., 2004). A decrease in BMD leads to osteoporosis, which can have manifestations, such as alveolar bone loss (Bergman et al., 1992). Different hypothesis can be proposed. First, as mentioned before, steroid hormones play an important role for the skeleton, but the bone parts can give different responses (Hill, 1998; Yilmaz et al., 2005), as well as different species and their bones can give varying responses. Second, the thyroid function has been shown to be influenced by PCB and, therefore, can lead to changes in BMD. That is supported by several studies investigating the thyroid function, which showed changes in the BMD (Sormo et al., 2005; Routti et al., 2008).

In the present study, a positive correlation between PCB concentrations and BMD was observed. The Baltic ringed seals were exposed to higher PCB concentrations for a longer time compared with those from Greenland, which leads to stronger effects on the health status of Baltic ringed seal. This underlines the changes in the BMD of the Baltic ringed seals compared to those from Greenlandic waters.

5. Conclusions

Skull BMD seemingly increased with increasing contaminant concentrations in the Baltic ringed seals, which can be explained by sterility of females. The increasing BMD can be an indirect effect from samples including a large proportion of female seals affected by sterility. Sterile females are expected to have higher BMD since they do not loose minerals during lactation as fertile females do. But this also happens, when contaminants lead to changes in the reproduction system and as a consequence, the females end up having a lower number of gestations. BMD levels during the pollution period (1958–1989) and the post-pollution period (1994–2019) were significantly higher than those found during the pre-pollution period (1829–1957), where the seals were not exposed to contaminants. The Baltic ringed seals show a significant higher BMD levels than Greenland ringed seals during the pollution and post-pollution period. Our large sample from Greenland ringed seal give a good baseline for natural variation in BMD in ringed seals, we could also show that males have higher BMD than in females and adults higher than juveniles. Changes in the BMD can be a proof for long-term damages.

Declaration of Competing Interest

None.

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