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Gene expression and trace elements in Greenlandic ringed seals (*Pusa hispida*)

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ABSTRACT

Marine top predators such as ringed seals biomagnify environmental contaminants; and with the increasing human activities in the Arctic, ringed seals are exposed to biologically significant concentrations of trace elements resulting in reproductive impairment, immunosuppression, and neurological damages. Little is known about the molecular effects of heavy metals on these vulnerable apex predators suffering from a rapidly changing Arctic with significant loss of sea-ice. In the present study, concentrations of cadmium (Cd), mercury (Hg) and selenium (Se) were measured in liver of sixteen Greenlandic ringed seals (nine adults and seven subadults) together with molecular biomarkers involved in bio-transformation, oxidative stress, endocrine disruption and immune activity in blood and blubber. The concentrations of trace elements increased in the following order: Hg > Se > Cd with levels of mercury and selenium being highest in adults. Aryl hydrocarbon receptor nuclear translocator (*ARNT*), peroxisome proliferator activated receptor alpha (*PPARα*, estrogen receptor alpha (*ESR1*), thyroid hormone receptor alpha (*TRα*) and interleukin – 2 (*IL-2*) mRNA transcript levels were highest in blubber, while heat shock protein 70 (*HSP70*) and interleukin – 10 (*IL-10*) were significantly higher in blood. There were no significant correlations between the concentrations of trace elements and mRNA transcript levels suggesting that stressors other than the trace elements investigated are responsible for the changes in gene expression levels. Since Hg seems to increase in Greenlandic ringed seals, there is a need to re-enforce health monitoring of this ringed seal population.

1. Introduction

Greenland ringed seals (*Pusa hispida hispida*) are associated with spring sea-ice for breeding, moulting and feeding (Lydersen et al., 2017; Bengtsson et al., 2020; Lydersen et al., 2017). They are generalist predators feeding on a variety of fish species, amphipods and euphausiids, with a preference for lipid rich Arctic cod (*Arctogadus glacialis*) and Polar cod (*Boreagadus saida*) (Siegstad et al., 1998; Wathne et al., 2000; Labansen et al., 2007; de la Vega et al., 2021). With the Arctic warming, sea ice is decreasing rapidly, providing new prey species for Arctic ringed seals because of borealization but also depleting breeding and resting grounds on the ice (AMAP, 2017; Nuttall, 2019; Fossheim et al., 2015; de la Vega et al., 2021). In Svalbard, ringed seals now haul out on shorelines exposing them to harsher climate and predators (Lydersen et al., 2017; Kovacs et al., 2021). The Greenlandic ringed seal is red listed as least concern by the International Union for Conservation of Nature (IUCN), mostly threatened by loss of sea-ice, and an annual catch of about 3000–5000/yr is considered sustainable (Rosing-Asvid et al., 2019). Subsistence hunting of seals for their skin and meat is legal in Greenland (Møller et al., 2010; Jenkins et al., 2013; Tryland et al., 2014; NAMMCO, 2018).

Contaminants such as mercury (Hg) are being transported long-range via atmospheric pathways to the Arctic, making this previously pristine environment a sink for pollutants that are bio-available to the resident species (Letcher et al., 2010; Vorkamp and Riget, 2014; Dietz et al., 2018, 2019). Some of the most environmentally important trace

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elements in the Arctic include arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and selenium (Se), whose concentrations have been increasing in resident marine mammals over recent decades (Brown et al., 2014, 2016; Dietz et al., 2011, 2020; Routti et al., 2011; AMAP 2017; Morris et al., 2022).

As long-lived aquatic top predators, ringed seals biomagnify contaminants in their tissues and can be used as bioindicators of chemical exposure (Das et al., 2002; Debier et al., 2003, 2006; Sonne et al., 2020; Boyi et al., 2022). The main route of entry for contaminants is through their diet, and concentrations can be influenced by foraging strategies and biological factors such as age and body condition (Das et al., 2002; Habran et al., 2011; Vorkamp et al., 2008; Lyytikainen et al., 2015). In pinnipeds, elevated levels of trace elements such as Hg and Cd can result in chronic health effects including reproductive impairment, neurological damage, immunotoxicity and impairment of organs like the central nervous system, liver and kidneys (Dietz et al., 2000; Desforges et al., 2021; Frouin et al., 2010; Krey et al., 2015; Kershaw and Hall, 2019).

It is difficult to establish relationships between contaminant exposure and adverse health effects at population level (Rodriguez-Estival and Mateo, 2019). One of the few cases where this has been established is on killer whales (*Orcinus orca*) with high levels of PCB exposure (Desforges et al., 2018). However, changes in mRNA expression levels have been correlated with exposure to contaminants and are used as essential tools to determine health effects on pinnipeds, e.g. when aryl hydrocarbon-, thyroid hormone- and estrogen receptors were related to POP burdens in harbour (*Phoca vitulina*) and ringed seals (Tabuchi et al., 2006; Routti et al., 2010; Lehnert et al., 2016; Boyi et al., 2022). The Arctic is rapidly changing, and it becomes crucial to understand the molecular adaptations of Greenlandic ringed seals to environmental change. Although transcript levels of some genes were measured in Arctic ringed seal populations, e.g. Canadian ringed seals (Brown et al., 2014, 2017) and ringed seals from Svalbard (Routti et al., 2010; Castelli et al., 2014), there is so far no information on ringed seals from Greenland.

The aim of the present study was to measure gene expression endpoints in blood and blubber of ringed seals from Qaanaaq, Greenland. For the first time, expression levels of genes related to detoxification of xenobiotics, immune response, endocrine function, and oxidative stress are analysed. Aryl hydrocarbon receptor nuclear translocator (*ARNT*), interleukins *IL-2* and *IL-10*, heat shock protein 70 (*HSP70*), peroxisome proliferator-activated receptor alpha (*PPARα*), thyroid hormone receptor alpha (*TRα*) and estrogen receptor alpha (*ESR1*) gene transcription was analysed in sixteen ringed seals sampled in Qaanaaq in 2018. Concentrations of environmentally relevant trace metals Hg, Cd and Se were measured in the liver. Understanding the molecular effects of these contaminants on Greenlandic ringed seals is essential for the timely protection and conservation of this population, as these changes are eventually seen at organismal and population levels. This is the first study to analyse gene expression levels in Greenlandic ringed seals and establishes an important baseline for future studies.

2. Materials and methods

2.1. Sample collection

Blood and tissue samples were collected from 16 ringed seals (Table S1) as part of the Inuit subsistence hunt in May 2018 in Qaanaaq, Greenland (Fig. 1) following the method described by Sonne et al. (2020) and Sonne-Hansen et al. (2001). 1 mL whole blood samples were collected from the intervertebral epidural vein of the seals post-mortem

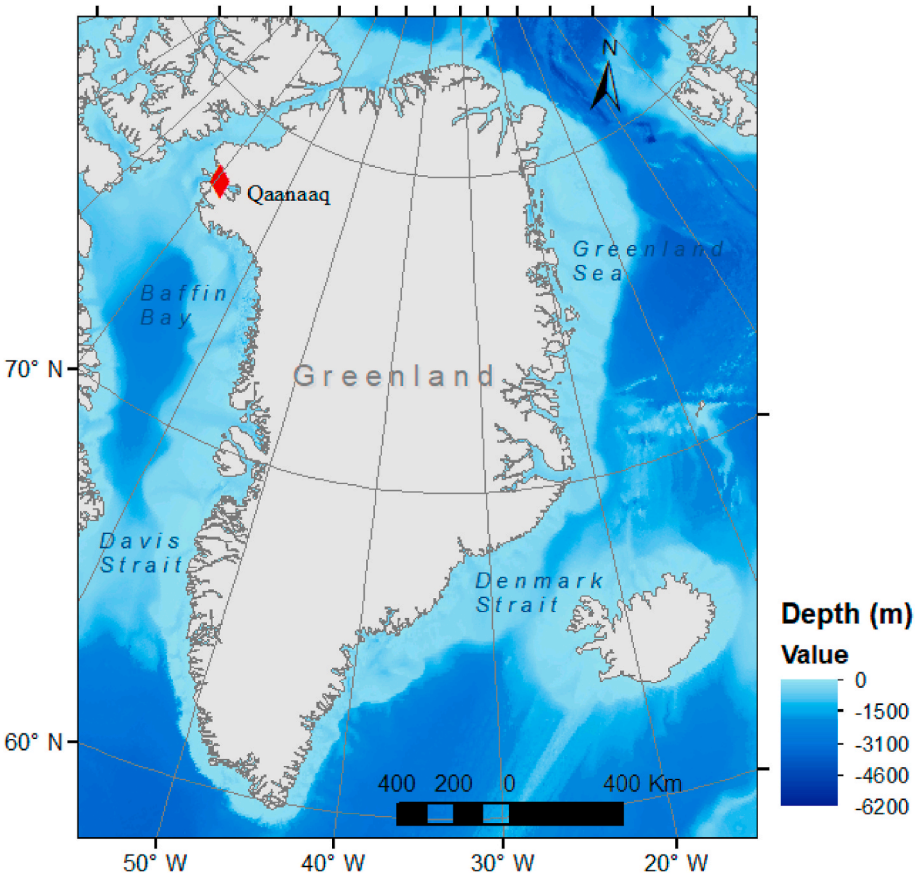

Fig. 1. Map showing the sampling location of 16 ringed seals in spring of 2018 from Qaanaaq Northwest Greenland, the red diamond represents Qaanaaq. The bathymetry is from GEBCO 2014.

in EDTA monovettes containing 3 mL RNAlater (Invitrogen, Germany). Blubber samples of 0.5 × 0.5 cm were collected in 2 mL Eppendorf tubes containing 1.5 mL of RNAlater. The samples were cooled at 4 °C overnight in the field and transported on dry ice to the laboratory where they were stored at −20 °C until RNA isolation. Liver samples were also collected in polyethylene plastic bags from the same animals for trace element analysis and stored at −70 °C in the laboratory. All tissue samples were collected within 24 h after the death of the animals.

2.2. Trace element analysis in ringed seals liver

Ringed seal liver samples were analysed for Hg, Se and Cd concentrations at the trace metal laboratory of the Department of Ecoscience Aarhus University, Roskilde, Denmark. Samples were freeze-dried. For Cd and Se analyses, 300 mg of freeze-dried sample and 4 mL of suprapur nitric acid were added to a Teflon container. The samples were heated for 12 h at 140 °C. After cooling and evaporation of the nitrous oxide, the samples were diluted with milli Q water to approximately 25 g. Cd and Se levels were determined by inductively coupled plasma-mass spectroscopy [(ICP-MS) PerkinElmer Inc.]. Multiple-element internal standards were added to each sample and calibration standard solutions. Quality control (QC) and quality assurance (QA) for ICP-MS included field blanks, method blanks and certified reference materials (CRMs) Seronorm L-3, DOLT-3, NIES-13 and BCR-063.

For the analysis of Hg concentrations, 2–10 mg of freeze-dried samples were thoroughly homogenized and analysed with a Milestone DMA-80 Direct Mercury analyzer. A 1000 ± 4 mg/L stock solution (Sigma Aldrich, Switzerland) was used for a calibration curve on which the sample concentrations were determined. Quality control and quality assurance included certified reference material DORM-4, blanks and aqueous controls (10 ng and 100 ng Hg) prepared from the stock solution and run every few samples. Following the analyses, sample concentrations were corrected for blank samples Hg levels and daily instrument drift. The measured recovery percentage (mean ± SD) of DORM-4 was 106 ± 4% (n = 41; certified Hg concentration = 0.410 ± 0.055 μg/g; measured Hg concentration = 0.439 ± 0.017 μg/g).

2.3. Expression levels of genes in blood and blubber samples

Total RNA was isolated from 500 to 700 μL of RNAlater blood using RiboPure Blood kit (Invitrogen, Germany) according to the manufacturers' protocol. From the blubber samples, total RNA was isolated using Qiagen Rneasy Fibrous Tissue Mini kit (Qiagen, Germany) with some modifications to the manufacturers' protocol as described in Boyi et al. (2022). 30 mg of blubber sample was lyzed by incubating in 180 μL of ATL buffer at 56 °C for 25 min. The completely homogenized lysate was incubated in 200 μL of AL buffer for 30 min at 56 °C. After this, the manufacturers' protocol was followed.

The quantity and purity of the isolated RNA were determined using a Thermo Scientific Nanodrop 2000 unit (Peqlab Biotechnologie GmbH). Samples of good quantity (approximately 20 ng/μL) and quality (260/280 ratio of 1.9–2.1) were selected for further downstream reactions. Murine reverse transcriptase (RT-PCR Core Kit™; Applied Biosystems, Weiterstadt, Germany) was used to reverse transcribe 80–100 ng of RNA from blood and blubber to cDNA. The amplification carried out in a thermocycler, had the following cycling conditions: 8 min at 25 °C, 15 min at 42 °C, 5 min at 96 °C and stored at 4 °C until the samples were removed and stored in the freezer at −20 °C. cDNA was stored at −20 °C until quantitative polymerase chain reactions (qPCR). The qPCR assays for all genes (Table S2) were carried out on an MX3000P QPCR System (Stratagene Europe, Amsterdam, Netherlands) using Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies) according to manufacturers' protocol. PCR reaction was initiated for 3 min at 95 °C, followed by 40 cycles with denaturation for 15 s at 95 °C and for 20 s at a primer specific annealing temperature. At the end of the annealing and the dissociation program, the fluorescence was measured at a wavelength of 530 nm. Excluding the measurement of nonspecific PCR products and of primer dimers and to determine true amplification, the dissociation program was run at 95 °C for 1 min, followed by one cycle starting with 30 s at 55 °C and ending with 30 s at 95 °C. Each primer pair showed a distinct peak in the dissociation curve ruling out the amplification of unspecific products or primer-dimer formation, and this was supported by Sanger sequencing a PCR product of each primer. Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ), Beta 2-microglobulin (ß2M), and ribosomal protein L8a (RPL8a) were used as reference genes. Reference genes were tested for stability using the geNorm software. The geomean of the reference gene copy numbers per μL was divided by the copy numbers per μL of the target gene for normalization.

2.4. Statistical analyses

All statistical analyses were performed using R (version 4.2.1). Shapiro-Wilk and Levene's tests were used to evaluate data normality and homogeneity of variances respectively. For the contaminant data, a Mann-Whitney *U* test was used to check for differences in concentrations between groups (sex and age). To test the difference in the mRNA expression levels between tissues, sexes and age classes, the data was log-transformed after which a Student's t-test was applied. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Trace elements concentrations in liver tissue

Among the three trace elements, Cd showed the lowest mean concentration (Table 1), while no significant difference between concentrations of the three trace elements were found in liver. For Hg and Se, adults had significantly higher concentrations compared to sub-adults, but Cd concentrations were similar between adults and sub-adults (Fig. 2). Sex-related differences were found for Cd with females having the highest concentrations (Fig. 3). Selenium to mercury molar ratio (Se:Hg) was 1.5 and 2.1 in adults and sub-adults, respectively (Table 1) while no significant correlation was found between trace elements and mRNA transcript levels.

3.2. Gene expression biomarkers

Amongst the genes involved in xenobiotic biotransformation, *ARNT* and *PPARα* levels were significantly higher in blubber than in blood ($p. < 0.05$) (Fig. 4). Contrastingly, for the immune relevant cytokines, mRNA transcript levels of pro-inflammatory cytokine *IL-2* were higher in blood than in blubber, while anti-inflammatory cytokine *IL-10* was more highly expressed in blubber than in blood (Fig. 5).

Transcript levels of endocrine related genes *ESR1* and *TRα* were significantly higher in blubber than in blood (Fig. 6). The acute phase protein *HSP70* mRNA transcript levels were significantly higher in blood than in blubber (Fig. 7).

No age or sex related differences were found in transcript levels of all investigated genes.

Table 1
Concentrations (μg/g ww) of trace elements (Cd, Hg and Se) in the liver of ringed seals (n = 16). Data are given as Mean ± SD (Min - Max).

	Cadmium	Mercury	Selenium	Molar ratio Se:Hg
Adults	2.8 ± 2.4 (0.4–7.5)	6.3 ± 11.1 (0.9–39.0)	3.9 ± 4.1 (1.1–15.6)	1.5
Sub adults	2.2 ± 1.5 (0.5–5.2)	2.8 ± 1.5 (1.5–5.6)	2.4 ± 0.7 (1.7–3.7)	2.1
Total	2.5 ± 2.0 (0.4–7.5)	4.8 ± 8.3 (0.9–39.0)	3.3 ± 3.1 (1.1–15.6)	1.8

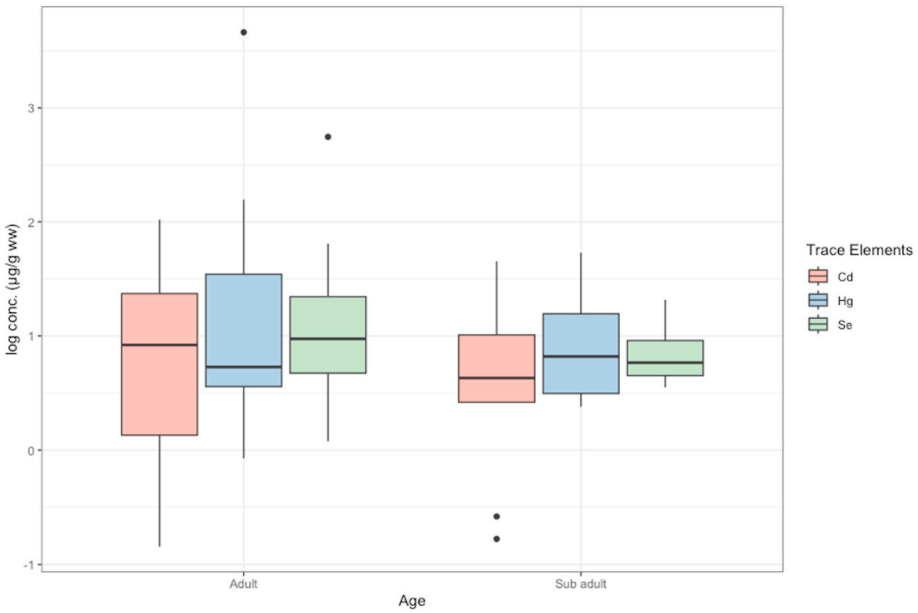

Fig. 2. Concentration (µg/g ww) of trace elements (cadmium (Cd), mercury (Hg), selenium (Se)) in different age class (Adult and Sub adult) of ringed seals from Northwest Greenland in 2018.

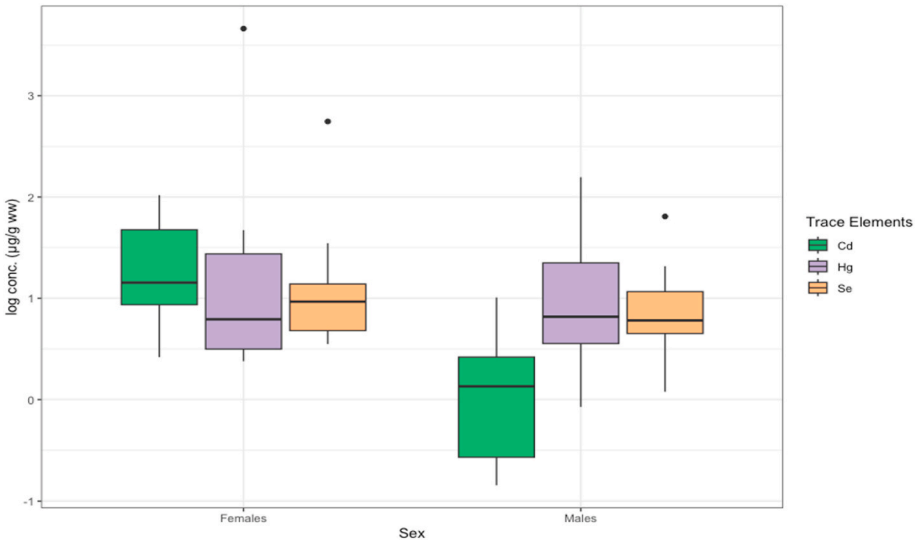

Fig. 3. Concentration (µg/g ww) of trace elements (cadmium (Cd), mercury (Hg) and selenium (Se)) in different sexes (Females and Males) of ringed seals from Northwest Greenland in 2018.

4. Discussion

4.1. Concentrations of trace elements

The trace metal concentrations measured in this study were in a similar range as those of ringed seals from Alaska, two times lower than in ringed seals of Sachs Harbour, Canada, and ten times lower than those of Baltic ringed seals, except Cd which was five-fold higher in the present study in Greenlandic seals (Dehn et al., 2006; Houde et al., 2020; Nyman et al., 2002). While a decrease in Hg concentrations in Baltic ringed seals in the last decade was observed (Boyi et al., 2022), Hg and Se concentrations measured in the present study were twice as high as previously measured in ringed seals from Northwest Greenland (Dietz et al., 1996; Rigét et al., 2012). Meanwhile, the concentration of Cd was lower (Dietz et al., 1996). Our results indicate a striking, on-going increase of Hg and Se concentrations in Arctic biota. Adult ringed seals showed higher Hg concentrations in their liver than sub-adults, reflecting the bioaccumulation of Hg over a lifetime (Das et al., 2002; Kakuschke and Prange, 2007; Ciesielski et al., 2010; Lyytikainen et al., 2015). Greenland ringed seals consume fishes like polar cod and sculpins with Hg concentrations above health effect thresholds (Dang et al., 2017; Dietz et al., 2019). Because the main source of trace elements for ringed seals is their diet, high concentrations of Hg in their tissue probably reflects biomagnification across the food chain (Dietz et al., 1996; Riget et al., 2007). Cd concentration was the lowest of the three trace elements analysed. However, the mean Cd concentrations found in ringed seals from this study were five times higher than in ringed seals from the Baltic and Sable Island (Nyman et al., 2002), indicating that the diet composition of ringed seals in these two locations is different. Greenlandic ringed seals from the 1990s (Dietz et al., 1996) had higher Cd levels than the present study, pointing at a decreased consumption of Cd rich food since then, which likely results from less invertebrates and

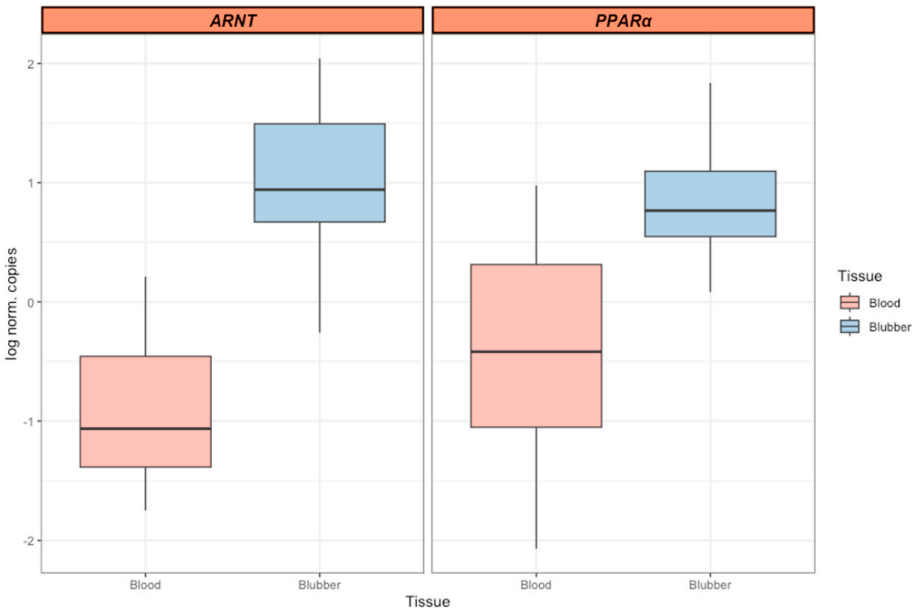

Fig. 4. Log transformed mRNA expression levels of *ARNT* and *PPARα* in blood and blubber of ringed seals from Northwest Greenland in 2018.

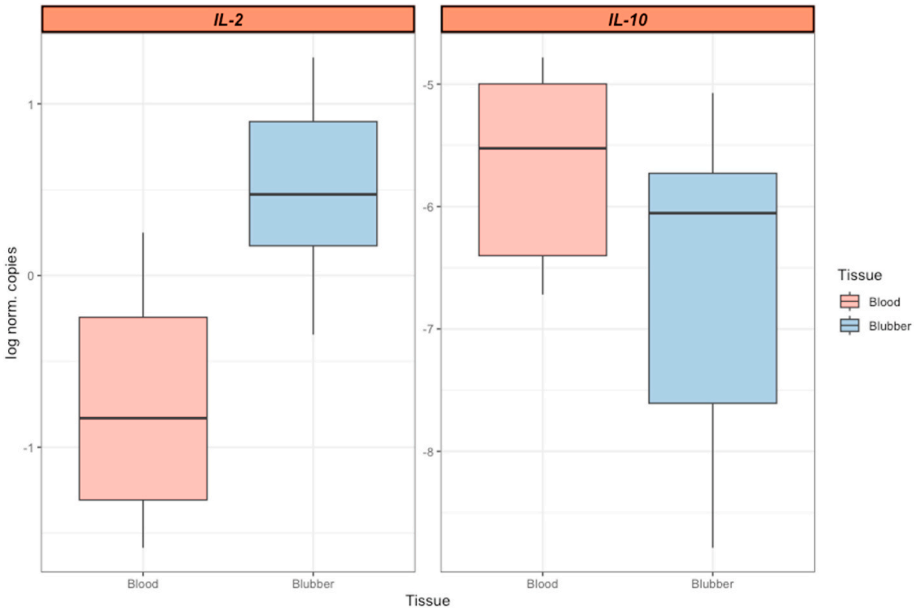

Fig. 5. Log transformed mRNA expression levels of cytokines (*IL-2* and *IL-10*) in blood and blubber of ringed seals from Northwest Greenland in 2018.

more fish, and also explains the higher Hg and Se concentrations in the ringed seals from 2018.

The main dietary source of Cd are invertebrates such as crustaceans and cephalopods, which Greenlandic ringed seals feed on to supplement their fish diet. These invertebrates contain high levels of Cd due to inhabiting a more saline environment (Szefer et al., 2002; Ciesielski et al., 2006). The over 1:1 selenium to mercury molar ratio in the investigated ringed seals depicts a coaccumulation between Se and Hg in this study and highlights Se as an antagonist of Hg (Nakazawa et al., 2011), hence the similar concentration patterns in our study. In the liver of marine mammals, organic Hg is demethylated via a unique Se mechanism, while inorganic Hg is immobilized and stored as mercuric selenide/Tiemannite (Dietz et al., 2000; Dehn et al., 2006; Riget et al., 2007; Loseto et al., 2008; Dietz et al., 2013). This mercuric selenide is an inert stable complex being generally non-toxic, protecting the animals from Hg poisoning (Wagemann et al., 2000; Nakazawa et al., 2011). Sex-related differences in concentrations were observed for Cd, pointing towards gestational and lactational transfer of trace elements from mother to pups as has been suggested in pinnipeds (Habran et al., 2011; Grajewska et al., 2019; Trukhanova et al., 2022).

4.2. Tissue specific gene expression

4.2.1. Xenobiotic related genes (ARNT and PPARα)

We found higher mRNA expression levels of xenobiotic related genes *ARNT* and *PPARα* in blubber than blood. The expression of *ARNT* is induced by environmental contaminants including organochlorine pollutants such as PCBs and dioxins (Chopra and Schrenk, 2011; Dietrich, 2016; Gardella et al., 2016). In long-lived marine mammals, the lipid-rich blubber tissue is the main storage site for these contaminants, where they can bio-accumulate over long periods of time (Tanabe et al., 1994; Debier et al., 2003; Nyman et al., 2003). Also, *ARNT* is involved in

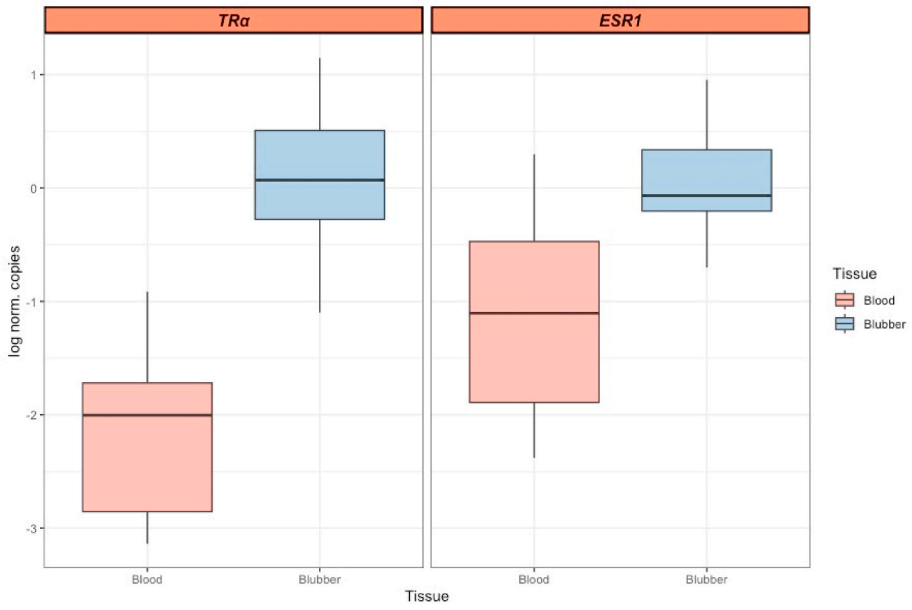

Fig. 6. Log transformed mRNA expression levels of *TRα* and *ESR1* in blood and blubber of ringed seals from Northwest Greenland in 2018.

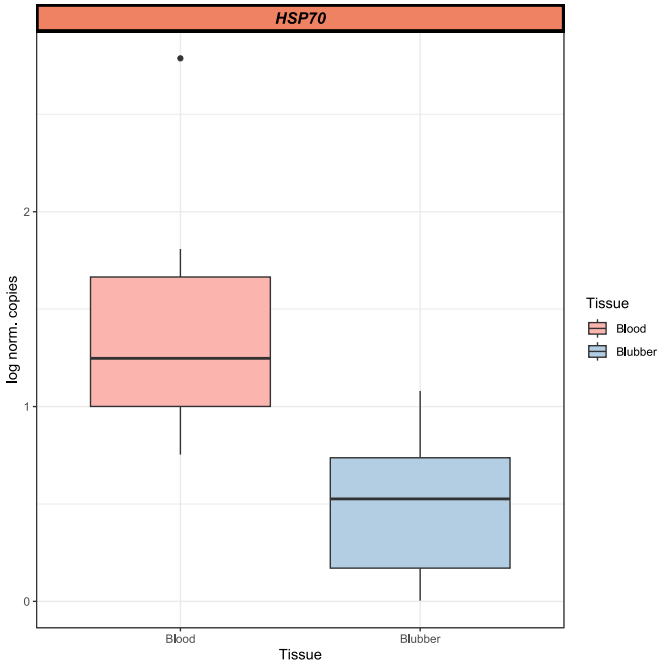

Fig. 7. Log transformed mRNA expression levels of *HSP70* in blood and blubber of ringed seals from Northwest Greenland in 2018.

adipogenesis and lipid metabolism by regulating glucose uptake and expression of glucose transporter genes (Shimba et al., 2005; Lee et al., 2011). In our study, no relationship between *ARNT* and trace element concentration was observed. The mechanism by which metals affect the transcription of *ARNT* and its dimerization partner *AHR* is not fully understood. However, in-vitro studies have shown that heavy metals such as Hg and Pb were able to induce expression of *AHR*-dependent genes such as cytochrome P450s in a concentration-dependent manner ((Korashy and El-Kadi, 2004; Korashy and El-Kadi, 2005). This modulation of *ARNT* and related genes by metals is however post-transcriptional (Elbekai and El-Kadi, 2007). Therefore, the protein levels of ARNT may show a better relationship with the trace element concentrations instead of the mRNA transcript levels. Similarly, Boyi et al., 2022 found no relationship between *ARNT* mRNA transcript levels and Hg concentrations in tissues of ringed seals from the Baltic Sea.

PPARα mRNA levels in pinnipeds were also shown to be regulated by organochlorines and per- and polyfluoroalkyl substances (PFASs) (Lehnert et al., 2016; Routti et al., 2019; Luhmann et al., 2020). *PPARα* is normally abundant in tissues with active fatty acid conversion, such as liver and heart, and functions in lipid metabolism and pro-inflammatory reactions (Feige et al., 2006; Lefebvre et al., 2006; Grabacka et al., 2021; Xu et al., 2022). The higher levels seen in the blubber could reflect their storage location for lipids. In ringed seals from the Baltic Sea, higher mRNA expression levels of *PPARα* were expressed in blubber than in liver and blood when those animals were not fasting (Boyi et al., 2022). During fasting, lipids are mobilised to other parts of the animal's body, thus increasing the expression levels of *PPARα* in other tissues (Desvergne et al., 2006; Khudyakov et al., 2017). mRNA expression levels of *PPARα* in Baltic ringed seals were related to fasting and moulting rather than pollutants (Castelli et al., 2014). *PPARα* was also suggested as a potential biomarker of diet in Antarctic seals (Lehnert et al., 2017).

4.2.2. *Acute phase protein (HSP70)*

In ringed seals investigated in this study, higher *HSP70* mRNA expression levels were found in the blood than in blubber. As the blood is a circulatory tissue and one of the main targets of oxidative stress (Mohanty et al., 2014; Maurya et al., 2015; Fujii et al., 2021), high *HSP70* expression is expected due to its higher metabolic rate compared to the relatively inert blubber tissue. Higher *HSP70* expression levels were measured in healthy grey seal pups compared to fasting individuals with lower metabolic rates (Bennett et al., 2014). The differences in mRNA levels of *HSP70* between blood and blubber of ringed seals could also be due to the different antioxidant capacities of these tissues. Blood has a more extensive antioxidant system (Fischer et al., 2005). There was no significant relationship between *HSP70* and trace element concentrations in the investigated ringed seals. Normally, *HSP70* mRNA expression levels are increased due to exposure to heavy metals to detoxify the metals or protect the tissues from detrimental effects (Nadeau et al., 2001; Deane and Woo, 2006). The lack of significant relationships between *HSP70* levels and trace element concentrations in our study could be because the concentrations of the trace elements were not high enough to elicit the adequate response or countering physiological effects. In harbour seals from the Wadden Sea, a positive correlation between trace elements in the blood and *HSP70* was

observed (Lehnert et al., 2016). In *in-vitro* fish studies, exposure of hepatocytes and fibroblasts cells to Cd caused an upregulation of *HSP70* mRNA expression (Boone and Vijayan, 2002; Deane and Woo, 2006).

4.2.3. Endocrine genes (TRα and ESR1)

ESR1 is an important growth mediator associated with adipose tissue metabolism (Anwar et al., 2001; Jia et al., 2015), this could be reflected in the higher *ESR1* mRNA transcript level in the blubber than in blood. It functions in regulating genes involved in growth, organ development and reproduction. In the present study, there was no significant difference in *ESR1* mRNA levels between the age classes. However, *ESR1* methylation, which results in the downregulation of *ESR1* gene expression increases with age (Pinzone et al., 2004). There was no relationship between *ESR1* mRNA transcript levels and trace elements measured in this study. But heavy metals such as Cd are known to have endocrine disruptive effects by mimicking estradiol and binding to the hormone-binding domain of the receptor whilst blocking the binding of estradiol (Stoica et al., 2000). Cd decreased *ESR1* protein and mRNA expression levels in breast cancer cells (Garcia-Morales et al., 1994; Lubovac-Pilav et al., 2013). *ESR1* upregulation could also influence *AHR*-regulated genes due to cross-talk between the two receptors (Matthews et al., 2007). In harbour seals and ringed seals from North America, significant positive correlations were found between *ESR1* mRNA transcript levels and PCBs concentration (Brown et al., 2014; Noël et al., 2017), whereas no relationship was found with contaminants in ringed seals from the Baltic Sea (Boyi et al., 2022).

Thyroid hormone is involved in the proliferation of adipocytes and lipid accumulation. The high levels of *TRα* expressed in the blubber of ringed seals in this study might be due to a disruption of the thyroid hormone signalling pathway. This disruption could compromise the blubber's integrity by affecting metabolism within adipocytes, resulting in thermoregulation and buoyancy problems in pinnipeds Tabuchi et al. (2006); Noël et al. (2017); Boyi et al. (2022) found higher levels of *TRα* mRNA transcript levels in the blood of ringed seals and related it to higher levels of circulatory thyroid hormones in the blood. Thyroid hormones act through thyroid hormone receptors to regulate metabolism, development, and growth, and a negative correlation was observed between *TRα* and thyroxine (TT4) in harbour seals (Tabuchi et al., 2006). Heavy metals such as Cd and Hg can bind to thyroid hormone receptor and block its activity, disrupting the expression of thyroid hormone responsive genes. The present study showed no relationship between *TRα* and trace elements investigated. In ringed seals from Labrador, no relationship between *TRα* mRNA levels and PCBs concentration was found (Brown et al., 2014) while in harbour seals, a positive correlation with PCBs was observed (Tabuchi et al., 2006). Exposure to trace elements such as Cd and Hg in fishes has resulted in downregulation of TRα gene expression (Arukwe and Jenssen, 2005).

4.2.4. Cytokines (IL-2 and IL-10)

The mRNA transcript levels of the pro-inflammatory cytokine *IL-2* were two-fold higher than the levels of anti-inflammatory cytokine *IL-10* in both tissues investigated in the present study. At the beginning of an infection, *IL-2* regulates inflammatory responses while *IL-10* suppresses T-cell function, preventing inflammatory and auto-immune reactions (Beineke et al., 2007; Weirup et al., 2013; Lehnert et al., 2017). They function as antagonists and so show reciprocal expression patterns. Higher levels of *IL-2* in the blubber than blood reflect a chronic infection state because of anthropogenic factors such as increased pollution and ocean noise (Altemus et al., 2001; Unal et al., 2018). A continued high expression of *IL-2* results in reduced immunocompetence, leaving the animals vulnerable to other infections and epizootics (Ross, 2002). The *IL-2* secreting T cells are also abundant in adipose tissue. Our study showed no relationship between the mRNA expression levels of cytokines measured and trace elements concentrations. This indicates that the immunological changes observed in the investigated seals might not be directly related to the selected trace elements. Also, the concentration of the investigated trace elements might not be high enough to result in immune suppression. *IL-2* expression levels were downregulated in seal lymphocytes exposed to methyl-mercury (Das et al., 2008) and seals in rehabilitation (Fonfara et al., 2008; Weirup et al., 2013), while there was a positive correlation between *IL-10* expression and Hg concentration in harbour seals from the North Sea and Ross seals (Lehnert et al., 2016, 2017). Also, in blubber of killer whales from Canada, PCBs concentrations were positively correlated with *IL*-10 mRNA expression (Buckman et al., 2011). Trace elements are not the only chemicals capable of disrupting cytokine expression. The cytokine expression levels of this study may be related to other environmentally important chemicals such as PCBs and PBDEs or other trace elements not measured in this study.

5. Conclusions

The present study showed that the levels of Hg and Se are increasing in Greenlandic ringed seals, especially in adults. Tissue-specific gene transcript levels highlighted blubber as a lipophilic store of contaminants having higher expression levels of contaminant-related genes and blood as an important oxidative stress target with higher levels of stress-related markers. However, gene transcription patterns were not directly linked to the selected trace elements, indicating that other factors besides the investigated metals might contribute to the observed biological effects. In the future, metal-induced markers such as metallothionein or RNA sequencing to identify differentially expressed genes modulated by the selected trace elements could be a promising research option. Nonetheless, the gene transcript levels provided by this study are the first for this population. They would serve as baselines and early warning signs for the health effects and biological impact of anthropogenic stress on the cellular level of aquatic wildlife.

Ethics statement

Ethical review and approval were not required for this study since the tissue samples were collected from the animals during the subsistence Inuit hunt in Greenland.

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CRediT authorship contribution statement

Joy Ometere Boyi: Writing - review & editing, Writing - original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Christian Sonne:** Writing - review & editing, Resources, Funding acquisition. **Rune Dietz:** Writing - review & editing, Resources, Funding acquisition. **Frank Rigét:** Methodology, Investigation, Data curation. **Ursula Siebert:** Writing - review & editing, Supervision, Resources, Project administration, Funding acquisition. **Kristina Lehnert:** Writing - review & editing, Writing - original draft, Visualization, Validation, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117839.

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