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Trace elements in blood of Baltic gray seal pups (*Halichoerus grypus*) from the Gulf of Riga and their relationship with biochemical and clinical parameters

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ABSTRACT

Trace elements are pollutants of both natural and anthropogenic origin which can influence negatively on ecosystem and wildlife health. We evaluated trace element in blood samples of gray seal (*Halichoerus grypus)* stranded in the Gulf of Riga and their influence on their health status through hematological and biochemical profiles. Zn showed the highest levels followed by Cu > Se > Pb > THg > As. Cr and Cd were not detected. Most trace element levels were generally comparable to those reported in seal species; however, high Pb values were observed in those sample showing detectable concentrations (<0.046–257.6 μg/kg ww). Significant positive correlations were found between trace elements concentrations and various biochemical parameters, including Se-ASAT, Se:Hg-ASAT, Cu-TP, Cu-ALB, Cu—Ca, Zn-ALAT, ZN-LDH, Zn—P, Zn-Segment neutrophils, and Pb-CK. Nevertheless, most relationships were not strong enough ($p > 0.04$) to assume a toxicological implication. Despite its limitations, this information could serve as the baseline for future research.

1. Introduction

Environmental pollution represents one of the main threats to global ecosystems conservation (Brusseau et al., 2019; Dietz et al., 2013). Pollutants are not homogeneously distributed in time and space and thus considerable regional and temporal variations in their concentration occur (Das et al., 2003). Metals and metalloids are persistent pollutants of natural and anthropogenic origin which are widely distributed in the environment (Selin, 2009; Tchounwou et al., 2012). Although some of these compounds are considered essential elements with biological functions in animals (e.g. selenium (Se), copper (Cu), and zinc (Zn)), prolonged or high-dose exposures can have detrimental health effects as well. On the other hand, trace elements such as lead (Pb), cadmium (Cd), arsenic (As), or mercury (Hg) can produce harmful even in low concentrations (Das et al., 2003; Polizzi et al., 2017). As non-essential elementals cannot be degraded biologically or chemically, they can only be modified or transformed from one oxidation state to another or from one organic complex to another (Pineda and Rodríguez, 2015). Once trace elements are absorbed, they are incorporated to the blood steam, from which they are distributed to different tissues and organs according to their psychochemical characteristics. Thus, their distribution and accumulation in the different organs differs according to the trace element but also to their chemical form (Bustamante et al., 2004; Das et al., 2003). The degree of accumulation of these trace elements in biota will depend on their accumulation rate and their elimination rate from the body (Ali et al., 2019). Some of them like Hg can bioaccumulate and biomagnify within the food webs especially affecting those organisms in the upper position like humans and marine mammals (Ali et al., 2019; Dietz et al., 2021; Jędruch et al., 2019).

The Baltic Sea is among the marine ecosystems with the highest reported pollutant concentrations globally (HELCOM, 2018a; Leppäranta and Myrberg, 2009). It is a shallow inland sea (mean depth 54 m) with a scarce water exchange with the North Sea (ICES, 2018). These characteristics combined with the discharge of five high-flowing rivers in the

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area contribute to the accumulation of environmental pollutants of agricultural, urban and industrial origin (Butrimavičienė et al., 2018). These conditions are even more pronounced in the Gulf of Riga, a semi-enclosed body of water within the semi-enclosed Baltic Sea. Notably, although the Gulf of Riga gathers several predisposing conditions for the accumulation of trace element pollution, this fact has been scarcely studied (HELCOM, 2010, 2018a).

Pinnipeds are identified as good sentinels due to certain intrinsic characteristics such as their longevity, large fat deposits, or their coastal habitat (Bossart, 2011). In addition, being in the upper trophic level, these species could be considered an important indicator of the health of ecosystems since they are more exposed to potentially toxic substances that biomagnify in the food web (López-Berenguer et al., 2020; Troisi et al., 2020). Since several of these pollutants can directly or indirectly affect humans, monitoring them through these sentinel animals can provide useful information to address efficient public health policies based on the "One Health" approach. The gray seal (*Halichoerus grypus*) is the most abundant pinniped in the Baltic Sea. The Baltic gray seal mating season is in February–March and parturitions in the middle of the following January (FAO Fisheries Series, 1978). The lactation period for the species lasts 21 days (Hall and Russell, 2018), followed by abrupt weaning, in which they fast until they learn to hunt. The pups are born with a white coat, called lanugo, which they lose after 1–2 months (Grajewska et al., 2019). Baltic gray seals show fidelity to the breeding colony and feeding areas, although some individuals make long migrations (Di Marzio, 2022; Graves et al., 2009; Oksanen et al., 2014). It is classified by the IUCN as "*least concern*" (LC), due to its increasing population (Bowen, 2016), after being heavily hunted at the beginning of the 20th century. However, the indicators of nutrition and reproduction do not reach the minimum threshold values to consider an optimal status of the population (HELCOM, 2018b, 2018c). Pollution has been proposed as possible cause for this situation (Das et al., 2003; Govind and Madhuri, 2014). The toxic effects of trace elements in vertebrates include immune system suppression, cancer, endocrine cycle disruption, failure of the reproductive process, reproductive impairment, and disruption of energy metabolism (Borrell et al., 2014; Polizzi et al., 2017; Sonne, 2010). Several studies have demonstrated that seals bioaccumulate high concentrations of trace elements in a variety of tissues, including blood (Gray et al., 2008; Kakuschke and Griesel, 2016). However, the pathophysiological effects of trace elements exposure in seals have not been well studied. Hematologic and blood biochemistry analysis can provide information about the functioning of different organs (Massanyi et al., 2014) and their alteration in response to different stressor, including trace elements (Berntssen et al., 2004; Rodrigues et al., 2010; Schaefer et al., 2011) Therefore, they are a valuable source on information for the assessment of pollutant-exposed wildlife's health. The interpretation of this information becomes complex due to the influence of factors such as the variable sensitivity to trace elements depending on the species (Dietz et al., 2013), the presence of concomitant pollutants, diseases and other stress factors, which could affect the parameters analyzed (Das et al., 2003).

In this work we used blood samples collected from stranded gray seal pups during the 2019 breeding period (January–April) to study blood trace element concentrations (Hg, Cd, Cu, Cr, Zn, Se, Pb, As) and their influence on the health status of the seals using hematology and serum biochemistry profiles.

2. Materials and methods

2.1. Species and sampling

During the breeding season (January–April), the Nature Conservation Agency (Republic of Latvia) constantly monitors the Latvian coasts, assessing the health status of the seals encountered. Animals in distress (mainly injured, malnourished pups) are carried to the facilities of Riga Zoo's gray seal rescue center, the only one in the Baltic republics (Fig. 1). After systematic veterinary control, blood samples are collected from the gray seal pups. A total of 16 pups, estimated age 1–2.5 months, were sampled during the 2019 breeding season (Table 1) for this work. Blood samples (3 mL in triplicate) were taken by puncture of the extradural intervertebral vein. Whole blood samples were immediately frozen and kept at −20 °C until trace element analysis. For hematological analysis

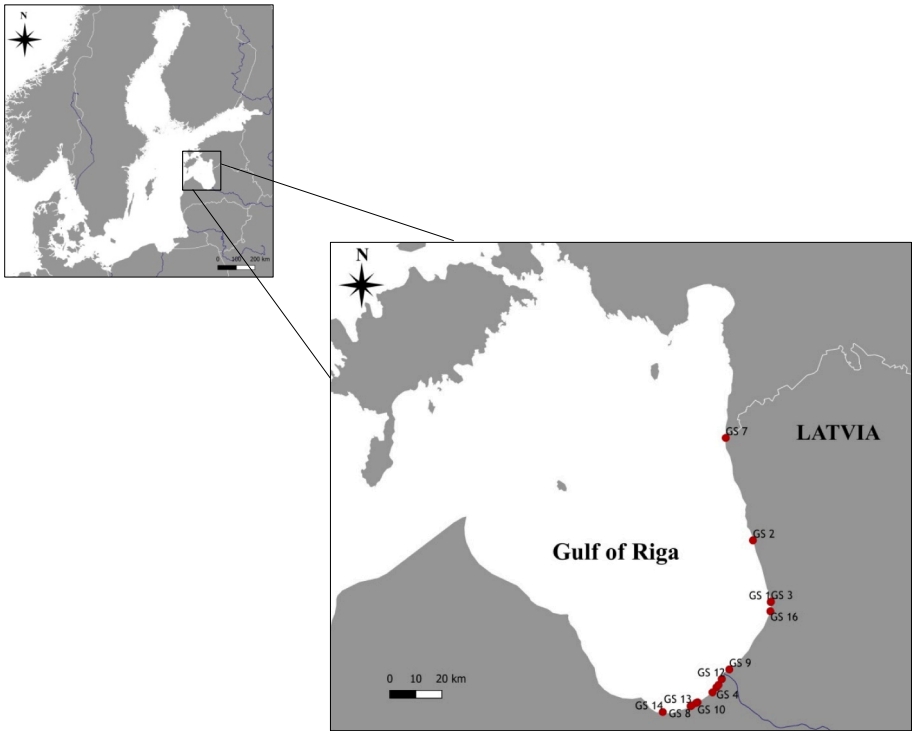

Fig. 1. Location of the 16 Gy seal pups stranded on the coast of the Gulf of Riga during the 2019 breeding period.

Table 1
Data collected (stranded date, location, sex, health status, and weight) of gray seal pups stranded on the coast of the Gulf of Riga during the 2019 breeding period.

Identification	Stranded date	Location	Sex	Health status	Weight (kg)
GS 1	07.03.2019	Saulkrasti	Male	Alive	12
GS 2	07.03.2019	Tūja	Male	Alive	11,8
GS 3	08.03.2019	Saulkrasti	Female	Death	10
GS4	14.03.2019	Vakarbuļļu	Male	Euthanized	9,5
GS 5	17.03.2019	Daugavgrīva	Female	Death	12
GS 6	17.03.2019	Vakarbuļļu	Female	Alive	11
GS 7	18.03.2019	Ainaži	Male	Alive	12
GS 8	23.03.2019	Dubulti	Female	Euthanized	11
GS 9	23.03.2019	Mangalsala	Female	Euthanized	12,6
GS 10	24.03.2019	Dzintari	Female	Alive	13
GS 11	24.03.2019	Dzintari	Male	Alive	15,8
GS 12	24.03.2019	Daugavgrīva	Female	Alive	14,2
GS 13	27.03.2019	Jūrmala	Female	Alive	9,9
GS 14	31.03.2019	Kanguri	Male	Alive	11,3
GS 15	14.04.2019	Lielupe	Male	Death	12,95
GS 16	16.04.2019	Saulkrasti	Male	Death	–

tubes with an anticoagulant reagent (EDTA) were used. For biochemical analysis, whole blood was centrifuged at 10000 rpm for 5 min to obtain plasma.

2.2. Trace elements analysis

The analysis of Cu, Zn, As, Se, Cr, Cd, and Pb was done following the trace element determination protocol for fish tissues with a microwave digestion system (MLS 1200 Mega, MPR 600/12, Milestone), and subsequent detection and quantification using inductively coupled plasma optic emission spectrometry (Agilent Technologies ICPMS. Model 7900). The Integrated Sample Introduction System (ISIS) was configured for discrete sampling. The Ultra High Matrix Introduction (UHMI) system was operated in robust mode. The 4th generation Octopole Reaction System (ORS4) was operated in helium (He) mode to reduce polyatomic interferences.

Then, 100 μL of blood were mineralized with 4.5 mL of concentrate nitric acid (Scharlau, for dithizone determinations) and 1 mL of hydrogen peroxide (30 %, stabilized for synthesis, Merck) in a closed Teflon PFA vessel. The curve of the calibration was prepared from a multi-element standard solution (CertiPUR, Merck). To check the purity of the reagents and contamination, two "blanks" (only reactive) were analyzed, and the accuracy was verified with the analysis of Standard Reference Material (Multi-element standard solution and Mercury ICP standard, both CertiPUR, Merck) in each run. After cooling, the solutions were transferred into 10 mL volumetric flasks. All Teflon vessels were washed with 3–4 mL ultrapure Milli-Q millipore (Gradient A 10) water. After that, it was filtered with a 0.45 μm filter and transferred to 15 mL threaded tubes identified with the sample code. The limit of detection, per trace element, was: 0.073 μg/kg (Cr), 0.292 μg/kg (Cu), 0.871 μg/kg (Zn), 0.023 μg/kg (As), 0.816 μg/kg (Se), 0.061 μg/kg (Cd) and 0.046 μg/kg (Pb).

The analysis of total Hg (THg) was performed by atomic absorption spectrophotometry using a direct Hg analyzer (DMA-80, Milestone®), with a detection limit of 0.005 ng. 0.2 ml of blood was used in nickel containers. The precision and accuracy of the method were previously evaluated using certified reference material (CRM, TORT-2, lobster hepatopancreas, National Research Council, Canada). A recovery percentage of 70 ± 0.7 % (mean ± standard deviation (SD)) and a coefficient of variation for repeatability of 1.11 % were obtained. In addition, controls were used for every three samples analyzed. The Hg detoxification process was also determined using the Se to Hg molar ratio. Se /Hg = ([Se] / 78.96) / ([Hg] / 200.59), where 78.96 g mol^{-1} and 200.59 g mol^{-1} are the atomic weight of Se and Hg, respectively.

2.3. Blood analysis

A complete blood cell count (CBC) was obtained using an automated hematology cell counter (Exigo Eos vet, Boule Medical AB, Sweden). Hematocrit was determined by centrifuging microcapillaries at 2200 g for 5 min and using a microhematocrit reader.

For the calculation of erythrocytes (RBC), mean corpuscular volume (MCV), leukocytes (WBC), thrombocytes (PLT), the impedance measurement method was used, and, for the calculation of hemoglobin (HGB), colorimetry was applied for the cyanide-free method 535 nm ± 5 nm. Hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated arithmetically. The differential count of white blood cells was performed manually by analyzing a blood smear with light microscopy (Nikon Eclipse E400, Leica DM5000 B), and staining was performed with the Bio-Optica MGG Quik Stain commercial kit.

Blood chemistry analysis was performed using the RX Daytona clinical chemistry analyzer (Randox UK, Japan). The following parameters were analyzed: Alanine-Aminotransferase, ALAT; Aspartate-Aminotransferase, ASAT; Lactate Dehydrogenase, LDH; Gamma-Glutamyl Transferase, GGT; Total Proteins, TP; Albumin, ALB; Urea; Creatinine, CREA; Cholesterol, CHOL; Creatine Kinase, CK; Calcium, Ca; Phosphorus, P; Sodium, Na and Potassium, K.

Table 2 shows the different techniques performed for the analysis of the biochemical parameters. The volume of serum used for each technique ranged from 2- to 16 μL.

2.4. Statistical analysis

Statistical analysis was performed using RStudio® for Windows® (version 4.1.0). The normality of trace elements concentration and the Se:Hg ratio, as well as the biochemical and hematological parameters, were verified with the Shapiro-Wilk test. Pearson's correlation analysis was used for normal variables, and Spearman's correlation analysis was used for variables that do not have a normal distribution. To compare categorical and numerical variables, the student's *t*-test and Wilcoxon's

Table 2
Biochemical parameters method and detection limit for each of the techniques.

Parameter	Method	Serum sample (μL)	Detection limit
ALAT	Alanine Aminotransferase Colorimetric IFCC Method,	13	9.7 - 3920.0 U/L
ASAT	Aspartate Aminotransferase Colorimetric IFCC UV Method	16	7.2 - 3750.0 U/L
LDH	Lactate Dehydrogenase Colorimetric DGKC Method	5	42.3 - 2875.0 U/L
GGT	Glutamyltransferase Colorimetric IFCC method according ECCLS	5	1.28 - 5580.0 U/L
TP	Total Protein Colorimetric Biuret Method	4	5.05 - 248.0 g/L
ALB	Albumin Colorimetric BCG Method	3	3.20 - 300.0 g/L
UREA	Urea Colorimetric Enzymatic Kinetic Method	4	0.87 - 113.0 mmol/L
CREA	Creatinine Colorimetric Kinetic Method with Picric acid	11	26.4 - 7821.0 μmol/L
CHOL	Cholesterol Colorimetric CHOD-PAP Enzymatic Endpoint Method	2	0.43 - 170.0 mmol/L
Ca	Calcium Colorimetric Method with Arsenazo III	3	0.09 - 6.45 mmol/L
P	Inorganic Phosphorus Colorimetric UV Method with phosphomolybdate complex	3	0.13 - 15.0 mmol/L
Na	Sodium Colorimetric Enzymatic Test	6	37.3 - 187.8 mmol/L
K	Potassium Colorimetric Enzymatic UV Method	5	2.46 - 11.2 mmol/L

test (parametric and non-parametric tests, respectively) were used. The level of significance was set at $\alpha = 0.05$.

3. Results

3.1. Trace elements analysis

Trace elements measured in blood samples of stranded pups of gray seals and the Se:Hg ratio are shown in Table 3. No significant differences in trace element concentrations were observed between the sexes. Therefore, males and females were pooled together. Detection rates were 100 % for THg, Se, Zn and Cu followed by Pb (44 %) and As with only one case detected. Cd and Cr were not detected in any of the analyzed blood samples. Zn showed the highest concentrations in all pups followed by Cu > Se > Pb > THg > As. Pb had the highest concentration of toxic trace elements measured in blood, with a mean of 45.5 μg/kg ww; however, it only appears in 7 of the samples analyzed. THg is the most abundant trace element although its concentrations are lower; being it is a mean of 22.2 μg/kg ww.

No statistically significant correlation was found between trace elements. No correlation was found between the weight of the seals evaluated and the concentrations of trace elements.

3.2. Blood analysis

Table 4 shows the results of the biochemical analysis and also CBC data. It should be noted that 2 samples with thrombocytopenia appeared, with platelet concentrations far below the mean (50×10^9/L and 67×10^9/L), possibly due to hemolysis that interfered with the results. There was also one seal that obtained an LDH concentration well below the rest (42.3 U/L).

Relationship between trace element concentration and blood parameters.

The analysis of the correlation between the different blood parameters and the concentrations of Cu, Zn, Se, THg, As, Cd, Cr, Pb, and the Se:Hg ratio is shown in Table 6. Regarding the biochemical analysis, ASAT was negatively correlated with the concentrations of Se and with the ratio Se:Hg (rho = −0.564; $p = 0.03$). Significant negative correlations were also found between TP (rho = −0.53; $p = 0.049$), Albumin (rho = −0.73; $p = 0.003$) and Calcium (rho = −0.60; $p = 0.02$) and the concentrations of Cu in the blood. Zn has a positive correlation with ALAT (rho = 0.54, $p = 0.045$) and negative correlations with LDH (rho = −0.59, p-value = 0.02) and P (rho = −0.61). Finally, a negative correlation appeared between CK and the concentration of Pb in blood (rho = −0.67; $p = 0.008$).

Concerning the study of hematological parameters, a negative correlation was observed between the WBC and Se (rho = −0.61; $p = 0.02$). A negative correlation was also found between segmented neutrophils and Zn (rho = −0.57; $p = 0.048$).

Table 4
Range, mean ± SD, and median of the biochemical* and hematological** parameters of the gray seals stranded on the coast of the Gulf of Riga in 2019.

Parameter	Range	Mean ± SD	Median
ALAT U/L	13.0–113.2	57.4 ± 27.8	52.4
ASAT U/L	14.8–423.5	140.7 ± 97.4	115.3
GGT U/L	3.9–148.2	33.7 ± 47.7	13.1
LDH U/L	42.3–9836.7	2962.0 ± 3233.7	1847.9
CHOL mmol/L	9.0–17.0	12.0 ± 2.4	11.7
TP g/L	56.1–86.8	68.4 ± 10.0	66.5
ALB g/L	23.1–43.5	33.1 ± 6.6	32.6
UREA mmol/L	14.6–21.50	17.7 ± 2.2	17.7
CREA μmol/L	36.8–121.0	57.8 ± 22.1	49.5
CK U/L	393.3–2473.8	1108.0 ± 630.3	839.1
P mmol/L	1.9–8.6	4.1 ± 2.2	3.3
Ca mmol/L	2.0–3.1	2.7 ± 0.3	2.7
K mmol/L	3.6–6.8	5.0 ± 0.8	4.8
Na mmol/L	130.9–166.8	152.7 ± 10.6	150.1
HCT %	41.4–62.3	52.6 ± 6.7	52.7
RBC ($\times 10^{12}$/L)	3.6–5.9	4.7 ± 0.7	4.6
MCV femtoL = 10^{-15} L	12.7–121.0	105.1 ± 28.07	113.3
HGB g/L	136–223	179 ± 24.44	180
MCH pg/cel	36.3–43.7	38.38 ± 1.92	37.9
MCHC g/L	321–361	340.2 ± 13.05	337
WBC ($\times 10^9$/L)	6.6–34.9	15.8 ± 6.89	15.6
Basophils ($\times 10^9$/L)	0–0.19	0.01 ± 0.05	0
Eosinophils ($\times 10^9$/L)	0–0.25	0.07 ± 0.09	0
Monocytes ($\times 10^9$/L)	0.37–2.84	1.48 ± 0.74	1.17
Segmented Neutrophils ($\times 10^9$/L)	4.42–29.32	12.23 ± 5.97	11.08
Neutrophils in band ($\times 10^9$/L)	0.07–2.09	0.88 ± 0.61	0.88
Lymphocytes ($\times 10^9$/L)	0.62–2.44	1.2 ± 0.53	1.09
PLT ($\times 10^9$/L)	50–535	317.4 ± 173.63	342

* $n = 15$, These parameters were not analized in one stranded animal for hemolyzed sample.

** $n = 14$, These parameters were not analized in two stranded animal for damaged sample.

Table 3
Values of Cu, Zn, Se, Hg, As, Cd, Cr, Pb, and Se:Hg ratio from blood samples of 16 Gy seal pups stranded on the coast of the Gulf of Riga during 2019 breeding season. Range, mean ± SD, and median (ww).

Seal code		Cu (μg/kg)	Zn (μg/kg)	Se (μg/kg)	THg (μg/kg)	As (μg/kg)	Cd (μg/kg)	Cr (μg/kg)	Pb (μg/kg)	Ratio Se:Hg
GS 1		581.0	1909.4	267.6	16.0	<0.023	<0.061	<0.073	257.6	42,4
GS 2		455.1	1034.0	206.2	14.1	2.7	<0.061	<0.073	<0.046	37,1
GS 3		863.1	1513.5	63.0	10.3	<0.023	<0.061	<0.073	<0.046	15,5
GS 4		468.1	1932.0	214.7	32.8	<0.023	<0.061	<0.073	<0.046	16,6
GS 5		394.3	3373.2	252.1	15.3	<0.023	<0.061	<0.073	<0.046	41,8
GS 6		488.7	2877.0	357.7	23.7	<0.023	<0.061	<0.073	<0.046	38,3
GS 7		636.5	2728.4	160.3	52.6	<0.023	<0.061	<0.073	123.7	7,7
GS 8		894.4	2096.4	495.4	5.7	<0.023	<0.061	<0.073	26.6	220,5
GS 9		763.6	1212.8	326.0	4.5	<0.023	<0.061	<0.073	<0.046	181,1
GS 10		699.0	2764.5	245.0	50.5	<0.023	<0.061	<0.073	149.4	12,3
GS 11		592.6	1750.6	170.4	38.3	<0.023	<0.061	<0.073	20.7	11,3
GS 12		580.5	2415.1	267.3	4.7	<0.023	<0.061	<0.073	143.4	144,0
GS 13		1178.1	2017.5	126.1	52.9	<0.023	<0.061	<0.073	6.7	6,0
GS 14		670.8	1707.1	107.0	9.9	<0.023	<0.061	<0.073	<0.046	27,3
GS 15		816.2	762.4	16.5	9.1	<0.023	<0.061	<0.073	<0.046	4,6
GS 16		801.6	1508.1	85.8	14.6	<0.023	<0.061	<0.073	<0.046	14,9
Totals	Range	394.3–1178.1	762.4–3373.2	16.5–495.4	4.6–52.9	<0.023–2.7	<0.061	<0.073	<0.046–257.6	4.6–220.5
	Mean ± SD	680.2 ± 201.9	1975.1 ± 714.9	210.1 ± 121.8	22.2 ± 17.5	–	–	–	45.5 ± 78.5	51.3 ± 67.4
	Median	653.7	1920.7	210.5	14.9	–	–	–	0	21.9

4. Discussion

4.1. Trace elements concentrations

Diet is the main route of exposure to trace elements for seals (Grajewska et al., 2019). For this study, seal pups recently rescued and in the post-weaning period (probably in prolonged fasting) were analyzed. They had a poor nutritional status (starvation). During this period, they also lose lanugo, which helps them excrete part of the trace elements that they have received from their mothers through the placenta and lactation (Grajewska et al., 2019; Habran et al., 2012). Although blood may reflect certain degree of bioaccumulation, it provides data on a limited temporal interval (Maceda-Veiga et al., 2015).

The absence of differences in trace element levels between males and females is consistent with the results of other seal studies, both in pups and adults (Griesel et al., 2008; Habran et al., 2013; Rea et al., 2020). We attribute to the homogeneity in weight the absence of significant differences on trace element concentrations between the study animals (Lian et al., 2021).

Zn showed the highest concentrations in all pups followed by Cu and

Table 5
Concentration values of Zn, Cu, Se, THg, Pb, As, Cd, and Cr in blood of pinnipeds. Mean ± SD, median, and range (μg/kg).

Sample	Area	Zn	Cu	Se	THg	Pb	As	Cd	Cr	Reference
Gray seal (pups) $n = 16$	Baltic Sea (Gulf of Riga)	1975.1 ± 714.9 1920.7 (762.4–3373.2)	680.2 ± 201.9 653.7 (394.3–1178.1)	210.1 ± 121.8 210.5 (16.5–495.4)	22.2 ± 17.5 14.9 (4.6–52.9)	104.0 ± 91.3 123.7 (6.7–257.6)	<LD[1]	<LD	<LD	Our study
Gray seal (pups) $n = 22$	Baltic Sea	–	–	– 700 (339–1052)	– 7 (6–11)	–	–	–	–	Grajewska et al., 2019
Harbor seal (adults) $n = 3$	Baltic Sea	– 2900 μg/L (2700–3160) μg/L	– 762 μg/L (703–807) μg/L	– 659 μg/L (585–773) μg/L	–	<LD	– 175 μg/L (148–633) μg/L		– 0.66 μg/L (0.59–0.88) μg/L	Kakuschke and Griesel, 2016
Harbor seal (adults) $n = 10$	North Sea	– 2670 μg/L (1100–3180) μg/L	– 711 μg/L (525–929) μg/L	– 809 (488–1063) μg/L	–	– – <LD–0.29 μg/L	– 209 μg/L (130–358) μg/L	–	– 0.41 μg/L (0.06–1.39) μg/L	Kakuschke and Griesel, 2016
Gray seal (pups) $n = 12$	North Sea	2900 ± 200 2900 (2600–3300)	850 ± 120 840 (730–1190)	2000 ± 700 2100 (700–2700)	21 ± 8 19 (13–36)	12 ± 5 11 (6–25)	–	<LD	2.5 ± 1.1 2.1 (1.7–5.3)	Habran et al., 2013
Harbor seal (pups) $n = 6$	North Sea	12,600 ± 520 – (12000–14,000)	4250 ± 510 – (3700–4800)	250 ± 20 – (150–310)	–	–	–	–	–	Kakuschke et al., 2009
Harbor seal (adults) $n = 28$	Wadden Sea	– – (2600–6200)	– – (527–1371)	– – (518–2261)	–	– – (<0.02–4.52)	– – (42–592)	– – (<0.12–5)	– – (1.52–84.9)	Griesel et al., 2008 Griesel et al., 2006
Harbor seal (pups) $n = 267$	California	–	–	–	190 ± 170 – (40–1130)	–	–	–	–	Lian et al., 2021
Harbor seal (pups) $n = 23$	California	–	–	–	93 ± 23 – –	–	–	–	–	Brookens et al., 2007
Harbor seal (pups) $n = 36$	California	–	–	–	166 ± 18 μg/L – (32–473) μg/L	–	–	–	–	Van Hoomissen et al., 2015
Harbor seal (≥1 year) $n = 73$	California	–	–	– – (480–1440)	– – (57–1190)	–	–	–	–	McHuron et al., 2014
Harbor seal (pups) $n = 79$	California	–	–	–	130 ± 130 – (20−1130)	–	–	–	–	McHuron et al., 2019
Elephant seal (pups) n = 22	California	2800 ± 200 2800 (2400–3200)	1060 ± 100 1030 (960–1440)	1700 ± 400 1800 (800–2400)	62 ± 17 62 (28–99)	22 ± 5 21 (15–32)	–	<LD	3 ± 1 3 (<2–6)	Habran et al., 2012
Elephant seal (adults) n = 6	Anctarctica	3130 ± 110 – –	1040 ± 40 – –	–	99.5 ± 15.4 – –	9.2 ± 2.15 – –	–	3.79 ± 1.23 – –	7.1 ± 1.79 – –	Baraj et al., 2001
Weddell seal (adults) n = 10	Anctarctica	371 ± 55.7 365 (319–502)	371 ± 180 323 (195–837)	233 ± 78.6 215 (137–440)	10.8 ± 7.10 11.3 (0–21.8)	0.21 ± 0.36 0.02 (0–0.91)	46.2 ± 23.5 44.7 (9.43–96.4)	0.62 ± 0.98 0.36 (0–3.2)	379 ± 43.6 357 (332–458)	Gray et al., 2008

[1] LD = limit of detection.

Se, in accordance with previous studies (Habran et al., 2012; Kakuschke et al., 2009; Kakuschke and Griesel, 2016). The Zn and Cu concentrations observed in the analyzed blood samples were similar to those reported by other authors in locations close to our study (Table 5). In the case of Se, our results were slightly lower than levels found from other investigations on seal pup blood (Table 5). Because of the implications of Se in the possible detoxification of Hg, it would be ideal to study it from this approach.

THg level in gray seal pups' blood samples was detected in the same range than other seal species from the North Sea and other areas of the Baltic Sea (Grajewska et al., 2019; Habran et al., 2013), but lower compared to the levels detected in seals from California (Table 5). It should be recalled that the latter is an area with high sources of Hg contamination (natural and anthropogenic) (Di Marzio et al., 2019). Although THg was detected in all samples from Riga Gulf, the maximum level detected was 52.91 μg/kg ww, lower than the toxic threshold established for THg in pinniped's blood (200 μg/kg ww; Li et al., 2020). Various studies have postulated a dilution effect of Hg concentrations in biota in an environment with a high overproduction of organic matter (Driscoll et al., 2012; Soerensen et al., 2016). However, more studies are required to evaluate such a model. Although the THg level detected in our study could be influenced by the eutrophication that the Baltic Sea has suffered for many years; it could also be caused by a lower pollution in comparison to other seas. In order to better interpret the data, it would be necessary to measure THg levels in primary producers or invertebrates and compare them with those of other seas.

Se has been suggested as a protective mechanism against Hg toxicity trough their linkage to form inert compounds. Then, a Se:Hg molar lower than 1 virtually indicates that there is not enough Se to counteract Hg toxicity (Martínez-López et al., 2019), which may impact animal health because of both the development of Hg-mediated toxicity and the absence of Se to perform other essential functions (Bjørklund, 2015). In our study, the majority of individuals showed a Se:Hg ratio largely above 1 (mean 51.3; Table 3), so Hg does not appear to be compromising the essential functions of Se. This ratio was lower than those values obtained in other studies with gray seal pups (142.8) (Grajewska et al., 2019) but above those provided for pups of other pinnipeds in California (mean 15.4; McHuron et al., 2014), a particularly polluted area.

On the other hand, although Pb was only detected in 44 % of samples (Table 5), we detected high levels of Pb in comparison to other studies. We hypothesize that these differences could come from higher Pb dietary concentrations in the mothers if the studied individuals, which could be mobilized to the milk during the lactation period (Habran et al., 2013).

The absence of Cd and Cr, as well as the extremely low detection rate for As (only in one sample), are compatible with the results of other studies of seal pup blood samples (Table 5). The age of the individuals studied, as well as the type of sample, could explain these results. For example, according to Habran et al. (2013), lanugo represents a better sample to assess maternal-fetal transfer of Cd.

No relationships were found between the different trace elements analyzed in gray seal pups in our study. However, it must be taken into account the existence on fluctuations of trace elements concentrations both for the mother and the seal pups according to their reproductive phase i.e. gestation/lactation/pos-weaning (Grajewska et al., 2019; Habran et al., 2013). Furthermore, the age and physiological variables of the dams are unknown, which could contribute to individual differences in trace elements concentrations in the offspring (Rea et al., 2020). The detection of these trace elements in recently weaned pups is evidence of trace elements transmission through the mother and its accumulation at such an early age.

4.2. Blood parameters

The scarce existent data on gray seal physiological blood parameters, together with the variation of such parameters according to several

Table 6
Correlation coefficient and significance between the concentration of THg, Se, ratio Se:Hg, Cu, Zn, Pb, As, Cd, and Cr in blood, and the biochemical and hematological parameters of the gray seals stranded on the coast of the Gulf of Riga during the 2019 breeding season.

Parameter	THg		Se		Se:Hg		Cu		Zn		Pb		As	Cd	Cr
Biochemistry	*p-Value*	rho [b]	*p-Value*	rho	*p-Value*	rho [b]	*p-Value*	rho	*p-Value*	rho	*p-Value*	rho[b]			
ALAT	0.64	0.13	0.62	−0.14 [a]	0.90	0.03	0.77	−0.08[a]	**0.045***	0.54[a]	0.98	−0.007	–	–	–
ASAT	0.11	0.44	**0.04***	−0.55 [b]	**0.04***	−0.54	0.97	0.01[b]	0.57	0.16[b]	0.23	−0.34	–	–	–
GGT	0.64	0.13	0.32	−0.28 [b]	0.27	−0.31	0.79	−0.07[b]	0.36	−0.26[b]	0.98	−0.007	–	–	–
LDH	0.06	−0.50	0.75	0.09 [b]	0.39	0.24	0.19	0.37[b]	**0.02***	−0.59[b]	0.60	−0.15	–	–	–
CHOL	0.66	0.12	0.73	0.10 [a]	0.65	0.12	0.37	−0.26[a]	0.25	0.32[a]	0.61	0.14	–	–	–
TP	0.80	−0.07	0.54	0.17 [a]	0.11	0.44	**0.049***	−0.53[a]	0.92	0.03[a]	0.66	−0.12	–	–	–
ALB	0.97	0.01	0.30	0.29 [a]	0.11	0.44	**0.003***	−0.73[a]	0.27	0.31[a]	0.50	0.19	–	–	–
UREA	0.79	−0.07	0.59	0.15 [a]	0.56	0.16	0.43	0.22[a]	0.67	0.12[a]	0.69	−0.11	–	–	–
CREA	0.71	−0.10	0.86	0.05 [b]	0.76	−0.09	0.22	0.35[b]	0.69	−0.11[b]	0.92	0.03	–	–	–
CK	0.27	−0.31	0.82	0.06 [a]	0.75	0.09	0.69	0.11[a]	0.28	−0.30[a]	**0.008***	−0.67	–	–	–
P	0.19	−0.36	0.90	−0.03 [b]	0.73	0.10	0.23	0.34[b]	**0.02***	−0.61[b]	0.13	−0.42	–	–	–
Ca	0.15	0.40	0.80	−0.07 [a]	0.70	0.11	**0.02***	−0.60[a]	0.28	0.30[a]	0.32	0.28	–	–	–
K	0.73	−0.10	0.68	0.11 [a]	0.49	0.20	0.59	0.15[a]	0.49	−0.20[a]	0.48	−0.20	–	–	–
Na	0.36	0.26	0.66	0.12 [a]	0.52	0.18	0.07	−0.49[a]	0.36	0.26[a]	0.20	0.36	–	–	–
Hematology															
RBC	0.87	−0.04	0.65	−0.13 [a]	0.93	0.02	0.55	−0.18[a]	0.46	0.22[a]	0.24	−0.34	–	–	–
HCT	0.99	0.005	0.84	−0.06 [a]	0.93	0.03	0.53	−0.19[a]	0.41	0.24[a]	0.28	−0.32	–	–	–
MCV	0.74	0.10	0.37	0.27 [b]	0.64	0.14	0.37	0.26[b]	0.32	0.29[b]	0.08	0.49	–	–	–
HGB	0.99	0.005	0.74	−0.10 [a]	0.49	0.21	0.44	−0.23[a]	0.58	0.17[a]	0.49	−0.21	–	–	–
MCH	0.61	−0.15	0.61	0.15 [b]	0.37	0.26	0.74	−0.09[b]	0.47	−0.22[b]	0.98	−0.005	–	–	–
MCHC	0.79	−0.08	0.64	−0.14 [a]	0.52	0.19	0.47	−0.21[a]	0.45	−0.22[a]	0.61	−0.15	–	–	–
WBC	0.59	−0.16	**0.02***	−0.61 [b]	0.14	−0.43	0.12	0.44[b]	0.07	−0.51[b]	0.27	−0.32	–	–	–
Basophils	0.44	−0.23	0.44	−0.23 [b]	0.91	0.03	0.80	−0.07[b]	0.80	−0.07[b]	0.40	−0.25	–	–	–
Eosinophils	0.98	−0.005	0.49	−0.20 [b]	0.55	0.17	0.39	−0.25[b]	0.54	−0.18[b]	0.91	−0.03	–	–	–
Monocytes	0.72	−0.11	0.80	−0.07 [a]	0.65	−0.13	0.78	0.08[a]	0.23	−0.35[a]	0.61	−0.15	–	–	–
Segmented neutrophils	0.31	−0.30	0.14	−0.43 [b]	0.30	−0.29	0.23	0.35[b]	**0.048***	−0.57[b]	0.32	−0.29	–	–	–
Neutrophils in band	0.79	0.09	0.09	−0.53 [a]	0.08	−0.47	0.22	0.40[a]	0.12	−0.48[a]	0.39	−0.28	–	–	–
Lymphocytes	0.15	0.42	0.21	−0.36 [b]	0.31	−0.29	0.85	0.05[b]	0.66	0.13[b]	0.72	−0.11	–	–	–
PLT	0.54	0.18	0.40	−0.25 [a]	0.30	−0.30	0.21	0.37[a]	0.53	−0.19[a]	0.55	−0.18			

a = Pearson's correlation coefficient, b = Spearman's correlation coefficient.
* Statistically significant correlation, where p-value <0.05.

physiological variables i.e. age, health status, stress, etc. (Desforges et al., 2016; Hall, 1998), difficult the interpretation of the obtained data. In general, biochemical parameters were similar to data from other studies in wild-caught gray seal pups. (Table 4; Erokhina et al., 2020; Hall, 1998). Only ALAT and particularly LDH exceeded the levels found by other authors in gray seal pups in the post-weaning period (57.4 U/L vs 16.7 U/L and 2962.0 U/L vs 1120.74 U/L, ALAT, and LDH respectively; Erokhina et al., 2020; Hall, 1998). The same occurred when comparing with adult individuals for some parameters (Nyman et al., 2003, adult mean ASAT 51 U/L, ALAT 30 U/L, GGT 9,5 U/L, LDH 1357 U/L vs our study, mean ASAT 140.7 U/L, ALAT 57.4 U/L, CGT 33.7 U/L, LDH 2962.0 U/L).

ALAT and ASAT can be increased due to hepatocellular injury (Barnett et al., 2007). According to Bossart et al. (2001), increases in ALAT are also associated with parasitism and liver and muscle trauma. Furthermore, ALAT is likely to be liver-specific in pinnipeds. However, ASAT may not be useful for detecting liver damage in seals, as handling can cause muscle damage leading to elevated levels of these enzymes (Bossart et al., 2001).

On the other hand, an increase in GGT may be indicative of obstructive liver disease and cholestasis; although it may also be increased by skeletal muscle injury (Bossart et al., 2001). LDH can be increased due to widespread tissue damage, as this enzyme is present in many body tissues (Barnett et al., 2007) Furthermore, due to its long half-life, LDH activity remains raised for some time after the initial damage (Kerr, 2001). It was postulated that in diving mammals, organs such as the brain or liver have adapted to long periods of hypoxia through the high activity of enzymes linked to the anaerobic mechanism such as LDH (Cantú Medellín, 2008).

Concerning CK, our detected levels were compatible with pups described by Barnett et al. (2007) suffering from some pathology (trauma, malnutrition, etc.). These authors described a CK mean value of 1073 U/L for these animals being our mean of 1108 U/L. In marine mammals, most increases in plasma CK occur due to skeletal muscle injuries associated with strenuous activity, transport, stranding, and seizures (Bossart et al., 2001). Therefore, it is reasonable that seals of our study will show such values in the mentioned enzymes (Table 4).

Regarding hematic values, our results (Table 4) largely agree with those found in the literature for gray seal pups (Hall, 1998; Hall et al., 2003; Lehnert et al., 2014). However, 40 % of our samples have slightly increased leukocytes and neutrophils (values $>18 \times 109$ leukocytes/L and $> 13 \times 109$ neutrophils/L) compared to the rest samples. An increase in the count of neutrophils and monocytes can be caused by different factors such as stress, inflammation, infection, or tissue damage (Kerr, 2001; Maceda-Veiga et al., 2015). Stranded animals like those in our study sometimes appear with infections or bites from other animals, which could contribute to these results.

Influence of trace elements on blood parameters.

Due to the absence of gray seal reference values for many of the parameters analyzed, the interpretation of the results had to be supported by the information available for other marine mammal species. The relationship detected in our study among Se and ASAT or that between the Se:Hg ratio and ASAT were not previously reported. This trend could have occurred due to the influence of other factors but also as a consequence of a low number of samples since there is a weak correlation (p-value $= 0.04$). Due to the protective effect of Se against the toxicity of Hg, we could consider the defense that Se provides to the liver since it seems to be the organ that accumulates the most Hg; however, this relationship must be supported by more research.

Schaefer et al. (2011) observed a positive relationship among band neutrophils and Hg concentrations in dolphin blood. Besides, in in vitro experiments, Lalancette et al. (2003) detected an alteration of phagocytosis due to exposure to Hg while Kerek et al. (2018) found a negative influence of Hg on red blood cells by lysis. However, we did not observe any relationship between THg and these blood parameters in pups of gray seals but a negative relationship between Se and leukocytes.

On the other hand, it is notable the negative relation between Pb concentrations and CK since data on the influence of Pb at this level was not previously reported. Studies in vitro suggested that Pb inhibits CK activity by interaction with their thiol group (Lepper et al., 2010). However, the toxicity of this trace element has been related mainly to alterations in the red blood cells, leading to anemia; in addition to kidney and liver damage and alterations in the nervous system (O'Hara and O'Shea, 2001).

Concerning essential trace elements, blood Cu binds to albumin and other proteins, which could explain the negative correlations between this trace element and TP and albumin. Tabrez et al. (2021), pointed out that trace elements can affect serum albumin and globulin concentrations but in this context, it does not seem to have any toxicological significance since Cu was not implicated as a potential toxin in marine mammals (O'Hara and O'Shea, 2001). About the relationship found between Zn and segmented neutrophils, studies in humans and rats showed that zinc modulates neutrophil functions by affecting neutrophil extracellular traps (NET) release and neutrophil degranulation, which could affect the immune system of animals. (Kuźmicka et al., 2021). The correlations found in this study between Zn and various biochemical parameters (LDH, ALAT and P) have not been previously reported on marine mammals. However, some studies have found associations between Zn and variations in the levels of LDH measured in muscle of fish (Kaustubh Bhagawati et al., 2016). Hu et al. (2020) found that serum zinc was positively associated with ALAT elevation in humans.

The appearance of alterations in the blood parameters mentioned in the previous section suggests that the seals stranded in the Gulf of Riga had some previous pathology. Although certain variations in blood parameters were strongly correlated with exposure to trace elements, it is difficult to link them to altered parameters in these individuals. In conclusion, we report information about trace elements levels in the blood of gray seals pups rescued in the Riga Gulf and their relationship with levels of biochemical and clinical parameters. The levels of non-essential trace elements in blood detected in our study do not seem to be of concern. However, their frequencies of detection as well as the Pb levels found in some individuals and the place where these species develop make it necessary to carry out continuous toxicological monitoring of these individuals. On the other hand, given the limited bibliography, our blood parameters data can be used as a baseline for future research, but many of the measured parameters are likely to vary naturally or may have been affected by rescue stress so it is also necessary to carry out more research.

CRediT authorship contribution statement

L Puchades and SE Gallego: formal analysis, writing-original draft and review; A di Marzio: conceptualization, resources, data curation, writing-review and supervision; E Martínez-López: Conceptualization, Data Curation, resources, funding acquisition, supervision, writing - review & editing.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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