



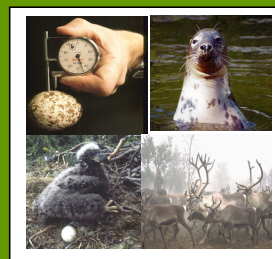
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A retrospective study of metals and stable isotopes in seals from Swedish waters

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Summary

Concentrations of Hg in Swedish marine systems are generally lower than in fresh water systems, but still often exceed environmental target levels. As top predators in many marine food webs, seals are exposed to heavy metals, like Hg and Cd, which are known to biomagnify with increasing trophic level in the food web. Therefore, studies of Hg concentrations over time are preferably conducted in relation to seal diet during the same period. By analysis of naturally occurring stable nitrogen and carbon isotopes it is possible to determine on which trophic level a certain organism is located and where it feeds. The aim of the present study was to try to establish background concentrations of Hg and some other metals in Swedish marine waters by analyses of seal bones from the 1840s and forward. Stable isotopes were analyzed in order to discover possible correlations between metal concentrations and trophic level, and changes over time in dietary intake of metals.

No significant change in metal concentrations was seen over time, except for a significant decrease in Zn concentrations in Baltic Grey seal. A significant difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between species and between Baltic and Skagerrak areas was discovered. No significant correlation between $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values and metals was found, however, the seal with the highest $\delta^{15}\text{N}$ value (Harbor seal) also had the highest Hg concentration. Even though the $\delta^{15}\text{N}$ values suggested an increase over time the increase was not statistically significant. No statistically significant change over time was found in $\delta^{13}\text{C}$ values.

It is important to keep in mind that this study was based on 44 individual seals spread over 126 years. Data on sex and age was lacking for almost half of the seals, which made statistical analysis on these parameters difficult to perform. Even though no obvious trends or differences in metal concentrations between areas and species were seen, the results from this study can serve as a base for further studies and provide valuable information for interpreting the actual contamination load in Swedish marine systems.

1. Introduction

1.1 Background

Heavy metals reach the marine ecosystem both from anthropogenic activities and from natural sources such as weathering of rocks. Even though metal discharges into the Gulf of Bothnia have decreased, contaminants are still stored in sediment and found in elevated concentrations in biota (Fant et al. 2001). Mercury (Hg) is a toxic element that easily transforms into different chemical forms and transports over long distances via the atmosphere. When deposited in aquatic ecosystems Hg bioaccumulates (Yang et al. 2008) and biomagnifies through the aquatic food web and it is known to affect productivity, reproduction and survival of coastal and marine animals (WHO 1989). High levels of mercury in Swedish freshwater systems are a well recognized environmental problem. In marine systems, concentrations are generally lower, but still often exceed the environmental target levels for prey species set by the EU at 20 ng/g fresh weight (2008/105/EC). Good Environmental Status within the Marine Strategy Framework Directive (MSFD) is defined as not exceeding target levels set to protect sensitive species from harmful effects. However, in order to allow for geographical differences within the EU, background concentrations can be added to the target level. In order to adjust the target level for Hg and other metals, knowledge of metal concentrations in seals during the last 160 years would provide valuable information for interpreting the actual contamination load in Swedish marine systems.

As top predators in many marine food webs, seals are exposed to numerous contaminants and hazardous substances (Frank et al. 1992; Olsson et al. 1992; Nyman et al. 2002; Routti et al. 2005). Some heavy metals, like Hg and cadmium (Cd) are known to biomagnify with increasing trophic level in the food web (Jarman et al. 1996). It has been shown that seals who feed on a higher trophic level contain higher concentrations of Hg than seals feeding on a lower trophic level (Jarman et al. 1996). Therefore, studies of time trends of Hg are preferably conducted in relation to dietary changes in the seals during the same period. Traditional dietary studies often use stomach analysis to describe animals feeding habits. However, analysis of naturally occurring stable isotopes is more and more being acknowledged as a powerful alternative, as stable isotopes reflect average dietary records and not just a dietary snapshot (DeNiro and Epstein 1981). By analysis of stable nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$) it is possible to determine on which trophic level a certain organism is located within the food web. Analysis of stable carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) can tell us about the spatial variation among or within species, for example if the animal is feeding inshore or offshore, benthic or pelagic, in marine, brackish or fresh water (Peterson and Fry 1987; Lawson et al. 2000).

Analysis of naturally occurring stable isotopes can be performed on most animal tissues, such as muscle, liver, feathers and blood. Different tissues have different protein turnover rates and thus reflect dietary records over different time spans (Greaves et al. 2004; Sinisalo et al. 2008). Tissues with high metabolic rates have a high protein

turnover (Welle et al. 1999), and it has been suggested that the low turnover rate of bone collagen averages out temporal variations from years to a lifetime in medium to large size animals (Chisholm et al. 1983; Tieszen et al. 1989; Lidén and Angerbjörn 1999; Dalerum and Angerbjörn 2005). As collagen can be extracted from old bones, it is also an ideal tissue to investigate long-term dietary changes (Ambrose, 1990).

1.2 Aim of the study

The aim of the present study was to try to establish background concentrations of mercury and some other metals in Swedish marine waters. In order to do this, metal concentrations were analyzed in seal bones, from different seal species and areas, from the 1840s and onward. The stable nitrogen and carbon isotope ratios were analyzed in order to discover possible correlations between metal concentrations and trophic level, or changes over time in dietary intake of metals. Differences between species and areas were taken into account.

2. Method

Bone samples from a total of 44 seals (Table 1) belonging to the Department of Vertebrate Zoology at the Museum of Natural History were analysed for naturally occurring stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and metal concentrations. The time series stretched from 1843 to 1969.

Table 1. Number of seals investigated.

	Harbor seal <i>Phoca vitulina</i>	Grey seal <i>Halichoerus grypus</i>	Ringed seal <i>Phoca hispida</i>	Total
Lake Ladoga, Russia	-	-	1	1
North Baltic	1	8	8	17
South Baltic	-	6	3	9
Skagerrak	16	1	-	17
Total	17	15	12	44

2.1 Stable isotope analysis

Since the measurements of stable isotopes should be made on as pure protein as possible (Dalerum and Angerbjörn 2005) lipids were extracted from the bone collagen. The isotope analysis was performed at the Stable Isotope Laboratory (SIL) at the Department of Geological Sciences, Stockholm University, using a Carlo Erba elemental analyzer (E1108 CHNS-O) connected to a Fison Optima isotope ratio mass spectrometer. The results are expressed as δ values as parts per thousand differences from a standard,

$$\delta X = \left[\left(\frac{R_{\text{SAMPLE}}}{R_{\text{STANDARD}}} \right) - 1 \right] \times 10^3$$

where X is ^{13}C and ^{15}N , and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The standards were air for nitrogen and Pee Dee Belemnite (PDB) for carbon.

2.2 Metal analysis

Seal bones were analyzed at ALS Scandinavia according to metal package M4, containing Arsenic (As), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Lead (Pb), Manganese (Mn), Mercury (Hg), Nickel (Ni) and Zinc (Zn). Analyses were conducted according to EPA-methods (modified) 200.7 (ICP-AES) and 200.8 (ICP-SMS), by solving 20 mg sample in 1 ml HNO₃ and then adding 9 ml MQ-water. Results were reported in ug/g dry weight.

2.3 Statistical analysis

For statistical analysis R version 2.12.1 (2010-12-16) was used, mainly ANOVA, Kruskal-Wallis and linear regressions.

3. Results and discussion

The mean metal concentrations and stable isotope values for species and areas for the whole time period were summarized in Table 2. For all graphs of metal concentrations over time, see Appendix 1. No statistically significant difference in metal concentrations between species within areas or between areas within species was noticed.

Table 2. Mean±SD for all metals over the whole time period, according to species and area, extreme values are included. Note that the two groups (species and area) contain the same 44 individual seals.

	n	Stable isotopes		Metals (ug/g) dry weight									
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Zn
<i>Species</i>													
Ringed seal	12	-19.2	14.5	242	0.08	0.07	2.11	3.34	0.19	8.59	376	6.92	259
		±0.89	±0.51	±821	±0.06	±0.04	±2.20	±2.39	±0.17	±5.17	±579	±6.29	±102
Grey seal	15	-17.2	16.6	4.65	0.09	0.05	1.12	4.12	0.12	3.38	621	6.99	200
		±0.92	±0.92	±9.61	±0.05	±0.03	±1.58	±4.08	±0.08	±1.22	±816	±10.7	±70
Harbor seal	17	-14.2	19.1	2.26	0.14	0.09	1.58	5.96	0.22	34.4	412	20.5	250
		±1.28	±1.17	±3.38	±0.15	±0.17	±1.73	±9.82	±0.30	±107.8	±532	±63.7	±71
<i>Area</i>													
Russia	1	-19.9	14.4	2850	0.05	0.05	3.23	2.87	0.18	12.3	343	12.1	330
North Baltic	17	-18.1	15.6	6.97	0.09	0.06	1.58	2.71	0.26	5.77	474	5.52	258
		±1.55	±1.73	±9.68	±0.07	±0.04	±2.06	±2.15	±0.31	±4.88	±747	±5.73	±83.3
South Baltic	9	-17.7	16.3	2.19	0.083	0.06	1.38	5.95	0.10	4.79	482	9.08	171
		±1.19	±1.55	±3.22	±0.05	±0.03	±1.69	±4.47	±0.06	±2.87	±732	±13.3	±77.4
Skagerrak	17	-14.3	18.9	1.46	0.14	0.09	1.56	5.92	0.14	34.4	476	20.6	242
		±1.34	±1.16	±1.40	±0.15	±0.17	±1.74	±9.85	±0.11	±108	±547	±63.7	±70.0

3.1 Metal concentrations and time trends

Fant et al. (2001) reported that in comparison with earlier studies on Baltic seals, the metal concentrations have remained at the same level since the 1980s. Frank et al. (1991) found no spatial differences in metal concentrations between Baltic areas and the Swedish west coast, except for Cr which was higher in Baltic harbor seals.

In this study, measured mean concentrations of Hg in the seals from northern Baltic were higher than levels from the other areas, but this difference was not statistically significant and could have arisen by chance. The only Harbor seal from the Baltic had the highest Hg level of all seals, reaching 1.3 $\mu\text{g/g}$. No significant change of Hg concentrations in seal bones was detected for the investigated time period (Fig. 1a). Further, Zn concentrations in the Ringed seal were the only metal that had a statistically significant change (decrease) over time (Fig. 1b). It is important to take into account that the statistics in this study are performed on means from values with a large temporal spread.

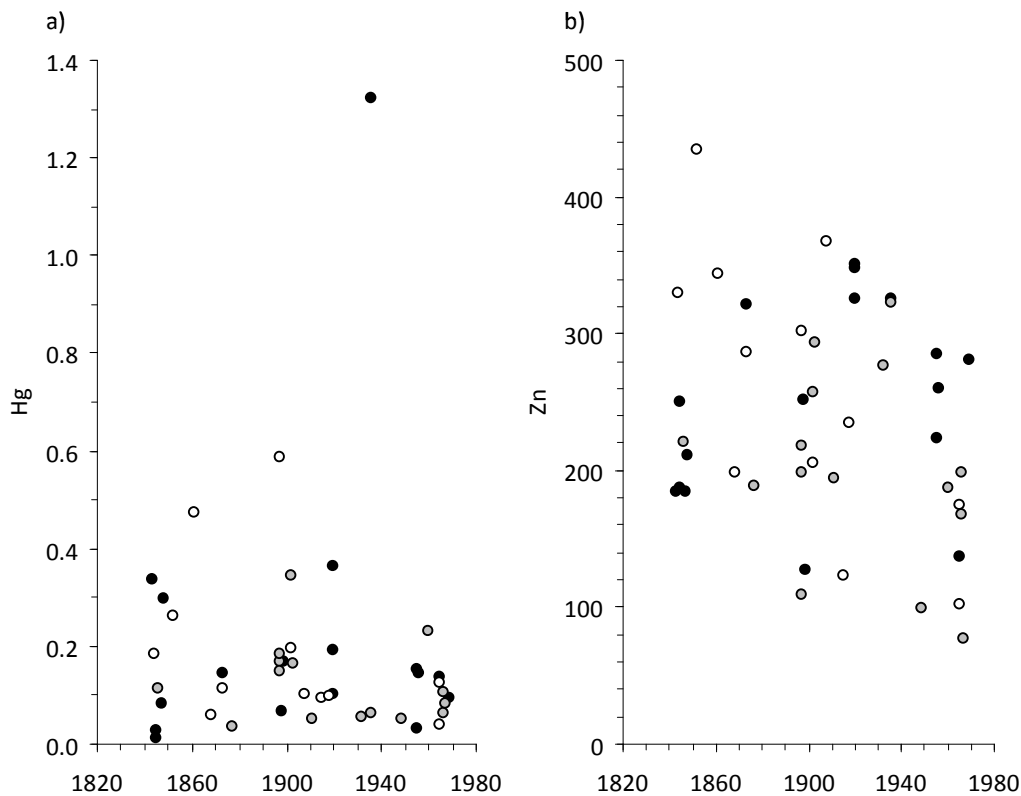


Figure 1. Concentrations ($\mu\text{g/g}$) of a) Hg and b) Zn in individual seal bones from Harbor seal (●), Grey seal (●) and Ringed seal (○, $p=0.007$, $R^2=0.69$) over time.

The Ringed seal from Lake Ladoga had a substantially higher As concentration (2.85 mg/g) than seals from the Skagerrak and the Baltic area. Lake Ladoga, today belonging to Russia, was at the time (yr 1844) shared between Russia and Finland. Except for the

Russian seal, seals from the northern Baltic had the highest As concentrations, however, the difference was not statistically significant. It is important to take into account that As has been used historically as a pest controller on museum animals and bones, why As contamination of the investigated bones is probable.

One Harbor seal from the Skagerrak area had the highest Pb concentration (266 ug/g), the highest Cu concentration (39.1 ug/g) and the highest Mn concentration (450 ug/g) of all seals from all species and areas. Another Harbor seal from the Skagerrak area had the highest concentration of Cd, reaching about 0.5 ug/g. The Cd concentrations in fish muscle and liver in the 80s (Swedish Contaminant Monitoring Program in Marine Biota (SCMPMB), 2010) are in a similar range as the concentrations in seal bones. However, many of the Baltic locations within the SCMPMB show increasing trends in Cd concentrations from the 80s and forward.

Between the 1840s and 1880s a possible decrease in Co concentrations was seen, maybe due to the cobalt mining decline in the 1860s, but the levels rose again in the 1900s and continued to fluctuate. Mn concentrations differed significantly between species with the extreme value included ($p < 0.01$), but not without it. No spatial or temporal trends regarding Cu were seen. The Cu concentrations in fish muscle and liver reported in the SCMPMB (2010) are slightly higher than the concentrations in seal bone in the present study.

The concentrations of Ni in seal bones in this study were high compared to levels in fish muscle and liver in the SCMPMB (2010). There was a peak in Ni levels around 1880-1900 that could have been due to the increased use of nickel in steel production.

Pb concentrations in liver of cod, perch and herring investigated in the SCMPMB (2010) are decreasing at the majority of the locations. The Pb concentrations in this study were higher than concentrations found in fish in the SCMPMB (2010). However, the concentration of Pb is known to be higher in bone tissue than in liver or muscle. Generally, there is a great variability in the accumulation of heavy metals among different tissues (Frank et al. 1992; Frodello and Marchand 2001; Garcia-Fernandez et al. 2009).

3.2 Stable isotopes and metals

A significant difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between species (Fig. 2) and areas, e.g. Baltic vs Skagerrak areas, was discovered (ANOVA, $p < 0.01$). No significant difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between northern and southern Baltic was found. In the present study, seals from early years were often lacking data on age and sex, resulting in a small test group.

Earlier studies have shown that there is a significant difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between seals from fresh and marine waters (Smith et al. 1996). It is suggested that seals from fresh water food webs have lower $\delta^{13}\text{C}$ values than seals from marine food webs, which corresponds with the results from this study. Smith et al. (1996) further claims that any enrichment in $\delta^{13}\text{C}$ from expected values would suggest a marine input to the diet. Generally seals from marine food webs tend to be more enriched in $\delta^{15}\text{N}$ than seals from fresh water food webs, mainly due to the more complex food webs in marine habitats.

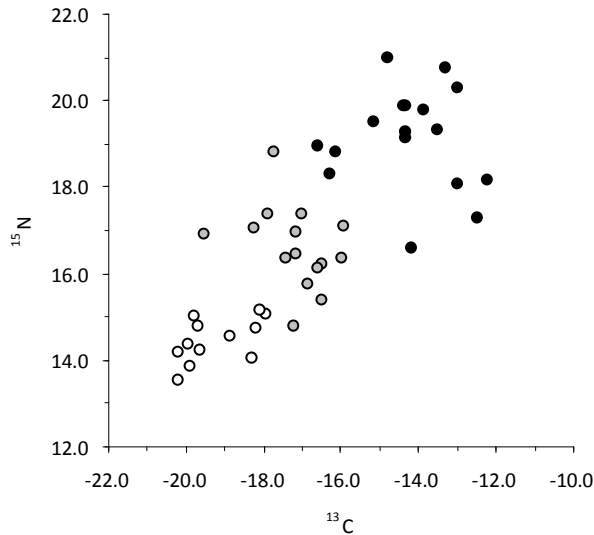


Figure 2. The relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for Harbor seal (●), Grey seal (◐) and Ringed seal (○).

In this study the difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ levels between species were significant. This could, at least partly, be explained by the different salinity (marine, brackish, fresh) in the species primary habitat, and thus the presence of different prey species. However, all three species are non-stationary, and in this study, there are no significant differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in seals from the northern and southern Baltic. In this study differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ levels are better explained by species than by area. The Ringed seal from Lake Ladoga in Russia had $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values that correspond well to its species counterparts in the Northern Baltic.

Even though the $\delta^{15}\text{N}$ values suggested an increase over time (Fig. 3a) the increase was not statistically significant. No statistically significant change over time was found in $\delta^{13}\text{C}$ values for the three species (Fig. 3b).

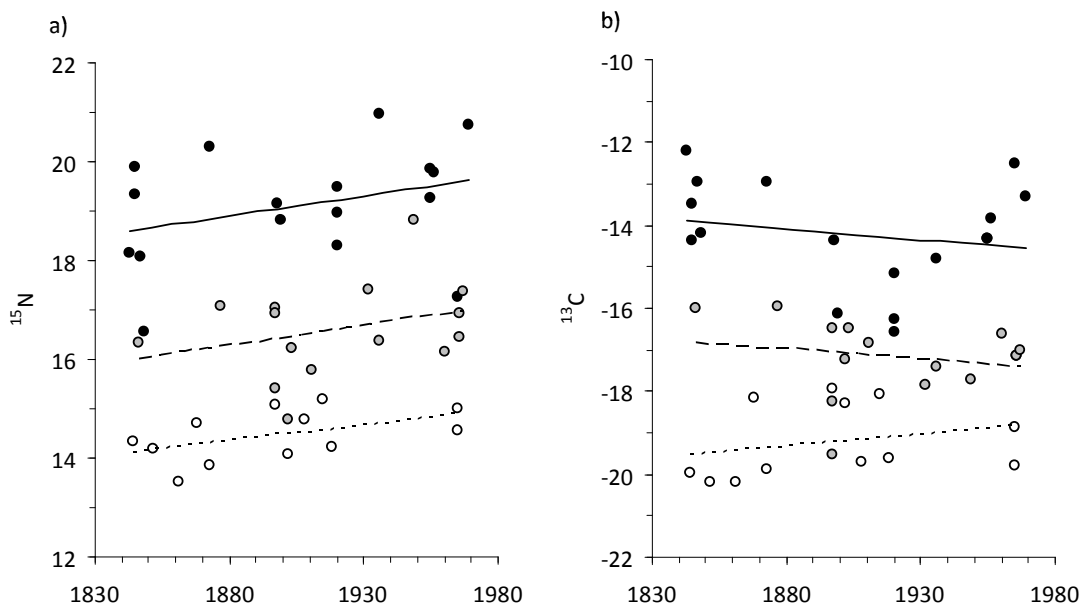


Figure 3. The a) $\delta^{15}\text{N}$ values and b) $\delta^{13}\text{C}$ values over time for Harbor seal (●), Grey seal (*) and Ringed seal (○).

No significant correlation between $\delta^{15}\text{N}$ values and metals was found, however, the one Baltic Harbor seal with the highest Hg level of all seals (1.3 ug/g) also had the highest $\delta^{15}\text{N}$, reaching 21. Further, no significant correlation between $\delta^{13}\text{C}$ values and metals was noticed. In accordance with Hobson et al. (1996), no significant difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between juveniles and adults or females and males was detected in this study. However, it has been shown that older seals feed on a slightly higher trophic level (Lawson et al. 2000) and that the mean metal concentrations are higher in older Baltic ringed seals than in young (Fant et al. 2001). Further, Lawson et al. (2000) found differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between age groups.

4. Concluding remarks

It is important to keep in mind that this study was based on 44 individual seals spread over 126 years. Data on sex and age was lacking for almost half of the seals, which made statistical analysis on these parameters difficult to perform. Even though no obvious trends or differences in metal concentrations between areas and species were seen, the results from this study can serve as a base for further studies and provide valuable information for interpreting the actual contamination load in Swedish marine systems. The time series in this study ends in the late 1960s, approximately when the earliest time series on metals in fish from Swedish marine systems begins (SCMPMB). More information on the background concentrations of Hg and other metals in Swedish marine systems could be gained by relating concentrations of metals in seals to the concentrations of metals in fish reported within the SCMPMB.

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Appendix 1

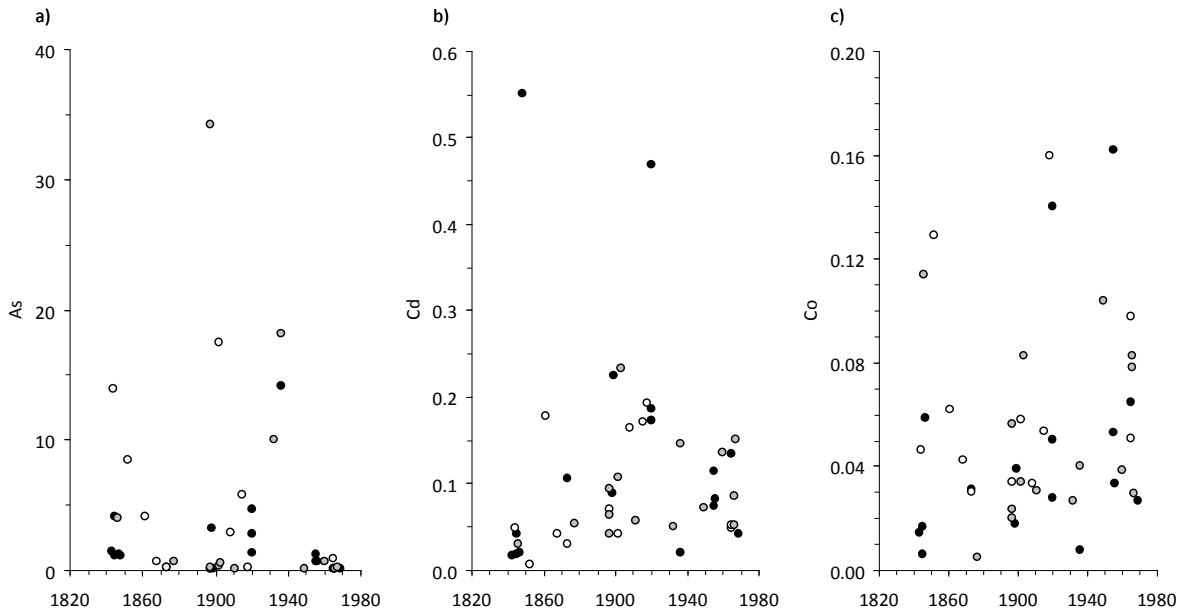


Figure 4. Concentrations ($\mu\text{g/g}$) of a) As (the Harbor seal, yr 1844, with a concentration of 2858 $\mu\text{g/g}$ is excluded), b) Cd and c) Co in seal bones from Harbor seal (●), Grey seal (●) and Ringed seal over time.

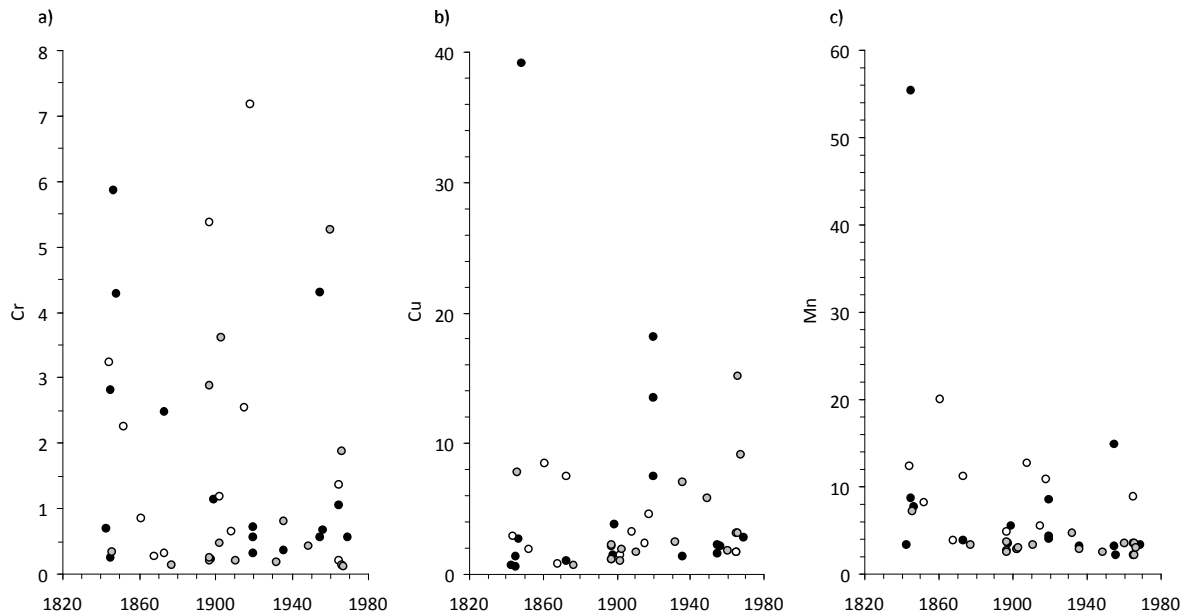


Figure 5. Concentrations ($\mu\text{g/g}$) of a) Cr, b) Cu and c) Mn (the individual seal, yr 1848, with a concentration of 450 $\mu\text{g/g}$ is excluded) in seal bones from Harbor seal (●), Grey seal (●) and Ringed seal over time.

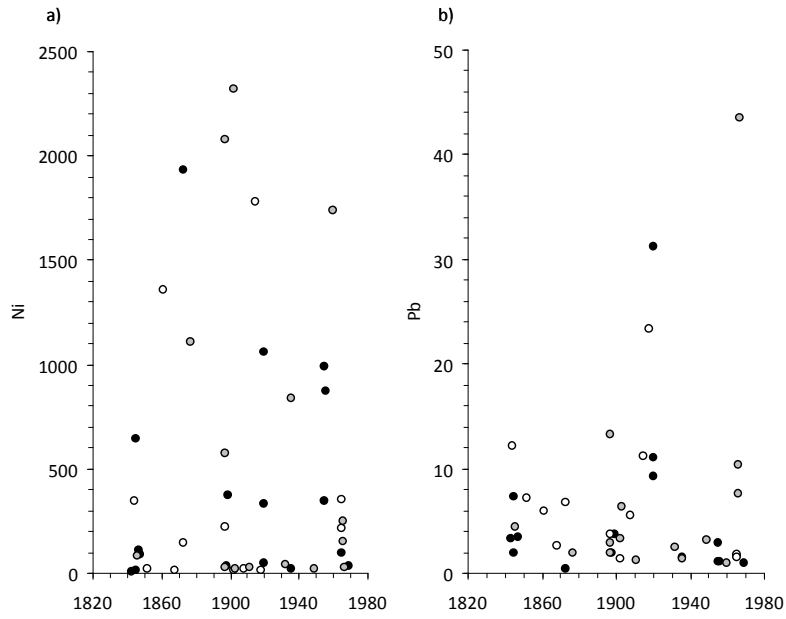


Figure 6. Concentrations ($\mu\text{g/g}$) of a) Ni and b) Pb (the individual seal, yr 1848, with a concentration of 266 $\mu\text{g/g}$ is excluded) in seal bones from Harbor seal (●), Grey seal (○) and Ringed seal over time.