

# Trace element bioaccumulation in grey seals *Halichoerus grypus* from the Faroe Islands

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**ABSTRACT:** Grey seals *Halichoerus grypus* were sampled in the Faroe Islands archipelago during the summers of 1993 to 1995. Concentrations of Cd, Cu, Hg, Se and Zn were measured in the liver, kidney and muscle of 68 seals. All elements except Zn exhibited lowest concentrations in the muscle. The liver contained the highest concentrations of Cu, Hg, Se and Zn, while the kidney contained the highest Cd concentrations. However, trace element concentrations within the tissues were influenced by sex and age. Thus, females had clearly higher Cd concentrations than males. Age was the most important factor influencing the concentration of Cd, Hg and Se in the liver, and of Cd and Hg in the kidney. A strong positive correlation between Cd, Hg and Zn in the kidney suggests the presence of a detoxification process involving metallothionein proteins. Similarly, a strong positive correlation between Hg and Se and a molecular Hg:Se ratio close to 1 in the liver suggests a demethylation process leading to the formation of mercuric selenide granules. High Hg concentrations could be related to fish consumption by the grey seal, but a piscivorous diet could not be responsible for the very high Cd concentrations. This suggests that the seals' diet changes during the year, and may include a significant proportion of cephalopods in seasons other than summer. Finally very high Cd concentrations in grey seal tissues compared to other grey seal populations also suggest that the Faroe Islands are subject to sub-arctic influences.

**KEY WORDS:** Heavy metals · Distribution · Detoxification · Marine mammals · Pinnipeds · Sub-arctic

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## INTRODUCTION

Situated in the Northeast Atlantic Ocean, the Faroe Islands are relatively far from major sources of anthropogenic pollution. Nevertheless, marine top predators from this area, such as the long-finned pilot whale *Globicephala melas* or the white-sided dolphin *Lagenorhynchus acutus*, exhibit cadmium (Cd) and mercury (Hg) concentrations in their tissues (Julshamn et al. 1987, Caurant et al. 1994, Dam 2001, Gallien et al. 2001) much higher than those reported for individuals from temperate areas which are subject to significant pollution from human activities (Mackey et al. 1995, Law et al. 2001). In fact, toxic metals in top marine predators from the Faroe Islands often reach the threshold level at

which metabolic disorders appear in humans (Nogawa 1984). However, such effects are difficult to detect in wild animals such as marine mammals. Recently, Gallien et al. (2001) investigated cellular alterations in relation to Cd concentrations in the kidney of white-sided dolphins from the Faroe Islands. Their study described calcium phosphate granules in this organ which may have resulted from an effective detoxification process of Cd, and might affect the functioning of the kidney. Cd can also be detoxified by proteins such as ferritin or metallothionein that regulate essential elements (e.g. Cu or Zn) (Das et al. 2000). In regard to Hg, other processes are involved in its co-precipitation with selenium that produces non-toxic mineral granules of tiemannite (Martoja & Berry 1980, Nigro & Leonzio 1996).

Top marine predators are mainly exposed to trace elements through their food (Aguilar et al. 1999). Thus, a marine mammal with a specialised diet is exposed either to very small or to very large amounts of toxic elements in its prey. For example, very high Cd concentrations in the kidney of pilot whales from the Faroe Islands have been related to their teutophageous diet (Caurant & Amiard-Triquet 1995, Bustamante et al. 1998a), and lactating pilot whales contain higher Hg concentrations, due to an increase in the proportion of fishes in their diet during the nursing period, since fishes are more energetically valuable than cephalopods (Caurant et al. 1994). In accordance with these conclusions, piscivorous marine mammals such as seals would be expected to have high Hg and low Cd concentrations in their tissues.

This study determined the concentration and distribution of 5 trace elements, Cd, Cu, Hg, Se and Zn, in a piscivorous marine mammal species from the Faroe Islands, the grey seal *Halichoerus grypus*. The primary objectives of this work were to investigate the influence of biological (sex, maturity stage and age), ecological (diet) and geographical factors on the bioaccumulation of these trace elements. Particular attention was paid to interactions between the toxic elements Cd and Hg, and the essential elements Cu, Se and Zn. Also, the trace-element concentrations in the grey seal were compared to those in other seal species and to grey seals from other areas.

## MATERIALS AND METHODS

**Sampling.** During the summers of 1993 to 1995, 68 grey seals were caught off the Faroe Islands (Fig. 1), in a co-operative study by the Museum of Natural History of the Faroe Islands and the Norwegian Institute of Fisheries and Aquaculture, with the aim of studying the diet of this poorly investigated population (Mikkelsen et al. 2002). Organs and tissues were sampled for metallic and organic contaminant investigations (Dam 2001). Samples for trace element analysis, i.e. the liver, kidney and muscle, were stored at  $-20^{\circ}\text{C}$  in individual plastic bags until use. Teeth were examined to determine age. Biological parameters (length, weight, blubber thickness, and age) for each sex and maturity stage are given in Table 1.

**Analytical procedure.** All equipment used in the sample processing was cleaned, and subsequently decontaminated for 24 h in a solution comprised of 35 ml  $\text{HNO}_3$  (65%) and 50 ml HCl (36%) to 1 l of Milli-Ro quality water.

Fresh samples were freeze-dried and ground to powder. The mean ratio between wet weight and dry weight was  $\sim 3.4$  for liver and muscle, and 4.1 for the kidney. We digested 2 aliquots of approx. 200 mg of each homogenised dry sample with 3.5 ml of 65%  $\text{HNO}_3$  at  $60^{\circ}\text{C}$  for 3 d. The digested contents were then diluted to 10 ml in Milli-Q quality water. Cd, Cu, Se and Zn content was assayed using a flame and graphite furnace atomic-absorption spectrophotometer (Varian 250 Plus) with deuterium background correction.

For Hg measurements, aliquots ranging from 0.5 to 10 mg of dried material were analysed directly for total Hg in an advanced mercury analyser spectrophotometer (Altec AMA 254). Hg determination involved evaporation of Hg by progressive heating to  $800^{\circ}\text{C}$  under oxygen atmosphere for 3 min, and subsequent amalgamation on a gold net. The net was subsequently heated to liberate the collected mercury and measured by UV atomic-absorption spectrophotometry.

Analytical accuracy was assessed using dogfish liver DOLT-2 (NRCC) and dogfish muscle DORM-2 (NRCC) as reference materials. Analyses were also validated by international intercalibration methods (e.g. Coquery et al. 2000). The standards were treated and analysed under the same conditions as the seal samples. Results were in good agreement with the certified values, and recoveries of the elements ranged from 93 to 105%.

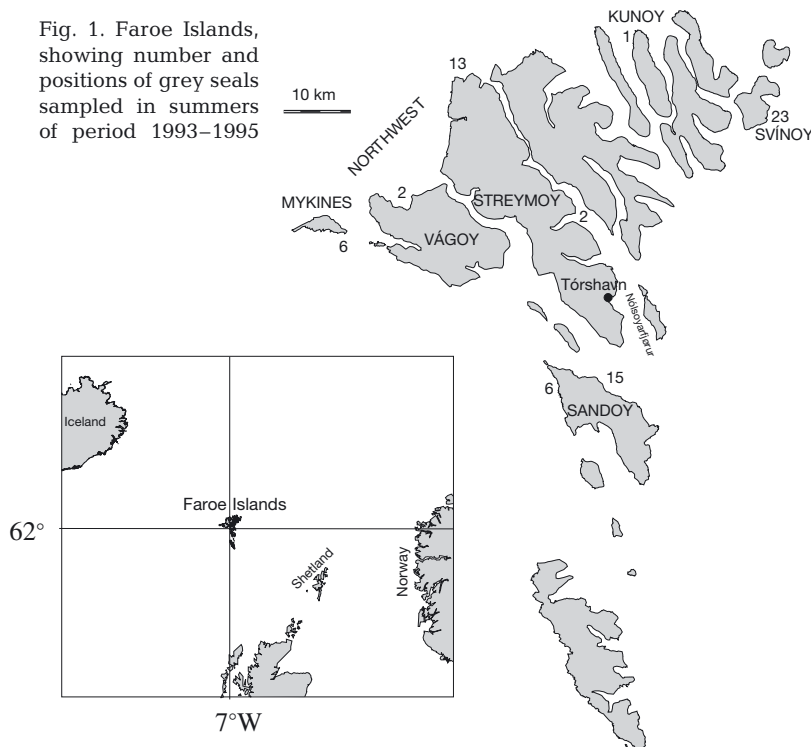


Table 1. *Halichoerus grypus*. Biological characteristics of individuals sampled in Faroe Islands in summers of the period 1993 to 1995. N: number of individuals

Sexual maturity	N	Length (cm)	Weight (kg)	Dorsal blubber thickness (mm)	Age (yr)
Male					
Mature	4	212.0 ± 11.9	229.3 ± 68.4	35.0 ± 10.0	17.0 ± 5.2
Immature	18	136.5 ± 15.7	56.8 ± 20.8	22.1 ± 4.7	1.9 ± 1.3
Total	22	150.3 ± 33.3	89.6 ± 76.6	24.9 ± 7.7	4.6 ± 6.4
Female					
Mature	23	176.4 ± 8.5	152.0 ± 20.8	39.7 ± 8.5	14.3 ± 4.5
Immature	23	133.7 ± 10.5	49.4 ± 11.4	22.6 ± 4.0	2.3 ± 1.1
Total	46	154.6 ± 23.6	99.6 ± 54.4	31.1 ± 11.1	8.3 ± 6.9
Total	68	153.2 ± 27.0	96.4 ± 61.9	29.1 ± 10.5	7.1 ± 6.9

Detection limits ( $\mu\text{g g}^{-1}$  dry wt) were 0.004 for Cd, 0.5 for Cu, 3 for Zn, 0.8 for Se, and 0.005 for total Hg. All trace element concentrations in seal tissues are reported as  $\mu\text{g g}^{-1}$  wet wt (WW).

**Statistical analysis.** Differences in concentrations of trace elements between species were tested by 1-way ANOVA (after log-transformation of data when necessary) followed by a Tukey multiple-comparison test with MINITAB 13.1 software. The hypothesis of normal distribution was tested with the Anderson–Darling test and equality of variance by the Bartlett test. The significance level for statistical analyses was always set at  $\alpha = 0.05$ .

## RESULTS

### Levels of trace elements and organotropism

Cd, Cu, Hg, Se and Zn concentrations in the liver, kidney and muscle for each sex and maturity stage are presented in Table 2. Cd and Hg concentrations varied greatly among tissues. The kidney exhibited the highest Cd concentrations ( $15.8 \pm 24.6 \mu\text{g g}^{-1}$  WW), followed by the liver ( $5.06 \pm 8.63 \mu\text{g g}^{-1}$  WW), with the muscle containing Cd concentrations several orders of magnitude lower ( $0.05 \pm 0.14 \mu\text{g g}^{-1}$  WW) (Table 2). As expected for a toxic element, the coefficients of variation for Cd were large in all tissues, i.e. 155% for the kidney, 170% for the liver and 280% for muscle. Among the 3 tissues analysed, the liver exhibited the highest Hg concentrations ( $59.7 \pm 70.4 \mu\text{g g}^{-1}$  WW) followed by the kidney ( $2.93 \pm 2.47 \mu\text{g g}^{-1}$  WW) and muscle ( $0.74 \pm 0.65 \mu\text{g g}^{-1}$  WW) (Table 2). The coefficients of variation for Hg were relatively high, particularly for the liver (118%) but also for the kidney and muscle (84 and 88%, respectively). The liver also exhibited the highest Se concentrations ( $23.0 \pm 26.2 \mu\text{g g}^{-1}$  WW), followed by the kidney ( $5.47 \pm 1.43 \mu\text{g g}^{-1}$  WW) and mus-

cle ( $0.27 \pm 0.10 \mu\text{g g}^{-1}$  WW). For Se, the coefficients of variation were lower than 40% in the kidney and muscle but higher than 100% in the liver.

The liver exhibited the highest Cu concentrations ( $38.7 \pm 18.1 \mu\text{g g}^{-1}$  WW), while concentrations were relatively low in the kidney and muscle ( $3.1 \pm 0.5$  and  $1.5 \pm 0.3 \mu\text{g g}^{-1}$  WW, respectively). As expected for an essential element, the coefficients of variation were low for Cu, i.e. 15, 25 and 47% in the kidney, muscle and liver, respectively. The liver also showed the highest Zn concentrations ( $57 \pm 10 \mu\text{g g}^{-1}$  WW), followed by the muscle ( $41 \pm 9 \mu\text{g g}^{-1}$  WW) and the kidney ( $27 \pm 7 \mu\text{g g}^{-1}$  WW). For Zn, the coefficients of variation were lower than 30% in all 3 tissues analysed.

### Influence of gender

Cd was the only element exhibiting a significant difference between sexes. Thus, Cd concentrations in the kidney were significantly higher ( $p < 0.001$ ) in adult females than in adult males (i.e.  $40.8 \pm 28.8$  and  $10.5 \pm 4.69 \mu\text{g g}^{-1}$  WW, respectively), resulting from 5 times higher Cd accumulation rates in females (Fig. 2). In the liver, males exhibited significantly ( $p < 0.001$ ) lower Cd concentrations of  $1.76 \pm 0.27 \mu\text{g Cd g}^{-1}$  WW, while females exhibited mean concentrations of  $13.2 \pm 11.0 \mu\text{g g}^{-1}$  WW, resulting from Cd accumulation rates that were 10 times higher than in males (Fig. 2).

No other significant differences between sexes were found for the other elements in grey seal tissues.

### Influence of maturity stage and age

Cd concentrations in the 3 tissues of mature individuals were higher than those in immature individuals ( $F = 47.40$ ,  $p < 0.001$ ) (Table 2). Moreover, Cd was significantly correlated with age in the kidney and

Table 2. *Halichoerus grypus*. Mean  $\pm$  SD and range of trace element concentrations ( $\mu\text{g g}^{-1}$  WW) in liver, kidney and muscle. N: number of individuals

Tissue	N	Cd		Cu		Hg		Se		Zn	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
<b>Liver</b>											
Male											
Mature	4	1.76 $\pm$ 0.27	1.50–2.09	31.2 $\pm$ 8.1	23.8–41.8	123 $\pm$ 70.9	46.4–199	41.2 $\pm$ 24.7	18.8–53.1	62 $\pm$ 14	50–76
Immature	18	0.59 $\pm$ 0.80	0.06–3.61	29.6 $\pm$ 18.2	5.5–79.6	10.3 $\pm$ 15.9	1.56–65.4	4.69 $\pm$ 5.63	1.43–23.7	53 $\pm$ 8	30–63
Female											
Mature	23	13.2 $\pm$ 11.0	2.29–51.9	51.6 $\pm$ 16.3	28.3–85.3	133 $\pm$ 58.0	34.2–238	50.6 $\pm$ 22.8	13.5–99.9	60 $\pm$ 8	46–75
Immature	23	1.04 $\pm$ 1.07	0.15–4.05	34.4 $\pm$ 14.0	7.2–61.1	14.0 $\pm$ 18.7	1.13–90.1	6.47 $\pm$ 7.15	1.17–35.6	56 $\pm$ 10	31–75
Total	68	5.06 $\pm$ 8.63	0.06–51.9	38.7 $\pm$ 18.1	5.5–85.3	59.7 $\pm$ 70.4	1.13–238	23.0 $\pm$ 26.2	1.17–99.9	57 $\pm$ 10	30–76
<b>Kidney</b>											
Male											
Mature	4	10.5 $\pm$ 4.69	6.30–15.3	2.5 $\pm$ 0.4	2.0–2.9	7.89 $\pm$ 5.86	2.85–15.9	6.49 $\pm$ 2.22	4.02–8.95	29 $\pm$ 4	23–31
Immature	18	2.07 $\pm$ 1.72	0.39–7.43	3.3 $\pm$ 0.5	2.6–4.6	1.77 $\pm$ 1.22	0.41–4.53	5.10 $\pm$ 1.24	2.72–6.87	23 $\pm$ 2	18–28
Female											
Mature	23	40.8 $\pm$ 28.8	8.91–155	3.0 $\pm$ 0.4	2.2–3.9	4.49 $\pm$ 1.28	1.63–7.15	5.93 $\pm$ 1.31	3.50–9.42	35 $\pm$ 6	26–53
Immature	23	2.52 $\pm$ 1.50	0.96–6.23	3.1 $\pm$ 0.4	2.4–3.6	1.41 $\pm$ 0.75	0.61–4.06	5.10 $\pm$ 1.40	2.63–7.15	23 $\pm$ 3	19–28
Total	68	15.8 $\pm$ 24.6	0.39–155	3.1 $\pm$ 0.5	2.0–4.6	2.93 $\pm$ 2.47	0.41–15.9	5.47 $\pm$ 1.43	2.63–9.42	27 $\pm$ 7	18–53
<b>Muscle</b>											
Male											
Mature	4	0.03 $\pm$ 0.02	0.01–0.05	1.1 $\pm$ 0.2	1.0–1.2	1.99 $\pm$ 1.86	0.59–4.61	0.27 $\pm$ 0.07	0.18–0.34	33 $\pm$ 9	25–44
Immature	17	0.01 $\pm$ 0.01	<0.01–0.04	1.7 $\pm$ 0.3	1.4–2.4	0.53 $\pm$ 0.28	0.18–1.21	0.29 $\pm$ 0.14	0.08–0.50	42 $\pm$ 9	24–57
Female											
Mature	23	0.12 $\pm$ 0.23	0.01–1.13	1.4 $\pm$ 0.3	1.1–1.9	1.00 $\pm$ 0.48	0.31–2.63	0.23 $\pm$ 0.05	0.14–0.36	44 $\pm$ 9	24–53
Immature	23	0.01 $\pm$ 0.01	<0.01–0.06	1.6 $\pm$ 0.4	1.2–2.9	0.44 $\pm$ 0.21	0.22–1.06	0.29 $\pm$ 0.10	0.11–0.51	40 $\pm$ 8	24–51
Total	67	0.05 $\pm$ 0.14	<0.01–1.13	1.5 $\pm$ 0.3	1.0–2.9	0.74 $\pm$ 0.65	0.18–4.61	0.27 $\pm$ 0.10	0.08–0.51	41 $\pm$ 9	24–57

liver (Fig. 2). In the former, the correlation was linear for both males and females ( $r = 0.947$ ,  $p < 0.001$ ;  $r = 0.818$ ,  $p < 0.001$ ; respectively). However, the correlation between hepatic Cd and age fitted a logarithmic pattern for males ( $r = 0.747$ ,  $p < 0.001$ ) and a linear pattern for females ( $r = 0.672$ ,  $p < 0.001$ ) (Fig. 2).

There were higher Hg concentrations in the 3 tissues in mature individuals than in immature seals ( $F = 23.91$ ,  $p < 0.001$ ). Hepatic and renal Hg concentrations increased linearly with increasing age ( $r = 0.882$ ,  $p < 0.001$ ;  $r = 0.733$ ,  $p < 0.001$ ; respectively) (Fig. 3).

Hepatic Se concentrations differed significantly ( $F = 82.48$ ,  $p < 0.001$ ) between immature ( $5.69 \pm 6.51 \mu\text{g g}^{-1}$  WW) and mature individuals of both sexes ( $49.2 \pm 22.9 \mu\text{g g}^{-1}$  WW). Se concentrations were correlated with age only in the liver ( $r = 0.857$ ,  $p < 0.001$ ). Furthermore, hepatic Cu concentrations were significantly lower ( $F = 6.86$ ,  $p < 0.002$ ) in immature ( $32.3 \pm 15.9 \mu\text{g g}^{-1}$  WW) than in mature ( $48.6 \pm 16.9 \mu\text{g g}^{-1}$  WW) seals, and renal Cu concentrations in both sexes were significantly higher ( $F = 6.02$ ,  $p < 0.004$ ) in immature ( $3.2 \pm 0.4 \mu\text{g g}^{-1}$  WW) than in mature ( $2.9 \pm 0.5 \mu\text{g g}^{-1}$  WW) seals. However, Cu concentrations in the tissues of grey seals were not correlated with age.

For both sexes, mature seals had significantly higher

Zn concentrations in the kidney ( $F = 55.36$ ,  $p < 0.001$ ) than immature individuals ( $34 \pm 7 \mu\text{g g}^{-1}$  WW and  $23 \pm 3 \mu\text{g g}^{-1}$  WW, respectively). Therefore, renal Zn increased linearly with increasing age ( $r = 0.796$ ,  $p < 0.001$ ). However, Zn concentrations in the liver and muscle were not correlated with age.

### Correlation between elements

Table 3 shows the correlation between trace elements in the 3 tissues. In the liver, Cd concentrations were correlated to Hg, Se and Zn ( $p < 0.001$ , Table 3). Hg and Se concentrations exhibited a linear relationship for both sexes ( $r = 0.989$ ,  $p < 0.001$ ) (Fig. 5). Thus, the calculated molar ratio between Hg and Se was also correlated with age ( $r = 0.642$ ,  $p < 0.001$ ), approached 1 (Fig. 5).

In the kidney, Zn was correlated with Cd ( $r = 0.907$ ,  $p < 0.001$ ) and Hg ( $r = 0.698$ ,  $p < 0.001$ ) (Fig. 4). Renal Hg concentrations were also strongly correlated with Se ( $r = 0.688$ ,  $p < 0.001$ ) and Cd ( $r = 0.773$ ,  $p < 0.001$ ) (Fig. 4).

In the muscle, only a negative correlation was found — between Cu and Hg (Table 3).

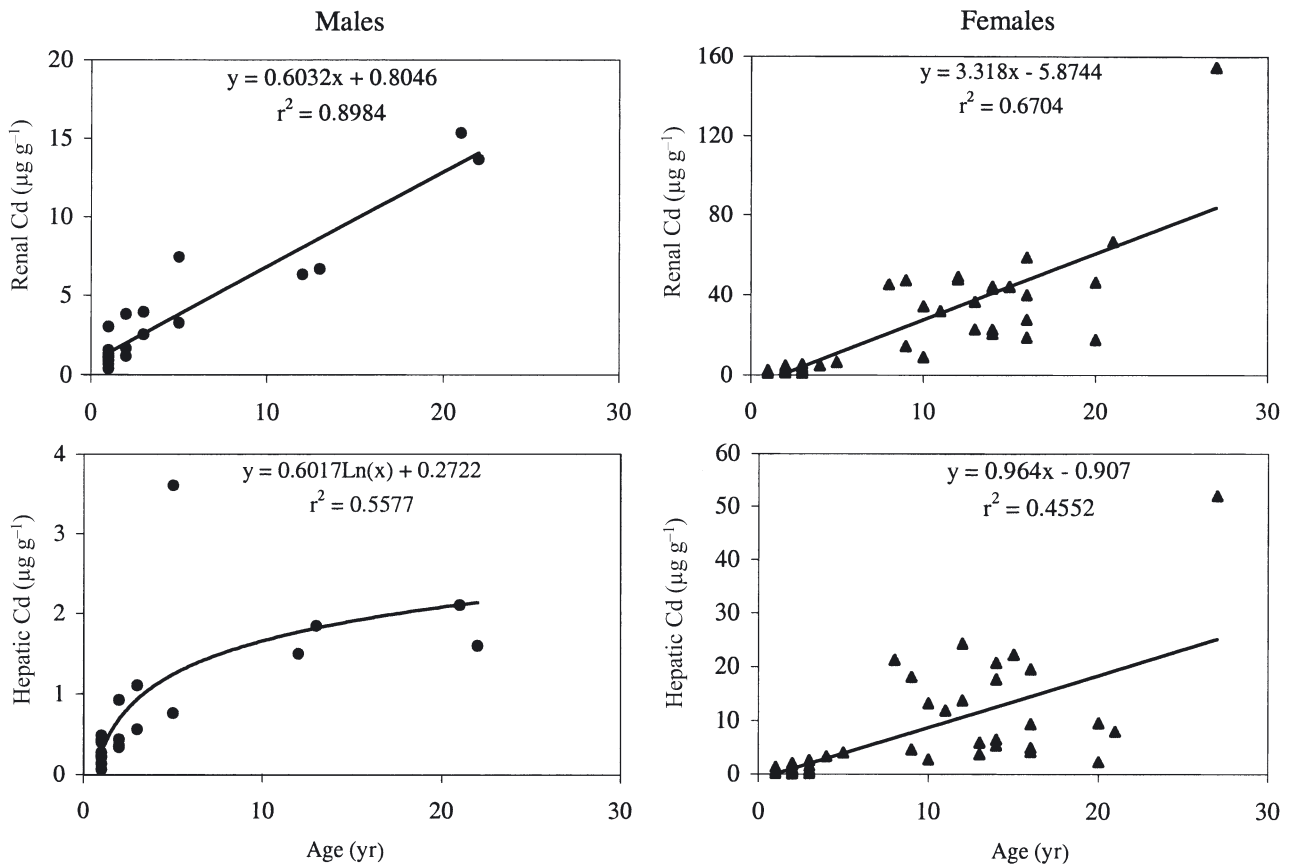


Fig. 2. *Halichoerus grypus*. Renal and hepatic Cd concentrations ( $\mu\text{g g}^{-1}$  WW) as a function of age in males and females. Note different scales of ordinates

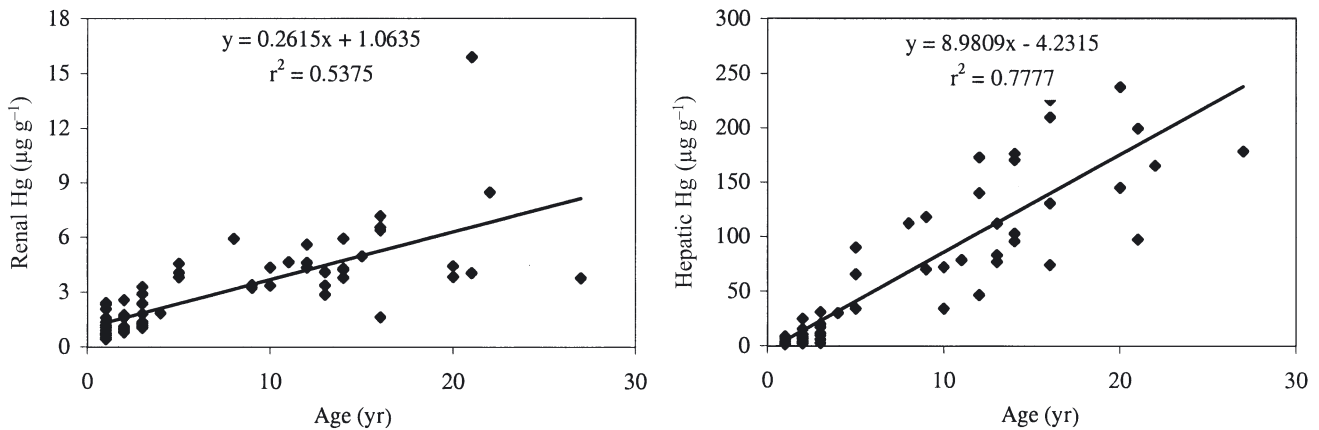


Fig. 3. *Halichoerus grypus*. Renal and hepatic Hg concentrations ( $\mu\text{g g}^{-1}$  WW) as a function of age

## DISCUSSION

In contrast to pinniped species, i.e. harp and ringed seals, that are traditionally hunted and which have been extensively studied for trace element and organic

compound bioaccumulation (see review by Muir et al. 1992), the grey seal has rarely been investigated, and then often using only a few individuals (Morris et al. 1989, Frank et al. 1992, Law et al. 1992, 1998, Teigen et al. 1999, Dam 2001, Nyman et al. 2002). This is proba-

Table 3. *Halichoerus grypus*. Correlation between trace elements in the different tissues. Underlined:  $p < 0.001$ ; other:  $p < 0.05$

Elements	Liver	Kidney	Muscle
Cd	<u>+Hg, +Se, +Zn</u>	<u>+Hg, +Zn</u>	
Cu	<u>+Hg, +Se, +Zn</u>	<u>-Hg</u>	- Hg
Hg	<u>+Cd, +Cu, +Se</u>	<u>+Cd, -Cu, +Se, +Zn</u>	- Cu
Se	<u>+Cd, +Cu, +Hg</u>	<u>+Hg,</u>	
Zn	<u>+Cd, +Cu</u>	<u>+Cd, +Hg</u>	

bly because most grey seal populations either live in protected areas or are not traditionally hunted. Generally, only stranded individuals, or those found in fishing by-catches, or individuals killed in small numbers with special authorisation are available for ecotoxicological analyses. Thus, the Faroe Island study of the grey seal diet in this archipelago provided a rare opportunity to investigate the bioaccumulation of trace elements in a presumably healthy marine mammal population.

As in other pinniped species (e.g. Wagemann et al. 1988, Dietz et al. 1996, de Moreno et al. 1997,

Julshamn & Grahl-Nielsen 2000, Fant et al. 2001), each trace element analysed was mainly concentrated in a specific tissue. Thus, the highest concentrations of Cu, Hg, Se and Zn were found in the liver, whereas the kidney contained the highest Cd concentrations (Table 2). However, within a given population, several factors such as sex, reproductive status, age and diet greatly influence the concentration of trace elements within tissues.

In marine mammals, trace elements are mainly incorporated into the body via their food, and thus diet is the main factor determining the trace-element load of a given species (Aguilar et al. 1999). Therefore, intake via food presumably represents the bulk of trace-element intake. Dietary examination of the same individuals used in this study showed that grey seals from the Faroe Islands feed exclusively on fishes during the summer period, with the stomach contents of males and females revealing a similar diet (Mikkelsen et al. 2002). However, the very high Cd concentrations present in their tissues was not in accordance with a piscivorous diet. Fish consumption would result in very low Cd levels, as most fish species generally contain

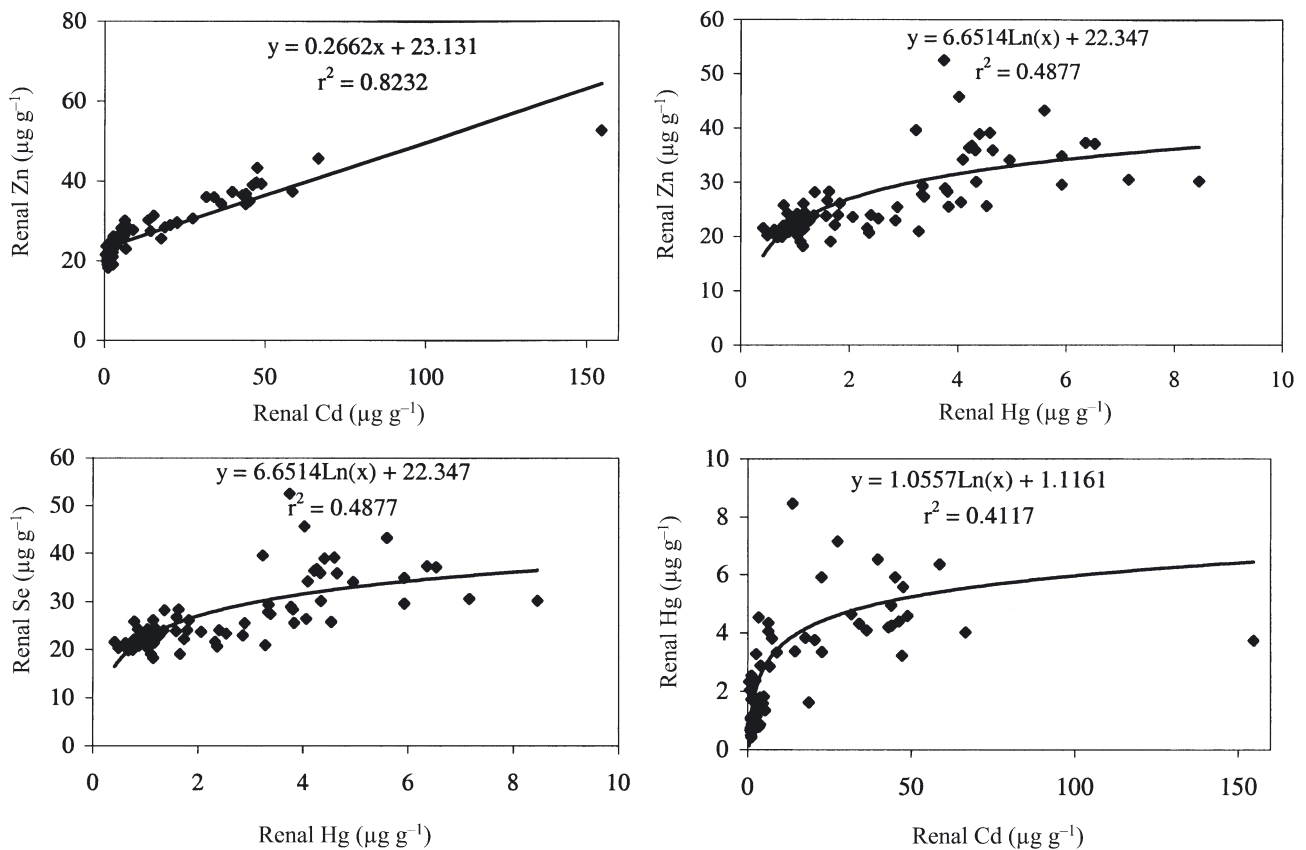


Fig. 4. *Halichoerus grypus*. Correlation between Cd and Zn, between Hg and Zn, between Hg and Se and between Cd and Hg concentrations ( $\mu\text{g g}^{-1}$  WW) in kidney



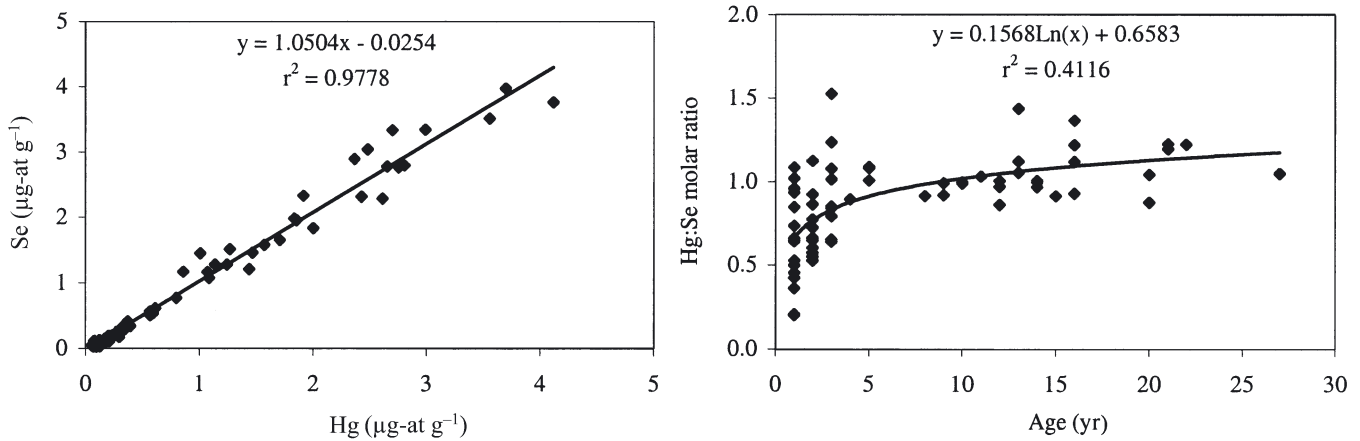


Fig. 5. *Halichoerus grypus*. Correlation between hepatic Hg and Se concentrations ( $\mu\text{g-at g}^{-1}$  dry wt) and molar Hg:Se as a function of age

relatively low Cd levels (Bustamante et al. 2003). In the light of the present results, it seems likely that the diet of grey seals in Faroese waters changes during the year, and includes a significant proportion of cephalopods, which strongly increases their exposure to Cd (Bustamante et al. 1998a). In fact, the diet of grey seals in northern temperate waters appears to be very dependent on season (Pierce et al. 1990, 1991, Hammond et al. 1994), and to be very similar to the diet described by Mikkelsen et al. (2002) for Faroe grey seals during the summer. However, in autumn, winter and spring, the grey seals of northern temperate waters increased the proportion of cephalopods in their diet. Such a shift in diet might occur in Faroe grey seals also, and would explain the high Cd concentrations found in their liver and kidney during the present study.

Although investigation of the grey seal diet in the Faroe Islands revealed no sex-related difference in diet (Mikkelsen et al. 2002), Cd concentrations in the liver and kidney displayed a significant gender-related difference. Thus, females exhibited significantly higher renal and hepatic concentrations than males (Table 2 & Fig. 1). Nyman et al. (2002) also reported higher Cd concentrations in the liver and kidney of female grey seals from the Baltic Sea and from Sable Island. Similar gender differences in the Antarctic fur seal *Arctocephallus gazella* and the Baikal seal *Phoca sibirica* were partly explained by different feeding habits of males and females (de Moreno et al. 1997, Watanabe et al. 1998). Such differences in metal concentrations are sometimes attributable to sexual dimorphism (Caurant et al. 1994, Watanabe et al. 1998), and this could be the case here, since grey seal males weigh up to 290 kg, while females do not exceed 191 kg. Moreover, female seals seem to have higher feeding rates than males (essentially because of the

energetic cost of reproduction, viz. gestation and lactation) while males drastically reduce their feeding during the mating and moulting seasons. For example, an adult male ringed seal weighing about 70 kg would ingest 1200 to 1300 kg food per year, whereas an adult female weighing about 60 kg would require 1600 kg per year (Ryg & Øritsland 1991). Since male and female grey seals feed on the same species, and probably have a similar feeding dimorphism to ringed seals, the uptake of Cd would be greater for females because of their higher ratios of food ingested:body weight. This also suggests that the transfer of Cd to the foetus through the placenta or to calves via milk is not an important way of excretion for females. It cannot be excluded that this difference between males and females is associated with a difference in metabolic pathways linked to the hormone cycle, which obviously differs between sexes (Caurant et al. 1994). An increase in trace element concentrations with increasing age was observed for Cd in the liver and kidney of both sexes, but gender differences in the accumulation rates occurred (Fig. 2). In the liver, the pattern of increasing Cd concentration with increasing age differed between the sexes, fitting a logarithmic trend only for males. A logarithmic pattern in Cd accumulation has previously been shown in the liver of the long-finned pilot whale from the Faroe Islands (Caurant et al. 1994). It may represent an equilibrium between the assimilation and excretion of Cd in the liver similar to that which occurs in humans of  $\geq 50$  yr of age (Friberg et al. 1974). In grey seals, such equilibrium would appear to occur from 12 yr onwards in males, whereas females accumulate Cd linearly with age. However, the present samples lacked very old females: the maximum age of our samples was 27 yr, whereas grey seals can attain more than 35 yr of age.

Table 4. Concentrations of renal Cd and hepatic Hg ( $\mu\text{g g}^{-1}$  WW) in various species of pinnipeds sampled in different regions and habitats. –: no data

Region	Species	Renal Cd			Hepatic Hg			Source
		N	Mean $\pm$ SD	Range	N	Mean $\pm$ SD	Range	
<b>Arctic</b>								
Greenland Sea	Harp seal	29	–	0.1–101	29	–	0.14–7.7	Julshamn & Grahl-Nielsen (2000)
Greenland Sea	Hooded seal	22	–	0.13–207	22	–	0.26–179	Julshamn & Grahl-Nielsen (2000)
Arctic Ocean	Harbour seal	–	–	–	38	–	0.2–66	Frank et al. (1992)
West arctic Canada	Ringed seal	144	21.1 $\pm$ 14.2	0.12–87.1	145	32.9 $\pm$ 35.2	0.2–2.2	Wagemann et al. (1996)
East arctic Canada	Ringed seal	35	47.7 $\pm$ 23.3	8.98–111	115	8.3 $\pm$ 7.0	0.4–38.7	Wagemann et al. (1996)
Eureka (Canada)	Ringed seal	17	15.6 $\pm$ 13.8	0.009–46.3	8	26.5 $\pm$ 36.9	0.4–143	Wagemann et al. (1996)
<b>Subarctic</b>								
Faroe Islands	Grey seal	68	15.8 $\pm$ 24.6	0.39–154.7	68	59.7 $\pm$ 70.4	1.13–237.7	This study
Baltic Sea	Grey seal	20	3.4 $\pm$ 1.7	1.4–7.6	20	78 $\pm$ 84	15–348	Nyman et al. (2002)
Baltic Sea	Grey seal	19	–	0.25–2.72	19	–	7.03–92	Frank et al. (1992)
Sable Island	Grey seal	20	1.8 $\pm$ 1.1	3.0–20	20	109 $\pm$ 72	27–278	Nyman et al. (2002)
Norway Oslofjord	Harbour seal	–	–	–	28	4.1	0.1–30	Skaare et al. (1990)
Norway southern coast	Harbour seal	–	–	–	26	16	0.2–89	Skaare et al. (1990)
Norway northern coast	Harbour seal	–	–	–	17	7.9	0.4–43	Skaare et al. (1990)
St. Laurent gulf	Harp seal	20	28 $\pm$ 9	9.5–46.3	20	10.4 $\pm$ 8.1	1.3–30.1	Wagemann et al. (1988)
Baltic Sea	Ringed seal	20	–	0.8–6	20	53	6.5–124	Fant et al. (2001)
<b>Northern temperate waters</b>								
Irish Sea	Grey seal	2	2.1	1.7–2.5	2	0.07	0.06–0.08	Morris et al. (1989)
Irish Sea	Grey seal	–	–	–	21	108 $\pm$ 132	0.5–430	Law et al. (1992)
Liverpool Bay	Grey seal	–	–	–	2	595	330–860	Law et al. (1992)
Irish Sea	Harbour seal	–	–	–	13	51 $\pm$ 58	1–170	Law et al. (1992)
<b>Lakes</b>								
Baikal	Baikal seal	40	1.8 $\pm$ 0.8	0.6–3.6	41	2.3 $\pm$ 2.6	0.2–9.1	Watanabe et al. (1998)

Cu and Zn concentrations in grey seals appear to be in the same order of magnitude as those reported for other pinniped species, probably because these essential elements are subject to homeostatic regulation in marine mammal tissues (Law 1996). However, Cd, Hg and Se levels in the present study were amongst the highest reported for pinnipeds in the literature. Thus, hepatic Se levels in grey seals from the Faroe Islands varied from 1.17 to 99.9  $\mu\text{g g}^{-1}$  WW and were comparable to the high hepatic Se concentrations (ranging from 1.30 to 65.3  $\mu\text{g g}^{-1}$  WW) in ringed seals from the western Canadian arctic (Wagemann et al. 1996). High Se concentrations in liver have also been reported for grey seals from the Baltic Sea (2.7 to 79.2  $\mu\text{g g}^{-1}$  WW) and from Sable Island (9.3 to 83  $\mu\text{g g}^{-1}$  WW) (Nyman et al. 2002). Cd and Hg concentrations are globally well documented in seals (Table 4). Generally, seals from polar areas exhibit high levels of renal Cd and hepatic Hg. Surprisingly for a species largely distributed in temperate waters, grey seals also generally have very high Hg concentrations in their liver. The present results for grey seals from the Faroe Islands follow this trend, with Hg concentrations in the liver varying from

1.13 to 238  $\mu\text{g g}^{-1}$  WW. As far as Cd is concerned, grey seals from the Faroe Islands appear to be atypical, compared to those from the Baltic Sea or Sable Island (Table 4), displaying Cd concentrations in the kidney comparable to those of seals from the Arctic, and clearly higher Cd concentrations than grey seals from the Baltic Sea or Sable Island.

In summary, compared to other pinnipeds, particularly those from lower latitudes, renal Cd and hepatic Hg concentrations in grey seals from the Faroe Islands are among the highest recorded (Table 4). Very high Hg concentrations in grey seals from the Irish Sea were related to feeding habits in a very contaminated area, Liverpool Bay, where their prey is enriched in Hg (Law et al. 1992). In the Faroe Islands, no such enrichment by industrial contamination exists. Consequently, the high Hg concentrations in the tissues of the grey seals must result from high background levels. In contrast, the reasons for the general Cd enrichment recorded in arctic waters remain unclear. Local enrichments have been noted for marine mammal populations throughout the Arctic but such local variations do not explain the phenomenon on a global scale (Wagemann et al.



1996). Several authors have proposed a phenomenon of Cd abnormality in polar and subpolar areas that leads to very high Cd concentrations in many invertebrates and vertebrates (Petri & Zauke 1993, Bargagli et al. 1996, Bustamante et al. 1998b, Zauke et al. 1999). Such an abnormality seems to occur in the Faroe Islands too. Indeed, similar to polar regions, soluble trace-element concentrations in the seawater appear to be very low in this area (Mart & Nürnberg 1984), but high Cd concentrations have been found in the cephalopods *Loligo forbesi*, *Todarodes sagittatus* and *Eledone cirrhosa* (up to  $9.06 \pm 3.38 \mu\text{g g}^{-1}$  WW) and in the queen scallop *Aequipecten opercularis* ( $6.55 \pm 1.97 \mu\text{g g}^{-1}$  WW) caught around the Faroes archipelago (Bustamante 1998, Bustamante et al. 1998a). Similarly, in coastal waters, the dog-whelk *Nucella lapillus* and the limpet *Patella* sp. displayed Cd concentrations of 28 and  $10 \mu\text{g g}^{-1}$  WW, respectively (Dam 2000); and other marine mammal species from the Faroe Islands, i.e. the long-finned pilot whale *Globicephala melas* and the white-sided dolphin *Lagenorhynchus acutus*, also had very high concentrations of Cd and Hg in their tissues (Julshamn et al. 1987, Caurant et al. 1994, Gallien et al. 2001, Olsen et al. 2003). Marine mammals are currently considered useful bioindicators of trace element contamination in the environment because of their high trophic level and their wide distribution. Differences in trace element concentrations between individuals from different areas may be due to different exposures through food rather than to metabolic differences. Consequently, the continued release of toxic metals such as Cd or Hg into the environment is of particular concern in these areas.

### Toxicological aspects

Processing of trace elements depends on the individual regulation and/or detoxification mechanisms of storage or excretion in the organism concerned. Thus extremely high levels of toxic Hg and Cd in mature grey seals may result in cellular and physiological damage to the storage organs, whereas renal dysfunction would be expected at Cd concentrations  $>50 \mu\text{g g}^{-1}$  WW in humans (Elinder & Järup 1996). In the present study, Cd concentrations in the kidney of mature grey seals were close to that threshold value ( $36.3 \pm 28.8 \mu\text{g g}^{-1}$  WW) with concentrations in the oldest females exceeding it by far (up to  $155 \mu\text{g g}^{-1}$  WW; Table 2). Furthermore, most mature grey seals in this study contained Hg levels exceeding the threshold of  $60 \mu\text{g g}^{-1}$  for liver damage in mammals (AMAP 1998). Nevertheless, their body mass and blubber thickness (Table 1) indicated that these seals were in good health, strongly suggesting that grey seals in the Faroe

Islands have developed a very efficient detoxification process that allows them to process high Cd and Hg concentrations in their tissues.

The liver and kidney of grey seals have been reported to contained high levels of metallothionein, which could trap toxic metals such as Cd (Olafson & Thompson 1974, Teigen et al. 1999). The primary role of metallothionein is to bind essential metals such as Cu and Zn and to serve as an intracellular protein in metal homeostasis (Vallee 1991). However, Cd shows a great affinity for metallothionein, leading to competition with Zn for binding sites and to a reduction of the toxic effects of Cd (Tsalev & Zaprianov 1983). Thus, displaced Zn would induce metallothionein synthesis, increasing the number of binding sites within the renal cells. If this hypothesis is true, then Cd and Zn would be accumulated simultaneously. Significant correlation between Cd and Zn (Fig. 4) in the kidney of grey seals from the Faroe Islands strongly suggests an effective detoxification process of Cd involving metallothionein in this organ.

In the grey seal liver, Hg concentrations were strongly positively correlated with Se during the life span (Fig. 5). Se has been reported to provide some protection against Hg toxicity, apparently preventing hepatic accumulation of this element by formation of mercuric selenide (HgSe) granules (Pelletier 1985, Cuvin-Aralar & Furness 1991, Nigro & Leonzio 1996). HgSe granules (tiemannite) have been identified in the liver of seabirds, marine mammals and humans (Martoja & Berry 1980, Pelletier 1985, Hansen et al. 1989, Nigro & Leonzio 1996). Moreover, many studies have reported Hg and Se to be correlated in a molar ratio of 1:1, as in HgSe (e.g. Koeman et al. 1973, Nielsen & Dietz 1990, Wagemann et al. 1998). The molar ratio of Hg:Se in the liver of grey seals from the Faroe Islands was  $\sim 1$  (Fig. 5), strongly suggesting the presence of tiemannite granules as a result of coprecipitation of Hg with Se. A  $\sim 1:1$  molar ratio between hepatic Hg and Se was found in mature males and females, which displayed the same kinetics of accumulation with age, indicating a similar detoxification process of Hg in the liver in both sexes. This also suggests that pregnancy and lactation are not efficient processes of heavy metal elimination in females. Moreover, the annual moult, which probably results in the elimination of some Hg, is not sufficient to counteract a high assimilation of Hg.

### Conclusion

Grey seals from the Faroe Islands had very high Cd and Hg concentrations in their tissues, much higher than in other grey seal populations. The concentrations in the oldest individuals were clearly higher than the

toxic threshold levels comprising a threat to their health. However, very efficient detoxification processes, involving metallothionein protein and co-precipitation of Hg with Se, probably account for the good condition of the analysed seals. The present results reveal that the Faroe Islands also display the Cd 'abnormality' previously reported for polar oceans. Finally, very high Cd contents of the tissue indicate important changes in the diet of the seals during the course of the year. Hence, seals may also feed on cephalopods during some months, accounting for the high Cd concentrations in the oldest individuals.

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