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Element concentrations, histology and serum biochemistry of arctic char (*Salvelinus alpinus*) and shorthorn sculpins (*Myoxocephalus scorpius*) in northwest Greenland

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ABSTRACT

The increasing exploratory efforts in the Greenland mineral industry, and in particular, the proposed rare earth element (REE) mining projects, requires an urgent need to generate data on baseline REE concentrations and their potential environmental impacts. Herein, we have investigated REE concentrations in anadromous Arctic char (*Salvelinus alpinus*) and shorthorn sculpins (*Myoxocephalus scorpius*) from uncontaminated sites in Northwest Greenland, along with the relationships between the element concentrations in gills and liver, and gill histology and serum biochemical parameters. Concentrations of arsenic, silver, cadmium, cerium, chromium, copper, dysprosium, mercury, lanthanum, neodymium, lead, selenium, yttrium, and zinc in gills, liver and muscle are presented. No significant statistical correlations were observed between element concentrations in different organs and gill histology or serum biochemical parameters. However, we observed positive relationships between age and histopathology, emphasizing the importance of including age as a co-variable in histological studies of fish. Despite no element-induced effects were observed, this study is considered an important baseline study, which can be used as a reference for the assessment of impacts of potential future REE mine sites in Greenland.

1. Introduction

Mining operations have existed in Greenland since the 1850s. Some older sites have caused severe contamination of the local environment, with elevated lead and zinc levels being the primary environmental contaminants (Nørregaard et al., 2018; Sonne et al., 2014). Presently, several mining projects are proposed in Greenland including two rare earth element (REE) mines (GME, 2015; Tanbreez, 2013), along with an iron mine in the Nuuk Fjord in Southwest Greenland and a lead-zinc mine in Citronen Fjord in North Greenland. Very few ecotoxicological studies on REEs exist and no information is available for Arctic fish species regarding risk assessment of REEs. The lack of such information makes it difficult to establish environmental guidelines or threshold concentrations (Gonzalez et al., 2014). Environmental monitoring of marine habitats in Greenland using fish has traditionally focused on shorthorn sculpins (*Myoxocephalus scorpius*), fourhorn sculpins (*M. quadricornis*), Greenland cod (*Gadus ogac*) and, in freshwater, landlocked and anadromous Arctic char (*Salvenius alpinus*) (Bach et al., 2014, 2016, Dang et al., 2017; Hansson et al., 2020, Søndergaard, 2013).

It is well documented that elemental pollution can have adverse

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effects on fish that range from molecular, sub-cellular, cellular, tissue, organ to organism levels (Gonzalez et al., 2014; van der Oost et al., 2003; Wood, 2011). In Greenland, histopathological alterations of the liver and gills in shorthorn and fourhorn sculpins have been reported to increase in parallel with organ concentrations of metals. Specifically, the presence of hyperplastic gill epithelium, hyperplasia of mucous and chloride cells in gills, hepatic deposits, parasites and necrosis in liver increased with the element concentrations in the organs of these two fish species (Nørregaard et al., 2018; Sonne et al., 2014). While there are no such studies for Arctic chars from Greenland, gill hypertrophy and hepatic necrosis have been shown in older, larger Arctic char from Canada with elevated mercury concentrations (Barst et al., 2019; Ribeiro et al., 2002).

Adverse environmental conditions, e.g., unsuitable temperature or presence of a toxic substance, can alter fish blood biochemistry. Because chemical parameters in plasma or serum reflect the physiological state of an animal, measurements of these variables are used to indicate general health status (Barcellos et al., 2004, Topić Popović et al., 2016). Indeed, exposures to heavy metals both *in situ* and under experimental conditions have been documented to change blood biochemical parameters such as concentrations of various enzymes and hormones, cholesterol, total protein, blood urea or ions in fish (Adams et al., 2010; Kim and Kang, 2015; Sastry and Sharma, 1980). Parameters such as concentrations of total protein, albumin, alkaline phosphatase (ALP) and alanine transaminase (ALT) have been shown to respond to chronic stress, and are indicative of liver lesions or disruption of normal liver functions (Al-Asgah et al., 2015; Firat and Kargin, 2010; Sastry and Sharma, 1980).

Our overall aim was to investigate the relationship between element concentrations with emphasis on REEs, and gill histology and serum biochemistry in marine Arctic char and shorthorn sculpin from Greenland, thus representing sentinel species in the marine coastal environment in Greenland. Specifically, we aimed to investigate whether: i) element concentrations in the liver, gills and muscle differed between Arctic char and shorthorn sculpins, ii) gill histological markers were related to element concentrations in gills and iii) hepatic element concentrations related serum biochemical parameters. The outcome of this study is important for establishing relevant environmental monitoring programs in marine coastal areas at potential future REE mining sites in Greenland.

2. Methods

2.1. Study site and fish capture

Anadromous Arctic char, *Salvenius alpinus*, and shorthorn sculpins, *Myoxocephalus scorpius,* were used as study species. Arctic char (*Salvelinus alpinus*) is a widespread fish in the Arctic and populates almost every lake and river in Greenland as either anadromous or landlocked populations. It is an important local traditional food resource in Greenland, typically caught with gill-nets, when the fish migrate back to freshwater systems from July to September. If mature, they will start spawning in November and then – from May to June – migrate to the sea. Here they will stay, usually within approximately 20 km of the system in which they overwinter (Muus et al., 1990).

Shorthorn sculpins are widespread in Greenland, benthic, relatively stationary and inhabit the coastal zones and fjords (Muus et al., 1990). Such characteristics make them ideal as indicator species, as they can be caught in any coastal zones where pollution is present, and accumulation gradients can, due to their sedentary nature, be established from e. g. point sources of pollution.

The fish were obtained from two locations in Laksebugten, Northwest Greenland (Fig. 1) in August 2015 to investigate the background levels in a high-Arctic Greenland ecosystem with no industrial activities.

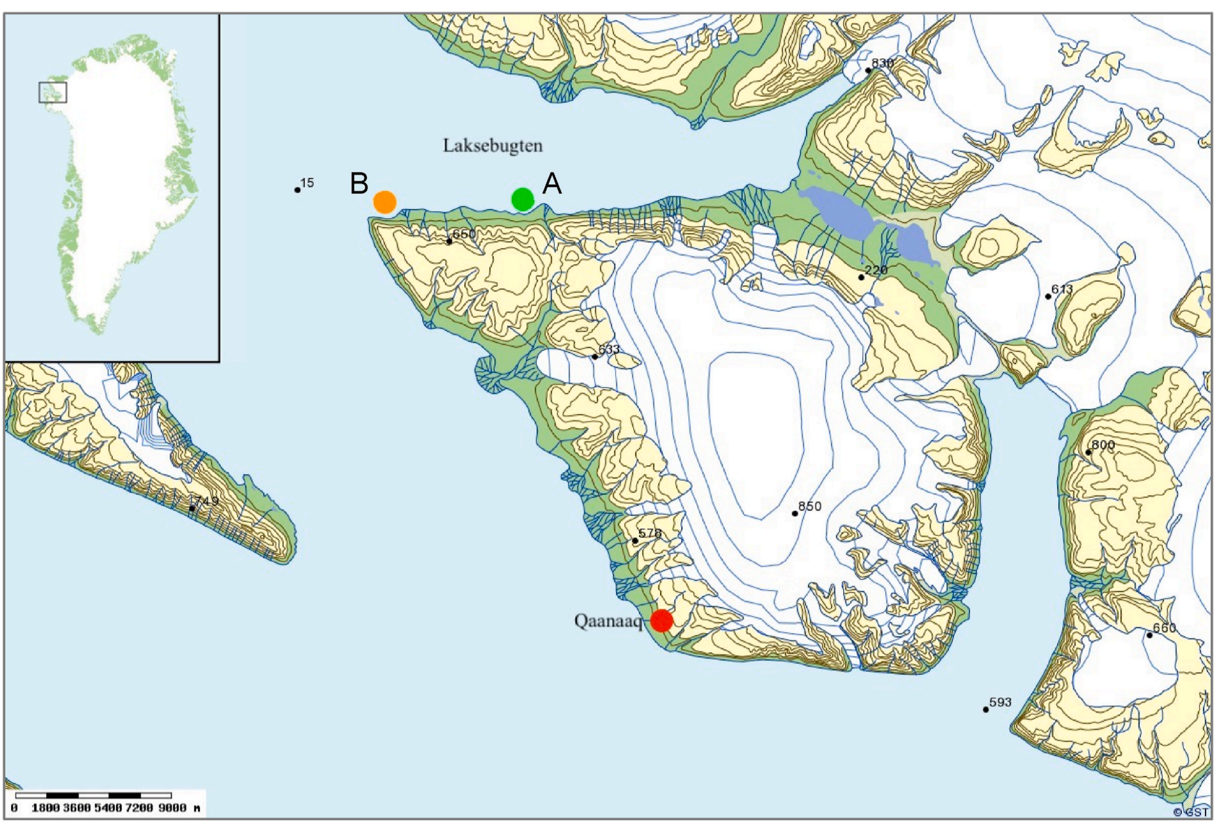

Fig. 1. Map of the sampling area, Laksebugten north of the settlement Qaanaaq in Northwest Greenland. The Arctic chars were caught at the green dot (A) while the shorthorn sculpins were caught at the orange dot (B). The red dot is Qaanaaq. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Arctic chars (n = 25) were caught by fishing nets set perpendicular to the beach, in the few hours after high tide. Shorthorn sculpins (n = 15) were caught by angling using fishing rods.

2.2. Sample collection

Upon capture, fish were placed and transported back to land in 20 L buckets containing fresh seawater, which was regularly refreshed to ensure optimum conditions of oxygen and temperature. Within 1 h after capture, the fish were handled in the field laboratory. Here approximately 1.5–3 mL of blood was collected from the cardinal vein using a sterile needle and syringe, and transferred to Eppendorf tubes and immediately frozen at −20 °C. The fish were then euthanized and handled according to the Greenland regulations by a FELASA certificated member (FELASA A/B/D accreditation No 32/10/288). All fish were weighed, total fish length measured (Table 1), and another blood sample was obtained by cardiac puncture with sterile single-use needles inserted into the heart of the fish. The blood samples were left to clot in a blood-clot vacutainer placed in a thermobox with sea ice for approximately 24 h before being spun down with a hand-centrifuge. The serum was then transferred to cryotubes and frozen. Sterile stainless-steel scalpels were used to dissect the fish, and the sampling environment was kept clean and wiped off between fish samples to avoid cross-contamination. For the histological assessments, the 2nd gill arch from the right gills of individual fish was carefully sampled and fixed in 4% buffered formalin. Samples of the posterior lobe of the liver, the right lateral muscle, and a gill arch from the right gills were sampled for element analyses. These samples were stored frozen at −20 °C in sterile polyethylene zip-lock bags until analysis.

2.3. Element analysis

All analyses were performed at the accredited trace element laboratory at Aarhus University, Roskilde. Samples of gills, liver and muscle were freeze-dried and the dry matter percentages were determined by weighing the samples before and after drying. Subsamples of gills (0.088–0.300 g dry weight), liver, and muscle (~0.300 g dry weight) were microwave digested (Anton Paar Multiwave 3000) in Teflon-lined reaction vessels in 4 ml/4 ml Merck Suprapure HNO_3/Milli-Q water. Digestion solutions were diluted with Milli-Q water to 5% HNO_3 prior to analyses. Digestion solutions were analyzed using an Agilent 7900 ICP-MS for c. 60 elements of which the following elements are reported in this study: chromium (Cr), zinc (Zn), copper (Cu), arsenic (As), selenium (Se), yttrium (Y), silver (Ag), cadmium (Cd), lanthanum (La), cerium (Ce), neodymium (Nd), dysprosium (Dy), mercury (Hg) and lead (Pb). The analytical quality was assured by analyzing blank samples and duplicate samples and the certified reference materials (CRMs): BCR-668, DOLT-5, DORM-4 and TORT-3 (muscle tissue, fish liver, fish protein and lobster, respectively) from the EU Joint Research Centre and the National Research Council Canada, respectively. A sample size of 0.2 g dry weight was used for the CRMs. Each digestion series of 16 vessels contained a least one blank, one duplicate, and one CRM. Detection limits for the elements were determined as 3 standard deviations (S.D.) on the blank samples. Duplicate samples typically showed a relative S.D. of <20% and individual measurements of the CRMs were all within the range of 77–118% of the certified values for the elements above. The detection limits for each element and the analytical results on the specific CRMs are shown in Table S8.

2.4. Gill histology and age determination

Gill samples were trimmed and processed using standard protocols for histology, then embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin (H&E). The prevalence of histological changes in the gills (congestions, telangiectasis, partial and complete epithelium lifting, necrosis, and epithelial hyperplasia, partial and complete fusion of the lamellae, mononuclear cell infiltrations and chondroplasia) were evaluated using low- and high-power light microscope fields (50–400 ×). Completely lifted epithelium and fused lamellae were categorized as such only when 100% of the lamellae were affected. Dilations in the lamellae capillaries, where the blood spaces are defined and separated by pillar cells was classified as telangiectasis, while the three-part wall structure of normal blood vessels, where such a dilation would be classified as an aneurism. The lesions were recorded as either "present" or "absent". For the purpose of modelling, if the gill element concentrations had any effect on the presence of lesions in the gills, the number of lesions for each fish was recorded and summarized (Table 2, Total Count). These numbers were then normalized against the number of lesions investigated, i.e., divided by the total number of potential lesions (Table 2, Normalized Total Count). For more information on the diagnoses and quantification of histological changes in Greenland sculpin studies see Sonne et al. (2014), Dang et al. (2017) and Verland et al. (2019). The age of the fish was estimated using the "break and burn method" described in Brogan and Anderl (2012), using a transversely sectioned otolith burned to enhance surface pattern.

2.5. Blood-biochemical analysis

Blood-serum analyses of clinical chemical parameters (BCCPs) included the following components: total protein, albumin, alkaline phosphatase and alanine transaminase. The analyses of blood clinical-chemical parameters were conducted using an automated spectrophotometrical analyser containing ion-selective electrodes (ADVIA 1800; Siemens Healthineers, Germany). All serum samples were analyzed following Sonne et al. (2008, 2010). Daily internal quality controls and quarterly external quality controls were performed on the selected assays. Only results from accepted analytical runs are reported in this study.

2.6. Statistics

The scaled mass index (SMI) model is a condition index method that allows to compare between species with different body shapes and was used to calculate species dependent condition indices (Peig and Green, 2009). Element concentrations were log-transformed prior to statistical analysis. To achieve normal distribution, the histopathological data were arc-tangent transformed. Modeling and statistical analysis was done using R-studio version 1.1.453 (RStudio Team, 2016).

2.6.1. Species differences in element concentrations

Species differences in tissue element concentrations were evaluated using linear models with species, age, sex and SMI as explanatory variables. Age and SMI were continuous variables. For each organ or tissue (i.e. liver, gills or muscle) a total of 14 linear models were applied, one

Table 1
Biometrics of the Arctic char and shorthorn sculpins caught in Laksebugten. Numbers are means with S.D. with min – max in parentheses.

Species	Gender	n	Weight (g)	Length (cm)	Liver weight (g)	Age (years)	SMI
Arctic char	F	18	1643 ± 392 (1052–2884)	45 ± 4 (40–53)	58 ± 20 (32–95)	8 ± 2 (4–11)	3762 ± 1771 (1626–7918)
	M	7	1542 ± 866 (223–2884)	43 ± 10 (24–54)	58 ± 34 (8–105)	7 ± 2 (3–9)	3856 ± 3197 (85–10156)
Shorthorn sculpin	F	7	448 ± 283 (142–880)	26 ± 6 (20–35)	60 ± 56 (11–147)	8 ± 2 (6–11)	998 ± 1091 (124–3073)
	M	8	190 ± 85 (61–300)	20 ± 4 (13–25)	10 ± 6 (1–17)	7 ± 2 (4–9)	161 ± 128 (11–389)

Table 2
Sum of counts of histopathological alterations of gills investigated in the Arctic char and shorthorn sculpins caught in Laksebugten. C = congestion, T = telangiectasis, ELP = part epithelial lift, ELC = complete epithelial lift, N = necrosis, EHP = epithelial hyperplasia, FP = part fusions of lamellae, CP = complete fusions of lamellae, CP = chondroplasia, MHP = mucus cell hyperplasia, MNCI = mononuclear infiltrations, P = *Trichodina* spp. Total Count = ∑found alterations in individual fish. Normalized Total Count = ∑Total Count/#investigated alterations. Total Count and Normalized Total Count numbers are mean ± S.D.

n	C	T	ELP	ELC	N	EHP	FP	FC	CP	MHP	MNCI	P	Total Count	Normalized Total Count
18	9	4	10	1	0	15	6	3	1	7	7	2	4 ± 2	0.30 ± 0.14
7	2	1	4	0	1	6	2	3	0	2	2	1	3 ± 1	0.29 ± 0.11
7	5	4	0	0	2	6	3	1	5	1	3	3	5 ± 2	0.39 ± 0.17
8	8	3	2	0	1	4	1	1	5	2	3	3	4 ± 2	0.34 ± 0.15

for each element (Cr, Zn, Cu, As, Se, Y, Ag, Cd, La, Ce, Nd, Dy, Hg and Pb). To reduce the risk of type I errors associated with multiple comparisons, model significance was evaluated based on Bonferroni-corrected p-values.

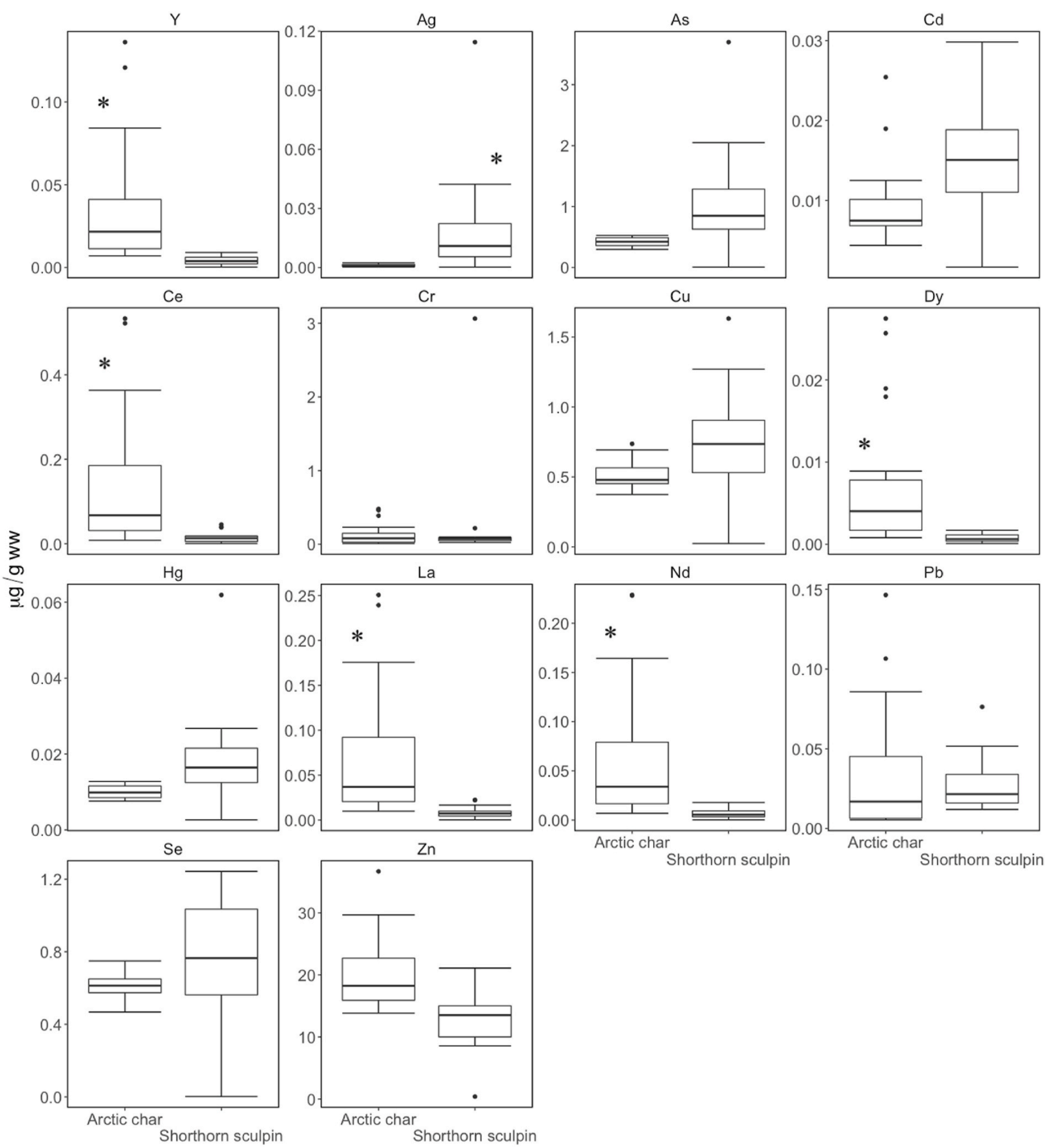

Fig. 2. Boxplot of element concentration (µg/g ww) in the gills of Arctic chars and shorthorn sculpins caught near Qaanaaq, Greenland. *: significantly higher concentration between the species. Bonferroni-corrected *p*-values: Ag = < 0.0001, Ce = 0.0001, Dy = 0.0001, La = < 0.0001, Nd = < 0.0001, Y = < 0.0001.

2.6.2. Principal component analysis

To reduce collinearity and the number of explanatory variables for the gill histopathological models, the element concentrations for gills and liver were grouped using principal components analyses (PCAs) after the concentrations were log-transformed and scaled. The elements were divided into 'common' elements (As, Ag, Cd, Cr, Cu, Hg, Pb, Se, Zn) and rare earth elements (Ce, Dy, La, Nd, Y), and a separate PCA was conducted for each group of elements for each organ and species (yielding a total of eight PCAs). Only the first two principal components (PC1 and PC2) were extracted for each PCA as these explained >50 percent of the variation in the original data in all PCAs. See supplementary Information for further information of PCA analyses.

2.6.3. Linear models

The influence of elements on BCCPs was investigated using eight linear models (four per species), with either total blood protein, albumin, alanine transaminase or alkaline phosphatase as dependent variable and the principal components as explanatory variables, along with the covariates age, sex and SMI. The influence of elements on gill histopathology was evaluated the same way, with two linear models (one per species), with the transformed histopathological data as dependent variables and principal components as explanatory variables, along with age, sex and SMI. Model significance was based on Bonferroni-corrected P-values.

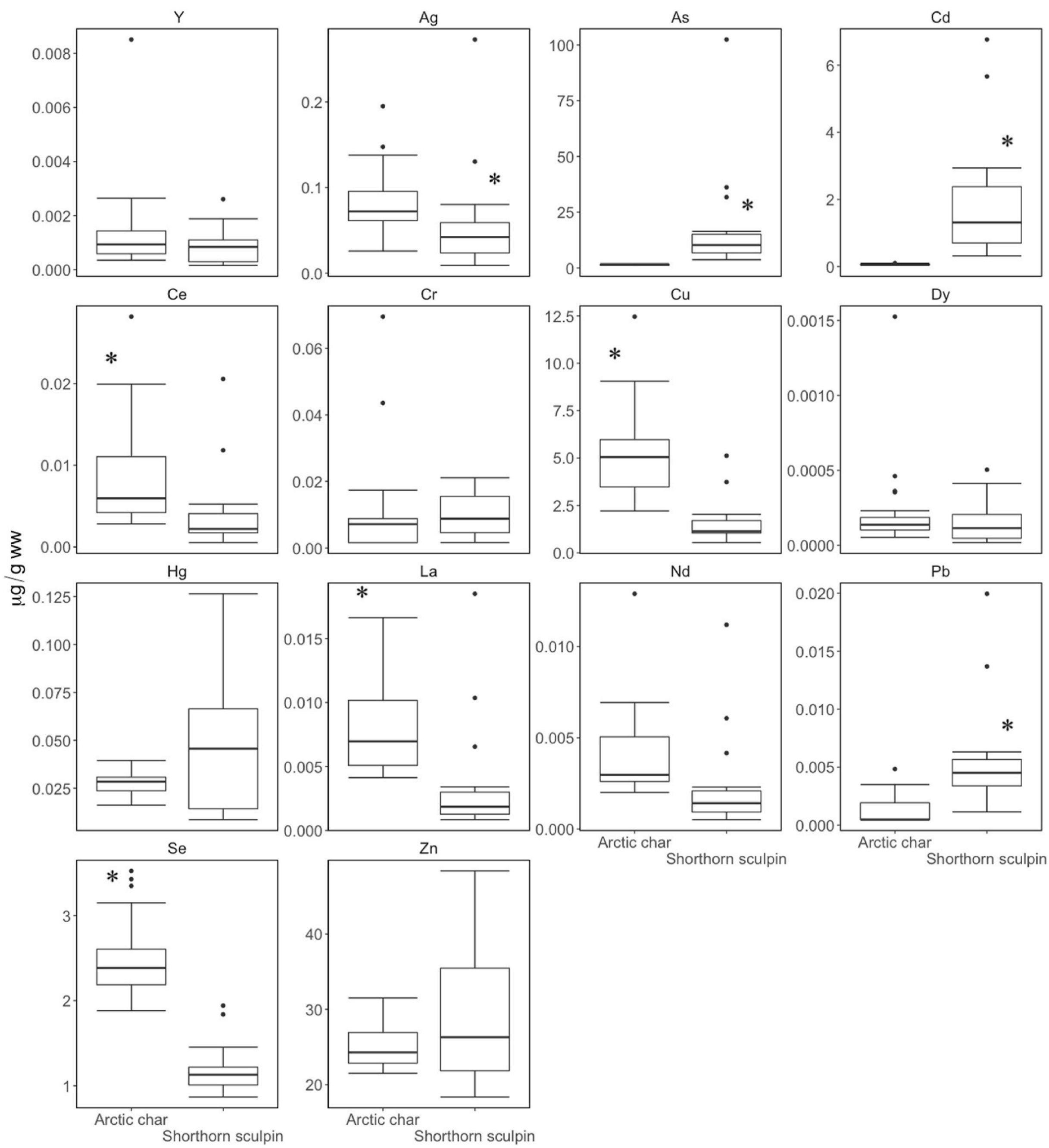

Fig. 3. Boxplot of element concentration (μg/g ww) in the livers of Arctic chars and shorthorn sculpins caught near Qaanaaq, Greenland. *: significantly higher concentration between the species. Bonferroni-corrected *p*-values: As = < 0.0001, Cd = < 0.0001, Ce = 0.0001, Cu = < 0.0001, La = < 0.0001, Nd = 0.0001, Pb = 0.0081, Se = < 0.0001.

3. Results

3.1. Biometrics

Biometrics of Arctic char (n = 25) and shorthorn sculpins (n = 15) are presented in Table 1. While no statistically significant differences were observed in the biometrics of the Arctic char, the male shorthorn sculpins were significantly shorter (p = 0.028) smaller and had lower weight (p = 0.021, One-way ANOVAs) than the female sculpins. Specifically, the mean SMI for the female sculpins was a factor 10 higher than the males. It should be noted, however, that the variation in SMI was large for both sexes for both species.

3.2. Element concentrations

Significant species-specific differences in element concentrations were observed for muscle, liver and gills. For gills this was particularly marked for Y, Ce, Dy, La and Nd that were higher in Arctic char compared to in the gills of shorthorn sculpins while Ag was higher in shorthorn sculpins compared to Arctic char (Fig. 2). For the liver, Ce, La, Nd, Cu, Se, and Ag were found at higher concentrations in Arctic char compared to in the liver of shorthorn sculpins. Contrary were As, Cd and Pb found in higher concentrations in shorthorn sculpins compared to Arctic char (Fig. 3). For the muscle tissue As, Cd, Hg and Zn were found in higher concentrations in shorthorn sculpins compared to Arctic char (Fig. 4). Concentrations of REEs were higher in Arctic chars than in shorthorn sculpins and "common" elements were typically found in

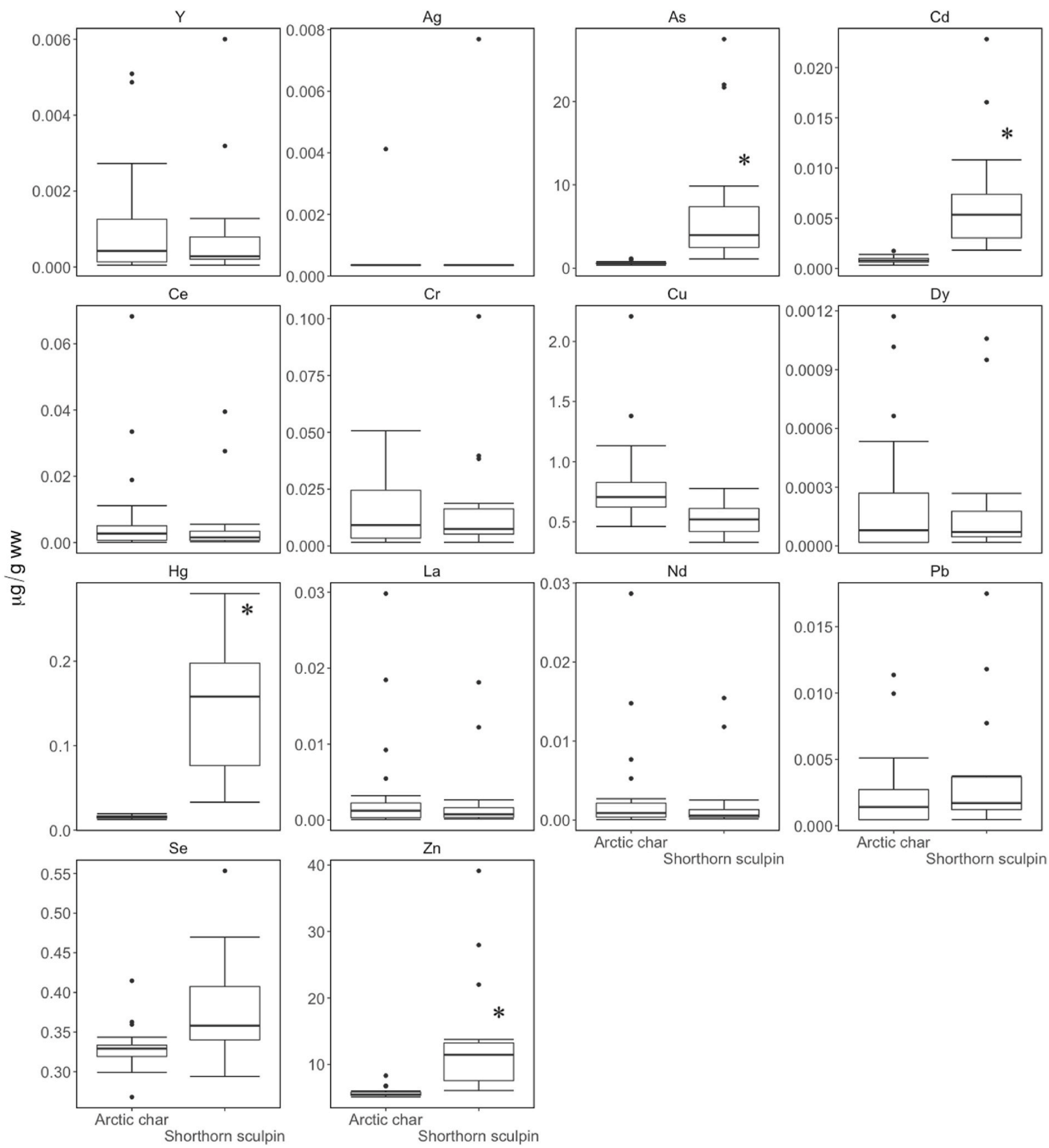

Fig. 4. Boxplot of element concentration (µg/g ww) in the muscle of Arctic char and shorthorn sculpins caught near Qaanaaq, Greenland. *: significantly higher concentration between the species. Bonferroni-corrected *p*-values: As = < 0.0001, Cd = < 0.0001, Hg = < 0.0001, Zn = 0.006566.

higher concentrations in shorthorn sculpins than in Arctic chars.

For both species, the REE were in general found in concentrations in an order of magnitude higher in the gills than in the liver and the muscle tissue, while Ag, Cd and Cu were found in the highest concentrations in the liver.

3.3. Gill histopathology

The most common lesions were partial epithelium lifting and congestion (Fig. 5A and B), which were present in 77 and 60% of the samples, respectively (Table 2). Other pathological changes including telangiectasis (Fig. 5C), complete epithelial lifting (Fig. 5A), fusion of lamellae (Fig. 5D) or necrosis (Fig. 5B) were only seen in small numbers of fish. The only obvious discrepancy between the two species were chondroplasia (Fig. 5E), which was present in 66% of the shorthorn sculpins, compared to one individual of Arctic char (4%).

The models for the relationship between gill element concentration and number of gill lesions found in the Arctic chars and shorthorn sculpins were not significant ($p = 0.082$ and 0.067, respectively), with R^2-values = 0.48 and 0.77, respectively (Table S3). The sum of histopathological changes found in the gills was not significantly related to any elements in either species (Table S3). Rather was the sum of histological changes found as a result of age, i.e. the age of the Arctic char significantly, correlated positively with the number of observed gill lesions ($p = 0.011$; Fig. 6). A similar, but less significant correlation, was found for shorthorn sculpin ($p = 0.058$).

3.4. Clinical chemical parameters

Mean serum concentrations of the measured clinical chemical parameters (BCCPs) are shown in Table 3. Note that the sample size is lower than the total number of fish caught. The models for the potential effects of liver element concentrations on the BCCPs were all affected by high p-values along with low R^2-values. Of the eight model runs, four for each species, the highest R^2-value was found in the shorthorn sculpin albumin model (Table S4), but none of the element or biometric predictors were significantly correlated with the blood serum albumin concentrations. The same was true for the other three models; total protein (Table S5), alanine transaminase (Table S6) and alkaline phosphatase (Table S7) for both species.

4. Discussion

This study is the first assessment of blood biochemical parameters in Greenland shorthorn sculpins and Arctic chars and documenting histological observations in the gills of Greenland Arctic chars. The data represent baseline measurements of elements including REEs, blood biochemistry and gill histology from pristine fish caught in Northwest Greenland.

We did not observe any statistically significant relationships between

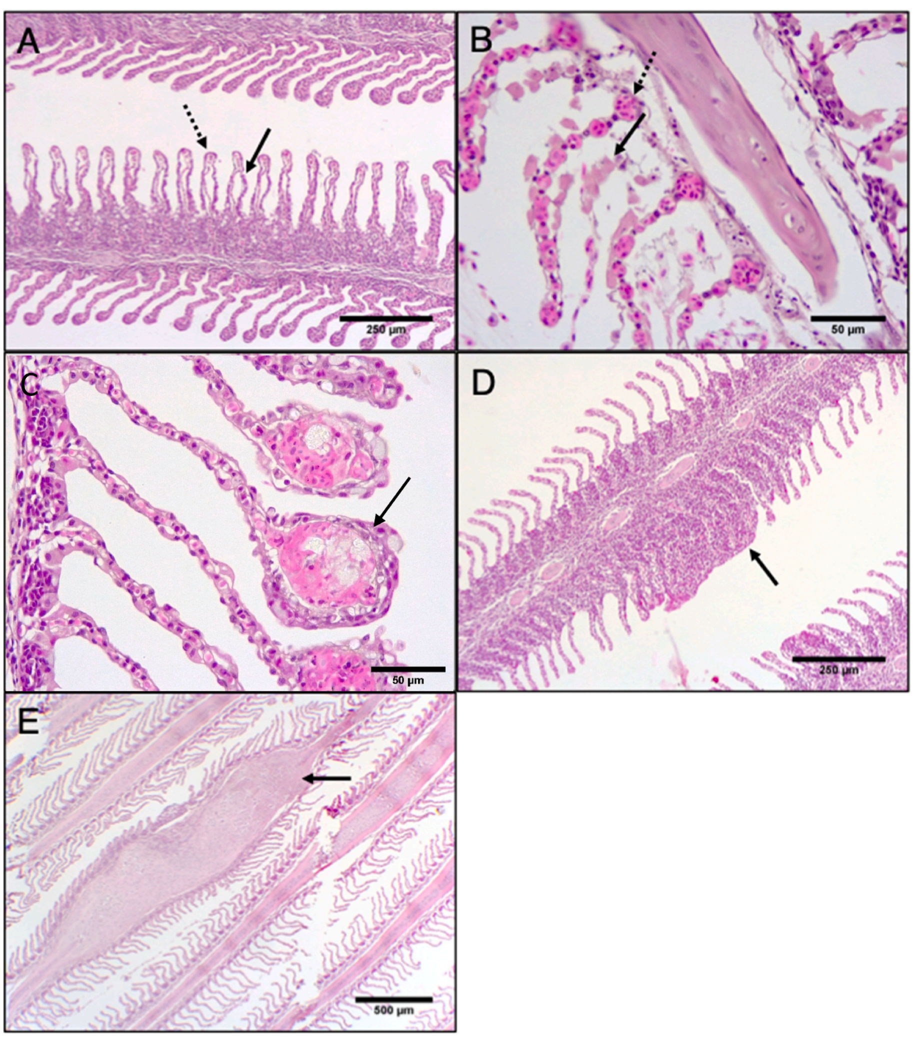

Fig. 5. Histopathology of the gills from Arctic chars and shorthorn sculpins (H&E). A: Partial (full arrow) and complete epithelial (dashed arrow) lifting. B: Necrotic epithelial tissue (full arrow) and basal congestion (dashed arrow). C: Telangiectasis (arrow). D: Complete lamellar fusion (arrow). E: Chondroplasia (arrow).

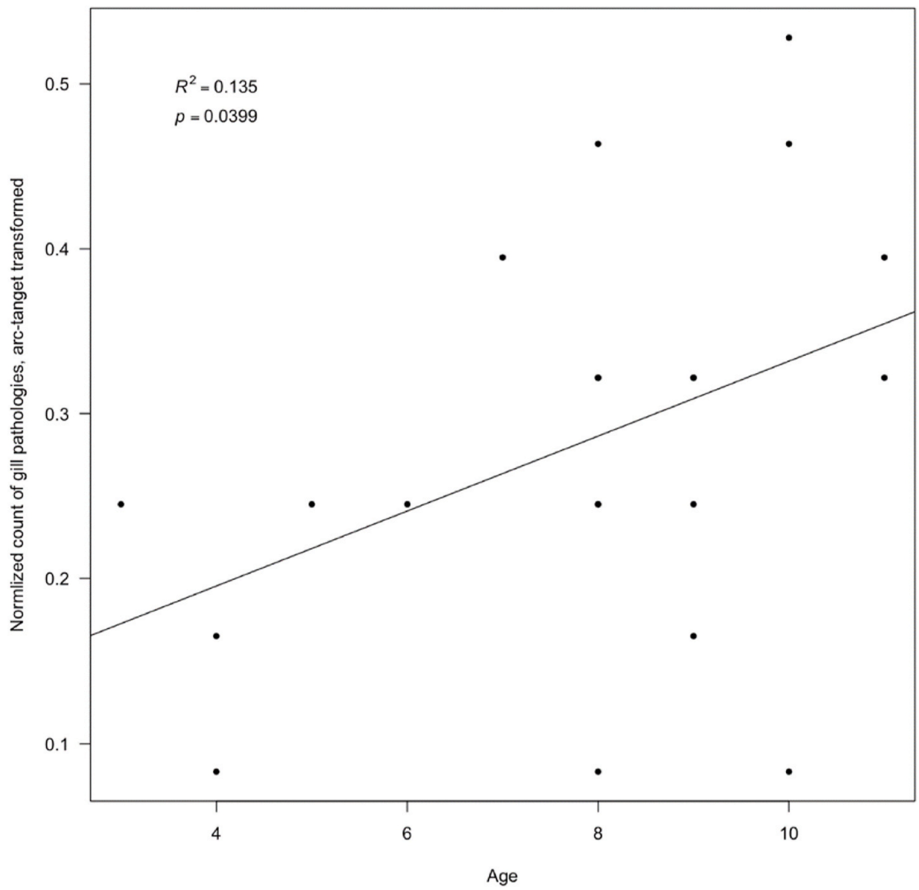

Fig. 6. Sum of normalized counts of histopathological alterations of gills investigated in the Arctic char as a function of age ($R^2 = 0.135$ and $p = 0.0399$).

Table 3
Concentrations of total proteins (g/L), albumin (g/L), alanine transaminase (U/L) and alkaline phosphatase (U/L) in blood serum of female (F) and male (M) Arctic char and shorthorn sculpins. Numbers are means with S.D. with min – max in parentheses. Note that, due to sampling difficulties, the number of replicates is lower than the number of fish collected.

Species	Gender	n	Total protein (g/L)	Albumin (g/L)	Alanine Transaminase (U/L)	Alkaline Phosphatase (U/L)
Arctic char	F	16	39.29 ± 5.86 (28.86–47.98)	19.13 ± 2.79 (14.29–23.67)	9.81 ± 11.97 (1.00–45.00)	518 ± 139 (323–878)
	M	5	38.91 ± 9.57 (25.25–47.75)	20.87 ± 3.13 (17.14–24.06)	9.40 ± 7.16 (0.00–19.00)	488 ± 170 (227–652)
Shorthorn sculpin	F	4	57.49 ± 5.42 (54.34–65.57)	18.61 ± 2.35 (16.07–21.54)	2.5 ± 1.73 (0.00–4.00)	292 ± 78 (234–406)
	M	1	31.25	12.54	2.00	105

element concentrations in gills, liver and muscle and histological changes in the gills and serum blood biochemistry in the two species. The cumulated gill pathologies correlated with age in the Arctic chars, and a similar trend was found for shorthorn sculpins. The age of fish is known to influence levels of pathological findings (Baumann et al., 1990; Baumann and Harshbarger, 1998; Okihiro et al., 2011; Stentiford et al., 2010), and this study underscores the importance of including age as a co-variable in studies involving histology of wild fish. The histopathological data along with the serum biochemical parameters suggested that the fish sampled in this study were healthy specimens and that they also originated from sites with little/no expected contamination.

An interesting finding is the high prevalence (66%) of chondroplasia in the shorthorn sculpins. Of the 25 Arctic char, only one individual showed this lesion. The high prevalence of chondroplasia in Greenland sculpins has been reported previously by Nørregaard et al. (2018) and Dang et al. (2021). In those studies, however, the lesion was found almost exclusively in fourhorn sculpins but not in shorthorn sculpins, and often in association with a cyst of unknown etiology. While these cartilage deformities certainly seem to be species specific, the specificity also seems to be related to the specific habitat of the fish within the geographic areas, evidenced through the high prevalence of chondroplasia found in the present study.

Two previous studies have reported levels of Hg in Arctic char in Greenland (Riget et al., 2000; Rigét et al., 2010). In Riget et al. (2000) a total of 72 anadromous Arctic chars were caught at three locations at Qaqortoq in Southwest Greenland. These studies reported an average Hg muscle concentration of 0.045 μg/g ww, which was about 3-fold higher than the average concentration measured in the present study (0.016 μg/g ww). Riget et al. (2000) also reported concentrations for a landlocked population at 70 km southwest of the capture site in Laksebugten. These fish had an average Hg muscle concentration of 0.231 μg/g ww, which is 15-fold higher than the present finding. This difference between Arctic char morphs is also well documented outside Greenland being related to the landlocked Arctic char's status as a top-level predator in these lakes combined with increased environmental concentrations due to natural and anthropogenic inputs (Dietz et al., 2013, Riget et al., 2000). The muscle Hg concentration measured in the present study is 30-fold lower than suggested threshold concentrations for tissue toxicity (0.5 μg/g ww) (Dietz et al., 2013; Barst et al., 2019). The concentrations of Pb and Cd in muscle tissue of Greenland Arctic char is Pb = 0.007–0.03 μg/g ww, Cd = 0.001–0.002 μg/g ww (AMAP, 2005). The Pb concentration in Arctic char (0.0028 μg/g ww) is in range of what is previously reported while Cd is 3.5-fold higher (0.0008 μg/g ww).

There are documented effects of REEs on bacteria, ciliates, macrophytes, crustaceans, nematodes, annelids and fish (Herrmann et al., 2016; Chen et al., 2000; Cui et al., 2012; Hua et al., 2017) f.x. at exposure concentrations at or above 0.04 mg/l for La (Hua et al., 2017); 0.07 mg/l for La and 0.06 mg/l for Yb (Cui et al., 2012) and 0.17 mg/l for Y; 0.11 mg/l for La and 0.10 mg/l for Gd (Chen et al., 2000). Though the exposure concentrations in the present study are unknown, the elements Ce, La and Y, have been found livers of Arctic chars in other areas of Greenland in ranges of 0.001 (±0.004) to 0.267 (±0.277); 0.008 (±0.004) to 0.481 (±0.52); and 0.002 (±0.003) to 0.019 (±0.011) μg/g ww, respectively (Nørregaard et al., 2019). Thus the concentrations found in the present study are within background levels in Greenland.

The present study documents unique differences in the element accumulation patterns between the shorthorn sculpin and Arctic char. In the shorthorn sculpins, the elements that were significantly higher compared to Arctic char were all "common" elements; Ag in the gills, As, Pb and Cd in the liver and Hg, Zn, As and Cd in the muscle. Contrary, the elements that were found in higher concentrations in Arctic chars compared to sculpins were almost entirely REEs (Y, Ce, La Nd, Dy in gills, Ce, La, Nd in liver).

There are a number of factors affecting element accumulation in fish, and there are distinct differences between the two species, all of which are known to affect element accumulation. Firstly, shorthorn sculpins are benthic fish and secondary to tertiary consumers, while the pelagic anadromous Arctic char is a predominantly tertiary consumer in the summer, when these fish were caught which is affecting exposure and bioaccumulation (Harwood and Babaluk, 2014, Couture and Pyle, 2011; Hussey et al., 2018; Muus et al., 1990). Secondly, the shorthorn sculpin is a purely marine species, while the Arctic char is anadromous. This implies a difference between these two species in the salinity-dependent ion regulatory system, which is known to play an important role in element accumulation and toxicity (Grosell et al., 2007). In addition, the overwinter stay in freshwater systems may affect the element mixture that the Arctic char is exposed to, for a larger part of the year, leading to an exposure pattern different from the shorthorn sculpins. This is due to differences in element bioavailability between salt and freshwater (Couture and Pyle, 2011; Wood, 2011), in addition to, the local geology impacting element concentrations in the habitat.

The results of the REE PCAs and biplots reveal an interesting pattern in the accumulation of elements in the two species. In the arctic chars and the shorthorn sculpins respectively, circa 95% and 80% of the variation in the gill and liver concentrations are explained by the first PC. Similarly, in the biplots, the REEs are tightly clustered, illustrating that the concentrations of the different REEs co-vary. This pattern has also been shown in the liver and muscle from pristine lake-residing brook trout (*Salvelinus fontinalis*) collected in Quebec, Canada (MacMillan et al., 2017). REEs are: i) chemically very similar, ii) are chemically relative inert, and iii) their bioavailability in marine environments are similar to each other (Munksgaard et al., 2003). It appears that this similarity also affects the uptake and excretion rates in Arctic chars and shorthorn sculpins leading to a proportional accumulation pattern.

Accumulation and derived toxic effects of element mixtures are difficult to model consistently due to the interactive nature of elements (Paquin et al., 2011), and this is clearly a topic that should be investigated further. Further, while this study describes baseline conditions in an area with no known major geological source of REEs, further studies of fish in areas with naturally elevated levels of REE near ore deposits such as those proposed for mining in South Greenland (Paulick et al., 2015) should also be studied prior to any mining activity in these areas.

5. Conclusions

There were no significant relationships between gill histology, blood biochemistry and the element concentrations in shorthorn sculpins and Arctic chars. Cumulated prevalence of lesions, however, was positively correlated with the age. This along with species specific differences in histopathology underscores the need to include these as co-variables in studies on effects of pollution on wild fish. For the element concentrations in organs and tissues, species differences were found. The group of REEs were, when significantly higher, always found highest in the Arctic char and the concentrations of REEs apparently co-varied in the gills, liver and muscle. The results in the study can be used as an important baseline to compare results from future environmental monitoring and impact assessments.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.112742.

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