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Otolith Chemistry of Common Sculpins (*Myoxocephalus scorpius*) in a Mining Polluted Greenlandic Fiord (Black Angel Lead-Zinc Mine, West Greenland)

**Jens Søndergaard · Norman Halden · Lis Bach ·
Kim Gustavson · Christian Sonne · Anders Mosbech**

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Abstract Sculpins are widely used as key species for monitoring heavy metal pollution near arctic mine sites. Typically, metal concentrations in liver and muscle tissue have been used as a proxy for metal exposure but such analyses lack temporal information of uptake and accumulation. Otoliths (ear bones) are considered metabolically stable and can potentially contain a complete record of the fish's metal exposure history. To investigate the otolith chemistry of sculpins and the potential of these as records of metal exposure, common sculpins (*Myoxocephalus scorpius*) were collected at five sites near a former Pb–Zn mine in West Greenland. Otoliths were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for 12 elements of which Mg, Mn, Sr, Ba, and Pb were detected. The highest Pb concentrations were found within the otoliths from the most Pb-polluted sites near the mine (up to 0.6 ppm), and decreasing concentrations were observed in a gradient away from the mine. Notably, Pb and Sr variations were closely correlated and showed an annual oscillatory pattern with peaks consistently found in the winter zones. It is not clear to what the extent high winter-time accumulation of Pb in the otoliths is due to high winter-time exposure of Pb through diet or water and/or to physiological processes such as growth in the sculpins. The study indicates that LA-ICP-MS analyses of sculpin otoliths have the potential to become a valuable method for assessing time-resolved metal loading near mine sites but also that more studies are required to investigate the links between metal sources, pathways, and processes affecting otolith metal deposition.

Keywords Sculpins · *Myoxocephalus scorpius* · Mining · Otoliths · LA-ICP-MS

1 Introduction

Metal pollution from mining activities is a well-known environmental concern, and detailed environmental monitoring before, during, and after mining is essential to continuously evaluate the pollution status of a mining area, identify pollution sources and pathways, and if needed initiate mitigation measures. In Greenland, methods for environmental monitoring near mine sites have traditionally been based on sampling of a selection of key monitoring organisms including seaweed, blue mussels, and fish (Søndergaard 2013). Sculpins have been used as the primary fish monitoring species for decades due to its wide abundance, non-migratory behavior, and ability to accumulate metals (Johansen et al. 1991; Søndergaard 2013; Sonne et al. 2014). In the sculpins, metal concentrations in the liver and muscle

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J. Søndergaard (✉) · L. Bach · K. Gustavson · C. Sonne · A. Mosbech
Arctic Research Centre, Department of Bioscience, Aarhus University, Frederiksborgvej 399, Roskilde DK-4000, Denmark
e-mail: js@bios.au.dk

N. Halden
Department of Geological Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

tissue have mainly been used to evaluate the mining pollution. These sample types can be used to assess recent metal pollution, but provide no temporal or historical information on metal exposure. This is especially a limitation at abandoned arctic mine sites where monitoring is usually based on sample collections during summer and where samples are often not taken on a yearly basis. A method of achieving a temporal information of metal loading at a mine site is to collect and analyze undisturbed sediment cores, but such cores are often challenging to collect, provide relatively low temporal resolution (using ^{210}Pb dating), and give little or no information about the bioavailability and biological uptake of the metals in biota (Elberling et al. 2002; Perner et al. 2010).

Advances in analytical techniques such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) have opened new opportunities for analyzing solid materials such as otoliths (fish ear bones) with a very fine spatial resolution (Halden and Friedrich 2008). Otoliths are tiny paired ear bones in all teleost fish used for the sensing of equilibrium and hearing (Payan et al. 2004). Otoliths consist of almost 100 % calcium carbonate in a protein matrix, primarily in the mineral form of aragonite, which has the ability to incorporate a range of other elements in the mineral structure (Halden and Friedrich 2008). LA-ICP-MS analyses of otoliths may provide an important new method for monitoring metal loading near mine sites as otoliths are considered metabolically stable, grow continuously during the lifetime of the fish, and may provide a complete time-resolved chemical record of fish's exposure history (Halden and Friedrich 2008; Friedrich and Halden 2011). Recent studies have highlighted the potential for fish otoliths as recorders of anthropogenic pollution with metals such as Cu, Zn, Pb, Ni, Cr, and Fe (Renaldi and Gagnon 2008; Friedrich and Halden 2010; Friedrich and Halden 2011).

Element incorporation into otolith aragonite, however, is a complex, multistage process, involving the movement of ions from the ambient water or food sources into the blood plasma via branchial or intestinal uptake, across inner ear membranes into the endolymph fluid surrounding the otoliths, and finally into the growing surface (Payan et al. 2004). Therefore, the chemical composition of the otolith will, at least in part, reflect the physicochemical conditions of the surrounding environment (Campana 1999). It is generally accepted that otolith chemical composition is influenced by both environmental and fish physiological factors, but the relative importance of these factors on the uptake of different chemical elements and differences between fish species are currently debated (Elsdon et al. 2008; Sturrock et al. 2012; Sturrock et al. 2014). Element discrimination from the environment to the otolith, ultimately affecting the otolith chemistry, can occur when elements are transferred across the several barriers/membranes (Sturrock et al. 2012).

This study is the first to investigate spatially resolved otolith chemistry of common sculpins and the accumulation and distribution of pollutants in sculpin otoliths near mine sites. The study is based on collection of sculpins from sites located on a distance-gradient away from the former Pb–Zn mine at Maarmorilik in West Greenland. Despite that the mine closed in 1990, the area is still heavily polluted by previous mining activities (Søndergaard et al. 2011a). The histopathology of the same sculpins as used in this study were examined by Sonne et al. (2014) who found that the sculpins at the most polluted sites near the mine were in a significantly worse health state (higher occurrence of gill and liver lesions and parasites) than the sculpins at the sites further away presumably due to uptake and accumulation of Pb, Hg, and As. During the time the sculpins were collected, the water quality at the sampling sites was assessed using diffusive gradients in thin films (DGT) samplers deployed in 9 days to provide a recent time-integrated measure of the bioavailable metal concentrations in the water (Davison and Zhang 1994). The DGT results have previously been published in Søndergaard et al. (2014). The hypothesis of this study was that otoliths from sculpins from the most polluted sites near the mine (as reflected by the DGT results) would contain higher concentrations of mine-related metals (such as Pb) than otoliths from sculpins from less- or non-polluted sites located further way. Furthermore, it was hypothesized that the distribution of metals in the otoliths originating from a point source such as the mine in Maarmorilik would provide evidence for the assumed non-migratory behavior of the sculpins in these areas (least in the scale of 10–20 km). Finally, the occurrence and spatial distribution of mine-related metals in the otoliths was considered to provide insight into the potential for using sculpin otoliths as temporal records of metal exposure at these sites.

2 Methods

2.1 Field Sites, Sampling of Common Sculpins, and In Situ Measurements of Dissolved 'Bioavailable' Metal Concentrations Using DGT Samplers

The Black Angel mine in Maarmorilik operated between 1973 and 1990, and the mining activities caused significant pollution of the surrounding area with mainly Pb, Zn, Cd, As, and Hg of which elevated levels can still be detected today more than 20 years after closure (Asmund et al. 1991; Søndergaard et al. 2011a; Sonne et al. 2014). Sources of pollution in Maarmorilik include subsea disposal of mine tailings and waste rock in the fiords, leaching of metals from waste rock located at the mountain slopes, and dispersion of metal-containing dust (Søndergaard et al. 2011a; Søndergaard et al. 2011b). The fiords near Maarmorilik can be described as stratified estuaries during summer. The stratification is caused by melt water from the rivers that flow on top of the heavier seawater creating a stagnant water mass in the bottom of the fiords. During winter/spring, however, a complete vertical mixing of the stratified water bodies has been reported to take place due to cooling and increased density of the upper surface water (Møller 1984). Hydrographical data from the area is sparse (ATV 1973; Møller 1984), but seawater temperatures have been reported between −1 and 10 °C and salinities between 26 and 34 psu. The tidal range is around 1 m and sea ice, 0.5–1 m thick, usually covers the fiords from November to April. A more detailed description of the area is provided in Søndergaard et al. (2011a).

Common sculpins were collected at five sites near the former Black Angel Pb–Zn mine (Fig. 1) in August 2012. Sites 1–3 are all located within 2 km of the former mining area, whereas sites 4 and 5 are located 12 and 40 km away from the mine, respectively. The five sampling sites for sculpins at Maarmorilik represent a gradient of pollution from the mining activities (Sonne et al. 2014; Søndergaard et al. 2014). Sites 1–3 are considered equally and highly polluted, whereas site 5 is not considered impacted by the mining activities and is regarded as an unpolluted reference site (Søndergaard et al. 2011a). Site 4 is only considered slightly polluted. A total of 35 sculpins, 7 individuals at each site, were sampled. All sculpins were caught near shore by angling using fishing rods and lures from boat and were caught near the bottom at depths ranging between 2 and 10 m. At the end of each sampling day, the fish were measured, weighed, and dissected. The total fish length, sex, total weight, and liver weight were recorded (Table 1). Otoliths and liver were collected from each individual and kept in polyethylene bags. Liver samples were kept frozen until analyses.

DGT samplers for measuring dissolved time-integrated 'bioavailable' metal concentrations in the water (www.dgtresearch.com) were installed near the bottom and left for 9 days at the sites during the period when the sculpins were collected. DGT samplers were inserted into pre-drilled holes in a piece of 20-cm-long and 9-cm-diameter PVC tube and held in place by plastic strips. The samplers were installed as triplicates with the membrane surface at the inside of the tube to minimize bio-fouling and sediment deposition at the surface. The tubes were tied to a nylon rope and were left hanging suspended from a plastic buoy with a weight tied to the end (1-L polyethylene bottle filled with sand). The buoy setup was anchored to the sea floor using a piece of rock. Three DGT samplers were selected as control blanks and not immersed in the seawater. Further details on the DGT treatment, analyses, and calculations are given in Søndergaard et al. (2014).

2.2 Analyses of Otoliths

Sculpin otoliths were analyzed for trace element distribution using LA-ICP-MS at the Department of Geological Sciences, University of Manitoba, Canada. To prepare for the analyses, otoliths were embedded in epoxy resin and cut transverse to create dorsoventral cross sections through the core of the otolith, exposing all annuli. Cross sections were sliced into 2-mm-thick sections, which were re-embedded in epoxy resin in 25-mm-diameter acrylic microprobe mounts (typically six otoliths per mount). The exposed otolith surfaces were ground (30- and 9-μm-wetted aluminum oxide lapping film), polished (0.3-μm-wetted aluminum oxide lapping film), and finally ultrasonically cleaned with Milli-Q water prior to analyses. Sectioned otoliths were analyzed by LA-ICP-MS using a Thermo Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd-YAG laser. Platinum cones, a 40-μm beam diameter and a scan speed of 3 $\mu m\ s^{-1}$ were used. The following isotopes were measured: ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{77}Se, ^{88}Sr, ^{111}Cd, ^{137}Ba, ^{202}Hg, and ^{208}Pb. Calcium was used as internal standard. External calibration was done using the certified reference materials NIST 610 glass (for all other elements but Hg) (www.

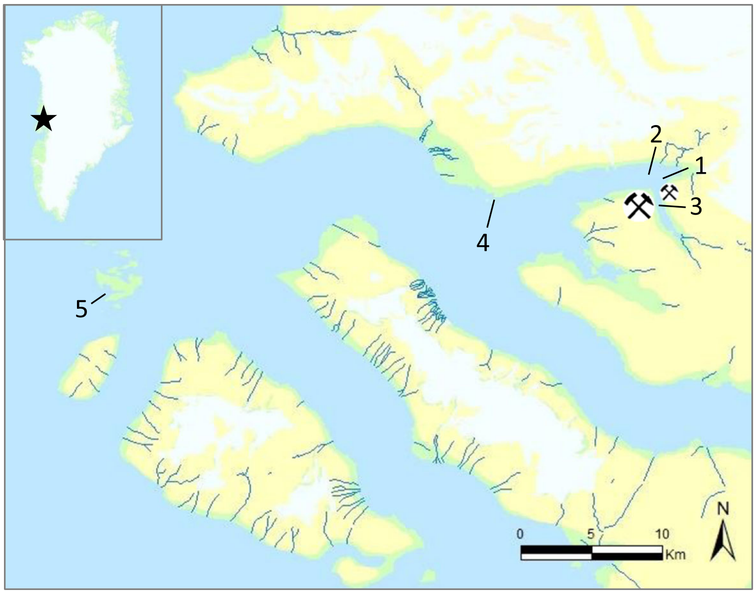

Fig. 1 Map showing the five sculpin sampling sites near the former Black Angel Pb–Zn mine (*marked with mining symbols*) in Maarmorilik, West Greenland

nist.gov) and USGS MACS-3 synthetic calcium carbonate pellet (for Hg) (www.usgs.gov). Scan lines were run in low-resolution mode for all elements. Iron contents in the otoliths were further evaluated based on spot sampling in medium-resolution mode. Scan lines were run either from edge to edge or from core to edge, at a high angle to the annuli. Standards were run at least every two samples. Element concentrations and detection limits (3 SD on background signal before and after each sample) were calculated using Iolite software (Paton et al. 2011). Detection limits for Mg, Mn, Sr, Ba, and Pb are given in Table 2. Detection limits for Fe, Ni, Cu, Zn, Se, Cd, and Hg were 6, 0.2, 0.2, 0.2, 0.2, 0.06, and 0.07 ppm, respectively (these elements were not found above detection limits in samples). Molar Sr/Ca ratios in the otoliths were calculated using the assumption that Ca concentrations in the otoliths were constant at 396 mg/g, corresponding to 99 % $CaCO_3$ (similar to Renaldi and Gagnon 2008). Before and after the LA-ICP-MS analyses, the embedded otoliths were viewed under a microscope using reflective light and the image captured using a digital camera. The age of the sculpins was determined based on assumed annuli counting from the core to the edge. Winter rings appeared as narrow

Table 1 Location of the sampling sites and biological information of the 35 common sculpins sampled in the Maarmorilik area during the summer 2012

Location	GPS positions	Sex	Number	Length (cm)		Total wet wt. (g)		Liver wt. (g)		Est. age (years)	
				Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Site 1	71° 13′ 37.2″ N	F	5	29–41	34±5	291–693	489±181	11–30	19.3±6.8	5–10	7±2
	51° 24′ 85.4″ W	M	2	26–28	27±1	212–270	241±41	3.4–4.5	4.0±0.8	5–7	6±1
Site 2	71° 14′ 48.8″ N	F	4	30–41	36±6	370–705	539±184	10–18	15.1±3.7	7–14	10±3
	51° 24′ 32.7″ W	M	3	25–28	27±2	175–248	215±37	2.4–5.5	3.8±1.6	6–8	7±1
Site 3	71° 12′ 14.4″ N	F	6	30–36	34±3	265–585	452±106	3.7–26	14.1±9.2	6–9	8±1
	51° 25′ 10.0″ W	M	1	25	–	188	–	3.8	–	6	–
Site 4	71° 09′ 99.6″ N	F	5	28–40	32±5	244–657	377±170	7.6–20	14.6±4.5	6–11	8±2
	51° 57′ 34.4″ W	M	2	28	28±0	216–236	226±14	3.3–8.7	6.0±3.8	5	5±0
Site 5	70° 99′ 66.5″ N	F	5	27–30	29±1	190–362	275±61	3.5–15	7.9±4.5	4–7	5±1
	52° 28′ 12.5″ W	M	2	23–27	25±3	138–223	181±60	1.8–10	6.0±6.0	3–4	4±1

Table 2 Concentrations of magnesium (Mg), manganese (Mn), strontium (Sr), barium (Ba), and lead (Pb) (ppm dry wt.) in sculpin otoliths from the sampling sites

Location		Otolith					Liver
		Mg	Mn	Sr	Ba	Pb	Pb
	Typical d.l.	1	0.09	0.2	0.06	0.006	0.03
Site 1	Site mean±SD	13±2	0.63±0.22	2500±190	2.2±0.6	0.017±0.014	0.51±0.59
	Typical scan range	5–30	<d.l.–2	1000–5000	1–5	<d.l.–0.2	0.1–1.4
Site 2	Site mean±SD	12±2	0.64±0.24	2400±230	2.2±0.6	0.031±0.026	1.00±0.71
	Typical scan range	5–25	<d.l.–2	1500–5000	1–5	<d.l.–0.2	0.2–1.8
Site 3	Site mean±SD	14±3	0.95±0.41	2400±290	2.2±0.5	0.026±0.023	0.31±0.25
	Typical scan range	5–30	<d.l.–2	1000–5000	1–5	<d.l.–0.2	0.02–0.8
Site 4	Site mean±SD	15±4	1.30±0.91	2500±370	2.2±0.3	0.006±0.004	0.09±0.09
	Typical scan range	5–30	<d.l.–5	1000–5000	1–10	<d.l.–0.05	<d.l.–0.2
Site 5	Site mean±SD	13±2	0.97±0.42	2000±220	2.4±0.5	<d.l.	0.03±0.04
	Typical scan range	5–30	<d.l.–5	1000–5000	1–10	<d.l.	<d.l.–0.09

The mean±SD otolith concentration for each site are calculated based on mean concentrations of LA-ICP-MS scans through the entire otoliths for all the individuals at the sites. The typical concentration range in a LA-ICP-MS scan through the entire otoliths for each site is also given. A range of other elements were analyzed in the otoliths (see Section 2) but were below the detection limit. Finally, concentrations of Pb (mean, SD, and range) in the liver (ppm wet wt.) are shown for comparison

d.l. detection limit determined as three standard deviations (SD) on background signals

and dark zones, whereas summer rings appeared as broad and light zones (Luksenburg and Pedersen 2002).

2.3 Analyses of Liver Samples

Liver samples were analyzed for Pb contents at the Department of Bioscience, Aarhus University, Denmark. Sub-samples (1 g wet wt.) were cut and microwave digested (Anton Paa Multiwave 3000) in Teflon bombs in 4 mL/4 mL Merck Suprapure HNO_3/Milli-Q water. Digestion solutions were diluted with Milli-Q water and analyzed for Pb using an Agilent 7500ce ICP-MS. The analytical quality was checked by analyzing blanks, duplicates, and the certified reference materials DOLT-4, DORM-4, and TORT-2 (fish liver, fish protein, and lobster, respectively) from the National Research Council Canada (www.nrc-cnrc.gc.com) along with the samples. The recovery percentage of Pb in the certified reference materials was between 95 and 111 % and the detection limit is shown in Table 2.

2.4 Data Treatment

Mean element concentrations in otolith scans were statistically compared between sites using Microsoft Excel software. A two-sample t test was used. Prior to the t test, datasets were tested for equal variances using an F test. Significance is used in cases where $p<0.05$. The correlation between Pb concentrations in the otoliths and Pb concentrations in the liver was investigated by means of a linear regression analysis using the "Analyses Toolpack" in Excel.

3 Results and Discussion

3.1 Strontium Distribution, Light//Dark Zonation, and Dating of the Otoliths

Strontium in the otoliths showed oscillatory variations correlating with the dark/light zonation or growth rings in the otoliths (Fig. 2). Peaks of Sr concentrations were consistently observed in the narrow dark zones in the otoliths and low Sr concentrations occurred in the broad light zones. The typical range of Sr concentrations in the otoliths was 1000–5000 ppm (Table 2) corresponding to Sr/Ca ratios between 1 and 6 μmol mol^{-1}. The otolith cross sections were scanned from edge to edge, and the scan results showed that distribution patterns of Sr and other elements in the growth rings were mirrored on

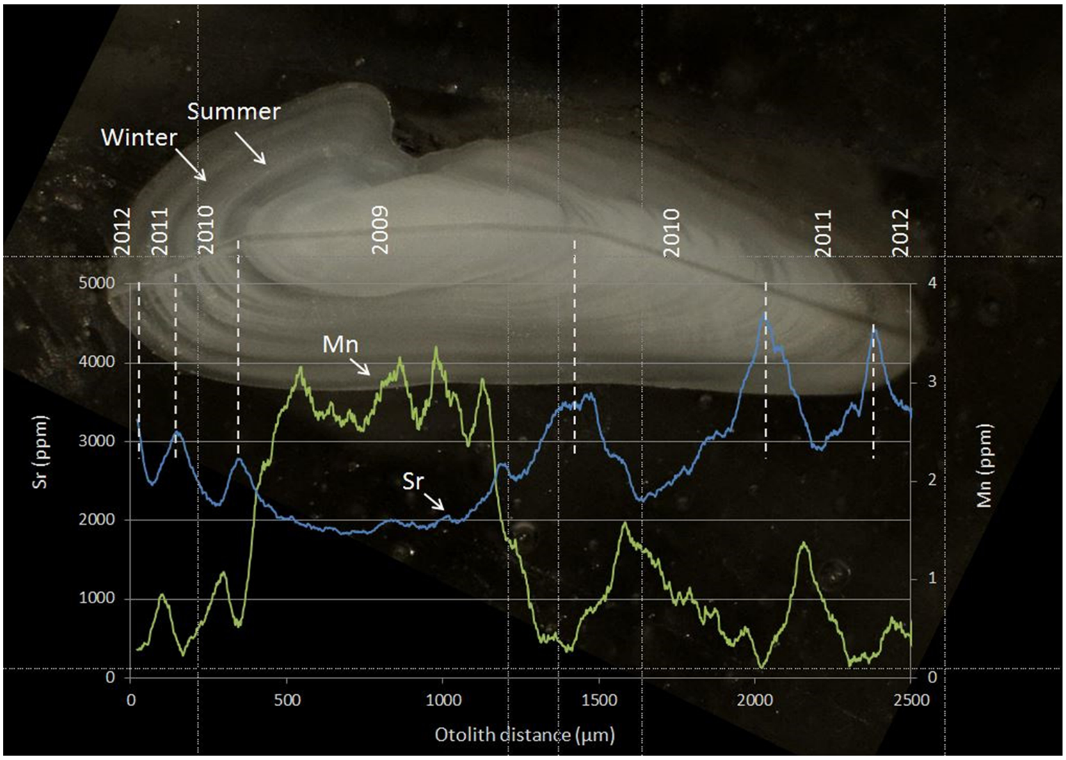

Fig. 2 Overlay of a LA-ICP-MS strontium (Sr) and manganese (Mn) distribution profile on an image of a sculpin otolith. Wide light and narrow dark zones in the otolith (viewed in reflected light) represent summer and winter growth layers, respectively. Years corresponding to each section of the otolith were determined by assigning annual growth zones from the year of capture (2012) backwards to the otolith primordia. Lines are 25-point running averages

both sides of the cores, but also that peak concentrations could vary markedly between the two sides (Fig. 2).

The range of Sr concentrations and Sr/Ca ratios found in sculpin otoliths in this study was within the range of Sr concentrations and Sr/Ca ratios reported in otoliths from other fish species in estuarine and marine areas (Dorval et al. 2007; Brown and Severin 2009; Morais et al. 2011). Oscillatory variations in otolith Sr concentrations were found in the marine fish species sablefish (*Anoplopoma fimbria*) (Brown and Severin 2009) and European flounder (*Platichthys flesus*) (Morais et al. 2011), but no attempt was made in these studies to correlate the variations with the growth increments of the otoliths. Growth rings of alternating dark and light zones in otoliths are widely used as a proxy for fish age and a single growth annulus typically consists of a narrow dark zone (when viewed in reflected light) deposited during the winter (slow growth) and a wide white zone deposited during the summer period (fast growth) (Renaldi and Gagnon 2008). In this study, Sr showed an oscillatory pattern corresponding with the dark/light zones in the otoliths, which indicate that both visual dating using dark/light zones and chemical dating using the Sr variations can be used as proxies to date the sculpins, at least for sculpins at these study sites. The sculpins collected during this study were estimated to be between 3 and ~14 years old (Table 1). The differences in absolute peak concentrations of Sr and other elements on both sides of the otolith cores sometimes observed in the edge to edge scans are mainly considered due to the geometry of the otolith, i.e., the orientation of the beam relative to the annuli. The beam penetrates 30–40 µm into the otolith and if the annuli are spaced close together and/or are not oriented perfectly perpendicular to the plane, the volume of material sampled may represent a mixture of annuli. In the following, core to edge scans on the widest side of the otoliths were used in order to minimize the effect (from core to the right on Fig. 2).

Oscillatory annual variations in Sr distribution in the sculpin otoliths can be due to several factors including variations in salinity, temperature, migration patterns, and physiological processes in the fish such as somatic growth (Campana 1999; Elsdon et al. 2008). No data was available on the variation in water salinity, temperature, and migration pattern of the sculpins at the sampling sites. However, high Sr concentration in otoliths during winter deposition was consistent with an expected higher salinity of the habitat during winter due to the lack of freshwater input. Also, low Sr concentrations during the summer period were in line with a high freshwater input due to snowmelt. However, the large variation in Sr (typically 1000–5000 ppm) and the fact that sculpins inhabit the sea bottom where salinity and temperature variations are expected to be lowest, indicate that factors other than salinity and temperature are likely to contribute to the variation. A number of studies have indicated that physiological processes may play an important role in regulating the deposition of Sr and other elements in the otoliths of marine fish (Kalish 1991; Sadovy and Severin 1992; Sadovy and Severin 1994; Brown and Severin 2009; Sturrock et al. 2014). However, processes and controls for element discrimination during otolith formation are not well understood (Elsdon et al. 2008). We hypothesize that the large annual Sr variation in the sculpin otoliths observed in this study was significantly influenced by physiological factors, i.e., variations in activity of internal processes and pathways controlling element uptake, transport, and deposition in the otolith (these being more or less selective towards Sr relative to Ca). Growth is considered the dominant physiological process in this regard, and growth rates of sculpins in this area are expected to display a distinct annual variation due low temperatures and decreased food availability during the winter period. Despite that the factors causing the annual variations in Sr in the sculpin otoliths in this study are being less well understood, the annual oscillatory pattern of Sr indicates that Sr oscillations can be used as a proxy for dating the sculpins and used as a timescale from the time of capture. Determination of fish age based on visual counting of growth increments is known to be markedly influenced by the reader and subject to uncertainty (Herbst and Marsden 2011; Dortel et al. 2013). Chemical patterns of elements displaying an annual oscillatory variation, in this case Sr, may provide a more accurate age determination or at least assist the visual dating to provide a more accurate age estimate.

3.2 Distribution of Mg, Mn, and Ba in the Otoliths

In addition to Sr, the elements Mg, Mn, and Ba were found in all sculpin otoliths. Like Sr, Mg showed annual oscillatory variations but variations were inversely correlated with those of Sr (Fig. 3). Consequently, peaks of Mg concentrations were observed in the light layers of the otoliths. Mn concentrations were typically highest in the core and showed oscillatory variations inversely correlated with Sr similarly to Mg (Fig. 2). Mean scan Mg and Mn concentrations in the otoliths did not differ significantly between sites. Ba showed distinct oscillatory variations that sometimes but not always correlated positively with variations in Sr (Fig. 3) suggesting that the timing of Ba uptake and/or exposure was different from that of Sr.

The reason for the observed Mg variations opposite of Sr in the sculpin otoliths is not clear. Sturrock et al. (2014) measured near-constant Mg concentrations in the blood plasma of European plaice (*Pleuronectes platessa*) despite significant variations in water Mg concentrations over a 1-year period. This could imply that the Mg variation found in the sculpin otoliths was probably not a result of Mg variations in water chemistry. Other studies have attempted to correlate otolith Mg variations with temperature, diet, growth rates, and intrinsic factors but with contradictory results (Sturrock et al. 2012). Physiological processes like growth may play an important role for the observed Mg distribution in the sculpin otoliths since Mg is an important element for a range of enzymatic reactions including proper bone formation.

Similar to the sculpin otoliths, high Mn concentrations in otolith cores were reported in a study of Atlantic herring (*Clupea harengus*) (Brophy et al. 2004). Brophy et al. (2004) hypothesized that the high Mn concentrations in the core area of the herring otoliths were either due to elevated Mn concentration in the egg during embryological development or variations in mineral structure of the calcium carbonate portion of the otolith core.

Barium in otoliths has been shown to reflect Ba in both the water and food sources (Doubleday et al. 2013; Buckel et al. 2004, Halden, unpublished data). In the present study, variations in Ba were generally, but not always positively correlated with Sr in the sculpin otoliths. Differences between Ba and Sr could be due to sudden changes in food availability and diets having different Ba contents relative to Sr.

3.3 Pb in Otoliths and Liver Tissue

Pb was observed in the sculpin otoliths above the detection limit but only at the four polluted sites near the mine

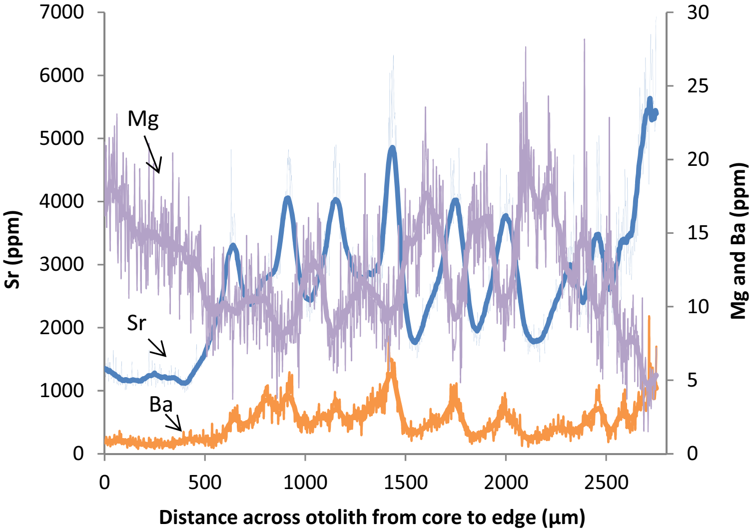

Fig. 3 Typical LA-ICP-MS barium (Ba, in *orange*), magnesium (Mg, in *purple*), and strontium (Sr, in *blue*) distribution profiles in sculpin otoliths. Smoothed lines are 25-point running averages

(Table 2). Sculpin otoliths from the unpolluted reference site (site 5) did not contain Pb above the detection limit. Mean LA-ICP-MS scan Pb concentrations in the otoliths from sites 1, 2, and 3, all located within 2 km from the mine, were significantly higher than those found at site 4, located 12 km away. Like Sr, variations in Pb concentrations showed an annual oscillatory pattern and variations in Pb correlated well with those of Sr (Fig. 4). Peaks of Pb concentrations in the otoliths were almost consistently found in the narrow dark winter zones of the otoliths.

The gradient in mean scan Pb concentrations in the sculpin otoliths decreasing with distance from the mine site in Maarmorilik indicates a significant Pb uptake and accumulation due to elevated Pb concentrations in the environment proximal to the mine. This is also evident from the DGT results showing dissolved 'bioavailable' Pb concentrations in the range of 0.30–0.81 μg L^{-1} at sites 1–3 decreasing to 0.01–0.03 μg L^{-1} at site 5 (Table 3). The peaks in Pb concentrations consistently observed within the dark zones of otoliths representing winter deposition are notable. It is unclear if the high winter Pb accumulation in the otolith is a result of physiological processes affecting internal regulation and subsequent deposition of Pb in the otoliths or due to a higher Pb exposure and uptake by the sculpins during winter. However, the consistency of the peaks and the good correlation with Sr indicate that it could be controlled by physiological processes superimposed on an environmental concentration gradient.

Peaks of Pb concentration in the otoliths during winter time varied up to tenfold between years indicating a significant inter-annual variation in otolith Pb accumulation. No consistent Pb distribution patterns were observed for the seven individual sculpins caught at each of the polluted sites in Maarmorilik (except that Pb peaks consistently occurred during winter time) showing high individual variability. Consequently, if absolute concentrations of metal ions in otoliths are to be used to evaluate the pollution status of a given site, a relative large sample size is needed to account for differences in life history stages, sex, variability in diet, etc. that could contribute to the variability in otolith metal accumulation.

In the sculpins, Pb was measured both in the otoliths and in the liver (Table 2). Sculpin liver samples have been included in environmental monitoring programs in Greenland for decades as a proxy for recent pollutant uptake (Johansen et al. 1991; Søndergaard 2013). Liver Pb concentrations (on wet wt. basis) were typically 10–30 times higher than mean otolith Pb concentrations in the LA-ICP-MS scans. Similar to the trend in otolith Pb concentrations, mean Pb concentrations in liver samples were significantly higher in sculpins from sites 1, 2, and 3 closest to the mine compared to sites 4 and 5 (Table 2). On an individual fish basis, Pb concentration in the otoliths correlated significantly with Pb concentration in liver but showed great variability. The best correlation was observed when using the mean otolith LA-ICP-MS scan Pb concentration in the most recent annulus versus the liver concentration ($r=0.49$; $p<0.01$). A slightly

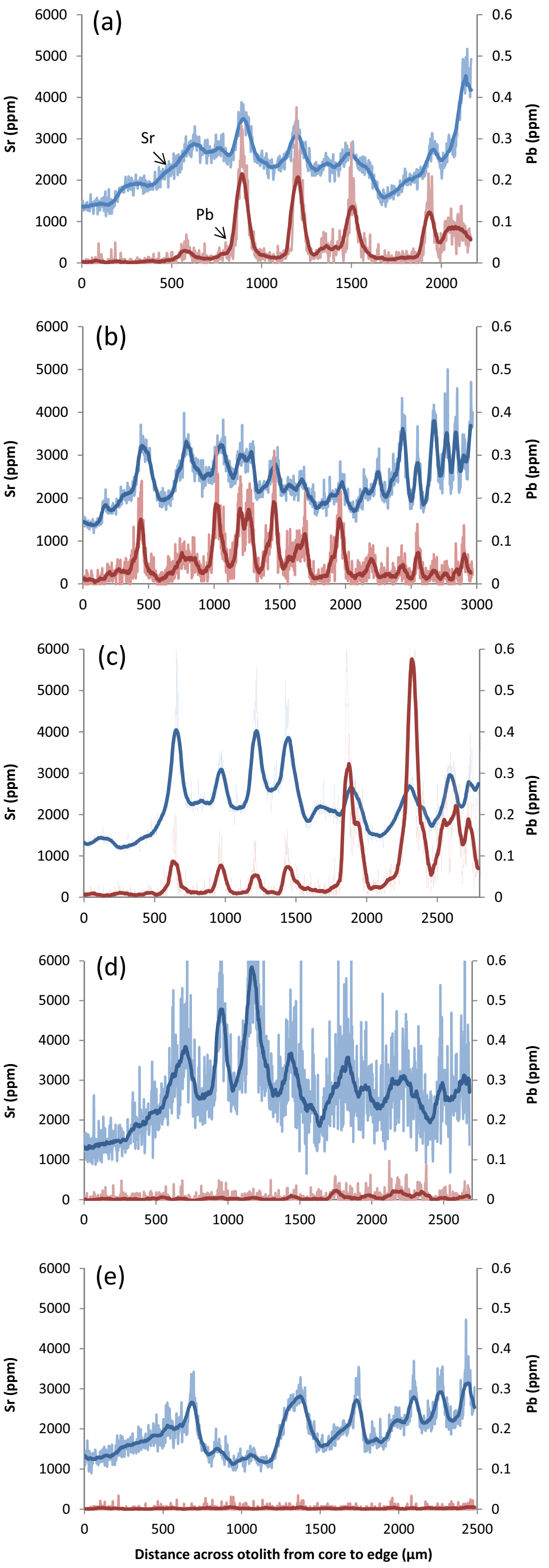

Fig. 4 Typical LA-ICP-MS strontium (Sr, in *blue*) and lead (Pb, in *red*) distribution profiles in sculpin otoliths from each of the five sampling sites near the former Black Angel lead-zinc mine in Maarmorilik, West Greenland. Sites 1, 2, and 3 (**a**–**c**, respectively) are located within 2 km of the mine site, while site 4 (**d**) and 5 (**e**) are located 12 and 40 km away from the mine, respectively. Smoothed lines are 25-point running averages

weaker correlation was obtained when correlating the mean otolith Pb scan concentrations in the entire otolith versus the liver concentration ($r=0.47$; $p<0.01$). Different pathways, regulations, and controls of Pb affecting accumulation in otoliths and liver of fish and processes like metabolic transformation and depuration, which can affect the sculpin's liver Pb content over time as opposed to otoliths that are regarded as metabolically stable, are considered important factors causing the variability between otolith and liver Pb contents.

3.4 The Potential for Sculpin Otoliths to Assess Time-Resolved Metal Pollution in Arctic Mining Areas

Accumulation of Pb in sculpin otoliths at Pb-polluted sites near the former Pb–Zn mine in Maarmorilik shows that application of sculpin otoliths can potentially be a valuable method to study time-resolved metal exposure, uptake, and accumulation by fish near mine sites. Application of otolith data could especially be relevant for environmental monitoring at infrequently visited former mine sites where other types of time-resolved data or samples are not available or accessible.

Our results showed that a timeline of up to ~14 years could be assessed from the sculpin otoliths. However, of the metals of concern to mining pollution in Maarmorilik (Pb, Zn, Cd, As, and Hg) (Søndergaard et al. 2011a; Sonne et al. 2014), only Pb could be measured above the detection limit in the otoliths. In contrast, detectable concentrations of all the abovementioned elements were observed in the liver samples (Sonne et al. 2014). Sonne et al. (2014) showed that only Pb, As, and Hg in liver samples differed between the sites in Maarmorilik and were related to the mining activity. Zn and Cd concentrations in the liver did not vary between sites indicating a high degree of physiological regulation of Zn and Cd in the sculpins. Since only Pb could be measured in the sculpin otoliths and only at the polluted sites, it indicates that the applicability of sculpin otoliths for monitoring metal loading is limited to relatively polluted sites and greatly selective to specific elements. Accumulation of Cu, Zn, Pb, Ni,

Table 3 Concentrations of dissolved 'bioavailable' metals in bottom water near the sampling sites in microgram per liter

	Cr	Fe	Ni	Cu	Zn	Ag	Cd	Pb
d.l.	0.019	0.1	0.02	0.15	0.31	0.0005	0.002	0.01
Site 1[a]	0.052–0.107	3.5–4.6	0.57–0.67	0.27–0.57	1.49–3.12	0.0007–0.0024	0.031–0.037	0.51–0.81
Site 2	0.030	2.9	0.52	0.30	1.23	0.0012	0.025	0.49
Site 3	0.027	2.9	0.46	0.17	0.76	0.0007	0.018	0.30
Site 4[b]	0.040	3.0	0.52	0.19	0.61	0.0009	0.015	0.14
Site 5[a]	0.054–0.135	2.4–2.7	0.77–0.90	0.32–0.66	0.37–0.83	0.0017–0.0032	0.025–0.025	0.01–0.03

Concentrations were measured using DGT samplers ($n=3$) suspended from buoys during a 9-day period at the time the sculpins were collected

d.l. detection limit determined as three SD on control blanks

[a] The values represent measurements from two buoys near sites 1 and 5, respectively

[b] The actual position of this buoy was between the original site 4 and sites 1–3 (Søndergaard et al. 2014)

Cr, and Fe in otoliths of various fish species due to anthropogenic pollution have previously been documented (Renaldi and Gagnon 2008; Friedrich and Halden 2010; Friedrich and Halden 2011). However, since uptake, regulation, and otolith accumulation of various metals varies from species to species (Campana 1999), it is unclear if sculpin otoliths could be used to monitor pollution of all these metals.

Sculpin is considered an adequate marine species for monitoring pollution from point sources such as arctic mine sites due to the widespread abundance in the Arctic region and relatively non-migratory behavior (Søndergaard 2013; Sonne et al. 2014). However, no specific information is available on the migration pattern of sculpins in Greenlandic fiords. The Pb concentrations measured in the sculpin otoliths from sites 1 to 3 (Fig. 4a–c) were consistently higher during the entire life of the fish than in the sculpin otoliths from site 4 (Fig. 4d) located 10–12 km away. This indicates that the distances they migrate are likely to be lower than that. Besides sculpins, other species could also be relevant to use. An example is Arctic char, which could provide information on both marine and freshwater environments due to its migration pattern. A relatively large sample size is needed if otoliths are to be used for monitoring of metal loading to account for differences in growth stages, sex, variability in diet, etc. that could contribute to the otolith metal accumulation. If sculpin otoliths are to be used to evaluate metal loading at a given site, it is important to include both young and old individuals in the sampling as recent layers in otoliths from old individuals are often spaced close together and difficult to separate making accurate dating of individual layers difficult (Fig. 4b). In contrast, otoliths from younger individuals will provide a more accurate but shorter timeline (Fig. 2). However, there are still important knowledge gaps if the otolith chemistry of sculpins or other marine fish species are to be used as a proxy for environmental conditions. A key point is that variations in physiological processes are likely to play a major role in controlling the otolith chemistry of marine fish (Kalish 1991; Brown and Severin 2009; Sturrock et al. 2014; this study). Consequently, there is a need of studies relating metal-specific concentrations in water and diet as well as changes in temperature, salinity, and fish metabolism to accumulation of metals in otoliths of marine fish.

4 Conclusions

This study is the first to highlight the potential for sculpin otoliths as time-resolved recorders of metal pollution near arctic mine sites. Pb was detected using LA-ICP-MS in sculpin otoliths from four polluted sites near the former Black Angel Pb–Zn mine in Maarmorilik in West Greenland. In contrast, no Pb could be detected in sculpin otoliths from an unpolluted reference site. In the otoliths from the polluted sites, a distinct seasonal pattern was observed for the Pb concentrations with the high concentrations occurring during winter and low concentrations occurring during summer. It is not clear to what extent the concentrations of Pb in the otoliths are controlled by physiological processes such as growth in the sculpins and/or to environmental exposure. The study shows that LA-ICP-MS analyses of otoliths have the potential to become a valuable method for assessing time-resolved metal exposure near mining areas, but also that more studies are required to investigate the links between metal sources, pathways, and processes affecting otolith metal concentration.

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References

Asmund, G., Johansen, P., & Fallis, B. W. (1991). Disposal of mine wastes containing Pb and Zn near the ocean: an assessment of associated environmental implications in the Arctic. *Chemistry and Ecology, 5*, 1–15.

ATV. (1973). *Recipient investigation of the Qaumarujuk and Agfardlikavsa fiords, July 1973 (in Danish) (original title: Recipientundersøgelse af Qaumarujuk og Agfardlikavsa, juli 1973)* (p. 169). Søborg, Denmark: Report from Vandkvalitetsinstituttet (ATV).

Brophy, D., Jeffries, T. E., & Danilowics, B. S. (2004). Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. *Marine Biology, 144*, 779–786.

Brown, R. J., & Severin, K. P. (2009). Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Canadian Journal of Fisheries and Aquatic Sciences, 66*, 1790–1808.

Buckel, J. A., Sharack, B. L., & Zdanowics, V. S. (2004). Effect on diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore. *Journal of Fish Biology, 64*, 1469–1484.

Campana, S. E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series, 188*, 263–297.

Davison, W., & Zhang, H. (1994). In situ speciation measurements of trace components in natural waters using thin-film gels. *Nature, 367*, 546–548.

Dortel, E., Massiot-Granier, F., Rivot, E., Million, J., Hallier, J.P., Morize, E., Munaron, J.M., Bousquet, N., Chassot, E., 2013. Accounting for age uncertainty in growth modeling, the case study of yellowfin tuna (*Thunnus albacares*) of the Indian Ocean. Plos One 8 (4), DOI: 10.1371/journal.pone.0060886

Dorval, E., Jones, C. M., Hannigan, R., & Montfrans, J. V. (2007). Relating otolith chemistry to surface water chemistry in a coastal plain estuary. *Canadian Journal of Fisheries and Aquatic Sciences, 64*, 411–424.

Doubleday, Z. A., Izzo, C., Woodcock, S. H., & Gillanders, B. M. (2013). Relative contributions of water and diet to otolith chemistry in freshwater fish. *Aquatic Biology, 18*, 271–280.

Elberling, B., Asmund, G., Kunzendorf, H., & Krogstad, E. J. (2002). Geochemical trends in metal-contaminated fiord sediments near a former lead-zinc mine in West Greenland. *Applied Geochemistry, 17*, 493–502.

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., Limburg, K. E., Secor, D. H., Thorrold, S. R., & Walther, B. D. (2008). Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and influences. *Oceanogranic Marine Biology., 46*, 297–330.

Friedrich, L. A., & Halden, N. M. (2010). Determining exposure history of northern pike and walleye to tailings effluence using trace metal uptake in otoliths. *Environmental Science and Technology, 44*, 1551–1558.

Friedrich, L. A., & Halden, N. M. (2011). Laser ablation inductively coupled plasma mass spectrometric analyses of base metals in arctic char (*Salvelinus alpinus*) otoliths collected from a flooded base metal mine. *Environmental Science and Technology, 45*, 4256–4261.

Halden, N. M., & Friedrich, L. A. (2008). Trace-element distributions in fish otoliths: natural markers of life histories, environmental conditions and exposure to tailings effluence. *Mineralogical Magazine, 72*, 593–605.

Herbst, S. J., & Marsden, J. E. (2011). Comparison of precision and bias of scale, fin ray, and otolith age estimates for lake whitefish (*Coregonus clupeaformis*) in Lake Champlain. *Journal of Great Lakes Research, 37*, 386–389.

Johansen, P., Hansen, M. M., Asmund, G., & Nielsen, P. B. (1991). Marine organisms as indicators of heavy metal pollution—experience from 16 years of monitoring at a lead zinc mine in Greenland. *Chemistry and Ecology, 5*, 35–55.

Kalish, J. M. (1991). Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus. Marine Ecology Progress Series, 74*, 137–159.

Luksenburg, J. A., & Pedersen, T. (2002). Sexual and geographical variation in life history parameters of the shorthorn sculpin. *Journal of Fish Biology, 61*, 1453–1464.

Møller, J. S. (1984). *Thesis: Institute of Hydrodynamics and Hydraulic Engineering (ISVA)* (p. 197). Denmark: Technical University of Denmark, Lyngby. Hydrodynamics of an Arctic Fiord.

Morais, P., Dias, E., Babaluk, J., & Antunes, C. (2011). The migration patterns of the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its distribution range: ecological implications and fishery management. *Journal of Sea Research, 65*, 235–246.

Paton, C., Hellstrom, J., Paul, B., Woodhead, J., & Hergt, J. (2011). Iolite: freeware for the visualisation and processing of mass spectrometric data. *Journal Analogy Atom Spectrom., 26*, 2508–2518.

Payan, P., Pontual, H. D., Bæuf, G., & Mayer-Gostan, N. (2004). Endolymph chemistry and otolith growth in fish. *Comptes Rendus Palevol, 3*, 535–547.

Perner, K., Leipe, T., Dellwig, O., Kuijpers, A., Mikkelsen, N., Andersen, T. J., & Harff, J. (2010). Contamination of arctic fiord sediments by Pb-Zn mining at Maarmorilik in central West Greenland. *Marine Pollution Bulletin, 60*, 1065–1073.

Renaldi, M. M., & Gagnon, M. M. (2008). Trace metal incorporation in otoliths of black bream (*Acanthopagrus butcheri* Munro), an indicator of exposure to metal contamination. *Water, Air, and Soil Pollution, 194*, 31–43.

Sadovy, Y., & Severin, K. P. (1992). Trace elements in biogenic aragonite: correlation of body growth rate and strontium levels in the otoliths of the white grunt, *Haemulon Plumieri* (Pisces: Haemulidae). *Bulletin of Marine Science, 50*, 237–257.

Sadovy, Y., & Severin, K. P. (1994). Elemental patterns in red hind (*Epinephelus guttatus*) otoliths from Bermuda and Puerto-Rico reflect growth-rate not temperature. *Canadian Journal of Fisheries and Aquatic Sciences, 51*, 133–141.

Søndergaard, J. (2013). Dispersion and bioaccumulation of elements from an open-pit olivine mine in Southwest Greenland assessed using lichens, seaweeds, mussels and fish. *Environmental Monitoring and Assessment, 185*, 7025–7035.

Søndergaard, J., Asmund, G., Johansen, P., & Rigét, F. (2011a). Long-term response of an arctic fiord system to lead-zinc mining and submarine disposal of mine waste (Maarmorilik, West Greenland). *Marine Environmental Research, 71*, 331–341.

Søndergaard, J., Johansen, P., Asmund, G., & Rigét, F. (2011b). Trends of lead and zinc in resident and transplanted *Flavocetraria nivalis* lichens near a former lead-zinc mine in West Greenland. *The Science of the Total Environment, 409*, 4063–4071.

Søndergaard, J., Bach, L., & Gustavson, K. (2014). Measuring bioavailable metals using diffusive gradients in thin films (DGT) and transplanted seaweed (*Fucus vesiculosus*), blue mussels (*Mytilus edulis*) and sea snails (*Littorina saxatilis*) suspended from monitoring buoys near a former lead-zinc mine in West Greenland. *Marine Pollution Bulletin, 78*, 102–109.

Sonne, C., Bach, L., Søndergaard, J., Rigét, F., Dietz, R., Mosbech, A., Leifsson, P. S., & Gustavson, K. (2014). Evaluation of the use of common sculpin (*Myoxocephalus scorpius*) organ histology as bioindicator for element exposure in the fiord of the mining area Maarmorilik, West Greenland. *Environmental Research, 133*, 304–311.

Sturrock, A. M., Trueman, C. N., Darnaude, A. M., & Hunter, E. (2012). Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? *Journal of Fish Biology, 81*, 766–795.

Sturrock, A. M., Trueman, C. N., Milton, J. A., Waring, C. P., Cooper, M. J., & Hunter, E. (2014). Physiological influences can outweigh environmental signals in otolith microchemistry research. *Marine Ecology Progress Series, 500*, 245–264.