Accumulation of lead (Pb) in brown trout (*Salmo trutta*) from a lake downstream a former shooting range

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Abstract

An environmental survey was performed in Lake Kyrtjønn, a small lake within an abandoned shooting range in the south Norway. In Lake Kyrtjønn the total water concentrations of Pb (14 µg/L), Cu (6.1 µg/L) and Sb (1.3 µg/L) were elevated compared to the nearby reference Lake Stitjønn, where the total concentrations of Pb, Cu and Sb were 0.76, 1.8 and 0.12 µg/L, respectively. Brown trout (*Salmo trutta*) from Lake Kyrtjønn showed very high levels of Pb in bone (104 mg/kg w.w.), kidney (161 mg/kg w.w.) and the gills (137 mg/kg d.w), and a strong inhibition of the ALA-D enzyme activity were observed in the blood (24 % of control). Dry fertilized brown trout eggs were placed in the small outlet streams from Lake Kyrtjønn and the reference lake for 6 months, and the concentrations of Pb and Cu in eggs from the Lake Kyrtjønn stream were significantly higher than in eggs from the reference. More than 90 % of Pb accumulated in the egg shell, whereas more than 80 % of the Cu and Zn accumulated in the egg interior. Pb in the lake sediments was elevated in the upper 2-5 cm layer (410-2700 mg/kg d.w), and was predominantly associated with redox sensitive fractions (e.g., organic materials, hydroxides) indicating low potential mobility and bioavailability of the deposited Pb. Only minor amounts of Cu and Sb were deposited in the sediments. The present work showed that the adult brown trout, as well as fertilized eggs and alevins, may be subjected to increased stress due to chronic exposure to Pb, whereas exposure to Cu, Zn and Sb were of less importance.

Keywords: Shooting range; brown trout; heavy metals; antimony; accumulation; ALA-D

1. Introduction

At small arms shooting ranges considerable amount of metals and metalloids from small arms ammunition are deposited. Some ranges have been in use for decades leaving behind tons of metals, especially lead (Pb), copper (Cu), antimony (Sb) and zinc (Zn), from bullets and cartridges. Between the years 2005 to 2008 it was estimated that 119 metric tons Pb, 74 tons Cu and 14 tons Sb were deposited annually at Norwegian small arms shooting ranges (Myhre et al. 2013). In the United States there are more than 3000 active military small arms shooting ranges, and it is estimated that approximately 70 000 metric tons of Pb are added to the berms annually (Larson et al., 2005). There are approximately 500 military shooting ranges for small arms in Norway, of which many are abandoned and subjected to remediation measures. The ranges are often located in recreational areas which are popular for game hunting and fishing.

The disposal of ammunition represents hot-spots of pollution, which may lead to considerable leakage of trace metal polluted water into streams and lakes. It is well documented that birds may be poisoned by ingestion of spent lead gunshot (e.g. Vyas et al., 2000; Fisher et al., 2006). Braun et al. (1997) showed that calves pasturing near a targeting area at a shooting range were poisoned by ingestion of Pb. Soil dwelling organisms may also be at risk for being affected by Pb and Cu deposited at shooting ranges (Migliorini et al., 2004). In several runoff streams from Norwegian small arms shooting ranges the Cu and Pb levels are regarded as toxic to aquatic organisms (Strømseng et al., 2009; Heier et al., 2009, Mariussen et al., 2012). Cu can be acutely toxic to aquatic organisms (Lydersen et al., 2002) and induces loss of Na and Cl through the gills with subsequent effects on ion balance and osmolarity, and cardiovascular effects (Playle et al., 1993). The acute toxicity of Pb occurs at higher concentrations than for Cu, and involves impact on the mechanism of ion regulation, such as uptake of Na, Cl and Ca via the gills (Rogers and Wood, 2004; Rogers et al., 2005).The toxicity of both metals is shown to be very dependent on the water chemistry, such

as pH, water hardness and presence of organic materials. Relatively few studies have, however, evaluated fish communities chronically exposed to Pb and Cu. Some studies have indicated that Cu may affect growth, behaviour and feeding pattern (McGeer et al., 2000). Pb accumulates in different fish tissues, such as bone (Spry and Wiener, 1991), and fish chronically exposed to Pb have shown deformities such as spinal curvatures and black-colored tails (Davies et al., 1976; Holocombe et al.. 1976). Fish from Pb contaminated lakes and streams have shown inhibition of δ-aminolevulinic acid dehydratase (ALA-D) activity in blood (Schmitt et al., 1984; Haux et al., 1986; Heier et al., 2009). Mager and Grosell (2011) showed that Fathead minnow exposed to lead had impaired swimming performance.

Gimlemoen small arms shooting range is an abandoned range located in the south of Norway. A small brown trout (*Salmo trutta*) bearing lake, Lake Kyrtjønn, within the range has been a recipient of metal polluted water from several shooting ranges located at or near the lake. The lake has elevated water levels of Pb, Cu and Sb. The objective of the present study was to assess the potential impact of the elevated trace metal concentrations in brown trout inhabiting the lake, and compare the results with data from the nearby reference lake, Lake Stitjønn, housing a mixed population of brown trout and perch (*Perca fluviatilis*). The assessment was based on trace metal levels in water, sediments and fish, including various organs, conditions factor and the levels of ALA-d in blood, a biomarker reflecting Pb exposure. The sensitivity of organisms to toxicants, such as Cu and Pb, depends substantially on factors affecting water quality, such as water hardness, pH, organic content and episodic events. A detailed survey of the water quality of the contaminated and reference lake was therefore performed. In addition, fertilized brown trout eggs were placed in the outlet stream of the two lakes in order to study survival and metal accumulation at early life history stages of brown trout.

2. Materials and Methods

2.1. Area description

Gimlemoen small arm shooting range is located near the city of Kristiansand in the south of Norway. The shooting range was established in 1864 and when it was abandoned in 2003 it covered an area of approximately 7 km^2. The bedrock in the area consists primarily of banded gneiss and granitic gneiss, but with marble lenses interbedded in the gneiss (Falkum 1982; Padget and Brekke 1996). The close proximity to the coast implies, in addition, presence of deposited marine sediments (Wright and Snekvik, 1978). Most of the shooting activity was performed near Lake Kyrtjønn (UTM 32 (Euref 89) coordinates: 6449583 North 440553 East) which covers an area of approximately 1 km^2. The lake itself covers an area of approximately 0.06 km^2. The lake receives contaminated drainage water from several small creeks (Forsvarsbygg, 2011). A small lake near the shooting range, Lake Stitjønn, (UTM 32 (Euref 89) coordinates: 6448844 North 441665 East), was used as a reference lake. This lake covers an area of approximately 0.03 km^2. Both lakes are fish bearing and popular for recreational fishing for the local communities. Because of the acidification of lakes in the southern part of Norway, due to long range transport of acid rain (Rosseland and Henriksen, 1991), both lakes have been subjected to liming. Lake Kyrtjønn was added 2.6 metric tons of lime annually in the years 2007-2009, and Lake Stitjønn was added 0.6 metric tons in 2007 and 2008. In Lake Kyrtjønn 150 cultivated juvenile brown trout was stocked into the lake in 2003 to sustain the brown trout population (County Governor of East Agder, Norway; personal communications). Both lakes are considered oligotrophic with some influence of humic acids from peat.

2.2. Sampling of fish

Fish was sampled with fishnet (Nordic Multi-mesh size gillnet series, Appelberg et al., 1995) placed at different sites in the two lakes 13-16th of September 2011. Sampling of fish was carried out in accordance to the EMERGE sampling protocol (Rosseland et al., 2001).The

sampled fish were sacrificed with a blow on their head and blood was immediately collected from the caudal vein with a heparinized syringe. One part of the blood was collected for analysis of metal concentration while another part of blood was centrifuged for 5 minutes (approximately 1000*g*) to separate blood cells from plasma. The red blood cells were snap frozen in liquid nitrogen and later analyzed for ALA-D activity. Fish weight and length were measured before organs were dissected out. Samples of gills (the second gill arch on the right side of the fish including gill filaments and gill arches), liver, kidney, brain, bone tissue and muscle were collected. The muscle tissue (the skin was removed) was taken from the left side of the fish just above the pectoral fin. A small part of the muscle and the intestine content was stored in separate vials on liquid nitrogen for ^{15}N, ^{14}N, ^{13}C and ^{12}C isotope analysis. If not otherwise stated the samples were stored cold on ice and then in laboratory kept in a freezer at -20°C. The age of the fish was estimated by counting the annuli on the fish otholiths and scales.

2.3 Analysis of stable isotopes

Stable isotopes of nitrogen ($^{15}N/^{14}N = \delta^{15}N$) and carbon ($^{13}C/^{12}C = \delta^{13}C$) in muscle tissue and intestine content were determined by IR-MS. Approximately 0.3 g of muscle tissue or stomach content was homogenized in 3 mL ultra-pure water, put on plastic vials, frozen and then freeze dried. A proportion of the freeze dried sample was then packed in tin capsules before $\delta^{15}N$ and $\delta^{13}C$ were determined using a Flash Elemental Analyzer (EA) and a continuous flow stable isotope ratio mass spectrometer (CF-IRMS, Finnigan Delta XP). The accuracy of $\delta^{15}N$ and $\delta^{13}C$ analyses was validated by standard materials from IAEA (IAEA-N1, IAEA-N2 and IAEA-CH6), and by measurements of an internal standard (brown trout) in between each 12^{th} sample. All measurements were within 1 SD of the certified references.

2.4 Calculation of trophic levels and condition factors

The trophic levels for the brown trout were determined as described in Fisk et al. (2001). Briefly, trophic levels were determined relative to the stomach content of the brown trout (eq. 2), which is assumed to occupy trophic level 2 (i.e. primary herbivore).

$$TL_{consumer} = 2 + \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{stomach})}{3.8} , \quad (2)$$

$TL_{consumer}$ is defined as the trophic level of the organism and 3.8 is the trophic enrichment factor (Hobson and Welch, 1992).

To evaluate the condition of the sampled fish the Fultons condition factor was estimated (Ricker, 1975; Sutton et al., 2000). Fulton condition factor (*k*) was estimated from the proportion between weight and length using the formula $k = (W \times 100)/L^3$, were W is the fish weight (g) and L is the fish length (cm). For brown trout, a $K \geq 1$ represents "good condition".

2.5 ALA-D-analysis

ALA-D (δ-aminolevulinic acid dehydratase) activity was determined according to Hylland (2004) in the live caught trout. Whole blood was homogenized 1:2 (v/v) with a teflon pestle in ice-cold phosphate buffer (0.1 M phosphate buffer, pH 6.2 containing 0.5 % (v/v) Triton X-100). The homogenates were centrifuged at 10,000 x *g* for 15 min (4°C) and the supernatant was transferred to a clean plastic tube (size 1.5 mL). The supernatant was diluted in ice-cold phosphate buffer (1:2) and distributed into 6 different tubes (size 1.5 mL), 50 µl in each. Three of the tubes were added 200 µL phosphate buffer (blanks) and 3 tubes were added 200 µL δ-aminolevulinic acid (ALA) reagent (phosphate buffer containing 5.1 mM ALA). After incubation of the samples at 25 °C for 2 h, 300 µL of precipitation reagent (4 % trichloro-acetic acid containing 10 mM mercury chloride) was added to each tube and incubated for 5 min, then centrifuged at 2500 x *g* at room temperature for 5 min. Supernatant was distributed

(150 µL) in duplicates to a 96-well microtiter plate and 150 µL modified Ehrlich's reagent (76 % (v/v) acetic acid and 13 % perchloric acid (v/v) containing 11.7 mM mercury chloride and 133 mM *p*-aminobenzoic acid) were added to all wells. The plate was incubated for 15 min at room temperature before absorbance was measured spectrophotometrically at 550 nm. Concentrations of resulting porphobilinogen (PBG) were calculated on the basis of standard curves for PBG and results normalized against protein levels determined by the Lowry method adapted to microtiter plates (Lowry et al., 1951).

2.6 Exposure of fertilized brown trout egg

Fertilized brown trout eggs were placed in the outlet streams from Lake Kyrtjønn and Lake Stitjønn. Eggs and sperm were collected in a local brown trout hatchery (Syrtveit Fiskeanlegg in The County of Aust Agder, Evje and Hornes community, Norway) in the morning (7^{th} of December 2011) and transported in a refrigerated box. The eggs were fertilized *in situ* and immediately after fertilization the eggs were placed into 24 wells polycarbonate cell culture dishes, either one or two eggs in each well. The dishes were sealed with a lid and put into a casket that was buried into the bottom gravel of the creeks. The swelling water was therefore natural creek water. To ensure that the eggs continuously received fresh oxygenated water, three bore holes (0.3 cm in diameter) were made at the bottom of each of the wells and also above each well in the lids. A thin mesh (0.8 mm) was placed between the eggs and the lid to prevent the eggs and alevins disappearing. In each locality, 1200 fertilized eggs distributed in 40 cell culture dishes were implanted at the sites. Brown trout eggs hatches after approximately 500 day degrees (dd), which are the product of the mean temperature in the water and number of days. The eggs were examined 4 times during the winter 2012: after 110 and 260 day-degrees, early in their development, and after 480 and 690 day degrees, near and after hatching. The numbers of fertilized egg, survival and accumulation of metals in the eggshell and the egg contents were measured. The egg content, which primarily consists of

the yolk and the embryo, was separated from the eggs by cutting a small section of the eggshell with a scalpel and then squeezing the content into a small plastic vial.

2.7 Sampling of water

The water in Lake Kyrtjønn and the reference water in Lake Stitjønn were fractioned *in situ* with respect to size (molecular mass) and charge properties as previously described (Teien et al., 2004; Salbu and Oughton, 1995). Size fractionation was performed using filtration (0.45 µm) and ultrafiltration (molecular mass cut off 10kDa), whereas charge fractionation was performed using ion chromatography on the 0.45 µm fraction. Amberlite IR-120 was used as a cation exchange resin and AG1-X8 was used as an anionic exchange resin. In each lake, *in situ* fractionation of water was performed from three or four different sites in the lakes. In addition total and filtered water samples from different streams (inlet/outlet) of the two lakes were collected. Water samples for ICP-MS analyses were preserved with HNO_3 (5% v/v). pH, conductivity and temperature were determined *in situ* (WTW, Hanna instruments).

2.8 Sampling of sediments

Sediment samples were taken from the upper 2-3 cm sediment layer along the waterside of the two lakes by a plastic spoon. Bottom sediments from Lake Kyrtjønn and Lake Stitjønn were, in addition, taken with a core sampler. The sediment cores were taken approximately in the middle of the lakes at a site with soft sediment. The cores were cut into 2 cm thick slices, which were subjected to extraction and element analysis. In addition 2 cm thick slices of the upper 6 cm of the bottom sediment from the two lakes were subjected to sequential extraction Sequential extraction was also performed on mineral soil from a nearby butt, heavily contaminated with ammunition residues from the shooting activity.

Sequential extraction of sediments was performed according to Tessier et al. (1979), modified by Oughten et al. (1992). The soil or sediment was air dried at approximately 40°C. An aliquot of soil was extracted into 6 different fractions according to Table 1S (Table 1

Supplementary) in a liquid to solid ratio of 10 (L/S 10). Samples were centrifuged for 25 min at 10.000 x g and the supernatants were decanted and filtered (Blue ribbon filter 110 mm, < 2µm pore size, Whatman). The soil residues from fraction F3, F4, F5 and F6 were washed with 10 ml deionized water and centrifuged for 15 minutes. The supernatants from the washing steps were filtered and mixed with the main fraction. All extracts were dried by heating on sand-bath (90°C), re-dissolved in 3 mL concentrated HNO_3 and re-dried. All extracts were then dissolved in 2.5 mL concentrated HNO_3, and diluted to 50 mL for analysis on ICP-MS.

2.9. Analysis

Aliquots of soil or biological materials were subjected to microwave assisted acid digestion using an UltraClave (Milestone, Leutkirch, Germany) at 200°C. Soil was digested using a mixture between HNO_3 and HF (5:1 v/v), whereas the biological materials were digested with HNO_3 only. After digestion, the samples were allowed to cool to room temperature and diluted with ultrapure water.

Total organic carbon (TOC) and dissolved organic carbon (DOC) were determined using Shimadzu TOC organic carbon analyzer (Japan). Chloride (Cl^-), nitrate (NO_3^-) and sulphate (SO_4^{2-}) were determined in unfiltered water samples by an Iachat IC5000 Ion chromatograph (Zellweger analytics Inc. USA). The trace elements Pb, Cu, Sb, Zn, calcium (Ca), magnesium (Mg), aluminum (Al), sodium (Na), potassium (K), iron (Fe), and manganese (Mn) in water, soil and sediments and biological samples were determined using ICP-MS (Perkin Elmer Sciex ELAN 6000). The samples were added internal standard for correct quantification. Certified reference material (NRCC-DORM-2, National Research Council of Canada, Dogfish muscle certified reference material for trace metals) was used as standard reference materials. Procedure blanks were regularly prepared to control background contamination.

2.10. Statistics

The number of fish and egg samples was too few to assess normal distribution in the data sets. Comparison between groups was, therefore, performed with the non-parametric Mann Mann-Whitney U test. For elements concentrations below the limit of detection (LOD) that were included in the statistical analyses, half the LOD for the respective elements in a sample was used. The statistical and descriptive analyses were performed with GraphPad Prism 5 and Microsoft Excel 2003.

3. Results and discussion

3.1. Characterization of water quality of the lakes

The concentrations of Na and Cl were relatively high (Table 1, 2S and 3S) in both lakes compared to other lakes in Scandinavia (Skjelkvåle et al., 2007), which probably reflects the lakes location close to the coast (Wright and Snekvik, 1978; Skjelkvåle et al., 2007). Elevated concentrations of major ions could also be due to weathering of deposited marine sediments (Wright and Snekvik, 1978). The water was low in conductivity and ionic strength with respect to Ca and Mg level. The TOC concentrations in the two lakes were about 10 mg C/L reflecting some influence from nearby bogs. Most of the organic materials were in the dissolved filtered (< 0.45 µm) fraction (Table 1). About 30% of the organic materials were identified as dissolved organic materials less than 10 kDa. Lake Kyrtjønn was somewhat more acidic than Lake Stitjønn, with a pH of 5.6. The difference in pH was even more pronounced during the brown trout egg experiment where the pH in the outlet stream of Lake Kyrtjønn varied between 4.9 and 5.8, whereas the pH in the outlet stream of Lake Stitjønn varied between 5.6 and 6.2 (Table 2S and 3S). Elevated concentrations of Pb, Cu and Sb was detected in Lake Kyrtjønn compared to the reference Lake Stitjønn, which is related to former deposition of small arms ammunition in the catchment area (Table 1, 2S and 3S). The lake receives input of from several streams draining the polluted area. Analysis of water samples collected in three of the streams during spring 2012 showed particularly high concentrations of Pb (67, 30 and 1.9 µg/L respectively), Cu (8.9, 9.7 and 1.9 µg/L respectively) and Sb (1.8, 10 and 0.2 µg/L respectively) in two of the streams (Table 4S). The concentrations of Pb and Cu are comparable to concentrations in rivers influenced by lead mining in the U.S. (Schmitt and Finger, 1982). The concentrations of Zn were slightly elevated compared to excepted background levels in Nordic surface levels in both lakes (Lydersen et al., 2002). Elevated concentrations of Zn in surface water have been attributed to low pH facilitating dissolution

of Zn, and input from air pollution (Lydersen et al, 2002). In Lake Kyrtjønn there will in addition probably be an input from residues of deposited small arms ammunition and cartridges.

Investigation of the size distribution of the elements in the water from Lake Kyrtjønn showed that the highest concentration of Pb and Cu was associated with the colloidal high molecular mass fraction (HMM), whereas most of Sb was associated with the low molecular mass (LMM) fraction. Zn was present in the colloidal HMM and LMM in approximately equal concentration (Table 2 and 5S). The reactivity of the elements, determined by ion exchange chromatography of the 0.45 µm fraction of the water from Lake Kyrtjønn, showed that approximately 50% and 40% of Pb was identified as positively charged species and neutrally charged species respectively (Table 2 and 5S). Cu was primarily identified as the negatively (~50%) and neutrally (~40%) charged species, Sb was primarily found as the negatively (~90%) charged species, whereas Zn primarily was detected as positively charged species (~90%). The average proportion of cationic Pb and Cu were higher in Lake Kyrtjønn than in Lake Stitjønn (Table 2), indicating a higher reactivity of these elements in Lake Kyrtjønn. The differences were, however, not significant (Mann Whitney U t-test). The chemical speciation of Pb, Cu and Zn in fresh water environment is complicated and dependent on several factors, where pH and the presence of organic materials are the most important. In fresh water with low ionic strength, pH below 6 and without organic materials, simple (hydrated) cations such as Pb^{2+}, Cu^{2+} and Zn^{2+} should be present in the water (Powell et al., 2007, 2009, 2013).These are regarded as the most toxic metal species. Cations, such as Pb and Cu have high affinity for functional groups associated with organic substances, which primarily are carboxylic acids and phenols (Stumm and Morgan, 1996). Humic substances in the water will have major implication of their toxic properties making them less bioavailable. The high fraction of Zn found as simple cation or as positively charged complexes indicates

less affinity to humic substances compared to Cu and Pb. These observations are in agreement with previous studies showing that the affinity of Cu, Pb and Zn to dissolved fulvic and humic substances are in the order Cu ≥ Pb > Zn (Cao et al., 1995; Christl et al., 2001, 2005; Chakraborty and Chakrabarti, 2008). Sb was primarily found as an anionic species. Previous works have showed that Sb is primarily found in freshwater as the negatively charged $Sb(OH)_6^-$, and probably has limited affinity to organic ligands in the water (Filella et al., 2002; Heier et al., 2009, 2010).

3.2. Characterization of sediments and soils

The sediment samples from Lake Kyrtjøn had high concentrations of Pb ranging from 410 mg/kg to 2700 mg/kg d.w. (Table 6S). The concentrations of Cu, Sb and Zn in sediment from Lake Kyrtjønn were in the range of 54-300 mg/kg, 3-36 mg/kg and 68-630 mg/kg d.w. respectively. Two sediment samples from Lake Stitjønn, the reference lake, had Pb concentrations of 130 mg/kg and 270 mg/kg d.w., Cu concentrations of 59 and 80 mg/kg d.w. and Zn concentrations of 130 and 490 mg/kg d.w. respectively (Table 6S). The concentration of Sb in the reference lake was low and only one sample had detectable amounts of less than 2 mg/kg d.w. (Table 6S). The amount of organic materials in the samples varied considerably. The top sediment layer along the water side was primarily a thin layer of deposited organic materials on top of what appeared to be a mix of mineral and organic soil. Analyses of core samples showed that the concentrations of Pb in the sediments decreased rapidly after only a few centimetres below the top sediment, from 780 mg/kg in the top sediment to 180 mg/kg at a depth between 4-6 cm in Lake Kyrtjønn (Table 7S). Similar was observed in the sediment core samples from Lake Stitjønn (Table 8S). Bearing in mind that this area has been used as a shooting range for decades it appears that there are little suspended particles in the water and a low sedimentation rate of contaminated materials. The water from Lake Kyrtjønn is acidic with a low ionic strength, which may lead to smaller organic aggregates of which the cationic

species are associated (Pédrot et al., 2008). However, most of the organic materials in the water were predominantly present in a dissolved state, which indicates that only a small fraction of this material will sediment within the lake, but could be transported further into downstream recipients. The expected background concentrations of Pb, Cu and Zn in lake sediments vary, and depend on the bedrock and long range atmospheric transport of pollutants. This site at the southern part of Norway is influenced by long range atmospheric transport form the European continent, which has led to acidification of Norwegian fresh water lakes. A survey of reference lake sediments in Finland showed mean Cu, Pb and Zn concentrations of 18, 121 and 134 mg/kg respectively (Iivonen et al., 1992), indicating that the concentration of Cu, Pb and Zn in the sediment of the control lake in our study is slightly elevated presumably from long range atmospheric transport or natural background levels. Nevertheless, these findings indicate that neither Cu, Sb nor Zn from munitions from small arms are particularly prone to be deposited in the lake sediments. Pb appears, however, to have a higher affinity to substances in the water subjected to sedimentation.

The binding properties of Pb, Cu and Zn in the sediment was investigated using sequential extraction technique on three core samples from the upper 6 cm of the bottom sediment from both lakes. Most of the Pb and Zn accumulated in the sediments were extracted by agents reflecting association to redox sensitive phases such as organic substances (~50%) or Fe/Mn-hydroxides (~20-30%) as shown in Fig. 1S (Fig. 1 Supplementary). Cu was particularly extracted by the strong redox sensitive fraction (~80%) associated with organic substances in addition to the mineral lattice (~20%). These findings indicate that deposited elements from the small arms ammunitions are relatively well associated with components in the sediments and are not particularly mobile or bioavailable. In contrast, sequential extraction of soil from a nearby berm (Fig. 2S) showed a much higher fraction of Pb, Cu and

Sb in the water soluble and pH sensitive fraction (fraction 1-3) indicating a high environmental mobility of these elements from deposited ammunition residues.

3.2. Condition factors and trophic levels of brown trout

In Lake Kyrtjønn, 8 brown trout were caught by net in which 7 were alive and 1 was dead at the time of sampling, whereas in Lake Stitjønn, 4 live and two dead brown trout were collected. In Lake Stitjønn approximately 30 small perches and one eel was caught in addition to the brown trout, indicating that the fish community in this lake was different from that in Lake Kyrtjønn. The brown trout from Lake Kyrtjønn were relatively large (weight: 650-1000g, length: 38-47 cm) while brown trout from Lake Stitjønn were smaller (weight :109 - 270 g, length: 24 to 36 cm) (Fig. 3S). The age distribution of brown trout from Lake Kyrtjønn was between 7 and 11 years, whereas the age distribution of brown trout from Lake Stitjønn was between 4 and 14 years (Fig. 3S). No brown trout of younger age (< 3 years) was caught. The brown trout from Lake Stitjønn had a significant (Man-Whitney t-test $p<0.001$) lower condition factor (0.78) compared to the fish from Lake Kyrtjønn being approximately 1.0 (Fig. 3S) indicating that the fish in Lake Kyrtjønn had satisfactory food conditions. The lower condition factor of the brown trout from Lake Stitjønn, as well as smaller size at same age classes, is most probably due to inter-species competition with the perch. The estimated trophic levels of the two brown trout populations, based on the relative difference in $\delta^{15}N$ between muscle and stomach content, resembles levels found in earlier studies and indicated that both populations feeds on a similar trophic level (Table 3). The $\delta^{13}C$-levels may give an indication of the fish preferred feeding habitat (France, 1995; Post, 2002). The $\delta^{13}C$ levels in the brown trout in this study (Table 3) were in the similar range as for fish eating herbivorous and soft bottom invertebrates (France, 1995, 1997; Eloranta et al., 2013; Karlsson and Byström, 2005). The stomach content of the fish were not identified with respect to species of

the pray, but contained primarily different insect species, indicating food search near the littoral zone as well.

3.3 Trace metal accumulation in the brown trout and ALA-D analysis of blood

The concentrations of Pb, Cu, Sb and Zn were determined in 7 different organs (Table 4). The concentrations of Pb in all the organs were significantly higher in the fish from Lake Kyrtjønn than in fish from the reference lake. The levels of Pb in the brown trout from Lake Kyrtjønn were particularly high in the bone tissue, kidneys and the gills. Similar accumulation pattern, but not as high concentrations, of Pb in fish is previously reported (e.g. Spry and Wiener, 1991). Stanskiene et al. (2006) found a median concentration of 0.17 mg/kg Pb w.w. in fish bone from different freshwater fish species from Lithuanian lakes. Rosseland et al. (2007) measured from 3-5 mg/kg Pb w.w. in gill, irrespective of age, in brown trout from Lochnagar, Scotland, whereas salmonids from eight other high mountain lakes in Europe had 0.1-1.5 mg/kg Pb w.w. in gills. Presuming moisture contents of approximately 80% in the gills (Fig 4S) the findings by Rosseland et al. (2007) imply dry weight concentrations of 0.5-25 mg/kg Pb in gills of brown trout from Lochnagar. In brown trout kidney from Lochnagar, Rosseland et al. (2007) found an increasing Pb concentration with age (3-11 years), from 7 to 28 mg/kg Pb w.w. These figures are in line with the concentrations of Pb found in gills and kidneys in brown trout from the reference lake in this study. Hodson et al. (1978) exposed rainbow trout (*Oncorhynchus mykiss*) to increasing concentrations of Pb (10-100 µg/L) for 32 weeks in hard water. They showed that the Pb accumulation in rainbow trout was a function of the Pb concentrations in the water and particularly high concentrations were observed in the bones (~100 mg/kg w.w.), gills (~10 mg/kg w.w.) and the kidneys (~10 mg/kg w.w.) from fish exposed to 100 µg/L for 32 weeks. In a more recent study Heier et al. (2009) exposed brown trout to stream water draining a shooting range. After 12 days the concentration of Pb in the gills reached a concentration of 15 mg/kg w.w. This is lower than the fish in the present study.

This was, however, only a time limited study and not a chronic exposure scenario. The level of Pb in the muscle tissue was low, confirming previous observations that Pb only to a little extent accumulates in muscle tissue. Previous analysis of Pb in muscle tissue from fish collected in Norway from non-polluted areas showed Pb-concentration of 0.1-0.2 mg/kg w.w. (Grande, 1987), similar as in our investigation. Amongst recreational fishermen there have been expressed concerns about the health risk of eating fish caught in lakes draining shooting ranges. The Norwegian Food safety Authorities have an upper limit for acceptable levels of Pb in fish-filet of 0.3 mg/kg, indicating that the Pb levels in brown trout muscle from Lake Kyrtjønn were within acceptable levels.

Acute effects of Pb exposure involve impact on ion regulation, such as uptake of Na, Cl and Ca via the gills. Lead acts as a Ca-analogue and may displace Ca at calcium binding sites in the gills, such as voltage independent Ca channels and the Ca-ATPase (Rogers and Wood, 2004). Pb may also affect the Na and Cl balance by inhibiting gill carbonic anhydrase and Na/K-ATPase activity (Rogers et al., 2005). An interesting observation is that the fish probably can adapt to elevated Pb concentrations. Grosell et al. (2006) exposed fathead minnows (*Pimephales promelas*) to sublethal concentrations of Pb for 30 days (17-269 µg/L). Ion-regulatory disturbances in the Na, K and Ca homeostasis were observed early in the experiment, but the fish appeared to recover later in the experiment. The mechanisms of toxicity after chronic Pb exposure appear to be more complex. The displacement of Ca is followed by an increased uptake of Pb through the gills which may lead to increased tissue and organ levels of Pb, particularly in blood and bone, and ultimately to chronic effects. One important symptom of chronic lead exposure is spinal deformities and darkening of the skin in the area caudal to the dorsal fin (Davies et al., 1976; Holocombe et al., 1976), which probably is attributed to extensive accumulation of Pb in the bone tissue. These effects may lead to atrophy of the tail region and interference with movement followed by death. In the study by

Hodson et al. (1978) it was observed black tails after 32 weeks of exposure to 100 µg/L. Davies et al. (1976) observed symptoms of Pb intoxication in soft water at a concentration of 8 µg/L. We did, however, not observe any of these morphological signs.

Accumulation of Pb is dependent on the water chemistry, such as the concentration of Ca, organic materials and pH. Reducing the pH can increase the bioavailability due to more lead present as simple LMM divalent ion (Merlini and Pozzi, 1977ab; Spry and Wiener, 1991; Köck and Hofer, 1998). Elevated calcium concentrations are protective primarily due to competitive transport with Pb (Davies et al., 1976; Holcombe et al., 1976; Rogers et al., 2003), and it is observed that acute gill accumulation of lead is negatively correlated with the calcium concentration in the water (Rogers and Wood, 2004; Bircenau et al., 2008). In a study by Grosell et al. (2006) on fathead minnows it was observed that the calcium concentration in the water was of less importance for whole body accumulation of lead after chronic exposure. Similar was observed by Mager et al. (2008) who showed that elevated calcium concentrations did not reduce accumulation of Pb after 160 days of exposure of fathead minnow to approximately 35 µg/L Pb. Similar is observed for other metals (McGeer et al., 2003) and the phenomenon is probably due to saturation of binding sites on transport proteins on the gills (Grosell et al., 2006). On the other hand, analysis of blood in different fish species from rivers contaminated from Pb-mining areas in Missouri USA, showed less accumulation of Pb than in our study (Schmitt et al., 2007). They found a maximum Pb concentration of 6.63 mg/kg d.w., which is equivalent with a w.w. concentration of 0.66 mg/kg (~90% water content). The watershed in Missouri was harder, with a Ca concentration of approximately 200 mg/L and a pH just below 8 (Besser et al., 2009; Brumbaugh et al., 2007) making Pb less bioavailable. The concentrations of Pb in liver from whitefish in a Pb contaminated lake (1.5-4.5 µg/L Pb) had a mean concentration of 7.1 mg/kg d.w., which is equivalent with a w. w. concentration of 2.1 mg/kg (~70% water content) (Haux et al., 1986). The lake water in the

study by Haux et al. (1986) was soft with a Ca concentration of approximately 20 mg/L and a pH between 6.5 and 7.0. The water pH is, therefore, a key factor for accumulation of Pb in fish leading to increased proportion of cationic Pb, which is considered most bioavailable. In an acidic lake with soft water, as Lake Kyrtjønn in this study, one would expect high bioavailability of Pb and increased susceptibility against Pb toxicity. The presence of organic materials, however, has been shown to reduce toxicity and Pb accumulation (Spry and Wiener, 1991; Richards et al., 2001; Grosell et al., 2006), due to complexation with Pb, which makes Pb less bioavailable. The very high concentrations of Pb in bone, gills and kidney indicate, however, that the brown trout in Lake Kyrtjønn could be subjected to significant stress due to Pb exposure. According to Davies et al. (1976) rainbow trout exposed as larvae or juveniles is particular vulnerable to chronic Pb exposure and may reduce their survival. This could support that not younger than three years old fish were caught in the lake.

The ALA-D analysis in blood from live caught brown trout from Lake Kyrtjønn showed a strong inhibition of the enzyme activity (Fig. 1). The enzyme activity was approximately 20% of what was observed in the blood from the fish from Lake Stitjønn. The ALAD-activity in the blood from the reference lake was within the similar range as observed for other field caught fish species (Ruus et al., 2003), but less than observed in farmed brown trout with presumably no history of any Pb exposure (Heier et al., 2009). ALA-D inhibition in blood is a strong indication of a chronic exposure to elevated levels of Pb both in fish and mammals (Hernberg and Nikkanen, 1970; Hodson, 1976; Schmitt et al., 1984; Haux et al., 1986). Haux et al. (1986) showed an inhibition of up to 88% of ALA-D in blood from whitefish (*Coregonus spp.*) from a low-alkalinity lake contaminated with Pb (0.5-4.5 µg/L Pb). Heier et al. (2009) observed approximately 50% inhibition of ALA-D activity in blood from brown trout after 12 days of exposure to Pb contaminated water (15-46 µg/L Pb) from a shooting range. In mammals the inhibition of ALA-D may lead to severe anaemia due to

inhibition of the heme synthesis (Warren et al., 1998). The significance of ALA-D inhibition in fish is unclear (Larsson, 1985; Hylland et al., 2006), but prolonged Pb exposure has been shown to affect haematological parameters in fish. In the study by Hodson et al. (1978) it was observed an increase in the number of red blood cells in rainbow trout, with a subsequent decrease in the red blood cell volume and iron content after exposure to 13 µg/L Pb for 32 days. No changes in the haematocrit or total iron level was observed, which indicate that the exposure to Pb result in decreased cell viability of the red blood cells followed by an increased production of cells to compensate for the loss.

With the exception of bone tissues, there were no significant differences in the Cu concentration in the different tissues (Table 4). In muscle tissue the concentration was approximately 0.3 mg/kg w.w., which is within expected background concentration of approximately 0.8 mg/kg w.w. (Grande, 1987, 1990). Rosseland et al. (2007) found mean concentration of 0.4 mg/kg Cu w.w. (2 mg/kg Cu d.w.) in gills of brown trout from Lochnagar, which is directly comparable with our results. Cu is acutely very toxic to fish and induce loss of Na and Cl via the gills with subsequent effects on ion balance and osmolarity, and cardiovascular effects (Playle et al., 1997). Less is known about the effects of chronic exposure to Cu. Some studies have shown effects on growth, changes in behaviour and feeding pattern, whereas other studies have only showed minor or no effects (McGeer et al., 2000). It appears that fish are able to adapt to increased Cu concentrations in the water provided they survive the first critical phase of exposure (McGeer et al., 2000). The toxicity of Cu is, however, very dependent on the water chemistry and is strongly reduced in the presence of organic materials, such as humic substances, and calcium. A Cu concentration of 4-7 µg/L and a TOC concentration of 10 mg C/L, as observed in Lake Kyrtjønn, are probably not sufficient to pose a serious threat to the adult fish in the lake.

The water concentrations of Zn were slightly elevated in both lakes. There were no significant differences in the Zn concentration in the tissues between the lakes (Table 4). The concentrations of Zn in liver and muscle were in the same range as observed in brown trout collected at a presumed noncontaminated site in a river in the southern Norway (Brotheridge et al., 1998). As for Cu, Zn is an essential metal and uptake and removal is regulated (Lydersen et al., 2002). A Zn concentration of approximately 5 µg/L has been reported toxic to yolk sac fry of brown trout in soft acidic water (Sayer et al., 1989). It has been shown that Zn may affect Ca transport through the gills (Spry and Wood, 1985; Hogstrand et al., 1995). The concentrations of Zn in both Lake Kyrtjønn and Lake Stitjønn were, however, below expected toxic levels of Zn in natural waters (Lydersen et al., 2002: Mebane et al., 2012).

The Sb-concentrations in the different fish tissue were low and are probably of no toxic concern (Table 4). Very few toxicological studies have, however, been performed on aquatic toxicity of Sb. The toxicity of Sb depends both on its oxidation state and type of antimony species and the trivalent organic forms of Sb are regarded as most toxic. In natural oxic waters Sb (V) predominates, primarily as $[Sb(OH)_6]^-$ (Filella et al., 2002). Antimony does not biomagnify in the food chain (Veenstra et al., 1998; Culioli et al., 2009). A few acute and sub-chronic studies on aquatic organisms have shown that Sb is not particularly toxic compared to other trace metals, such as Cu and Pb. Estimated LC_{50} of $SbCl_3$ for 3-day tilapia larvae (*Oreochromis mossambicus*) in a 96-h study is approximately 35.5 mg/L (Lin and Hwang, 1998). Estimated LC_{50} of $SbCl_3$ for juvenile common carp (*Cyprinus* carpio) in a 96-h study was shown to be 14.05 mg/L (Chen and Yang, 2007). Takayanagi (2001) exposed juvenile red seabrem (*Pargus major*) for $SbCl_3$, $SbCl_5$ and $K[Sb(OH)_6]$ with 24-h LC_{50} values of 15.5 mg/L, 0.93 mg/L and 6.9 mg/l respectively.

3.3. Fertilized brown trout eggs in the creeks

Both eggs and water samples were collected totally four times during the exposure period of approximately 6 months. The most pronounced difference in the water qualities between the two streams was elevated concentrations of Cu, Pb and Sb in the outlet stream from Lake Kyrtjønn with mean concentrations of 4.0, 12 and 0.94 µg/L, respectively (Table 2S and 3S). In addition, the stream from Lake Kyrtjønn was more acidic with a pH ranging from 4.9 to 5.8, whereas the pH in the creek from Lake Stitjønn ranged from 5.6 - 6.4. Live eggs or alevins from the two sites were sampled for chemical analysis after 110, 260, 480 and 690 day degrees. The concentrations of Pb in both eggs (after 110 and 260 day degrees) and alevins (after 580 and 690 day degrees) from Lake Kyrtjønn were significantly higher than in Lake Stitjønn (Table 5). The concentrations of Cu were significantly higher in eggs (after 110 and 260 day degrees) from Lake Kyrtjønn compared to Lake Stitjønn. There were no differences in the Zn concentrations in the eggs and alevins from the two sites. Interestingly, more than 90% of the Pb accumulated in the egg shell, whereas more than 80% of the Cu and Zn accumulated inside the egg (Table 6, Fig. 5S). This indicates that Pb in the water is not available for transport through the egg shell membrane, but that Pb can displace Ca in the egg shell. This could probably be reflected in the low Pb concentration observed in the alevins after 480 day degrees, where the egg shell is removed (Table 6).

After approximately 110 and 260 day degrees the mortality of the eggs from the outlet stream of Lake Kyrtjønn was 18% and 10% respectively, whereas the egg mortality in the outlet stream of Lake Stitjønn was 10% and 9% respectively (Fig. 6S). The difference in mortality of eggs between the two lakes was not significant. There was a large variation in mortality of eggs between the boxes put into the same location, which indicate that the eggs are very sensitive to how and where they are placed in the stream. The micro environment between the eggs may differ, such as local differences in in the water stream velocity and oxygen saturation. A mortality of approximately 10% of fertilized eggs in an acidic creek

with hard water is, however, in good accordance with a study by Sayer et al. (1991) who showed that pH and calcium was more critical for survival of eggs than exposure to metals such as Cu (5 µg/L), Pb (10µg/L) and Zn (20 µg/L). A study by Edwards and Gjerdrem (1979) shown that a reduction in pH from 6.4 to 5.2 and 4.7 dramatically increased the mortality of fertilized brown trout eggs from approximately 25% mortality to approximately 70% and 80% mortality.

After 480 day-degrees the eggs started to hatch and an increase in mortality was observed, particularly in the stream of the reference water, Lake Stitjønn, with a mortality of 75%. After 690 day-degrees, all the surviving eggs had hatched and the mortality was 60% in Kyrtjønn and close to 100% amongst the eggs from Lake Stitjønn (Fig. 6S). The high mortality of the eggs from Lake Stitjønn was probably due to the physical conditions in the stream. The stream velocity in this creek was relatively low which led to a higher deposition of particles on the boxes containing the eggs and alevins probably leading to reduced oxygen saturation and alevin mobility. The growth condition was therefore probably not optimal. The high mortality of the alevins in Lake Kyrtjønn could be ascribed to the low pH and Ca concentrations and high Al concentration in the stream, rather than elevated concentrations of metals from ammunition (Skogheim and Rosseland, 1984; Sayer et al., 1991; Gjedrem and Rosseland, 2012). Sayer et al. (1991) investigated the effect of trace metals and calcium on mortality of brown trout eggs and alevins in reconstituted lake water without organic materials at different pH levels. In water with low pH (4.5) and calcium (20 µmol/L, 0.8 mg/L) mortality of approximately 80 % was reached during the first 100 day-degrees, irrespectively of the presence of the metals. However, at high concentrations of calcium (200µmol/L, 8 mg/L) no mortality was observed. The presence of trace metals may however, be an additional stress factor, and in acidic soft water, it was shown that trace metals may increase the mortality of alevins (Sayer et al., 1989, 1991) even at very low concentrations. In

the study by Sayer et al. (1991) it was also showed that Cu (5 µg/L) reduced the protective effect of calcium in alevins. These studies therefore show that even low concentrations of Pb, Cu and Zn may be an additional stress factor for alevins in acidic soft water.

4. Conclusions

An environmental survey was performed in the small Lake Kyrtjønn in Norway located within an abandoned shooting range, and in the nearby reference, Lake Stitjønn, having quite similar water chemistry. The brown trout from Lake Kyrtjønn showed very high concentrations of Pb accumulated in bone, kidney and the gills, and ALA-D analysis showed a strong inhibition of the enzyme activity in the blood. The low pH of the water in Lake Kyrtjønn appeared to be the most important factor for the high Pb accumulation in the fish tissue. Neither Cu, Zn nor Sb accumulated to a greater extent in Lake Kyrtjønn in any of the brown trout tissues that were analysed. The condition factors of the brown trout in Lake Kyrtjønn indicated satisfactory food conditions. Our results indicated that the brown trout may be subjected to increased stress due to chronic exposure to Pb. Pb was selectively adsorbed to the egg shells of fertilized brown trout eggs that were placed in output creeks from the two lakes, whereas Cu and Zn primarily accumulated in the egg content. Substantial mortality was observed on the fertilized brown trout eggs and the most important factor for survival of eggs and alevins in Lake Kyrtjønn was probably the low pH-level and Ca concentration in the water, as well as high Al concentration. In addition, the considerable amounts of organic materials in the water probably reduced the potential toxicity of the metals, especially Cu. Analysis of the lake sediments from both lakes showed that Pb accumulated primarily in the upper 2-5 cm layer. Most of the Pb was associated with redox sensitive fractions in the sediments, such as organic substances or to hydroxides of for instance Fe and Mn, indicating a low mobility and bioavailability of the deposited Pb. Only

minor amounts of Cu and Sb were deposited in the sediments, indicating that most of these metals were transported downstream from the lake.

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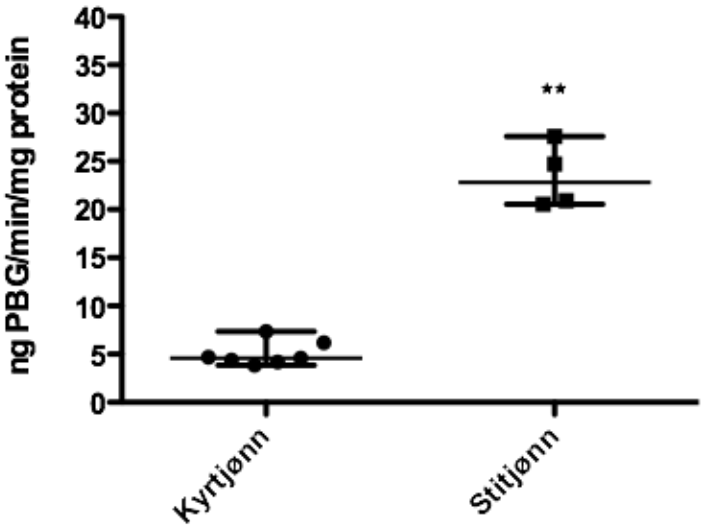

Fig 1. The ALA-D activity in blood from brown trout (median and range).The ALA-D activity in blood from brown trout from Lake Kyrtjønn was significantly different from brown trout collected from Lake Stitjønn (Man-Whitney U t-test *** $p<0.001$).

Table 1. Water chemistry of Lake Kyrtjønn and Lake Stitjønn. The water was sampled 14-16 August 2011. The results are shown as mean (± SD) from 3 or 4 separate samples taken from different places near the shore line of the lakes. In brackets, the concentrations of some of the elements and ions are shown as µeq/L. Other metals are shown in total concentrations.

	Lake Kyrtjønn	**Lake Stitjønn**
pH	5.6	5.9
Conductivity (µSi/cm)	30	42
Pb total (µg/L)	14 ± 1.9	0.76 ± 0.09
Cu total (µg/L)	6.1 ± 0.64	1.8 ± 0.09
Sb total (µg/L)	1.3 ± 0.15	0.12 ± 0.02
Zn total (µg/L)	12 ± 1.2	8.7 ± 0.11
Cl (mg/L)	4.4 ± 0.02 (124)	6.2 ± 0.06 (175)
SO_4 (mg/L)	2.3 ± 0.06 (48)	3.1 ± 0.02 (65)
NO_3(mg/L)	0.07 ± 0.004 (1.2)	0.05 ± 0.001 (0.7)
TOC (mg/L)	10 ± 0.32	8.9 ± 0.06
DOC < 0.45µm	11 ± 0.44	9.0 ± 0.04
DOC <10KDa	3.1 ± 0.12	3.2 ± 0.27
Al (mg/L)	0.67 ± 0.01	0.52 ± 0.13
Ca (mg/L)	1.9 ± 0.35 (95)	2.3 ± 0.58 (115)
Fe (mg/L)	0.59 ± 0.011	0.22 ± 0.06
K (mg/L)	0.32 ± 0.04 (8.2)	0.50 ± 0.08 (13)
Mn (mg/L)	0.03 ± 0.002	0.030 ± 0.008
Na (mg/L)	4.5 ± 0.14 (196)	5.8 ± 1.3 (252)
Mg (mg/L)	0.87 (72)	1.04 (86)

Table 2. Size and charge distribution of the water sampled from Lake Kyrtjøn (Kyr) and Lake Stitjønn (Sti). The table shows the mean proportion (% ± SD) of Pb, Cu, Sb and Zn in the particulate fraction (> 0.45 µm), HMM fraction (0.45 µm – 10kDa) and LMM fraction (< 10 kDa), and the proportion of the cationic, anionic and neutral species (% ± SD) as determined by ion exchange chromatography of filtered samples (0.45µm).

Kyr (n=4)	Particulate	HMM	LMM	Cation	Anion	Neutral
Pb	14 ± 6.0	69 ± 6.0	17 ± 1.0	50 ± 7.0	12 ± 6.7	39 ± 2.3
Cu	7.9 ± 4.4	65 ± 3.6	27 ± 1.1	10 ± 3.1	48 ± 15	42 ± 12
Sb	0.16 ± 3.2	23 ± 4.8	77 ± 4.6	-1.2 ± 1.5	89 ± 8.5	13 ± 7.6
Zn	3.0 ± 12	44 ± 6.1	52 ± 5.9	93 ± 5.9	-0.74 ± 3.1	7.8 ± 8.1
Sti (n=3)	Particulate	HMM	LMM	Cation	Anion	Neutral
Pb	14 ± 11	63 ± 7.0	23 ± 5.7	28 ± 6.9	32 ± 11	40 ± 15
Cu	8.2 ± 7.3	59 ± 3.9	33 ± 3.7	-0.8 ± 5.9	80 ± 9.5	21 ± 14
Sb	0.0 ± 26	27 ± 40	75 ± 17	2.2 ± 13	91 ± 4.1	7.2 ± 15
Zn	1.7 ± 5.3	31 ± 7.6	67 ± 7.3	94 ± 1.0	0.31 ± 2.5	6.0 ± 1.8

Table 3. $\delta^{15}N$ and $\delta^{13}C$ in the brown trout and their stomach content, and estimated trophic levels (TL) of the brown trout from Lake Kyrtjønn and Lake Stitjønn. The results are presented as mean ± SD.

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	TL	N
Brown trout-Lake Kyrtjønn	6.9 ± 0.8	-26 ± 0.8	3.7 ± 0.4	8
Stomach- Lake Kyrtjønn	1.3 ± 1.3	-29 ± 0.8		8
Brown trout-Lake Stitjønn	5.7 ± 0.9	-25 ± 1.4	3.6 ± 0.4	6
Stomach- Lake Stitjønn	0.30 ± 0.80	-28 ± 1.4		6

Table 4. The mean concentration (± SD, median in brackets) of Pb, Cu, Zn, Sb in blood, gills, liver, kidney, brain, bone and muscle tissue in brown trout collected from Lake Kyrtjønn and Lake Stitjønn. The concentrations are presented as mg/kg wet weight, with the exception of gills, which are presented as mg/kg dry weight. Statistical significant differences between brown trout from the two lakes are shown with asterisks (Man-Whitney t-test *p<0.05, **p<0.01, *** p<0.001).

Element	Organ	Lake Kyrtjønn	Lake Stitjønn	n*
Pb	Blood	5.4 ± 4.5 (2.7)**	0.13 ± 0.03 (0.13)	7/4
	Gill	137 ± 77 (170)**	2.7 ± 1.1 (2.4)	8/6
	Liver	6.7 ± 3.0 (6.5)***	0.13 ± 0.06 (0.10)	8/6
	Kidney	161 ± 134 (152)***	1.0 ± 0.65 (0.80)	8/6
	Brain	0.79 ± 0.60 (0.66)**	< 0.05	8/5
	Bone	104 ± 78 (105)**	2.4 ± 0.78 (2.1)	8/6
	Muscle	0.10 ± 0.08 (0.08)*	< 0.05	8/6
Cu	Blood	0.36 ± 0.05 (0.37)	0.32 ± 0.02 (0.32)	7/4
	Gill	1.6 ± 0.24 (1.6)	1.8 ± 0.30 (1.8)	8/6
	Liver	636 ± 573 (519)	228 ± 151 (213)	8/6
	Kidney	3.1 ± 0.98 (3.3)	2.5 ± 0.29 (2.6)	8/6
	Brain	2.6 ± 0.57 (2.5)	2.4 ± 0.92 (1.9)	8/5
	Bone	0.29 ± 0.06 (0.24)*	0.47 ± 0.31 (0.35)	8/6
	Muscle	0.32 ± 0.12 (0.30)	0.28 ± 0.09 (0.20)	8/6
Zn	Blood	14 ± 3.3 (14)	17 ± 2.8 (18)	7/4
	Gill	449 ± 176 (445)	715 ± 370 (645)	8/6
	Liver	50 ± 12 (47)	53 ± 18 (51)	8/6
	Kidney	52 ± 11 (54)	87 ± 59 (58)	8/6
	Brain	10 ± 1.8 (9.7)	11 ± 3.0 (10)	8/5
	Bone	131 ± 29 (135)	150 ± 32 (150)	8/6
	Muscle	3.5 ± 0.88 (3.1)*	4.7 ± 1.1 (4.9)	8/6
Sb	Blood	< 0.005	< 0.005	7/4
	Gill	0.04 ± 0.02 (0.03)	0.03 ± 0.01 (0.03)	8/6
	Liver	< 0.005	< 0.005	8/6
	Kidney	0.040 ± 0.02 (0.04)**	0.009 ± 0.008 (0.002)	8/6
	Brain	< 0.005	< 0.005	8/5
	Bone	0.04 ± 0.02 (0.03)	0.03 ± 0.01 (0.03)	8/6
	Muscle	< 0.005	< 0.005	8/6

*First number refers to samples from Lake Kyrtjønn, second number refers to Lake Stitjønn

Table 5. The mean concentrations (± SD, median in brackets) of Pb, Cu, Zn and Sb in whole eggs and alevins from Lake Kyrtjønn and Lake Stitjønn. The concentrations are presented as mg/kg dry weight. Statistical significant differences between brown trout from the two lakes are shown with asterisks (Mann Whitney t- test, *p<0.05, **p<0.01).

	Pb (mg/kg d.w.)	Cu (mg/kg d.w.)	Zn (mg/kg d.w.)	Sb (mg/kg d.w.)
Kyr 110 dd	5.1 ± 0.29 (5.2)**	4.0 ± 0.20 (4.0)*	63 ± 7.1 (60)	0.01 ± 0.01 (0.02)
Sti 110 dd	1.4 ± 0.28 (1.4)	3.5 ± 0.32 (3.4)	62 ± 6.0 (62)	< 0.003
Kyr 260 dd	6.8 ± 1.2 (7.0)**	4.0 ± 0.22 (4.0)**	57 ± 3.0 (56)	0.02 ± 0.02 (0.03)
Sti 260 dd	1.6 ± 0.45 (1.6)	3.3 ± 0.28 (3.4)	65 ± 10 (60)	< 0.003
Kyr 480 dd	0.27 ± 0.15 (0.27)**	3.0 ± 0.34 (3.0)	56 ± 2.9 (56)	< 0.003
Sti 480 dd	< 0.03	2.9 ± 0.19 (2.9)	67 ± 12 (65)	< 0.003
Kyr 690 dd	5.9 ± 2.7 (5.5)**	3.1 ± 0.29 (3.0)	75 ± 17 (69)	< 0.003±
Sti 690 dd	0.66 ± 0.73 (0.64)	2.8 ± 0.27 (2.7)	67 ± 17 (69)	< 0.003

Table 6. The mean concentrations of Pb, Cu, Sb and Zn (± SD, median in brackets) in the egg contents from fertilized trout eggs from Lake Kyrtjønn and Lake Stitjønn. The concentrations are presented as mg/kg dry weight.

	Pb (mg/kg d.w.)	Cu (mg/kg d.w.)	Zn (mg/kg d.w.)	Sb (mg/kg d.w.)
Kyr 110 dd	< 0.03	3.1 ± 1.4 (3.6)	51 ± 7.9 (50)	< 0.003
Sti 110 dd	< 0.03	3.3 ± 0.16 (3.3)	54 ± 7.1 (52)	< 0.003
Kyr 260 dd	0.54 ± 0.22 (0.60)	3.5 ± 0.15 (3.4)	61 ± 6.7 (59)	< 0.003
Sti 260 dd	< 0.03	2.7 ± 0.20 (2.8)	57 ± 5.6 (56)	< 0.003