NWSF Project Number: 206-16-01

Project Title: Metal-Related Oxidative Stress and DNA Damage in Ringed Seal Population from the Strathcona Sound (Arctic Bay, Nunavut)

Project Leader: Solomon Amuno, PhD

Summary: This project investigated the effects of residual mining derived contaminants (heavy metals) on health of ringed seals in the vicinity of the former Nanisivik lead-zinc mine in Strathcona Sound, Arctic Bay, Nunavut. The project focused on answering the following research questions:

1. Does ringed seal population from the Strathcona Sound carry a higher burden of heavy metals in comparison with population from reference location (s)?
2. To what extent have metal-related stress, including oxidative damage to lipids, DNA and proteins occurred in ringed seal population from the Strathcona Sound in comparison with population from reference location (s)?
3. To what extent have exposure to metals caused immunosuppression and decreased resistance to diseases in population from the Strathcona Sound and in comparison with population from reference location (s)?

Project Objectives:

The project objectives are three-fold:

1. To determine the current level of contaminant load (arsenic, cadmium, mercury and lead) in selected organ tissues (liver, kidneys, gonads and bones) of ringed seals population inhabiting the Strathcona Sound, and in reference locations. We compared contaminant load in population near the former mining area, with those farther away from the site, to determine if significant differences exist between the two groups.
2. Utilize selected biomarkers to assess the potential effects that these metals bear on oxidative stress biomarkers (lipid peroxidation), antioxidant capacity and genotoxicity (DNA damage) in population from the Strathcona Sound and in reference locations.
3. The third was to compare and assess immunosuppression and disease susceptibility of ringed seal population from the study area, with those from reference locations.

Material and Methods:

We collaborated with the Ikajutit Hunters and Trappers Association in Arctic Bay to capture a total of 8 ringed seals, although we had initially planned for 30 ringed seals for our study. We were unable to get additional seals from the study area due to the degradation of sea-ice at the time of the sampling, and other unforeseen logistical challenges. Field work was led by PhD student (Ankur Jamwal), University of Saskatchewan and a local hunter in Arctic Bay. Sampling of seals commenced between August 3-15, 2016. Appropriate research permits, including animal care handing protocols were obtained prior to research activities from the Nunavut Research Institute and Fisheries and Oceans Canada. A high-powered rifle was used to shoot ringed seals within and around the vicinity of Strathcona sound/Admiralty Inlet and Adam Sound. Animals died instantly. The GPS coordinates of sampling locations as well as gender of each animal were recorded immediately. Each animal were dissected for the removal of target organs, such as kidney, liver, eyes, testicles, bones and fatty tissues. Tissue samples were stored in appropriate polyethylene vials for metal analyses at University of Saskatchewan, and also in histology jars containing formalin for preservation of samples for histopathological studies. All biological samples were stored in an electric cooler to facilitate transportation of tissues for analyses. No significant disturbance to the ecosystem resulted from the research.

The following were the specific analyses conducted on the tissue samples:

1. Biomarkers of oxidative stress: measurement of lipid peroxidation (a good marker of oxidative damage in cells);
2. Enzyme Assays: catalase, superoxide dismutase, Glutathione peroxidase, Glutathione, Redox status expressed as GSH:GSSG ratio;
3. DNA damage: a DNA ladder assay was used to determine extent of DNA strand breaks in liver tissues of the sampled seals;
4. Heavy metal analyses: concentrations of selected trace metals (arsenic, cadmium, copper, lead, selenium and zinc) were determined in liver, kidney, gonad and bone tissues of the sampled seals;
5. Histopathology: Ultrastructural changes were also determined in eye globes and livers of the seals for assessing potential tissue abnormalities or damage due to contaminant exposure. Optical coherence tomography (OCT) was specifically conducted on all eye tissues to assess integrity of ocular tissues of the seals; and
6. Bone densitometry/X-ray: bone mineral density (BMD), including radiological assessment of the rib bones were also examined to monitor skeletal mineralization and other potential bone abnormalities.

Project Schedule: We had anticipated starting the project around May 2016, however due to extenuating circumstances that were beyond our control, we could only commence the project in August 2016. The timing made seal hunting a challenge, in addition to coinciding with narwhal hunting season in the community.

Preliminary Results and Discussion:

Below are the major highlights of the results of the study:

1. The concentration of selected metals (As, Cd, Cu, Pb, Se and Zn) in renal and bone tissues of the seals from the mine area was significantly higher compared with the seal from the reference location. Renal and bone concentration of cadmium was at least in the range of 3 to 33 times higher in the case animals from the mine area compared to the reference seal.
2. Bone mineral content, including bone mineral density of the reference seal was significantly higher in comparison with the case seals; however, bones from both locations showed varying severities of abnormal bone mineralization, porosity and microfractures.
3. Two seals from the mine area were also observed with lens opacification (cataracts), and one with iris atrophy. This is the first time cataracts and iris atrophy are reported in wild population (such lesions are typically seen in captive seals), so this is a significant discovery.
4. Significant hepatic changes were only noted in the reference seal likely age-related, and with lesions comprising of steatosis, mild changes in normal

appearance of the central vein, hepatocytes and sinusoids, in addition to portal expansion with fibrous septa.

5. Renal concentration of As, Cd, Cu, Se and Zn in ringed seals obtained in this present study was significantly lower compared to the baseline bioaccumulation data collected in 1989, suggesting a decreasing trend of metal input into the local food chain following mine closure.
6. Seals from both locations showed no evidence of DNA fragmentation; however, most seals from the mine area exhibited increased levels of lipid peroxidation and reduced antioxidants suggesting oxidative stress responses due to metal exposure.
7. The results of this present study indicate that chronic heavy metal exposure may be a potential risk factor likely to induce skeletal demineralization, as well as ocular and hepatic lesions in exposed ringed seals from the study area.
8. This preliminary study is the first study to report the occurrence of cataracts, skeletal demineralization and hepatic lesions in free-ranging ringed seals exposed to mining derived contaminants in the Canadian high arctic.

Please see the attached publication for more details (images of pathology are after the reference section). We are currently preparing a second publication entitled "Biomarkers of metal-induced oxidative stress and DNA damage in seven ringed seals (Phoca hispida) breeding near the former Nanisivik lead-zinc mine in the Canadian arctic: Part II". We would forward this publication to the NWMB as soon as it is peer-reviewed and ready for publication.

Reporting to Communities and Resource Users: The project leader (Solomon Amuno) communicated the results of the study to the Arctic Bay Hunters and Trappers Association in January and February 2017. The HTO are greatly appreciative of the study, and recommended that we undertake further monitoring studies with more seal samples in order to assess the changing health of marine mammals near Strathcona sound and Admiralty Inlet.

Interim Financial Report

NWSF Project Number: 206-16-01

Project Title: Metal-Related Oxidative Stress and DNA Damage in Ringed Seal Population from the Strathcona Sound (Arctic Bay, Nunavut)

Project Leader: Solomon Amuno, PhD

Original Project Budget: Project leader received $30,000 from NWSF.

Original Contributions: $30,000

Explanation of Changes: There were no major changes in the budget as originally proposed except for repriotizing items for expenditure based on field conditions and laboratory capacity.

Financial Report

Proposed budget in 2016

Budget Item	Budgeted	Disbursed	Variance
Metal analyses	$10,500		
Enzyme assays	$4800		
Histology	$2382		
Lab technician	$3000		
Travel	$8000		
Truck rental	$2000		
Total	$30,682	$30,000	

Actual Budget after completion of research

List of Expenses	Cost ($)	Recipient
Flight: SK to Ottawa	678.94	Ankur Jamwal (PhD student)
Flight: Ottawa to NU	6907.97	Ankur Jamwal (PhD student)
Electric Cooler	198	Ankur Jamwal (PhD student)
Stipend for grad student	500	Ankur Jamwal (PhD student)
Hotel Ottawa for graduate student	161.78	Ankur Jamwal (PhD student)
Hunter in Arctic Bay	5300	Mathew Akikulu
Hotel arctic bay	2100	Hotel Arctic Bay
DNA damage	1400	University of SK
Bone X-ray and densitometry	340	McGill University
Shipment of bones (McGill to SK)	120	UPS
Shipment of bones (SK to McGill)	120	UPS
Translation	100	Mr. Morgan (Pond Inlet)
Surfer 10	1200	Golden Surfer
Ocular histology and OCT	552.52	University of SK

Lab technician	2,500	University of SK
Additional lab analyses (trace metals and enzymes)	3560	University of SK
Trip to Iqaluit for retrieval of samples	1,656	Canadian North
Hotel in Iqaluit and YK (3 days)	600	Accommodations by the sea (Iqaluit) and Super 8 (YK)
Travel to SETAC meeting to seek advice from toxicologist regarding bone and eye pathology	3,030	Society for Toxicology and Environmental Chemistry (SETAC)-Nov
Total	31,025	

Explanation of variances:

We still remained within budget, although with some expenses that were not anticipated which are briefly described below:

1. Purchase of Surfer 10 software: This was intrinsically part of the project as the software was used for modelling trace metals dispersion in Strathcona Sound and Admiralty Inlet.
2. Trip to Iqaluit: This was necessary in order for the project leader to meet with Baffin hunters transiting through Iqaluit for potential additional sampling as we only captured 8 seals.
3. Travel to SETAC meeting: During analyses, we detected some abnormal pathologies of the eyes (cataracts and iris atrophy), and bones (severe demineralization) of ringed seals which have not been previously reported. I had to attend toxicology sessions with leading arctic biologists to discuss our observed pathologies, and get expert advice prior to finalizing the research.

Verification of Information provided: I certify that this is an accurate statement of the Board project funs received and disbursed in accordance with the joint contribution agreement.

Chronic heavy metal exposure induces ocular anomalies, skeletal demineralization and hepatic changes in ringed seals (*Phoca hispida*) captured near the vicinity of a former lead-zinc mine: A report of seven cases from the Canadian high arctic

S. Amuno[a*1], A. Jamwal[b], S. Osinchuk[c], B. Grahn[c], S. Niyogi[b]

[a] School of Environment and Sustainability, University of Saskatchewan, Saskatoon, Canada
[b] Department of Biology, University of Saskatchewan, Saskatoon, Canada
[c] Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada

Abstract

The current study was undertaken to evaluate the role of chronic heavy metal exposure in the etiology of ocular anomalies, skeletal demineralization and hepatic alterations in seven (7) free-ranging ringed seals (Phoca hispida) captured near the vicinity of the former Nanisivik Pb-Zn mine in the Canadian high arctic. The affected case animals were compared with one (1) adult ringed seal captured in Adam Sound, approximately 50 kilometres from the mine area. Trace metal analyses included measurement of As, Cd, Cu, Pb, Se and Zn in tissues, and histopathological analyses included evaluation of hepatic and ocular lesions. Osteodensitometry was also conducted on the rib samples of the seals to determine changes in bone mass and bone microstructure, including degree of bone mineralization. The concentration of selected metals in renal and bone tissues of the seals from the mine area was significantly higher compared with the seal from the reference location. Renal and bone concentration of Cd was at least in the range of 3 to 33 times higher in the case animals compared to the reference seal. Bone mineral content, including bone mineral density of the reference seal was significantly higher in comparison with the case seals; however, bones from both locations showed varying severities of abnormal bone mineralization, porosity and microfractures. Two seals from the mine area were also observed with lens opacification, and one with iris atrophy. Significant hepatic changes were only noted in the reference seal likely age-related, and with lesions comprising of steatosis, mild changes in normal appearance of the central vein, hepatocyctes and sinusoids, in addition to portal expansion with fibrous septa. Renal concentration of As, Cd, Cu, Se and Zn in ringed seals obtained in this present study was significantly lower compared to the baseline bioaccumulation data collected in 1989, suggesting a decreasing trend of metal input into the local food chain following mine closure. The results of this present study indicate that chronic heavy metal exposure may be a potential risk factor likely to induce skeletal demineralization, as well as ocular and hepatic lesions in exposed ringed seals from the study area. This preliminary study is the first study to report the occurrence of cataracts, skeletal demineralization and hepatic lesions in free-ranging ringed seals exposed to mining derived contaminants in the Canadian high arctic.

Keywords: heavy metals; ringed seals; mining; arctic and contaminants

Introduction

The Nanisivik lead and zinc mine operated from 1976 to 2002, and was located on the Borden Peninsula of northern Baffin Island, Nunavut, Canada. During the operation of the mine, community members from the Inuit community of Arctic Bay, approximately 30 kilometres from the mine, expressed serious health concerns due to wind-blown tailings to the surrounding area, and contamination of the natural environment with complex mixtures of contaminants, particularly heavy metals (Wagemann,1989). Marine pollution concerns were also raised by different stakeholders in subsequent years during mine operations with respect to the disposal of mine tailings containing metals such as zinc, lead, cadmium, iron and arsenic into the adjacent Twin Lake Creeks, which drained directly into the Strathcona Sound (Asmund et. al, 1997; Bohn, 1979). Furthermore, there were also reported incidences of accidental spillage of lead and zinc ore concentrates into the marine ecosystem of the Strathcona Sound during loading of ore

*Corresponding Author: Email: solomon.amuno@gmail.com; Telephone: +18679834982
1 Note: Dr. Amuno's participation in this study was undertaken independently and apart from his work with the Nunavut Impact Review Board. The analysis and views expressed in the study remain solely those of the authors and do not constitute the views of the Nunavut Impact Review Board.

concentrates into ship vessels for transportation out of the mine site (Wagemann, 1989). Despite closure of the Nanisivik mine in 2003, and subsequent decommissioning of mine infrastructures in 2006, Inuit engaged in hunting and fishing around the Strathcona sound continue to express concerns about residual contamination, and potential contaminant bioaccumulation in exposed ringed seals breeding near the former mine site. In addition, there were also observation of liver lesions in the seals from the mine area, and speculations that these pathological changes were likely induced by long-term exposure to mining derived contaminants and infectious diseases (Fallis, 1982; Furgal et al, 2002). Several studies have shown that heavy metals can trigger the loss of genetic diversity, and suppress the function of the immune system in a wide range of organisms, causing population bottlenecks, and increasing the susceptibility of organisms to opportunistic parasites and diseases (Poulsen and Escer, 2012).

During the operational phase of the Nanisivik mine, Wagemann (1989) reported elevated levels of heavy metals in tissues of ringed seals from the Strathcona Sound; but since cessation of mining activities no study has evaluated the status of metal bioaccumulation, including exposure-related effects in ringed seals from the former mining area. Previous studies monitoring metal accumulation in ringed seals from the Canadian arctic have solely relied on the measurement of selected trace metals in organ tissues of animals as means to evlaute contaminant exposure (Wagemann et. al, 1983; Wagemann et. al, 1996; Norstrom et. al, 1986). While this approach has provided useful data on contaminant bioaccumulation, it does not explicitly identify whether contaminants has had toxic effects. Monitoring sub-lethal effects including histopathological changes can provide additional information regarding the toxic effects of contaminant exposure in wildlife population (Sonne et. al, 2008; Amuno et. al, 2016).

The purpose of this research project is three-fold. The first is to determine the current level of trace metals (arsenic, cadmium, lead, copper, zinc and selenium) in renal and bone tissues of ringed seals captured from Strathcona Sound, near the vicinity of the former Nanisivik mine, and in a reference location approximately 50 kilometres from the mine area. We are specifically interested in comparing trace metal load in seals captured near the mining area, with that farther away from the site, to determine if significant differences exist between the two groups, and to determine whether current accumulation differs from the baseline obtained in 1989 (Wagemann, 1989). The second is to utilize histopathological markers to evaluate ocular and hepatic lesions, as well as osteodensitometry to assess the extent and severities of skeletal demineralization in ringed seals from the study area.

Materials and Methods

Prior to field sampling, a Nunavut Research Licence, and Nunavut Wildlife Research Permit were obtained respectively from the Nunavut Research Institute, and Department of Environment, Government of Nunavut (GN), Canada. Through collaboration with the Ikajutit Hunters and Trappers Association, Arctic Bay, Nunavut (Canada), and pursuant to the requirement of the Wildlife Research Permit, a total of 8 ringed seals were captured for our study. Seals (n=7) were captured within and around the vicinity of the former Nanisivik lead-zinc mine area (Figure 1), and euthanized similar to the procedures outlined in Wagemann (1983). Adam Sound, an unindustrialized location near to the community of Arctic Bay was selected as the background area for the reference seal (n=1). Additional reference animals were not obtained for sampling due to field constraints and dangerous weather condition at the time of the fieldwork. The specific GPS location of capture and gender of each animal was recorded immediately. Tissue samples were collected from each animal and stored in prepared vials for metal analyses at University of Saskatchewan, and in histology jars containing formalin for preservation of samples for histopathological studies. All biological samples were stored in cooler to facilitate transportation of tissues for analyses.

Samples Collection and Analyses

Selected trace metals (As, Cd, Fe, Pb and Zn) were determined in kidney and bone tissues of ringed seals using a graphite furnace atomic absorption spectrometer (Analyst 800, Perkin Elmer, USA). Due to limited number of samples collected for this preliminary study (n=8), animals from each site were not segmented into age classes for ease of further analyses. Of the total animals, 7 seals were captured near the mine area, while 1 seal proximal to the community of Arctic Bay. The kidney tissue samples (~1.0 g) were digested in 15mL polyethylene vials 1N nitric acid (15.8N) (Ultrapure, Merck, Canada), with a tissue weight to acid volume ratio of 1:5, at 60^0C for 48h (Amuno et al., 2016). The bone samples were digested similarly in concentrated nitric acid (15.8N) (Ultrapure, Merck, Canada) in 20 mL borosilicate glass vials with polypropylene screws (Metal free, EPA certified, VWR, Canada) in room temperature. The concentrations of metals in digested tissues were measured after appropriate dilutions with 0.2% nitric acid. The quality control and quality assurance of the analytical method were maintained by using certified standards for each metal, the standard addition and recovery procedure, and a certified reference material (DOLT-4; National Research Council of Canada). The reference material was digested and analyzed concurrently with the tissues samples, and the recovery of metals varied between 94 and 103%. All tissue metal data are presented on a wet weight basis.

Tissue collection for light microscopy and optical coherence tomography

Seals were shot with a rifle and globes and liver sections where collected promptly in the field and were immersed in 10% formalin at 10:1 fluid to tissue ratio. Sections of liver were dehydrated through a series of alcohols and xylene and then sectioned and embedded into paraffin wax. Blocks were sectioned and routinely stained with hematoxylin and eosin, and were examined with light microscopy. Following formalin fixation, the globes were examined grossly and they were measured with a caliper. The corneas were also measured. The globes were sectioned with a brain blade in a vertical plane. Each half of the globe was examined grossly and abnormalities were recorded and photographed. One half was selected for sectioning and a calotte was prepared by removing a section of the scleral cap to allow complete paraffin embedding. Six micron sections were harvested and stained routinely with hematoxylin and eosin and periodic Schiff and Luxol fast blue stains. Light microscopy was completed on all stained sections. Formalin fixed eye cups were placed in front of the optical coherence tomograph (Heidelberg Engineering Spectralis OCT; Heidelberg Engineering, Germany; HRA/Spectralis Image Capture Module software version 5.7) fitted with the anterior segment module. Horizontal volume scans were collected through retina adjacent to the optic nerve as well as multiple areas in the peripheral retina. Images were evaluated for evidence of retinal pathology. Scans consisted of 758 A scans with an automated real time tracking of 60 repeats per image.

Radiographic analysis and densitometric measurements

Radiological bone assessment was conducted at the Centre for Bone and Periodontal Research, McGill University, Quebec Canada. A single X-ray of the rib bones was taken using a Kubtec Xpert 80 radiography system with automatic calibration. Densitometric measurement was also obtained using an animal densitometer (Luna Piximus) for assessment of bone mineral density. The results were provided in terms of bone mineral density (g/cm^2), bone mineral content (g), fat and lean tissue content (g).

Results

Trends of metal concentration in renal and bone tissues:
The concentration of As, Cd, Cu, Pb, Se and Zn were determined on wet weight basis in renal tissues, of seven ringed seals captured from the vicinity of the former Nanisivik mine, and compared with one adult ringed seal caught approximately 50km from the mine area. In addition, Cd and Pb levels were also determined in rib samples of seals from both locations. As indicated in table 1 below, the concentration of cd in renal tissues of seals from the Nanisivik mine area ranged from 2.4µg/g to 31.5µg/g, while that from the control seal was 1.16µg/g, suggesting that the animals from the mine area carry a higher cadmium burden, likely as a result of increased cd input into the local marine environment from natural and

anthropogenic sources due to previous mining activities. The concentration of bone cd in ringed seals from the mine area was significantly increased, in the range of 3 to 33 times (0.023 μg/g to 0.25 μg/g) more than the control adult seal (0.0074 μg/g), which suggest that seals from the mine area may be chronically exposed to elevated cd in their habitats and from dietary sources. Renal concentration of Pb in seals from the mine area ranged from 0.1 μg/g to 0.24 μg/g, at least doubling Pb concentration in kidney of the reference seal (0.049 μg/g). Unlike cadmium, similar concentration of bone Pb was noted in seals from both the mine area (0.24 μg/g to 0.46 μg/g) and reference site (0.23 μg/g), which suggest that seals from both locations are likely to be exposed to similar background levels of environmental Pb. Renal concentration of Cu in ringed seals from the mine area ranged from 6.03 μg/g to 14.7 μg/g, while that from the referenced seal was 4.08 μg/g. Renal concentration of As in the reference seal was 0.35 μg/g, while those from seals at the mine area ranged from 0.21 μg/g to 0.46 μg/g. Zn concentration in renal tissue of seals from the mine area ranged from 12.6 μg/g to 17.1 μg/g, while that in the reference site was 15.3 μg/g. Se levels in the reference seal was 0.95 μg/g, while those in seals from the mine area ranged from 1.04 μg/g to 1.87 μg/g. In general, most trace metals except for Cd and Pb were in similar concentrations in renal and bones tissues of the seals from the mine area and reference location.

Bone densitometry and analysis of x-ray bone anatomy

Radiological assessment of seal ribs, including densitometric measurement conducted on 8 ribs of sampled ringed seals to evaluate bone mineral density, bone mineral content, percentage of fat, bone area and lean tissue content. The data from table 2 suggest that case and control seals significantly differ in the measured bone parameters. As observed, the bone mineral density of the reference seal was 0.24 gm/cm^2, while those from the mine area ranged from 0.064 gm/cm^2 to 0.18 gm/ cm^2. Further, the bone mineral content of the reference seal was 1.1grams (g), while that from the study area ranged from 0.078g to 0.47g. In addition, the bone area of the reference seal was $4.4cm^2$, significantly higher than those observed from the study area (1.22 cm^2 to 2.54 cm^2). It is likely that age-related differences between the case animals, which mainly comprised of sub-adult seals and the adult reference seal may have contributed to the differential levels of bone mineral content and bone mineral density as observed in the study. Detailed evaluations of the radiological assessment of the bones are presented below:

Seal 1 (control sample):

Figure 2 shows the bone scan of an adult ringed seal captured approximately 50 km from the mine site. The x-ray pattern shows severe loss of the normal bony anatomy due to massive sclerosis of the rib, as well as abnormal deposition of calcium with simultaneous loss of the osteoclastic activity. The distal part of the rib and other two-third of the bone show multiple disfiguring fractures with total loss of the normal bony anatomy. Pathological ossification was observed not to follow a uniform pattern. The fractured areas in the bone are compatible with areas of increased bone turnover activity, which may have likely resulted in a weaker cortical bone in this reference seal. While this reference seal was found to carry a lower concentration of Cd and Pb in renal and bone tissues compared to the case animals; however, bone resorption and anatomical changes including disturbed mineralization observed in this bone may have been influenced by chronic exposure to low levels of Cd and Pb, in addition to other persistent organic compounds during its lifetime. It is likely that the decrease in bone mineral density, collagen maturity, mineral crystallinity, and altered mineral/matrix ratios may have been influenced by metal exposure, and resulted in a weaker, fragile and fractured bone as observed in the distal part of the rib. In addition, the proximal part of the bone was observed to be extremely sclerotic due to disturbed osteoclastic activity.

Seals 2, 3, 4, 7:

Figure 3 is a representative bone scan observations for seals 2, 3, 4 and 7 from the mine area. The x-ray of the bones generally show remnants of the fragmented bone interspersed with abnormal crystals of pathological calcium deposition. The observed pattern is typically reflective of disturbed bone turnover activity and possibly abnormal mineralization due to chronic metal exposure, including accumulation of Cd and Pb in the bone matrix. The pattern of fragmentation and friability of the bones are more pronounced within the distal part of the ribs. Areas of pathological calcium deposition and or lead/cadmium particles along the friable bone are also apparent. As seen it is likely that lead or cadmium is mineralizing the bone tissue which in fact was not uniformly distributed in the bone matrix. It is possible for heavy metals such as lead and cadmium to accumulate in bone regions undergoing the most active calcification at the time of exposure.

Seal 5:
Figure 4 show evidence of complete loss of the normal bony anatomy. Areas of high density (sclerotic-longitudinal bands) were similarly observed likely as a result of abnormal deposition of calcium secondary to accumulation of lead or cadmium alternating with areas of fragmentations and multiple micro-fractures, which may have influenced the complete loss of the normal bony architecture.

Seal 6:
Figure 5 shows multiple microfractures due to extreme sclerosis of the rib including the streaks of longitudinal sclerotic bands alternating with areas of hypomineralization. Hypersclerotic bands are known to be mostly related to chronic heavy metal exposure, particularly lead and cadmium poisoning. The rib sclerosis as observed in this scan appears not to take the uniform pattern of abnormal ossification, which may be as a result of disturbed osteoclastic activity.

Seal 8
Figure 6 show severe friability and fragmentations with areas of radio-opacity which mostly represent the abnormal calcium deposition due to accumulated lead in the bone matrix. A pathological feature which is encountered in chronic lead exposure.

In general, the strong correlative relationships of BMDs/BMCs and the pathological features of dystrophy like features in these ribs are suggestive of disruption which may have been caused by long term exposure to heavy metals such as lead and cadmium and other contaminants.

Gross, light microscopic and OCT findings of the globes
Minimal ocular abnormalities were detected in the 8 ringed seals that were examined grossly, histologically and with optical coherence tomography. Seal numbers 6 and 7 (Figures 9 and 10) had acute subretinal hemorrhage that most likely developed secondary to the gunshot. Seals 7 and 8 had small subcapsular anterior cortical cataracts and lens epithelial metaplasia (Figures 10 and 11). Seal 4 had iridial atrophy (Figure 8). Multiple OCT figures were reviewed from the posterior segment of each globe and compared to the single histologic sections of each seal. Retinal images demonstrated normal architecture in all animals. Artifacts due to fixation and autolysis included vitreous debris, wrinkling of the retina, vitreous detachment and artefactual retinal detachment were also noted in the samples. These findings were confirmed on light microscopy. Generally, the globes were marginally fixed given the field enucleation and emersion in formalin. This is due minimal scleral and corneal formalin penetration. This created multiple retinal separations and wrinkles and debris noted on the OCTs. Multiple OCT sections are provided with the gross and histologic sections of the retina, choroid and sclera. Aside from the two seals with subretinal hemorrhage, no other abnormalities were detected.

Liver Pathology
Ultrastructural alterations of hepatic tissues were only noted to be common in the adult ringed seal from the reference location. Central vein, hepatocytes and sinusoid showed mild to severe changes in normal appearance and with fat vacuoles observed. Mild infiltration of portal tract by mononuclear cells and hepatocycte swelling was also observed in addition to portal expansion with fibrous septa. No major histological changes of pathological significance were observed in the liver of the ringed seals from the mine area. Photomicrograph of the hepatic changes noted in the reference seal is presented in Figure 12 below.

Discussion

<u>Trends of metal bioaccumulation in seals inhabiting mining areas of the arctic:</u>
The results of trace metals bioaccumulation in this present study represent the first post-mining baseline data for ringed seals in the Canadian high arctic. Prior to this current investigation, only one study had specifically reported the bioaccumulation pattern for heavy metals in tissues of ringed seals from the Nanisivik mine area during its operation (Wagemann, 1989). Ringed seals are known to have extensive seasonal home ranges across the arctic and can potentially accumulate contaminants from different sources during their lifetime (Quakenbush and Citta, 2009); notwithstanding, recent studies have

indicated that ringed seals show fidelity to specific sites for breeding, as such are particularly vulnerable to contaminant exposure from point-sources (Kelly et. al. 2010; Niemi et. al, 2013). In addition, the traditional knowledge gathered from Inuit hunters in Arctic Bay similarly confirm that the vicinity of the mine area, specifically Strathcona Sound and the adjacent Admiralty Inlet are key breeding sites for ringed seals and other marine mammals. Based on the results presented in Table 2 below, it is evident that the mean concentration of As, Cd, Cu, Se and Zn in renal tissues of seals from the Nansivik mine was significantly higher in 1989 compared to the results obtained in this current study. The decreasing trend of metal bioaccumulation may be indicative of the reduced input of toxic metals into the local food chain following mine closure, or removal of other point source of pollution into marine area. The study conducted by Johansen et. al (2008) observed that the concentration of renal Pb in seals from Kong Oscars Fjords near the vicinity of a lead-zinc mine in Mestervig, East Greenland ranged from <0.04 µg/g to 0.06 µg/g, which was significantly lower compared to results of our study. Furthermore, Cd accumulation in renal tissues from this present study was also compared with baseline data for North Baffin Island seals sampled in 1976 and 1977 respectively, including western Greenland in 1979 (Wagemann and Muir, 1984). Again, a similar trend of decreasing Cd accumulation was generally noted, suggesting declining bioavailability to ringed seal population in the north Baffin region. The results of renal Pb obtained from this present study was significantly higher compared to the concentration of Pb noted in North Baffin Island seals sampled in 1976, 1977 and in Greenland in 1979. The higher trend of Pb accumulation observed in this study may be indicative of increasing Pb input into the arctic marine environment from local anthropogenic sources, in addition to long-range atmospheric transport.

Effect of heavy metals on bone pathology of ringed seals:

There has been no published work regarding the potential for chronic heavy metal exposure to induce bone disorders in ringed seals from the Canadian arctic. The only research work on bone pathology similar to our investigation was the study conducted by Sonne et. al (2002) regarding cadmium-induced nephropathy and osteodystrophy in ringed seals from Qaanaaq, northwest Greenland. In the study, Sonne et. al (2002) argued that if cadmium induced renal damage affected calcium metabolism in ringed seals, leading to osteopenia, then decalcification of the skeleton system would be evident in older seals and that seals with kidney damage would have significantly lower measures of bone mineral density. The study noted that the cases of nephropathy in the seals examined could neither be linked to the degree of mineralisation of the skeleton nor to Cd concentrations, and that the composition of the ringed seals diet, which contains high levels of vitamin D, calcium, phosphorus, zinc, selenium and protein were most likely to counteract any potential cadmium-induced damage. The study also concluded that despite high levels of Cd accumulation in renal tissues, none of the ringed seals sampled showed any signs of Cd induced nephropathy or osteodystrophy, and on that basis the authors speculated that ringed seals are not particularly vulnerable to osteodystrophy due to their continuous growth (bone mineralization) throughout life. Although the evaluation of renal osteodystrophy due to Cd exposure was not the specific focus of this present study, however, results from our current study area does show a pattern of decreased bone mineral density, including skeletal demineralization despite a relatively low level of Cd accumulation in renal tissues of exposed ringed seals. The results of our study agree with other empirical studies that have shown that chronic exposure to low-level Cd is associated with decreased bone mineral density and could increase the risk of osteoporosis and osteomalacia in exposed populations (Alfvén et. al, 2000; Staessen et. al, 1999).

Based on the review of existing literature, including the work of Sonne et. al (2002), there seem to be more scientific focus given to low bone mineral density due to increased risk fractures, with little or no attention given to the pathological implications of high bone mineral density. In practice, high bone mineral density is also considered an alarming sign for underlying degenerative bone pathology as it may reflect a long list of sclerosing disorders that may have been acquired as seen in metabolic bone diseases or genetically determined (Chan et. al, 2003; Laway et. al, 2006). Furthermore, the studies conducted by Theppeang et. al (2008) and Campbell et. al (2004) similarly confirm that subjects with high Pb exposure show a significantly higher bone mineral density than subjects with low lead exposure. In the context of our study, it is noteworthy to mention that the combined effects from long-term exposure to heavy metals, particularly Cd and Pb, in addition to other persistent organic pollutants, such as dioxins, organochlorines and flame retardant chemicals originating from long-range atmospheric transport may also play a role in the etiology of bone damage in the exposed ringed seals from the study area. Due to limited sample size, we are unable to conclusively determine whether the pathology observed in the bones are only unique to the Nanisivik area, or prevalent across the Canadian arctic.

Effects of heavy metal exposure on ocular pathology of ringed seals:

Ridgeway et. al (1975) reported that the primary ocular lesions frequently observed in pinnipeds include conjunctivitis, cataracts, cornea ulceration, cornea edema and scaring, but the etiology of these conditions are yet to be fully understood. Most studies investigating lens opacification in pinnipeds have majorly focused on the potential roles of viral infection (Colitz et. al, 2010), prolonged exposure to sunlight (Gage, 2011) as risk factors for cataracts, but studies evaluating the potential role of contaminants in inducing cataracts are scarce. A growing number of empirical studies have shown that chronic exposure to Cd and Pb may induce abnormalities in epithelial cells causing structural protein change, and may result to lens opacity (Racz and Erdöhelyi, 1988; Shearer et al, 1983). Other studies have also found elevated levels of Cd and Pb in lenses of humans and animals with cataracts, and have implicated these metals in the pathogenesis of cataracts (Schaumberg et al, 2004; Cekic,1998; Tamada et. al, 2001). The only ocular lesions noted in our present study were focal anterior cortical cataracts with lens epithelial metaplasia and iris atrophy in two seals from the mine area. This could be a significant finding in a wild population of seals, however the number in this study precludes meaningful data analysis. In addition, histologic examination is generally a poor method of cataract detection compared to biomicroscopy, which should be considered in future investigations of these seals.

Conclusions

Results of this study confirm that ringed seals captured within the vicinity of the mine carry a relatively higher tissue concentration of Cd and Pb, but at levels still considered relatively low compared with baseline data for the area in 1989. Generally, renal levels for most metals except Cd and Pb appear to be higher in seals from the mine area compared to that from the reference location. Based on the pattern of metal distribution noted in this present study, it is likely that seals from the Strathcona Sound area, near the vicinity of the former Nanisivik mine are still exposed to relatively higher levels of heavy metals compared to the reference seal, captured approximately 50km from the mine area. While it its recognized that ringed seals have large home-range and may have accumulated contaminants from other sources apart from the mine area, it is likely that fidelity of seals to habitats within and around the Strathcona Sound may have induced chronic exposures to metal contaminants. All the seals captured from the mine area showed evidence of skeletal demineralization, including hepatic and ocular alterations such as cataracts and iris atrophy. Given that this is a preliminary study, and that the sample size utilized for post-mine monitoring study was relatively small; the associated data should be interpreted with caution, and treated as a baseline for monitoring long-term effects of metal contaminants in marine mammals inhabiting the vicinity of other operational or deactivated mines. There is also need for collection and analysis of larger sample from the study area in order to generate more conclusive results regarding the toxic effects of mining derived pollutants in exposed marine mammals in the Canadian arctic.

Acknowledgement

The authors acknowledge the financial assistance of the Nunavut Wildlife Management Board for funding this research, in addition to the logistical support received from the Arctic Bay Hunter and Trappers Organization. We also thank Dr. Al Kaissi Ali for providing expert advise regarding the bone pathology of the seals. We also appreciate the assistance of Dr. Yongjun Xiao and Prof David Goltzman for helping to analyze the bones, as well as Prof. Fabiano Montiani-Ferreira for providing additional expert advise on ocular pathology.

Conflict of Interest/Disclaimer

The views and opinions expressed are solely those of the authors and are not the views of the Nunavut Impact Review Board.

References

Alfvén, T., Elinder, C. G., Carlsson, M. D., Grubb, A., Hellström, L., Persson, B., ... & Järup, L. (2000). Low-level cadmium exposure and osteoporosis. Journal of Bone and Mineral Research, 15(8), 1579-1586.

Amuno, S., Niyogi, S., Amuno, M., & Attitaq, J. (2016). Heavy metal bioaccumulation and histopathological alterations in wild Arctic hares (Lepus arcticus) inhabiting a former lead-zinc mine in the Canadian high Arctic: A preliminary study. *Science of the Total Environment*, *556*, 252-263.

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(1-2), 1-15.

Bohn, A. (1979). Trace metals in fucoid algae and purple sea urchins near a high Arctic lead/zinc ore deposit. Marine Pollution Bulletin, 10(11), 325-327.

Campbell, J. R., Rosier, R. N., Novotny, L., & Puzas, J. E. (2004). The association between environmental lead exposure and bone density in children. Environmental health perspectives, 1200-1203.

Chan, F. K., Tiu, S. C., Choi, K. L., Choi, C. H., Kong, A. P., & Shek, C. C. (2003). Increased bone mineral density in patients with chronic hypoparathyroidism. The Journal of Clinical Endocrinology & Metabolism, 88(7), 3155-3159.

Colitz, C. M., Saville, W. J., Renner, M. S., McBain, J. F., Reidarson, T. H., Schmitt, T. L., ... & Mejia-Fava, J. C. (2010). Risk factors associated with cataracts and lens luxations in captive pinnipeds in the United States and the Bahamas. Journal of the American Veterinary Medical Association, 237(4), 429-436.

Cekic, O. (1998). Copper, lead, cadmium and calcium in cataractous lenses. Ophthalmic research, 30(1), 49-53.

Fallis, B. (1982). Trace metals in sediments and biota from Strathcona Sound, NWT; Nanisivik marine

Furgal, C. M., Innes, S., & Kovacs, K. M. (2002). Inuit spring hunting techniques and local knowledge of the ringed seal in Arctic Bay (Ikpiarjuk), Nunavut. Polar Research, 21(1), 1-16.

Gage, L. J. (2011). Captive pinniped eye problems, we can do better. J Mar Anim Ecol, 4, 25-28.

Johansen, P., Asmund, G., Aastrup, P. & Tamstorf, M. 2008: Environmental Impact of the Lead-Zinc Mine at Mestervig, East Greenland. National Environmental Research Institute, University of Aarhus, Denmark. 30 pp. – Research Notes from NERI no. 241. http://www.dmu.dk/Pub/AR241.pdf

Kelly, B. P., Badajos, O. H., Kunnasranta, M., Moran, J. R., Martinez-Bakker, M., Wartzok, D., & Boveng, P. (2010). Seasonal home ranges and fidelity to breeding sites among ringed seals. Polar Biology, 33(8), 1095-1109.

Laway, B. A., Goswami, R., Singh, N., Gupta, N., & Seith, A. (2006). Pattern of bone mineral density in patients with sporadic idiopathic hypoparathyroidism. Clinical endocrinology, 64(4), 405-409.

Niemi, M., Auttila, M., Viljanen, M., & Kunnasranta, M. (2013). Home range, survival, and dispersal of endangered Saimaa ringed seal pups: Implications for conservation. Marine Mammal Science, 29(1), 1-13.

Norstrom, R. J., Schweinsberg, R. E., & Collins, B. T. (1986). Heavy metals and essential elements in livers of the polar bear (Ursus maritimus) in the Canadian Arctic. Science of the Total Environment, 48(3), 195-212.

Poulsen, A., & Escher, B. (2012). Chemically induced immunosuppression and disease susceptibility in marine wildlife: A literature review. National Research Centre for environmental Toxicology (Entox), The University of Queensland, Brisbane, Queensland. 110p.

Quakenbush, L., & Citta, J. J. (2009). Trace element concentrations in bearded seals (Erignathus barbatus) near Red Dog Mine compared to other locations in Alaska. Journal of Marine Biology, 2009.

Racz, P., & Erdöhelyi, A. (1988). Cadmium, lead and copper concentrations in normal and senile cataractous human lenses. Ophthalmic research, 20(1), 10-13.

Ridgway, S. H., Geraci, J. R., & Medway, W. (1975). Diseases of pinnipeds. Rapports et Proces-Verbaux des Reunions (Denmark).

Sonne, C., Wolkers, H., Leifsson, P. S., Jenssen, B. M., Fuglei, E., Ahlstrøm, Ø., ... & Jørgensen, E. (2008). Organochlorine-induced histopathology in kidney and liver tissue from Arctic fox (Vulpes lagopus). Chemosphere, 71(7), 1214-1224.

Shearer, T. R., Anderson, R. S., & Britton, J. L. (1983). Influence of selenite and fourteen trace elements on cataractogenesis in the rat. Investigative ophthalmology & visual science, 24(4), 417-423.

Sonne-Hansen, C., Dietz, R., Leifsson, P. S., Hyldstrup, L., & Riget, F. F. (2002). Cadmium toxicity to ringed seals (Phocahispida): an epidemiological study of possible cadmium-induced nephropathy and osteodystrophy in ringed seals (Phoca hispida) from Qaanaaq in Northwest Greenland. Science of the Total Environment, 295(1), 167-181.

Schaumberg, D. A., Mendes, F., Balaram, M., Dana, M. R., Sparrow, D., & Hu, H. (2004). Accumulated lead exposure and risk of age-related cataract in men. Jama, 292(22), 2750-2754.

Staessen, J. A., Roels, H. A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., & Fagard, R. (1999). Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. The Lancet, 353(9159), 1140-1144.

Tamada, Y., Fukiage, C., Mizutani, K., Yamaguchi, M., Nakamura, Y., Azuma, M., & Shearer, T. R. (2001). Calpain inhibitor, SJA6017, reduces the rate of formation of selenite cataract in rats. Current eye research, 22(4), 280-285.

Theppeang, K., Glass, T. A., Bandeen-Roche, K., Todd, A. C., Rohde, C. A., Links, J. M., & Schwartz, B. S. (2008). Associations of bone mineral density and lead levels in blood, tibia, and patella in urban-dwelling women. Environmental health perspectives, 116(6), 784.

Wagemann, R. (1989). Comparison of heavy metals in two groups of ringed seals (Phoca hispida) from the Canadian Arctic. Canadian Journal of Fisheries and Aquatic Sciences, 46(9), 1558-1563.

Wagemann, R., Snow, N. B., Lutz, A., & Scott, D. P. (1983). Heavy metals in tissues and organs of the narwhal (Monodon monoceros). Canadian Journal of Fisheries and Aquatic Sciences, 40(S2), s206-s214.

Wagemann, R., & Muir, D. C. G. (1984). Concentrations of heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation (Vol. 1279). Canada: Western Region, Department of Fisheries and Oceans.

Wagemann, R., Innes, S., & Richard, P. R. (1996). Overview and regional and temporal differences of heavy metals in Arctic whales and ringed seals in the Canadian Arctic. Science of the Total Environment, 186(1-2), 41-66.

Figure 1: Map showing study area and location of sampled seals

Seal ID	Gender	GPS coordinates	Comments	Distance to mine	Bones		Kidneys					
					Cd	Pb	Cd	Cu	As	Pb	Zn	Se
S 1	Male	72º58'008''N 084º58'44.4"W	Approx. 15 years old caught in Adams Sound	53.7 km	0.0074	0.2327	1.164	4.080	0.135	0.049	15.348	0.956
S2	Male	73º02'27.4''N 83º52'52.1"W	Less than 1 year old caught in Strathcona sound	21km	0.0234	0.4603	2.422	6.867	0.241	0.101	12.972	1.190
S3	Male	73º03'15.8''N 84º07'22.6"W	Sub-adult caught in Strathcona sound	13.8km	0.0247	0.3491	1.798	6.309	0.259	0.107	12.648	1.279
S 4	Female	73º03'15.8''N 84º07'22.6"W	Sub-adult caught at the same location as #3	13km	0.1024	0.3722	5.388	6.030	0.249	0.188	13.692	1.042
S5	Female	73º05'00.1''N 84º20'26.2"W	Adult caught in Strathcona sound	6.8km	0.2517	0.2483	31.524	10.371	0.217	0.136	14.388	1.488
S 6	Female	73º04'11.1''N 84º32'09.4"W	Adult caught very close to the Nanisivik mine area.	0.33km	0.1015	0.3118	26.928	14.727	0.433	0.112	13.932	1.878
S7	Female	73º04'10.2''N 84º32'11.2"W	Sub-adult caught in. Strathcona sound	0.3km	0.1327	0.2517	7.497	6.465	0.263	0.244	14.232	1.249
S 8	Female	73º08'40.0''N 85º26'43.4"W	Adult caught in Admiralty Inlet.	30.65km	0.0433	0.3055	11.238	8.838	0.265	0.232	17.076	1.192

Table 1: Trace metal concentration in bones and renal tissues (µg/g wet wt.) of ringed seals from Nanisivik mine area

Sample ID	BMD(grams/cm2)	BMC(grams)	B Area(cm2)	T Area(cm2)	% Fat
S 1	0.2490	1.1054	4.44	7.16	31.53
S2	0.0721	0.0900	1.25	3.92	21.77
S3	0.0644	0.0785	1.22	3.38	25.24
S 4	0.0669	0.0881	1.32	5.02	17.77
S5	0.1869	0.4748	2.54	5.34	28.58
S 6	0.1361	0.2482	1.82	3.27	43.14
S7	0.1109	0.1787	1.61	3.95	25.42
S 8	0.0755	0.1115	1.48	4.09	28.09

Table 2: Osteodensitometry parameters of Ringed seals from Nanisivik mine and reference site

Locations	Year	As	Cd	Cu	Pb	Se	Zn	Reference
Nanisivik (mine area) - (n=13)	1989	1.49-0.05 (0.36)	222-2.0 (76.7)	178-15.2 (38.7)	1.58-0.02 (0.17)	12-4.92 (7.65)	356-120 (216)	Wagemann (1989)
Nanisivik (reference area) - (n=15)	1989	2.6-0.006 (1.31)	608-2.0 (208)	64.9-12.7	0.2-<0.002	11.5-7.27 (9.35)	441-104 (203)	Wagemann (1989)
North Baffin (n=1)	1976	N/A	65.9	4.18	<0.02	N/A	39.6	Fallis (Unpublished)
North Baffin (n=1)	1977	N/A	27.9	N/A	<0.004	N/A	41.1	Fallis (Unpublished)
Umanak, Western Greenland (n=29)	1979	N/A	146.2-9.01 (37.4)	21.8-4.95 (10.6)	0.48- <0.004 (0.05)	N/A	78-27.9 (46.2)	Johansen et. al (1980)
Alaska, USA (n=17)	2001	0.14	26.01	7.84	0.01	2.51	46.36	Woshner et. al (2001) **

Table 3: Baseline renal concentration of trace metals (μg/g dry weight) in ringed seals from Nanisivik and similar locations

NA: Not applicable; ** μg/g wet weight

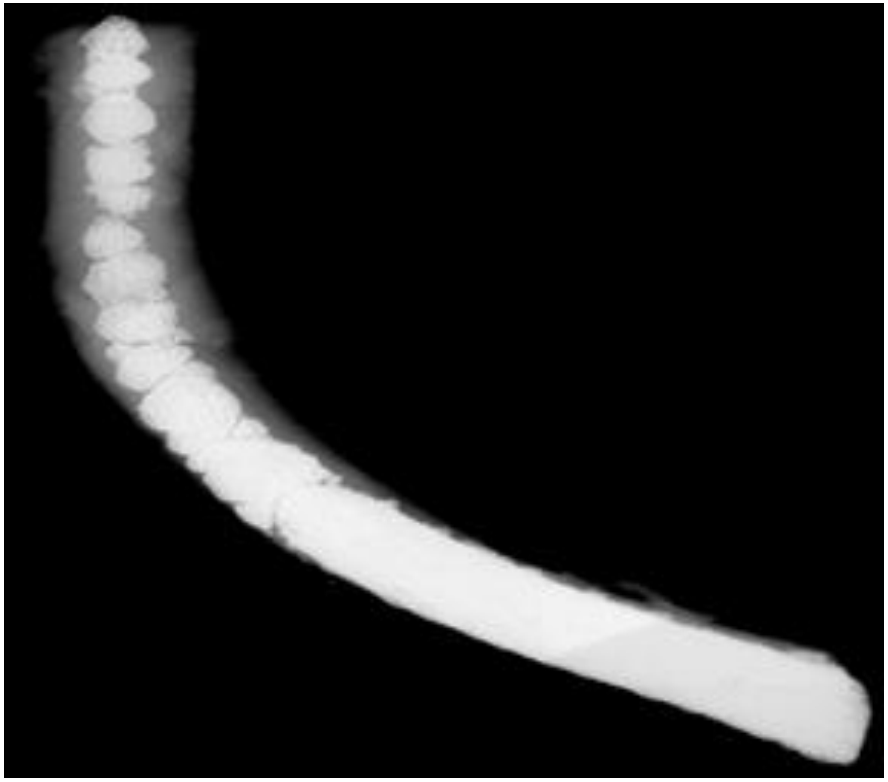


Figure 2: Bone X-ray of seal 1 caught from Adam Sound

Fig 3: Representative bone X-ray of seals 2, 3, 4, 7 from mine area

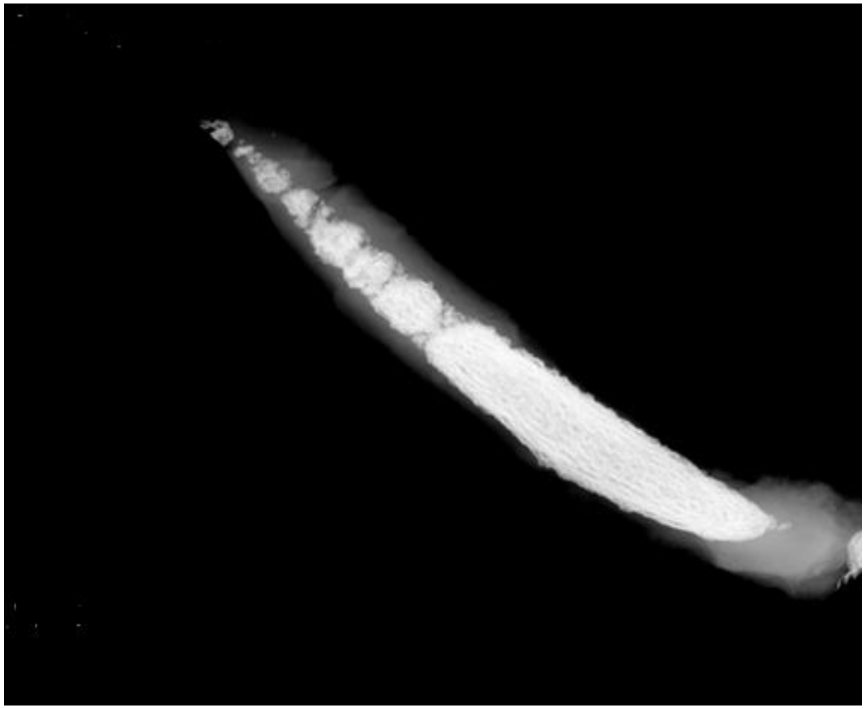


Figure 4: Bone X-ray of seal 5 from mine area

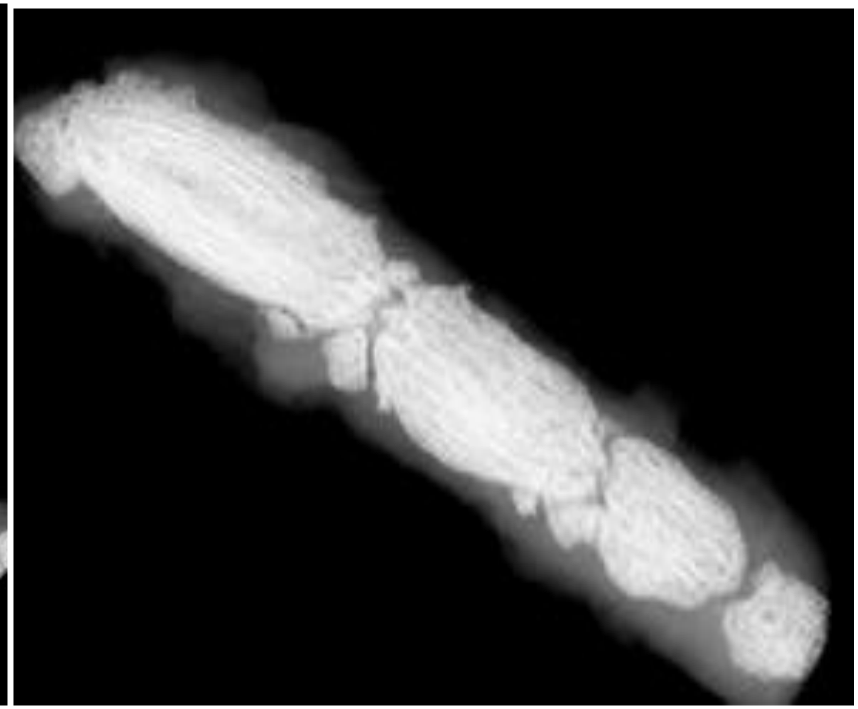


Figure 5: Bone X-ray of seal 6 from mine area

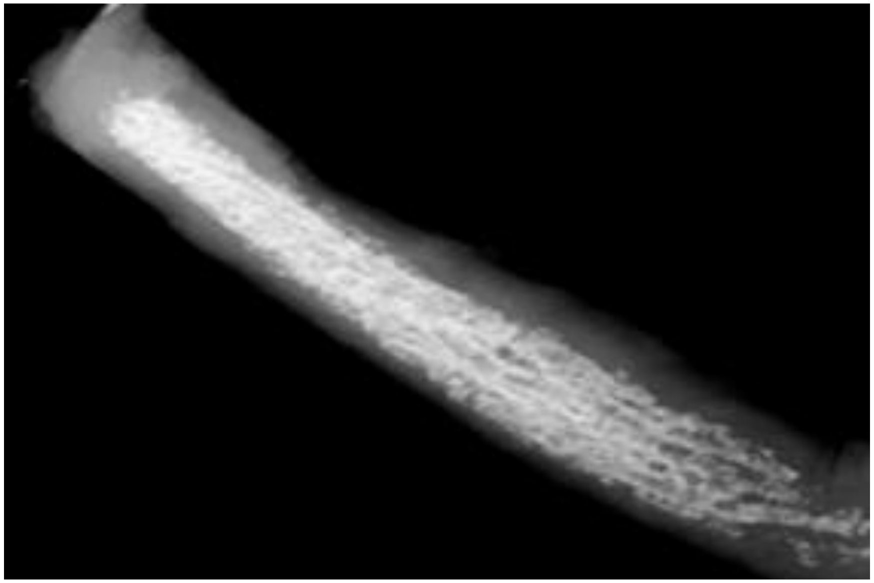
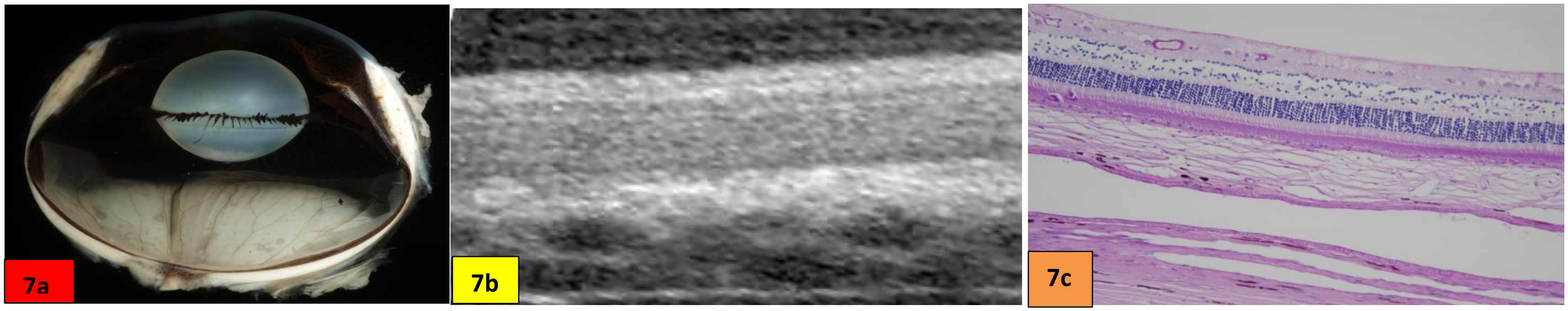
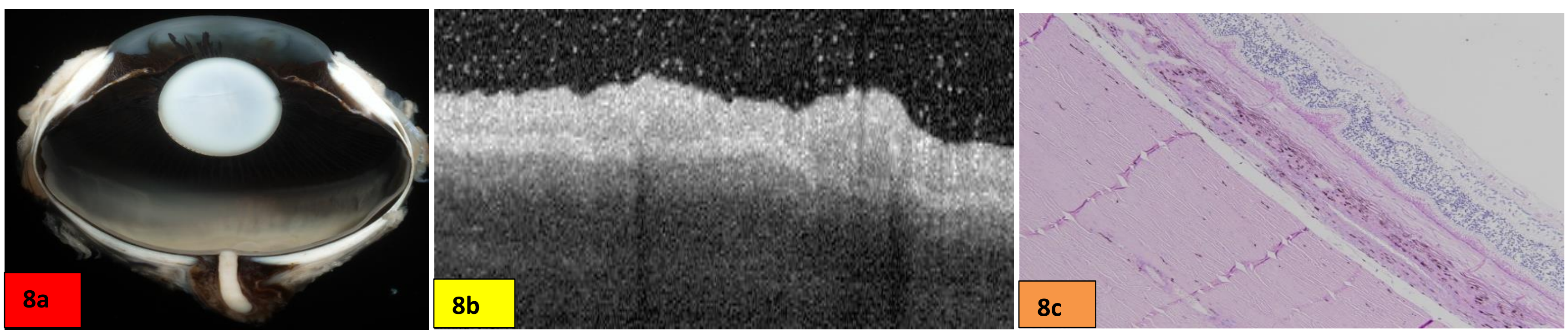


Fig 6: Bone X-ray of seal 8 from mine area

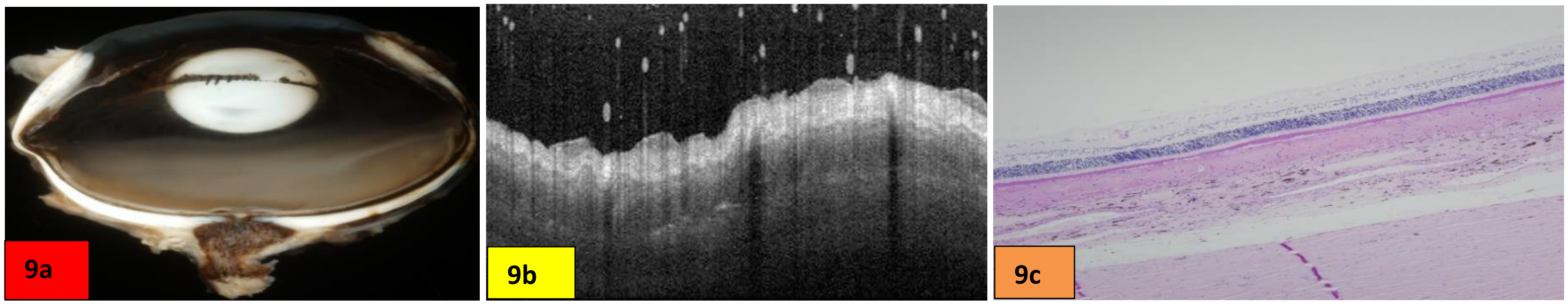
Seal 1 (Control). Gross measurements (7a): Globe 42mm X40mm; Cornea 30mm X30mm. Focal retinal detachment noted likely due to artifact of fixation and sectioning, in addition to pale grey retinal patches which are visible on gross section but not on histology examination, likely fixation artifact. Optical Coherence Tomography (7b): All layers present in retina, no significant abnormalities noted. Debris artifact from autolysis Histology (7c): No abnormalities noted in cornea or sclera, uvea, retina retinal pigment epithelium or lens and vitreous. Fixation was noted to be good on this eye.



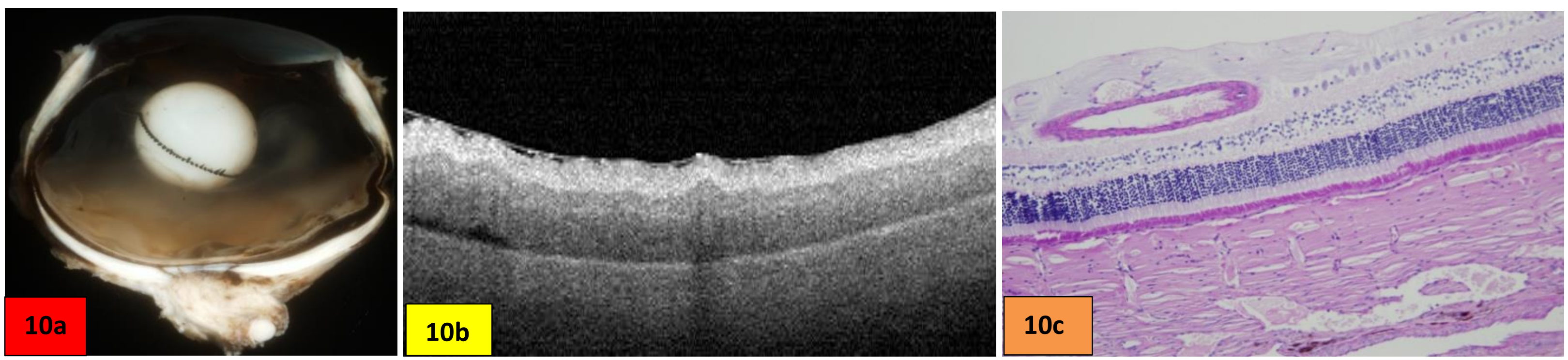
Seal 4. Gross measurements (8a): Globe 36mm X38mm; Cornea28mm X25mm. Note the brown strands in anterior chamber, on histologic examination it appears to be pars plicata (ciliary processes) as well as iris atrophy. Optical coherence tomography (8b): No abnormalities noted as all retinal layers present. Wrinkling and debris artifact due to autolysis. Histology (8c). No abnormalities noted on H&E PAS LFB on cornea and sclera, uvea, retina and retinal pigment epithelium or lens and vitreous. However, fixation was poor as noted on photoreceptors.

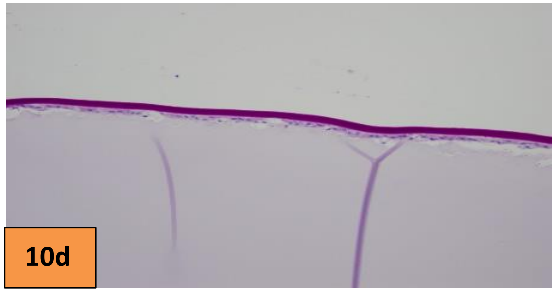


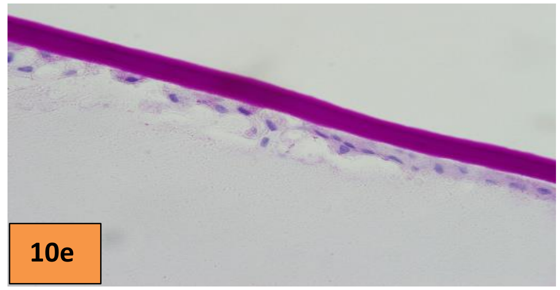
Seal 6. Gross measurements (9a): Globe 36mmX35mm; Cornea 27mmX25mm. Gross findings include focal peripapillary retinal detachments most likely artefactual, no abnormalities noted on this specimen. Optical coherence tomography (9b): All retinal layers are present. Vitreous detachment from the inner retina is noted. Histology (9c): Peripapillary subretinal bleed likely agonal given no RPE hypertrophy or outer retinal atrophy, matches the gross photo and the OCT. Cornea, sclera, uvea and lens and vitreous are normal.

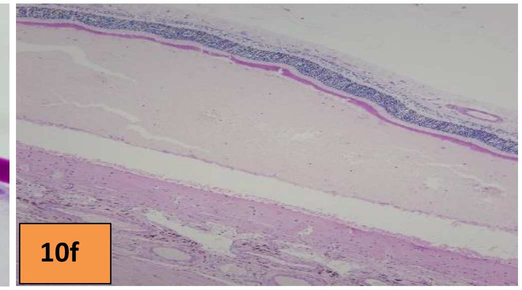


Seal 7. Gross measurements (10a): Gross globe measurements 37mmX 37mm; Cornea 27mmX25mm. Gross findings multiple subretinal red fluid accumulations, including agonal retinal detachments. Optical coherence tomography (10b): All retinal layers present. Artifactual detachment of the retina due to autolysis. Histology (10c-f): No abnormal findings in Cornea sclera, uvea or vitreous, however, focal subcapsular cataracts present under anterior lens capsule and focal subretinal hemorrhage likely agonal. Focal lens epithelial metaplasia and superficial cortical liquefaction were noted in this seal

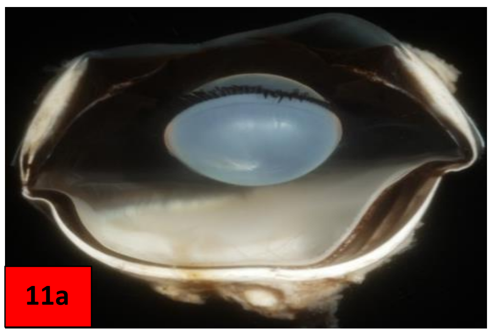


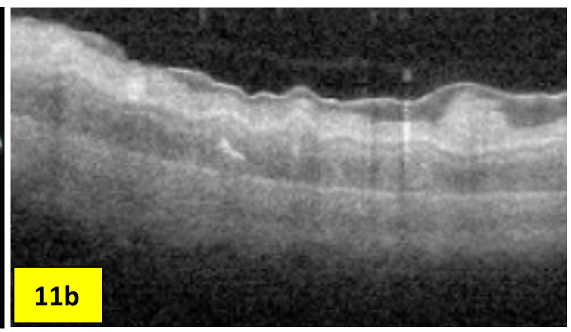


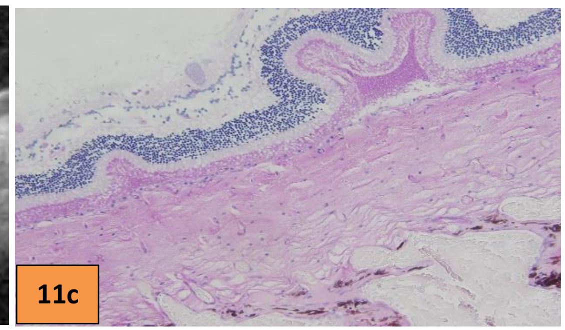




Seal 8. Gross measurements (11a): Gross globe measurements 38mmX35mm; Cornea 27mmX26mm. Gross artificial retinal separations and what appears to be a posterior subcapsular cataract no other abnormalities. Optical coherence tomography (11b): All retinal layers intact. Minor vitreous detachment. Debris artifact from autolysis. Histology (11c): Generally, a poorly fixed specimen with autolytic change note photoreceptors in this histology section. No abnormalities in the cornea or sclera, uvea retina, retinal pigment epithelium and no cataracts detected histologically.







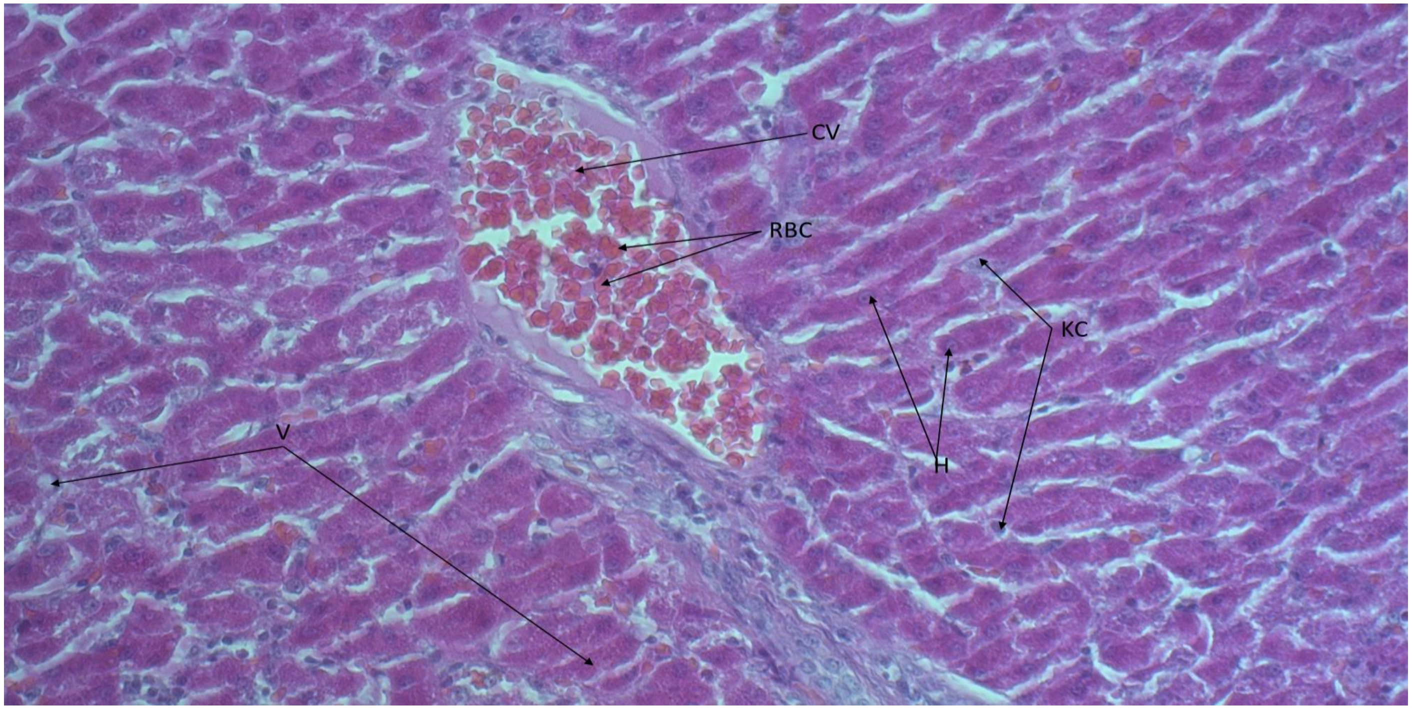


Figure 12: Liver pathology of seal 1: Age-related hepatic changes include steatosis, mild changes in normal appearance of the central vein, hepatocyctes and sinusoids, in addition to portal expansion with fibrous septa.
(CV: Central Vein. RBC: Red Blood Cells: KC: Kupffers Cells. V: Vacuolization (Fat deposition).