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Do mercury, selenium, cadmium and zinc cause oxidative stress in common eiders (Somateria mollissima) from Svalbard?

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Summary

The levels of mercury have shown to increase in the Arctic environment as a cause of human activities. Few studies have examined the antioxidant system as a response to heavy metals in Arctic seabirds. Levels of the elements mercury (Hg), selenium (Se), cadmium (Cd) and zinc (Zn) were analyzed in hepatic tissues of female common eiders (*Somateria mollissima)* collected in July 2008 and 2009 from Kongsfjorden (KF) and Liefdefjorden (LF), Svalbard. The molar ratio of Hg relative to Se (ratio Hg:Se) was also calculated. The two fjord systems are dominated by inflow of different water masses (Atlantic vs. Arctic), which are suggested to vary in the abundance of contaminants. As an indicator of heavy metal exposure, antioxidants in the defense against reactive oxygen species (ROS) were analyzed. These were total reduced glutathione (tGSH), oxidized glutathione (GSSG) and their ratio (tGSH:GSSG), together with its unique enzymes glutathione reductase (GR) and glutathione peroxidase (GPx). Other proteins quantified included metallothionein (MT) and catalase (CAT). As a measure of oxidative damage the levels of lipid peroxidation (TBARS) were analyzed.

Se, ratio Hg:Se, GSSG, ratio tGSH:GSSG, GPx and CAT were found to be significantly different between the locations; however this was only in 2008. No parameter was found significantly different between the fjords in 2009. Differences were mainly thought to be caused by seasonal changes between the locations and years rather than various inflows of Atlantic and Arctic waters in the two fjords. The common eiders seems to be less affected by the examined elements compared to seabirds at higher trophic position, except with respect to Se, which were found in relative high concentrations. In the general linear models (GLM) Hg was a strong predictor of levels of GR and MT. In addition, Se was found to correlate with GPx and Zn correlated strongly with MT. However, the metals revealed fewer relationships with enzyme activity compared to previous studies. The present study suggests that several physiological and ecological factors are more important than element burden in explaining differences in status of the antioxidant defense system. This especially involves the female common eider which goes through a prolonged period of incubation fast.

Table of contents

Acknowledgements......I
Summary......II
Table of contents......III

1. Introduction......1

2. Material and methods......8
2.1 Field work......8
2.2 Metal quantification......8
Freeze drying......8
Acid digestion and metal quantification of the samples.......9
2.3 Protein quantification......9
Tissue homogenization......9
Glutathione (tGSH/GSSG) assay......10
Glutathione peroxidase (GPx) assay......11
Glutathione reductase (GR) assay.......12
Catalase (CAT) quantification.......12
Metallothionein (MT) assay.......13
Total protein quantification.......14
2.4 Lipid peroxidation (TBARS)......14
2.5 Statistics......16

3. Results......17
3.1 Metals......17
3.2 Molar ratio Hg:Se......18
3.3 Antioxidant and enzyme activity......19
The glutathione system......19
Catalase.......23
Metallothionein.......23
3.4 Lipid peroxidation (TBARS)......23
3.5 Correlated variables......25

4. Discussion......27
4.1 Levels of Hg, Cd, Zn and geographical differences......27
4.2 Se and the molar ratio Hg:Se......32
4.3 The antioxidant system......33
The glutathione system......34
Catalase......37
Metallothionein......38
Lipid peroxidation (TBARS)......38
4.4 Physiological and ecological factors influencing the antioxidant system......39

5. Conclusion......42
References......43

1. Introduction

The Arctic is considered a clean and unaffected environment. However, there are great concerns about the increasing levels of mercury (Hg) in the biota despite few anthropogenic sources in the Arctic regions. Hg exists naturally in the environment, but the levels are increasing as a result of human activities. The emissions are thought of being deposited to environmental compartments at significantly greater scale than natural processes (AMAP, 2005). Release of Hg into the environment is a result of volcanic activity, mining, coal burning, incineration of waste and industrial production. Even though a huge effort has been done to reduce the emissions, the amounts released are increasing as a consequence of enhanced industrial activity in Asia, mainly China and India (Gabrielsen and Sydnes, 2009).

Hg is transported to the Arctic via ocean currents, riverine and atmospheric transport, and migratory animals (AMAP, 2005). The long-range atmospheric transport and Arctic mercury depletion events (AMDE) are considered the most important source of Hg found in the Arctic (Arya et al., 2004). Through AMDE, gaseous elemental mercury (GEM) becomes oxidized to reactive gaseous mercury (RGM, Hg^{2+}) by reactive halogens and becomes deposited to snow and ice. The Hg^{2+} is not very bioavailable. However, bacteria at the snow surface convert the species to methylmercury (MeHg) as it enters the food chain (Steffen et al., 2008). The process is mostly limited to the arctic springtime and the highest levels of MeHg are found in the snowpack in May. The influx of Hg in to the Arctic is therefore highly seasonal, depending on the variations of riverine and atmospheric transport. By these global processes the Arctic has lately been recognized as an important sink for Hg, which is also confirmed by the recent increase in accumulation in Arctic marine biota (AMAP, 2005).

Hg exists in several inorganic and organic forms with varying degrees of stability and toxicity. It is volatile, undergoes biological transformations and bioaccumulates. The species MeHg is known to be the most bioavailable and toxic to wildlife (Wolfe et al., 1998). Hg exerts a broad spectre of toxicities including loss of coordination, reduction in the field of vision, numbness of the extremities, and decline in mental activity and awareness. Reproductive toxicities involve reduced hatching success and number of laid eggs (Thompson, 1996). The most toxic form, MeHg has the ability to cross biological barriers such as the blood-brain and placenta (Liu et al.,

2008). Seabirds are widely used to monitor trace element levels due to their wide distribution and high position in the food chain (Furness, 1997). Generally seabirds exhibit higher levels of Hg than terrestrial birds since the marine food chain tend to have higher levels of Hg (Scheuhammer, 1987). Thus seabirds are usually more tolerate to high concentrations of Hg before toxic effects occur (Thompson, 1996). Through the processes of bioaccumulation contaminant levels tend to be higher with increasing trophic position in the food chain (Braune et al., 2005; Jæger et al., 2009). Excretion of Hg is limited, but involves pathways such as moulted feathers, eggs and excretion or egestion (Lewis and Furness, 1991). The half life of Hg in seabirds is estimated to be 60 days. The intestinal absorption of inorganic Hg is low compared to MeHg which is nearly complete (Wolfe et al., 1998).

The present study also includes the elements cadmium (Cd), selenium (Se), and zinc (Zn). Similar to Hg, Cd seems to have to no biological function, and these metals have therefore received most attention (Gabrielsen and Sydnes, 2009). Cd usually occurs associated with Zn, and they are both released in to the environment by many of the same mechanisms as described for Hg. Cd bioaccumulates mainly in the liver and kidney, and have an extremely strong affinity to bind to sulfhydryl groups (Nordheim and Nordheim, 2002).

Se and Zn are categorized as essential elements, as they both have multiple roles in biological systems. In animal tissues the main form of Se is selenocysteine (SeCys) (Himeno and Imura, 2002). The nutritional status of Se has shown to be an important factor in modulating the toxicities for other elements, such as Hg (Khan and Wang, 2009). Regardless of being essential in biological systems, Se can exert toxic properties at elevated levels (Himeno and Imura, 2002). Zn participates in a broad array of metabolic processes, and is usually tightly correlated with other essential elements. In fact, Zn deficiency is more common than Zn toxicity (Liu et al. 2008).

Metals have the ability to induce the formation of free radicals, which can induce various modifications to DNA bases, increase level of lipid peroxidation and enhance calcium and sulfhydryl homeostasis (Valko et al., 2005). Free radicals are formed as a product of normal aerobic metabolism, but can be produced at elevated levels under influence of toxic substances,

such as metals, or physical states that disrupt the normal balance (Halliwell and Gutteridge, 2007). Oxidative stress is a condition where there is an imbalance between the formation of oxidants and the cellular antioxidant defence system (Sies, 1997). As a result of aerobic respiration, molecular oxygen in an organism will be reduced to H_2O, a reduction involving four electrons that are sequentially added. During the process the electron intermediates are released at low amounts and include the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (*OH). These radicals are known as reactive oxygen species (ROS) (Stohs and Bagchi, 1995). By having an unpaired electron they tend to be extremely reactive in biological systems, with the hydroxyl radical being the most reactive (Fridovich, 1998). Even though metals exert different degrees of toxicity, which is related to parameters such as solubility, absorbability, transport and chemical reactivity, they all exhibit the same basic mechanisms producing ROS (Stohs and Bagchi, 1995). The metals can be divided into redox-active metals, which includes among others iron (Fe), copper (Cu) and chromium (Cr), and redox-inactive metals such as Hg, Cd, Zn and lead (Pb) (Ercal et al., 2002). The redox-active metals, also known as transition metals, induce their formation of ROS through redox cycling where metal ions accepts an electron from a cofactor such as NADPH, and in the presence of O_2 the unpaired electron of the ion will react and yield O_2^- (Valko et al., 2005). A transition metal ion can also react with H_2O_2 and generate the *OH radical and an oxidized metal ion. The efficiency of the different metals to form ROS depends mostly on their oxidation/reduction potential (Leonard et al., 2004*)*. Redox-inactive metals binds strongly to sulfhydryl containing endogenous antioxidants like glutathione (GSH), resulting in depleted levels of this important molecule, hence increasing the level of oxidative stress (Koivula and Eeva., 2010). Hg is classified as a redox-inactive metal even though oxidised Hg has ability for redox cycling (Valko et al., 2005).

The antioxidant system is recognized by its ability to scavenge free radicals and reduce oxidation and damage by ROS in biological systems (Sies, 1997). A basic overview of the system is shown in Figure 1. The most important antioxidants are GSH, flavonoids, vitamin C, uric acid and carotenoids. The system also includes antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-s-transferase (GST) (Halliwell and Guttridge, 2007). These essential enzymatic and non-enzymatic antioxidants maintain the redox balance in tissue of the organism (Monteiro et al., 2009), and are

suggested biomarkers of oxidative stress (Koivula and Eeva, 2010). CAT is responsible for the reduction of H_2O_2 succeeded by the formation of H_2O, whereas SOD uses O_2^- as substrate in the generation of H_2O_2 (Fridovich, 1998) (Fig. 1). Glutathione exists in reduced (GSH) or in oxidized (GSSG) state, and it is the most important low-molecular-mass thiol in plants and animals (Halliwell and Guttridge, 2007). Total glutathione (tGSH) is the total amount of both bound and free GSH (Sies, 1997). Similar to CAT enzymes, GPx is involved in the reduction of H_2O_2 where the electron is transferred to the GSH, functioning as a co-factor, yielding two parts GSSG (Halliwell and Guttridge, 2007. Se is an important cofactor provided in the GPx molecule. GR on the other hand, reduces GSSG back to its original state (GSH) making use of NADPH as reductant (Fridovich, 1998) (Fig. 1).

Another important molecule is metallothionein (MT), a cystein rich and low molecular-mass protein known to detoxify, scavenge and bind to metals by its sulfhydryl groups. Its properties as a metal chelating agent are assumed to protect organisms from ROS (Vanparys et al., 2008). It is known to bind to Zn, Cd, Cu, Hg and Ag in increasing order of affinity. Exposure of metals induces the synthesis of thionein in the liver, and to a lesser extent, in the kidney. The process continues until most of the exogenous metal has been bound (Brady, 1982).

Under conditions of compromised antioxidant status, one consequence can be lipid peroxidation as a relevant mechanism for cell injury (Halliwell and Gutteridge, 2007). Similar to proteins, carbohydrates and nucleic acids, lipids are targets for ROS which become oxidized to various detectable reactive end products like the thiobarbituric acid (TBA) and malondialdehyde (MDA) known as thiobarbituric acid reactive substances (TBARS) (Niki, 2009).

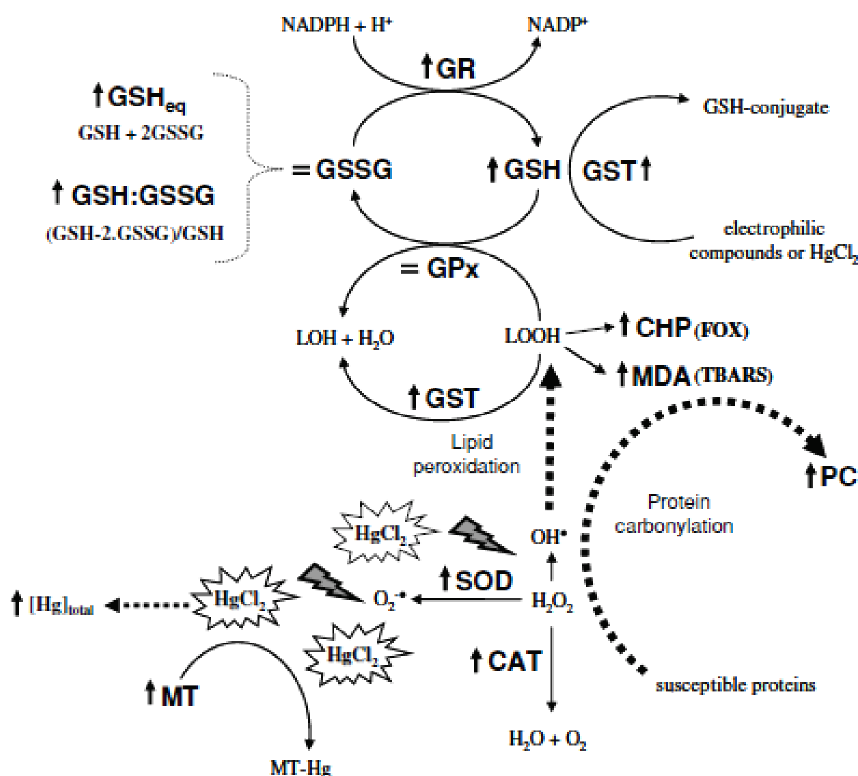


Figure 1: Overview of the antioxidant defense system (Monteiro et al., 2009). Refer to the text for details.

The target species used in the present study was common eider (*Somateria mollissima)* (Fig. 2). The common eider is a seabird species belonging to the duck family that has a circumpolar distribution, breeding in the arctic and boreal zones of the northern hemisphere (Anker-Nilssen et al., 2000). An adult eider is between 50-71 cm long, weighting approximately 1200-2800 gram. The highest known age of an eider duck is 22 years. In Europe it breeds along the coast from north of France to Russia, in addition to the arctic areas like Svalbard, Greenland, Iceland and the Franz Josef lands. The Svalbard population is estimated to 8000-14000 individuals and winters along the Norwegian coast and on Iceland (Kovacs and Lydersen, 2006). The common eider feed at the lower levels of the food chain

*Figure 2: Female eider duck (*Somateria mollissima*) (photo: Ida Beathe Øverjordet).*

having a diet mostly consisting of benthic organisms like mussels and amphipods. They breed in colonies, where the nest being located on the ground, usually on islands isolated from mammalian predators. Before the incubation period the female gains relatively huge fat reserves in order to cope with the incubation fast, which last for 24-26 days (Gabrielsen et al., 1991). The common eider population has been stable in the Barents region (Gabrielsen and Sydnes, 2009), whereas the population in North-America has declined (Wayland et al., 2002).

Alterations in the Arctic environment induced by the climate changes are thought to affect contaminant pathways, since contaminants enter the Arctic trough global systems like wind and ocean currents (Macdonald et al., 2005). The changes are complicated to predict because of the uncertainties regarding the physics of the atmosphere, the Arctic ecosystems and effects of the contaminants and their interactions (AMAP, 2005). The two sampling areas of common eiders, Kongsfjorden (KF) and Liefdefjorden (LF) (Fig. 3) are characterized by their influence on Atlantic (warm) and Arctic (cold) water masses, respectively. The western part of Svalbard is dominated by the northernmost extension of the warm Gulf Stream, carrying warm and salty waters (Atlantic water). In contrast, the arctic water is colder and characterized by less salty melt waters (Svendsen et al., 2002). KF is a glacial fjord located on the west coast of Svalbard (79°N, 12°E) (Fig. 3. This fjord represents an area between the Atlantic and Arctic bio-geographical zones (Hop et al., 2002) and has therefore been extensively used as a reference site measuring impacts of climate changes and transport of contaminants. During the last five winters KF has been ice free and largely dominated by Atlantic water masses (Hallanger, 2010). The biodiversity and animal populations in the fjord are strongly structured by the different physical factors, in terms of Atlantic and glacial influences, that affect the fjord from both ends (Hop et al., 2002). LF is located at the northern part of Svalbard (79°N, 13°E) (Fig. 3), and is a fjord-branch of Woodfjorden. Few studies on physical oceanography and biodiversity have been provided from this area. The fjord was selected as Arctic reference site due to its position (Hallanger, 2010). Water sampling by CTD (Conductivity, Temperature and Density) during the fieldwork confirmed that the fjord was dominated by Arctic waters.

Figure 3: Overview over the north-western part of the island of Spitsbergen, Svalbard. The sample areas, Kongsfjorden and Liefdefjorden, are labeled with red circles. (Norsk polarinstitutt, 2010).

The present study is a part of the ongoing project COPOL (Contaminants in Polar Regions) where the aim is to study uptake and effects of environmental contaminants in Arctic food chains and the influence of climate changes on transport and uptake of contaminants. COPOL is a project financed by the International Polar Year (2007-2009).

The aim of this master thesis has been to examine the distribution of metals, mainly Hg, and levels of proteins involved in the cellular defence against metal-mediated oxidative stress in common eiders, breeding in two fjords exposed to warm (KF) and cold (LF) water masses. The study was conducted during two consecutive years in order to study the annual influence of oxidative stress. In the present study I also studied the relationships between the investigated metals (Hg, Se, Cd and Zn), and the antioxidants/antioxidant enzymes (tGSH/GSSG, GPx, GR, CAT and MT), together with the biochemical responses of lipid peroxidation (TBARS).

2. Material and methods

2.1 Field work

The field work at Svalbard was carried out in 2008 and 2009 from the 12th to 28th of July. Stationed at Ny-Ålesund and on RV Lance. The sampling included a total number of 40 eider ducks taken from KF (n=20), near Ny-Ålesund, and from LF (n=20). The birds were shot by shotgun from close distance (Fig. 4). Liver were cut out in field and put in liquid nitrogen to preserve the enzyme activity in the tissue. Field measurements included sex, wing length (WL,mm), length of tarsus (TL, mm), beaklenghts (BK, mm), length of head (HL, mm) and weight (g). The rest of the dissection was carried out in the laboratory of Ny-Ålesund. Samples were put in plastic tubes, and placed in the freezer (-80). The liver samples, for the measurement of the enzyme activity, were all dissected from the bottom right side of the liver.

Figure 4: Eider duck hunting along a migratory path (photo: Halvor Saunes).

2.2 Metal quantification

The process of metal analysis involved freeze drying, digestion and metal quantification of the liver tissue. The final analysis of metal content was performed by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS).

Freeze drying

Frozen samples were cut into pieces (0.6-1 g) and put in acid washed plastic tubes. All handling of the samples were done on a chopping board wrapped in plastic foil, and the scalpel blade was changed before every new sample. All samples were randomized and given a new number according to analyze sequence. The cap of each tube was screwed half open to have the sample in equilibrium with the air of the surroundings of the capsule of the machine. Samples were freeze dried (CIT, Leybold-Heraeus, Köln, Germany) until the content was completely dry (20 hours).

Acid digestion and metal quantification of the samples

Samples were digested before running the HR-ICP-MS. This was done using UltraClave, a high pressure microwave system (Milestone, Shelton, CT, USA) over 2 hours in temperature up to 250°C and pressure of 160 bar. Samples (0.1-1.5 g), Scanpure nitric acid (HNO_3, 14.44 M, 4.2±0.2 g) and distilled water (Milli-Q H_2O, 3±0.2 g) were added to acid washed Teflon tubes designed for the UltraClave. Three blank samples together with six samples of reference material consisting of oyster (NIST 1566b) and chicken tissue (GBW-10018) were included. After digestion, the samples were diluted with 60 mL ion exchanged MQ-water with a final concentration at 0.6 M.

The total content of metals was measured by HR-ICP-MS (Thermo Electronic Corporation, Waltham, MA, USA) at the Department of Chemistry at NTNU. The order of the samples was randomized. ICP-MS is an extremely precise instrument for quantification of trace elements. The process involves mass spectrometry (MS) of the elements atomized in inductively coupled plasma. A torch generates the plasma of the samples, which becomes decomposed to natural elements and ionized. The MS separates the ions by mass and charge through the mass filter. The level of the respective element is calculated against a similar certified metal in the machine (Montaser, 1998). All operational parameters are available in appendix A.

2.3 Protein quantification

Tissue homogenization

Samples of liver tissue from all individuals (n=40) were cut in pieces, weighted (0.12-0.18 g) and added a 1:9 weight ratio triz-HCl buffer (20 mM, pH=7.4). The homogenization was performed by a Potter-Elvhjelm's homogenizer. Samples designated for the TBARS assay were added phosphate buffer saline (PBS) combined with Butylated hydroxytoluene (BHT) instead of triz-HCl (See TBARS assay). The samples were centrifuged by an Eppendorf table centrifuge at 1000 G for 10 minutes. The liver tissue supernatant was dispersed to new tubes ready to be used for the respective enzyme assay. All homogenization were performed on ice to

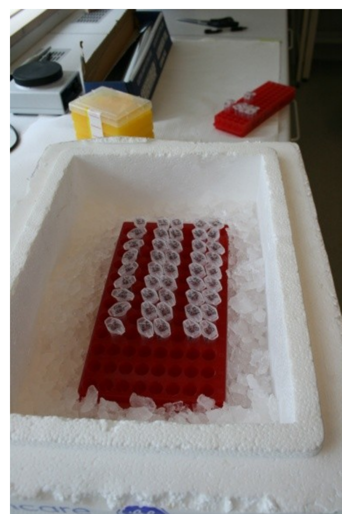


Figure 5:Homogenizated samples on ice (photo: Halvor Saunes).

prevent degradation of the enzyme activity (Fig. 5). All analyzes required 100 µl, except the CAT assay which only needed 10µl of sample. After homogenization, the samples were stored on freezer (-80). All chemicals used in the following protein analysis were produced by Sigma-Aldrich, St. Louis, MO, USA, if nothing else is mentioned.

Glutathione assay (tGSH/GSSG)

The tGSH/GSSG assay was based on the methods according to Høiberg (2006), Baker et al. (1990) and Vandeputte et al. (1994). Standards of GSH (500 µM) and GSSG (100 µM) stock solutions were made by dilution of MQ-water. The GSSG stock was added 2-vinylpyridine (VP, C_7H_7N, 0.35M) and triethanolamine (TEA,$C_6H_{15}NO_3$, 7 mM), and mixed for 45 minutes for derivitization and neutralization of GSSG. Both solutions were transferred to 30 different 1.5 ml tubes and placed in the freezer (-80), one tube was used for each plate. The buffer (pH=7.5) consisted of dipotassium phosphate (K_2HPO_4, 1M) and ethylenediaminetetraacetic acid (EDTA, 1mM) ($C_{10}H_{16}N_2O_8$, Fluka chemie, Buchs, Switzerland) and was made in quantity and stored at 4 ^{o}C. EDTA was used to bind the potassium, thus preventing degradation of proteins. A solution containing the dipotassium phosphate buffer (1M), 5.5'-Dithio-Bis 2-Nitrobenzoic Acid (DTNB, 3.23 mM) and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH, 0.24mM) was made daily, and used in all wells to initiate the enzymatic reaction. To avoid interference with sulfhydryl groups, proteins were precipitated by diluting the sample 1:11 with 5% sulfosalicylic acid (SSA, $C_7H_6O_6S$). The samples were then stored on ice for 15 minutes followed by a centrifugation at 1000 G over 5 minutes (Microcentrifuge, eppendorf, Germany). Samples designated for the GSSG assay were added VP and TEA, and mixed for 45 minutes. A standard curve was made for each plate prepared from the pre-made GSH (Tab. 1) and GSSG (Tab. 2) stock solutions.

Table 1: Dilutions scheme for the standard curve in the tGSH assay. These contained 6 known concentrations of tGSH between 0-50 µM used to generate a linear slope for the determination of tGSH in the samples.

Tube	tGSH (µl)	5% SSA (µl)	tGSH (µM)
A	0	1000	0
B	3	1497	1
C	10	990	5
D	10	490	10
E	10	240	20
F	10	132	35
G	10	90	50

Table 2: Concentration of GSSG in the standards. These contained 7 known concentrations of GSSG between 0-37.5 µM. The slope created from the standards represents the average increase in the concentration and was used to calculate the concentration in each respective sample.

Tube	GSSG (µl)	5% SSA (µl)	GSSG (µM)
A	0	1000	0
B	10	1590	0.63
C	10	790	1.25
D	20	438	4.37
E	20	300	6.26
F	50	350	12.50
G	50	150	25.00
H	100	167	37.50

Seconds before reading the absorbance, a GR solution was added, thus initiating the enzymatic recycling process. The sulfhydryl groups of GSH react with DTNB oxidising the molecule to GSSG. Simultaneously GSSG is reduced back to GSH by GR and NADPH. The reduction of DNTB by GSH creates a yellow dye, 5-thio-2-nitrobenzoic acid (TNB), which is proportional to the concentration of tGSH or GSSG. The plate reader was run at 405 nm over two minutes, which included 18 readings (Elx 808 IU Ultra Microplate Reader, Biotek Intstuments Inc, Winooski, VF, USA). The linear standard curve together with the total dilution factor was used to calculate the amount of tGSH and GSSG in the samples. Samples ranged between 4.03- 40.43 µM tGSH (Tab. 1), and 3.52-25.28 µM GSSG on the standard curve (Tab. 2). The ratio between tGSH and GSSG (tGSH:GSSG) was calculated using formula 1 (Oxford biomedical research, 2009). One molecule of tGSH equals two molecules of GSSG.

$$Ratio\ tGSH\!:GSSG = tGSH - \frac{2GSSG}{GSSG} \quad (1)$$

Glutathione peroxidase (GPx) assay

The GPx assay was conducted according to the Cayman chemicals protocol (2010), based on the methods by Paglia and Valentin (1967). The whole assay, including the chemicals, was manufactured by Cayman chemicals company, Ann Arbor, MI, USA. The measurement of GPx is based on the indirectly coupled reaction of GR. Oxidised glutathione (GSSG) is generated through the reduction of hydroperoxide by GPx, and is recycled to its reduced state (GSH) by GR and NADPH. The measurement of the oxidation of NADPH to $NADP^+$ is followed by a

decrease in the absorbance. Since GPx is the rate limiting substrate the decrease in absorbance will be directly proportional to the amount of GPx activity. Samples were added 1:6 times buffer (pH=7.6) containing triz-HCl (500 mM), EDTA (50 mM) and bovine serum albumin (BSA, 10mg/ml). The buffer containing triz-HCl and EDTA, was added to a microtiter plate together with a solution of NADPH, GSH and GR. The samples were added as triplets, and cumene hydroperoxide ($C_6H_5C(CH_3)_2OOH$) was added to initiate the final reaction. Blank and GPx control were included in order to calculate the activity. The absorbance of the reaction kinetics was read at 340nm (Ascent, Multiscan, Labsystems) every minute over a time period of 5 minutes. The five readings formed the basis of the linear slope which estimated the total amount of GPx, multiplied by the dilution factor (6).

Glutathione reductase (GR) assay

The GR-assay was performed according to the commercial kit booklet by Cayman chemicals, (2008), based on the principles to Carlberg and Mannerheim (1985). The method measures the kinetics of the oxidation of NADPH by GR. The oxidation of NADPH to $NADP^+$ is followed by a decrease in absorbance and is proportional to the amount of GR in the sample. Hence, one unit of GR activity is defined as the quantity of enzyme that catalyzes the reaction of 1 µmol of NADPH. The samples were diluted 1:6 by buffer (pH=7.5) containing potassium phosphate (K_2HPO_4, 50 mM) and, EDTA ($C_{10}H_{16}N_2O_8$, 1mM,)(Fluka chemie, Buchs, Switzerland). The assay was conducted on a microtiter plate, each sample run as triplets. On the plate samples were added GSSG and potassium phosphate buffer containing BSA (10mg/ml). The plate included 3 blanks and 3 positive GR controls. The reaction was initiated by the addition of NADPH. The plate was read at 340 nm (Ascent, Multiscan, Labsystems) over a time period of 5 minutes, having one reading per minute. The values of the first minute were not used in the calculations because of the unstable reaction kinetics. The decrease in absorbance was plotted as a function of time (min) which created a linear slope explaining the rate of NADPH oxidized per minute multiplied by the dilution factor (6).

Catalase (CAT) assay

The CAT assay was performed according to the Cayman chemicals kit (2009a), a method described by Johansson and Borg (1988). The assay was performed on a microtiter plate adding

the samples and standards as triplets. The samples were added methanol (CH_3OH, E.Merck, Germany) and hydrogenperoxide (H_2O_2, 35 mM), which resulted in the formation of formaldehyde (CH_2O) through the CAT enzyme. This reaction continued over 20 minutes and was followed by an addition of potassium hydroxide (KOH, 10 M) terminating the reaction. Formaldehyde were detected by adding purpald ($C_2H_6N_6S$, 5mg/mL HCl), a reaction that created purple colour. The staining was stopped by adding potassiumperiodate (KIO_4, 65.2 mM). The standards consisted of known concentration of formaldehyde (CH_2O, 4.25 mM) (Tab. 3). The intensity of dye represents the concentration of formaldehyde and was relative to the amount of CAT in each sample. The absorbance was read at 540 nm on a plate reader (Elx 808 IU Ultra Microplate Reader, Biotek Intstuments Inc, Winooski, VF, USA), and the activity of CAT in each sample was determined by relating the absorbance to the standard curve multiplied by the total dilution factor (70).

Table 3: Standards for the CAT assay, describing the amount of formaldehyde, sample buffer and total concentration of formaldehyde in each tube.

Tube	Formaldehyde (µl)	Sample buffer (µl)	Formaldeyde concentration (µM)
A	0	1000	0
B	25	975	8.854
C	50	950	17.708
D	100	900	35.426
E	150	850	53.125
F	200	800	70.833
G	250	750	88.542

Metallothionein (MT)

The MT quantification was performed using the Cd-chelex method described by Bartsch et al. (1990). The assay is based on Cd-saturation of the MT molecule. Known concentrations of both radioactive $^{109}Cd^{2+}$ and Cd^{2+} were added to the samples. The Cd has high affinity for binding to the MT-molecule, and replaces other metals bound to MT. Acetonitrile (C_2H_3N, Merck KGaA, Darmstadt, Germany) was used to precipitate larger proteins. Two fixed tubes were designated blank and total. These were added Buffer-A containing triz-HCl (10 mM) and NaCl (85 mM) (Ph=7.4). Chelex (Bio-Rad Labratories, Hercules, CA, USA) was added, which has the ability to bind to free excess Cd-ions that were precipitated during the centrifugation. The portions of

free/bound Cd were separated by the centrifugation (12000, 5min, 4^oC). The amount of Cd left in the sample after centrifugation was relative to the amount of MT. Two parallels were run for each individual. The supernatant was allocated on new tubes and the radioactivity determined using a gamma counter (Cobra II Auto-Gamma, Packard Instruments Company, Dowers Grove, IL, USA). The MT-concentrations were calculated using formula (2). The CPM_s is counts per minute in the sample, CPM_{bg} is counts per minute in the blank and CPM_t is total CPM in sample without chelex. 1/7 means that 7 binding sites of Cd-atoms combine with each MT molecule. The numbers 10 and 1.49 is the dilution factors during homogenization and implementation of the assay.

$$MT(nmol/g \ \ wet\ weight) = \frac{CPM_S - CPM_{Bg}}{CPM_T} * 263\, nmol/mL * \frac{1}{7} * 10 * 1.49 \qquad (2)$$

Total protein quantification

The total amount of proteins was determined by using the Bradford protein assay (Bio-Rad, 2007). The results were used to normalize the enzyme activity in the concerned tissue to the protein content including GR, GPx and CAT. 6 solutions of known concentrations of BSA were made, ranging between 0-80 µl/ml, and used to generate the standard curve. Each sample was diluted 1:400 with MQ-water and added to a microtiter plate, succeeded by the addition of Bio-RAD dye reagent containing methanol (CH_3OH) and phosphoric acid (H_3PO_4) (Bio-Rad, Hercules, CA, USA). After incubation for 5 minutes, the absorbance was read at 595 nm on a plate reader (Elx 808 IU Ultra Microplate Reader, Biotek Intstuments Inc, Winooski, VF, USA). The samples were compared to the albumin standards for calculation of the results.

2.4 Lipid peroxidation (TBARS)

The thiobarbituric acid reactive substances (TBARS) assay was performed according to the Cayman chemicals kit booklet (2009b) and is based on the methods by Yagi (1998). All chemicals were supplied by Cayman chemicals Ann Arbor, MI, USA. The method measures the amount of malondialdehyde (MDA) and thiobarbituric acid (TBA) in the samples, which are products of lipid hydroperoxide decomposition. The procedure involved thiobarbituric acid (TBA) reacting with malondialdehyde (MDA) creating TBA-MDA adduct that creates

fluorescent. Unsaturated lipids will yield higher levels of MDA than lipids containing a lower ratio of saturation. Samples for the TBARS-assay were homogenized 1:9 with PBS buffer (pH=7.4), together with BHT. The BHT was used as an antioxidant preventing further oxidation of the lipids in the sample. The lipophilic properties of BHT made it difficult to dissolve in the PBS solution; 40 mg of BHT were added 10 ml ethanol to get the crystals fully dissolved. The BHT–ethanol solution was added slowly to the PBS in the ratio 1/100. A standard curve was made of 7 dilutions of Malondialdehyde (MDA) (125 μM) stock solution and MQ-water (Tab. 4).

Table 4: Dilution scheme consisting of MDA and water for the standards. By measuring the concentration of MDA colorimetrically the resulting data gave a regression slope explaining the concentration MDA-TBA adducts in the samples.

Tube	MDA (μl)	Water (μl)	MDA (μM)
A	0	1000	0
B	5	995	0.625
C	10	990	1.25
D	20	980	2.5
E	40	960	5
F	80	920	10
G	200	800	25
H	400	600	50

The colour agent was created by mixing the substrates acetic acid (CH_3COOH), sodium hydroxide (NaOH) and TBA. Each tube was added standard or sample followed by the addition of sodium dodecyl sulphate solution (SDS). After mixing, the tubes were added colour agent and placed in a boiling water bath for one hour. During the boiling the MDA-TBA adducts were created. The coloured agent is the condensation of 2 moles of TBA and 1 mole of MDA. After boiling, the tubes were immediately put on ice for 10 minutes to stop the reaction. The tubes were centrifuged at 1600 g in 10 minutes (Heraeus instruments, Buckinghamshire, UK). The samples were stable for 30 minutes and they were, together with the standards added in triplicates to the microtiter plate. The absorbance was read at 540 nm on the plate reader (Elx 808 IU Ultra Microplate Reader, Biotek Intstuments Inc, Winooski, VF, USA). The TBARS concentration ranged between 23.34-11.77 μM MDA on the linear standard curve (Tab. 4).

2.5 Statistics

The statistics were performed using Sigmaplot 11 (Systat software Inc., Chicago, USA), except for all regression analysis which were generated using SPSS (PAWS statistics, Somers, NY, USA). All data were checked for normality using Shapiro-Wiik. The data was log transformed, if necessarily, to obtain normal distribution and t-test were used for univariate comparisons to compare groups by location and year separately. Man-Whitney U was used as non-parametric test. General linear models (GLM) were used for the multivariate regression analysis to test simultaneous categorical (location, year) and continuous variables (metals, enzyme activity). The method is based on the principles of ANOVA. By using back-wards elimination, non-significant variables were ruled out in order to create the best model fit. In the models where the factors (year, location) were excluded, a separate analyzes of multiple linear regression were conducted to test the significant x-variables for multicolinearity. The variation inflation factor (VIF) was set to 2. Levene's test was used to check each model for homogeneity of variance. To examine the strength and negative/positive relationship of the predictor variables determined by the GLM, a separate Pearson correlation test was performed for each variable. The P-value was set to <0.05 for all statistical comparisons.

3. Results

The results consisted of several parameters measured in liver samples from 40 common eiders collected in KF and LF during the summers of 2008 and 2009. These samples were analyzed for the levels of the metals Hg, Se, Cd and Zn. The hepatic concentration of tGSH, GSSG, GPx, GR, CAT, MT and TBARS were also quantified in liver samples of the same eiders. Regression analyses were conducted in order to study the relationship between metals and antioxidant/enzyme activity. The samples were divided by location (KF/LF) and year (2008/2009), using 10 individuals from each group, if nothing else is mentioned. The individuals were all females, which exclude the variations that might be introduced by gender. All values of metals are presented as µg metal/g tissue dry weight (dw), whereas MT, tGSH/GSSG and TBARS are given in wet weight (ww). GPx, GR and CAT were normalized to protein content. All results are given in mean ± SD. Values for the descriptive and statistical data are presented in Table 5-7 in appendix C and D.

3.1 Metals

The hepatic levels of Hg are shown in Figure 6. The liver Hg concentration in the eider ducks from KF was 1.82±0.99 µg/g and 1.74±0.63 µg/g in 2008 and 2009, respectively. The mean levels in samples from LF were 1.34±0.33µg/g and 1.55±0.93 µg/g in 2008 and 2009, respectively. There were no significant differences in Hg levels between KF and LF in 2008 (T-test, P= 0.170) or 2009 (T-test, P=0.391). Some outliers influenced the Hg results, although these values are within natural variation. There were no significant differences in Hg levels when making comparisons in KF 2008 and 2009 (T-test, P=0.099) or making comparisons of samples from 2008 and 2009 in LF (T-test, P=0.767).

The hepatic levels of Se, Cd and Zn are presented in Table 5. The table summarizes the concentrations of the metals from KF and LF (2008 and 2009). Se is the only metal that revealed a significant difference between the KF and LF (T-test, P=0.038). However, this is only with regard to the 2008 samples. No significant differences were found when comparing samples from 2008 against 2009 samples for any of the metals presented in the Table 5 (see appendix D for statistical data).

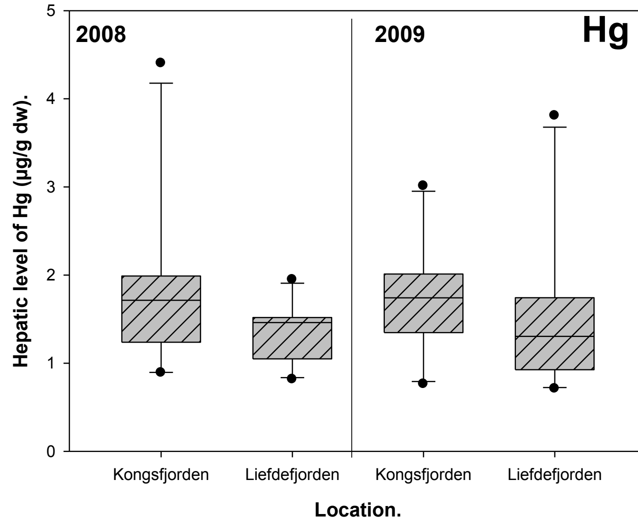


Figure 6: Levels of Hg (µg/g dry weight) in liver samples from Somateria mollissima *collected in Kongsfjorden and Liefdefjorden during the summers of 2008 and 2009. The median values are represented by the horizontal line in each box while the upper and lower sides of the box represent the 75-and 25 percentiles. The outliers above the 90-percentile whiskers are marked with black dots.*

Table 5: Hepatic levels of Se, Cd and Zn (µg/g dw) in samples from Somateria mollissima *from Kongsfjorden and Liefdefjorden during the summers of 2008 and 2009 (shown as mean ± SD, median, minimum and maximum values). Significant differences between groups are denoted by * (p<0.05).*

Element	Group	Mean ± SD	Median	Min	Max
Se	KF 2008*	18.28±9.47	17.68	8.16	35.46
	LF 2008*	26.91±7.14	26.05	20.14	43.93
	KF 2009	22.76 ±4.47	22.16	16.23	30.07
	LF 2009	25.51±17.43	21.20	9.87	64.79
Cd	KF 2008	18.25 ±9.39	15.55	7.98	40.45
	LF 2008	21.70±9.55	22.09	7.69	34.29
	KF 2009	17.36±13.62	14.34	3.42	51.43
	LF 2009	24.39±19.46	19.31	7.04	75.61
Zn	KF 2008	178.28±48.80	160.35	119.19	266.17
	LF 2008	190.32±58.11	175.68	115.93	318.40
	KF 2009	182.90±43.48	173.52	123.27	247.10
	LF 2009	169.52±53.94	155.16	110.59	278.85

3.2 Molar ratio Hg:Se

The molar ratio Hg:Se can be used quantitatively to measure the relative abundance of Hg compared to Se. A low ratio Hg:Se indicate a lower potential for Hg to promote adverse effects. The levels of Hg and Se were calculated to mol/g to equalize importance of both metals. The results of hepatic molar ratio of Hg:Se are presented in Figure 7. The levels from KF were

0.047±0.027 and 0.031±0.013 in 2008 and 2009, respectively. The common eiders from LF had a mean ratio Hg:Se at 0.021±0.009 and 0.030±0.019 in 2008 and 2009, respectively. A clear significant difference (T-test, P=0.005) in Hg:Se levels was found between KF and LF in 2008. However, this was not the case in 2009 (T-test, P=0.911). No significant differences were found in common eiders from KF or LF when making comparisons between 2008 (T-test, P= 0.119) and 2009 (T-test, P = 0.305). Figure 7 shows that the ratio Hg:Se are below 0.1, which is a result of excess Se in the liver compared to Hg.

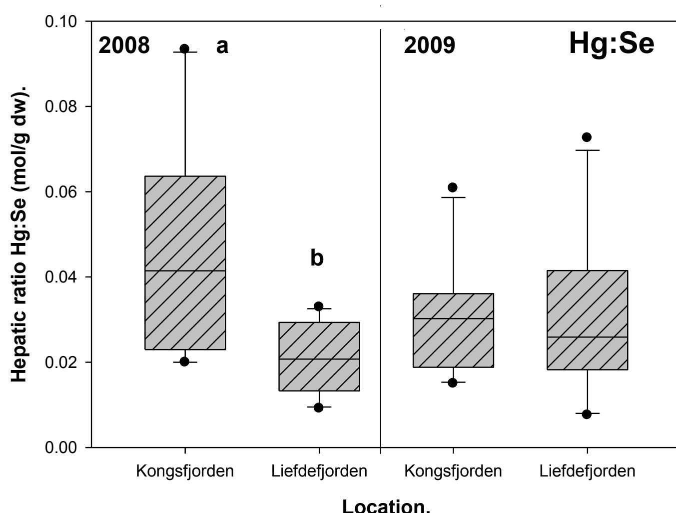


Figure 7: Hepatic ratio Hg:Se of molar basis in tissue of Somateria mollissima *from Kongsfjorden and Liefdefjorden 2008 and 2009. Significant variations between groups are denoted by different letters (p<0.05). The median values are represented by the horizontal line in each box while the upper and lower sides of the box represent the 75-and 25 percentiles. The outliers above the 90-percentile whiskers are marked with black dots.*

3.3 Antioxidant and enzyme activity

The glutathione system

Both tGSH and GSSG are presented in mmol/g. Total glutathione (tGSH) levels in liver samples in common eider ducks from KF and LF in 2008 and 2009 are shown in Table 6. This is the total amount of bound and free glutathione in the reduced (GSH) state. No group stood out from the others, thereby no significant differences were found between the fjords (2008: T-test, P=0.799, 2009: T-test, P=0.788) or with regard to year (KF: T-test, P = 0.876, LF: T-test, P = 0.905).

The hepatic levels of oxidized glutathione (GSSG) in common eiders from KF and LF, from

2008 and 2009 are shown in Table 6. In 2008 there was a higher average level of GSSG in KF when compared to LF (T-test, P=0.033), whereas no such difference were found in 2009 (Mann-Whitney, P=0.241). No differences were found when comparing year for any of the locations (KF: T-test, P=0.313, LF: Mann-Whitney, P=0.427).

Table 6: Hepatic levels of total glutathione (tGSH) and its oxidized dimer (GSSG) (mmol/g) in tissue samples from female Somateria mollissima. *Samples were collected from the breeding colonies at Kongsfjorden and Liefdefjorden in 2008 and 2009. The table shows mean ± SD, median, minimum and maximum values. Significant differences between groups are denoted by * (p<0.05)*

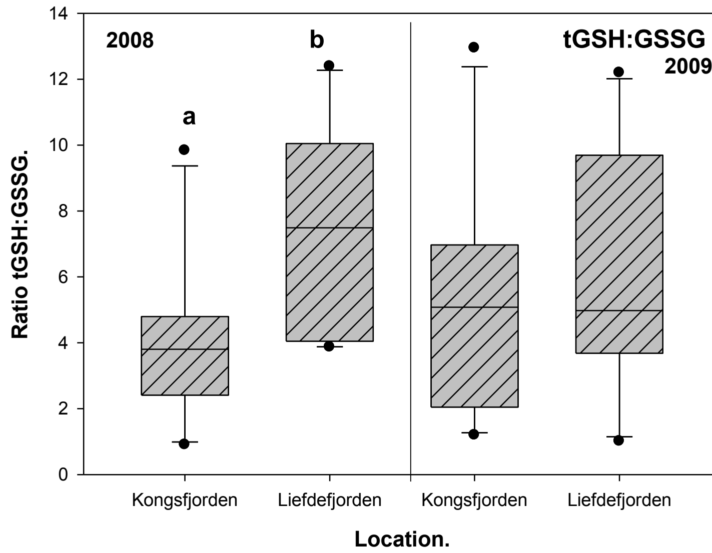
Glutathione	Group	Mean ± SD	Median	Min	Max
tGSH	KF 2008	2.17±1.47	1.83	0.35	5.12
	LF 2008	2.33±1.20	2.19	0.35	4.26
	KF 2009	2.27±1.30	2.09	0.62	4.43
	LF 2009	2.25±1.64	1.57	0.69	5.39
GSSG	KF 2008*	0.60±0.33	0.47	0.16	1.09
	LF 2008*	0.33±0.16	0.36	0.08	0.68
	KF 2009	0.48±0.14	0.52	0.25	0.73
	LF 2009	0.41±0.25	0.39	0.07	1.01

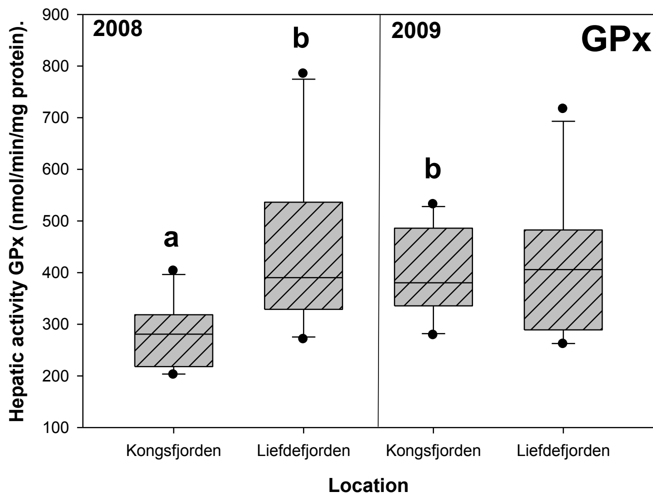
The ratio between total and oxidized glutathione (tGSH:GSSG) can be used quantitatively to measure the oxidative status of glutathione system in the organism. A high level of tGSH:GSSG indicates a higher potential to reduce ROS. The tGSH:GSSG ratio in common eiders are shown in Figure 8. The ratio tGSH:GSSG from KF is 3.98±2.43 in 2008 and 5.14±3.45 in 2009. At LF the tGSH:GSSG ratio were 7.30±3.20 in 2008 and 6.36±3.70 in 2009. The result shows a higher mean ratio tGSH:GSSG in eiders from LF than KF in 2008 (T-test, P=0.018). In 2009, no difference were found with respect to tGSH:GSSG between KF and LF (T-test, P=0.456), even though some definite values were exceedingly higher for LF (Fig. 8). No differences were found when comparing 2008 against 2009 for any of the fjords (KF: T-test, P=0.50, LF:T-test, P=0.553).

The hepatic activity of GPx in common eiders are shown in Figure 8. The results are presented in nmol/min/mg protein. The eiders from KF had an activity of GPx at 282.31±61.82 and 399.88±83.54 nmol/min/mg in 2008 and 2009, respectively. For the KF groups the GPx activity was 443.17±167.79 and 414.75±135.91 nmol/min/mg in 2008 and 2009, respectively. In 2008

the activity were significantly lower in LF compared to KF (T-test, P=0.004), whereas no significant variation was observed in 2009 (T-test, P=0.772). The relative low GPx activity in KF from 2008 show a significant difference (T-test, P=0.002) between the years. No such trend was observed with regards to eiders from LF when comparing 2008 and 2009 (T-test, P=0.712).

The hepatic activity of the GR in common eiders is shown in Figure 8. The eiders from KF had a hepatic activity mean of GR at 70.26±15.92 (n=9) and 82.81±19.06 nmol/min/mg in 2008 and 2009, respectively. In LF, the average GR activity was 71.79±15.92 and 64.43±20.68 nmol/min/mg in 2008 and 2009, respectively. There was observed no variation of GR activity in eiders between the fjords in 2008 (T-test, P=0.837). No differences were found when comparing years for any of the locations (KF: T-test, P=0.140, LF: T-test, P=0.385).





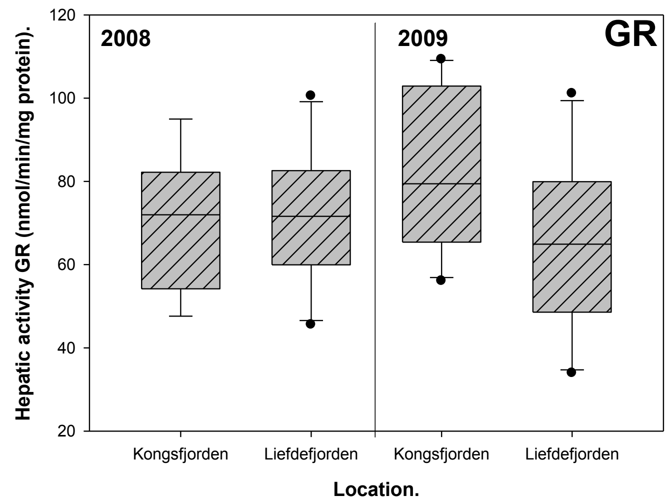


Figure 8: The ratio of tGSH:GSSG (above), together with concentrations of Glutathione peroxidase (left) and Glutathione reductase (right) (nmol/min/mg protein) in liver samples from Somateria mollissima*, collected in Kongsfjorden and Liefdefjorden in 2008 and 2009. The median values are represented with the horizontal line in each plot. The upper and lower sides of the plots represent the 75-and 25 percentiles. The whiskers express 90- and 10 percentiles, outliers exceeding the 90-percentile are represented with block dots. Significant variations are denoted by different letters ($p<0.05$).*

Catalase

The hepatic activity of CAT in common eiders is shown in Figure 9. The activity of the enzyme is given in µmol/min/mg of total protein quantity. The CAT activity in eiders from KF was 376.50±135.59 and 396.70±196.23 µmol/min/mg in 2008 and 2009, respectively. The eiders from LF had a mean level at 564.99±114.37 and 458.90±185.86 µmol/min/mg in 2008 and 2009, respectively. There was a significantly lower CAT activity in the eiders from KF when compared with eiders from LF in 2008 (T-test, P=0.003). No significant difference was revealed when comparing the locations in 2009 (T-test, P=0.371). Although not significant, eiders from LF had higher mean levels of CAT than eiders from KF in 2009, indicating the same findings as shown for eiders collected in 2008. No significant differences in CAT activity were observed in eiders between 2008 and 2009 for any of the locations (KF: T-test, P=0.858, LF: T-test, P= 0.077).

Metallothionein

The MT results from common eiders are shown in Figure 9. The level of MT in eiders from KF were 0.70±0.29 µg/g and 0.69±0.32 µg/g in 2008 and 2009, respectively. LF birds had a MT level of 0.85±0.33 µg/g and 0.85±0.38 µg/g in 2008 and 2009, respectively. Even though the overall level of MT in the eiders from LF was higher when compared to KF, there was no significant difference between the two locations in 2008 (T-test, P=0.313) or 2009 (Man-Whitney, P = 0.140). The MT levels were also very similar in eiders from same fjord with respect to both years (KF: T-test, P=0.895 and LF: T-test, P= 0.993).

3.4 Lipid peroxidation (TBARS).

The levels of lipid peroxidation in common eiders are expressed in Figure 9. The hepatic TBARS level in eiders from KF was 18.50±2.61 nmol/mg and 17.37±2.99 nmol/mg in 2008 and 2009, respectively. The eiders from LF had a TBARS liver concentration at 18.00±1.48 and 15.18 ±1.82 nmol/mg (n=9) in 2008 and 2009, respectively. No statistical differences in TBARS levels were found between eiders from KF and LF in 2008 (T-test, P=0.611) or in 2009 (T-test, P=0.075). Likewise, no differences were found when comparing TBARS levels in KF 2008 to 2009 (T-test, P=0.380). However, a significant difference was found in TBARS level in eiders from LF between 2008 and 2009 (P = 0.002).

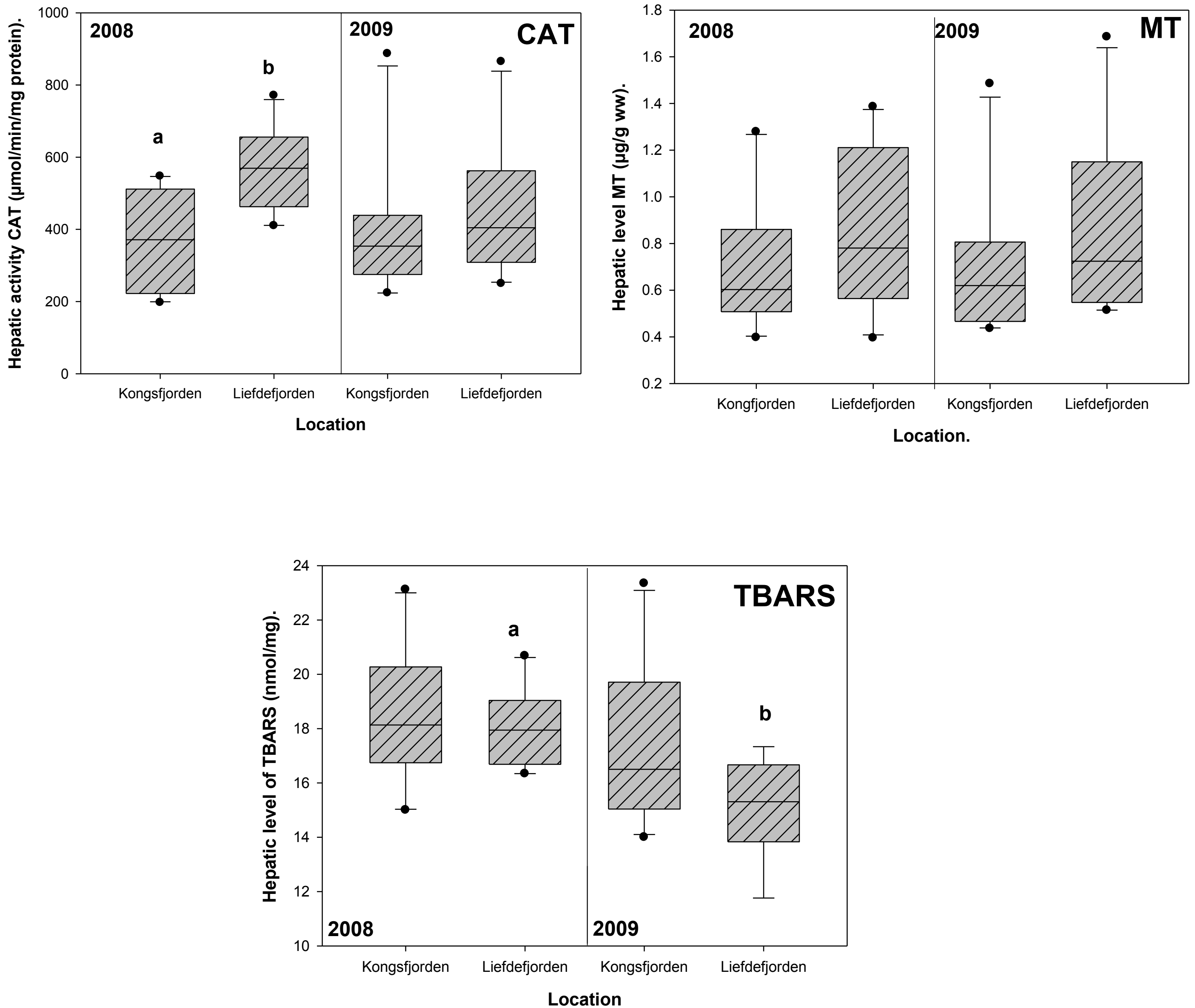


Figure 9: Catalase activity(above right) (µmol H_2O_2/ min/ mg protein ww), metallothionein (µg/g ww) (above left) and lipid peroxidation (TBARS)(nmol/mg ww) in liver tissue from Somateria mollissima *collected in Kongsfjorden and Liefdefjorden during summers 2008 and 2009. The median values are represented with the horizontal line in each plot. The upper and lower sides of the plot represent the 75-and 25 percentiles. The whiskers express 90- and 10 percentiles, outliers exceeding the 90-percentile are represented with block dots. Significant variations are denoted by different letters (p>0.05).*

3.5 Correlated variables

General linear models (GLM) univariate analysis was used to test for the strongest predictors of each response variable in the common eiders. Each model examined the relationship between protein levels, enzyme activity, metals, and the factors of location (KF/LF) and year (2008/2009). Each response variable was tested against all possible covariates (Hg, Se, Cd, Zn, ratio Hg:Se, tGSH, GSSG, ratio tGSH:GSSG, GPx, GR, CAT. MT and TBARS). Location (KF/LF) and year (2008/2009) were included in the models to see if these factors were important predictors of explaining the relationships. All regression coefficients and p-values are given in Table 7.

Table 7: Results from general linear models analysis testing proteins (MT, tGSH, GSSG, GR, GPx), metals (Hg, Cd, Zn, Cu, Se) and lipid peroxidation (TBARS) in liver tissue of Somateria mollissima *taken from Kongsfjorden and Liefdefjorden, 2008 and 2009. Each model represents the most important predictor with respect to the response variable. The table presents the* R^2, $F_{df,error}$, *and p-value (p<0.05) for each model.*

Response variable		Corrected model (model fit)	Covariates		
	R^2 =0.143		**GSSG**		
tGSH	$F_{1, 38}$	-	6.336		
	P	-	**0.016**		
GSSG	R^2 =0.236		**CAT**		
	$F_{1, 38}$	-	11.87		
	P	-	**0.001**		
	R^2 =0.640		**Se**	**CAT**	
GPx	$F_{1,37}$	12.43	6.78	9.937	
	P	**0.0001**	**0.013**	**0.003**	
	R^2 =0.279		**Hg**	**GPx**	
GR	$F_{1,36}$	6.968	4.994	7.545	
	P	**0.003**	**0.032**	**0.009**	
	R^2 =0.487		**Cd**	**GPx**	
CAT	$F_{1,36}$	11.39	10.17	26.35	
	P	**0.0001**	**0.003**	**0.0001**	
	R^2 =0.503		**Location**	**Hg**	**Zn**
MT	$F_{1, 35}$	12.13	7.68	10.108	7.961
	P	**0.0001**	**0.009**	**0.003**	**0.007**

Year (2008/2009) was consistent in all models, thus this factor was removed. Location (KF/LF) was excluded from all groups, except for MT. In the model for MT the different fjords were strong predictors of the variables Hg and Zn in the common eiders. No significant variables were found with respect to ratio Hg:Se, ratio tGSH:GSSG and TBARS. Since location and year were insignificant for all groups (KF/LF, 2008/2009), except for MT, these groups were combined in the next analysis. The Pearson product-moment correlation test was run in order to examine the strength and positive/negative influence of the response variables and covariates separately. All correlated variables and descriptive data are given in Table 8. The correlation test confirmed the predictions by the GLM-models, except for one variable. The Cd-CAT interaction was not significant when combining the groups of location and year.

Table 8: Correlated variables. Strength and positive/negative relationships between the variables examined in the GLM-models run separately by use of Pearson correlation test. Parameters included were the response variables tGSH, GSSG, GPx, GR, CAT, MT and the variables GSSG, CAT, GR, Se, Hg, Cd and Zn in hepatic tissue of Somateria mollissima*. All groups (KF/LF, 2008/2009) were combined in cases these factors were removed in the GLM models. Except for MT where each location was run separately. The table shows the respective variables, correlation coefficient, p-value and number of individuals.*

Correlated variables		**Correlation coefficient (r_s)**	**p-value**	**n**
tGSH	GSSG	0.378	0.016	40
GSSG	CAT	-0.488	0.001	40
GPx	CAT	0.541	0.001	40
	Se	0.491	0.001	40
GR	Hg	0.358	0.025	39
	GPx	0.423	0.007	39
CAT	Cd	0.305	0.055	40
MT (KF)	Hg	0.679	0.001	20
MT (LF)	Hg	0.522	0.018	20
MT (KF)	Zn	0.541	0.013	20
MT (LF)	Zn	0.583	0.007	20

4. Discussion

4.1 Levels of Hg, Se, Cd, Zn and geographical differences

The toxicity threshold of Hg in liver tissues of marine seabirds is approximately 20 µg/mg wet weight (AMAP, 2005). The threshold concentrations are not precise, and depend on the species used in the study, their physiology and other environmental stressors (Thompson, 1996). In this study the liver concentrations of Hg (1.61±0.76 µg/g dw) was found to be below the threshold levels connected to acute toxicity in seabirds (Fig. 6).

Savinov et al. (2003) examined liver tissue of several seabird species, including common eider, northern fulmar (*Fulmaris glacialis)*, black-legged kittiwake (*Rissa tridactyla)*, razorbill (*Alca torda)*, Brünnich's guillemot (*Uria lomiva)* and Atlantic puffin (*Fratercula arctica)* for the metals Hg, Se, Cd and Zn. Their sampling was carried out at several locations around the Barents Sea in July and August of 1991, and May and August of 1992. The levels of Hg in common eider ducks from KF in 1991 (1.80±0.22 µg/g dw) were slightly higher compared to the eiders sampled in KF in 2008-2009 (1.63±0.71 µg/g dw) (Fig. 6). Savinov et al. (2003) concluded that the Hg levels did not exceed levels which indicated increased environmental exposure and that the levels were natural as a result of physiological processes in the birds. Studies have shown that seabirds inhabiting KF have slightly higher levels of metals compared to other locations in the Barents Sea (Savinov et al., 2003; Jæger et al., 2007). It has been suggested that this is linked to the potential local sources from the former mining activity at Ny-Ålesund (Savinov et al., 2003). No other study has provided data on metal exposure in birds from LF. However, the Hg levels (1.54±0.67 µg/g dw) are comparable to the levels found in other bird studies from the Barents Sea area (Tab. 9). The lower levels reported in 2008-2009 as compared to 1991 can be related to the recent year's effort to reduce the emissions (AMAP, 2005). Hg levels in seabirds from the Barents Sea are generally low compared to the Canadian and Siberian Arctic (AMAP, 2005). Metal levels in hepatic tissues of common eider were examined by Stout et al. (2002). They collected their samples between May and August in 1991 to 1995 in the Canadian Arctic detecting Hg levels at 1.59 µg/g dw (range 0.56-5.0 µg/g dw) in female eider ducks. This reflects the levels found in the eiders from the present study (Fig. 6). In the same study they found levels up to 7.61 µg/g dw in the Steller's eider (*Polysticta stelleri).* In summary, the levels of Hg found

in liver of female common eiders were within the same range that has been reported in previous studies from Arctic areas.

The Cd levels reported in the present study were higher (20.42±13.40 µg/g dw) (Tab. 5) compared to the levels found in common eiders from KF by Savinov et al. (2003) (17.2±5.57 µg/g dw). This may indicate an ongoing increase of Cd in biota. In the study by Savinov et al. (2003) all maximum mean levels of Cd were found in seabirds collected in KF. This could be due to the local sources at Ny-Ålesund. High Cd levels might also be a result of its feeding behavior, mainly on blue mussels (*Mytilus edulis)* in their wintering grounds. No Cd threshold levels have been established for seabirds (Furness, 1996). The concentrations of Se (23.37±10.87 µg/g dw) in the present study were more than twice as high (Tab. 5) as the levels found in livers of common eider collected by Savinov et al. (2003) (9.21±0.90 µg/g dw). In their study they found the highest concentrations in northern fulmars inhabiting KF (19 µg/g dw). They also found that Se was the element that varied most in tissue concentration of all seabird species. According to Heinz (1996) is Se levels below 10 mg/kg wet weight (approximately 30 µg/g dry weight) regarded as background levels. Some of the maximum Se levels found in the present study exceed this criterion. Even though Se is an essential element known as an important component in many enzymatic processes, it is also an element able to induce several toxic effects at elevated levels (Franson et al., 2007). The levels of Zn (180.25±49.94 µg/g dw) (Tab. 5) in this study were lower than those reported in common eiders by Savinov et al. (2003) at 281±29.1 µg/g dw. They found that hepatic Zn levels in seabirds from the northern Barents Sea were higher compared to the seabirds collected further south. In the present study all samples exhibited normal physiological levels of this essential element. Zn is an essential element which is more regulated by normal physiological processes in the liver compared to the non-essential elements (Liu et al., 2008). Borgå et al. (2006) showed that the regulation of essential elements in liver of Arctic seabirds was a more important explanatory factor for the levels than their respective diet. The result from the present study shows a few high maximum values of Zn, the highest at 318.40 µg/g dw (Tab.5), that probably is caused by loss of body mass during the incubation (Savinov et al., 2003). In summary, Zn was within the range that has been reported previously from the Barents Sea area. However, levels of Se and Cd showed an increasing trend compared to common eiders sampled in 1991.

Table 9: Levels of Hg, Cd, Se and Zn in liver and muscle tissue in a selection of Arctic sea bird species (µg/g dry weight).

Species	Sampling location, month, year	Tissue	Hg	Cd	Se	Zn
Common eider (*Sommateria mollissima*)						
	Kongsfjorden, Svalbard, Jul, 2008/2009[1]	Liver	1.63±0.71	19.10±10.81	22.56±7.91	183 ±48.9
	Liefdefjorden, Svalbard, Jul, 2008/2009[1]	Liver	1.54±0.67	21.51±14.56	25.06±10.97	180 ±51.1
	Kongsfjorden, Svalbard, Jul, 2008/2009[2]	Muscle	0.42±0.18	NM	NM	NM
	Liefdefjorden, Svalbard, Jul, 2008/2009[2]	Muscle	0.36 ±0.13	NM	NM	NM
	Kongsfjorden, Svalbard, Jul/Aug 1991 [3]	Liver	1.80±0.22	17.2±5.57	9.21±0.90	281±29.1
	Kongsfjorden, Svalbard, Jul/Aug 1991 [3]	Muscle	0.40±0.16	0.43±0.26	3.17±1.16	36.4±2.8
	YK Delta, Canada Jul/Aug 1991-1995[4]	Liver	1.59 (0.56-5.00)	79.9 (14.0-507)	7.85 (2.50-44.0)	139 (78.8-415)
	Nunavut, Canada Jul 2000 [6]	Liver	3.3 (1.5-9.8)	164.6 (74.1-389.1)	16.2 (6.5-47.5)	NM
Northern fulmar (*Fulmaris glacialis)*						
	Kongsfjorden, Svalbard, Jul/Aug 1991 [3]	Liver	5.72±0.83	48.0±18.6	15.1±4.17	268±87.0
	Kongsfjorden, Svalbard, Jul/Aug 1991 [3]	Muscle	0.72±0.26	8.71±5.69	8.60±2.90	69.3±8.1
	Bjørnøya, Jul/Aug 1991 [3]	Liver	1.95±1.29	36.6±19.3	10.2±1.22	142±18.8
	Kongsfjorden, Svalbard, Jun/Jul 2005 [5]	Liver	2.57±0.17	NM	NM	NM
	Prince leopold Is. Canada, Jul 1993 [7]	Liver	8.12	39.4	34.4	179
Black-legged kittiwake (*Rissa triactyla)*						
	Kongsfjorden, Svalbard, Jul/Aug 1991 [3]	Liver	1.95±0.44	48.0±18.6	15.1±4.17	126±16.8
	Kongsfjorden, Svalbard, Jun/Jul 2005 [5]	Liver	0.94±0.008	NM	NM	NM
	Prince leopold Is. Canada, Jul 1993 [7]	Liver	3.05	24.2	36.2	171
Atlantic Puffin (*Fratercula actica)*						
	Kongsfjorden, Svalbard, Jul/Aug 1991 [3]	Liver	1.12±0.32	9.77±3.21	11.9±3.48	101±6.7
	Hornøya, Barents Sea, Jul/Aug 1991 [3]	Liver	1.22±0.35	2.60±1.0	9.18±1.68	84.6±11.9
			MeHg	**% MeHg of tot.Hg**		
Brünich's guillemot (*Uria lomivia)*	Kongsfjorden/ Jul 2005, 2006 [5]	Liver	0.37±0.04	81.2		
Black-legged kittiwake (*Rissa triactyla)*	Kongsfjorden/ Jul 2005, 2006 [5]	Liver	0.94±0.08	71.9		
Northern Fulmar (*Fulmaris glacialis)*	Kongsfjorden/ Jul 2005, 2006 [5]	Liver	2.57±0.17	32.4		
			Molar ratio Hg:Se			
Northern Fulmar (*Fulmaris glacialis)*	North Pacific 1985 [8]	Liver	0.07			
White-chinned Petrel (*Procellaria aequinoctialis)*	North Pacific 1985 [8]	Liver	0.15			
Black-footed Albatross *(Phoebastria nigripes)*	North Pacific 1985 [8]	Liver	1.02			

1) This study (mean ± SD) 2) Slåtsveen (2010) (mean ± SD) 3) Savinov et al. 2003 (mean ± SD) 4) Stout et al. 2002 (mean, range) 5) Jæger et al. 2009 (mean ± SD) 6) Wayland et al. 2003 (mean, range) 7) Braune and Scheuhammer (2008) (pooled) 8) Kim et al. (1996b) (pooled). NM=Not measured.

There were no significant differences in levels of hepatic Hg between the fjords for any of the years (Fig. 6). This is in contrast to the findings by Savinov et al. (2003) and Slåtsveen (2010). Savinov et al. (2003) found a significant geographical difference in Hg levels between KF, Franz Josef Land and Bjørneøya in northern fulmar, black-legged kittiwake and Brünnich's guillmot. In addition, the master thesis by Slåtsveen (2010) on the benthic food chain, when comparing KF and LF she found a significant difference of Hg in muscle tissues from the same common eiders (E41-E80) used in the present study. The hepatic Hg levels reported in this study were generally higher (1.61±0.76 μg/g dw) compared to the Hg muscle levels (0.38±0.17 μg/g dw). This is normal in tissues of seabirds, since liver is the primary organ involved in the metabolism of nutrients and essential elements (Thompson, 1996). Slåtsveen (2010) also found differences in Hg content in sediments between the two fjords. She concluded that the observed differences between KF and LF were caused by several fjord specific characteristics in ice cover and various dominance of Atlantic and Arctic water masses in the two fjords. The same factors were also suggested by Hallanger (2010) who found significant differences in concentration of persistent organical pollutions (POPs) in seabirds and zooplankton from the two fjords. The present master thesis suggests that the fjord specific factors influencing Hg levels in muscle tissue of common eider cannot be applied to the concentrations found in the liver of the birds. This may be a result of a higher rate of element regulation and sequestering or organ specific distribution of various species of Hg, such as MeHg. Naturally, muscle tissue exhibits a more stabile contaminant level because of lower metabolic activity (Kim et al.1996a). There may also be a possibility that the liver reflects the short-term uptake, whereas muscle reflects a long-term which originates from its overwintering grounds. Overall, in contrast to muscle tissue, liver does not seem to show any evidence of different Hg exposure between KF and LF.

Though there were few significant differences in levels of the examined metals between the two breeding fjords, this may be explained by the relative short distance between the locations. The common eider is a migratory bird, and therefore not bound to one fixed location. Body burden of metals might also reflect the influence from an unknown source in its overwintering grounds. The metal levels may therefore be a result of feeding on contaminated food in more polluted coastal areas further south (Savinov et al., 2003). The Svalbard population is known to migrate to the coasts of Norway and Iceland at wintertime (Kovacs and Lydersen, 2006). Birds from

different colonies at Svalbard are known to winter at the same areas in North-Norway (Mehlum, 1991). Regardless of being a migratory bird, the female common eiders are known to be fairly permanently present at their breeding grounds during summertime (Anker-Nielsen et al., 2000).

Inter-species variation in body burden of metals is mainly linked to dietary differences and Hg has shown to biomagnify and bioaccumulate with increasing trophic position (Borgå et al., 2006; Jæger et al., 2009). For example, seabirds having a diet consisting of zooplankton, such as the little auk (*Alle alle)*, tend to have low levels of Hg (Savinov et al., 2003). Contrary, Savinov et al. (2003) found the highest hepatic concentration of Hg in tissues of northern fulmar and razor bill (Tab. 9). This was assumed to be a result of the dietary dominance of fish. Element burden in eider duck is thought to be a useful indicator of the local environmental concentrations (Norheim and Kjos-Hanssen, 1984). The prey items for common eiders are benthic organisms, which absorbs metals directly via the sediments entering the food chain. Thus, common eiders usually have higher body burden of metals compared to pelagic birds at the same trophic position (Braune, 1987; Dietz et al., 1996). However, Lavoie et al. (2010) suggested that the transfer of Hg in the benthic food chain was inferior compared to the pelagic food chain. This was also confirmed by Slåtsveen (2010) who did not find any relationship between trophic position, using stable nitrogen isotopes ($\delta^{15}N$) and Hg levels in the benthic food chain. She suggested that the low rate of biomagnification was mainly a result of the high bioaccumulation in the benthic organisms compared to the muscle tissue in the common eiders.

The toxicity of Hg is connected to its chemical form. MeHg is known to have greater toxicity and bioaccumulation compared to the inorganic Hg (Wolfe et al., 1998). The percentage MeHg relative to the total level of Hg in hepatic tissues of seabirds is highly variable (Jæger et al., 2009) (Tab. 9). A high proportion of inorganic Hg in liver compared to muscle is mainly a result of demethylation of MeHg in the liver (Eagles-Smith et al., 2009). In the study by Jæger et al. (2009) they concluded that 81.2% of the total Hg in liver from Brünnich's guillemot was in its methylated form. The lowest percentage MeHg of total Hg was found in northern fulmar (32.4%). They also reported that the levels of MeHg decreased with increasing levels of total Hg in the liver samples. No such study has been provided with regards to MeHg in common eiders from Svalbard. It is reasonable to believe that the percentage MeHg of total Hg is lower

compared to pelagic species, since more MeHg are associated with the pelagic food chains (Wolfe et al., 1998).

The variations in hepatic element levels between seabird species, and within the examined groups of common eiders, are linked to several decisive factors such as excretion, age and nutritional status (Borgå et al., 2006). Excretion pathways for Hg are mainly moulting of feathers and egg laying (Wolfe et al., 1998). These pathways can affect seasonal Hg levels. The time when female common eiders are moulting is variable. It may occur when the chicks are fledged, or in failed breeders, just after losing eggs in case of nestling failure (Parker and Mehlum, 1991). Thus, levels of Hg in female common eiders might be highly connected to breeding success. The age is impossible to determine in adult birds (Gabrielsen and Sydnes, 2009). Such information has therefore not been included in this study, even though it is assumed to be an important factor influencing body burden of heavy metals. In the Barents Sea the highest hepatic levels of Hg is found in northern fulmars, which is primarily linked to its high trophic position and longevity. (Knudsen et al., 2007; Gabrielsen and Sydnes, 2009). Many of the same mechanisms described for Hg can also be applied to Cd.

4.2 Se and the molar ratio Hg:Se

In the present study, Se was the only element which was significantly different between the two fjords, although this was only in 2008 (Tab. 5). Even though it is difficult to draw any firm conclusions, a possible explanation is that LF is more dominated by local geological sources. There are also reasons to believe that the difference is caused by Se's role as an essential element with a high physiological importance. An increased metabolism, physiological strain, for example during the incubation period and exposure to a non-measured contaminant, will increase the demand for Se. In 2008, the Arctic spring was earlier in KF than usual (pers.comm, G.W.Gabrielsen, Norwegian Polar Institute). It may be suggested that the KF eiders were more affected by the incubation fast than birds incubating further north. Although the Se levels were relatively high compared to other studies (Tab. 9), no relationship was found between Se and any of the measured biochemical parameters, except for GPx where Se functions as a cofactor (Toppo et al., 2009).

The observed difference in molar ratio Hg:Se in common eiders between KF and LF in 2008 (Fig. 7) is mainly a result of the significant difference observed for Se (Tab. 5). During the incubation period, the physiological demands for Se will increase, followed by an increase of non-essential elements such as Hg and Cd (Wayland et al., 2002). Se has normally shown to have antagonistic effects on Hg, but even so, a low ratio does not exclusively diminishes the toxic potential of Hg (Hoffman and Heinz, 1998). The 1:1 molar ratio of Hg:Se is normal in marine mammals (Dietz, 2000). When the 1:1 ratio is exceeded it is believed to increase the toxic properties of Hg. Arctic seabirds tend to have variable ratio of Hg:Se, but normally in surplus of Se (Dietz, 2000; Borgå et al., 2006). The results (0.032±0.019 molar ratio Hg:Se) (Fig. 7) indicate that the common eiders are well protected against Hg toxicity due to the excess Se in their tissue. Kim et al. (1996b) studied the molar ratio Hg:Se in liver tissue of several seabird species in the north of Pacific (Tab. 9). The species that accumulated the most Hg were the ones having the highest ratio, such as the black-footed Albatross (*Diomedea nigripes)*, which had a molar ratio Hg:Se of 1.02. Other species such as northern fulmar had a molar ratio Hg:Se at 0.07. The variable results were assumed being caused by the essential and nonessential properties of Se and Hg, together with the excretion of MeHg during the molting period. They found correlations between Hg and Se only in the birds having the highest Hg levels. No such correlation was found in the present analysis. Since Hg levels were low compared to Se, it is reason to believe that their molar ratio Hg:Se was inferior to cause any interactions. The mechanisms behind the Hg/Se interaction are unclear (Khan and Wang, 2009). There are several proposed mechanisms for the interaction between these elements. This include Se-aided demethylation of MeHg, formation of inorganic Hg:Se compounds, Se inhibition of radicals from Hg, redistribution of inorganic Hg by Se and Hg-induced Se deficiency (Khan and Wang, 2009). The fact that Hg has an affinity to Se 10^7 times higher than sulphur, an important metal chelator, is most likely the key factor in the Hg:Se interaction (Ralston et al., 2009).

4.3 The Antioxidant system

The balance of ROS and the antioxidant defense system is continually maintained in healthy organisms. When the balance becomes disturbed, ROS-mediated damage is likely to occur (Halliwell and Gutteridge, 2007). An activation of the antioxidant defense system is considered as a subclinical effect of metal exposure (Berglund et al., 2007). Most other studies have

examined the responses from only one single metal. Halliwell and Gutteridge (1999) concluded that there is no universal biomarker for oxidative stress. The most desirable would be to include as many parameters of antioxidants and contaminants as possible to get an overview of this intricate system and interactions. They suggest that the most relevant biomarker depends mostly on the study question and analysis that is possible to obtain from the species studied. In this study the antioxidants and enzymes included were tGSH, GSSG, GPx, GR, CAT and MT. TBARS were included as a measure of oxidative damage in the hepatic tissue.

The glutathione system

GSH reacts to a broad selection of ROS creating GSSG (Stohs and Bagchi, 1995). Hegseth et al. (2011) found that the level of tGSH in black-legged kittiwake from KF was 2.75±0.63 mmol/g. This is only slightly higher compared to values obtained in female common eiders in the present study (2.25±1.36 mmol/g) (Tab. 6). Contrary, when compared to the tGSH levels in pied flycatcher (*Ficedula hypoleuca)* (4.6±0.33 mmol/g) (Berglund et al. 2007) the tGSH levels in the common eiders are considerably lower.

GSSG was significantly different in common eiders from KF and LF in 2008 (Tab. 6).The difference in levels of GSSG 2008 is also the main contributor for the observed variations in the ratio tGSH:GSSG in 2008 (Fig. 8). The levels of tGSH were very similar for all groups (KF/LF, 2008/2009) of common eiders (Tab. 6). This confirms the observation by Isaksson et al. (2005) that induction of GSSG and ratio tGSH:GSSG are better indicators of oxidative stress than tGSH. Isaksson et al. (2005) suggested that tGSH indicate a more long-term up-regulation of the glutathione system rather than oxidative stress. The ratio tGSH:GSSG is known as an highly potent indicator of the glutathione system (Koivula and Eeva, 2010). The ratio tGSH:GSSG in hepatic tissue (5.70±3.35) (Fig.8) in the common eiders was a little higher compared to ratio found in the kidney tissue of the same birds (E61-E70) by Jansen (2010), which were 4.54±1.65. Hegseth et al. (2010) found the highest tGSH:GSSG ratio in northern fulmar (6.66±2.0). The higher ratio of northern fulmar compared to common eiders shows that fulmars have higher proportion of reduced glutathione and thereby a better ability to scavenge ROS. This might also be a result of natural adaption because of its higher trophic position in the food chain.
Several exposure studies have examined the relationship between metals and glutathione

metabolism in birds (Hoffman and Heinz, 1998; Kenow et al., 2002; Hoffman et al., 2005). These have shown a strong relationship between Hg and altered glutathione metabolism. Hoffman and Heinz (1998) found that mallard ducks (*Anas platyrhyncos)* fed with Hg displayed levels of GSSG that increased drastically, whereas Se and Hg combined did not increase levels of GSSG. This observation suggest that the common eiders in the present study do not have an increased level of GSSG due to the high molar Hg:Se ratio. In an exposure study by Kenow et al. (2008) examining the responses on the glutathione metabolism in Common loon (*Gavia immer)* chicks fed on different doses of MeHg (0.4-1.2 µg/g wet fish), they found evidence to suggest that an altered glutathione metabolism was induced at ecologically relevant dietary Hg levels (0.4 µg/g ww). However, in this study no metal was found to significantly interact with any of the forms of glutathione in hepatic tissue of common eider. One reason might be that the eiders were adult birds and therefore more adapted to environmental exposures. Contrary to this, the tGSH and GSSG levels might have been unaffected by the metals by the availability of precursor compounds like cystein and glutamate, which are important components in the glutathione molecule (Forman et al., 2009). Even though GSH is a cofactor to the GPx enzymes, it have important roles in many other metabolic processes as well, such as ascorbate metabolism, cell communication and preventing protein SH-groups from oxidizing and cross-linking (Halliwell and Gutteridge, 2007). The positive correlation between tGSH and GSSG (Tab. 8) shows an up-regulation of the glutathione system as possible a mechanism of increased sulfhydryl concentration or exposure to a non-measured contaminant. GSH is recognized as an important detoxifying constituent. Despite these findings, recent studies of the glutathione system have suggested that properties of the thiol group as a metal chelating agent promotes the distribution of Hg, helping metals cross biological barriers (Rooney, 2007).

GPx is neutralizing H_2O_2 by the oxidation of GSH to GSSG, and is a family of phylogenetically related proteins (Toppo et al., 2009). The levels of GPx activity in the common eiders (385.02±130.79 nmol/min/mg protein) (Fig. 8) is comparable to what is found in other bird studies. In an exposure study, using great egret nestlings (*Ardea alba*) controls, the mean hepatic activity of GPx was 404±26 nmol/min/mg protein (Hoffman et al., 2005). They found that the GPx activity decreased significantly in birds fed with Hg-contaminated food. In the kidney tissue, from the same common eiders used in the present study (E61-E70), Janssen (2010)

reported that the total GPx activity was 114.36±28.42 nmol/min/mg protein. The reduced activities in kidney are not surprising, since liver is the most active organ for glutathione metabolism (Halliwell and Gutteridge, 2007). Hegseth et al. (2011) found that the GPx levels in black-legged kittiwake were 305.83±54.56 µmol/min/mg protein, which is considerably higher compared to the common eiders. Se levels were positively correlated to the activity of GPx (Tab. 8). This was expected since Se is an important subunit providing selenocysteine to a majority of the GPx enzymes (Halliwell and Gutteridge, 2007). Thus, Se has the ability to directly affect the GPx activity. The same relationship was found by Wayland et al. (2010) in glaucous gulls (*Larus hyperboreus)* from the Canadian Arctic. These birds also had lower levels of Se (range 0.08-5.2 µg/g dw) compared to the eiders used in the present study (Tab. 5). The positive correlation between GPx and CAT (Tab. 8) may indicate an elevated level of H_2O_2 in the hepatic tissues of the eiders. These are separate enzyme systems, but have quite similar function in reducing H_2O_2. No other metal than Se was found to correlate with GPx. This may indicates that the H_2O_2 production might have been induced by a natural physiological increase or a non-measured contaminant. An increased level of this radical will trigger the synthesis of these enzymes and prevent further oxidation (Halliwell and Gutteridge, 2007).

The GR enzymes are responsible for the reduction of GSSG back to GSH (Halliwell and Gutteridge, 2007). In the study by Berglund et al. (2007) on pied flycatcher nestlings, the GR activity in the reference group was 17±1.9 nmol/min/mg protein. Similar, Hegseth et al. (2011) found that the GR activity in black-legged kittiwake was 16.3±5.1 nmol/min/mg protein. This is relative low compared to the common eiders (72.37±18.63 nmol/min/mg protein) (Fig. 8) and may indicate that the eiders have a high ability to reduce GSSG. A positive correlation was found between GR and Hg (Tab. 8), which indicates that the levels of Hg influence the activity of GR in the hepatic tissue of the common eiders. Kenow et al. (2008) found in their exposure study of MeHg on common loon (*Gavia immer)* chicks that increased Hg levels in blood were positively correlated with increased activity of GR. Even though the Hg levels in the present study are considered as quite similar to other studies, one cannot rule out that Hg induce GR activity even at normal physiological levels. Not surprisingly, GR was strongly correlated whit GPx in the common eiders (Tab. 8). An increased level of GPx activity forming GSSG demands a higher activity of GR to reduce GSSG back to GSH. The correlation indicates an up regulation of the

enzyme system, as a possible indicator of increased exposure to ROS. No negative relationship between GR and GSSG was found in the present study, which would have been expected, since more GR would reduce GSSG. The same observation was applied by Hegseth et al. (2011) who concluded that GR was probably not affected by the mechanisms regulating glutathione.

In summary, no metal were found to interact with any of the forms of glutathione. However, GSSG seems to rise as an increasing level of tGSH, which might involve increased sulfhydryl concentration or a non-measured contaminant. Of the antioxidant enzymes GPx was highly connected to Se, mainly because of being a cofactor for the enzyme, and GR seemed to be influenced by the levels of Hg.

Catalase

Similar to the GPx, CAT enzymes catalyses the decomposition of H_2O_2 to O_2 and H_2O and are mainly localized in the peroxisomes. (Halliwell and Guttridge, 2007). The CAT activity was higher and displayed a much wider range (449.27±172.16 µmol/min/mg protein) (Fig. 9) when compared to other Arctic bird species examined. In the master thesis by Kongsrud (2009), the activity of CAT in black-legged kittiwakes from KF in 2007 ranged between 10.33- 40.81 µmol/min/mg protein, which was assumed to be very low. Low levels of CAT in kittiwakes were also confirmed by Hegseth et al. (2010) who found considerably higher levels of CAT in northern fulmar (284.49±84.28 µmol/min/mg) and herring gull (224.72±31.88 µmol/min/mg). This is in contrast to the findings from pied flycatcher nestlings in which the CAT activity ranged between 175-275 mmol/min/g (Berglund et al., 2007), which is exceedingly higher when compared to the common eiders (Fig.9). This may indicate that Arctic seabirds have less activity of CAT due to the fact that some of the species are less exposed to contaminants. It also confirms the theory that normal rate of H_2O_2 production is maintained by GPx, but at elevated production of H_2O_2 CAT becomes more important (Halliwell and Gutteridge, 2007). GSSG and CAT was negatively correlated (Tab. 8), even though they have no joint coupled mechanism. However, both contribute to the reduction of H_2O_2. The negative correlation between GSSG and CAT might explain their overlapping function. An increased level of GSSG might have reduced the need of CAT for the removal of H_2O_2. In summary, the CAT activity was higher compared to other Arctic seabirds, although considerably lower than those found in terrestrial birds further

south.

Metallothionein

MT is highly important in detoxifying and maintaining homeostasis of metals, and has therefore been recognized as a useful bioindicator for elevated metal concentrations (Braune and Scheuhammer, 2008). Its metal ion binding capacity is contributed by the high level of cystein-SH groups (Park et al., 2001; Halliwell and Gutteridge, 2007). The levels of MT in common eiders were lower (1.04±0.48 µg/g ww) (Fig. 9) compared to the levels found in several seabird species by Braune and Scheuhammer (2008) in the Canadian Arctic. These were 1.75±1.25 µg/g and 1.37±0.72 µg/g wet weight in hepatic tissues of black-legged kittiwakes and in northern fulmar, respectively. In addition, these bird species had higher levels of most metals (Tab. 9). In the GLM analysis (Tab. 7), the respective location turned out to be a strong predictor of the correlation of MT to Hg and Zn. MT was the only response which differed significantly between the locations in the GLM models. The stronger correlation of MT to Hg in KF (Tab. 8) is probably a result of the slightly higher levels of Hg and other metals in birds from KF. The strong correlation between MT and Zn is not surprising. MT has a high affinity for Zn and is known to play an important role in homeostasis and distribution of this essential element (Kang, 2008). Of the measured response variables, MT was the strongest indicator of elevated metal exposure in common eiders. Because of its role in maintaining metal homeostasis, MT is very sensitive to even low levels of metals. Thus, this response does not exclusively indicate evidence of increased ROS. Park et al. (2001) demonstrated that Cd was the most important of all metals affecting MT-sequestering. In the present study no such relationship was found. This indicates that the levels of Hg and Zn were more influential in determining MT than the levels of Cd.

Lipid peroxidation (TBARS)

An increased level of lipid peroxidation is a result of the oxidation of polyunsaturated fatty acids (Halliwell and Gutteridge, 2007). It can be used to measure the rate of oxidative damage in the birds. The process yields several secondary, highly damaging byproducts such as malondialdehyde (MDA) detectable by the TBARS assay (Monaghan et al., 2009). In common eiders the TBARS levels were lower (17.31±2.55 nmol/mg) (Fig. 9) compared to lung tissues from Great tits (*Parus major)* which was 25.12±1.36 nmol/mg (Isakson et al., 2009). No metals

were found to be predictors of the TBARS levels in the GLM-models (Tab. 7). It is worth mentioning that other studies, which show interaction between metals and TBARS, had higher levels of the examined metals compared to the common eiders (Berglund et al., 2007; Kamiǹski et al., 2009). There is a lot of controversy with respect to the specificity of the TBARS-assay. Monaghan et al. (2009) claims the specificity is low due to the fact that TBA reacts with other substrates apart from MDA. Also, the TBARS-assay does not give the whole picture, since the oxidation of lipids creates several non-detectable by products (Halliwell and Gutteridge, 2007).

4.4 Physiological and ecological factors influencing the antioxidant system

Significant interactions between metals and enzyme/antioxidant activity in birds have proven to be considerably more prominent in areas affected to more severe pollution (Ji et al., 2006; Berglund et al., 2007; Kamiǹski et al., 2007; Issakson et al., 2009). Since the measured metal concentrations, except for Se, did not vary significantly between the breeding colonies (KF and LF), it is reasonable to believe that other factors might responsible for the observed differences in antioxidant/enzyme levels. Levels of parameters measured in the antioxidant defence system are not constant, but highly dependent on several factors such as gender, time of year, migratory pattern, diet, life stage and health status (Constantini, 2008). It is assumed that several of the measured enzymatic and non-enzymatic antioxidants were highly influenced by many of the same physiological and ecological factors, and are therefore discussed together as a whole. The parameters measured in the present study that show significant differences between KF and LF include GSSG (Tab. 6), tGSH:GSSG (Fig. 8), GPx (Fig. 8) and CAT (Fig. 9), all of which were collected in 2008. This indicates that some explanatory factors specific to 2008 made it a particularly important year in explaining the differences. This is also supported by the significant differences found with respect to the same fjord in between different year (2008 and 2009), including GPx (Fig. 8) in KF and TBARS (Fig. 9) in LF. The activity and biological production in the Arctic areas are highly influenced by climatic parameters during the year. A possible explanation is that the Arctic springtime occurred earlier than usual in KF in 2008. KF had been ice free most of the winter 2008 (pers.comm, G.W.Gabrielsen, Norwegian Polar Institute). The sampling in the present study was conducted intensively during the month of July to exclude some of the seasonal variations. Regardless, differences might be a result of seasonal variations between the locations and years. In addition, springtime usually occurs later in LF because it

situated further north (Hallanger, 2010). It is reason to believe that the eiders in LF obtained the seasonal effects later (start to breed later) than the eiders from KF. Nests are usually initiated after the ice break-up. In years when the ice break-up is late, females are forced to delay their egg laying for several weeks (Mehlum, 1991). The birds sampled in KF in 2008 were probably in their late stages of the incubation and thereby more affected by the fasting compared to the eiders sampled in 2009. During incubation the female eider duck goes through a long period of fasting, for 24 to 26 days (Gabrielsen et al. 1991). The reproduction is an energetically demanding activity, and the birds go through extreme changes in basal and field metabolic rates. The birds lose up to 30-45% of their body mass (Parker and Holm, 1990; Gabrielsen et al. 1991). Fasting thus results in a poor health status. Essential components in the diet will be sequestrated for reproduction and reducing the amount of these constituents in other metabolic activity (Constantini, 2008). Also, the elevated metabolic activity will increase the natural production of ROS (Alonso-Alvarez et al., 2004). Studies have for example shown that fasting lower the activity of the H_2O_2 scavenger GPx (Altan et al., 2005). The lipid mobilization are known to increase levels of contaminants drastically in blood of common eider during incubation fast (Henriksen et al. 1996; Wayland et al., 2005; Bustnes et al., 2010). This rapid release of contaminants during incubation will influence several enzyme systems to withstand the stressors. This condition was confirmed by the fact that the three highest levels of Hg were found in birds incubating, having a brood patch on their chest. In summary, the differences in antioxidant and enzyme activity between the fjords seem to be connected to the reductions in adipose tissue during the incubation fast in female common eiders.

Different feeding ecology and composition of prey species is an important factor that may influence the hepatic levels of the measured biochemical parameters. Intake of micronutrient antioxidants and essential dietary components is important for the activity of the antioxidant system (Cohen et al., 2009). Slåtsveen (2010) found that the trophic position ($\delta^{15}N$) measured in muscle tissues of common eiders was significantly different between the fjords. Trophic position and composition of prey items will affect intake of essential nutrients and also accumulation and distribution of contaminants, including POPs (Hegseth et al., 2011). The antioxidant defense system has proven to be susceptible to exposure to these highly lipophilic chemicals (Jansen, 2010; Wayland et al., 2010; Hegseth et al., 2011). Janssen (2010) found that the level of POPs

and fasting were strong predictors of reduced total oxidative scavenging capacity (TOSC) in kidney tissues of Herring gull chicks. Wayland et al. (2010) showed that high concentration of POPs reduced the levels of sulfhydryl in hepatic tissues of glaucous gull. It is reason to believe that the eiders were affected by increasing levels of POPs during the incubation, as shown by Bustnes et al. (2010). Hegseth et al. (2011) studied the antioxidant responses in relation to body burden of polychlorinated biphenyls (PCB) in liver tissue in northern fulmar, black-legged kittiwake and herring gull (*Larus argentatus)*. She suggested that the total body burden of PCB could explain the inter-species differences in status of the antioxidant defense system. The activity of the antioxidant system has shown to be highly species-specific (Berglund et al., 2007; Koivula and Eeva, 2010; Hegseth et al., 2011). It is therefore difficult to compare antioxidant/enzyme levels to other studies because of extreme variations in levels and activity. In such comparisons it is also important to take into account the differences in methods applied.

Overall, due to the relatively low levels of the investigated metals in common eiders, strong evidence of metal induced oxidative stress is scarce. The present study shows a weak relationship between oxidative stress caused by Hg, increasing the activity of GR and MT. Nevertheless, these interactions between metal-antioxidant/enzyme activities have shown to be more extensive in areas affected by more serious pollution. Several exposure studies have also found numerous evidence of increased oxidative stress in birds exposed to heavy metals (Hoffman and Heinz, 1998; Hoffman et al., 2005; Kevow et al., 2008). The differences found in eiders from the KF and LF is most likely a consequence of the lipid mobilization during incubation fast. The common eiders from KF 2008 seem to be the most susceptible group to oxidative damage by the depletion of several antioxidant/enzyme systems. The present study suggests that the physiological and ecological factors involved are even stronger predictors of antioxidant/enzyme activity and makes it hard to conclude about metal induced oxidative stress. This problem involves especially birds that go through extreme physiological changes during the incubation fast, such as the female common eiders. Several of the enzyme/antioxidant interactions are common mechanisms that do not exclusively indicate metal induced stress. In addition, no metals were shown to induce oxidative damage in terms of lipid peroxidation. The antagonistic property of Se and the low molar Hg:Se ratio is probably also an important factor explaining the few metal-antioxidant/enzyme interactions.

5. Conclusion

The hypothesis that Arctic and Atlantic water masses, together with fjord specific factors such as ice-cover and glacier meltdown, influence the hepatic levels of Hg was not proved. Since eiders belong to the lower levels of the food chain, they seem less affected by trace elements than birds at a higher trophic position. Of the investigated elements Se was the element of greatest concern. It was found to be near the toxicity thresholds for marine birds. However, none of the examined antioxidant or enzyme systems seemed to be influenced by Se. The antagonistic properties of Se are also thought of contributing towards a reduced toxicity of Hg. The Hg levels were in the range that has been showed in previous studies from Svalbard. Even though it has been raised concerned about the increasing levels of certain elements in the biota, Svalbard is still generally considered a clean environment. In a field study like this it is complicated to determine the effects of age, migration, excretion and reproduction, due to the lack of data. Future studies examining the antioxidant defense system in birds in field should include physiological and ecological factors such as health, diet and breeding effort, together with body burden of POPs.

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APPENDIX

Contents

A. Operational parameters HR-ICP-MS…………………………………………….II

B. Raw data...III-IV

C. Descriptive data...V-VI

D. Statistical comparisons...VII-VIII

A. Operational parameters HR-ICP-MS

*Table 1: Detection limits for the HR-ICP-MS metal analysis in hepatic tissue of common eider (*Somateria mollissima). *Resolution given in low (Lr), mediam (Mr) and high (Hr) and intern detection limit (IDL).*

Element	Isotope	Resolution	IDL-25% µg/l	Liver µg/g
Hg	202	Lr	0.0010	0.0120
Se	82	Hr	0.1500	1.800
Cd	114	Lr	0.0020	0.024
Zn	66	Mr	0.0250	0.300

Table 2: Element concentrations in reference oyster and chicken material in the HR-ICP-MS analysis. Values are given in certified value (CV) and mean value (MV) ± SD.

Element		Chicken (n=4)	Oyster (n=3)
Mercury (µg/g)	CV ± SD	0.0036±0.0015	0.0370±0.0013
	MV ± SD	0.0039±0.0005	0.0350±0.0009
Selenium (µg/g)	CV ± SD	0.490±0.06	2.06±0.15
	MV ± SD	0.62±0.01	2.22±0.10
Cadmium (µg/g)	CV ± SD	NC	2.48±0.08
	MV ± SD	0.004±0.002	2.67±0.07
Zinc (µg/g)	CV ± SD	26.00±1.00	1.42±46.00
	MV ± SD	28.21±0.61	1.49±22.29

NC=not certified

B. Raw data

Table 3: Raw data used in the results. All metals are given in dry weight.

Sample ID	Date	Location	Sex	Wing (cm)	Tars (mm)	Head (mm)	Weight (gr)	Hg (µg/g dw)	Se (µg/g)	Cd (µg/g)	Zn (µg/g)	Hg:Se (mol/g)
E41	2008	Kongsfjorden	Female	29.10	62.40	122.70	1486	2.141	35.469	40.454	254.250	0.024
E42	2008	Kongsfjorden	Female	30.00	61.40	123.40	1560	1.358	9.959	14.029	119.199	0.054
E43	2008	Kongsfjorden	Female	29.60	65.00	118.70	1440	1.939	8.177	23.501	149.474	0.093
E44	2008	Kongsfjorden	Female	30.00	65.50	119.40	1364	1.341	11.583	7.982	209.572	0.046
E45	2008	Kongsfjorden	Female	28.90	60.50	116.80	1812	1.612	23.346	9.244	166.168	0.027
E46	2008	Kongsfjorden	Female	29.40	*	118.10	1206	4.403	31.134	22.344	266.173	0.056
E47	2008	Kongsfjorden	Female	27.80	59.80	117.10	1732	1.865	19.677	20.917	150.972	0.037
E48	2008	Kongsfjorden	Female	29.00	62.70	121.80	1740	1.814	8.161	17.086	166.447	0.087
E49	2008	Kongsfjorden	Female	28.30	62.60	117.90	1848	0.929	17.758	13.563	154.537	0.021
E50	2008	Kongsfjorden	Female	29.00	61.20	116.50	1576	0.892	17.606	13.475	146.028	0.020
E51	2008	Liefdefjorden	Female	30.00	67.40	122.70	*	1.486	28.959	10.754	166.369	0.020
E52	2008	Liefdefjorden	Female	29.00	57.40	120.10	*	1.537	20.666	34.295	181.540	0.029
E53	2008	Liefdefjorden	Female	30.10	63.80	117.80	*	1.499	20.142	24.727	158.928	0.029
E54	2008	Liefdefjorden	Female	29.40	61.30	116.70	*	1.437	26.612	30.309	236.395	0.021
E55	2008	Liefdefjorden	Female	29.90	60.60	108.90	*	1.023	43.938	19.454	318.401	0.009
E56	2008	Liefdefjorden	Female	29.20	64.90	120.20	*	1.058	30.815	17.012	141.357	0.014
E57	2008	Liefdefjorden	Female	30.20	68.00	123.50	*	1.098	29.019	7.698	115.938	0.015
E58	2008	Liefdefjorden	Female	29.10	63.40	119.40	*	1.512	20.182	13.014	170.460	0.029
E59	2008	Liefdefjorden	Female	28.10	59.00	109.40	*	0.816	25.494	26.236	180.906	0.013
E60	2008	Liefdefjorden	Female	28.00	*	*	*	1.948	23.321	33.576	232.921	0.033
E61	2009	Kongsfjorden	Female	28.60	64.00	117.20	1221	3.011	19.488	25.934	219.565	0.061
E62	2009	Kongsfjorden	Female	30.20	64.00	115.80	1377	2.400	26.924	13.568	195.239	0.035
E63	2009	Kongsfjorden	Female	29.90	62.20	115.20	1657	1.780	20.072	20.649	150.366	0.035
E64	2009	Kongsfjorden	Female	28.90	66.30	117.70	1791	0.763	16.228	8.080	145.403	0.018
E65	2009	Kongsfjorden	Female	29.60	63.30	119.50	1746	1.704	23.733	10.792	123.265	0.028
E66	2009	Kongsfjorden	Female	29.10	63.00	123.90	1736	1.864	18.791	51.431	190.679	0.039
E67	2009	Kongsfjorden	Female	29.00	63.00	122.80	1794	1.444	30.066	3.418	156.352	0.019
E68	2009	Kongsfjorden	Female	29.60	62.70	119.10	1788	1.547	20.584	8.483	155.931	0.030
E69	2009	Kongsfjorden	Female	29.40	64.90	124.90	1896	1.883	23.989	15.116	247.104	0.031
E70	2009	Kongsfjorden	Female	29.50	62.80	127.10	1883	1.058	27.812	16.137	245.182	0.015
E71	2009	Liefdefjorden	Female	29.40	62.30	88.20	1710	0.953	31.455	15.426	146.326	0.012
E72	2009	Liefdefjorden	Female	30.50	62.90	122.00	2150	3.808	64.790	33.221	278.853	0.023
E73	2009	Liefdefjorden	Female	28.40	60.10	120.60	1710	1.344	12.063	9.494	110.590	0.044
E74	2009	Liefdefjorden	Female	28.30	59.80	114.70	1690	1.488	24.986	19.569	168.839	0.023
E75	2009	Liefdefjorden	Female	29.70	61.60	119.40	1820	1.224	11.832	7.039	131.025	0.041
E76	2009	Liefdefjorden	Female	29.90	67.10	124.00	2030	1.409	17.962	25.580	236.737	0.031
E77	2009	Liefdefjorden	Female	28.70	61.50	122.30	2210	0.848	44.080	19.046	152.598	0.008
E78	2009	Liefdefjorden	Female	30.20	68.90	121.10	1910	2.512	13.626	18.830	114.761	0.073
E79	2009	Liefdefjorden	Female	29.00	63.40	119.20	1820	0.711	9.865	75.607	157.718	0.028
E80	2009	Liefdefjorden	Female	29.50	60.70	117.00	1650	1.265	24.437	20.110	197.760	0.020

*=Not measured

Table 4: Raw data for the measured antioxidants and enzymes. Total GSH, GSSG, MT and TBARS are given in wet weight, whereas activity of GR, GPx and CAT are normalized to protein content.

Sample ID	Date	Location	tGSH (mmol/g)	GSSG (mmol/g)	Ratio tGSH/GSSG	GR (nmol/min/ mg protein	GPx (nmol/min/ mg protein)	CAT (µmol/min/ mg protein)	MT (µg/gww)	TBARS (nmol/g)
E41	2008	Kongsfjorden	1.414	0.447	3.163	88.160	201.931	325.759	1.641	15.001
E42	2008	Kongsfjorden	1.511	0.861	1.754	69.422	337.642	334.050	0.646	17.224
E43	2008	Kongsfjorden	2.026	0.394	5.149	76.232	217.996	222.676	0.994	15.308
E44	2008	Kongsfjorden	5.128	1.096	4.679	75.539	261.545	221.089	1.062	17.588
E45	2008	Kongsfjorden	4.362	1.076	4.056	54.015	278.197	197.035	0.771	17.337
E46	2008	Kongsfjorden	1.035	0.292	3.548	94.986	309.299	465.834	1.793	19.044
E47	2008	Kongsfjorden	0.351	0.389	0.903	47.628	218.101	501.197	0.793	19.721
E48	2008	Kongsfjorden	2.088	0.502	4.164	54.386	402.928	542.152	0.734	18.680
E49	2008	Kongsfjorden	2.205	0.838	2.631	*	312.049	408.271	0.896	23.118
E50	2008	Kongsfjorden	1.646	0.167	9.834	71.984	283.489	547.014	0.556	21.928
E51	2008	Liefdefjorden	2.696	0.383	7.044	100.504	784.827	770.932	1.131	18.004
E52	2008	Liefdefjorden	1.689	0.213	7.932	55.670	351.464	509.248	1.248	17.147
E53	2008	Liefdefjorden	1.728	0.199	8.691	45.555	332.694	655.383	1.058	17.882
E54	2008	Liefdefjorden	2.906	0.235	12.385	77.387	339.884	593.386	1.668	20.673
E55	2008	Liefdefjorden	0.353	0.087	4.072	75.702	683.122	657.521	1.943	20.136
E56	2008	Liefdefjorden	2.664	0.689	3.867	67.489	317.403	422.342	0.739	18.671
E57	2008	Liefdefjorden	4.261	0.379	11.237	81.139	429.230	409.852	0.554	16.779
E58	2008	Liefdefjorden	1.571	0.394	3.984	61.363	487.439	545.564	0.809	18.004
E59	2008	Liefdefjorden	1.466	0.352	4.159	66.249	270.644	476.144	0.999	16.411
E60	2008	Liefdefjorden	3.985	0.413	9.650	86.886	434.976	609.559	1.789	16.336
E61	2009	Kongsfjorden	0.622	0.520	1.195	72.548	278.740	223.643	1.279	15.040
E62	2009	Kongsfjorden	1.124	0.576	1.951	92.681	531.799	360.277	2.082	17.671
E63	2009	Kongsfjorden	2.917	0.225	12.945	84.726	367.521	378.938	0.719	15.669
E64	2009	Kongsfjorden	1.421	0.431	3.301	109.291	362.470	548.437	0.882	17.337
E65	2009	Kongsfjorden	2.765	0.528	5.235	64.304	344.415	303.590	0.611	23.342
E66	2009	Kongsfjorden	1.098	0.528	2.079	107.058	434.416	886.705	1.081	15.404
E67	2009	Kongsfjorden	4.439	0.610	7.278	65.756	393.350	291.683	0.879	14.001
E68	2009	Kongsfjorden	4.176	0.735	5.683	56.083	309.142	224.712	0.858	15.040
E69	2009	Kongsfjorden	1.751	0.356	4.923	101.523	493.432	346.509	0.649	19.339
E70	2009	Kongsfjorden	2.438	0.355	6.864	74.110	483.460	402.507	0.656	20.825
E71	2009	Liefdefjorden	0.891	0.372	2.397	33.932	474.888	423.113	0.916	17.003
E72	2009	Liefdefjorden	1.490	0.300	4.966	101.090	716.474	864.536	1.579	15.308
E73	2009	Liefdefjorden	1.022	1.011	1.011	84.318	482.983	249.761	0.738	11.763
E74	2009	Liefdefjorden	2.659	0.545	4.884	72.750	482.632	604.259	1.094	*
E75	2009	Liefdefjorden	1.655	0.402	4.113	41.696	261.340	546.755	0.720	16.336
E76	2009	Liefdefjorden	0.691	0.073	9.460	69.376	412.430	548.602	1.714	17.337
E77	2009	Liefdefjorden	0.883	0.177	4.989	50.888	293.826	315.824	0.778	13.101
E78	2009	Liefdefjorden	4.216	0.406	10.389	60.466	348.614	287.455	2.363	16.132
E79	2009	Liefdefjorden	3.641	0.394	9.251	51.339	274.940	386.170	0.937	14.572
E80	2009	Liefdefjorden	5.393	0.442	12.193	78.473	399.346	362.547	1.117	15.040

*=Not measured.

C. Descriptive data.

Table 5: Descriptive data material from liver tissues of Somateria mollissima from Kongsfjorden and Liefdefjorden in 2008. The table shows the levels of the metals Hg, Se, Cd and Zn (µg/g dw), the molar ratio of Hg:Se, tGSH (mmol/g ww), GSSG (mmol/g ww), ratio tGSH:GSSG, GPx (nmol/min/mg protein ww), GR (nmol/min/mg protein ww), CAT (µmol/min/mg protein ww), MT (µg/g ww) and TBARS (nmol/g ww). Values are given as mean, standard deviation, maximum, minimum and 0.25% and 0.75% percentiles.

Data material 2008	Group	n	Mean	Std Dev.	Std. Dev/mean	Max	Min	Median	0.25%	0.75%
Hg	KF	10	1.83	1.00	0.55	4.40	0.89	1.71	1.34	1.94
	LF	10	1.34	0.33	0.25	1.95	0.82	1.46	1.06	1.51
Se	KF	10	18.29	9.47	0.52	35.47	8.16	17.68	9.96	23.35
	LF	10	26.92	7.14	0.27	43.94	20.14	26.05	20.67	29.02
Cd	KF	10	18.26	9.40	0.52	40.45	7.98	15.56	13.48	22.34
	LF	10	21.71	9.56	0.44	34.30	7.70	22.09	13.01	30.31
Zn	KF	10	178.28	48.80	0.27	266.17	119.20	160.35	149.47	209.57
	LF	10	190.32	58.11	0.31	318.40	115.94	175.68	158.93	232.92
Ratio Hg:Se	KF	10	0.047	0.027	0.572	0.093	0.020	0.041	0.024	0.056
	LF	10	0.021	0.009	0.400	0.033	0.009	0.021	0.014	0.029
tGSH	KF	10	2.18	1.47	0.68	5.13	0.35	1.84	1.41	2.21
	LF	10	2.33	1.20	0.52	4.26	0.35	2.20	1.57	2.91
GSSG	KF	10	0.61	0.33	0.55	1.10	0.17	0.47	0.39	0.86
	LF	10	0.33	0.17	0.49	0.69	0.09	0.37	0.21	0.39
Ratio tGSH:GSSG	KF	10	3.99	2.43	0.61	9.83	0.90	3.80	2.63	4.68
	LF	10	7.30	3.20	0.44	12.39	3.87	7.49	4.07	9.65
GPx	KF	10	282.32	61.82	0.22	402.93	201.93	280.84	218.10	312.05
	LF	10	443.17	167.79	0.38	784.83	270.64	390.35	332.69	487.44
GR	KF	9	70.26	15.92	0.23	94.99	47.63	71.98	54.29	79.21
	LF	10	71.79	15.96	0.22	100.50	45.56	71.60	61.36	81.14
CAT	KF	10	376.51	135.59	0.36	547.01	197.04	371.16	222.68	501.20
	LF	10	564.99	114.37	0.20	770.93	409.85	569.48	476.14	655.38
MT	KF	10	0.71	0.30	0.42	1.28	0.40	0.60	0.52	0.76
	LF	10	0.85	0.33	0.39	1.39	0.40	0.78	0.58	1.19
TBARS	KF	10	18.50	2.61	0.14	23.12	15.00	18.13	17.22	19.72
	LF	10	18.00	1.48	0.08	20.67	16.34	17.94	16.78	18.67

Table 6: Descriptive data material for measured parameters in hepatic tissue of Somateria mollissima from Kongsfjorden and Liefdefjorden in 2009. The table shows the levels of the metals Hg, Se, Cd and Zn (µg/g dw), the molar ratio of Hg:Se, tGSH (mmol/g ww), GSSG (mmol/g ww), ratio tGSH:GSSG, GPx (nmol/min/mg protein ww), GR (nmol/min/mg protein ww), CAT (µmol/min/mg protein ww), MT (µg/g ww) and TBARS (nmol/mg ww). Values are given as mean, standard deviation, maximum, minimum and 0.25% and 0.75% percentiles.

Data material 2009	**Group**	**n**	**Mean**	**Std Dev.**	**Std. Dev/ mean**	**Max**	**Min**	**Median**	**0.25%**	**0.75%**
Hg	KF	10	1.75	0.64	0.36	3.01	0.76	1.74	1.44	1.88
	LF	10	1.56	0.93	0.60	3.81	0.71	1.30	0.95	1.49
Se	KF	10	22.77	4.47	0.20	30.07	16.23	22.16	19.49	26.92
	LF	10	25.51	17.43	0.68	64.79	9.87	21.20	12.06	31.46
Cd	KF	10	17.36	13.62	0.78	51.43	3.42	14.34	8.48	20.65
	LF	10	24.39	19.46	0.80	75.61	7.04	19.31	15.43	25.58
Zn	KF	10	182.91	43.48	0.24	247.10	123.27	173.52	150.37	219.57
	LF	10	169.52	53.94	0.32	278.85	110.59	155.16	131.03	197.76
Ratio Hg:Se	KF	10	0.031	0.013	0.421	0.061	0.015	0.030	0.019	0.035
	LF	10	0.030	0.019	0.617	0.073	0.008	0.026	0.020	0.041
tGSH	KF	10	2.28	1.31	0.58	4.44	0.62	2.10	1.12	2.92
	LF	10	2.25	1.65	0.73	5.39	0.69	1.57	0.89	3.64
GSSG	KF	10	0.49	0.15	0.30	0.74	0.23	0.52	0.36	0.58
	LF	10	0.41	0.25	0.61	1.01	0.07	0.40	0.30	0.44
Ratio tGSH:GSSG	KF	10	5.15	3.45	0.67	12.95	1.20	5.08	2.08	6.86
	LF	10	6.37	3.71	0.58	12.19	1.01	4.98	4.11	9.46
GPx	KF	10	399.88	83.54	0.21	531.80	278.74	380.44	344.42	483.46
	LF	10	414.75	135.91	0.33	716.47	261.34	405.89	293.83	482.63
GR	KF	10	82.81	19.06	0.23	109.29	56.08	79.42	65.76	101.52
	LF	10	64.43	20.68	0.32	101.09	33.93	64.92	50.89	78.47
CAT	KF	10	396.70	196.23	0.49	886.71	223.64	353.39	291.68	402.51
	LF	10	458.90	185.86	0.41	864.54	249.76	404.64	315.82	548.60
MT	KF	10	0.69	0.32	0.46	1.48	0.44	0.62	0.47	0.77
	LF	10	0.85	0.38	0.45	1.69	0.51	0.72	0.55	1.13
TBARS	KF	10	17.37	2.99	0.17	23.34	14.00	16.50	15.04	19.34
	LF	9	15.18	1.82	0.12	17.34	11.76	15.31	14.20	16.50

D. Statistical comparisons

Table 7: Results from the statistical comparisons showing the each group, respective test, p-value, test variable, normality, equality of variance, power of test and transformation. 4 tests were conducted for each variable (KF and LF), (2008 and 2009), (KF 2008 and KF 2009) (LF 2008 and 2009).

Data material	Test	Groups	P-value	Test variable	Normality	Equal Variance	Power	Transformation
Hg	t-test	**2008:** Kongsfjorden, Liefdefjorden	**P = 0.170**	t = 1.429 df=18	P = 0.115	P = 0.288	**0.152**	log
	t-test	**2009:** Kongsfjorden, Liefdefjorden	**P = 0.391**	t = 0.879 df=18	P = 0.555	P = 0.613	**0.050**	log
	t-test	**Kongsfjorden:** 2008, 2009	**P = 0.966**	t = 0.0430 df=18	P = 0.569	P = 0.678	**0.050**	log
	t-test	**Liefdefjorden:** 2008, 2009	**P = 0.767**	t = -0.301 df=18	P = 0.374	P = 0.273	**0.050**	log
Se	Mann-Whitney Rank Sum Test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0,038**	T = 77,000	P < 0,050			-
	Mann-Whitney Rank Sum Test	**2009:** Kongsfjorden, Liefdefjorden	**P = 0,734**	T = 110,000	P < 0,050			-
	t-test	**Kongsfjorden:** 2008, 2009	**P = 0.193**	t = -1.353 df=18	P = 0.427	P = 0.092	**0.131**	-
	Mann-Whitney Rank Sum Test	**Liefdefjorden:** 2008, 2009	**P = 0.345**	T = 118.000	P < 0.050			-
Cd	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0,427**	t = -0,814 df=18	P = 0,532	P = 0,562	0.050	-
	Mann-Whitney Rank Sum Test	**2009**: Kongsfjorden, Liefdefjorden	**P = 0,273**	T = 90,000	P < 0,050			-
	Mann-Whitney Rank Sum Test	**Kongsfjorden:** 2008, 2009	**P = 0.623**	T = 112.000	P < 0.050			-
	Mann-Whitney Rank Sum Test	**Liefdefjorden:** 2008, 2009	**P = 0.791**	T = 109.000	P < 0.051			-
Zn	Mann-Whitney Rank Sum Test	**2008:** Kongsfjorden, Liefdefjorden	**P = 0.521**	T = 96.000	P < 0.050			-
	t-test	**2009**: Kongsfjorden, Liefdefjorden	**P = 0.549**	t = 0.611 df=18	P = 0.133	P = 0.899	0.050	-
	Mann-Whitney Rank Sum Test	**Kongsfjorden:** 2008, 2009	**P = 0.850**	T = 102.000	P < 0.050			-
	t-test	**Liefdefjorden:** 2008, 2009	**P = 0.418**	t = 0.830 df=18	P = 0.055	P = 0.946	0.050	-
Ratio Hg:Se	t-test	**2008:** Kongsfjorden, Liefdefjorden	**P = 0.005**	t = 3.165 df=18	P = 0.366	P = 0.343		log
	t-test	**2009:** Kongsfjorden, Liefdefjorden	**P = 0,911**	t = 0,113 df=18	P = 0,094	P = 0,454	0.050	-
	t-test	**Kongsfjorden:** 2008, 2009	**P = 0.119**	t = 1.637 df=18	P = 0.071	P = 0.062	0.220	-
	t-test	**Liefdefjorden:** 2008, 2009	**P = 0.305**	t = -1.055 df=18	P = 0.842	P = 0.467	0.059	log
tGSH	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0.799**	t = -0.258 df=18	P = 0.128	P = 0.972	0.050	-
	t-test	**2009**: Kongsfjorden, Liefdefjorden	**P = 0.788**	t = 0.272 df=18	P = 0.422	P = 0.602	0.050	log
	t-test	**Kongsfjorden**: 2008, 2009	**P = 0.876**	t = -0.158 df=18	P = 0.064	P = 0.835	0.050	-
	t-test	**Liefdefjorden**: 2008, 2009	**P = 0.905**	t = 0.121 df=18	P = 0.152	P = 0.516	0.050	-
GSSG	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0,033**	t = 2,310 df=18	P = 0,431	P = 0,073	0.497	-

	Mann-Whitney Rank Sum Test	**2009**: Kongsfjorden, Liefdefjorden	**P = 0,241**	T = 121,000	P < 0,050			-
	t-test	**Kongsfjorden**: 2008, 2009	**P = 0.313**	t = 1.039 df=18	P = 0.726	P = 0.055	0.056	-
	Mann-Whitney Rank Sum Test	**Liefdefjorden**: 2008, 2009	**P = 0.427**	T = 94.000	P < 0.050			-
Ratio tGSH:GSSG	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0,018**	t = -2,606 df=18	P = 0,139	P = 0,159	0.624	-
	t-test	**2009**: Kongsfjorden, Liefdefjorden	**P = 0,456**	t = -0,762 df=18	P = 0,579	P = 0,693	0.050	-
	t-test	**Kongsfjorden**: 2008, 2009	**P = 0.503**	t = -0.683 df=18	P = 0.397	P = 0.624	0.050	log
	t-test	**Liefdefjorden**: 2008, 2009	**P = 0.553**	t = 0.605 df=18	P = 0.270	P = 0.823	0.050	-
GPx	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0.004**	t = -3.246 df=18	P = 0.362	P = 0.223	0.846	log
	t-test	**2009**: Kongsfjorden, Liefdefjorden	**P = 0,772**	t = -0,295 df=18	P = 0,213	P = 0,329	0.050	-
	t-test	**Kongsfjorden**: 2008, 2009	**P = 0.002**	t = -3.577 df=18	P = 0.781	P = 0.300	0.916	-
	t-test	**Liefdefjorden**: 2008, 2009	**P = 0.712**	t = 0.375 df=18	P = 0.210	P = 0.781	0.050	log
GR	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0,837**	t = -0,209 df=17	P = 0,866	P = 0,789	0.050	-
	t-test	**2009:** Kongsfjorden, Liefdefjorden	**P = 0,053**	t = 2,066 df=18	P = 0,687	P = 0,908	0.390	-
	t-test	**Kongsfjorden:** 2008, 2009	**P = 0.140**	t = -1.547 df=17	P = 0.254	P = 0.557	0.189	-
	t-test	**Liefdefjorden:** 2008, 2009	**P = 0.385**	t = 0.891 df=18	P = 0.972	P = 0.351	0.050	-
CAT	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0,003**	t = -3,360 df=18	P = 0,252	P = 0,376	0.873	-
	t-test	**2009:** Kongsfjorden, Liefdefjorden	**P = 0.371**	t = -0.918 df=18	P = 0.417	P = 0.871	0.050	log
	t-test	**Kongsfjorden** 2008, 2009	**P = 0.859**	t = -0.180 df=18	P = 0.442	P = 0.442	0.050	log
	t-test	**Liefdefjorden**: 2008, 2009	**P = 0.077**	t = 1.876 df=18	P = 0.968	P = 0.089	0.310	log
MT	t-test	**2008:** Kongsfjorden, Liefdefjorden	**P = 0,313**	t = -1,039 df=18	P = 0,051	P = 0,595	0.056	-
	Mann-Whitney Rank Sum Test	**2009:** Kongsfjorden, Liefdefjorden	**P = 0.140**	T = 85.000		P < 0,050		-
	t-test	**Kongsfjorden:** 2008, 2009	**P = 0.895**	t = 0.134 df=18	P = 0.101	P = 0.852	0.050	log
	t-test	**Liefdefjorden:** 2008, 2009	**P = 0.993**	t = -0.00859 df=18	P = 0.064	P = 0.921	0.050	-
TBARS	t-test	**2008**: Kongsfjorden, Liefdefjorden	**P = 0,611**	t= 0,518	P = 0,594	P = 0,133	0.050	-
	t-test	**2009:** Kongsfjorden, Liefdefjorden	**P = 0,075**	t = 1,898	P = 0,434	P = 0,312	0.318	-
	t-test	**Kongsfjorden**: 2008, 2009	**P = 0.380**	t = 0.899 df=18	P = 0.196	P = 0.677	0.050	-
	t-test	**Liefdefjorden**: 2008, 2009	**P = 0.002**	t = 3.729 df=17	P = 0.920	P = 0.352	0.050	-