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THE ROLE OF OXIDATIVE STRESS IN ENVIRONMENTAL RESPONSES OF FENNOSCANDIAN ANIMALS

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ABSTRACT

All aerobic organisms have to deal with the toxicity of oxygen. Oxygen enables more efficient energy production compared to anaerobic respiration or fermentation, but at the same time reactive oxygen species (ROS) are being formed. ROS can also be produced by external factors such as UV-radiation and contamination. ROS can cause damage to biomolecules such as DNA, lipids and proteins and organisms try to keep the damage as small as possible by repairing biomolecules and metabolizing ROS. All ROS are not harmful, because they are used as signaling molecules. To cope against ROS organism have an antioxidant (AOX) system which consists both enzymatic and non-enzymatic AOX defense. Some AOX are produced by the organism itself and some are gained via diet.

In this thesis I studied environmentally caused changes in the redox regulation of different wild vertebrate animals to gain knowledge on the temporal, spatial and pollution-derived-effects on the AOX systems. As study species I used barn swallow, ringed seal and the Baltic salmon. For the barn swallow the main interest was the seasonal fluctuation in the redox regulation and its connection to migration and breeding. The more contaminated ringed seals of the Baltic Sea were compared to seals from cleaner Svalbard to investigate whether they suffered from contaminant induced oxidative stress. The regional and temporal variation in redox regulation and regional variation in mRNA and protein expressions of Baltic salmon were studied to gain knowledge if the salmon from different areas are equally stressed. As a comparative aspect the redox responses of these different species were investigated to see which parts of the AOX system are substantial in which species.

Certain parts of AOX system were connected to breeding and others to migration in barn swallows, there was also differences in biotransformation between birds caught from Africa and Finland. The Baltic ringed seal did not differ much from the seals from Svalbard, despite the difference in contaminant load. A possible explanation to this could be the enhanced AOX mechanisms against dive-associated oxidative stress in diving air-breathing animals, which also helps to cope with ROS derived from other sourses. The Baltic salmon from Gulf of Finland (GoF) showed higher activities in their AOX defense enzymes and more oxidative damage than fish from other areas. Also on mRNA and proteomic level, stress related metabolic changes were most profound in in the fish from GoF.

Mainly my findings on species related differences followed the pattern of mammals showing highest activities and least damage and birds showing lower activities and most damage, fish being intermediate. In general, the glutathione recycling-related enzymes and the ratio of oxidized and reduced glutathione seemed to be the most affected parameters in all of the species.

TIIVISTELMÄ

Kaikkien happea käyttävien eliöiden pitää tulla toimeen hapen myrkyllisyyden kanssa. Happi mahdollistaa paljon tehokkaamman energiantuotannon kuin hapettomat hengitys- tai käymisreaktiot, mutta samalla prosessissa vapautuu reaktiivisia happiradikaaleja (ROS). ROS:a voi muodostua myös ulkoisten tekijöiden, kuten UV-säteilyn ja saastumisen seurauksena. ROS:t voivat vaurioittaa biomolekyylejä kuten DNA:ta, rasvoja sekä proteiineja ja eliöt pyrkivätkin pitämään vaurioiden määrän niin pienenä kuin mahdollista, korjaamalla biomolekyylejä ja käsittelemällä ROS:ja. Kaikki ROS:t eivät ole vahingollisia, koska osa toimii mm. tiedonvälitysmolekyyleinä. Jotta eliöt pärjäisivät ROS:ja vastaan, on niille kehittynyt antioksidanttipuolustusjärjestelmä (AOX), johon kuuluvat entsymaattiset ja ei-entsymaattiset systeemit. Osan AOX:ta eliö tuottaa itse ja osa saadaan ravinnosta.

Olen tutkinut väitöskirjassani ympäristön aiheuttamia muutoksia villien selkärankaisten hapetus-pelkistys säätelyyn saadakseni tietoa ajallisten, paikallisten ja saasteiden aiheuttamien muutosten vaikutusta AOX puolustukseen. Tutkimuslajeina käytin haarapääskyjä, Itämeren norppia ja Itämeren lohia. Haarapääskytutkimuksessa suurin mielenkiinto kohdistui hapetus-pelkistyssäätelyn vuodenaikaisvaihteluun ja sen yhteyteen lintujen muuttoon ja lisääntymiseen. Itämeren norppia verrattiin puhtaammissa oloissa eläviin Huippuvuorten norppiin, jotta saataisiin tietoa kärsivätkö ne saasteiden aiheuttamasta hapetusstressistä. Itämeren lohella tutkittiin sekä ajallista, että paikallista vaihtelua hapetus-pelkistys säätelyssä ja eroja mRNA- ja proteiinituotannossa, jotta saataisiin selville ovatko eri alueiden lohet yhtä stressaantuneita. Lisäksi näiden kolmen eri lajin hapetus-pelkistys vasteita vertailtiin, jotta tiedettäisiin mitkä osat AOX puolustuksesta ovat tärkeitä milläkin lajilla.

Haarapääskyllä tietyt osat AOX systeemissä liittyivät lisääntymiseen ja toiset muuttoon, lisäksi Afrikasta pyydettyjen lintujen myrkkypuolustuksessa oli eroa Suomesta pyydettyihin lintuihin nähden. Itämeren norpat eivät eronneet paljoakaan Huippuvuorten norpista huolimatta suurista eroista saastepitoisuuksissa. Yksi mahdollinen syy tähän saattaisi olla sukeltavien, mutta ilmaa hengittävien eliöiden AOX puolustusentsyymeiden kohonnut tehokkuus, joka auttaa niitä selvitymään paremmin sukeltamiseen liittyvästä hapetusstressistä, sekä muista syistä johtuvasta liiallisesta ROS tuotannosta. Suomenlahden lohien AOX entsyymien aktiivisuudet olivat kohonneita muiden alueiden lohiin verrattuna. Lisäksi Suomenlahden lohilla havaittiin stressiaineenvaihduntaan liittyviä muutoksia mRNA- ja proteiinitasolla.

Pääasiassa tulokseni eri lajienvälisistä eroista noudatti jo aiemmin havaittua linjaa: nisäkkäillä oli korkeimmat entsyymiaktiivisuudet ja vähiten vaurioita biomolekyyleissä, kun taas linnuilla oli matalimmat aktiivisuudet ja eniten vaurioita. Kalat puolestaan olivat tältä väliltä. Yleisesti ottaen glutationin hapetus-pelkistusreaktioihin liittyvien entsyymien aktiivisuudet, sekä glutationin pelkistyneen ja hapettuneen muodon suhde muuttuivat eniten kaikilla lajeilla.

ABBREVIATIONS

AhR	Aryl hydrocarbon receptor
AOX	Antioxidant
ARNT	AhR nuclear translocator
BS	Bothnian Sea
CAT	Catalase
CBS	Central and Southern Baltic Sea
CIA	Co-inertia analysis
CYP	Cytochrome P450
EROD	Ethoxyresorufin-*O*-deethylase
ETC	Electron transport chain
GoF	Gulf of Finland
GO	Gene ontology
GP	Glutathione peroxidase
G6PDH	Glucose-6-phosphate dehydrogenase
GR	Glutathione reductase
GSH	Glutathione, reduced form
GSSG	Glutathione, oxidized form
GSH/GSSG	The ratio between reduced and oxidized glutathione
GST	Glutathione-S-transferase
LPO	Lipid peroxidation
MLSP	Maximum life span
NADPH	Nicotinamide adenine dinucleotide phosphate
NS	Reactive nitrogen species
PAH	Polycyclic aromatic hydrocarbons
PCA	Principal component analysis
PCB	Polychlorinated biphenyls
ROS	Reactive oxygen species
SOD	Superoxide dismutase
totGSH	Both reduced and oxidized glutathione
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1

LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and manuscripts referred to in the text by their Roman numerals:

I Raja-aho S*, Kanerva M*, Eeva T, Lehikoinen E, Suorsa P, Gao K, Vosloo D & Nikinmaa M. 2012. Seasonal variation in the regulation of redox state and some biotransformation enzyme activities in the barn swallow (*Hirundo rustica* L.). *Physiological and Biochemical Zoology*, 85 (2) 148-158. *SR & MK contributed equally to this article

II Kanerva M*, Routti H*, Tamuz Y, Nyman M & Nikinmaa M. 2012. Antioxidative defense and oxidative stress in ringed seals (*Pusa hispida*) from differently polluted areas. *Aquatic Toxicology*, 114-115, 67-72. *MK & HR contributed equally to this article

III Vuori K A, Kanerva M, Ikonen E & Nikinmaa M. 2008. Oxidative stress during Baltic salmon feeding migration may be associated with yolk-sac fry mortality**.** *Environmental Science & Technology*, 42:2668-2673.

IV Kanerva M, Vehmas A, Nikinmaa M & Vuori K A. Spatial variation in gene expression of Atlantic salmon during feeding migration in the Baltic Sea. Manuscript accepted to *Environmental science and trechnology*.

1. INTRODUCTION

1.1. Oxygen

Over 2.2 billion years ago oxygen started to appear in significant amounts in the Earth's atmosphere (Frei et al., 2009). This was mainly due to the appearance of photosynthesis by cyanobacteria (Kasting, 1993). The rise in atmospheric O2 helped the formation of ozone (O3) layer in the stratosphere, by making the Earth more inhabitable in many ways, and the removal of ferrous (Fe2+) from the aquatic environment (Frei et al., 2009), which aided to prevent Fenton chemistry, where organic molecules are oxidized in the presence of H2O2 and Fe2+ (Halliwell and Gutteridge, 2007). Despite the advantages of increased O2 levels, it also placed severe stress on the existing organisms (Halliwell and Gutteridge, 2007). Some of them continued to use anaerobic respiration or fermentation for their energy synthesis, but remained restricted to places without O2. Other organisms began to evolve defense systems against the toxicity of O2 and started using O2 for efficient energy production allowing the development of multicellular organism (Lane, 2002). In fact the aerobic glucose metabolism produces about 15 times more energy than anaerobic glucose metabolism per molecule utilized (Halliwell and Gutteridge, 2007).

About 80% of energy in animal cells is produced in mitochondria (Halliwell and Gutteridge, 2007). These cell organelles have a system called the electron transport chain (ETC) where O2 is reduced to H2O and energy is being produced. This energy is used for phosphorylation of ATP, the most important energy storage molecule of most cells. In the oxidation-reduction processes of mitochondria oxygen radicals, called reactive oxygen species (ROS) and other oxidizing products are being formed and some of them may leak outside the ETC system (Beckman and Ames, 1998). Other main sources of ROS in the cells are the peroxisomal fatty acid oxidation, the "respiratory burst" of phagocytic cells and the cytochrome P450 reactions (Beckman and Ames, 1998). The phagocytic cells, that are part of the immune system, use ROS and other oxidants to kill pathogens (Droge, 2002; Finkel and Holbrook, 2000). The cythochrome P450 enzymes metabolize e.g. xenobiotic compounds, cholesterol, vitamin D3 and retinoic acid in various oxidation and reduction processes, thus producing ROS (Coon, 2005). Endogenous processes are not the only things that cause ROS and other oxidants to occur. External sources such as UV light, ionizing radiation, and xenobiotics can increase the amount of ROS in organisms (Balaban et al., 2005; Finkel and Holbrook, 2000).

1.2. Reactive oxygen species (ROS) and their effects

Reactive oxygen species (ROS) is a broad term for various oxygen radicals and non-radical derivatives of O_2, such as the hydrogen peroxide, hypochlorous acid and ozone. They belong to the group of free radicals, which are basically any species that are capable of independent existence and contains one or more unpaired electrons (Halliwell and Gutteridge, 2007). The reactivity of ROS varies a lot; some are more selective in their reactivity with biological molecules than other and hence are less harmful. The endogenous ROS are the greatest threat to organisms and even though it

seems that ROS from exogenous sources can attack biomolecules in some circumstances, the menace is not comparable to in vivo production (Balaban et al., 2005).

In biological context the most important oxygen free radicals are superoxide, hydroxyl radical and nitric oxide (Finkel and Holbrook, 2000). These radicals are very unstable and persist only for micro- or nanoseconds, but they trigger chain reactions and pass reactivity on to other, possibly more dangerous compound. The most common non-radicals are hydrogen peroxide, hypochlorous acid and singlet oxygen. They show higher stability, from minutes to hours and can therefore cause oxidative damage to biomolecules (Surai, 2002). Reactive nitrogen species (NS) are also known to cause damage, but they are less studied and considered to be less of a threat, when compared to ROS. In vitro studies have shown that 1-2% of consumed oxygen is funneled into ROS generation but in vivo this is a lot less, probably around 0.2%, which is still a considerable amount (Balaban et al., 2005).

Not all of the ROS production has a negative effect on organisms. Many higher organisms use NS and ROS also as signaling molecules for physiological functions. These include monitoring of oxygen tension in the control of ventilation, erythropoietin production, regulation of vascular tone and signal transduction from membrane receptors in various physiological processes. In these cases NS and ROS are typically generated by tightly regulated enzymes such as NS synthase and NAD(P)H oxidase isoforms (Droge, 2002). Because ROS can be involved in signaling, ROS effects can occur even when the changes are not large enough to cause measurable direct damage.

1.2.1. Damage caused by ROS

ROS can cause damage to biomolecules such as DNA, proteins and lipids and this can accelerate for example cell senescence and cell death (Halliwell and Gutteridge, 2007). Organisms try to keep the damage as small as possible by metabolizing ROS and by repairing the occurring damage, although this is not always possible (Halliwell and Gutteridge, 2007).

It has been thought that the oxidation and methylation of DNA bases have the most serious consequences on phenotype (Falnes et al., 2007) and that about 10 000 base modifications per day are caused by ROS (Ames et al., 1991). Mitochondrial DNA seems to be especially vulnerable to damage caused by ROS, because of the close proximity of most produced ROS and lower level of repair than in nuclear DNA (Finkel and Holbrook, 2000). In addition to causing point mutations to DNA (Evans et al., 2004), ROS can also reduce the length of telomeres, the chromosome caps, that are important for genome stability. This shortening of telomeres has been associated with hastened cell senescence (Richter and von Zglinicki, 2007).

ROS can cause damage to lipids and this can have a significant effect on membrane structure and properties and can lead to disruption of membrane-bound proteins (Beckman and Ames, 1998). Polyunsaturated fatty acids are more vulnerable to oxidation compared to monounsaturated or saturated fatty acids and their proportion in

membrane composition can affect the rate of oxidative damage (Hulbert et al., 2007). Oxidized lipids can also trigger chain reactions where the end products may act as mutagens or inactivate enzymes; they can also cause damage to DNA and proteins (Beckman and Ames, 1998).

Oxidative damage to proteins can impair the function of receptors, antibodies, transport proteins and enzymes (Halliwell and Gutteridge, 2007). ROS induce the oxidation of amino acid residue side chains, formation of protein-protein cross-linkages and breakdown of peptide ponds, which results in protein fragmentation (Berlett and Stadtman, 1997). Some amino acids are more vulnerable to oxidation than others; examples are tryptophan, tyrosine, histidine and cysteine. Oxidation can also alter the secondary and tertiary structure of proteins leading to functional problems (Droge, 2002).

1.3. The antioxidant (AOX) defense system

The simplest defense against O_2 toxicity is to avoid it. This has been accomplished by packing redox constituents together in electron transport chains, so that leaking of O_2 is less likely. Also lowering the amount of O_2 in parts of the organism helps to keep the problems smaller (Halliwell and Gutteridge, 2007). Another line of defense is structural, so that the most sensitive or important structures are more resistant to ROS as a consequence of their amino or fatty acid composition (Hulbert et al., 2007). However these defense mechanisms are not enough for organisms to prevent the oxidation by ROS and therefore specific non-enzymatic and enzymatic antioxidants have evolved to counteract this. Antioxidants can be described as substances that can, at relatively low concentrations, delay, prevent or remove oxidative damage to target molecule (Halliwell and Gutteridge, 2007).

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide $O_2^{\bullet-}$ to H_2O_2 and oxygen (O_2). In vertebrates there are two forms of SOD, MnSOD and CuZnSOD (Fridovich, 1995). There are some differences between these two forms, CuZnSOD is inhibited by CN^- or diethyldithiolcarbamate and it is inactivated by prolonged exposure to H_2O_2, whereas MnSOD is not (Halliwell and Gutteridge, 2007). Both products of SOD catalyzed reaction (Fig. 1), H_2O_2 and singlet oxygen, are potential ROS that can cause damage. The generated H_2O_2 can be removed by two types of enzymes; the catalases (CAT) and the peroxidases. CAT catalyzes the direct decomposition of H_2O_2 to ground state O_2 and water, whereas peroxidase enzymes remove H_2O_2 by using it to oxidize another substrate (Chance et al., 1979). Examples of peroxidases are glutathione peroxidases (GP), peroxiredoxins and thioredoxin. When the concentrations of H_2O_2 is high, CAT has a greater role, whereas GP, of which there are at least five types, deals with lower concentrations (Behne and Kyriakopoulos, 2001; Halliwell and Gutteridge, 2007).

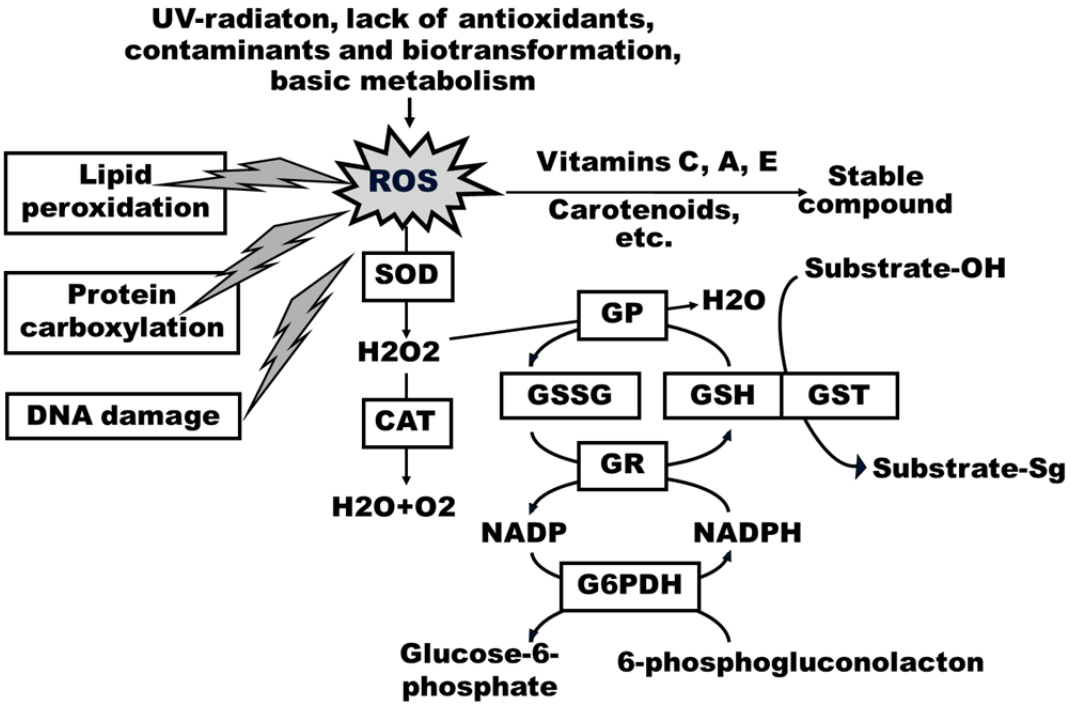

Figure 1. Production of reactive oxygen species (ROS), the antioxidant (AOX) defense system and the damage done to biomolecules. ROS are produced especially in the mitochondria of cells, but contaminants and biotransformation processes, UV-radiation etc. can cause ROS to accumulate. Several enzymatic and chemical ROS production processes seem to be pO_2 dependent; higher amounts of ROS are formed when the pO_2 increases. ROS are handled by the antioxidant system which consist the non-enzymatic part, such as vitamins and other small molecules that scavenge ROS and enzymatic part represented partially in here. Superoxide dismutase (SOD) transforms $O_2^{\bullet-}$ to H_2O_2 and O_2, which is then handled either by catalase (CAT) or glutathione peroxidase (GP) and turned into water. In this reaction CAT does not need any other substrate, but GP oxidizes glutathione in this process. Glutathione reductase (GR) and glucose-6-phosphate-dehydrogenase (G6PDH) can be considered as parts of the antioxidant system since the former reduces glutathione back to its active form and the latter produces the energy (NADPH) for this process. Glutathione-S-transferase (GST) is part of the biotransformation system, but it is closely connected to antioxidant system since it both enhances and reduces oxidative stress by consuming glutathione in biotransformation processes, but it can also metabolize oxidized lipids.

Glutathione (GSH) is one of the most important and active non-enzymatic AOX in biological systems (Schafer and Buettner, 2001). This tripeptide acts as cofactor for GP enzymes and as an AOX that can react with ROS independently (Chance et al., 1979). GSH acts as a reducing agent by donating a reducing equivalent, the remaining glutathione radical combines with another glutathione radical, forming a dimer; GSSG. GSSG can be reduced back by glutathione reductase (GR) enzymes in a NADPH powered reaction. Although GR doesn't deal with ROS directly it's often considered as a part of the antioxidant system, along with the NADPH producing glucose-6-

phosphate dehydrogenase, the first enzyme of the pentose phosphate pathway, because of the recycling of GSSG back to reduced form of GSH (Halliwell and Gutteridge, 2007). The ratio between the reduced and oxidized forms of glutathione has been considered an important indicator of the redox status of cells (Wu et al., 2004). GSH is also a part of xenobiotic metabolism, by acting as a conjugant in a reaction catalyzed by glutathione S-transferase (GST) enzymes (Hayes et al., 2005). There are seven classes of GSTs of which alpha, mu and pi are most abundant in mammals (Hayes and Pulford, 1995). Some GSTs can catalyze the reaction of organic peroxides with GSH, thus preventing lipid peroxidation (Prohaska and Ganther, 1977). Xenobiotic metabolism can enhance oxidative stress in two ways, by producing more ROS in the oxidation-reduction processes of cytochrome P450 (CYPs) and by depleting GSH in the conjugation reactions (Halliwell and Gutteridge, 2007).

Metal chelating proteins like metallothionein can be considered as a part of the AOX system since by chelating metals they decrease metal-induced ROS generation (Koivula and Eeva, 2010). Many other antioxidants are derived from the diet, such as vitamins and carotenoids. Several studies have shown that vitamins C, E and A, and carotenoids have ROS scavenging properties, but often there are not enough supporting in vivo data to assure this. For example vitamin C has been noted to have good antioxidant capacity in vitro but its reactivity *in vivo* has not been determined yet (Halliwell and Gutteridge, 2007). Vitamin E is considered to be one of the most important inhibitors of lipid peroxidation, but just like with vitamin C, there is no solid *in vivo* proof (Halliwell and Gutteridge, 2007).

The damaged biomolecules can be repaired or removed. For DNA damage there are several studied repair mechanisms related to cell cycle (Kastan and Bartek, 2004). For damaged lipids and proteins the repair and destruction systems are less well known (Halliwell and Gutteridge, 2007).

1.4. Oxidative stress

Oxidative stress is defined as an imbalance between ROS and AOX and it can be caused either by the excess production of ROS, depletion of AOX or both of these (Fig 2.) (Scandalios, 2005). In the evaluation of whether organism is suffering from oxidative stress prior knowledge of the basal level of redox balance is required. Measurements of ROS production or AOX defense alone are not sufficient to tell about the balance, but combined information of these and the damage caused by ROS are needed (Monaghan et al., 2009).

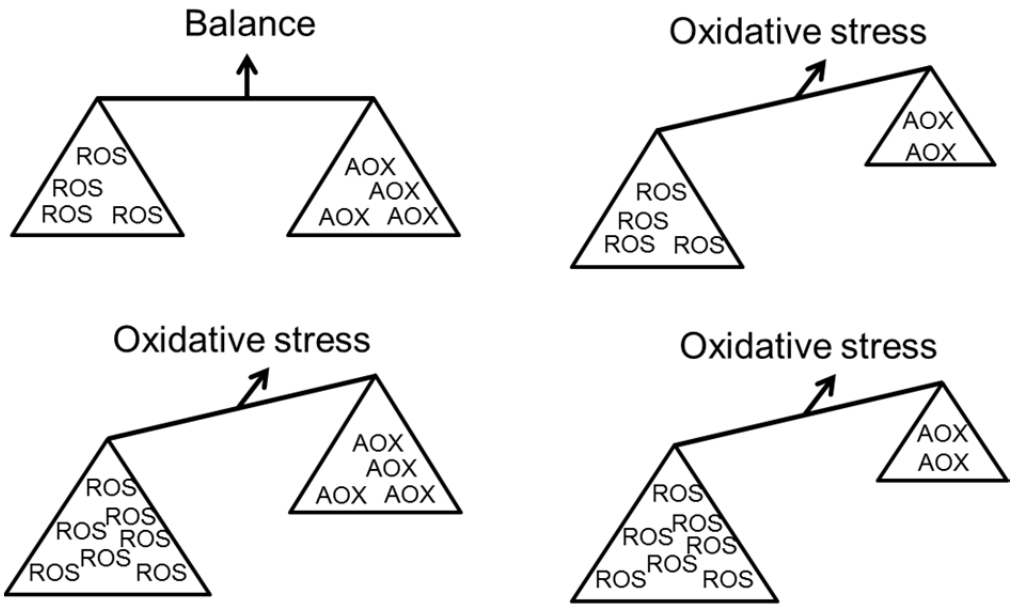

Figure 2. Oxidative stress is the imbalance between reactive oxygen species (ROS) and antioxidants (AOX). It can be caused by the lack of AOX, the excess production of ROS, or the combination of these. Modified from (Scandalios, 2005).

1.5. Life history and oxidative stress

1.5.1. External effects

Both lower and higher temperatures than the optimum of an organism can cause oxidative stress and this has been noticed both in endo- and ectothermic animals (Sahin and Gumuslu, 2007; Selman et al., 2000; Bagnyukova et al., 2007a; Bagnyukova et al., 2007b; Heise et al., 2006a; Heise et al., 2006b). The change in temperature, or being outside the organism temperature optimum, usually increases metabolic rate and thereby ROS production. This does not necessarily mean more oxidative damage, but more effective AOX defense (Beamonte-Barrientos and Verhulst, 2013; Lamarre et al., 2009; Malek et al., 2004). In aquatic systems cold temperature increases the solubility of oxygen, but lower its' conductance. Despite the slower metabolic rate in ectotherms living in the cold, ROS production per mg of protein is similar to animals living in temperate and warm areas (Abele and Puntarulo, 2004).

For aquatic organisms, especially for water breathing ones, changes in salinity can affect oxidative stress levels. Besides affecting the osmoregulatory processes, it has been noted that increase or decrease in salinity could enhance ROS production (Liu et al., 2007; Martinez-Alvarez et al., 2002). There are also studies where the effects of salinity changes have been studied in co-exposure with, for example, metals. Salinity can affect the toxicity of metals either enhancing or suppressing the oxidative stress (Baysoy et al., 2012; Loro et al., 2012). There have also been cases where changes in

salinity have no effect and this might be because the organism is adapted to changing salinity (Adeyemi and Klerks, 2012).

Trace metals and organic pollutants found in the environment can cause oxidative stress to organism by enhancing the generation of ROS (Halliwell and Gutteridge, 2007). Trace metals (As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se, V and Zn) can generate ROS because of their ability to lose electrons and to catalyze Haber-Weiss and Fenton reactions (redox active metals), where the oxidation state of the metal changes and oxygen radicals are formed (Halliwell and Gutteridge, 2007). Metals can also deplete AOX such as GSH (redox inactive metals) thereby enhancing the onset of oxidative damage (Regoli, 2012). Organic pollutants like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), halogenated hydrocarbons, dioxin, and dioxin-like chemicals cause enhancement in the intracellular ROS production by induction of cytochrome P450 system (Stegeman and Lech, 1991). Most of the aromatic chemicals induce cytochrome P450 via the Aryl hydrocarbon receptor (AhR), which is a cytosolic xenobiotic binding protein. When AhR binds to a ligand it is activated and the ligand-AhR-complex then translocates into nucleus and dimerizes with AhR nuclear translocator (ARNT). This complex acts as a transcription factor by binding to specific DNA regions, DREs (dioxin response elements) or nowadays more commonly called the xenobiotic response element (Denison et al., 2002; Nikinmaa, 2014). Other genes, besides the cytochrome P450, that are induced by AhR include for example UGT1A1, UDP glucuronosyltransferase 1 family, polypeptide A1 and GST. Cytochrome P450 is a multigene family of enzymes that catalyze several oxidative reactions, called the phase I reactions (Meunier et al., 2004). In these reactions the polarity of the xenobiotic is increased by addition of, for example, hydroxyl group (Meunier et al., 2004). These reactions are usually fast, but sometimes, for example, the slow oxidation of certain xenobiotics cause the release of ROS (Schlezinger et al., 2006). After the oxidation the xenobiotics can be either eliminated without further modifications or conjugated with various endogenous compounds, such as GSH, to further decrease their lipophility (Jancova et al., 2010) to increase the ease of their elimination in bile. These reactions are called the phase II reactions and are catalyzed for example by GSTs. The metabolites of some PAH compounds can be pro-oxidants and can generate $O_2^{\bullet-}$ in the reactions with other intracellular compounds (Regoli, 2012). The metabolism of xenobiotics can thus cause oxidative stress in different ways, as by increasing the ROS production and by depleting antioxidants.

Some of the AOX defense is gained through diet, so it is possible that the lack of, e.g., vitamins in the diet can cause oxidative stress. However since the AOX defense is composed of multiple factors a lack in one substance can cause the synthesis and activation of others (Monaghan et al., 2009). Also the absorption of dietary AOX can be enhanced and the animal might be selective on the food source, favoring AOX-rich food (Catoni et al., 2008). The relevance of different antioxidants, both dietary and endogenous, in different taxonomic groups and life history traits varies. An example of this is vitamin C which can be produced endogenously by many birds and mammals but not by primates and most passerines (Catoni et al., 2008).

1.5.2. Internal effects

Reproduction is demanding for an animal, but the knowledge of how much oxidative stress it can cause, is scarce (Speakman, 2008). In general it is thought that reproduction causes oxidative stress. Oocyte production in birds generates ROS (Murdoch et al., 2005), also the capability to fight against ROS has been noticed to diminish the more eggs the female has laid (Bertrand et al., 2006). In mammals the elevated mitochondrial activity and ROS production in placenta can lead to oxidative stress (Myatt and Cui, 2004) and in human studies it has been found that gestating women have more oxidative damage (lipid hydroperoxides in plasma) than non-gestating women (Toescu et al., 2002). Also rearing the offspring can generate oxidative stress. An experiment with zebra finch showed that individuals that were made to rear enlarged broods had weaker resistance against ROS (Alonso-Alvarez et al., 2004). Oxidative stress can also constrain reproduction and this has been mainly studied in the context of oxidative stress-producing contaminants in wild animals (Reglero et al., 2009; Subramanian et al., 2006). For wild animals from unpolluted sites the role of oxidative stress in constraining reproduction is not well known (Metcalfe and Alonso-Alvarez, 2010), but at least for birds there seems to be species differences in the AOX defense (Berglund et al., 2014).

Increase in physical activity can cause higher ROS production due to increased mitochondrial activity for ATP production (Leeuwenburgh and Heinecke, 2001), this link, however, is not straightforward (Metcalfe and Alonso-Alvarez, 2010). It is known that the ADP state of mitochondria is altered in moderate activation to produce less ROS (Navarro et al., 2004). Most of the information about oxidative stress and exercise comes from human studies and some of this information applies to other animals as well, but there are animals with different traits such as long distance migration where the adaptation to ROS production caused by exercise is different (Monaghan et al., 2009). Intermediate regular exercise often elevates the AOX system (Chandwaney et al., 1998) and this seems to be mediated by ROS themselves (Powers and Jackson, 2008).

1.6. The different redox characteristics of different vertebrate groups

Most of the information on redox characteristics in different vertebrate groups comes from studies of aging and they are concentrated mainly on mammals and birds. The connection between maximum life span (MLSP), body size, ROS productions and AOX defense has been under research (Hulbert et al., 2007). It can be generalized that in all vertebrate groups the MLSP increases when the body size of the animal increases, although there are some exceptions to this, like humans, bats and albatrosses that have a much longer MLSP than expected (Hulbert et al., 2007). In general it has been noted that in long-lived species ROS production is smaller than in short living species, the antioxidant enzyme activities are also lower or there is no correlation to longevity, and the non-enzymatic AOX are not correlatied or negatively connected to MLSP (Perez-Campo et al., 1998). Besides ROS production the membrane fatty acid composition has been connected to MLSP, with lower degree of polyunsaturated fatty acids, compared to monounsaturated and saturated fatty acids and better resistance to lipid peroxidation in longer living animals (Hulbert et al., 2007).

When comparing MLSP of similarly sized mammals, birds and ectothermic vertebrates, birds have higher life span expectancy than the other groups. Their ROS production is generally lower and membrane lipid peroxidation resistance is higher (Brown et al., 2009; Pamplona et al., 1999a; Pamplona et al., 1999b), but opposite findings on ROS production also exist (Brown et al., 2009; Montgomery et al., 2011). The temperature of the environment affects the metabolic rate of ectothermic animals more than that of birds and mammals which makes comparisons more difficult. When comparing the resting metabolic rate in ectothermic vertebrates in their optimal temperature to that of birds and mammals, it has been noted to be 5-10 times less. However, this does not seem to affect the MLSP which is similar especially to mammals (Hulbert et al., 2007).

1.7. Animals used in the thesis

1.7.1. Barn swallow

The barn swallow (*Hirundo rustica*) is a small insect-eating passerine bird that migrates long distances. The barn swallows breeding in Finland, Sweden and the Baltic countries usually winter in the eastern parts of South Africa (Ambrosini et al., 2009; Szep et al., 2006). They usually arrive to Finland in the beginning of May, breed in June and start their autumn migration in late August or early September. The barn swallows molt completely during their wintering time.

1.7.2. Ringed Seal

Ringed seal (*Pusa hispida*) is a circumpolar arctic marine mammal that is on a high level of the food chain. The average weight is from 50 to 70kg, length is about 1.5 m. The main food is fish such as cod and herring and invertebrates such as crustaceans. The Baltic Sea population is isolated from the Atlantic population and due to higher contaminant levels in the Baltic Sea it accumulates a higher concentration of for example persistent organic pollutants (Nyman et al., 2003; Nyman et al., 2002). The ringed seal population in Svalbard is considered one of the healthiest in the world (Krafft et al., 2006; Tryland et al., 2006) compared to populations found in the industrialized areas (Nyman et al., 2002) and can thus be used as reference population when studying contamination.

1.7.3. The Baltic salmon

The Baltic salmon is an isolated population of Atlantic salmon (*Salmo salar*) and it differs genetically from it. The Baltic salmon is an anadromous fish and its lifecycle contains both freshwater and brackish water periods. The salmon migrate from sea to rivers to spawn in the late autumn and the eggs hatch in the spring. Salmon spends one to four years in the river as a parr after which it migrates back to the sea for feeding as a smolt. The area of feeding migration depends at least partially on the river of origin (Ikonen, 2006; Vuori et al., 2012). After one to four years in the sea salmon returns to its origin river to spawn.

2. AIMS OF THE STUDY

The overall objective of this thesis was to study environmentally caused changes in the redox regulation of differed wild animals and to gain knowledge on the temporal, spatial and pollution derived effects on the antioxidant systems. One key point for analyzing AOX enzymes, ROS related damage and the broader scale transcriptome and proteome was to know the suitability of these measurements as biomarkers of environmentally caused stress of the animal. Also the differences between species were a point of interest.

The specific aims of the different studies were:

I) To gain knowledge of the seasonal fluctuation and the effects of different stages of life cycle such as breeding and migration on the ROS-related enzymatic activities and redox status of a long distance migratory bird, barn swallow.

II) To obtain information if changes in redox status of ringed seal are associated with high pollution level causing health problems affects. Further, it was asked, if the life strategy of the seal, repetitive ischemia reperfusion, which naturally produces ROS, affects the capacity to handle oxidative stress.

III) To study the regional and temporal differences in the redox status of Baltic salmon.

IV) To evaluate how regional differences affect the gene expression of Baltic salmon that have different oxidative stress statuses, by analyzing the differences in the mRNA and proteome of the fish and to see what are the main response pathways affected by these differences.

3. MATERIALS AND METHODS

3.1. Liver sampling

3.1.1. Barn swallow

Birds were caught with mist nets, after which they were killed and the livers were excised, frozen immediately in liquid nitrogen, and stored at -80 °C until measurements were done. Basic measurements such as body weight and wing length were taken before killing the bird, also the age and the sex of the bird were determined. The wintering barn swallows were caught before spring migration in Potchefstroom, South Africa (26°42'S, 27°06'_E; ~100 km from Johannesburg). Barn swallows that were arriving in spring, breeding in summer, and migrating in autumn were captured from the common roosts in Petteby, Finland (60°17'_N, 22°_11'_E). All the barn swallows were caught during the year 2007 and the licenses for animal experiments were granted by the Southwest Finland Regional Environment Centre (LOS-2006-L-416-254 and LOS-2007-L-22-254), the Central Animal Laboratory of the University of Turku (1661/06), and the South African Department of Agriculture, Conservation, Environment and Tourism (000377NW-06 and 000054NW-07).

3.1.2. Ringed seals

The Baltic ringed seals were shot in April and May 1997, 2002, 2006 and 2007 (65°_10'_N, 24°20'_E) with the permission of the Finnish Game and Fisheries granted by the Ministry of Forestry and Agriculture in Finland. The seals from the west coast of Svalbard, Norway (77°_50'_N to 79°_00'_N, 12°_00'_E to 17°_00'_E) were shot in May and June 1996 and 2007 with the special permission granted to the Norwegian Polar Institute by the Governor of Svalbard and during the local hunting season under local hunting law of Svalbard. All the seal samples were collected after the weaning period during the moulting season. Liver samples for analysis of redox parameters were frozen in liquid nitrogen in the field and stored at -80 °C until analysis. The rest of the seal was used in contaminant analyses and hormonal effect related research.

3.1.3. Baltic salmon

The feeding salmon were caught from International Council for the Exploration of the Sea (ICES) subdivisions 25 (Bornholm deep), 28 (Gotland deep), 30 (Bothnian Sea), and 32 (Gulf of Finland) during the late autumn 2006/winter 2007 (November-January) with the help of Finnish fishermen. The fish were killed and the livers were excised, frozen immediately in liquid nitrogen, and stored at -80 °C until measurements were done. Also the weight and the length of the fish were measured and some scales were stored for determination of the age of the fish.

3.2. Sample processing for redox measurements (I, II & III)

For enzyme activity measurements glutathione reductase (GR), glutathione peroxidase (GP), Glutathione-S-transferase (GST), glucose-6-phosphate-dehydrogenase (G6PDH), catalase (CAT) and superoxide dismutase (SOD) a piece of liver was homogenized in

potassium phosphate. For the GSH/GSSG ratio and total glutathione measurements the homogenate was treated with 1-methyl-2-vinylpyridinium-trifluoromethanesulfonate to prevent GSH from oxidizing. To measure lipid peroxidation a preweighed piece of liver was homogenized in methanol to remove proteins and to dissolve the fats.

3.3. Redox enzyme activities and small molecule measurements (I, II & III)

The parameters were measured in triplicate using 96– and 384–well microplates, which in most cases required reducing reagent volumes as compared to method instructions. Activities if GR, GP, GST and CAT were measured with Sigma kits (Sigma Chemicals, St. Louis, USA). The method for measuring GR activity is described by Smith et al. (1988). The GP activity was measured using H_2O_2 as the substrate (Lorentzen et al., 1994). The inhibition rate of SOD was measured using a Fluka kit (Fluka, Buchs, Germany) and it is based on the colorimetric reaction of tetrazolium salt and superoxide anion (Peskin and Winterbourn, 2000; Ukeda et al., 1999). GST measurement was done according to Habig et al. (1974). FOR CAT activity measurement, the catalase reaction was stopped using NaN_3 (Deissero.A and Dounce, 1970) and the remaining H_2O_2 was detected using a colorimetric reaction (Fossati et al., 1980). G6PDH activity was measured according to Noltmann et al. (1961) and EROD activity was measured according to Burke and Mayer (1974). All the measurements were done in room temperature.

The ratio between reduced and oxidised glutathione (GSH/GSSG-ratio) and the total glutathione content, with both the reduced and oxidised forms of glutathione (tot GSH) were measured with Oxisresearch kit (OxisResearch, Portland,USA) and it is based on the method described by Tietze (1969).

The lipid hydroperoxides were measured using the FOXII assay modified from the protocols described by Eymard and Genot (2003) and Bou et al. (2008) and it is based on the ferrous oxidation of xylenol orange.

The protein consentration was determined with the Bradford method (Bradford, 1976) using BioRad protein assay (BioRad, Espoo, Finland) with bovine serum albumin (Sigma Chemicals, St. Louis, USA) as a standard.

All the measurements were done with Envision plate reader (Perkin-Elmer, Turku, Finland), except for protein content determinations in work I, which were performed with a Victor 1 plate reader (Wallac, Turku, Finland).

3.4. RNA sample preparation and microarray (IV)

Total RNA was isolated from liver tissue using TRI-reagent (Molecular Research Center, OH, USA) and an additional purification of the extracted RNA was performed using Nucleospin RNA II (Machinery-Nagel, Germany) according to the manufacturer's instructions. RNA concentration was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, DE, USA), and RNA quality was assessed using an Agilent 2100 bioanalyzer RNA 6000 Nano kit (Agilent

Technologies, CA, USA). The mean RNA integrity number (stdev) of all samples was 9.64 (0.20).

RNA labeling, hybridizations, and scanning were performed by the Finnish DNA Microarray Centre. Briefly, total RNA (200 ng) was amplified and Cy3-labeled with Agilent's Low Input Quick Amp Labeling Kit, One Color (Agilent), along with Agilent's One-Color RNA Spike-in Kit following the manufacturer's protocols. After the labeling, the cRNA was examined with the Nanodrop ND-1000 and the Agilentin 2100 bioanalyzer RNA 6000 Nano kit to assess the concentration and quality of the labeling. Each sample was hybridized to the Agilent's 4x44K Salmon array (Design ID 020938) at 65°C overnight using Agilent's Gene Expression Hybridization kit. Washes were conducted as recommended by the manufacturer using Agilent's Gene Expression Wash Pack and using Agilent's stabilization or drying solution. Arrays were scanned with Agilent Technologies Scanner, model G2565CA. Spot intensities and other quality control features were extracted with Agilent's Feature Extraction Software version 10.7.1.

3.5. Proteomic sample isolation and mass spectrometry (IV)

The proteins were isolated from liver tissue using acetone precipitation. The samples were digested into peptides using trypsin and extracted with Empore C18 solid phase extraction cartridges (Sigma-Aldrich, MO, USA). Finally the samples were eluted to 60% acetonitrile/0.1% HCOOH mixture for the analysis.

The liver peptide samples were analyzed by microcapillary liquid chromatography electrospray ionisation – tandem mass spectrometry (μLC/ESI-MS/MS) on an ESI-hybrid Ion Trap-Orbitrap mass spectrometer (LTQ Orbitrap Velos; Thermo Fisher Scientific, Bremen, Germany) coupled to an Easy Nano LC nano-liquid chromatography (nLC) system (Thermo Fisher Scientific, Bremen, Germany).

The samples were analyzed in Orbitrap Velos in Data Dependent Acquisition (DDA) mode, where the 15 most intense doubly or triply charged parent masses were automatically selected for fragmentation. Mass scanning (MS1) was performed in positive-ion mode in the Orbitrap mass analyzer, where a low resolution preview scan and subsequent survey scan (MS) was performed. Precursor ions were selected for fragmentation (MS/MS) by collision induced dissociation (CID) in the ion trap mass analyzer.

The database searches for spectrum files were performed in Proteome Discoverer (version 1.4.0.288 Thermo Fisher Scientific) using the Mascot algorithm. The spectra were searched against a Uniprot salmon database supplemented with common contaminants (10039 sequences, accessed July-2011).

3.6. Data analysis

Barn swallow data (I) were analysed using generalized linear models (later GLMM) with the GLIMMIX-procedure of SAS (version 9.2) (SAS, 2008) and principal

component analysis (later PCA) with PASW statistics 18.0. 95% confidence intervals were calculated to establish bounds for the true effect (Steidl and Thomas, 2001). First, seasonal variation in enzyme activities was analysed with GLMM the. Second, PCA was used to discriminate the patterns of variation in the measured variables. PCA was performed using all individual enzyme activities, residual mass and fat score as input variables. Finally, the relationship between different body condition variables and the biomarkers were tested separately for each season (spring, summer, autumn, winter). Sex, residual mass, fat score, gonad size (mm) and completion of moult (in wintering birds only) were used as explanatory variables in the models.

Statistical analyses on seal data (II) were carried using R version 2.11.1. Linear models were used to test the effect of geographical area on oxidative stress parameters. The stress parameters were log transformed for normality. Time period (period 1: 1997–1998; period 2: 2002–2006) was set as a cofactor for all models, because the samples of the two different time periods were analyzed separately for the oxidative stress parameters. Also, sex and age were included as cofactors in the models. The interaction between time and area was included in the initial models and non-significant interaction terms were omitted. In addition, the relationships between contaminant concentrations and oxidative stress parameters using time period as a cofactor were tested. Parameter estimates for the geographical differences were obtained from the linear models. The bootstrapped, bias-corrected and accelerated 95% confidence intervals were used to investigate whether the parameter estimates differed significantly ($p<0.05$) from zero. Calculations were made using parametric bootstrapping with 2000 replicates. Means and parameter estimates in the text are given with 95% confidence intervals.

For salmon data in work III, SPSS 12.0.1 software was used for all statistical analyses. In general, the enzyme activity data were not normally distributed (Shapiro-Wilk's test for normality), so both parametric and nonparametric (Kruskal–Wallis) ANOVAs were used. Tamhane's *T*2 test and Mann–Whitney's *U*-test were used as post hoc tests. When both tests gave equal results the statistical significances are reported from Tamhane's *T*2 test. Principal component analysis (PCA) was conducted to emphasize the differences between populations and years.

For article IV the array quality was assessed through the use of Agilent control features as well as spike-in controls (Agilent One-Color RNA Spike-in Kit). The Chipster open source platform (Kallio et al., 2011) was used to further analyze the gProcessedSignals, the end result of standard Agilent normalization and background correction procedures, obtained from the Feature Extraction Software (v 10.7.1. The arrays were normalized using the quantile method (Smyth and Speed, 2003). Probes with missing values and probes that varied least across all observations were filtered away. Bayesian procedure (Smyth, 2004) was used to identify the differentially expressed transcripts between samples collected from different sea.

In the proteomic analysis raw spectral data and identification data from the mass spectrometry runs were imported to Progenesis 4.0 for feature detection and

quantification. All LC-MS maps were aligned together and the feature detection was done by automatic peak picking. The peptide-feature matches that had less than two hits or had precursor mass tolerance higher than 5 ppm were removed in Progenesis. The analysis was restricted to the linear section of the gradient and the normalization was done against all features.

Associations between multivariate descriptors of mRNA and protein expression were inferred via co-inertia analysis (CIA), using the 'ade4' package (Dray and Dufour, 2007) implemented in R. CIA is a multivariate method that identifies trends or co-relationships in multiple datasets (Dray et al., 2003),

Blast2Go (Conesa et al., 2005) was used to determine if differentially transcribed genes or expressed proteins were significantly over- or underrepresented by particular functional categories. Reference set for transcriptomic results analyses consisted of top blast results of all the microarray's probe sequences obtained from NCBI's non-redundant databases. Proteins from *Salmo salar* UniProt were used as reference sets for proteomics results (Papakostas et al., 2012). For analyzing significantly enriched gene ontology (GO)-categories and their relationships ClueGO, a Cytoscape plug-in (Bindea et al., 2009), was used. The results were visualized with Cytoscape (Shannon et al., 2003). Because of the limited annotation information available, salmon transcripts and proteins were matched to their human orthologs using BLAST searches from EMBL database for ClueGO analyses.

Human orthologs of salmon transcripts and proteins were used for interaction network analyses. Models were inferred with PPI and R spider (Antonov et al., 2009; Antonov et al., 2010). Significant models ($p<0.05$), assessed according to a Monte Carlo simulation procedure (Antonov et al., 2009; Antonov et al., 2010), of sub-networks with zero or one intermediate ('missing') nodes between list members were chosen for further examination and the models were visualized with Cytoscape (Shannon et al., 2003).

4. RESULTS AND DISCUSSION

4.1. Temporal changes and the effect of life stage

There were indications of oxidative stress in the wintering barn swallows when compared to birds caught in other seasons. Their biotransformation enzymes (EROD and GST) had the highest activities and they had the lowest GSH/GSSG ratio and the most LHPs. The birds caught in the spring and summer (when preparing for and during breeding) seemed to have intermediate stress with low levels of redox and biotransformation enzyme activities. The birds preparing for migration showed very little oxidative stress (low LPH and high GSH/GSSG ratio) despite high activities of some enzymes (SOD, GR and G6PDH). These findings suggest that part of the oxidative stress seen in the wintering birds could be caused by pollution. Unfortunately we were unable to measure the toxic compounds/organic pollutants in the birds. Higher contaminant levels can be expected in the birds from Africa compared to those of Finland since the capture area was along a river that runs through rural areas with intensive agriculture and has several goldmines in the upstream.

Contamination is the most probable explanation for the elevated EROD activities and PAHs and PCBs have been found to cause increase EROD activity of birds in previous studies as well (Bishop et al., 1999; Custer et al., 2001; Custer et al., 2006; Kuzyk et al., 2003). The high GST activities could be explained by pollution related phase II biotransformation and/or the molting of the wintering birds. This is suggested by the connection of both GST and lipid peroxidation (LPO) to molting phase with lower activity and amount in the birds that had nearly completed their moult. GST can metabolize organic hydroperoxides by using GSH (Halliwell and Gutteridge, 2007; Hayes et al., 2005) and one hypothesis is that during feather growth LHPs are formed and GST is handling them. However, no connection between lipid metabolites and molting was found in the study of (JenniEiermann and Jenni, 1996).

The elevated activities of SOD, GR and G6PDH are most likely connected to preparation of migration, since high activities were found both in the winter and autumn birds. One possible explanation for the elevation of GR and G6PDH activities is the attempt to increase GSH/GSSG ratio for maintaining the redox balance in the oxidative conditions of high caloric intake (Halliwell and Gutteridge, 2007), related to the premigratory fuelling of fats (McWilliams et al., 2004). The spring birds did not differ from the summer birds and this indicates that the migratory flight or reproduction are not so costly for the barn swallow that it would cause much oxidative stress. However the lack of difference could also be caused by the fact that some of the birds may have been in Finland for 2-3 weeks at the time of capture in the spring and therefore have recovered from the migration. The situation is hard to compare to previous studies since most of them have been made on birds not accustomed to long-distance flights (Costantini et al., 2008; Larcombe et al., 2010; Larcombe et al., 2008). Also the exact breeding status, breeding or not, number of chicks etc., of the summer birds could not be determined.

The total amount of glutathione (totGSH, contains both reduced and oxidized form) and GSH/GSSG ratio were connected to testis size in barn swallow with higher amount and ratio in males with larger testis. Testosterone have been noticed to elevate oxidative stress in zebra finch (Alonso-Alvarez et al., 2007) and red legged partridge (Alonso-Alvarez et al., 2008). The size of the testis does not necessarily mean higher testosterone production (Moore et al., 2002), but it can indicate more effective sperm production (Moller, 1988). This could suggest that the male barn swallows with higher GSH/GSSG ratio to cope against ROS could have better reproduction success than the individuals with lower GSH/GSSG ratio.

Baltic Salmon caught from the Eastern Baltic Sea (EBS) in the year 1999 suffered from more oxidative stress than salmon from the year 2006. This can be seen as the significantly elevated activities of most of the redox enzymes (SOD, GR and G6PDH) and lower totGSH and GSH/GSSG ratio in the fish from 1999. Biotransformation-related parameters DRE and GST activities were also significantly higher in 1999, but surprisingly the EROD activity was lower than in 2006, even though levels of several contaminants and eutrophication have been higher in the 1990s (Bignert et al., 2010; HELCOM, 2009, 2010). Usually EROD activity is increased by various organic pollutants (Whyte et al., 2000), but it can also be decreased after a chronic severe contamination in natural environment (Couillard et al., 2005). Since organic pollutants can cause oxidative stress e.g. (Bukowska, 2004; Schlezinger et al., 2006) they are one likely explanation for the elevated stress levels in the year 1999, another affecting thing or additional reason can be the changes in the food web, such as lower concentrations of AOX in the salmon diet (Gasiunaite et al., 2005; Ronnestad et al., 1998) and abiotic factors such as salinity, temperature and oxygen levels.

4.2. Spatial changes

Seals from the Baltic Sea had significantly higher GR and GP activities than seals from Svalbard. There were no differences in any of the other measured parameters (SOD, CAT, G6PDH and totGSH, vitamin E for 2002-2007 individuals, GST for 1996-1998 individuals). The amount of GSSG was below detection limit, so the GSH/GSSG-ratio could not be calculated. Also for many individuals the amount of lipid hydroperoxides was under the detection limit and therefore no statistical analyses were done. However the percentage of samples above the minimum level of quantification was approximately the same in Baltic seal individuals in both year groups (78% for 1996-1998 and 71% for 2002-2007) and similar to seals from Svalbard 1996-1998 (80%), but a lot lower for seals from Svalbard from 2002-2007 (43%). The lack of greater differences and signs of oxidative stress in highly contaminated Baltic seals was somewhat unexpected since several studies done on various species indicate oxidative stress after exposure to PCBs or polybrominated diphenyl ethers (Bukowska, 2004; Fernie et al., 2005; Frouin et al., 2010; Hoffman et al., 1996; Jin et al., 2001; Lai et al., 2010) and is opposite to finding on Baikal seals (*Pusa sibirica*) (Hirakawa et al., 2011).

The previous studies done with the same seals report contaminant-related EROD activity induction (Nyman et al., 2000; Routti et al., 2008). On the other hand there are studies that report no signs of oxidative stress or activation of the antioxidant defense

after EROD induction (Ait-Aissa et al., 2003; Gravato et al., 2006; Porte et al., 2000). However the activities of GR and GP and the concentration of totGSH increased with hepatic sum of contaminants and this suggests pollution-related enhancement in the redox cycle of the Baltic seals although the situation does not seem to lead to oxidative stress. One possible explanation for the lack of signs of oxidative stress could be the enhanced protective mechanisms against dive-associated oxidative stress in diving mammals (Hindle et al., 2010; Righetti et al., 2014; Vazquez-Medina et al., 2012; Wilhelm et al., 2002) and in other air-breathing diving animals like turtles (Hermes-Lima and Zenteno-Savin, 2002) and penguins (Corsolini et al., 2001). The adaptations to dive-associated oxidative stress, which is caused by the oxygen-derived free radicals generated in ischemia followed by post-dive reperfusion, have been studied previously on ringed seals (Elsner et al., 1998; Johnson et al., 2005; Vazquez-Medina et al., 2006, 2007; Zenteno-Savin et al., 2002). The GSH concentration and the enzyme activities of glutathione redox cycle along with CAT and SOD have been reported to be important protection mechanisms against dive-associated oxidative stress.

The fish from central and southern Baltic Sea (combined as CBS) are less stressed than the fish from Gulf of Finland (GoF) and the Bothnian Sea (BS). Especially the fish from GoF show signs of oxidative stress with significantly higher SOD, GP, GR and G6PDH activities and lower CAT activity and GSH/GSSG ratio than fish from the central and southern Baltic. There are also signs of elevated biotransformation since the DNA-binding of dioxin responsive element, EROD and GST activities were significantly higher in GoF salmon than in the CBS. On mRNA level there was increase of *GP* and *CYP19A1* in GoF when compared to CBS, decrease of *GP* and *GR* and increase of two forms of *GST* and *CYP1A* when compared to BS. A significant increase in the levels of SOD, CAT, Cytochrome P450 and several forms of GST proteins were found in GoF when compared to CBS and in SOD and CAT proteins when compared to BS. The fish from the BS have also elevated enzyme activities of SOD, GP, G6PDH and GST when compared to CBS and SBS, but for example the GSH/GSSG-ratio does not differ from the fish of CBS and SBS. There were no differences between CBS and BS on mRNAs of genes encoding same redox and biotransformation enzymes. However at the protein level a form of SOD, G6PDH and two forms of GST were increased in BS and CAT and other two forms of GST were decreased when compared to CBS. These findings show that there is no straightforward correspondence between the mRNA, protein and enzyme activity levels for some genes involved in the regulation of redox balance and biotransformation. This may indicate posttranscriptional, e.g. splicing, and posttranslational, e.g. addition of functional groups, regulation, which enables more rapid responses to unpredictable situations than just transcriptional regulation.

GO enrichment tests and interaction network analyses gave a better overall picture on the differences between sites, rather than looking only the individual expressions of certain genes. Stress, such as oxidative stress, detoxification, DNA damage and cell death, related mRNA and protein levels were altered according to the sampling area, with salmon from GoF showing the most elevations, BS being intermediate and CBS showing the lowest amount of responses. Even though we do not have contaminant

levels of the salmon used in our study it can be assumed from previous organochlorine measurements from salmon from the same areas of the Baltic Sea that the fish from GoF are the most contaminated (Kiljunen et al., 2008). Similar pollution-related elevated stress responses in mRNA and/or proteomic level have been found in goldfish (*Carassius auratus*) (Wang et al., 2007; Wang et al., 2008), flounder (*Platichtys flesus*) (Falciani et al., 2008; Galland et al., 2013; Williams et al., 2011) and eelpout (*Zoarces viviparous*) (Asker et al., 2013) caught from the wild.

Another category with clear spatial differences was the respiratory chain and ATP synthesis-related genes. Again, salmon from GoF showed both mRNA and protein level induction when compared to CBS, but besides induction there was some mRNA level repression as well when compared to BS. There were almost no differences between salmon from BS and CBS. Elevated energy demand by enhanced xenobiotic metabolism has been suggested as one reason behind the upregulation of respiratory chain genes in polluted environments (Williams et al., 2011). Since the salinity and oxygen levels were almost the same between different sites they probably did not have a very big effect on respiratory chain related genes.

Several studies done on natural populations from contaminated environments show mainly induction in the transcriptional and translational levels (Asker et al., 2013; Falciani et al., 2008; Galland et al., 2013; Wang et al., 2007). Contamination can also explain the decrease in RNA synthesis and processing and translation and protein folding in the salmon form GoF when compared to fish from CBS. The decrease can be seen especially at proteomic level, where it was also significant when compared to fish from BS. The level of proteins involved in ribosome biogenesis were decreased and the transcription of ribosomal proteins altered also in CBS vs BS comparison, but not as much as in CBS vs GoF comparison. These findings are in line with the results of others on fish from contaminated sites (Asker et al., 2013; Falciani et al., 2008; Wang et al., 2007; Williams et al., 2011). The expression of genes involved in amino acid, lipid and carbohydrate metabolism was dissimilar in salmon from different areas. In general the changes of gene expression were more distinct in the final step, i.e.,protein level than at the intermediate step, mRNA expression. There are several factors that can lead to variation in amino acid, lipid and carbohydrate metabolism related gene expression such as pollution (De Wit et al., 2008; Falciani et al., 2008; Wang et al., 2007; Wang et al., 2008), diet (Morais et al., 2012) and the origin of the fish regarding wild vs. hatchery stock (Morais et al., 2012; Normandeau et al., 2009) and different rivers of origin. The salmon from GoF originate mainly from different genetic lineage compared to salmon feeding in CBS and BS (Koljonen, 2006; Vuori et al., 2012) and it has been shown that salmon from different sampling locations have differences in the trophic positions and diets (Kiljunen et al., 2008). Immune response-related gene expression was also affected in all comparisons. The differences in immune function-related transcripts may be a result of general differences in environmental conditions and pathogen exposure, but also affected by degree of exposure to environmental stressors, as contamination has been found to affect the transcription of immune function related genes (Asker et al., 2013; Falciani et al., 2008; Wang et al., 2007; Williams et al., 2011).

4.3. Comparison of redox system between vertebrate groups

When comparing the three species used in these studies different strategies in the redox responses can be seen. Relative activities and amounts of redox parameters are presented in Figure 3. Because the amount of GSSG was below detection limit in the ringed seals the GSH/GSSG ratio would be extremely high. For visualization purposes the ratio was set to 600. For the Barn swallow an average of the four seasons was calculated for each parameter but the extremely high values from the autumn GSH/GSSG ratio (higher than 1000 µM/mg total protein) were omitted. The ringed seal has the highest values in several parameters (SOD, GR, totGSH and GSH/GSSG-ratio) and the lowest in the amount of LPO, suggesting that the seals have more effective antioxidant system than barn swallows or salmon. Seals seem to rely on effective enzymatic defense against ROS with active circulation of glutathione and this leads to minimal damage.

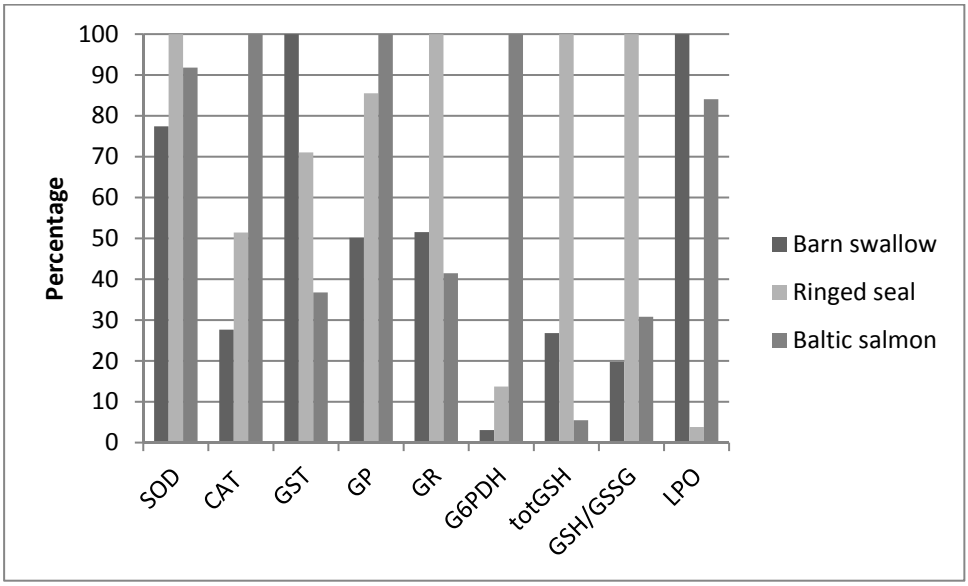

Figure 3. The relative activities and amounts of studied redox parameters in the liver tissue of three focal species. The highest value (concentration, activity or ratio) has been marked as 100% and other results have been proportioned to that. The GSH/GSSG ratio for ringed seal has been set to 600 to represent a high value, this was due to GSSG values that were below detection limit and therefore the ratio becomes extremely high. The result is an average from all seasons for barn swallow, all areas and years for ringed seal and all areas for Baltic salmon.

The barn swallows have the lowest values in 5 parameters (SOD, CAT, GP, G6PDH and GSH/GSSG ratio) and highest in 2 (GST and LPO) which could indicate according to previous studies (Barja et al., 1994; Herrero and Barja, 1998) that their ROS production is low and therefore they do not need high antioxidant enzyme activities. The high LPO and low GSH/GSSG ratio suggest that some ROS related damage is occurring, although most of it was detected from the African birds and this might indicate that they do not tolerate environmental contamination well. If the

extremely high values of GSH/GSSG ratio of barn swallow are taken into account in the average of the ratio it rises above salmon.

The Baltic salmon has equal amount of high, low and intermediate values in the studied parameters, 3 highest values (CAT, GP and G6PDH) and 3 lowest (GST, GR and totGSH). According to the highest CAT and GP activities it can be suggested that the production and/or concentrations of H_2O_2 are higher in salmon than in other groups. Despite the often seen and here existing correlation of GR and G6PDH (Halliwell and Gutteridge, 2007) the Baltic salmon has very low GR activity. This means that NADPH is not diverted appreciably to GSSG reduction but used in other pathways like biotransformation. Although it is impossible to draw conclusions for the whole class with only one species in each class, some comparisons to previous studies can be made (which similarly often face the same problem). Also the annual fluctuation and the effects of different environmental factors in each parameter, that are generally not very well known cause difficulties in comparative studies. In general the trend seems to be higher activity and glutathione values for mammals, followed by birds and lowest values for fishes, lizards and amphibians and vice versa for lipid related damage (Lopeztorres et al., 1993; Page et al., 2010; Venditti et al., 1999). For GR activity and totGSH content, our study follows the trend of previous studies for mammals, birds and fish. For SOD, CAT and GP activities and LPO it is the same for mammals and birds, but salmon result does not fit this trend with either highest (CAT and GP) or intermediate (SOD and LPO) values. One of the reasons for dissimilarities could be that most of the comparative research has been done on laboratory or captive animals whilst ours were collected from the nature and therefore are exposed to seasonal variation, pollutants etc.

5. CONCLUSIONS

In this thesis I study how environmental changes and life history affect the redox status of different vertebrates. I found temporal changes throughout a year and between years and there were differences in animals from different locations. Some of the effects were expected and some of them were not, like the absence of damage in Baltic ringed seals and the lack of difference between just arrived and breeding barn swallows.

Contamination had an effect in all of the studied species, but the level of response varied. Unfortunately ringed seals were the only animals whose contaminant levels were measured, but it is very probable that the barn swallows caught from Africa were more exposed to pollutants compared to the birds caught from Finland. Also the Baltic salmon from the year 1999 from central Baltic proper and from 2006 from Gulf of Finland had likely higher contaminant levels than the other fish. In all species contamination increased EROD activity and often antioxidant enzyme activities. Despite this, signs of oxidative stress (low GSH/GSSG-ratio, lot of LPO) were not found in ringed seal, even though these indicators were clear in barn swallows and Baltic salmon. However, glutathione recycling increased. This is probably due to the adaptation to diving, where seals have to face repetitive ischemia and reperfusion which produces ROS. This leads to enhanced protection mechanism, such as more effective glutathione recycling, which can be utilized in all sorts of ROS-related metabolisms, not only diving. This doesn't mean that seals are not affected by contamination, but they don't suffer from oxidative stress as easily as other species. Similar findings have been made with other air-breathing diving animals (Corsolini et al., 2001; Hermes-Lima and Zenteno-Savin, 2002; Vazquez-Medina et al., 2012). Therefore redox parameters are not probably the best options used as biomarkers for contaminant studies in seals, however they can give valuable information on responses to relatively short term anoxia.

In barn swallows some of the enzymes with high activity, such as SOD, GR and G6PDH, were most likely connected more to preparation of migration than to contamination, since similar elevation could be seen in both migratory groups, spring and autumn. The time of sampling can have a big effect on the results and should be taken into consideration. This study showed also that all increases in the redox enzyme activities do not necessarily indicate stress, but are part of normal life cycle fluctuation.

In Baltic salmon stress-related GO categories were the most enriched and especially the fish from GoF had elevated signal when compared to other areas. This reveals that they have problems in a larger scale than just oxidative stress, but these stress markers seem to work as relatively good biomarkers of the condition of the fish. Contamination didn't only affect stress-related metabolism in Baltic salmon, but for example respiratory chain, RNA synthesis and translation were altered as well.

Comparative studies on different vertebrates would need more species per group to emphasize the group differences instead of special characteristics of certain species. However, the results from this thesis seem to agree relatively well with those from

earlier studies. Combining results from different research groups can be problematic since there is a vast variety of oxidative stress-related measurements that are not necessarily correlated and the tissues used for measurements can be different. In this thesis it has not only been important to know if the animals are stressed or not, but to investigate the antioxidant system more in-depth to know which parts are more affected. In general, the glutathione recycling-related enzymes and the ratio of oxidized and reduced glutathione seemed to be the most affected parameters. They were also found important in the meta-analysis done by (Isaksson, 2010). If choosing only few biomarkers for monitoring purposes it would be important to measure as much as possible at first and then evaluate the most important ones and there should always be some measure of ROS production/AOX defense and damage, because elevated defense does not necessarily lead to oxidative stress just like the lack of damage does not mean that the redox system is unaffected by the environment.

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REFERENCES

Abele, D., and S. Puntarulo, 2004, Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish: Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, v. 138, p. 405-415.

Adeyemi, J. A., and P. L. Klerks, 2012, Salinity acclimation modulates copper toxicity in the sheepshead minnow, Cyprinodon variegatus: Environmental Toxicology and Chemistry, v. 31, p. 1573-1578.

Ait-Aissa, S., O. Ausseil, O. Palluel, E. Vindimian, J. Garnier-Laplace, and J. M. Porcher, 2003, Biomarker responses in juvenile rainbow trout (Oncorhynchus mykiss) after single and combined exposure to low doses of cadmium, zinc, PCB77 and 17 beta-oestradiol: Biomarkers, v. 8, p. 491-508.

Alonso-Alvarez, C., S. Bertrand, G. Devevey, J. Prost, B. Faivre, and G. Sorci, 2004, Increased susceptibility to oxidative stress as a proximate cost of reproduction: Ecology Letters, v. 7, p. 363-368.

Alonso-Alvarez, C., S. Bertrand, B. Faivre, O. Chastel, and G. Sorci, 2007, Testosterone and oxidative stress: the oxidation handicap hypothesis: Proceedings of the Royal Society B-Biological Sciences, v. 274, p. 819-825.

Alonso-Alvarez, C., L. Perez-Rodriguez, R. Mateo, O. Chastel, and J. Vinuela, 2008, The oxidation handicap hypothesis and the carotenoid allocation trade-off: Journal of Evolutionary Biology, v. 21, p. 1789-1797.

Ambrosini, R., A. P. Moller, and N. Saino, 2009, A quantitative measure of migratory connectivity: Journal of Theoretical Biology, v. 257, p. 203-211.

Ames, B. N., M. K. Shigenaga, and E. M. Park, 1991, DNA damage by endogenous oxidants as a cause of aging and cancer: Oxidative Damage & Repair: Chemical, Biological and Medical Aspects, p. 181-187.

Antonov, A. V., S. Dietmann, I. Rodchenkov, and H. W. Mewes, 2009, PPI spider: A tool for the interpretation of proteomics data in the context of protein-protein interaction networks: Proteomics, v. 9, p. 2740-2749.

Antonov, A. V., E. E. Schmidt, S. Dietmann, M. Krestyaninova, and H. Hermjakob, 2010, R spider: a network-based analysis of gene lists by combining signaling and metabolic pathways from Reactome and KEGG databases: Nucleic Acids Research, v. 38, p. W78-W83.

Asker, N., E. Kristiansson, E. Albertsson, D. G. J. Larsson, and L. Forlin, 2013, Hepatic transcriptome profiling indicates differential mRNA expression of apoptosis and immune related genes in eelpout (Zoarces viviparus) caught at Goteborg harbor, Sweden: Aquatic Toxicology, v. 130, p. 58-67.

Bagnyukova, T. V., S. I. Danyliv, O. S. Zin'ko, and V. I. Lushchak, 2007a, Heat shock induces oxidative stress in rotan Perccottus glenii tissues: Journal of Thermal Biology, v. 32, p. 255-260.

Bagnyukova, T. V., O. V. Lushchak, K. B. Storey, and V. I. Lushchak, 2007b, Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23 degrees C: Journal of Thermal Biology, v. 32, p. 227-234.

Balaban, R. S., S. Nemoto, and T. Finkel, 2005, Mitochondria, oxidants, and aging: Cell, v. 120, p. 483-495.

Barja, G., S. Cadenas, C. Rojas, R. Perezcampo, and M. Lopeztorres, 1994, Low mitochondrial free-radical production per unit O-2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic-rate in birds: Free Radical Research, v. 21, p. 317-327.

Baysoy, E., G. Atli, C. O. Gurler, Z. Dogan, A. Eroglu, K. Kocalar, and M. Canli, 2012, The effects of increased freshwater salinity in the biodisponibility of metals (Cr, Pb) and effects on antioxidant systems of Oreochromis niloticus: Ecotoxicology and Environmental Safety, v. 84, p. 249-253.

Beamonte-Barrientos, R., and S. Verhulst, 2013, Plasma reactive oxygen metabolites and non-enzymatic antioxidant capacity are not affected by an acute increase of metabolic rate in zebra finches: Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology, v. 183, p. 675-683.

Beckman, K. B., and B. N. Ames, 1998, The free radical theory of aging matures: Physiological Reviews, v. 78, p. 547-581.

Behne, D., and A. Kyriakopoulos, 2001, Mammalian selenium-containing proteins: Annual Review of Nutrition, v. 21, p. 453-473.

Berglund, A. M. M., M. J. Rainio, M. Kanerva, M. Nikinmaa, and T. Eeva, 2014, Antioxidant status in relation to age, condition, reproductive performance and pollution in three passerine species: Journal of Avian Biology, v. 45, p. 235-246.

Berlett, B. S., and E. R. Stadtman, 1997, Protein oxidation in aging, disease, and oxidative stress: Journal of Biological Chemistry, v. 272, p. 20313-20316.

Bertrand, S., C. Alonso-Alvarez, G. Devevey, B. Faivre, J. Prost, and G. Sorci, 2006, Carotenoids modulate the trade-off between egg production and resistance to oxidative stress in zebra finches: Oecologia, v. 147, p. 576-584.

Bignert, A., S. Danielsson, and E. Nyberg, 2010, Comments Concerning the National Swedish Contaminant Monitoring Programme in Marine Biota, *in* S. E. P. Agency, ed., Swedish Museum of Natural History, p. 156.

Bindea, G., B. Mlecnik, H. Hackl, P. Charoentong, M. Tosolini, A. Kirilovsky, W.-H. Fridman, F. Pages, Z.

Trajanoski, and J. Galon, 2009, ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks: Bioinformatics, v. 25, p. 1091-1093.

Bishop, C. A., N. A. Mahony, S. Trudeau, and K. E. Pettit, 1999, Reproductive success and biochemical effects in tree swallows (Tachycineta bicolor) exposed to chlorinated hydrocarbon contaminants in wetlands of the Great Lakes and St. Lawrence River basin, USA and Canada: Environmental Toxicology and Chemistry, v. 18, p. 263-271.

Bou, R., R. Codony, A. Tres, E. A. Decker, and F. Guardicila, 2008, Determination of hydroperoxides in foods and biological samples by the ferrous oxidation-xylenol orange method: A review of the factors that influence the method's performance: Analytical Biochemistry, v. 377, p. 1-15.

Bradford, M. M., 1976, Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding: Analytical Biochemistry, v. 72, p. 248-254.

Brown, J. C. L., G. B. McClelland, P. A. Faure, J. M. Klaiman, and J. F. Staples, 2009, Examining the mechanisms responsible for lower ROS release rates in liver mitochondria from the long-lived house sparrow (Passer domesticus) and big brown bat (Eptesicus fuscus) compared to the short-lived mouse (Mus musculus): Mechanisms of Ageing and Development, v. 130, p. 467-476.

Bukowska, M., 2004, 2,4,5-T and 2,4,5-TCP induce oxidative damage in human erythrocytes: the role of glutathione: Cell Biology International, v. 28, p. 557-563.

Burke, M. D.; Mayer, R. T., 1974, Ethoxyresorufin—Direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metabolism and Disposition, v. 2 p. 583–588.

Catoni, C., A. Peters, and H. M. Schaefer, 2008, Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants: Animal Behaviour, v. 76, p. 1107-1119.

Chance, B., H. Sies, and A. Boveris, 1979, Hyperoxide metabolism in mammalian organs: Physiological Reviews, v. 59, p. 527-605.

Chandwaney, R., S. Leichtweis, C. Leeuwenburgh, and L. L. Ji, 1998, Oxidative stress and mitochondrial function in skeletal muscle: Effects of aging and exercise training: Age, v. 21, p. 109-117.

Conesa, A., S. Gotz, J. M. Garcia-Gomez, J. Terol, M. Talon, and M. Robles, 2005, Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research: Bioinformatics, v. 21, p. 3674-3676.

Coon, M. J., 2005, Cytochrome P450: Nature's most versatile biological catalyst: Annual Review of Pharmacology and Toxicology, v. 45, p. 1-25.

Corsolini, S., M. Nigro, S. Olmastroni, S. Focardi, and F. Regoli, 2001, Susceptibility to oxidative stress in Adelie and emperor penguin: Polar Biology, v. 24, p. 365-368.

Costantini, D., G. Dell'Ariccia, and H. P. Lipp, 2008, Long flights and age affect oxidative status of homing pigeons (Columba livia): Journal of Experimental Biology, v. 211, p. 377-381.

Couillard, C. M., M. Lebeuf, M. G. Ikonomou, G. G. Poirier, and W. J. Cretney, 2005, Low hepatic ethoxyresorufin-O-deethylase activity correlates with high organochlorine concentrations in Atlantic tomcod from the Canadian East Coast: Environmental Toxicology and Chemistry, v. 24, p. 2459-2469.

Custer, T. W., C. M. Custer, K. Dickerson, K. Allen, M. J. Melancon, and L. J. Schmidt, 2001, Polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, trace elements, and monooxygenase activity in birds nesting on the North Platte River, Casper, Wyoming, USA: Environmental Toxicology and Chemistry, v. 20, p. 624-631.

Custer, T. W., C. M. Custer, B. L. Goatcher, M. J. Melancon, C. W. Matson, and J. W. Bickham, 2006, Contaminant exposure of barn swallows nesting on Bayou D'Inde, Calcasieu Estuary, Louisiana, USA: Environmental Monitoring and Assessment, v. 121, p. 543-560.

De Wit, M., D. Keil, N. Remmerie, K. van der Ven, E.-J. van den Brandhof, D. Knapen, E. Witters, and W. De Coen, 2008, Molecular targets of TBBPA in zebrafish analysed through integration of genomic and proteomic approaches: Chemosphere, v. 74, p. 96-105.

Deissero.A, and A. L. Dounce, 1970, Catalase – Physical and chemical properties, mechanism of catalysis, and physiological role: Physiological Reviews, v. 50, p. 319-&.

Denison, M. S., A. Pandini, S. R. Nagy, E. P. Baldwin, and L. Bonati, 2002, Ligand binding and activation of the Ah receptor: Chemico-Biological Interactions, v. 141, p. 3-24.

Dray, S., D. Chessel, and J. Thioulouse, 2003, Co-inertia analysis and the linking of ecological data tables: Ecology, v. 84, p. 3078-3089.

Dray, S., and A.-B. Dufour, 2007, The ade4 package: Implementing the duality diagram for ecologists: Journal of Statistical Software, v. 22, p. 1-20.

Droge, W., 2002, Free radicals in the physiological control of cell function: Physiological Reviews, v. 82, p. 47-95.

Elsner, R., S. Oyasaeter, R. Almaas, and O. D. Saugstad, 1998, Diving seals, ischemia-reperfusion and oxygen radicals: Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology, v. 119, p. 975-980.

Evans, M. D., M. Dizdaroglu, and M. S. Cooke, 2004, Oxidative DNA damage and disease: induction, repair and significance: Mutation Research-Reviews in Mutation Research, v. 567, p. 1-61.

Eymard, S., and C. Genot, 2003, A modified xylenol orange method to evaluate formation of lipid

hydroperoxides during storage and processing of small pelagic fish: European Journal of Lipid Science and Technology, v. 105, p. 497-501.

Falciani, F., A. M. Diab, V. Sabine, T. D. Williams, F. Ortega, S. G. George, and J. K. Chipman, 2008, Hepatic transcriptomic profiles of European flounder (Platichthys flesus) from field sites and computational approaches to predict site from stress gene responses following exposure to model toxicants: Aquatic Toxicology, v. 90, p. 92-101.

Falnes, P. O., A. Klungland, and I. Alseth, 2007, Repair of methyl lesions in DNA and RNA by oxidative demethylation: Neuroscience, v. 145, p. 1222-1232.

Fernie, K. J., J. L. Shutt, G. Mayne, D. Hoffman, R. J. Letcher, K. G. Drouillard, and I. J. Ritchie, 2005, Exposure to polybrominated diphenyl ethers (PBDEs): Changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (Falco sparverius): Toxicological Sciences, v. 88, p. 375-383.

Finkel, T., and N. J. Holbrook, 2000, Oxidants, oxidative stress and the biology of ageing: Nature, v. 408, p. 239-247.

Fossati, P., L. Prencipe, and G. Berti, 1980, Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid-4-aminophenazone chromogenic system in direct enzymatic assay of uric-acid in serum and urine: Clinical Chemistry, v. 26, p. 227-231.

Frei, R., C. Gaucher, S. W. Poulton, and D. E. Canfield, 2009, Fluctuations in Precambrian atmospheric oxygenation recorded by chromium isotopes: Nature, v. 461, p. 250-U125.

Fridovich, I., 1995, Superoxide radical and superoxide dismutases: Annual Review of Biochemistry, v. 64, p. 97-112.

Frouin, H., M. Lebeuf, M. Hammill, S. Masson, and M. Fournier, 2010, Effects of individual polybrominated diphenyl ether (PBDE) congeners on harbour seal immune cells in vitro: Marine Pollution Bulletin, v. 60, p. 291-298.

Galland, C., C. Dupuy, C. Capitaine, M. Auffret, L. Quiniou, J. Laroche, and V. Pichereau, 2013, Comparisons of liver proteomes in the European flounder Platichthys flesus from three contrasted estuaries: Journal of Sea Research, v. 75, p. 135-141.

Gasiunaite, Z. R., A. C. Cardoso, A. S. Heiskanen, P. Henriksen, P. Kauppila, I. Olenina, R. Pilkaityte, I. Purina, A. Razinkovas, S. Sagert, H. Schubert, and N. Wasmund, 2005, Seasonality of coastal phytoplankton in the Baltic Sea: Influence of salinity and eutrophication: Estuarine Coastal and Shelf Science, v. 65, p. 239-252.

Gravato, C., M. Teles, M. Oliveira, and M. A. Santos, 2006, Oxidative stress, liver biotransformation and genotoxic effects induced by copper in Anguilla anguilla L. - the influence of pre-exposure to beta-naphthoflavone: Chemosphere, v. 65, p. 1821-1830.

Habig, W. H., M. J. Pabst, and W. B. Jakoby, 1974, Glutathione S-Transferases - First enzymatic step in mercapturic acid formation: Journal of Biological Chemistry, v. 249, p. 7130-7139.

Halliwell, B., and J. M. C. Gutteridge, 2007, Free Radicals in Biology and Medicine: Oxford, Oxford University press, 851 p.

Hayes, J. D., J. U. Flanagan, and I. R. Jowsey, 2005, Glutathione transferases: Annual Review of Pharmacology and Toxicology, v. 45, p. 51-88.

Hayes, J. D., and D. J. Pulford, 1995, The glutathione S-Transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance: Critical Reviews in Biochemistry and Molecular Biology, v. 30, p. 445-600.

Heise, K., S. Puntarulo, M. Nikinmaa, D. Abele, and H. O. Portner, 2006a, Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout Zoarces viviparus L: Journal of Experimental Biology, v. 209, p. 353-363.

Heise, K., S. Puntarulo, M. Nikinmaa, M. Lucassen, H. O. Portner, and D. Abele, 2006b, Oxidative stress and HIF-1 DNA binding during stressful cold exposure and recovery in the North Sea eelpout (Zoarces viviparus): Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, v. 143, p. 494-503.

HELCOM, 2009, Eutrophication in the Baltic Sea – An integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region, Balt. Sea Environ. Proc.

HELCOM, 2010, Hazardous substances in the Baltic Sea – An integrated thematic assessment of hazardous substances in the Baltic Sea, Balt. Sea Environ. Proc.

Hermes-Lima, M., and T. Zenteno-Savin, 2002, Animal response to drastic changes in oxygen availability and physiological oxidative stress: Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, v. 133, p. 537-556.

Herrero, A., and G. Barja, 1998, H2O2 production of heart mitochondria and aging rate are slower in canaries and parakeets than in mice: sites of free radical generation and mechanisms involved: Mechanisms of Ageing and Development, v. 103, p. 133-146.

Hindle, A. G., J. M. Lawler, K. L. Campbell, and M. Horning, 2010, Muscle aging and oxidative stress in wild-caught shrews: Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, v. 155, p. 427-434.

Hirakawa, S., D. Imaeda, K. Nakayama, M. Udaka, E.-Y. Kim, T. Kunisue, M. Ogawa, T. Matsuda, S. Matsui, E. A. Petrov, V. B. Batoev, S. Tanabe, and H. Iwata, 2011, Integrative assessment of potential effects of dioxins and related compounds in wild Baikal seals (Pusa sibirica): Application of microarray and biochemical analyses: Aquatic Toxicology, v. 105, p. 89-99.

Hoffman, D. J., M. J. Melancon, P. N. Klein, C. P. Rice, J. D. Eisemann, R. K. Hines, J. W. Spann, and G. W.

Pendleton, 1996, Developmental toxicity of PCB 126 (3,3',4,4',5-pentachlorobiphenyl) in nestling American kestrels (Falco sparverius): Fundamental and Applied Toxicology, v. 34, p. 188-200.

Hulbert, A. J., R. Pamplona, R. Buffenstein, and W. A. Buttemer, 2007, Life and death: Metabolic rate, membrane composition, and life span of animals: Physiological Reviews, v. 87, p. 1175-1213.

Ikonen, E., 2006, The role of the feeding migration and diet of Atlantic salmon (Salmo salar L.) in yolk-sack-fry mortality (M74) in the Baltic Sea: Helsinki :, Finnish Game and Fisheries Research Institute, 34, 60 s p.

Isaksson, C., 2010, Pollution and Its Impact on Wild Animals: A Meta-Analysis on Oxidative Stress: Ecohealth, v. 7, p. 342-350.

Jancova, P., P. Anzenbacher, and E. Anzenbacherova, 2010, Pahse II drug metabolizing enzymes: Biomedical Papers-Olomouc, v. 154, p. 103-116.

JenniEiermann, S., and L. Jenni, 1996, Metabolic differences between the postbreeding, moulting and migratory periods in feeding and fasting passerine birds: Functional Ecology, v. 10, p. 62-72.

Jin, X. L., S. W. Kennedy, T. Di Muccio, and T. W. Moon, 2001, Role of oxidative stress and antioxidant defense in 3,3 ',4,4 ',5-pentachlorobiphenyl-induced toxicity and species-differential sensitivity in chicken and duck embryos: Toxicology and Applied Pharmacology, v. 172, p. 241-248.

Johnson, P., R. Elsner, and T. Zenteno-Savin, 2005, Hypoxia-inducible factor 1 proteomics and diving adaptations in ringed seal: Free Radical Biology and Medicine, v. 39, p. 205-212.

Kallio, M. A., J. T. Tuimala, T. Hupponen, P. Klemela, M. Gentile, I. Scheinin, M. Koski, J. Kaki, and E. I. Korpelainen, 2011, Chipster: user-friendly analysis software for microarray and other high-throughput data: Bmc Genomics, v. 12.

Kastan, M. B., and J. Bartek, 2004, Cell-cycle checkpoints and cancer: Nature, v. 432, p. 316-323.

Kasting, J. F., 1993, Earts early atmosphere: Science, v. 259, p. 920-926.

Kiljunen, M., H. Peltonen, R. I. Jones, H. Kiviranta, P. J. Vuorinen, M. Verta, and J. Karjalainen, 2008, Coupling stable isotopes with bioenergetics to evaluate sources of variation in organochlorine concentrations in Baltic salmon (Salmo salar): Canadian Journal of Fisheries and Aquatic Sciences, v. 65, p. 2114-2126.

Koivula, M. J., and T. Eeva, 2010, Metal-related oxidative stress in birds: Environmental Pollution, v. 158, p. 2359-2370.

Koljonen, M.-L., 2006, Annual changes in the proportions of wild and hatchery Atlantic salmon (Salmo salar) caught in the Baltic Sea: Ices Journal of Marine Science, v. 63, p. 1274-1285.

Krafft, B. A., C. Lydersen, and K. M. Kovacs, 2006, Serum haptoglobin concentrations in ringed seals (Pusa hispida) from Svalbard, Norway: Journal of Wildlife Diseases, v. 42, p. 442-446.

Kuzyk, Z. Z. A., N. M. Burgess, J. P. Stow, and G. A. Fox, 2003, Biological effects of marine PCB contamination on black guillemot nestlings at Saglek, Labrador: Liver biomarkers: Ecotoxicology, v. 12, p. 183-197.

Lai, I., Y. T. Chai, D. Simmons, G. Luthe, M. C. Coleman, D. Spitz, W. M. Haschek, G. Ludewig, and L. W. Robertson, 2010, Acute toxicity of 3,3 ',4,4 ',5-pentachlorobiphenyl (PCB 126) in male Sprague-Dawley rats: Effects on hepatic oxidative stress, glutathione and metals status: Environment International, v. 36, p. 918-923.

Lamarre, S. G., N. R. Le Francois, W. R. Driedzic, and P. U. Blier, 2009, Protein synthesis is lowered while 20S proteasome activity is maintained following acclimation to low temperature in juvenile spotted wolffish (Anarhichas minor Olafsen): Journal of Experimental Biology, v. 212, p. 1294-1301.

Lane, N., 2002, Oxygen: the Molevule That Made the World: Oxford, UK, Oxford University Press.

Larcombe, S. D., J. S. Coffey, D. Bann, L. Alexander, and K. E. Arnold, 2010, Impacts of dietary antioxidants and flight training on post-exercise oxidative damage in adult parrots: Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, v. 155, p. 49-53.

Larcombe, S. D., C. A. Tregaskes, J. S. Coffey, A. E. Stevenson, L. Alexander, and K. E. Arnold, 2008, The effects of short-term antioxidant supplementation on oxidative stress and flight performance in adult budgerigars Melopsittacus undulatus: Journal of Experimental Biology, v. 211, p. 2859-2864.

Leeuwenburgh, C., and J. W. Heinecke, 2001, Oxidative stress and antioxidants in exercise: Current Medicinal Chemistry, v. 8, p. 829-838.

Liu, Y., W.-N. Wang, A.-L. Wang, J.-M. Wang, and R.-Y. Sun, 2007, Effects of dietary vitamin E supplementation on antioxidant enzyme activities in Litopenaeus vannamei (Boone, 1931) exposed to acute salinity changes: Aquaculture, v. 265, p. 351-358.

Lopeztorres, M., R. Perezcampo, C. Rojas, S. Cadenas, and G. Barja, 1993, Maximum life-span in vertebrates – Relationship with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true malondialdehyde, in vivo H2O2, and basal and maximum aerobic capacity: Mechanisms of Ageing and Development, v. 70, p. 177-199.

Lorentzen, M., A. Maage, and K. Juhlshamn, 1994, Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon *(Salmo salar)*: AQUACULTURE, v. 121, p. 359-367.

Loro, V. L., M. B. Jorge, K. R. da Silva, and C. M. Wood, 2012, Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in a euryhaline teleost Fundulus heteroclitus: Protective effects of salinity: Aquatic Toxicology, v. 110, p. 187-193.

Malek, R. L., H. Sajadi, J. Abraham, M. A. Grundy, and G. S. Gerhard, 2004, The effects of temperature reduction on gene expression and oxidative stress in skeletal muscle from adult zebrafish: Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, v. 138, p. 363-373.

Martinez-Alvarez, R. M., M. C. Hidalgo, A. Domezain, A. E. Morales, M. Garcia-Gallego, and A. Sanz, 2002, Physiological changes of sturgeon Acipenser naccarii caused by increasing environmental salinity: Journal of Experimental Biology, v. 205, p. 3699-3706.

McWilliams, S. R., C. Guglielmo, B. Pierce, and M. Klaassen, 2004, Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective: Journal of Avian Biology, v. 35, p. 377-393.

Metcalfe, N. B., and C. Alonso-Alvarez, 2010, Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death: Functional Ecology, v. 24, p. 984-996.

Meunier, B., S. P. de Visser, and S. Shaik, 2004, Mechanism of oxidation reactions catalyzed by cytochrome P450 enzymes: Chemical Reviews, v. 104, p. 3947-3980.

Møller, A. P., 1988, Testes size, ejaculate quality and sperm competition in birds: Biological Journal of the Linnean Society, v. 33, p. 273-283.

Monaghan, P., N. B. Metcalfe, and R. Torres, 2009, Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation: Ecology Letters, v. 12, p. 75-92.

Montgomery, M. K., A. J. Hulbert, and W. A. Buttemer, 2011, The long life of birds: the rat-pigeon comparison revisited. PLoS One 6.8: e24138.

Moore, I. T., N. Perfito, H. Wada, T. S. Sperry, and J. C. Wingfield, 2002, Latitudinal variation in plasma testosterone levels in birds of the genus Zonotrichia: General and Comparative Endocrinology, v. 129, p. 13-19.

Morais, S., T. Silva, O. Cordeiro, P. Rodrigues, D. R. Guy, J. E. Bron, J. B. Taggart, J. G. Bell, and D. R. Tocher, 2012, Effects of genotype and dietary fish oil replacement with vegetable oil on the intestinal transcriptome and proteome of Atlantic salmon (Salmo salar): Bmc Genomics, v. 13.

Murdoch, W. J., E. A. Van Kirk, and B. M. Alexander, 2005, DNA damages in ovarian surface epithelial cells of ovulatory hens: Experimental Biology and Medicine, v. 230, p. 429-433.

Myatt, L., and X. L. Cui, 2004, Oxidative stress in the placenta: Histochemistry and Cell Biology, v. 122, p. 369-382.

Navarro, A., C. Gomez, J. M. Lopez-Cepero, and A. Boveris, 2004, Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer: American Journal of Physiology-Regulatory Integrative and Comparative Physiology, v. 286, p. R505-R511.

Nikinmaa, M., 2014, An Introduction to Aquatic Toxicology, v. 1: United States of America, Academic Press, 240 p.

Noltmann, E., C. J. Gubler, and S. A. Kuby, 1961, Glucose 6-phosphate dehydrogenase (Zwischenferment) .1. Isolation of crystalline form yeast: Journal of Biological Chemistry, v. 236, p. 1225-&.

Normandeau, E., J. A. Hutchings, D. J. Fraser, and L. Bernatchez, 2009, Population-specific gene expression responses to hybridization between farm and wild Atlantic salmon: Evolutionary Applications, v. 2, p. 489-503.

Nyman, M., M. Bergknut, M. L. Fant, H. Raunio, M. Jestoi, C. Bengs, A. Murk, J. Koistinen, C. Backman, O. Pelkonen, M. Tysklind, T. Hirvi, and E. Helle, 2003, Contaminant exposure and effects in Baltic ringed and grey seals as assessed by biomarkers: Marine Environmental Research, v. 55, p. 73-99.

Nyman, M., J. Koistinen, M. L. Fant, T. Vartiainen, and E. Helle, 2002, Current levels of DDT, PCB and trace elements in the Baltic ringed seals (Phoca hispida baltica) and grey seals (Halichoerus grypus): Environmental Pollution, v. 119, p. 399-412.

Nyman, M., H. Raunio, and O. Pelkonen, 2000, Expression and inducibility of members in the cytochrome P4501 (CYP1) family in ringed and grey seals from polluted and less polluted waters: Environmental Toxicology and Pharmacology, v. 8, p. 217-225.

Page, M. M., J. Richardson, B. E. Wiens, E. Tiedtke, C. W. Peters, P. A. Faure, G. Burness, and J. A. Stuart, 2010, Antioxidant enzyme activities are not broadly correlated with longevity in 14 vertebrate endotherm species: Age, v. 32, p. 255-270.

Pamplona, R., M. Portero-Otin, J. R. Requena, S. R. Thorpe, A. Herrero, and G. Barja, 1999a, A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria of the longevous pigeon than in the short-lived rat: Mechanisms of Ageing and Development, v. 106, p. 283-296.

Pamplona, R., M. Portero-Otin, D. Riba, F. Ledo, R. Gredilla, A. Herrero, and G. Barja, 1999b, Heart fatty acid unsaturation and lipid peroxidation, and aging rate, are lower in the canary and the parakeet than in the mouse: Aging-Clinical and Experimental Research, v. 11, p. 44-49.

Papakostas, S., A. Vasemagi, J.-P. Vaha, M. Himberg, L. Peil, and C. R. Primmer, 2012, A proteomics approach reveals divergent molecular responses to salinity in populations of European whitefish (Coregonus lavaretus): Molecular Ecology, v. 21, p. 3516-3530.

Perez-Campo, R., M. Lopez-Torres, S. Cadenas, C. Rojas, and G. Barja, 1998, The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach: Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology, v. 168, p. 149-158.

Peskin, A. V., and C. C. Winterbourn, 2000, A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1): Clinica Chimica Acta, v. 293, p. 157-166.

Porte, C., E. Escartin, L. M. Garcia, M. Sole, and J. Albaiges, 2000, Xenobiotic metabolising enzymes and antioxidant defences in deep-sea fish: relationship with contaminant body burden: Marine Ecology-Progress Series, v. 192, p. 259-266.

Powers, S. K., and M. J. Jackson, 2008, Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production: Physiological Reviews, v. 88, p. 1243-1276.

Prohaska, J. R., and H. E. Ganther, 1977, Glutathione peroxidase-activity of glutathione-S-transferases purified from rat-liver: Biochemical and Biophysical Research Communications, v. 76, p. 437-445.

Reglero, M. M., M. A. Taggart, P. Castellanos, and R. Mateo, 2009, Reduced sperm quality in relation to oxidative stress in red deer from a lead mining area: Environmental Pollution, v. 157, p. 2209-2215.

Regoli, F., 2012, Chemical pollutants ant the mechanisms of reactive oxygen species generation in aquatic organisms, *in* D. V.-M. Abele, José Pablo. Zenteno-Savín, Tania, ed., Oxidative stress in aquatic ecosystems, v. 1, Wiley-Blackwell, p. 308-316.

Richter, T., and T. von Zglinicki, 2007, A continuous correlation between oxidative stress and telomere shortening in fibroblasts: Experimental Gerontology, v. 42, p. 1039-1042.

Righetti, B. P. H., P. C. Simoes-Lopes, M. M. Uhart, and D. Wilhelm Filho, 2014, Relating diving behavior and antioxidant status: Insights from oxidative stress biomarkers in the blood of two distinct divers, Mirounga leonina and Arctocephalus australis: Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, v. 173, p. 1-6.

Ronnestad, I., S. Helland, and O. Lie, 1998, Feeding Artemia to larvae of Atlantic halibut (Hippoglossus hippoglossus L) results in lower larval vitamin A content compared with feeding copepods: Aquaculture, v. 165, p. 159-164.

Routti, H., R. J. Letcher, A. Arukwe, B. van Bavel, N. G. Yoccoz, S. G. Chu, and G. W. Gabrielsen, 2008, Biotransformation of PCBs in Relation to Phase I and II Xenobiotic-Metabolizing Enzyme Activities in Ringed Seals (Phoca hispida) from Svalbard and the Baltic Sea: Environmental Science & Technology, v. 42, p. 8952-8958.

Sahin, E., and S. Gumuslu, 2007, Stress-dependent induction of protein oxidation, lipid peroxidation and anti-oxidants in peripheral tissues of rats: Comparison of three stress models (immobilization, cold and immobilization-cold): Clinical and Experimental Pharmacology and Physiology, v. 34, p. 425-431.

SAS, 2008, SAS/STAT ® 9.2 User's Guide, Cary, NC, Sas Institute.

Scandalios, J. G., 2005, Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses: Brazilian Journal of Medical and Biological Research, v. 38, p. 995-1014.

Schafer, F. Q., and G. R. Buettner, 2001, Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple: Free Radical Biology and Medicine, v. 30, p. 1191-1212.

Schlezinger, J. J., W. D. J. Struntz, J. V. Goldstone, and J. J. Stegeman, 2006, Uncoupling of cytochrome P450 1A and stimulation of reactive oxygen species production by co-planar polychlorinated biphenyl congeners: Aquatic Toxicology, v. 77, p. 422-432.

Selman, C., J. S. McLaren, M. J. Himanka, and J. R. Speakman, 2000, Effect of long-term cold exposure on antioxidant enzyme activities in a small mammal: Free Radical Biology and Medicine, v. 28, p. 1279-1285.

Shannon, P., A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski, and T. Ideker, 2003, Cytoscape: A software environment for integrated models of biomolecular interaction networks: Genome Research, v. 13, p. 2498-2504.

Smith, I. K., T. L. Vierheller, and C. A. Thorne, 1988, Assay of glutathione-reductase in crude tussue-homogenates using 5,5'-dithiobis(2-nitrobenzoic acid): Analytical Biochemistry, v. 175, p. 408-413.

Smyth, G. K., 2004, Linear models and empirical bayes methods for assessing differential expression in microarray experiments: Statistical applications in genetics and molecular biology, v. 3, p. Article3-Article3.

Smyth, G. K., and T. Speed, 2003, Normalization of cDNA microarray data: Methods, v. 31, p. 265-273.

Speakman, J. R., 2008, The physiological costs of reproduction in small mammals: Philosophical Transactions of the Royal Society B-Biological Sciences, v. 363, p. 375-398.

Stegeman, J. J., and J. J. Lech, 1991, Cytochrome P-450 monooxygenase systems in aquatic species – Carcinogen metabolism and Biomarkers for carcinogen and pollutant exposure: Environmental Health Perspectives, v. 90, p. 101-109.

Steidl, R. J., and L. Thomas, 2001, Power analysis and experimental design, *in* S. M. Scheiner, and J. Gurevitch, eds., Design and analysis of ecological experiments: New York, Oxford University Press, p. 14-36.

Subramanian, S., G. Rajendiran, P. Sekhar, C. Gowri, P. Govindarajulu, and M. M. Aruldhas, 2006, Reproductive toxicity of chromium in adult bonnet monkeys (Macaca radiata Geoffrey). Reversible oxidative stress in the semen: Toxicology and Applied Pharmacology, v. 215, p. 237-249.

Surai, P., F, 2002, Natural Antioxidants in Avian Nutrition and Reproduction, v. 1, Nottingham University Press, 616 p.

Szep, T., A. P. Moller, S. Piper, R. Nuttall, Z. D. Szabo, and P. L. Pap, 2006, Searching for potential wintering and migration areas of a Danish Barn Swallow

population in South Africa by correlating NDVI with survival estimates: Journal of Ornithology, v. 147, p. 245-253.

Tietze, F., 1969, Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione – Applications to mammalian blood and other tissues: Analytical Biochemistry, v. 27, p. 502-&.

Toescu, V., S. L. Nuttall, U. Martin, M. J. Kendall, and F. Dunne, 2002, Oxidative stress and normal pregnancy: Clinical Endocrinology, v. 57, p. 609-613.

Tryland, M., B. A. Krafft, C. Lydersen, K. M. Kovacs, and S. I. Thoresen, 2006, Serum chemistry values for free-ranging ringed seals (Pusa hispida) in Svalbard: Veterinary Clinical Pathology, v. 35, p. 405-412.

Ukeda, H., D. Kawana, S. Maeda, and M. Sawamura, 1999, Spectrophotometric assay for superoxide dismutase based on the reduction of highly water-soluble tetrazolium salts by xanthine-xanthine oxidase: Bioscience Biotechnology and Biochemistry, v. 63, p. 485-488.

Vazquez-Medina, J. P., T. Zenteno-Savin, and R. Elsner, 2006, Antioxidant enzymes in ringed seal tissues: Potential protection against dive-associated ischemia/reperfusion: Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, v. 142, p. 198-204.

Vazquez-Medina, J. P., T. Zenteno-Savin, and R. Elsner, 2007, Glutathione protection against dive-associated ischemia/reperfusion in ringed seal tissues: Journal of Experimental Marine Biology and Ecology, v. 345, p. 110-118.

Vazquez-Medina, J. P., T. Zenteno-Savin, R. Elsner, and R. M. Ortiz, 2012, Coping with physiological oxidative stress: a review of antioxidant strategies in seals: Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology, v. 182, p. 741-750.

Venditti, P., C. M. Daniele, M. Balestrieri, and S. Di Meo, 1999, Protection against oxidative stress in liver of four different vertebrates: Journal of Experimental Zoology, v. 284, p. 610-616.

Vuori, K., M. Kiljunen, M. Kanerva, M. L. Koljonen, and M. Nikinmaa, 2012, Stock-specific variation of trophic position, diet and environmental stress markers in Atlantic salmon Salmo salar during feeding migrations in the Baltic Sea: Journal of Fish Biology, v. 81, p. 1815-1833.

Wang, J., Y. Wei, X. Li, M. Xu, and J. Dai, 2007, Identification of differentially expressed genes from contaminant and thermal exposed goldfish Carassius auratus in Gaobeidian Lake in Beijing, China: Ecotoxicology, v. 16, p. 525-532.

Wang, J., Y. Wei, D. Wang, L. L. Chan, and J. Dai, 2008, Proteomic study of the effects of complex environmental stresses in the livers of goldfish (Carassius auratus) that inhabit Gaobeidian Lake in Beijing, China: Ecotoxicology, v. 17, p. 213-220.

Whyte, J. J., R. E. Jung, C. J. Schmitt, and D. E. Tillitt, 2000, Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure: Critical Reviews in Toxicology, v. 30, p. 347-570.

Wilhelm, D., F. Sell, L. Ribeiro, M. Ghislandi, F. Carrasquedo, C. G. Fraga, J. P. Wallauer, P. C. Simoes-Lopes, and M. M. Uhart, 2002, Comparison between the antioxidant status of terrestrial and diving mammals: Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology, v. 133, p. 885-892.

Williams, T. D., N. Turan, A. M. Diab, H. Wu, C. Mackenzie, K. L. Bartie, O. Hrydziuszko, B. P. Lyons, G. D. Stentiford, J. M. Herbert, J. K. Abraham, I. Katsiadaki, M. J. Leaver, J. B. Taggart, S. G. George, M. R. Viant, K. J. Chipman, and F. Falciani, 2011, Towards a System Level Understanding of Non-Model Organisms Sampled from the Environment: A Network Biology Approach: Plos Computational Biology, v. 7.

Wu, G. Y., Y. Z. Fang, S. Yang, J. R. Lupton, and N. D. Turner, 2004, Glutathione metabolism and its implications for health: Journal of Nutrition, v. 134, p. 489-492.

Zenteno-Savin, T., E. Clayton-Hernandez, and R. Elsner, 2002, Diving seals: are they a model for coping with oxidative stress?: Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, v. 133, p. 527-536.