Turun yliopisto
University of Turku

STRESS INDUCED ALTERATIONS IN REDOX STATUS AND TELOMERE LENGTH AS A POTENTIAL MECHANISM OF BIOLOGICAL AGEING

Janina Stauffer

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja – ser. A II osa – tom. 345 | Biologica – Geographica – Geologica | Turku 2018

Turun yliopisto
University of Turku

STRESS INDUCED ALTERATIONS IN REDOX STATUS AND TELOMERE LENGTH AS A POTENTIAL MECHANISM OF BIOLOGICAL AGEING

Janina Stauffer

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. A II osa - tom. 345 | Biologica - Geographica - Geologica | Turku 2018

University of Turku

Faculty of Science and Engineering
Department of Biology
Division of Physiology and Genetics
University of Turku Graduate School (UTUGS)
Doctoral Programme of Biology, Geography and Geology (BGG)

Supervised by

Dr. Petteri Ilmonen
Department of Biology
University of Turku
Finland

Dr. Tiina Henttinen
Department of Biology
University of Turku
Finland

Reviewed by

Professor Dennis Hasselquist
Department of Biology
Lund University
Lund, Sweden

Dr. Michaël Beaulieu
Zoological Institute and Museum
University of Greifswald
Greifswald, Germany

Opponent

Dr. Sylvie Massemin
Department of Ecology, Physiology and Ethology
Institut Pluridisciplinaire Hubert Curien
Strasbourg, French

ISBN 978-951-29-7361-3 (PRINT)
ISBN 978-951-29-7362-0 (PDF)
ISSN 0082-6979 (PRINT)
ISSN 2343-3183 (PDF)
Painosalama Oy - Turku, Finland 2018

Dedicated to my other half, Timo Vannesluoma.

Abstract

Biological ageing, i.e. the loss of physiological functioning leading to increased frailty and mortality risk with advancing age, is influenced both by genotype and environmental factors as well as their interactions. The leading evolutionary hypothesis is that the resource allocation from self-maintenance and repair to growth and reproduction is the cause for this detrimental process. In this thesis I studied redox metabolism and telomere attrition, which have been suggested to be molecular mechanisms of biological ageing across species. Redox status is a dynamic state composed of reactive species (RS) induced oxidative damage and antioxidant (AO) defense. In healthy tissues most of the RS are produced by normal metabolism, however, several stressors can enhance RS production. Telomeres are protective DNA sequences at the end of chromosomes, which shorten with each cell division and advancing age. Telomeres are vulnerable to RS and short telomere length (TL) is linked with increased mortality and age-related disease risk.

In the first two studies, the main interest was to elucidate the impacts of different stressors on TL and redox status in free-living vertebrates. In the first study, the effects of heavy metal pollution on TL and on redox status were investigated in adult and nestling great tits and pied flycatchers. The results varied remarkably among the species and age groups. Both of these passerine species showed pollution induced redox status alterations, but great tits, especially at an early age were more vulnerable to pollution induced oxidative stress and telomere attrition. In addition to pollution, within-brood competition induced growth stress was also found to cause TL attrition and alterations in AO-defense in great tit nestlings. In the second study, the potential roles of TL and AO defense in pathogen resistance and disease severity were investigated in *Tetracapsuloides bryosalmonae*-parasite infected brown trout. Parasite load was not directly associated with either AOs or with TL, but some AO-activities were lower in fish with severe disease symptoms than in fish with modest symptoms. It seems that TL may reflect individuals' ability to tolerate infection, as the fish which were less sensitive to parasite-induced impaired growth had longer TLs and lower levels of certain AOs than the more sensitive fish.

In the last two studies, experimentally produced selection lines of wild house mice with divergent TLs were used to obtain better understanding of the relationship between redox status and TLs in constant laboratory conditions. In the third study, the age and sex-depended alterations in AOs in relation to TL were examined with a longitudinal approach. Most of the measured AOs increased from the age of 8 weeks to 6 months, and females had, in general, higher AO-levels than males. TL was also found to correlate positively with some of the AOs. In addition,

there was a delayed negative effect of sibling competition on females' AO defense in later life. In the last study, the differences in metabolism and redox status were examined in relation to experimentally selected divergent TLs. Half of the experimental mice were exposed to short-term exercise in order to magnify the possible among TL selection group differences. Males in the long TL group had considerably lowered AO-defense although their cell respiration was relatively high and comparable to control TL males. Short TL group males showed drastic reduction in cell respiration, and yet high AO defense activity.

Overall, the results indicate that a variety of environmental stressors can accelerate biological ageing via oxidative stress and telomere attrition. Furthermore, unfavorable early life experiences, like growth stress can have long-lasting negative consequences for individuals' future fitness and survival. Taken together the results highlight that changes in redox status and telomeres are highly dependent on the species, sex, the phase of life and the experienced stress. The very same factors determine which parts of the AO defense system are used for self-maintenance.

Tiivistelmä

Biologinen ikääntyminen, eli ikääntymisen myötä tapahtuva fysiologisten toimintojen heikkeneminen ja kuolleisuusriskin kasvu, määrittyy perimän ja ympäristötekijöiden, sekä näiden yhdysvaikutusten seurauksena. Biologisen ikääntymisen evolutiivisena syynä pidetään resurssien allokaatiota elimistön ylläpidosta ja korjausmekanismeista kasvuun ja lisääntymiseen. Tässä väitöskirjassa tutkin redox-tasapainoa ja telomeerejä, joiden oletetaan toimivan biologisen ikääntymisen molekyylitason mekanismina. Redox-, eli hapetus-pelkistys-tasapaino on dynaaminen tila, joka koostuu vapaiden radikaalien (*engl.* reactive species, RS) aiheuttamista oksidatiivisista vaurioista ja antioksidanttipuolustuksesta (*engl.* antioxidant, AO). Terveissä kudoksissa RS syntyvät pääasiassa normaalin aineenvaihdunnan sivutuotteena, mutta useat eri stressitekijät voivat lisätä RS tuotantoa. Telomeerit ovat suojaavia alueita kromosomien päissä, jotka lyhenevät jokaisen solunjakautumisen ja ikääntymisen myötä. Telomeerit ovat alttiita oksidatiivisille vaurioille ja lyhyiden telomeerien on havaittu olevan yhteydessä lisääntyneeseen kuolleisuusriskiin ja kasvaneeseen alttiuteen sairastua ikääntymiseen liittyviin sairauksiin.

Kahdessa ensimmäisessä osatyössä kiinnostuksen kohteena olivat erilaisten stressitekijöiden vaikutus telomeerien pituuteen ja redox-tasapainoon luonnonvaraisilla selkärankaisilla. Ensimmäisessä osatyössä tutkin talitiais- ja kirjosiepponaarailla sekä poikasilla raskasmetallien vaikutuksia telomeerien pituuteen ja redox-tasapainoon. Tuloksissa ilmeni merkittäviä lajien ja ikäryhmien välisiä eroja. Saasteet aiheuttivat molemmilla varpuslinnuilla muutoksia redox-tasapainossa, mutta talitiaiset olivat, erityisesti poikasina, herkempiä saasteiden aiheuttamalle oksidatiiviselle stressille sekä telomeerien lyhenemiselle. Saastevaikutusten lisäksi havaitsin, että poikueen sisäisestä kilpailusta johtuva kasvustressi aiheutti talitiaispoikasilla telomeerien lyhenemistä ja muutoksia AO-puolustuksessa. Toisessa osatyössä selvitin taimenilla telomeerien pituuden ja antioksidanttipuolustuksen mahdollista yhteyttä *Tetracapsuloides bryosalmonae* loisinfektioon ja infektio-oireiden ilmenemisen vakavuuteen. Loisen määrä ei vaikuttanut suoraan AO:eihin, eikä telomeerien pituuteen, mutta eräät AO-aktiivisuudet olivat matalampia kaloilla, joilla oli vakavia infektio-oireita verrattuna kaloihin, joilla oli lievempiä oireita. Telomeerien pituus saattaa heijastaa yksilön kykyä selviytyä loisinfektiosta huolimatta, sillä kaloilla joiden kasvu ei hidastunut loisista huolimatta oli myös pidemmät telomeerit ja vähemmän tiettyjä AO:eja verrattuna loisinfektiolle herkempiin kaloihin.

Ymmärtääkseni paremmin redox-tasapainon ja telomeerien välistä yhteyttä käytin kahdessa viimeisessä osatyössäni villeistä kotihiiristä telomeerin pituuteen kohdistuneen valinnan avulla tuotettuja hiiriä kontrolloidussa laboratorio-ympäristössä. Kolmannessa osatyössä selvitin iästä ja sukupuolesta riippuvia

telomeerien pituuden vaikutuksia redox-tilaan yksilöiden sisällä tehdyissä AO-toistomittauksissa. Useimmat mitatut AO:it lisääntyivät 8-viikon iästä 6-kuukauden ikään. Lisäksi naaraiden AO-tasot olivat korkeampia kuin urosten. Havaitsin myös että telomeerien pituus korreloi positiivisesti osan AO:ien kanssa. Tämä osatyö osoitti myös poikueen sisäisen sisaruskilpailun aiheuttavan naarailla myöhemmällä iällä ilmeneviä negatiivisia vaikutuksia AO-puolustukseen. Viimeisessä osatyössä tutkin telomeerien pituudesta riippuvia muutoksia hiirien aineenvaihdunnassa ja redox tasapainossa tarkastelemalla eroja keinotekoisen valinnan avulla tuotettujen toisistaan telomeerien pituudeltaan eroavien hiirien välillä. Toteutin lyhytkestoisen rasituskokeen puolella koehiiristä voimistaakseni mahdollisia telomeeri-valintaryhmien välisiä eroja metaboliassa ja AO-tasoissa. Pitkä telomeeri-ryhmän uroshiirillä oli huomattavasti madaltunut AO-puolustus, huolimatta siitä että niiden aineenvaihdunta oli suhteellisen korkea ja samalla tasolla kuin kontrolli-uroksilla. Lyhyt telomeeri-ryhmän uroksilla oli huomattavasti alentunut aineenvaihdunta, mutta siitä huolimatta korkea AO-aktiivisuus. Toisin kuin uroksilla, naaraiden telomeerivalintaryhmien välillä ei ollut eroja metaboliassa tai AO-aktiivisuudessa.

Kokonaisuudessaan väitöskirjan tulokset tukevat käsitystä, että erilaiset ympäristön stressitekijät voivat kiihdyttää biologista ikääntymistä oksidatiivisen stressin ja telomeerien lyhenemisen seurauksena. Lisäksi, varhaisiän epäedulliset kokemukset, kuten kasvustressi, voivat aiheuttaa muutoksia, jotka voivat pidemmällä aikavälillä heikentää yksilöiden jäljellä olevan elinajan kelpoisuutta ja elossasäilyvyyttä. Kaiken kaikkiaan tämän väitöskirjan tulokset korostavat, että muutokset redox-tasapainossa ja telomeereissä ovat lajikohtaisia ja vaihtelevat lisäksi sukupuolesta, elinkierron vaiheesta sekä koetusta stressistä riippuen. Samat tekijät vaikuttavat myös siihen, mitkä eri AO-puolustuksen osat ovat käytössä elimistön ylläpidossa.

Table of Contents

List of Original Publications and Author Contributions

This thesis is a summary of the following publications and a manuscript, referred to in the text by their Roman numerals.

I. Stauffer J, Panda B, Eeva T, Rainio M, Ilmonen P. 2017. *Telomere damage and redox status alterations in free-living passerines exposed to metals.* Science of the Total Environment, 575, 841-848.

II. Stauffer J, Bruneaux M, Panda B, Visse M, Vasemagi A, Ilmonen P. 2017. *Telomere length and antioxidant defense associate with parasite-induced retarded growth in wild brown trout.* Oecologia, 185, 365-374.

III. Stauffer J, Panda B, Ilmonen P. 2018. *Telomere length, sibling competition and development of antioxidant defense in wild house mice.* Mechanisms of ageing and development, 169, 45-52.

IV. Stauffer J, Panda B, Ilmonen P. *Selection for divergent telomere lengths leads into altered metabolism and redox status in wild house mice.* Manuscript.

Contributions to the original publications.

	I	II	III	IV
Study design	TE, MR, PI	AV, PI	JS	JS, PI
Experiment/field work	TE, MR	MB, MV, AV	JS, BP, PI	JS
Laboratory	JS, BP	JS, BP, MV	JS, BP	JS, BP
Data analysis	JS	JS	JS	JS
Manuscript	JS, PI, BP, TE, MR	JS, PI, AV, MB, BP, MV	JS, PI, BP	JS, PI, BP

Abbreviations

AO	Antioxidant
ARP	Aldehyde reactive probe
BCA	The bicinchoninic acid assay
BSA	Bovine serum albumin
CAT	Catalase
CDNB	1-chloro-2,4-dinitrobenzene
DNA	Deoxyribonucleic acid
DTNB	5,5-dithiobis(2-nitrobenzoic acid)
ETC	Electron transport chain
FOX-2	The ferrous oxidation in xylenol orange version 2
G6-P	D-glucose 6-phosphate sodium salt
G6PDH	Glucose 6-phosphate dehydrogenase
GP	Glutathione peroxidase
GR	Glutathione reductase
GSH/GSSG	The ratio between reduced and oxidized glutathione
GSH_{tot}	Total amount of glutathione
GST	Glutathione S-transferase
ICP-MS	Inductively coupled plasma mass spectrometry
IGF-1	Insulin like growth factor 1
LP	Lipid peroxidation
MT	Metallothioneins
mTOR	The mechanistic target of rapamycin
NADPH	β-Nicotinamide Adenine Dinucleotide Phosphate
NTC	Non-template control
PC	Principal component
PCA	Principal component analyses
PKD	Proliferative kidney disease
qPCR	Quantitative real-time polymerase chain reaction
RBC	Red blood cells
ROS	Reactive oxygen species
RPMI	Roswell park memorial institute medium
RS	Reactive species, pro-oxidants, free-radicals
SOD	Superoxide dismutase
TL	Telomere length
TPP	Triphenylphosphine
TRF	Time-resolved fluorescence
WST-1	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt
WBC	White blood cell
18S rRNA	18S ribosomal RNA
36B4	Ribosomal protein, large, P0

1 Introduction

1.1 Biological ageing

Biological ageing (i.e. senescence) is inevitable, age-dependent somatic deterioration and loss of physiological functioning, leading to decline of fertility and increase of mortality risk at older age. Ageing has received increasing attention among researchers over the centuries producing a plethora of theories explaining the age-dependent physiological deterioration of organisms. Evolutionary, ultimate hypotheses try to explain why we age, whereas mechanistic, proximate hypotheses try to solve the molecular mechanisms underlying the ageing process. Based on the current knowledge, organismal senescence is due to accumulated damage in somatic cells as a consequence of resource allocation from self-maintenance and repair to growth and reproduction (Kirkwood, 2002; Kirkwood and Rose, 1991). To date, current studies suggest that stress resistance, oxidative damage, telomere shortening, and particular signaling pathways e.g. insulin/IGF-1, sirtuin, and mTOR pathways are prominent mechanisms that are involved in biological ageing (Gems and Partridge, 2013; Monaghan and Haussmann, 2006; Monaghan et al., 2009). Although the proximate mechanisms of biological ageing still remain rather unclear, there is a general consensus that ageing is a result of multifactorial and complex processes, which interact with each other leading to considerable variation in the rate of ageing and expected lifespan among species and individuals (e.g. Kirkwood, 2011; Lopez-Otin et al., 2013; Ricklefs, 2010).

1.1.1 The Evolutionary hypotheses of ageing at the organism level

Ageing is a result of evolution due to natural selection. In addition to intrinsic mortality factors (i.e. biological ageing), the organismal life-span is limited due to extrinsic mortality factors e.g. predators, infectious diseases and accidents. Due to these extrinsic mortality factors only a few individuals survive to very old age in nature, and therefore alleles that act late in life have a low chance of ever being expressed. Because of this “selection shadow”, the force of natural selection declines with advancing age. Based on this fundamental concept of declining force of natural selection Medawar (1952) reasoned that alleles that are neutral (i.e. have no effect) in early life, but detrimental in late life are not efficiently eliminated by natural selection, and thus accumulate in populations over time and contribute to the evolution of biological ageing. Medawar’s Mutation Accumulation theory was developed further and complemented by Williams (1957) in the Antagonistic Pleiotropy theory by realizing that the alleles that are beneficial in early life, but detrimental in late life are not eliminated, but in fact, favored by natural selection, and consequently accumulate in populations and contribute to the evolution of ageing. Thus, under Williams' hypothesis, the evolution of ageing can be seen as a maladaptive byproduct of selection favoring high and early fecundity at the expense of survival at old age (Fabian and Flatt, 2011). Williams (1957) also brought out the idea of the allocation dilemma between maintenance and reproduction.

According to the life-history theory, there are trade-offs between investment in competing traits in the context of resource allocation (Stearns, 1992). In other words, beneficial adaptations in one trait are balanced by costs in another trait. Natural selection favors individuals that allocate limited resources with an optimal balance between costs and benefits, thereby maximizing lifetime reproductive success. Optimal allocation depends on a variety of interacting organismal (e.g. behavioral, physiological and morphological traits) and environmental variables (e.g. food resources and temperature) (Figure 1). The disposal soma theory of ageing suggests that resources allocated to growth and reproduction deplete resources available for self-maintenance and repair (Kirkwood, 1977; Kirkwood and Holliday, 1979). Size and growth has played a major but controversial role in theories of ageing. Large species tend to live longer than smaller ones, but within the species it seems to be the opposite (de Magalhaes and Faragher, 2008; Metcalfe and Monaghan, 2003; Rollo, 2002). Fast growth is linked to reduced longevity (Hector and Nakagawa, 2012; Metcalfe and Monaghan, 2003; Monaghan and Ozanne, 2018) and growth is expected to be optimized via a number of life-history trade-offs (e.g. Arendt and Wilson, 1997; Metcalfe and Monaghan, 2001). Since costs in self-maintenance and repair are the cause for organismal senescence, mechanistic theories have tried to solve how this damage accumulation in somatic cells develops at the cellular level.

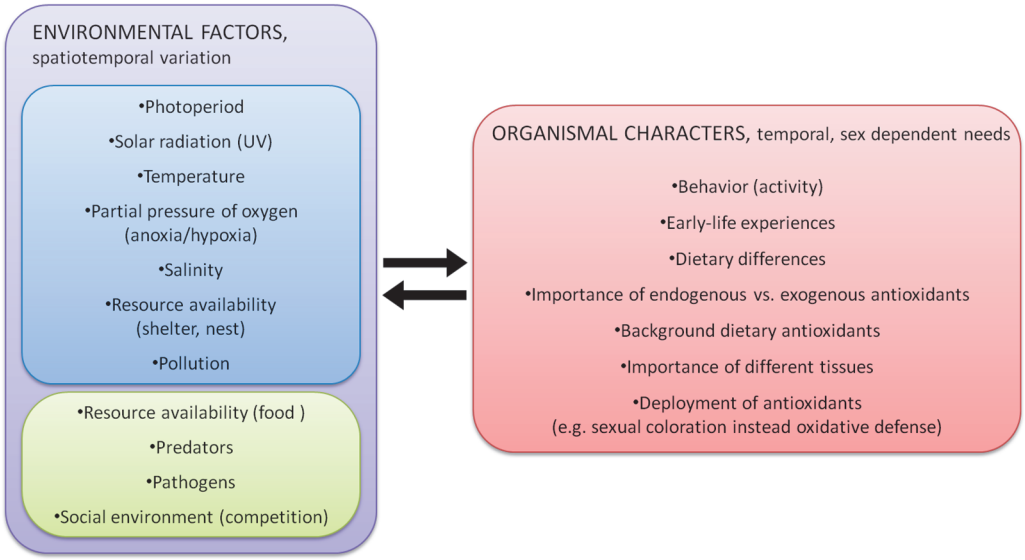


Figure 1. Variation in biological ageing, among and within species, depends on organismal characters (red background) and prevailing abiotic (blue background) and biotic (green background) environmental factors (purple background). Individual temporal needs, based on physiological stage depending on sex, phase of life and earlier experiences are in interaction with spatiotemporal variation in environmental factors resulting in different life-histories and lifespans. Furthermore, each of the organismal and environmental factors might have additive, antagonistic or synergistic effects with each other.

1.1.2 The Mechanistic hypotheses of ageing at the cellular level

Physiological pathways and molecular mechanisms that regulate energy production and expenditure, immune responses and hormones mediate trade-offs at the organismal level and thus are relevant in every aspect of life-histories and longevity. The importance of energy production and expenditure in ageing was initially presented in the rate of living theory, which was based on the observation that the maximum lifespan of species is dependent on a mass-specific rate of metabolism i.e. bigger animals have lower metabolic rate and live longer (Pearl, 1928). Metabolism is the main source of reactive species (RS) that cause oxidative damage in macromolecules. The free radical theory of ageing suggest that the accumulation of oxidative damage due to RS production drives cells to senescence and finally to degradation of tissues and disturbed body functions, leading to biological ageing (Beckman and Ames, 1998; Harman, 1956). RS are known to be harmful and cause oxidative stress, decrease fitness and accelerate biological ageing (Finkel and Holbrook, 2000). However, it has been shown that neither the amount of RS nor oxidative damage are directly proportional to metabolic rate, and in addition, increase in metabolism does not always reduce lifespan (Hulbert et al., 2007; Speakman, 2005). The current oxidative-stress theory of ageing has complemented the free radical theory, suggesting that several mechanisms, e.g. antioxidant defense, fatty-acid profiles and uncoupling proteins affect resistance to RS induced oxidative stress and ageing (Brand, 2000; Hulbert, 2005; Pamplona et al., 2002; Speakman et al., 2004). Despite their destructive activity, RS function as well-described secondary messengers in a variety of cell signaling pathways (Droge, 2002; Thannickal and Fanburg, 2000). Whether RS act as damaging or beneficial signaling molecules depends on the equilibrium between the production and scavenging of RS. It has been shown that mild stress can have a positive effect through hormetic mechanisms and even extend lifespan (Costantini et al., 2012; Lagisz et al., 2013; Yang and Hekimi, 2010). Response to mild mitochondrial stress appears to induce a wide-ranging cytoprotective state resulting in long-lasting metabolic and biochemical changes in the cells. Rather than being deleterious, these changes may hinder cellular ageing and reduce susceptibility to disease (Yun and Finkel, 2014).

The cell senescence telomere hypothesis of ageing (Weinert and Timiras, 2003) is based on the limited replicative capacity of cells (Hayflick, 1965), which is driven by gradual telomere shortening in each replication event (Harley et al., 1990). Eventually, telomeres reach a critical length which leads to cell senescence (Haussmann and Marchetto, 2010; Hornsby, 2003). It might be that only a few telomeres in the cell must reach this critical length to induce the senescence process (Campisi and di Fagagna, 2007). Telomere dysfunctions have been found to be associated with accelerated ageing and increased risk of ageing related diseases (Blackburn et al., 2006). However, the rate of telomere shortening is highly dependent on the phase of life and stress (Epel et al., 2004; Heidinger et al., 2012). The cell senescence telomere hypothesis is compatible with the oxidative stress

theory of ageing, as oxidative stress has been shown to accelerate telomere attrition both *in vitro* and *in vivo* (Reichert and Stier, 2017; von Zglinicki, 2002). Overall, it seems that all of the suggested hypotheses of ageing are linked with each other, producing a dynamic network of multiple molecular mechanisms (Figure 2).The relative role of these mechanisms for biological ageing vary depending on multiple factors such as the tissue type, species, life history stage and environmental conditions (Kirkwood, 2011; Lopez-Otin et al., 2013). Although the role of redox status in ageing is not completely understood, it represents a prominent physiological mechanism linking environmental stressors, susceptibility to diseases and the rate of ageing.

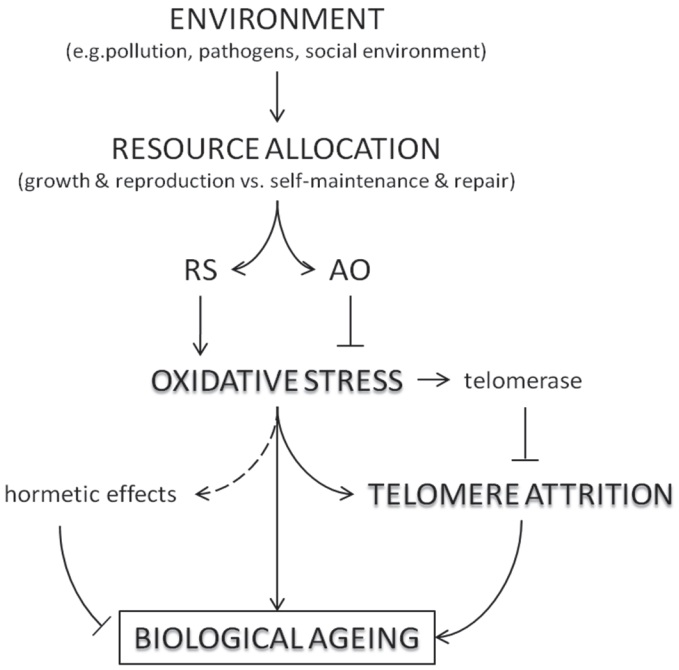


Figure 2. The mechanism of ageing is a multidimensional network of several molecular processes, regulated by environment induced resource allocation between life-history traits. RS predominance over antioxidant activity leads to oxidative stress and damage in macromolecules including telomeres and their repair mechanisms (e.g. telomerase). Accumulation of damage is a proximate mechanism of biological ageing. However, mild stress can have hormetic effects and extend lifespan. RS=reactive species, AO=antioxidant.

1.2 Redox status: the balance between reactive species and antioxidants

Cells of aerobic organisms are exposed to RS as part of normal metabolism, which affects redox balance. Unfavorable environmental conditions and exposure to various life-stresses can increase RS production. Cells maintain redox balance through elimination of RS and oxidative damage with antioxidant (AO) defense mechanisms. Oxidative stress can be defined as the imbalance between RS

production and AO capability. Therefore, insufficient AO defense in relation to RS production leads to oxidative stress and damage in different macromolecules like lipids, proteins and DNA (von Zglinicki, 2002). Several molecules and signaling pathways affect the redox balance, and organisms have a variety of behavioral and physiological adaptations to maintain the balance (Costantini, 2014). Therefore, different species and even populations have differences in susceptibility to oxidative damage and in the relative importance of specific endogenous and exogenous AOs. Resistance to oxidative stress, i.e. the ability to maintain redox balance, promotes longevity (reviewed in Balaban et al., 2005; Barja, 2004; Reichert and Stier, 2017). Long-lived endothermic vertebrates have lower RS production and higher level of AOs, or molecules that are resistant to oxidative damage (Hulbert et al., 2007; Lambert et al., 2007; Lopez-Torres et al., 1993; Pamplona and Costantini, 2011; Perez-Campo et al., 1994; Sasaki et al., 2008). However, determination of the oxidative stress or redox balance of the body is somewhat challenging due to its continuously changing multivariate nature. Firstly, the low AO levels might be a result of high chronic stress (worn out defense) or low stress (low defense is enough). Secondly, the variation in the magnitude and duration of stress exposures at different phases of life change the responses (Beaulieu and Costantini, 2014; Costantini et al., 2014).

1.2.1 Sources of reactive species

Reactive species (RS), also called free radicals or pro-oxidants, are derivates of reactive oxygen species (ROS) or other elements such as nitrogen. RS contain one or more unpaired electrons and are thus highly reactive with biological molecules. The biologically most relevant RS are superoxide, hydroxyl radical and nitric oxide, which can trigger chain reactions and pass reactivity on to other compounds (Finkel and Holbrook, 2000). The primary source of RS in healthy tissues is aerobic metabolism by the electron transport chain (ETC) in mitochondria (Beckman and Ames, 1998; Halliwell and Gutteridge, 2007). Electrons leaked at complexes can reduce oxygen and give rise to superoxide anions. ETC complexes I and III are considered to be the main sites of RS production, and any structural modification of complex I or III may alter the RS production process (Aledo, 2014). Therefore, RS production is highly affected by the metabolic rate and e.g. fast growth has been found to be associated with high oxidative stress both in laboratory and field studies (Smith et al., 2016). The other endogenous and external sources of RS are the peroxisomal fatty acid oxidation, the activity of phagocytic cells (oxidative burst), the metabolism of xenobiotics (cytochrome reactions, redox active metals), and the exposure to UV-light or ionizing radiation (Balaban et al., 2005; Beckman and Ames, 1998; Finkel and Holbrook, 2000; Meunier et al., 2004).

1.2.2 Antioxidant defense

AOs are any substances, mechanisms or structures, which delay, prevent or remove oxidative damage of its target molecule. AOs can be endogenous or dietary e.g. vitamin E, carotenoids, and polyphenols. There is a large variety of AO molecules and pathways, and many of them are highly conserved across species (Pamplona and Costantini, 2011). AO machinery maturates postnatally (Costantini et al., 2006) and therefore the effectiveness of AO defenses increases with age (Blount et al., 2003; Robles et al., 2001). AOs can prevent oxidative damage directly or indirectly by interacting synergistically with other AOs. This provides high plasticity in stress responses as the defense is not solely dependent on a single molecule or mechanism. The AO defense is also closely associated with other physiological mechanisms. For example, short-term increases in glucocorticoids, i.e. stress hormones, enhance AO-activity, whereas prolonged exposure to glucocorticoids results in reduced AO defense (Birnie-Gauvin et al., 2017; Costantini et al., 2011). In addition, redox inactive metals can deplete major AOs (Ercal et al., 2001) and the AO defense is altered due to immune response and RS-production of macrophages (Fialkow et al., 2007).

AOs are divided into enzymatic and non-enzymatic molecules. The various AOs measured for this thesis and their associations are presented in Figure 3. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), glutathione reductase (GR), glutathione S-transferase (GST), and glucose 6-phosphate dehydrogenase (G6PDH) are enzymatic sources of AOs. Glutathione (GSH) is one of the most important non-enzymatic AOs and the most abundant non-protein thiol in cells. In cells GSH is mainly in the reduced form. GSH can be oxidized with GR or GST, while protecting the other molecules from oxidation. The oxidation of GSH leads to the formation of glutathione disulfide (GSSG). Therefore, the ratio between GSH and GSSG (GSH/GSSG) is an indicator of the overall state of a cell and it is often used to measure oxidative stress in general (Halliwell and Gutteridge, 2007; Valko et al., 2005). GR can recycle GSSG back to the reduced form GSH with β-Nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate (Halliwell and Gutteridge, 2007). NADPH is generated by the pentose phosphate pathway, where G6PDH has a major role (Halliwell and Gutteridge, 2007). GST participates in biotransformation processes transforming xenobiotics into less harmful forms (Jancova et al., 2010) and is also important in host immune responses during parasitic infection (Skalova et al., 2007). Besides glutathione metabolism and recycling, SOD and CAT are two important AO-enzymes. SOD catalyzes the dismutation of the superoxide radical anions into hydrogen peroxide (H_2O_2) and oxygen, and CAT catalyzes the decomposition of H_2O_2 to water and oxygen (Halliwell and Gutteridge, 2007). In addition, GP also has a role in H_2O_2 elimination, but whereas CAT has low affinity and is effective when H_2O_2-concentrations are high, GP has high affinity and is significant with low H_2O_2-concentrations (Hulbert et al., 2007).

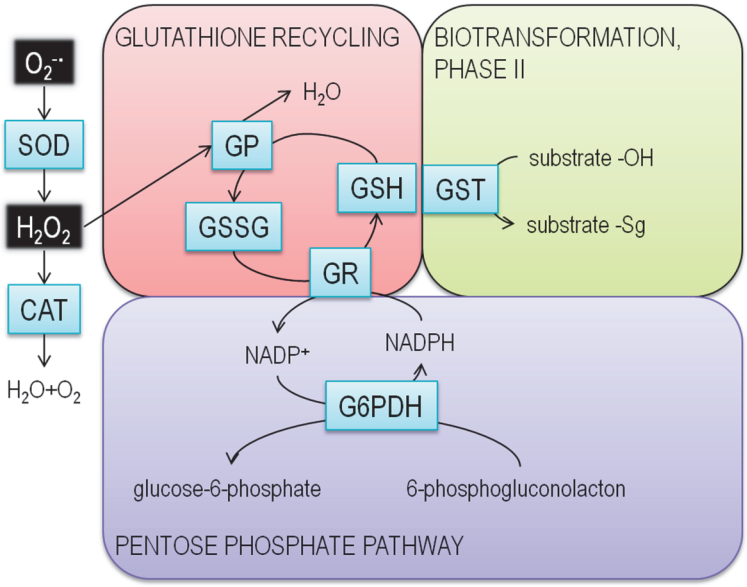


Figure 3. A variety of AO molecules (blue boxes) and pathways interact with RS (black boxes) production and define the redox status. Glutathione recycling (red background) and metabolism are essential parts of AO defense and directly related to the pentose phosphate pathway (purple background). The pentose phosphate pathway also has a role in immune defense. In addition, redox status is sensitive to toxicological responses, since biotransformation (green background) is linked to glutathione recycling.

1.2.3 Oxidative stress-mediated damage to macromolecules

The rate and severity of oxidative damage to macromolecules varies, because the sensitivity to oxidation, and the irreversibility or function of the repair mechanisms are highly dependent on the molecules (Hulbert et al., 2007). RS induced oxidative damage can appear in proteins (protein carbonylation), lipids (hydroperoxides, malondiaaldehyde, 4-hydroxy-2-nonenal and isoprostanes) or in DNA (8-hydroxyguanosine, 8-hydroxy-2'-deoxyguanosine and telomere damage) (von Zglinicki et al., 2001).

The biologically and functionally relevant type and level of oxidative damage leading to cell deterioration, senescence and increased risk of disease and mortality, is not clear. In any case, long-living species have developed many different strategies to avoid oxidative damage. For example, certain polyunsaturated fatty acids which are more vulnerable to oxidation are less abundant in long-living species (Hulbert et al., 2007; Pamplona, 2008). In addition, some long-living species e.g. naked mole rats (Perez et al., 2009) and bats (Salmon et al., 2009) have proteins that are resistant to oxidative damage. In general, cysteine and methionine are the most sensitive amino acids to oxidative damage (Stadtman et al., 2003) and long-lived species have less of these amino acids in mitochondrial respiratory chain complexes than short-lived species (Carlos Aledo et al., 2011; Moosmann and Behl, 2008). The vulnerability of DNA to oxidative damage is also related to its structure.

Guanine is the most easily oxidized nucleotide (Bjelland and Seeberg, 2003; Kovacic and Wakelin, 2001). On the other hand, DNA sequences with high AT-content are susceptible to mutations because of high free energy between strands, exposing them to spontaneous opening of the double strands of DNA (Samuels, 2005).

1.3 Telomeres

Telomeres are tandem repeats of short non-coding DNA sequences (TTAGGG) at the ends of chromosomes (Blackburn and Gall, 1978). Telomere length (TL) declines with advancing age, and both short TL and high telomere attrition rate have been linked to increased mortality and age-related disease risk (Armanios and Blackburn, 2012; Aubert and Lansdorp, 2008; Sanders and Newman, 2013; Tricola et al., 2018; Wilbourn et al., 2018). Eventually TL reaches a critical point leading to cell senescence and apoptosis, which cause problems in tissue renewal and organismal function (Monaghan and Haussmann, 2006; van Deursen, 2014). TL is heritable and genetically determined, but also shaped by environmental factors (Houben et al., 2008). TL is highly species specific, and it varies within species and among individuals at the same age (Dugdale and Richardson, 2018). This intraspecific variation in TL within the same age class is associated with environmental factors and fitness related traits. Therefore, TL has been suggested to be a potential biomarker for individual viability, which might reflect the environmental challenge that an individual faces, or has faced, but also the capacity to deal with it (Bateson, 2016; Epel et al., 2004; Monaghan, 2010; Pauliny et al., 2006).

1.3.1 Telomere shortening and maintenance

The telomere system of chromosome protection and the mechanisms contributing to telomere shortening are highly conserved across eukaryotes (Gomes et al., 2010). Telomeres protect genome integrity during cell division by protecting chromosomes from end-to-end fusion and degradation (Blackburn, 2005). Telomeres shorten with each cell division (replicative senescence), due to the incomplete replication of the 3’ ends of DNA strands (end-replication problem) (Blackburn, 2005; Hayflick, 1965). In addition, high stacked content of guanine bases in the telomere sequences makes them highly vulnerable to oxidative damage (Ludlow et al., 2014; Petersen et al., 1998; Sarkar and Liu, 2016; von Zglinicki, 2002). Reduced RS production in mitochondria, as well as AOs, reduces telomere shortening (Glade and Meguid, 2015; Houben et al., 2008; von Zglinicki, 2002). Telomerase enzyme activity is the most important telomere repair and maintenance mechanism in all vertebrates (Monaghan and Haussmann, 2006). Oxidative stress can also influence TL indirectly by interfering with telomerase activity (Beery et al., 2012; Borras et al., 2004; Haendeler et al., 2004; Kurz et al., 2004). The vulnerability of telomeres and their repair mechanisms to oxidative damage could explain why telomere attrition can be accelerated due to metabolic

dysfunctions, stress hormones, inflammation, and environmental stressors (Angelier et al., 2018; Glade and Meguid, 2015; Ilmonen et al., 2008; Kotrschal et al., 2007; Metcalfe and Monaghan, 2013; Monaghan, 2014; Price et al., 2013; Reichert and Stier, 2017; Zhang et al., 2016).

Telomerase is a reverse transcriptase enzyme, an RNA-dependent DNA polymerase synthesizing telomere DNA sequences (Taylor and Delany, 2000). Different telomerase activities are documented among species and cell types. In most somatic cells of endotherms (birds and mammals) telomere restoration is limited, because telomerase is suppressed (Gomes et al., 2010). On the contrary, many ectotherms (e.g. fish) show somatic telomerase activity throughout life (Gomes et al., 2010; Olsson et al., 2018). These notable differences in telomerase-activity are possibly based on different growth strategies and differences in tissue regeneration potential (Gomes et al., 2010). Most ectotherms are indeterminate growers, which continue to grow throughout life, whereas endotherms (e.g. birds and mammals) prefer a determinate growth strategy with early-life growth to sexual maturity and final size (Sebens, 1987). In addition, telomerase suppression in the somatic cells of endotherms has been suggested to prevent cancer (Wright and Shay, 2000). Therefore telomere shortening has been proposed to be an important sentinel system for removal of damaged cells, preventing the accumulation of damage in tissues (Gomes et al., 2010; von Zglinicki, 2002).

Both telomere shortening rate and telomerase activity varies depending on phase of life (Beery et al., 2012; Hatakeyama et al., 2016). Adverse early-life experiences induce telomere loss, which in turn can reduce future fitness and survival (Boonekamp et al., 2014; Fairlie et al., 2016; Watson et al., 2015). In addition, the rate of telomere loss is greatest during early life, when growth is fast leading to high cell proliferation and energy expenditure (Allsopp et al., 1995; Bhattacharyya and Lustig, 2006; Foote et al., 2011; Heidinger et al., 2012; Salomons et al., 2009). In general, the relationship between growth and TL is negative, however, it still remains unclear how exactly size, energy allocation and metabolism are associated with telomere dynamics and longevity (Monaghan and Ozanne, 2018). Furthermore, there is an ongoing debate on whether the TL or rate of telomere attrition matter the most.

2 Aims of the Thesis

The aim of this thesis is to investigate associations between redox status and TL in different vertebrates. One of the main interests was to elucidate the impacts of different stressors (heavy metal pollution, parasite infection and sibling competition) on alterations in redox status and TL depending on life stage or sex. As metabolism is one of the main sources of RS, one goal was to find out how TL, redox status and metabolic rate are connected with each other. As explained above, oxidative stress and telomere dynamics are thought to be involved in biological ageing and the evolution of life-histories. Therefore, the purpose of this thesis in the wider scope is to understand the complex network of biological ageing.

The specific objectives of the different studies are:

I. Does exposure to heavy metal pollution influence redox status and TL in two free-living passerine species? Are there any among-species or age-dependent differences in responses to heavy metals?

   Do subdominant nestlings show TL attrition and oxidative stress as a result of within-brood competition induced growth stress?

II. What are the potential roles of AO defense and TL in pathogen resistance and tolerance in *T. bryosalmonae*-infected fish?

III. How do different AO activities or the amount of glutathione change between the ages of 8 weeks and 6 months in house mice? More specifically, does sex, within-litter competition, or TL at 8 weeks of age have any impact on the AO levels, or on the age-dependent changes in AOs.

IV. Are there differences in metabolism or redox status between mouse lines that have been experimentally selected for divergent TLs?

3 Material and Methods

3.1 Study species and experimental designs

The role of oxidative stress in longevity has been widely studied among different species (Hulbert et al., 2007). The basic system of telomere dynamics and redox status are highly conservative across eukaryotes, and there are commonalities in senescence in a variety of species (Ricklefs, 2010). However, the details vary between and within species and among tissues, and are highly affected by the environment and its interaction with life stage (Reichert and Stier, 2017). In order to understand factors that cause biological ageing, and interactions between these factors, it is of great interest to study the relationships of molecular level mechanisms of ageing under different environmental conditions in a wide range of taxa. Wild animals provide genetically diverse and more reliable models in the context of normal ageing compared to many laboratory animal models, which are highly inbred and kept in unrealistically favorable conditions over dozens or even hundreds of generations (Ricklefs, 2010). Laboratory strains of mice have also extraordinary long, hypervariable TLs when compared to wild mice (Hemann and Greider, 2000; Manning et al., 2002), which raises concerns about the generality of the results for their wild counterparts, humans and other vertebrates. For the aforementioned reasons, free-living birds and fish in their natural environments (I &II) and wild mice under controlled laboratory conditions were used (III & IV) as study species in this thesis.

3.1.1 Passerine birds: Great tit (*Parus major*) and Pied flycatcher (*Ficedula hypoleuca*) (I)

The great tit and the pied flycatcher were used for the pollution effect study (I). Birds have a higher size related life span expectancy than other vertebrates (Hulbert et al., 2007), which makes them interesting targets of ageing research. Both great tits and pied flycatchers are abundant small insectivorous hole-breeding passerines, which live in forests, woodlands and towns (Hoyo et al., 2007; Lundberg et al., 1992). Despite many similarities, these two species differ in some life-history traits, providing a valuable opportunity to evaluate the conservativeness of the mechanisms of ageing. The great tit is a mostly resident, territorial species, which migrates only due to extremely harsh conditions, whereas the pied flycatcher is a migratory bird that overwinters in Africa (Hoyo et al., 2007; Lundberg et al., 1992). Mainly due to their differences in migratory behavior, these two species also have differences in their diets. Insects are available year-round for pied flycatchers (Bibby and Green, 1980), whereas great tits have been adapted to use a wider range of food sources in winter, including mainly seeds and to some extent even small mammals and birds (Estok et al., 2010). In addition, there are also some differences in their diets during the breeding season. Great tits prefer caterpillars and gastropods, and use gleaning feeding-strategy, whereas pied flycatchers feed mostly

on flying insects (Hoyo et al., 2007; Lundberg et al., 1992). Because of their migratory behavior, pied flycatchers also have a shorter breeding season (June to August) compared to great tits (March to September) (Hoyo et al., 2007; Lundberg et al., 1992). Therefore it is common that great tits can have two broods (clutch size 6-12) (Perrins and McCleery, 1989) and pied flycatchers only one (clutch size 4-8) per season (Lundberg et al., 1992). These two species also have differences in their mating strategies. Great tits are monogamous (Krebs, 1984), whereas pied flycatchers have a mixed mating system of monogamy and bigamy (Lundberg et al., 1992). The survival of small passerines in nature is relatively low and only a small proportion of fledglings survive to breed in the next breeding season. In general great tits live 3-4 years and pied flycatchers 2 years (Robinson, 2018). However, according to ringing data, pied flycatchers can reach even 9 years and great tits almost 14 years in age (Robinson, 2018).

To study pollution effects on TLs and redox status, great tits and pied flycatchers were caught from five nest box sites near a pollution source (within 2 km), a Ni/Cu-smelter (61°20' N, 22°10' E, Harjavalta, Finland), and five nest box sites further away (>9 km) in a non-polluted control-zone. This area has been studied for decades, providing broad ecological background information on the pollution effects on the ecosystems around Harjavalta (e.g. Eeva et al., 2012; Eeva et al., 2006; Eeva et al., 1997; Koivula et al., 2011). In this study, the adult females were caught while incubating (great tits n=23 and pied flycatchers n=22). In addition, one randomly selected nestling per brood (great tits n=21 and pied flycatchers n=24) was sampled (pied flycatchers: 8-11 days old and great tits: 8-15 days old). Morphological measurements (weight and wing length) were taken and after the birds were sacrificed, liver samples were excised and placed immediately into liquid nitrogen. Later on the samples were stored at -80 °C. The study was done under the license of the Regional Environmental Centre (LOS-2008-L-224-254). A variety of redox variables and TL were measured from the livers. In addition, the individual data of liver heavy metal concentrations and metallothioneins (MT), a biomarker for metal exposure (Koivula and Eeva, 2010), were available for this study. Heavy metal (As, Pb, Cd, Cu, Ni) and calcium (Ca) concentrations (mg/kg, dry mass) were measured with ICP-MS [Elan 6100 DCR+ from PerkinElmer-Sciex, for the details, see Berglund et al. (2011)]. The amount of MTs was measured spectrophotometrically (412 nm) using Ellman's reaction and a calibration curve of reduced glutathione (GSH) was utilized to quantify the MT content (Viarengo et al., 1997).

3.1.2 Brown trout (*Salmo trutta*) (II)

Salmonid species are relatively widely studied due to their commercial importance as a food source, and some recent studies have also focused on biological ageing and telomere dynamics (McLennan et al., 2016; McLennan et al., 2018; Näslund et al., 2015). As fish are indeterminate growing ectotherms, using the brown trout as study species (II) in addition to birds and mammals, extends the understanding of

biological ageing and the mechanisms of senescence. Brown trout is an opportunistic carnivore living in cold streams, rivers and lakes and feeding on different invertebrates and small fish depending on life stage and season (Klemetsen et al., 2003). Usually, a part of the brown trout population is anadromous and another part stays as residents (Klemetsen et al., 2003). In the spring or summer, depending on the latitude, the eggs hatch and the smolts from the previous generations start to migrate to the sea for the first time (Elliott, 1994). After at least a year and a half at sea, anadromous fish migrate back to rivers to reproduce in the late autumn (Elliott, 1994).

Juvenile brown trout were used to study the TL and AO dynamics in a native host-parasite system. Myxozoan *Tetracapsuloides bryosalmonae* infection is relatively well documented in the chosen brown trout population (Dash and Vasemagi, 2014). *T. bryosalmonae* infection causes proliferative kidney disease (PKD), which causes high mortality highly relevant for both farmed and natural brown trout populations (Burkhardt-Holm et al., 2005) and also other salmonids (Hedrick et al., 1993). A subset (n=52) of samples of juvenile, young-of-the-year fish caught from Vainupea river (Estonia) for earlier study (Bruneaux et al., 2017) were used for AO and TL measurements. The experiment was performed according to the animal experimentation permit no. 53 issued by the Estonian Ministry of Agriculture (issued on 17.11.2010, valid 17.11.2010-31.12.2014). Fish were caught at the end of September, and due to contemporary hatching (Elliott, 1984), they were simultaneously exposed to *T.bryosalmonae* infection. After euthanasia the fork length, from snout to the end of the middle caudal fin was measured and tissues were collected for further analysis. The relative parasite load i.e. the amount of *T. bryosalmonae* in the kidneys were quantified with qPCR, and PKD related traits; kidney hyperplasia, hematocrit and leucocyte formula were assessed as described in detail in (Bruneaux et al., 2017). The amount of different blood cells is highly dependent on hematocrit, and thus thrombocytes *per* 10000 RBCs (red blood cells), and relative proportions of each leucocyte category were used in statistical analysis.

Recent host-parasite studies have highlighted the importance of tolerance, i.e. the individual ability to minimize the fitness costs of infection, in addition to resistance, i.e. the ability to limit parasite load (Gomez et al., 2014; Medzhitov et al., 2012). Furthermore, previous studies have found high variation both in resistance to and tolerance of *T. bryosalmonae* infection in brown trout based on high in-between individual variation in physiopathological symptoms, e.g. anemia and kidney hyperplasia and in parasite load (Bruneaux et al., 2017; Debes et al., 2017; Grabner and El-Matbouli, 2009). Thus, the parasite load adjusted fork length, which reflects fishes' ability to grow despite the parasite load, was used as a measure of tolerance.

3.1.3 House mouse (*Mus musculus musculus*) (III & IV)

House mice are small mammals, and they are abundant worldwide. Mice are important laboratory model organisms in biology and medicine. The spatial

distribution of the commensal house mouse has led to several subspecies (e.g. *Mus musculus musculus* and *Mus musculus domesticus*) and their hybrids in nature (Wilson and Reeder, 2005). Omnivorous mice eat mainly plant matter in nature. House mice are territorial and live in polygamous populations of a dominant male and cooperatively breeding females (Phifer-Rixey and Nachman, 2015). Females can have 5 to 10 litters (3-14 pups per litter) per year. A fast and effective reproduction strategy is needed because the usual life expectancy of a mouse is less than a year due to high predation risk and low survival. Although, in favorable environments, mice can live to 3-4 years age.

House mice from a unique breeding program for divergent leukocyte TLs were used for studies III and IV. First, wild mice were live-trapped from four different locations in Vienna, Austria, and two rounds of cross breeding among the sub-populations were performed under constant colony conditions (Konrad Lorenz Institute of Ethology, University of Veterinary Medicine Vienna, Austria) to increase the genetic heterogeneity. Second, founders for the artificial selection experiment were chosen from these outbred descendants (141 mice from 25 pairs) based on their white blood cell (WBC) TLs at the age of 8 weeks. First, control TL group breeders (15 females and 15 males) were randomly chosen, irrespective of their TLs, and thereafter the mice with the most extreme TLs were assigned into a long TL (15 females and 15 males) and a short TL group (15 females and 15 males). Finally, these mice were transferred to the Turku Animal Experimentation Centre (University of Turku, Finland) to continue the selective breeding program with a similar selection scheme applied in each generation, which led into increasing TL differences between the selection lines with each subsequent generation (Figure 4).

3-week-old pups were weaned and ear marked for identity, weighed, determined for sex and housed with 1-3 females or one male per cage. At the age of 8 weeks, all mice were weighed again and blood sampled for WBC TL-measurement. The blood sample was taken from the tail vein by using non-heparinized capillaries (Duran, Hirschmann, Eberstadt, Germany) and stored in -80 °C for further analysis. Mice were kept on a 12 h: 12 h light cycle at 22.5 °C (humidity 40%) with food (CRM (E) Standard diet, Special Diets Services, England) and water available *ad libitum.* Plastic cages (height 14 cm, length 22 cm, depth 16 cm) were used with bedding (GLP Aspen Bedding, Tapvei, Harjumaa, Estonia), nest material (GLP Aspen Nesting Material, Tapvei, Harjumaa, Estonia) and cardboard rolls to mimic hiding places in natural environments. The experiments were approved by the Animal Experiment Board of Finland (ESAVI/3175/04.10.03/2012).

A subset of both female and male mice (n=61) from the 2nd generation of TL selection was used to study the development of the AO-system in relation to TL (III). Blood samples at the age of 8 weeks and 6 months provided a valuable longitudinal approach to observe the redox dynamics within individuals with different TLs. Unfortunately, the TLs of available mice did not differ statistically significantly among selection groups, and therefore TL was treated as a continuous

variable. In addition, sibling competition was taken into account by using the relative size of pups within a litter as a proxy for natural within-litter competition induced growth stress. Thus, a relative deviation (%) of the weight of each 3-week old mouse from the mean body mass of the litter was calculated. To further understand the effects of experimentally increased and reduced TLs on redox status and metabolism, a subset of 5th generation mice (n=124) from each TL selection group (except females long TL group) were chosen for a short-term exercise experiment to increase possible among TL group differences in redox status and metabolism (IV). Adult mice (8 months old) of both sexes and from each TL selection group were divided into exercise and control treatments. The mice in exercise treatment were forced to swim (tails of floating mice were pinched) in round containers (diameter 33 cm) during the treatment (30 min, 35 min, 0 min, 40 min and 45 min respectively on each day). The day after the end of the treatment, mice were weighed and euthanized. The spleen was dissected and homogenized into a single-cell suspension for O_2 consumption measurement. In addition, liver-tissue was collected for redox measurements, snap-frozen in liquid nitrogen and stored in -80 °C.

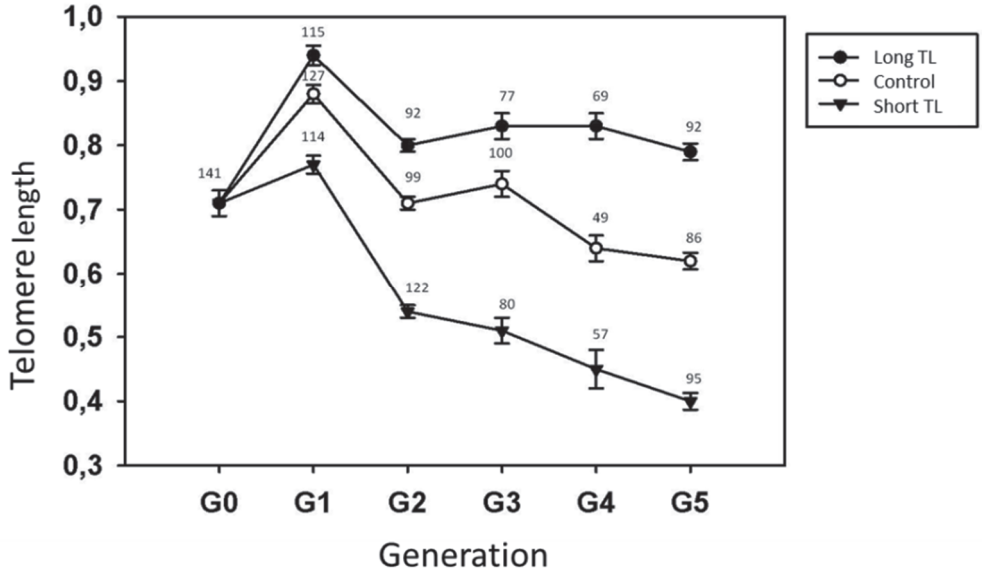


Figure 4. Selective breeding program led into increasing WBC TL differences in long, control, and short TL groups of wild mice. Means and standard errors for TL are presented and the sample sizes are given above the bars. A subset of G2 mice were used in study III and a subset of G5 mice in study IV.

3.2 RT-qPCR for telomere length

A real-time quantitative PCR assay (Cawthon, 2002) was used to determine the relative TL. Suitability and specificity of primers were tested as recommended by Smith et al. (2011) and thereafter, primers for telomeric sequences, Tel-1b and Tel-2b (Epel et al., 2004) and primers for a non-variable single-copy genes, 18S rRNA

(Maeda et al., 2002) (I & II), and *36B4* (Callicott and Womack, 2006) (III & IV), were chosen. The total genomic DNA was extracted for TL measurements from liver (I & II) with a salt extraction method (Aljanabi and Martinez, 1997), and from blood (III & IV) with the Qiagen DNeasy Blood and Tissue kit. Sample triplicates were used and each reaction contained 10 ng DNA, 300 nM of each primer, 10 µl SensiFAST™ SYBR Hi-Rox (Bioline Reagents Ltd.), and ultra-pure PCR-grade H_2O to make up the total reaction volume to 20 µl on a 96-well plate. Plates were run with the ABI 7900HT fast Real-Time PCR system (Applied Biosystem) and calculations were done with LinRegPCR version 2012.0 developed by Ruijter et al. (2009). Control DNA samples were used to normalize the relative TL of all experimental samples and to estimate the inter-plate variation. In addition, non-template control (NTC) was used to secure the absence of non-specific amplification for both TL and single-copy gene primers. The final TL was calculated using an equation described in (Pfaffl, 2001). Further details of the method are reported in the original publications (I-IV).

3.3 Redox measurements

A variety of approaches have been used to estimate oxidative stress. Some studies measure pro-oxidants like chemical exposure (indirect measure of oxidative stress) and others actual circulating levels of RS and/or different parameters of oxidative damage. In addition there are methods to estimate the total AO capacity and others for each specific AO separately. In this thesis, the aim was to achieve as comprehensive assessment of redox status as possible by measuring several different AOs as well as oxidative damage of macromolecules.

3.3.1 Sample processing (I-IV)

In studies I, II and IV, redox status was measured from liver tissue, and in study III from blood. Animals were dissected and livers were immediately frozen in liquid nitrogen and stored further in -80 °C. Frozen tissues were crushed to pieces with a cooled mortar and further homogenized with TissueLyzer II Bead mill (Qiagen, Austin, USA). For AO samples 0.1 M K_2HPO_4 + 0.15 M KCl buffer (pH 7.4) was used. After homogenization, samples were centrifuged for 15 min at 10 000 x g at + 4°C and thereafter, the supernatants were divided into aliquots and frozen for further use. For lipid peroxidation (LP) measurement, the liver pieces were weighed before homogenization with methanol. Samples were centrifuged for 10 min at 5000 x g at + 4°C and the supernatant was divided into two 45 µl aliquots per measurement. Blood samples were diluted with 0.9 % NaCl to obtain optimal volume for all assays. All samples were divided into aliquots to avoid exposing samples to a freeze and thaw cycle. During the preparation, the samples were always kept on ice and thereafter snap-frozen in liquid nitrogen and stored at -80°C.

3.3.2 Antioxidants (I-IV)

Methods were modified for small sample volumes from original instructions. The methods used and their modifications are listed in Table **1** and described in detail in the original publications. Samples were measured in triplicates (intra-assay coefficient of variability [CV] < 15 % in all cases) in 96 or 384-well microplates. All values are expressed per protein content measured with the Bradford (I-III) or the BCA (bicinchoninic acid assay) (IV) method (Bradford, 1976; Smith et al., 1985; respectively). Samples were randomly placed on the plates and aliquots of the same three control samples (*Salmo salar* liver) were used in each method on every plate to correct for interassay variation (max 20 %) with the ratio specific to the particular plate. All the measurements were done with an EnVision plate reader (Perkin-Elmer, Turku, Finland).

Table 1. Original protocols and modifications for small sample volumes for AO methods.

AO	**Original protocol**	**Plate formate**	Sample volume/concentration: **I** liver	**II** liver	**III** blood	**IV** liver
GSH_{rati}, GSH_{tot}	Glutathione Fluorescent Detection Kit (K006-F1), Arbor Assays, Ann Arbor, MI, USA	384-well	1:30	1:30	1:30	1:30
CAT	Sigma CAT100 Sigma Chemicals, St. Louis, USA	96-well	0.3 mg/ml	0.3 mg/ml	0.6 mg/ml	0.3 mg/ml
GP	Sigma CGP1 Sigma Chemicals, St. Louis, USA	384-well	2 µl	3 µl	3 µl	1 µl
GR	Sigma GR-SA Sigma Chemicals, St. Louis, USA	384-well	2 µl	4 µl	4 µl	2 µl
GST	Sigma CS0410 Sigma Chemicals, St. Louis, USA	384-well	2 µl	3 µl	6 µl	1 µl
SOD	Fluka 19160 SOD determination kit Fluka, Buchs, Germany	384-well	0.3 mg/ml	0.1 mg/ml	1 mg/ml	0.3 mg/ml
G6PDH	(Noltmann et al. 1961)	384-well	-	5 µl	6 µl	-

All of the methods, except the GSH/GSSG-ratio and GSH_{tot} method, were absorbance based. The CAT-assay was based on stopping the reaction of CAT and H_2O_2 with 15 mM NaN_3 (Deisseroth and Dounce, 1970) and detecting the remaining H_2O_2 via colorimetric reaction at 520 nm (Fossati et al., 1980). The GP-assay was based on the oxidation of glutathione catalyzed by GP, which is thereafter reduced back utilizing GR and NADPH. The indirect method measures the decrease in NADPH absorbance at 340 nm, which is indicative of GP-activity as it is the limiting factor of the coupled reactions (Mannervik, 1985). The GR-assay was based on the reduction of glutathione by NADPH in the presence of GR. Because DTNB [5,5-dithiobis(2-nitrobenzoic acid)] reacts with the GSH formed, the activity of GR can be measured

as an increase in absorbance at 412 nm caused by the reduction of DTNB (Smith et al., 1988). The GST-assay is based on the conjugation of the thiol group of glutathione to the CDNB (1-chloro-2,4-dinitrobenzene) substrate. The change in absorbance at 340 nm is directly proportional to the GST-activity, since GST catalyzes the conjugation (Habig et al., 1974; Mannervik and Danielson, 1988; Wilce and Parker, 1994). The measurement of SOD inhibition rate was based on the ability of SOD to catalyze the dismutation of the superoxide anion (O_2-). Colorimetric reaction of WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], which produces a water-soluble formazan dye upon reduction with O_2- that absorbs light at 450 nm was used to determine SOD-activity (Peskin and Winterbourn, 2000; Ukeda et al., 1999). The rate of the reduction with O_2- is linearly related to xanthine oxidase activity, and is inhibited by SOD. Thus, the higher the inhibition rate percentage, the higher the SOD activity. The G6PDH-activity method is based on the change in absorbance at 340 nm (Noltmann et al., 1961), when G6PDH catalyzes the reduction of NADP to NADPH and the oxidation of G6-P (Kruger and von Schaewen, 2003).

The GSH/GSSG-ratio and GSH_{tot} method utilizes a proprietary non-fluorescent molecule, a ThioStar that covalently bind to the free thiol group on GSH to yield a highly fluorescent product. The fluorescence was measured at an excitation of 405 nm and an emission of 510 nm to determine GSH concentration. First, free GSH was measured, and thereafter the reaction mix was added to the wells to determine the GSH_{tot} (GSH+GSSG).

3.3.3 Oxidative damage: lipid peroxidation (I & IV) and protein carbonylation (IV)

Lipid peroxidation (lipid hydroperoxides, LP) were measured with the FOX-2 method (the ferrous oxidation in xylenol orange version 2) originally described by Bou et al. (2008) and Eymard and Genot (2003) and further modified e.g. for birds by Raja-aho et al. (2012). The method is based on the ferrous oxidation of xylenol orange. Cumene hydroperoxide was used as a standard. First 5 µl of either 10 mM triphenylphosphine (TPP) or methanol was added to sample duplicates (45 µl each) and after 30 min incubation, 950 µl of FOX reagent (1:9, 2.5 mM ammonium iron (II) sulfate in 0.25 M H_2SO_4 and 0.111 mM xylenol orange in methanol) was added to all samples and standards. After 2h incubation in dark, the samples were pipetted in triplicate (intra-assay coefficient of variability [CV] < 10 %) to 96-well plates and the absorbance was measured at 570 nm with an EnVision plate reader (Perkin-Elmer, Turku, Finland). Finally, the results were normalized with the weights of the liver pieces.

A plate-based time-resolved fluorescence (TRF) method was used to quantify the carbonylation damage in proteins. Standard curves were done by mixing different volumes of reduced and oxidized bovine serum albumin (BSA) prepared according to Alamdari et al. (2005). The carbonyl content of oxidized and reduced BSA for

standard was measured with a colorimetric assay at an absorbance of 375 nm with an EnSpire plate reader (Perkin-Elmer, Turku, Finland). The samples and standards were diluted to a protein concentration of 4 mg/ml based on BCA protein assay measurements (Smith et al., 1985) with an EnVision microplate reader (Perkin-Elmer, Turku, Finland). Aldehyde reactive probe (ARP) (Aldehyde reactive probe, Cayman Immunodiagnostics, Finland) was used to detect protein carbonyls in samples. Because ARP recognizes carbonylated sites also in nucleic acid strands, streptomycin sulfate treatment was used to precipitate nucleic acids. ARP was conjugated with proteins in the samples and standard, and bound to high binding DELFIA plates (Perkin-Elmer, Turku, Finland). Thereafter, a europium labeled streptavidin label (PerkinElmer, 1244-360) was added into the wells. The label binds via a strong non-covalent affinity bond to the biotin-tag of ARP and produces an amplified fluorescent signal measured with an EnVision plate reader (Perkin-Elmer, Turku, Finland) (excitation 340 nm, emission 615 nm), which is proportional to the amount of carbonylation in the sample. The method is described in detail in original publication IV.

3.4 Cell respiration (IV)

Oxygen consumption of intact spleen cells was measured with a high-resolution oxygraph (O2k, Oroboros Instruments, Innsbruck, Austria). Primary spleen cells were isolated immediately after euthanasia. Red blood cells were lysed and splenocytes were suspended in growth media (RPMI containing 5 % fetal bovine serum). Isolated cells were counted with Brücker-chamber and diluted to $2.5x10^6$/ml or, in case of milder samples, used as raw suspension for respirometry to measure O_2 consumption. The titrations with rotenone and antimycin were used to determine the residual oxygen consumption, which was subtracted from routine respiratory flux. O2k-Protocols (Oroboros Instrument, Innsbruck, Austria) were followed to measure the routine respiratory flux of the cells and the data was analyzed with DatLab 6 software (Oroboros Instruments, Innsbruck, Austria). The samples with less than $1x10^6$/ml cells were excluded from the final dataset.

3.5 Statistical analyses

The data in publication I and II was analyzed with IBM SPSS Statistic software 22 and the data in publications III and IV with a later version, IBM SPSS Statistic software 24 (IBM SPSS Statistics. New York: IBM Corporation).

In order to reduce the number of pollution and AO variables for further analyses and to describe the general level of AO status and heavy metal accumulation, two principal component analyses (PCA) were done in the first study (I). The PCA for the pollution resulted in two components; $PC1_{MET}$ (positive loadings from As and Pb) and $PC2_{MET}$ (negative loading from Cd and positive loadings from Cu and Ni), and the PCA for AOs in three components; $PC1_{AO}$ (positive loadings from GR, GST, GSH_{ratio}, GSH_{tot}), $PC2_{AO}$ (positive loadings from CAT) and $PC3_{AO}$ (positive loading

from SOD and negative loading from GP). Both study species, age groups, and zones were used in the same PCA-analysis. In addition to variables used in PCAs, TL, Ca, MT and LP were log-transformed to obtain normality (Shapiro-Wilk), before further analysis. Correlations and t-tests (between zone differences) were conducted separately for both study species and age groups. Each nestling's relative deviation (%) from the mean body mass of the brood was calculated to describe growth stress due to sibling competition.

Spearman correlations were used to evaluate relationships among TL, redox status and parasite infection in study II. In addition, for a parasite infection tolerance variable, unstandardized residuals were calculated from linear regression between the square-root-transformed parasite load and fork length of the fish. Therefore higher residuals indicate that the fish has been able to grow relatively large when compared to a fish with similar parasite burden, but lower residuals.

For the longitudinal data in study III, generalized Linear Mixed Models with random intercepts for subject ID number to account for repeated AO-measures, were used to test whether sex, TL, or relative size have an effect on AO-levels, or on AO-change. The relative size of each mouse at the age of 3 weeks to indicate sibling competition was calculated as relative deviation from the mean body mass of the litter. TL and relative size were examined in different models because preliminary observation (e.g. non-significant Spearman correlation between TL and relative weight) indicates their independency. Non-significant terms were dropped one-by-one from the models. In case of significant interaction among three variables (sex, age, TL or relative size), further analyses were done with Spearman correlations separately within each categorical group.

In the last study (IV), as the TL selection groups were different between sexes (short, control and long TL groups for males, short and control TL groups for females) generalized linear models were used separately for males and females to examine the main effects and interactions of TL selection group and exercise treatment on redox status and cell respiration. The possible impacts of body size on cell respiration and redox variables were controlled by using log-transformed body weight as a covariate. Non-significant terms were dropped sequentially from each model. Pairwise comparisons were carried out with Sidak adjustment for multiple comparisons. Pearson correlations were used to test the possible associations between cell respiration, oxidative damage and AOs.

4 Main Results and Discussion

4.1 Pollution and within-brood competition induced redox change and TL attrition (I)

Based on the between-zone differences in individual heavy metal concentrations, the main pollution effects were due to arsenic (As) and lead (Pb) exposure. Both great tits and pied flycatchers showed alterations in redox status due to pollution, and in addition, great tit nestlings in the polluted zone had significantly shorter telomeres than those in the unpolluted control zone. Adult females of both species and pied flycatcher nestlings did not show any pollution effects on TL. Earlier studies have also shown As and Pb to induce oxidative stress and telomere shortening in humans and rodents (Bhattacharjee et al., 2013; Pottier et al., 2013). The found between-species difference in pollution-induced telomeric DNA damage is in good agreement with earlier findings. Eeva et al. (2006) found that great tits close to two different point pollution sources had increased mutation rates when compared to control sites, whereas the opposite effect was observed in pied flycatchers, indicating that great tits might be less efficient in handling toxic compounds.

Adult pied flycatchers had statistically significantly shorter telomeres than nestlings. This is in good agreement with the general finding that telomeres get shorter with advancing age, and thus adults can be expected to have shorter telomeres than their offspring. However, when the TLs of great tits were studied, the age-dependent shortening of telomeres was not observed. Salmon et al. (2017) found that great tits early-life TL predicted post-fledging survival in urban and rural areas, and in urban habitat it led to selective disappearance of birds with shorter TLs. It could be that under high stress telomeres might shorten to a critical point already at juvenile stage before reaching reproduction age, with consequent increase of mortality in individuals with short TLs (Fairlie et al., 2016).

The pollution effect on redox status was remarkable different among species and age groups. SOD and GP seem to be the most important first line defense AOs for great tits, whereas pied flycatchers had more alterations in glutathione related AOs. In great tits, high As and Pb concentrations affected the enzyme activities of SOD and GP ($PC3_{AO}$). The effect was different between great tit nestlings and adult females as nestlings had lower SOD and higher GP activities due to pollution, and *vice versa* in adults. Adult great tit females with higher As and Pb concentrations had also more oxidative damage as demonstrated by increased LP. This is consistent with the fact that adult great tit females are exposed for the longest and the most continuous period to pollution when compared to nestlings or migratory pied flycatchers.

The redox status in adult great tits and pied flycatchers in the polluted zone differed from those breeding more than 9 km (control zone) from the Ni/Cu-smelter. Adult

great tits in the polluted zone had higher SOD- and lower GP-activities ($PC3_{AO}$) than in control zone, while adult pied flycatchers in the polluted zone had lower GST and GR-activities, less glutathione and lower GSH/GSSG ($PC1_{AO}$) than in the control zone. These species specific responses are likely due to differences in diets and migratory behavior. Similar species specific features in AO responses to pollution have been documented earlier, even in more taxonomically related passerine birds, by Rainio et al. (2013), Berglund et al. (2014) and Salmon et al. (2018). Moreover, it is impossible to control all environmental factors when natural populations are studied in the wild. It might be that pied flycatchers were less stressed in the polluted zone than in the control zone. First, the low GST and GR-activities in the polluted zone probably indicate low stress, since the previous study with pied flycatchers has reported opposite AO activities at a similar point pollution source (Berglund et al., 2007). Second, pied flycatcher nestlings in the polluted zone had higher calcium concentrations than in the control zone. Calcium prevents heavy metal absorption (Scheuhammer, 1991) and is essential for bone and feather development (Dawson and Bidwell, 2005; Sillanpää et al., 2010). Therefore, it can be suggested that the control zone might be a more stressful environment for pied flycatchers than the polluted zone due to other environmental stressors. One significant source of stress might be the investment in reproduction and parental effort. My findings are consistent with the study published by Eeva and Lehikoinen (2015), which reported that in this study area pied flycatchers raise larger broods in the control zone than in the polluted zone.

Great tit nestlings were more vulnerable to within-brood competition than pied flycatchers, probably due to between-species differences in reproduction. Previously it has been shown that great tits have larger broods and higher variation in nestling size due to higher within-brood hatching asynchrony than pied flycatchers (Cramp et al., 1993). This was also the case in our study. Great tits had a larger within-brood standard deviation of body mass than pied flycatchers. Subdominant great tit nestlings which were suffering from higher within-brood competition, measured as relative size, had also shorter TLs and lower SOD-activity and higher GP-activity ($PC3_{AO}$) compared to the great tit nestlings that were on top of the within-brood hierarchy. Pied flycatcher nestlings did not show similar within-brood competition effects on AOs or TL. Several earlier studies on passerines have reported similar within-brood competition or hatching order induced effects on TL and oxidative damage (Nettle et al., 2013; Nettle et al., 2015; Stier et al., 2015). Taken together, these results suggest that the trade-off between the number and quality of offspring could be mediated via oxidative stress induced telomere attrition.

Ecotoxicological studies on telomere dynamics are still rare and only few studies have investigated pollution effects on TL in wild birds (Blévin et al., 2016; Ibanez-Alamo et al., 2018; Salmon et al., 2016; Salmon et al., 2017; Salmon et al., 2018; Sletten et al., 2016), however, none of these have focused on heavy metal pollution. The results of this thesis study show that wild birds, especially in early life are

vulnerable to negative impacts of heavy metal pollution even in relatively low concentrations. The results also underline the need to take into account also other potential sources of stressors, such as growth stress and parental effort, which can also alter redox status and telomere dynamics, and therefore confound the interpretation of results.

4.2 Redox status and telomere length in relation to parasite infection (II)

Associations between TL and redox status with *T. brysalmonae* infection were more evident in relation to disease symptoms and tolerance than simply parasite load (i.e. resistance). In fact, parasite load did not correlate either with redox status or TL. However, the fish with severe proliferative kidney disease (PKD) symptoms were smaller and had lower AOs (SOD, CAT, GST) than fish with milder symptoms. This is in good accordance with the previous results from a study that compared AOs in healthy versus PKD infected rainbow trout (*Oncorhynchus mykiss*) (Elia et al., 2009). It seems that fish that are able to maintain higher AOs do not suffer as severe kidney hyperplasia as fish with lower AOs irrespective of parasite load.

It is generally expected that within species fast growth and bigger body size accelerate telomere loss, which in turn could be a mechanism mediating the trade-off between growth and life-span (reviewed in Monaghan and Ozanne, 2018). However, it seems that telomere attrition rate due to growth is highly dependent on exposure to additional environmental stressors (McLennan et al., 2016; Mizutani et al., 2016; Reichert et al., 2015). This could well explain why some studies fail to find any associations (Giroud et al., 2014; Izzo et al., 2014; Meillere et al., 2015; Parolini et al., 2015; Vedder et al., 2017), or why the correlation between growth and TL is positive (Izzo et al., 2014; Parolini et al., 2015; Ringsby et al., 2015; Young et al., 2017), as was also found in this thesis, i.e. the smaller fish had shorter TLs (r_s=0.341, n=51, p=0.014). In good agreement with our results in *T. bryosalmonae* infected brown trout, several types of myxosporean parasite infections have been reported to be associated with impaired growth in fish (Golomazou et al., 2009; Sitja-Bobadilla et al., 2008). Furthermore, in our study more tolerant fish, which were able to grow despite the parasite load, also had longer TL, lower GR and GST-activity and less GSH_{tot} compared to fish that were less tolerant to infection induced retarded growth.

In summary, the results of this thesis chapter suggest that TL might reflect quality in terms of individuals' ability to grow despite parasitic infection. In addition, the different stressors seemed to influence different parts of AO systems. Disease severity was associated with low SOD, CAT and GST-activities, whereas growth related stress affected glutathione metabolism. The research on telomere dynamics in host-parasite systems is still surprisingly rare. Previous studies on infection induced telomere attrition have been limited to inflammatory diseases, bacterial infections and blood parasites (Asghar et al., 2016; Cohen et al., 2013; Hau et al.,

2015; Ilmonen et al., 2008; Karell et al., 2017; Raymond et al., 2014). Parasites, which are widespread and pervasive, should not be underestimated when thinking about the factors that influence biological ageing.

4.3 The effects of TL, age and sex on redox status (III)

Age specific differences in redox status were observed between nestlings and adult birds already in the first thesis study, and therefore age specific AO alterations were studied in more detail in mice with longitudinal data. The results of this study show that AOs increase within individuals from the age of 8 weeks to 6 months. All AOs, except the GR-activity and GSH/GSSG increased statistically significantly. Earlier studies have shown that AO-defense maturates post-natally (Barja, 2002; Robles et al., 2001), but it seems to be highly species and tissue specific whether AOs increase or decrease with advancing age (Carrillo et al., 1992; Jacob et al., 2013). Most likely AO defense first elevates during maturation and finally becomes weaker at old age as reviewed by Barja (2002) and documented in detail in rats (Tsay et al., 2000). For example, GSH/GSSG ratio has been found to decline with old age in several tissues (Calabrese et al., 2004; Jones et al., 2002; Suh et al., 2004). Six-month-old mice, which were used in this study, are relatively young, and based on the unaffected GSH/GSSG, it seems that they still have been able to maintain redox balance.

In addition to age, it is clear that there are remarkable between sex differences both in physiology and life-history strategies. In accordance with earlier knowledge (e.g. Jankowiak et al., 2015; Lindström, 1999; Vina et al., 2006), female mice in this study had higher or equal activity or amount of all measured AOs than males, irrespective of the age. Female and male sex hormones have opposite effects on redox status (Halliwell and Gutteridge, 2007). The sex differences in redox status, among other factors (e.g. telomere shortening rate and body size), could explain why females usually live longer than males (Barrett and Richardson, 2011; Stindl, 2004). The sex difference in this thesis study was statistically significant only for GP-activity and GSH_{tot}, most likely due to the relatively early life stage of mice. Female mice suffering from higher sibling competition had lower SOD activity in later life. This is in agreement with the general suggestion that early growth conditions can, in addition to immediate negative impacts, also have lingering long-term consequences for individuals' fitness and life expectancy (e.g. Angelier et al., 2018). The studies on direct associations between redox status and sibling competition in small mammals are scarce. However, an earlier experimental study in wild house mice has also shown that sibling competition increases oxidative stress, and has sex-specific negative fitness consequences after maturation (Gibson et al., 2015). In addition, litter size manipulation and supplementary maternal diet experiments with wild meerkats (*Suricata suricatta*) show that an increase in the number of pups is associated with shorter TLs in pups, but only if the mother did not receive improved nutrition (Cram et al., 2017). In accordance with this, we failed to find any

TL effect due to sibling competition in benign laboratory conditions. Still, if we had measured TL change in later life, we may well have found negative impacts also on TL, especially among the female mice that showed delayed negative impact of sibling competition on SOD.

Interestingly, mice with longer TL in early life had more GSH_{tot} and higher SOD-activity in both sexes, indicating better AO-defense compared to mice with shorter TLs. Effective AO defense may have prevented TL attrition directly (Lee et al., 2009) or via telomerase-activity (Borras et al., 2004; Makino et al., 2011). The first two studies in this thesis failed to find any direct associations between TL and redox status in birds (I) or fish (II) in nature. The association between redox status and TLs is probably more straight-forward in benign environments, like in study III, whereas simultaneous exposure to several different stressors in natural environments might mask or confound the relationship.

Although the dynamic nature of redox status is widely recognized, studies using a longitudinal approach for redox status variables in relation to TL are uncommon. This study together with age group comparisons in passerine birds (I), clearly underlines that redox status is highly dependent on age and life stage. Therefore, when comparing telomere dynamics among individuals and especially in interpretations among studies, it is crucial to take into account the phase of life.

4.4 TL dependent differences in metabolism and redox status (IV)

The TL selection line mice provided an unique model to examine redox status and metabolism in relation to increased or reduced TLs. Male mice with increased TLs showed higher cell respiration, lower AO-defense (CAT, SOD) and larger body mass than males with decreased TLs. However, the males with increased TLs had similar cell respiration level than controls, and furthermore, males with decreased TLs had similar AO-activities than controls despite their low metabolism. This indicates that extreme TLs, short or long, might interfere differently with mitochondrial function, which in turn could hinder or accelerate growth and metabolism, and also suppress or elevate AO defense. Both male and female mice with more oxidative damage (protein carbonylation) had also higher AOs. However, there were no statistically significant associations either between cell respiration and AOs, or between lipid peroxidation and AOs.

The experimental short-term exercise experiment was an attempt to magnify the possible among TL group differences in metabolism and redox status. However, the exercise treatment was likely too mild to magnify any measurable TL effect on respiratory metabolism or consequent ROS-production. However, females lost some weight due to swimming and had also higher GSH/GSSG-ratio, indicating suppressed oxidative stress compared to controls. Activity and exercise may also result in hormetic activation of a number of molecular pathways. Prolonged or

heavy exercise increase oxidative stress and could induce harmful effects (Alessio, 1993; Ji, 1999; Powers and Jackson, 2008). On the contrary, moderate activity has a positive effect as anti-stress and anti-inflammatory responses and repair mechanisms are activated (Gomez-Cabrera et al., 2008; Ji et al., 2006; Radak et al., 2008; Rattan, 2008). Anyhow, it should be noted that in general the effects of exercise differ depending on the duration and intensity of the activity, and whether it is voluntary and independent from social factors or not. Most likely the wild house mice grown-up in benign colony conditions were able to maintain their physiological homeostasis despite the exercise. This interpretation is in accordance with lack of any differences in measured oxidative damage between the exercise treatment and control group or among the TL groups in both sexes.

Unfortunately, the cell respiration and redox status variables were measured from different tissues, and there is no data on ROS production. Therefore the interpretations on the underlying background mechanisms remain allusive. Anyway, these suggestions are in accordance with the finding that telomere dysfunction is associated with impaired mitochondrial function and increased RS production (Sahin et al., 2011). Mice in the long TL group were larger than in the short TL groups, contrary to the findings in most of the previous studies (reviewed in Monaghan and Ozanne, 2018). However, studies where growth rate has been manipulated for example by maternal diet, growth hormones or brood size hierarchy in order to test the impact of growth and body size on TL remain inconclusive (Monaghan and Ozanne, 2018). It seems that telomere maintenance in relation to growth is highly dependent on resource-based trade-offs and canalization of resource allocation (Cram et al., 2017; Vedder et al., 2017).

Similar to findings in the first mouse study (III) in this thesis, TL was associated with certain AOs. However, the results are somewhat inconsistent and the link between TL and redox status remains ambiguous, mainly because in the first study (III) the AOs were measured from blood and in study IV from liver. The male mice in the long TL group had lower CAT- and SOD-activities in liver than males in the control and short TL groups, whereas the opposite, positive correlation between TL and blood AOs (SOD and GSH_{tot}) was found in both sexes (III). The life stage of the studied mice in both studies (III, IV) was almost the same (6 versus 8 months old, respectively). For this reason, it can be suggested that these contradictory results are partly due to tissue difference, and partly due to the dynamic nature of AOs. However, it seems that SOD-activity is the main AO involved in telomere dynamics.

5 Conclusions

In this thesis, I investigated how TL and redox status, both of which are expected to be involved in biological ageing, are connected to organismal characters and the environmental stress factors (Figure 1) in different vertebrates. I found that short TL and redox status alterations were linked with pollution exposure (I), poor tolerance to parasitic infection (II), natural within-brood (I) or within-litter (III) competition, small size (II-IV) and low metabolism (IV). I show that responses in redox status and TL were highly dependent on species, type of experienced stress, phase of life and sex, i.e. there is no single universal pattern of redox regulation and telomere erosion. Therefore research on redox status alterations and telomeres requires careful planning and one should be cautious when interpreting the results. Ideally, the choice of measured biomarkers of redox status should be based on *a priori* knowledge on which of the markers can be expected to give the best overall picture of oxidative stress in particular conditions. First, elevated AO defense does not necessarily reflect increased oxidative stress if further oxidative damage is prevented by the enhanced defense. Second, the lack of oxidative damage does not necessarily indicate that the redox status is unaffected, but rather that the AO-defense is sufficient enough. Furthermore, longitudinal TL measurements should be favored because it allows estimating the effects of both TL and shortening rate. In addition, simultaneous measurements of ROS and telomerase activity would provide valuable background information on telomere dynamics and efficiency of telomere repair under oxidative challenges.

Due to a rapidly changing environment and the ageing of human populations, understanding the mechanisms of biological ageing is beneficial, not only for a biologist trying to understand the huge diversity of life-histories of all living organisms on earth, but rather for all of us. This thesis shows that human impact on the environment might indirectly accelerate biological ageing in free-living vertebrates (I-II). Humanity is continuously polluting indispensable resources like air, water, and soil and ruining delicate ecosystems. Increased production of pollutants results in challenges for all living organisms. In addition to pollution, human activities play an important role in the spread of diseases through transport and climate change (Beaulieu and Costantini, 2014). Therefore, the potential impacts of infectious agents on biological ageing via somatic deterioration deserve more attention. Studies on genetically diverse model organisms in their natural environments provide a good approach for trying to understand how variation in physiological systems and life history strategies result in diverse patterns of biological ageing. Despite the methodological limitations, redox status together with telomere dynamics provide a promising tool to estimate species' and populations' capability to resist and tolerate novel environmental challenges, and to evaluate their ability to survive in an unpredictably changing world (Beaulieu and Costantini, 2014; Costantini, 2018; Dupoue et al., 2017).

Acknowledgements

This work was carried out at the University of Turku, Department of Biology between 2012 and 2018 mainly in the Laboratory of Animal Physiology. The work was supported by the Academy of Finland, the Finnish Cultural Foundation, Turku University Foundation, and the Ella and Georg Ehrnrooth Foundation. Final writing of the thesis was made possible financially by the Doctoral Programme in Biology, Geography and Geology (BGG). I gratefully acknowledge all the funding sources. Furthermore, various people have contributed to this work and impacted the completion of the thesis. I would like to thank them all and express my deepest gratitude for their support and friendship during these past years.

First of all I would like to thank my main supervisor Petteri Ilmonen for giving me the opportunity of doing this thesis, and welcoming me to the ongoing project. I admire your devotion and I am grateful for all of your support. You were always willing to help and easy to approach with my questions. Thank you for long and inspiring converstations and helping me see the significance of my work, when I was not sure about it. I also sincerely thank my co-supervisor Tiina Henttinen for great advice and support. You would always help me forward and give me a fresh perspective.

I would also like to express my gratitude to Doctor Michaël Beaulieu and Professor Dennis Hasselquist for valuable comments and taking the time to pre-examine my thesis.

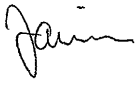
I would like to express my gratitude to all the collaborators and co-authors who have contributed to this work. First, I am grateful to Tapio Eeva and Anti Vasemägi for advice and collaboration with wild birds and fish. I also warmly thank Tapio for being my custos. I am very thankful to all of people who have collected samples in the field and helped with laboratory work, especially to Miia Rainio, Matthieu Bruneaux and Marko Visse who also shared their time during the preparation of the manuscripts. Second, the work done with wild house mice would not been possible without the support and proficiency of the personnel of Konrad Lorenz Institute of Ethology (Vienna) and Turku Animal Center. I would like to thank Dustin Penn and Michaela Thoss for all the work with selection line founder mice in Vienna, and Ulla-Marjut Jaakkola for advice and support with animals at Turku. Furthermore, the most sincere thanks to my fellow group-members; Bineet Panda for his enormous work on telomere measurements, and Annika Andtsjö for taking good care of our mice and providing assistance with sampling and experiments whenever it was needed.

I am grateful to the Department of Biology, with special thanks to Division of Physiology and Genetics, Professor Harri Savilahti and Professor Mikko Nikinmaa. Thanks to all former and present members of the Laboratory of Animal Physiology: Jenni Prokkola, Johanna Iljamo-Wihervuori, Katja Anttila, Kristiina Vuori, Mario Lewis, Minna Vainio, Mirella Kanerva, Miriam Götting, Niina Santio, Nina Aro, Päivi

Koskinen-Kajuutti, Tomi Streng and all the others for great company and creating friendly working atmosphere. Whenever I have needed advice, assistance at lab or help with animal care, there has always been people around who I can count on. Additional thanks to Katja for sharing an office with me. I really appreciate your support and friendship. I could always trust on your help in any issue. In addition, I would like to thank Pablo Sanchez Virosta, Pauliina Järvistö, Sandra Ruiz, Silvia Espin, Suvi Ruuskanen, Thomas Lilley and Toni Laaksonen from Section of Ecology for great discussions, companionship and support. Having collaborators from different fields has been a priviledge and it has given me more insight than I could have hoped for. Furthermore, special thanks to Mirella and Thomas who first introduced me into the field of ecophysiology. Your example and the skills you taught me during my master's studies have helped me considerably to accomplish this work. I will always be inspired by your optimistic and broad-minded attitude!

I would also like to thank my family and friends, who have shared the ups and downs during my never-ending studies. Thanks to friends from the beginning of my academic journey, as well as friends outside the University for being there for me. Special thanks to man's best friends, Oiva and Ainu and all likeminded dog people for bringing so much joy into my life and taking my mind off the work. I would not have managed to survive as well as I did without you. My warmest thanks and love to my family: mom, Johanna, Janette, my grandparents and other relatives, as well as Timo's family, for the support. You were always there to help and never lost your belief in me. It meant a lot to me! Finally, I am grateful to Timo for your love, incredible patience and encouragement. I could not have done this work without your support: you are the best clownfish ever! In addition, thanks to our daughter Minja for reminding me of what life is actually all about. You gave me the final motivation to finish this thesis.

Paimio, August 2018



References

Alamdari DH, Kostidou E, Paletas K, Sarigianni M, Konstas AGP, Karapiperidou A, et al. High sensitivity enzyme-linked immunosorbent assay (ELISA) method for measuring protein carbonyl in samples with low amounts of protein. Free Radical Biology and Medicine 2005; 39: 1362-1367.

Aledo JC. Life-history Constraints on the Mechanisms that Control the Rate of ROS Production. Current Genomics 2014; 15: 217-230.

Alessio HM. Exercise-induced oxidative stress. Medicine and Science in Sports and Exercise 1993; 25: 218-224.

Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research 1997; 25: 4692-4693.

Allsopp RC, Chang E, Kashefiaazam M, Rogaev EI, Piatyszek MA, Shay JW, et al. Telomere Shortening Is Associated with Cell Division in-Vitro and in-Vivo. Experimental Cell Research 1995; 220: 194-200.

Angelier F, Costantini D, Blevin P, Chastel O. Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. General and Comparative Endocrinology 2018; 256: 99-111.

Arendt JD, Wilson DS. Optimistic growth: Competition and an ontogenetic niche-shift select for rapid growth in pumpkinseed sunfish (*Lepomis gibbosus*). Evolution 1997; 51: 1946-1954.

Armanios M, Blackburn EH. The telomere syndromes. Nature Reviews Genetics 2012; 13: 693-704.

Asghar M, Palinauskas V, Zaghdoudi-Allan N, Valkiunas G, Mukhin A, Platonova E, et al. Parallel telomere shortening in multiple body tissues owing to malaria infection. Proceedings of the Royal Society B-Biological Sciences 2016; 283.

Aubert G, Lansdorp PM. Telomeres and aging. Physiological Reviews 2008; 88: 557-579.

Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell 2005; 120: 483-495.

Barja G. Rate of generation of oxidative stress-related damage and animal longevity. Free Radical Biology and Medicine 2002; 33: 1167-1172.

Barja G. Free radicals and aging. Trends in Neurosciences 2004; 27: 595-600.

Barrett ELB, Richardson DS. Sex differences in telomeres and lifespan. Aging Cell 2011; 10: 913-921.

Bateson M. Cumulative stress in research animals: Telomere attrition as a biomarker in a welfare context? Bioessays 2016; 38: 201-212.

Beaulieu M, Costantini D. Biomarkers of oxidative status: missing tools in conservation physiology. Conservation Physiology 2014; 2.

Beckman KB, Ames BN. The free radical theory of aging matures. Physiological Reviews 1998; 78: 547-581.

Beery AK, Lin J, Biddle JS, Francis DD, Blackburn EH, Epel ES. Chronic stress elevates telomerase activity in rats. Biology Letters 2012; 8: 1063-1066.

Berglund AMM, Koivula MJ, Eeva T. Species- and age-related variation in metal exposure and accumulation of two passerine bird species. Environmental Pollution 2011; 159: 2368-2374.

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

Berglund AMM, Sturve J, Förlin L, Nyholm NEI. Oxidative stress in pied flycatcher (*Ficedula hypoleuca*) nestlings from metal contaminated environments in northern Sweden. Environmental Research 2007; 105: 330-339.

Bhattacharjee P, Banerjee M, Giri AK. Role of genomic instability in arsenic-induced carcinogenicity. A review. Environment International 2013; 53: 29-40.

Bhattacharyya MK, Lustig AJ. Telomere dynamics in genome stability. Trends in Biochemical Sciences 2006; 31: 114-122.

Bibby CJ, Green RE. Foraging behavior of migrant pied flycatchers, ficedula-hypoleuca, on temporary territories. Journal of Animal Ecology 1980; 49: 507-521.

Birnie-Gauvin K, Peiman KS, Larsen MH, Aarestrup K, Willmore WG, Cooke SJ. Short-term and long-term effects of transient exogenous cortisol manipulation on oxidative stress in juvenile brown trout. The Journal of Experimental Biology 2017.

Bjelland S, Seeberg E. Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 2003; 531: 37-80.

Blackburn EH. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. Febs Letters 2005; 579: 859-862.

Blackburn EH, Gall JG. Tandemly repeated sequence at termini of extrachromosomal ribosomal-rna genes in tetrahymena. Journal of Molecular Biology 1978; 120: 33-53.

Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, *Tetrahymena* and yeast to human cancer and aging. Nature Medicine 2006; 12: 1133-1138.

Blount JD, Metcalfe NB, Arnold KE, Surai PF, Devevey GL, Monaghan P. Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. Proceedings of the Royal Society B-Biological Sciences 2003; 270: 1691-1696.

Blévin P, Angelier F, Tartu S, Ruault S, Bustamante P, Herzke D, et al. Exposure to oxychlordane is associated with shorter telomeres in arctic breeding kittiwakes. Science of The Total Environment 2016; 563–564: 125-130.

Boonekamp JJ, Mulder GA, Salomons HM, Dijkstra C, Verhulst S. Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. Proceedings of the Royal Society B-Biological Sciences 2014; 281: 7.

Borras C, Esteve JM, Vina JR, Sastre J, Vina J, Pallardo FV. Glutathione regulates telomerase activity in 3T3 fibroblasts. Journal of Biological Chemistry 2004; 279: 34332-34335.

Bou R, Codony R, Tres A, Decker EA, Guardicila F. 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 2008; 377: 1-15.

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

Brand MD. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Experimental Gerontology 2000; 35: 811-820.

Bruneaux M, Visse M, Gross R, Pukk L, Saks L, Vasemägi A. Parasite infection and decreased thermal tolerance: impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. Functional Ecology 2017; 31:216-226.

Burkhardt-Holm P, Giger W, Güttinger H, Ochsenbein U, Peter A, Scheurer K, et al. Where have all the fish gone? Environmental Science & Technology 2005; 39: 441A-447A.

Calabrese V, Scapagnini G, Ravagna A, Colombrita C, Spadaro F, Butterfield DA, et al. Increased expression of heat shock proteins in rat brain during aging: relationship with mitochondrial function and glutathione redox state. Mechanisms of Ageing and Development 2004; 125: 325-335.

Callicott RJ, Womack JE. Real-time PCR assay for measurement of mouse telomeres. Comparative Medicine 2006; 56: 17-22.

Campisi J, di Fagagna FD. Cellular senescence: when bad things happen to good cells. Nature Reviews Molecular Cell Biology 2007; 8: 729-740.

Carlos Aledo J, Li Y, Pedro de Magalhaes J, Ruiz-Camacho M, Antonio Perez-Claros J. Mitochondrially encoded methionine is inversely related to longevity in mammals. Aging Cell 2011; 10: 198-207.

Carrillo MC, Kanai S, Sato Y, Kitani K. Age-related-changes in antioxidant enzyme-activities are region and organ, as well as sex, selective in the rat. Mechanisms of Ageing and Development 1992; 65: 187-198.

Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Research 2002; 30: e47.

Cohen S, Janicki-Deverts D, Turner RB, Casselbrant ML, Li-Korotky HS, Epel ES, et al. Association Between Telomere Length and Experimentally Induced Upper Respiratory Viral Infection in Healthy Adults. Jama-Journal of the American Medical Association 2013; 309: 699-705.

Costantini D. Oxidative stress and hormesis in evolutionary ecology and physiology: a marriage between mechanistic and evolutionary approaches. Berlin: Springer, 2014.

Costantini D. Nothing in modern biology makes sense except in the light of ecology and biodiversity conservation. Conservation Physiology 2018; 6.

Costantini D, Casagrande S, De Filippis S, Brambilla G, Fanfani A, Tagliavini J, et al. Correlates of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 2006; 176: 329-337.

Costantini D, Marasco V, Moller AP. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. Journal of Comparative Physiology B-Biochemical

Systemic and Environmental Physiology 2011; 181: 447-456.

Costantini D, Monaghan P, Metcalfe NB. Early life experience primes resistance to oxidative stress. Journal of Experimental Biology 2012; 215: 2820-2826.

Costantini D, Monaghan P, Metcalfe NB. Prior hormetic priming is costly under environmental mismatch. Biology Letters 2014; 10.

Cram DL, Monaghan P, Gillespie R, Clutton-Brock T. Effects of early-life competition and maternal nutrition on telomere lengths in wild meerkats. Proceedings of the Royal Society B-Biological Sciences 2017; 284.

Cramp S, Perrins C, Duncan B. The Birds of the Western Palearctic, Volume 7: Old World Flycatchers to Shrikes: Oxford University Press, 1993.

Dash M, Vasemagi A. Proliferative kidney disease (PKD) agent *Tetracapsuloides bryosalmonae* in brown trout populations in Estonia. Diseases of Aquatic Organisms 2014; 109: 139-148.

Dawson RD, Bidwell MT. Dietary calcium limits size and growth of nestling tree swallows *Tachycineta bicolor* in a non-acidified landscape. Journal of Avian Biology 2005; 36: 127-134.

de Magalhaes JP, Faragher RGA. Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity. Bioessays 2008; 30: 567-578.

Debes PV, Gross R, Vasemägi A. Quantitative Genetic Variation in, and Environmental Effects on, Pathogen Resistance and Temperature-Dependent Disease Severity in a Wild Trout. The American Naturalist 2017; 190: 244-265.

Deisseroth A, Dounce AL. Catalase - physical and chemical properties, mechanism of catalysis, and physiological role. Physiological Reviews 1970; 50: 319.

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

Dugdale HL, Richardson DS. Heritability of telomere variation: it is all about the environment! Philosophical Transactions of the Royal Society B-Biological Sciences 2018; 373.

Dupoue A, Rutschmann A, Le Galliard JF, Clobert J, Angelier F, Marciau C, et al. Shorter telomeres precede population extinction in wild lizards. Scientific Reports 2017; 7.

Eeva T, Belskii E, Gilyazov AS, Kozlov MV. Pollution impacts on bird population density and species diversity at four non-ferrous smelter sites. Biological Conservation 2012; 150: 33-41.

Eeva T, Belskii E, Kuranov B. Environmental pollution affects genetic diversity in wild bird populations. Mutation Research-Genetic Toxicology and Environmental Mutagenesis 2006; 608: 8-15.

Eeva T, Lehikoinen E. Long-term recovery of clutch size and egg shell quality of the pied flycatcher (*Ficedula hypoleuca*) in a metal polluted area. Environmental Pollution 2015; 201: 26-33.

Eeva T, Lehikoinen E, Sunell C. The quality of pied flycatcher (*Ficedula hypoleuca*) and great tit (*Parus major*) females in an air pollution gradient. Annales Zoologici Fennici 1997; 34: 61-71.

Elia AC, Dorr AJM, Zippilli L, Prearo M. Antioxidant response of farmed *Oncorhynchus mykiss* affected by proliferative kidney disease (PKD). Ecotoxicology research developments. 2009: 187-203.

Elliott JM. Numerical Changes and Population Regulation in Young Migratory Trout *Salmo trutta* in a Lake District Stream, 1966-83. Journal of Animal Ecology 1984; 53: 327-350.

Elliott JM. Quantitative ecology and the brown trout. Oxford series in ecology and evolution. Oxford University Press, 1994.

Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, et al. Accelerated telomere shortening in response to life stress. Proceedings of the National Academy of Sciences of the United States of America 2004; 101: 17312-17315.

Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. Current Topics in Medicinal Chemistry 2001; 1: 529-539.

Estok P, Zsebok S, Siemers BM. Great tits search for, capture, kill and eat hibernating bats. Biology Letters 2010; 6: 59-62.

Eymard S, Genot C. 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 2003; 105: 497-501.

Fabian D, Flatt T. The Evolution of Aging. Nature Education Knowledge, 2011; 3: 10: 9.

Fairlie J, Holland R, Pilkington JG, Pemberton JM, Harrington L, Nussey DH. Lifelong leukocyte telomere dynamics and survival in a free-living mammal. Aging Cell 2016; 15: 140-148.

Fialkow L, Wang YC, Downey GP. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radical Biology and Medicine 2007; 42: 153-164.

Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239-247.

Foote CG, Gault EA, Nasir L, Monaghan P. Telomere dynamics in relation to early growth conditions in the wild in the lesser black-backed gull. Journal of Zoology 2011; 283: 203-209.

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

Gems D, Partridge L. Genetics of Longevity in Model Organisms: Debates and Paradigm Shifts. Annual Review of Physiology 2013; 75: 621-644.

Gibson AB, Garratt M, Brooks RC. Experimental evidence that litter size imposes an oxidative challenge to offspring. Journal of Experimental Biology 2015; 218: 3911-3918.

Giroud S, Zahn S, Criscuolo FO, Chery I, Blanc S, Turbill C, et al. Late-born intermittently fasted juvenile garden dormice use torpor to grow and fatten prior to hibernation: consequences for ageing processes. Proceedings of the Royal Society B-Biological Sciences 2014; 281.

Glade MJ, Meguid MM. A glance at ... telomeres, oxidative stress, antioxidants, and biological aging. Nutrition 2015; 31: 1447-1451.

Golomazou E, Athanassopoulou F, Karagouni E, Kokkokiris L. The Effect of Seasonality on the Health and Growth of a Newly Recorded *Myxobolus* Species Infecting Cultured Sharp Snout Seabream (*Diplodus puntazzo C.*). Turkish Journal of Veterinary & Animal Sciences 2009; 33: 1-5.

Gomes NMV, Shay JW, Wright WE. Telomere biology in Metazoa. Febs Letters 2010; 584: 3741-3751.

Gomez D, Bartholomew J, Sunyer JO. Biology and mucosal immunity to myxozoans. Developmental and Comparative Immunology 2014; 43: 243-256.

Gomez-Cabrera MC, Domenech E, Vina J. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. Free Radical Biology and Medicine 2008; 44: 126-131.

Grabner DS, El-Matbouli M. Comparison of the susceptibility of brown trout (*Salmo trutta*) and four rainbow trout (*Oncorhynchus mykiss*) strains to the myxozoan *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD). Veterinary Parasitology 2009; 165: 200-206.

Habig WH, Pabst MJ, Jakoby WB. Glutathione s-transferases - first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 1974; 249: 7130-7139.

Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM, et al. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. Circulation Research 2004; 94: 768-775.

Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press, 2007.

Harley CB, Futcher AB, Greider CW. Telomeres shorten during aging of human fibroblasts. Nature 1990; 345: 458-460.

Harman D. Aging - a theory based on free-radical and radiation-chemistry. Journals of Gerontology 1956; 11: 298-300.

Hatakeyama H, Yamazaki H, Nakamura KI, Izumiyama-Shimomura N, Aida J, Suzuki H, et al. Telomere attrition and restoration in the normal teleost *Oryzias latipes* are linked to growth rate and telomerase activity at each life stage. Aging-Us 2016; 8: 62-76.

Hau M, Haussmann MF, Greives TJ, Matlack C, Costantini D, Quetting M, et al. Repeated stressors in adulthood increase the rate of biological ageing. Frontiers in Zoology 2015; 12.

Haussmann MF, Marchetto NM. Telomeres: Linking stress and survival, ecology and evolution. Current Zoology 2010; 56: 714-727.

Hayflick L. Limited in vitro lifetime of human diploid cell strains. Experimental Cell Research 1965; 37: 614-&.

Hector KL, Nakagawa S. Quantitative analysis of compensatory and catch-up growth in diverse taxa. Journal of Animal Ecology 2012; 81: 583-593.

Hedrick RP, MacConnell E, de Kinkelin P. Proliferative kidney disease of salmonid fish. Annual Review of Fish Diseases 1993; 3: 277-290.

Heidinger BJ, Blount JD, Boner W, Griffiths K, Metcalfe NB, Monaghan P. Telomere length in early life predicts lifespan. Proceedings of the National Academy of Sciences of the United States of America 2012; 109: 1743-1748.

Hemann MT, Greider CW. Wild-derived inbred mouse strains have short telomeres. Nucleic Acids Research 2000; 28: 4474-4478.

Hornsby PJ. Replicative senescence of human and mouse cells in culture: significance for aging research. Mechanisms of Ageing and Development 2003; 124: 853-855.

Houben JMJ, Moonen HJJ, van Schooten FJ, Hageman GJ. Telomere length assessment: Biomarker of chronic oxidative stress? Free Radical Biology and Medicine 2008; 44: 235-246.

Hoyo Jd, Elliott A, Christie D. Handbook of the birds of the world. Vol. 12, Picathartes to tits and chickadees. Lynx, 2007.

Hulbert AJ. On the importance of fatty acid composition of membranes for aging. Journal of Theoretical Biology 2005; 234: 277-288.

Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. Life and death: Metabolic rate, membrane composition, and life span of animals. Physiological Reviews 2007; 87: 1175-1213.

Ibanez-Alamo JD, Pineda-Pampliega J, Thomson RL, Aguirre JI, Diez-Fernandez A, Faivre B, et al. Urban blackbirds have shorter telomeres. Biology Letters 2018; 14.

Ilmonen P, Kotrschal A, Penn DJ. Telomere Attrition Due to Infection. Plos One 2008; 3.

Izzo C, Bertozzi T, Gillanders BM, Donnellan SC. Variation in Telomere Length of the Common Carp, *Cyprinus carpio* (*Cyprinidae*), in Relation to Body Length. Copeia 2014: 87-94.

Jacob KD, Noren Hooten N, Trzeciak AR, Evans MK. Markers of oxidant stress that are clinically relevant in aging and age-related disease. Mechanisms of Ageing and Development 2013; 134: 139-157.

Jancova P, Anzenbacher P, Anzenbacherova E. Phase ii drug metabolizing enzymes. Biomedical Papers-Olomouc 2010; 154: 103-116.

Jankowiak D, Pilarczyk R, Drozd R, Pilarczyk B, Tomza-Marciniak A, Wysocka G, et al. Activity of antioxidant enzymes in the liver of wild boars (*Sus scrofa*) from a selenium-deficient area depending on sex, age, and season of the year. Turkish Journal of Biology 2015; 39: 129-138.

Ji LL. Antioxidants and oxidative stress in exercise. Proceedings of the Society for Experimental Biology and Medicine 1999; 222: 283-292.

Ji LL, Gomez-Cabrera MC, Vina J. Exercise and Hormesis Activation of Cellular Antioxidant Signaling Pathway. Understanding and Modulating Aging 2006; 1067: 425-435.

Jones DP, Mody VC, Carlson JL, Lynn MJ, Sternberg P. Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. Free Radical Biology and Medicine 2002; 33: 1290-1300.

Karell P, Bensch S, Ahola K, Asghar M. Pale and dark morphs of tawny owls show different patterns of telomere dynamics in relation to disease status. Proceedings of the Royal Society B-Biological Sciences 2017; 284.

Kirkwood TBL. Evolution of aging. Nature 1977; 270: 301-304.

Kirkwood TBL. Evolution of ageing. Mechanisms of Ageing and Development 2002; 123: 737-745.

Kirkwood TBL. Systems biology of ageing and longevity. Philosophical Transactions of the Royal Society B-Biological Sciences 2011; 366: 64-70.

Kirkwood TBL, Holliday R. Evolution of aging and longevity. Proceedings of the Royal Society Series B-Biological Sciences 1979; 205: 531-546.

Kirkwood TBL, Rose MR. Evolution of senescence - late survival sacrificed for reproduction. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 1991; 332: 15-24.

Klemetsen A, Amundsen PA, Dempson JB, Jonsson B, Jonsson N, O'Connell MF, et al. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecology of Freshwater Fish 2003; 12: 1-59.

Koivula MJ, Eeva T. Metal-related oxidative stress in birds. Environmental Pollution 2010; 158: 2359-2370.

Koivula MJ, Kanerva M, Salminen J-P, Nikinmaa M, Eeva T. Metal pollution indirectly increases oxidative stress in great tit (*Parus major*) nestlings. Environmental Research 2011; 111: 362-370.

Kotrschal A, Ilmonen P, Penn DJ. Stress impacts telomere dynamics. Biology Letters 2007; 3: 128-130.

Kovacic P, Wakelin LPG. Review - DNA molecular electrostatic potential: novel perspectives for the mechanism of action of anticancer drugs involving electron transfer and oxidative stress. Anti-Cancer Drug Design 2001; 16: 175-184.

Krebs JR. Citation classic - territory and breeding density in the great tit, parus-major L. Current Contents/Agriculture Biology & Environmental Sciences 1984: 14-14.

Kruger NJ, von Schaewen A. The oxidative pentose phosphate pathway: structure and organisation. Current Opinion in Plant Biology 2003; 6: 236-246.

Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. Journal of Cell Science 2004; 117: 2417-2426.

Lagisz M, Hector KL, Nakagawa S. Life extension after heat shock exposure: Assessing meta-analytic evidence for hormesis. Ageing Research Reviews 2013; 12: 653-660.

Lambert AJ, Boysen HM, Buckingham JA, Yang T, Podlutsky A, Austad SN, et al. Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. Aging Cell 2007; 6: 607-618.

Lee MS, Yaar M, Eller MS, Runger TM, Gao Y, Gilchrest BA. Telomeric DNA induces p53-dependent reactive oxygen species and protects against oxidative damage. Journal of Dermatological Science 2009; 56: 154-162.

Lindström J. Early development and fitness in birds and mammals. Trends in Ecology & Evolution 1999; 14: 343-348.

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The Hallmarks of Aging. Cell 2013; 153: 1194-1217.

Lopez-Torres M, Perez-Campo R, Rojas C, Cadenas S, Barja G. Maximum life span in vertebrates: Relationship with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true malondialdehyde, in vivo H_2O_2, and basal and maximum aerobic capacity. Mechanisms of Ageing and Development 1993; 70: 177-199.

Ludlow AT, Spangenburg EE, Chin ER, Cheng WH, Roth SM. Telomeres Shorten in Response to Oxidative Stress in Mouse Skeletal Muscle Fibers. Journals of Gerontology Series a-Biological Sciences and Medical Sciences 2014; 69: 821-830.

Lundberg A, Alatalo RV, Pärt T. The pied flycatcher. Poyser, London, 1992.

Maeda S, Miyauchi T, Iemitsu M, Tanabe T, Yokota T, Goto K, et al. Effects of exercise training on expression of endothelin-1 mRNA in the aorta of aged rats. Clinical Science 2002; 103: 118S-123S.

Makino N, Maeda T, Oyama J, Sasaki M, Higuchi Y, Mimori K, et al. Antioxidant therapy attenuates myocardial telomerase activity reduction in superoxide dismutase-deficient mice. Journal of Molecular and Cellular Cardiology 2011; 50: 670-677.

Mannervik B. Glutathione-peroxidase. Methods in Enzymology 1985; 113: 490-495.

Mannervik B, Danielson UH. Glutathione transferases - structure and catalytic activity. Crc Critical Reviews in Biochemistry 1988; 23: 283-337.

Manning EL, Crossland J, Dewey MJ, Van Zant G. Influences of inbreeding and genetics on telomere length in mice. Mammalian Genome 2002; 13: 234-238.

McLennan D, Armstrong JD, Stewart DC, McKelvey S, Boner W, Monaghan P, et al. Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. Molecular Ecology 2016; 25: 5425-5438.

McLennan D, Armstrong JD, Stewart DC, McKelvey S, Boner W, Monaghan P, et al. Links between parental life histories of wild salmon and the telomere lengths of their offspring. Molecular Ecology 2018; 27: 804-814.

Medawar PB. An Unsolved Problem of Biology. H. K. Lewis, London London, 1952.

Medzhitov R, Schneider DS, Soares MP. Disease Tolerance as a Defense Strategy. Science 2012; 335: 936-941.

Meillere A, Brischoux F, Ribout C, Angelier F. Traffic noise exposure affects telomere length in nestling house sparrows. Biology Letters 2015; 11.

Metcalfe NB, Monaghan P. Compensation for a bad start: grow now, pay later? Trends in Ecology & Evolution 2001; 16: 254-260.

Metcalfe NB, Monaghan P. Growth versus lifespan: perspectives from evolutionary ecology. Experimental Gerontology 2003; 38: 935-940.

Metcalfe NB, Monaghan P. Does reproduction cause oxidative stress? An open question. Trends in Ecology & Evolution 2013; 28: 347-350.

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

Mizutani Y, Niizuma Y, Yoda K. How Do Growth and Sibling Competition Affect Telomere Dynamics in the First Month of Life of Long-Lived Seabird? PLOS ONE 2016; 11: e0167261.

Monaghan P. Telomeres and life histories: the long and the short of it. Annals of the New York Academy of Sciences 2010; 1206: 130-142.

Monaghan P. Organismal stress, telomeres and life histories. Journal of Experimental Biology 2014; 217: 57-66.

Monaghan P, Haussmann MF. Do telomere dynamics link lifestyle and lifespan? Trends in Ecology & Evolution 2006; 21: 47-53.

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

Monaghan P, Ozanne SE. Somatic growth and telomere dynamics in vertebrates: relationships, mechanisms and consequences. Philosophical Transactions of the Royal Society B-Biological Sciences 2018; 373.

Moosmann B, Behl C. Mitochondrially encoded cysteine predicts animal lifespan. Aging Cell 2008; 7: 32-46.

Nettle D, Monaghan P, Boner W, Gillespie R, Bateson M. Bottom of the Heap: Having Heavier Competitors Accelerates Early-Life Telomere Loss in the European Starling, *Sturnus vulgaris*. Plos One 2013; 8: 8.

Nettle D, Monaghan P, Gillespie R, Brilot B, Bedford T, Bateson M. An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. Proceedings of the Royal Society B-Biological Sciences 2015; 282.

Noltmann E, Gubler CJ, Kuby SA. Glucose 6-phosphate dehydrogenase (Zwischenferment) 1. Isolation of crystalline enzyme from yeast. Journal of Biological Chemistry 1961; 236: 1225-&.

Näslund J, Pauliny A, Blomqvist D, Johnsson JI. Telomere dynamics in wild brown trout: effects of compensatory growth and early growth investment. Oecologia 2015; 177: 1221-1230.

Olsson M, Wapstra E, Friesen C. Ectothermic telomeres: it's time they came in from the cold. Royal Society Philosophical Transactions Biological Sciences 2018; 373: 20160449-20160449.

Pamplona R. Membrane phospholipids, lipoxidative damage and molecular integrity: A causal role in aging and longevity. Biochimica Et Biophysica Acta-Bioenergetics 2008; 1777: 1249-1262.

Pamplona R, Barja G, Portero-Otin M. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span - A homeoviscous-longevity adaptation? Increasing Healthy Life Span: Conventional Measures and Slowing the Innate Aging Process 2002; 959: 475-490.

Pamplona R, Costantini D. Molecular and structural antioxidant defenses against oxidative stress in animals. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 2011; 301: R843-R863.

Parolini M, Romano A, Khoriauli L, Nergadze SG, Caprioli M, Rubolini D, et al. Early-Life Telomere Dynamics Differ between the Sexes and Predict Growth in the Barn Swallow (*Hirundo rustica*). Plos One 2015; 10.

Pauliny A, Wagner RH, Augustin J, Szep T, Blomqvist D. Age-independent telomere length predicts fitness in two bird species. Molecular Ecology 2006; 15: 1681-1687.

Pearl R. The Rate of Living. University of London Press, London, 1928.

Perez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, et al. Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. Proceedings of the National Academy of Sciences of the United States of America 2009; 106: 3059-3064.

Perez-Campo R, Lopez-Torres M Fau - Rojas C, Rojas C Fau - Cadenas S, Cadenas S Fau - Barja G, Barja G. Longevity and antioxidant enzymes, non-enzymatic antioxidants and oxidative stress in the vertebrate lung: a comparative study. J. Comp Physiol B 1994.

Perrins CM, McCleery RH. Laying dates and clutch size in the great tit. Wilson Bulletin 1989; 101: 236-253.

Peskin AV, Winterbourn CC. A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1). Clinica Chimica Acta 2000; 293: 157-166.

Petersen S, Saretzki G, von Zglinicki T. Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. Experimental Cell Research 1998; 239: 152-160.

Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research 2001; 29.

Phifer-Rixey M, Nachman MW. Insights into mammalian biology from the wild house mouse *Mus musculus*. Elife 2015; 4.

Pottier G, Viau M, Ricoul M, Shim G, Bellamy M, Cuceu C, et al. Lead Exposure Induces Telomere Instability in Human Cells. Plos One 2013; 8.

Powers SK, Jackson MJ. Exercise-induced oxidative stress: Cellular mechanisms and impact on

muscle force production. Physiological Reviews 2008; 88: 1243-1276.

Price LH, Kao H-T, Burgers DE, Carpenter LL, Tyrka AR. Telomeres and Early-Life Stress: An Overview. Biological Psychiatry 2013; 73: 15-23.

Radak Z, Chung HY, Koltai E, Taylor AW, Goto S. Exercise, oxidative stress and hormesis. Ageing Research Reviews 2008; 7: 34-42.

Rainio MJ, Kanerva M, Salminen J-P, Nikinmaa M, Eeva T. Oxidative status in nestlings of three small passerine species exposed to metal pollution. Science of the Total Environment 2013; 454: 466-473.

Raja-aho S, Kanerva M, Eeva T, Lehikoinen E, Suorsa P, Gao K, et al. Seasonal Variation in the Regulation of Redox State and Some Biotransformation Enzyme Activities in the Barn Swallow (*Hirundo rustica* L.). Physiological and Biochemical Zoology 2012; 85: 148-158.

Rattan SIS. Hormesis in aging. Ageing Research Reviews 2008; 7: 63-78.

Raymond AR, Norton GR, Harden LM, Woodiwiss AJ, Brooksbank RL. Chronic inflammation reduces cardiac relative telomere length without altering left ventricular chamber function. International Journal of Cardiology 2014; 175: 367-369.

Reichert S, Criscuolo F, Zahn S, Arrive M, Bize P, Massemin S. Immediate and delayed effects of growth conditions on ageing parameters in nestling zebra finches. Journal of Experimental Biology 2015; 218: 491-499.

Reichert S, Stier A. Does oxidative stress shorten telomeres in vivo? A review. Biology Letters 2017; 13.

Ricklefs RE. Insights from comparative analyses of aging in birds and mammals. Aging Cell 2010; 9: 273-284.

Ringsby TH, Jensen H, Parn H, Kvalnes T, Boner W, Gillespie R, et al. On being the right size: increased body size is associated with reduced telomere length under natural conditions. Proceedings of the Royal Society B-Biological Sciences 2015; 282.

Robinson RA. BirdFacts: profiles of birds occurring in Britain & Ireland (BTO Research Report 407). British Trust for Ornithology, BTO BirdFacts https://www.bto.org/ (visited 2.5.2018)

Robles R, Palomino N, Robles A. Oxidative stress in the neonate. Early Human Development 2001; 65: S75-S81.

Rollo CD. Growth negatively impacts the life span of mammals. Evolution & Development 2002; 4: 55-61.

Ruijter JM, Ramakers C, Hoogaars WMH, Karlen Y, Bakker O, van den Hoff MJB, et al. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Research 2009; 37.

Sahin E, Colla S, Liesa M, Moslehi J, Muller FL, Guo MR, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 2011; 470: 359-365.

Salmon AB, Leonard S, Masamsetti V, Pierce A, Podlutsky AJ, Podlutskaya N, et al. The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. Faseb Journal 2009; 23: 2317-2326.

Salmon P, Nilsson JF, Nord A, Bensch S, Isaksson C. Urban environment shortens telomere length in nestling great tits, *Parus major*. Biology Letters 2016; 12.

Salmon P, Nilsson JF, Watson H, Bensch S, Isaksson C. Selective disappearance of great tits with short telomeres in urban areas. Proceedings of the Royal Society B-Biological Sciences 2017; 284.

Salmon P, Stroh E, Herrera-Duenas A, von Post M, Isaksson C. Oxidative stress in birds along a NOx and urbanisation gradient: An interspecific approach. Science of the Total Environment 2018; 622: 635-643.

Salomons HM, Mulder GA, van de Zande L, Haussmann MF, Linskens MHK, Verhulst S. Telomere shortening and survival in free-living corvids. Proceedings of the Royal Society B-Biological Sciences 2009; 276: 3157-3165.

Samuels DC. Life span is related to the free energy of mitochondrial DNA. Mechanisms of Ageing and Development 2005; 126: 1123-1129.

Sanders JL, Newman AB. Telomere Length in Epidemiology: A Biomarker of Aging, Age-Related Disease, Both, or Neither? Epidemiologic Reviews 2013; 35: 112-131.

Sarkar J, Liu Y. The origin of oxidized guanine resolves the puzzle of oxidation-induced telomere-length alterations. Nature Structural & Molecular Biology 2016; 23: 1070-1071.

Sasaki T, Unno K, Tahara S, Shimada A, Chiba Y, Hoshino M, et al. Age-related increase of superoxide generation in the brains of mammals and birds. Aging Cell 2008; 7: 459-469.

Scheuhammer AM. Effects of acidification on the availability of toxic metals and calcium to wild

birds and mammals. Environmental Pollution 1991; 71: 329-375.

Sebens KP. The ecology of indeterminate growth in animals. Annual Review of Ecology and Systematics 1987; 18: 371-407.

Sillanpää S, Salminen J-P, Eeva T. Fluctuating asymmetry in great tit nestlings in relation to diet quality, calcium availability and pollution exposure. Science of the Total Environment 2010; 408: 3303-3309.

Sitja-Bobadilla A, Calduch-Giner J, Saera-Vila A, Palenzuela O, Alvarez-Pellitero P, Perez-Sanchez J. Chronic exposure to the parasite *Enteromyxum leei* (*Myxozoa : Myxosporea*) modulates the immune response and the expression of growth, redox and immune relevant genes in gilthead sea bream, *Sparus aurata* L. Fish & Shellfish Immunology 2008; 24: 610-619.

Skalova L, Krizova V, Cvilink V, Szotakova B, Storkanova L, Velik J, et al. Mouflon (*Ovis musimon*) *dicrocoeliosis*: Effects of parasitosis on the activities of biotransformation enzymes and albendazole metabolism in liver. Veterinary Parasitology 2007; 146: 254-262.

Sletten S, Bourgeon S, Badrdsen B-J, Herzke D, Criscuolo F, Massemin S, et al. Organohalogenated contaminants in white-tailed eagle (*Haliaeetus albicilla*) nestlings: An assessment of relationships to immunoglobulin levels, telomeres and oxidative stress. Science of the Total Environment 2016; 539: 337-349.

Smith IK, Vierheller TL, Thorne CA. Assay of glutathione-reductase in crude tissue-homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). Analytical Biochemistry 1988; 175: 408-413.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al. Measurement of protein using bicinchoninic acid. Analytical Biochemistry 1985; 150: 76-85.

Smith S, Turbill C, Penn DJ. Chasing telomeres, not red herrings, in evolutionary ecology. Heredity 2011; 107: 372-373.

Smith SM, Nager RG, Costantini D. Meta-analysis indicates that oxidative stress is both a constraint on and a cost of growth. Ecology and Evolution 2016; 6: 2833-2842.

Speakman JR. Body size, energy metabolism and lifespan. Journal of Experimental Biology 2005; 208: 1717-1730.

Speakman JR, Talbot DA, Selman C, Snart S, McLaren JS, Redman P, et al. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging Cell 2004; 3: 87-95.

Stadtman ER, Moskovitz J, Levine RL. Oxidation of methionine residues of proteins: Biological consequences. Antioxidants & Redox Signaling 2003; 5: 577-582.

Stearns SC. The evolution of life histories. Oxford University Press, Oxford, UK, 1992.

Stier A, Massemin S, Zahn S, Tissier ML, Criscuolo F. Starting with a handicap: effects of asynchronous hatching on growth rate, oxidative stress and telomere dynamics in free-living great tits. Oecologia 2015; 179: 999-1010.

Stindl R. Tying it all together: telomeres, sexual size dimorphism and the gender gap in life expectancy. Medical Hypotheses 2004; 62: 151-154.

Suh JH, Shenvi SV, Dixon BM, Liu HL, Jaiswal AK, Liu RM, et al. Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proceedings of the National Academy of Sciences of the United States of America 2004; 101: 3381-3386.

Taylor HA, Delany ME. Ontogeny of telomerase in chicken: Impact of downregulation on pre- and postnatal telomere length in vivo. Development Growth & Differentiation 2000; 42: 613-621.

Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. American Journal of Physiology-Lung Cellular and Molecular Physiology 2000; 279: L1005-L1028.

Tricola GM, Simons MJP, Atema E, Boughton RK, Brown JL, Dearborn DC, et al. The rate of telomere loss is related to maximum lifespan in birds. Philosophical Transactions of the Royal Society B-Biological Sciences 2018; 373.

Tsay HJ, Wang P, Wang SL, Ku HH. Age-associated changes of superoxide dismutase and catalase activities in the rat brain. Journal of Biomedical Science 2000; 7: 466-474.

Ukeda H, Sarker AK, Kawana D, Sawamura M. Flow-injection assay of superoxide dismutase based on the reduction of highly water-soluble tetrazolium. Analytical Sciences 1999; 15: 353-357.

Valko M, Morris H, Cronin MTD. Metals, toxicity and oxidative stress. Current Medicinal Chemistry 2005; 12: 1161-1208.

van Deursen JM. The role of senescent cells in ageing. Nature 2014; 509: 439-446.

Vedder O, Verhulst S, Bauch C, Bouwhuis S. Telomere attrition and growth: a life-history framework and case study in common terns. J Evol Biol 2017.

Viarengo A, Ponzano E, Dondero F, Fabbri R. A simple spectrophotometric method for metallothionein evaluation in marine organisms: An application to Mediterranean and Antarctic molluscs. Marine Environmental Research 1997; 44: 69-84.

Vina J, Borras C, Gomez-Cabrera MC, Orr WC. Part of the series: from dietary antioxidants to regulators in cellular signalling and gene expression - Role of reactive oxygen species and (phyto)oestrogens in the modulation of adaptive response to stress. Free Radical Research 2006; 40: 111-119.

von Zglinicki T. Oxidative stress shortens telomeres. Trends in Biochemical Sciences 2002; 27: 339-344.

von Zglinicki T, Burkle A, Kirkwood TBL. Stress, DNA damage and ageing - an integrative approach. Experimental Gerontology 2001; 36: 1049-1062.

Watson H, Bolton M, Monaghan P. Variation in early-life telomere dynamics in a long-lived bird: links to environmental conditions and survival. Journal of Experimental Biology 2015; 218: 668-674.

Weinert BT, Timiras PS. Theories of aging. Journal of Applied Physiology 2003; 95: 1706-1716.

Wilbourn RV, Moatt JP, Froy H, Walling CA, Nussey DH, Boonekamp JJ. The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. Philosophical Transactions of the Royal Society B-Biological Sciences 2018; 373.

Wilce MCJ, Parker MW. Structure and function of glutathione s-transferases. Biochimica Et Biophysica Acta-Protein Structure and Molecular Enzymology 1994; 1205: 1-18.

Williams GC. Pleiotropy, natural-selection, and the evolution of senescence. Evolution 1957; 11: 398-411.

Wilson DE, Reeder DM. Mammal species of the world: a taxonomic and geographic reference. Johns Hopkins University Press, 2005.

Wright WE, Shay JW. Telomere dynamics in cancer progression and prevention: fundamental differences in human and mouse telomere biology. Nature Medicine 2000; 6: 849-851.

Yang W, Hekimi S. A Mitochondrial Superoxide Signal Triggers Increased Longevity in Caenorhabditis elegans. PLoS Biology 2010; 8: e1000556.

Young RC, Welcker J, Barger CP, Hatch SA, Merkling T, Kitaiskaia EV, et al. Effects of developmental conditions on growth, stress and telomeres in black-legged kittiwake chicks. Molecular Ecology 2017; 26: 3572-3584.

Yun J, Finkel T. Mitohormesis. Cell Metabolism 2014; 19: 757-766.

Zhang J, Rane G, Dai X, Shanmugam MK, Arfuso F, Samy RP, et al. Ageing and the telomere connection: An intimate relationship with inflammation. Ageing Research Reviews 2016; 25: 55-69.

Annales Universitatis Turkuensis

Turun yliopisto
University of Turku

Painosalama Oy, Turku, Finland 2018

ISBN 978-951-29-7361-3 (PRINT)
ISBN 978-951-29-7362-0 (PDF)
ISSN 0082-6979 (PRINT) | ISSN 2343-3183 (PDF)