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Multidimensionality of aging in a long-lived seabird

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Press

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Author's contribution to the papers ('*' denotes a moderate contribution, '**' denotes a high contribution, '***' denotes a leading role)

	I	**II**	**III**	**IV**	**V**
Original idea	*	*		***	*
Study design		**		***	*
Data collection	***	***	**	***	***
Data analysis		***	**	***	**
Manuscript preparation	**	***	**	***	**

1. INTRODUCTION

1.1. Life history theory, pace-of-life, and senescence

Organismal aging, or senescence, can be defined as a progressive, irreversible loss of function that results in declines in fertility and survival (Holmes and Martin 2009). This definition restricts senescence to age-related deterioration that occurs after organisms reach maturity. Hence, classical evolutionary theory does not refer to senescence as a state of senility in very late adulthood rather it predicts that senescence should begin at the age of sexual maturity and progress from that point as the force of natural selection weakens (Williams 1957, Hamilton 1966). Senescence includes processes that can be detrimental to reproductive success and, therefore, are relevant to fitness trade-offs (Holmes and Martin 2009).

There are multiple evolutionary theories of senescence (reviewed by Nussey et al. 2013). Two of those theories: antagonistic pleiotropy (Williams 1957) and disposable soma theory (Kirkwood 1977) are considered to be life-history theories of aging (Partridge and Barton 1996). According to life-history theory, an organism must divide its limited resources (e.g. energy) between somatic maintenance and reproductive effort in order to maximize its lifetime fitness (Williams 1966). As stated by both antagonistic pleiotropy and disposable soma theory, senescence can hence be viewed as a result of natural selection favouring greater investment into reproduction early in life at the expense of somatic maintenance. That allocation pattern itself is determined by the species ecological context, with a more stable environment and low extrinsic mortality leading to a greater investment into self-maintenance and a longer lifespan. Since greater investment into somatic maintenance automatically results in diminished investment into reproduction, one can place species onto a fast-slow life-history continuum (Gaillard et al. 1989, Bielby et al. 2007), with species in the “slow” end of the spectrum experiencing slow growth rate, increased size at maturity, late maturation, reduced number of offspring, long lifespan and low adult and juvenile mortalities.

The pace-of-life syndrome (POLS) hypothesis suggests that species should also differ in physiological traits that have co-evolved with the life-history particularities of each species (Ricklefs and Wikelski 2002, Wikelski et al. 2003a). Hence the idea of POLS is closely related to classic r- and K- strategy axis (Pianka 1970) as well as extends the fast-slow life-history continuum (Gaillard et al. 1989, Bielby et al. 2007). Figure 1. illustrates the potential variation of traits along the pace-of-life continuum.

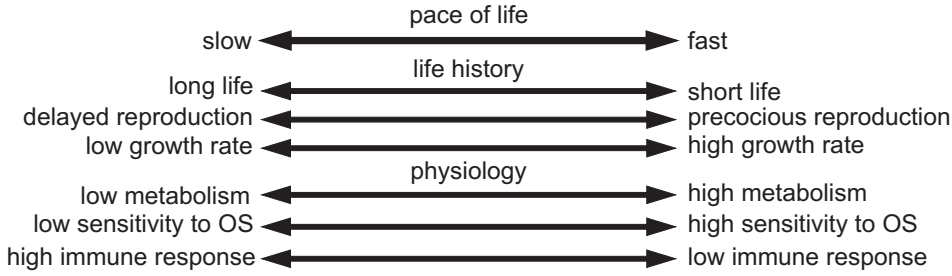

Figure 1. Schematic of the potential integration of different traits along a pace-of-life continuum. Double arrows illustrate presumed continuous variation in life-history strategies among species. Adapted from Reale *et al.* (2010)

Of course, there are notable exceptions to POLS. For example birds are considered to be long-lived slowly aging animals despite their high metabolic rate and small size. In fact, they can live up to three times longer than similarly sized mammals, despite their metabolic rates being 1.5–2.5 times higher (Holmes and Ottinger 2003). This inconsistency is explained by their ability to fly. Organisms with higher mortality rates undergo natural selection for early rapid maturation, early reproduction, and hence shorter life-span, while organisms with effective protection against predators, like flying ability, are expected to invest more into soma. Consistent with this view is the fact, that flying and gliding mammals also experience slower aging and longer lifespans, than predicted by their metabolism (Holmes and Ottinger 2003). Therefore birds might have "private" physiological mechanisms enabling them to combat the senescence effects usually associated with fast metabolism (reviewed by Holmes and Martin 2009). Furthermore, since birds are considered long-lived endotherm species, those mechanisms could be more easily transferable to human systems, than research carried out on traditional model species (e.g. *Caenorhabditis elegans*, *Drosophila melanogaster* and lab mouse). All the aforementioned reasons combined make avian systems interesting models for determining and validating the physiological trade-offs causing aging in long-lived species.

1.2. Multidimensionality of aging

According to Williams (1957), senescence should be synchronous across the physiological systems, since it would not be beneficial for the organism if one aspect of its physiology had a catastrophic failure before the others. For example, there is no benefit in organism maintaining a viable germ line, if somatic senescence has progressed to the point that prevents successful reproduction (Kirkwood and Shanley 2009). However empirical data from humans and laboratory model organisms suggests that aging asynchrony is commonplace and health- and lifespan are inherently uncoupled (Herndon et al.

2002, Burger and Promislow 2006, Martin et al. 2007, Bansal et al. 2015). Of course one might theorize that since humans and laboratory model organisms experience life in benign and protected conditions, their life-span might be unnaturally high, which might disrupt the coupling of senescence.

Although there are multiple studies investigating senescence rates across phenotypic traits (Nussey et al. 2008, Massot et al. 2011), studies investigating the uncoupling of survival and fecundity senescence in natural populations remain scarce (Nussey et al. 2013). Female post-reproductive survival has been documented in a few long-lived mammal species (Croft et al. 2015). There is also evidence from wild ungulates that reproductive senescence may start later and progress more rapidly, than age-related declines in survival probability (Nussey et al. 2013). Therefore, evidence suggests, that in long-lived species, faster reproductive senescence could be a cost of slower somatic senescence (Croft et al. 2015, Griesser et al. 2017). The same could be said about bird species, for, as stated earlier, most of them have abnormally long lifespans for their size. However, since there is a scarcity of studies comparing physiological and reproductive senescence in the same system, fundamental gaps remain in our understanding of the nature of asynchrony in the senescence of these traits.

1.3. Aims of the thesis

Aging research has been traditionally conducted on short-lived laboratory organisms (Holmes and Martin 2009). Since long-lived organisms differ in their senescence patterns from short-lived ones (Lemaitre et al. 2015) and senescence effects on fitness cannot be reliably measured in sterile laboratory conditions (Speakman et al. 2015), there is a need to integrate more long-lived wild populations into biogerontological research. Hence, the main aims of this thesis were to (1) assess the different aspects of senescence in a long-lived seabird and to (2) reveal the potential trade-offs leading to senescence in long-lived wild bird species.

In order to assess the fitness effects of senescence, one must first determine if the population actually ages. There has been a persistent fallacy in biology that natural populations do not senesce, due to them succumbing to the unpredictable natural environment before the negligible effects of senescence can occur (Medawar 1952). However, there is an accumulating amount of evidence, showing, that in nature, senescence does indeed occur (reviewed by Nussey et al. 2013), further supported by studies on the common gull model system employed for the present thesis, indicating reproductive senescence (Rattiste 2004). To further test this notion, I aimed to measure aging, using two widely used physiological age markers (erythrocyte telomere length and skin pentosidine concentration). Both of these markers have previously been shown to correlate with chronological age and have been hypothesised to be affected by oxidative stress (OS), so the setup also enabled me to test the relevance of OS for senescence (**paper I**).

Since aging has been functionally linked to oxidative stress (OS) (Harman 1956), there is a multitude of studies investigating associations between OS and aging (reviewed by Holmes and Martin 2009, Costantini et al. 2010), however most of them have only focused on a few markers of oxidative damage or antioxidant defence (Monaghan et al. 2009). To properly assess the associations between aging and OS, however, one must use a multitude of markers (Hõrak and Cohen 2010, Speakman et al. 2015). I aimed to determine if OS relates to aging and if older organisms are more prone to oxidative damage, using several markers of oxidative damage, antioxidant protection and nutritional state (**paper II**).

To further explore the multidimensionality of aging, I intended to determine, if there is detectable senescence in reproductive traits. Many gull species poses wing ornamentation (Coulson et al. 1982, Allaine and Lebreton 1990), which is under sexual selection (Andersson 1994). I measured common gull wing tip ornamentation in order to find out if they were sexually dimorphic, correlated with age and predicted longevity (**paper III**).

Studies of senescence in the wild have traditionally been focused on traits like fecundity and survival, while traits related to self-maintenance remain understudied in the context of aging. The uropygial or preen gland is a holocrine gland exclusive to birds, directly linked to self-maintenance. To assess the senescence of self-maintenance mechanisms, I measured the size of uropygial glands of common gulls in a cross-sectional manner in relation to aging (**paper IV**).

Finally, I intended to explore, if maternal investment depended upon age. For that purpose, I measured egg yolk carotenoid content and composition, as well as testosterone and vitamin A and E contents, from eggs of differently aged mothers (**paper V**). Carotenoids, vitamins A and E and testosterone have all been linked to offspring quality, so I expected them to reveal signs of maternal senescence through differences in maternal allocation patterns.

2. MATERIALS AND METHODS

2.1. Model system

The biology of aging, or biogerontology, is broadly focused on understanding basic processes responsible for variation in animal life spans and aging patterns, including evolutionary forces as well as physiological and molecular mechanisms (Holmes and Martin 2009). Most biogerontological research has traditionally been conducted on short-lived, inbred laboratory model organisms, rather than wild, free-living animals (Holmes and Martin 2009), although recent studies have also started to incorporate long-lived species, in both organismal (Edrey et al. 2011) and cellular levels (Alper et al. 2015). Since long-lived and short-lived organisms have different life-histories (Pianka 1970, Gaillard et al. 1989, Lemaitre et al. 2015) and underlying physiological mechanisms (Lambert et al. 2007, Galvan et al. 2015, Piersigilli and Meyerholz 2016), and physiological trade-offs inducing senescence cannot be reliably measured in laboratory conditions (Speakman et al. 2015), there is a need to incorporate more long-lived natural animal populations into the field of biogerontology. Birds live remarkably long for their body size compared to mammals and in general, are expected to senesce at slower rates (Williams 1957, Holmes and Austad 1995, Ricklefs and Scheuerlein 2001). Seabirds, in particular, are among the longest-lived of all birds and constitute excellent models for research into both the evolutionary ecology and physiological basis of aging (Ricklefs 1998, Holmes et al. 2001, Monaghan and Haussmann 2006).

The common gull (*Larus canus*) is a monogamous long-lived seabird breeding mainly in colonies. Adult birds weigh around 430 g and have a wingspan of about 120 cm. Breeding season starts in late April, with males arriving at the colony ahead of females and securing a nesting site. Common gull has a fixed clutch size of three eggs, with the third egg being considerably lighter. About 10% of all eggs produce recruits. Eggs are laid 2–3 days apart and incubation starts after the third egg has been laid. Both male and female birds contribute equally into hatching the eggs and rearing the young. Recruits start breeding at 3–4 years of age and breeding lasts on average 5–6 years (Rattiste 2004), although some individuals are capable of breeding for over 30 years. Nonetheless, after the 10th breeding year, breeding success declines as reproductive senescence starts to emerge (Rattiste 2004). As there is no variation in clutch size, laying date is presumably a key reproductive trait (Brommer and Rattiste 2008), with more successful birds breeding earlier. Since breeding success is dependent upon laying date and finding a new partner is time-consuming, it is crucial for the birds to form long-lasting pairs with high-quality individuals. Pair bonds persist on average for 2–3 years (mainly due to high divorce rate among inexperienced individuals and mortality in senescent age classes), with the longest pair lasting for 21 years (Rattiste and Lilleleht 1986, Rattiste personal observations). Like many other gull species

(Coulson et al. 1982, Allaine and Lebreton 1990) common gulls also exhibit wing ornamentation reflecting individual quality (**paper III**) as well as display conspicuous carotenoid-based coloration on their bill and legs.

Studies for the current thesis were conducted on a common gull colony located on Kakrarahu islet in Matsalu National Park (west coast of Estonia, 58°46'N, 23°26'E). The colony has been continuously monitored since 1962, so the population structure is well-known, with over 50% of the males and 10% of the females returning to their birth colony to breed and less than 3% switching colonies between breeding attempts (Rattiste 2004). From the late 1970s onwards, competition and nest predation by herring gulls (*Larus argentatus*) in other colonies have made Kakrarahu the preferred breeding site for common gulls in western Estonia. As a result, the number of breeding pairs is steadily increasing and actual return rates might now be even higher than previously reported. Birds in the colony are ringed as chicks and fitted with unique alpha-numerically coded PVC bands at their first breeding attempt, simplifying later identification. Adult birds are caught (for ringing as well as for studies comprising this thesis) from nests after the tenth day of incubation, using spring traps. Breeding success is recorded by daily inspections of the nests as each egg's laying date and mass is recorded as well as hatching order of the chicks. For the aforementioned reasons, the study system enables the collection of longitudinal individual-based data from the first breeding attempt to the last, perfect for conducting aging research.

2.2. Methodology

2.2.1. Measuring erythrocyte telomere length and skin pentosidine concentration

For the first paper in this thesis, we measured erythrocyte telomere length and skin pentosidine concentration. For telomere length measurement, we collected 50 µl of whole blood into an Eppendorf tube and mixed it with SET puffer (0.15 M NaCl, 20 mM TrisHCl, 1 mM EDTA, pH 8.0). Telomere length was measured using real time quantitative polymerisation chain reaction (qPCR) on a Mx3000P q-PCR system (Stratagene) as described by Asghar *et al.* (2014). For the telomere region, we used primers described by Criscuolo *et al.* (2009) and for the control region we used primers described by Asghar *et al.* (2011). We calculated a relative telomere length (T/S ratio) value, by dividing the (plate-adjusted) qPCR value for the telomere length (T) with the (plate-adjusted) qPCR value for the single copy nuclear sequence.

In order to measure skin pentosidine concentration, we took a skin biopsy from the inner patagium and placed it into an Eppendorf tube with distilled water. We processed the skin samples and analysed 20µl of each sample using the hydroxyproline (OH-proline) analysis as described by Cooey *et al.* (2010).

We used simple regressions to analyse associations between erythrocyte telomere length and age, skin pentosidine concentration and age and telomere length and skin pentosidine. All analyses were performed using Statistica 10 (Statsoft 2010).

2.2.2. Measuring biomarkers of oxidative damage, antioxidant defence and nutritional state

For the second paper in this thesis, we measured several markers of antioxidant defence, oxidative damage and nutritional state in three consecutive years. In order to assess oxidative stress levels and nutritional state, we measured markers of antioxidant defence, oxidative damage and nutritional state. We measured three different antioxidants as well as plasma total antioxidant capacity (TAC). These antioxidants were erythrocyte glutathione, plasma carotenoids and plasma uric acid. We measured glutathione, one of the main intracellular antioxidants (Galván and Alonso-Alvarez 2008), from erythrocytes as described by Galván and Alonso-Alvarez (2008). Concentrations were expressed in micromoles per gram of blood pellet (a solid pellet consisting mainly of erythrocytes achieved by centrifugation).

Carotenoids are fat-soluble carbohydrates linked with fundamental redox pathways (although their importance in those pathways remains to be determined; see Hill and Johnson 2012). We measured carotenoids from 15µl of plasma diluted in acetone as described by Tummeleht et al. (2006).

Plasma uric acid is the main end product of nitrogen metabolism in birds and due to its abundance, it is thought to be one of the main antioxidants in birds (Tsahar et al. 2006).We measured uric acid concentration spectrophotometrically from 5 mL of plasma using a standard kit (Human GmbH kit, Weisbaden, Germany).

As described earlier, in addition to individual antioxidants, we also measured TAC, a measure of water-soluble antioxidants in serum. We measured TAC spectrophotometrically from 5 µl of plasma as described by Erel (2004).

We also measured lipid peroxidation (LPO) as an indicator of oxidative cell damage (Niki 2009). LPO was measured spectrophotometrically using a standard kit (Bioxytech LPO-586, OxisResearch). The method has been previously described by Hõrak et al. (2007).

In addition to markers of oxidative status, two biochemical markers of nutritional condition were measured. These markers were total concentrations of protein and triglycerides in plasma, both of which are expected to reflect nutritional status (Jenni-Eiermann and Jenni 1998). The markers were measured from 5 and 2.5 µl of plasma for total protein and triglycerides, respectively, using a standard kit (Human GmbH kit).

We analysed the data with three different methods. Firstly, for analyzing age-related patterns in physiological and reproductive variables on the basis of cross-sectional data, we relied on generalized mixed models (PROC GLIMMIX,

SAS/STAT, ver. 9.2, SAS), with breeding year (an approximation of age) as a continuous independent variable, individual identity as a random factor and focal physiological or breeding parameter as a dependent variable. To describe possible parabolic relations of measured parameters and age, all models were also tested for significance of the square of breeding year.

Secondly, to distinguish between cross-sectional and longitudinal patterns, as described by Herborn et al. (2016), we partitioned age into within- and between-individual components and substituted these two new fixed effects for age in the original model. The between-individual component was the average age at which each bird was sampled. The within-individual component was (age – average age). We included identity and the slope of the within-individual component of age correlated with identity as random effects. These analyses were conducted using R, version 3.2.2, and the package nlme (R Development Core Team 2015).

Thirdly, To test whether a bird's physiological or breeding parameters were different in its last year of life, we used the discrete factor "terminal breeding year" as a predictor variable in generalized mixed models with individual identity as a random factor. To test whether any of the recorded variables predicts longevity, we used the mixed-effects Cox model using R, version 3.2.2, and the package survival (Therneau 2015).

2.2.3. Measuring wing tip pattern, -abrasion, and preen gland size

For the third and fourth papers in this thesis we measured the size of white wing patches (an ornamental trait), wingtip abrasion and preen gland size (an indicator of somatic maintenance). To characterize the individual wing tip pattern, we summed the measurements of the areas of the white spots on five to six (some birds had a white spot on the 6th feathers, some did not) outermost primaries on the right wing of the bird. As the wing-patches were measured on two different periods, separated by more than ten years, two different techniques were used.

On the first period (1997), the area of spots was measured as follows: the wing was placed in its natural position on the flat surface so that the edges of all white spots on the feathers were visible. Transparent plastic sheet with a grid of 5×5 mm cells was placed on the wing and the areas of spots were estimated as the number of squares with the precision of 1/4 cell, which was thereafter multiplied by 25 to obtain the measurement in square millimetres.

On the second period (2007), all birds were photographed with a ruler placed next to their right wing for scale. Patch size was measured as the white area in mm^2 from digital photographs using IMAGEJ software (<http://rsbweb.nih.gov>). Since there were photographs from the first period, it enabled us to assess correlation between the two methods. The correlation was extremely strong ($r = 0.98$, $p = 0.0001$).

Wingtip abrasion level was measured by was estimated on a scale of 0–4, with 0 representing no visible abrasion and 4 representing missing feather tips on several feathers. Feather abrasion was estimated independently by four persons with the repeatability of 0.67 ($p = 0.0001$, $F_{212,671} = 6.7$).

Uropygial gland was measured with a dialled calliper to the nearest 0.1 mm. Since uropygial glands of gulls are round-shaped (personal observations; see also (Salibian and Montalti 2009)), only one measure for diameter (as opposed to length + width) was taken. Repeatability of gland diameter was 0.86 ($F_{16,19}=14.25$, $p<0.0001$). For birds with multiple measurements, average gland diameter was used in the analyses.

For analyzing age-related patterns of wing patch size we used linear mixed-effects models, with individual identity as a random factor and wing patch size as a dependent variable. Models were fit using maximum likelihood. Type III tests were used for testing fixed effects. Breeding year (as a proxy of age) was included in models as a continuous independent variable to describe associations between measured parameters and age. To describe possible parabolic relations of measured parameters and age, all models were also tested for significance of the square of breeding year. Models were ran using R ver. 3.2.2 and the package nlme. We used Cox proportional hazard models to test whether any of the recorded variables predicts survival, using R ver. 3.2.2 and the package survival (Therneau 2015).

For the preen gland analysis, we used t-tests for comparing trait values between sexes, ANCOVA-s for testing the sex-specific associations between breeding age, its square or uropygial gland size vs dependent variables. The analyses were performed in Statistica v10 (Statsoft 2010).

2.2.4. Measuring yolk testosterone and carotenoids

For the fifth paper in this thesis, we measured yolk androgen and carotenoid contents. We measured yolk testosterone concentration by radioimmunoassay after yolk steroid extraction following previously established protocol (Okuliarova et al. 2011).

We measured the concentration of yolk carotenoids by adapting previously established high-performance liquid chromatography (HPLC) methods (McGraw et al. 2002). This method allowed us to separate different types of carotenoids. We calculated the concentration of each compound, in μg/ml, by comparing absorbance values to previously prepared standard reference curves.

We used multiple regression analyses to analyse associations between egg parameters (egg mass and testosterone and carotenoid contents) and maternal age. To test for possible parabolic relations between egg parameters and age, we included the square of maternal age as a predictor variable and to control for possible age-independent sifferences in maternal quality, we included laying date as a predictor. The final models had maternal age, its square and laying date as independent variables and the specific egg characteristic as dependent variable. All analyses were performed using Statistica 10 (Statsoft 2010).

2.3. Ethics of the experiments

The experiments, that were carried out for this thesis comply with the current law of the Republic of Estonia and were approved by Animal Procedures Committee of the Estonian Ministry of Agriculture (decision #5, issued on 20 April 2013 and decision #106, issued on 24 April 2017). These licenses granted permission to:

- Catch common gulls from their nests using spring traps
- Collect blood and skin samples in amounts previously reported not to be harmful for the species in question to assess telomere length and skin pentosidine concentration
- Collect freshly laid eggs for sampling to determine the eggs' hormonal and carotenoid content

The studies complied with the organisational conditions of the experiments stated in the licenses.

3. RESULTS AND DISCUSSION

3.1. Do common biomarkers of aging predict chronological age? (I)

Birds do not possess many of the phenotypic traits associated with aging, present in mammals (i.e. wrinkling of the skin, whitening of the muzzle or wear of teeth), complicating our ability to quantify aging in birds (Chaney et al. 2003). Reliably measuring age in wild bird populations could play an important role in population management or conservation of endangered species (Anders and Marshall 2005, Cooey et al. 2010, Mills 2016). Since bird banding studies often take a long time to acquire usable demographic data (Cooey et al. 2010) and can produce a bias in estimating a population's age structure (Mills 2016), there is a need for universal aging biomarkers for birds. It is also necessary to validate those markers on a wide variety of species. In the common gull, there is a previously documented noticeable senescent decline in both breeding success (Rattiste 2004) and annual fitness (Brommer et al. 2010) however, it has not been previously established if the same decline is also detectable in biochemical markers of aging.

In paper I, I aimed to validate two commonly used independent biomarkers of aging. For that purpose, we caught 47 male birds of known ages (2–33 years) in 2013 breeding season in order to measure their erythrocyte telomere length and skin pentosidine concentration. Telomeres are highly conserved protective DNA sequences at the ends of chromosomes that progressively shorten in proliferative cells. Their length has been shown to correlate with chronological age. The association with age is different for short-lived and long-lived species (Haussmann et al. 2003) and both negative and positive correlation with age has been found in cross-sectional studies (Haussmann and Mauck 2008, Holmes and Martin 2009). Pentosidine is a marker of both oxidative and glycative damage to proteins. Glycation theory of aging suggests that modification of proteins by glucose leads to production of advanced glycation endproducts (AGEs), such as pentosidine. AGEs cause gradual crosslinking in collagen that is characteristic of aging and leads to deterioration of tissues (Miyata et al. 1998). Pentosidine is found in many different tissues and organs and formed continuously under natural conditions. For several bird species, skin pentosidine content has been shown to correlate with chronological age (Chaney et al. 2003, Cooey et al. 2010).

As in previous studies of the same study system (Rattiste and Lilleleht 1986, Rattiste 2004), the age of the males was related to their partners' laying dates in a concave manner, suggesting a senescent decline in breeding success. Neither of the physiological aging markers, however, correlated with chronological age. Moreover, there was no correlation between telomere length and skin pentosidine concentration, suggesting that there is no common physiological factor regulating aging throughout different tissues of the organism.

To my knowledge, this was the first study that failed to find a correlation between skin pentosidine levels and chronological age. An explanation for such an absence could be, that there is only a weak correlation between physiological and chronological age in common gulls. In many species there exists significant functional and survival variation among like-aged elderly individuals (Collier and Coleman 1991, Nussey et al. 2013), so similarly aged individuals might be in very different physiological condition – have different physiological ages. One might hypothesize, that in the common gull oldest individuals do not comprise a random sample of individuals but are more viable, than the average bird in the population and thus appear physiologically younger for their chronological age. If viability relates to an ability to slow down or offset the processes that cause accumulation of pentosidine with age, then the correlation between pentosidine levels and age would be weak or absent. The uncoupling of physiological and chronological ages in the study system is also supported by the fact, that erythrocyte telomere length, a marker associated with cellular senescence due to oxidative stress (Monaghan and Haussmann 2006), also had no associations with age. Furthermore, there was more variability of telomere length in older individuals, suggesting a relaxed selection on the trait, which could indicate an ability to combat cellular senescence through various physiological mechanisms, one example of them being the telomerase enzyme (Haussmann et al. 2007). Lack of correlation between telomere length and skin pentosidine levels also suggests that neither OS nor any other physiological mechanism affects the rate of aging in this species since both markers have been associated with susceptibility to oxidative damage (Wellsknecht et al. 1995, Monaghan and Haussmann 2006). What is more, it has been shown, that different traits of an organism senesce at different rates (Nussey et al. 2013), so it is possible, that mechanisms responsible for telomere length and skin pentosidine accumulation senesce at a leisurely rate, while reproductive mechanisms senesce in a faster pace. Of course, caution must be taken in interpreting the results. Since it was a cross-sectional study, I cannot exclude the possibility of a cohort effect eclipsing the within-individual senescent pattern with between-individual variability. Finally, it should be noted, that we had an 80% power to detect a significant positive correlation above $r = 39$ between skin pentosidine and age, so I cannot exclude the possibility of existence of weaker (but still biologically meaningful) association between these traits.

3.2. No evidence for OS affecting somatic senescence (II)

Free radical theory of aging (Harman 1956) suggests that senescence is caused by the accumulation of damage caused by free radicals, whether by direct damage to biomolecules by reactive oxygen species (ROS) (Kirkwood and Kowald 2012) or through membrane fatty acids susceptibility to ROS attack and subsequent lipoxidation of important macromolecules (Galvan et al. 2015). The free radicals responsible for aging are mainly produced in the mitochondria

during ATP production. Those radicals are usually produced in minuscule amounts, but production may increase in damaged or aged mitochondria (Buffenstein et al. 2008). Since this effect is often cumulative, an organism might get more susceptible to oxidative damage with advancing age. Though there is no shortage of studies relating OS to senescence, they have mostly been conducted on short-lived laboratory organisms (Costantini et al. 2010) and only recently have natural populations been included (Nussey et al. 2009, Bize et al. 2014). Mostly those studies focus on a few markers of oxidative stress or antioxidant defence, assuming that they provide adequate information about OS (Monaghan et al. 2009). However, to measure OS accurately, one must utilize a multitude of biomarkers (Hõrak and Cohen 2010, Speakman et al. 2015).

I aimed to test longitudinally, whether senescence increases susceptibility to OS using several markers of oxidative damage and antioxidant protection. Antioxidants measured over three year period included plasma uric acid, carotenoids, total antioxidant capacity (TAC), and erythrocyte glutathione (GSH). From oxidative damage markers, lipid peroxidation (LPO) was measured. Overall nutritional condition was assessed by measuring plasma protein and triglycerides concentrations.

The results did not indicate any senescence-related increase or decrease in any of the measured markers. However, erythrocyte GSH concentration predicted the longevity of female gulls, with birds having lower concentrations living longer. None of the other markers predicted lifespan. Age-related decline in markers of reproductive success (laying date and clutch mass) was still observed.

Since none of the measured markers of oxidative stress correlated with age, the results do not support the idea of OS being the key mechanism inducing senescence in common gulls (as was also evident in **paper I**). These results also do not support the notion of an age-related decline in resistance to OS reported in some studies (Gil et al. 2006, Devevey et al. 2010). Indeed more and more results suggest, that the relationship between aging, lifespan, and OS might not be as clear-cut as previously hypothesised (reviewed by Buffenstein et al. 2008, Speakman and Selman 2011). Alternatively, it is possible, that since organismal senescence is asynchronous among traits (Nussey et al. 2013), mechanisms responsible for maintaining redox balance have negligible senescence in long-lived species like the common gull, for whom there already is some support for this notion (**paper I**).

GSH was the only marker that showed any associations with lifespan, with females having higher GSH levels also having greater mortality risk. Since GSH is often considered the main intracellular antioxidant (Galván and Alonso-Alvarez 2008), one should expect that in accordance with the free radical theory of aging, the results would be opposite. However, elevated GSH levels might indicate a compensatory upregulation of antioxidant defences in response to a past oxidative insult. Indeed a study on greenfinches indicated, that induction of severe OS by administration of paraquat elevated the subjects' GSH levels (Meitern et al. 2013). The results do provide limited support for OS relating to longevity. However, caution must be taken in interpreting the results, since only

one antioxidant form a single tissue showed any mortality related variation. To fully prove or dismiss OS as a determinant of aging and a shaper of life history, there is a need to encompass more definitive OS markers and tissue types, since oxidative damage in different tissues may not correlate (Yang et al. 2013). Hence there is a chance of oxidative damage in other tissues, some of which may be more vital for organismal functioning, than the one measured. Finally as discussed earlier, long-lived organisms could have "private" mechanisms enabling them to negate the effects of OS on senescence altogether.

3.3. Senescence of ornamental traits in a long-lived species (III)

Since Darwin (1871) first refined his views on sexual selection, the function of ornamental traits has been amongst the most thoroughly studied topics in behavioural ecology. According to the Zahavian handicap principle (Zahavi 1975), those ornaments should be costly to the bearer and therefore directly linked to organismal fitness. However, there is little theoretical consensus under which conditions ornamental traits should correlate with fitness (Kokko 1997, Hoglund and Sheldon 1998, Getty 2006, Ercit and Gwynne 2015). Furthermore, not much is known about associations between colour-based signals and lifespan, a notable fitness component. Studies in short-lived bird species have indicated either positive (Hõrak and Männiste 2016), negative (Moore et al. 2015) or stabilising (Gregoire et al. 2004) survival selection on ornaments. Even less is known about viability selection on ornamentation in long-lived monogamous bird species, for which lifespan is often the most important determinant of lifetime reproductive success (Rattiste 2004). Moreover, there is no consensus, on whether an individual should invest more into ornamentation with advancing age as suggested by classical life-history theory (Williams 1966), or the investment into ornaments should depend upon an organisms current and future condition as predicted by the condition-based approach to life-history theory (McNamara et al. 2009).

Wingtip patterns of several gull species are highly variable, age-dependent and sometimes sexually dimorphic (Coulson et al. 1982, Allaine and Lebreton 1990) as expected from sexually selected traits (Andersson 1994). I intended to test on the common gull, whether the size of the white wing patches is sexually dimorphic, changes and covaries with age and predicts lifespan. I also tested, if those wing patches were costly to the bearer by determining if the size of the white wing patch correlated with wingtip abrasion. White wing patch area and black wingtip size (a utilitarian trait not expected to be under sexual selection) were measured from photographs from a sample of 446 birds caught in either 1997 or 2007. 35 of the birds caught in 1997 were also in the 2007 sub-sample, allowing for a semi-longitudinal approach. Wingtip abrasion was assessed from the same photographs using a five-unit scale and pooling the assessments of four independent evaluators.

Females had smaller wing patches than males ($t=-6.2$, $p<0.0001$), this together with a relatively high variability (CV=0.18 in males and 0.23 in females) in the trait suggests that those patches are indeed sexually selected for (Andersson 1994). Size of the white wing patches was also individually highly repeatable (females, $F_{18,19}=17.7$, $R=0.89$; males, $F_{15,16}=11.4$, $R=0.84$) over the ten year period, indicating, that the ornament can reliably demonstrate long-term fitness. Furthermore, since females with smaller white wing patches had more abraded wings ($r_s=-0.21$, $p=0.028$), it also appears to be a Zahavian handicap, at least for females. That notion is further supported by the fact, that irrespective of sex, white wing patch size positively predicted survival for both sexes, while the absolute and relative size of black wing tips was negatively associated with survival. Since black wing-tip area also correlated negatively with white wing patch size, they may have evolved as reverse components of a singular trait. Most of the associations between ornaments and fitness were similar between the sexes, so it is feasible, that as a long-lived monogamous species, the common gull practises mutual mate choice.

In a cross-sectional dataset white wing patch size correlated with age in a concave manner for both males and females. This finding is in accordance with previous studies finding a similar concave relationship between age and breeding success in the colony (Rattiste 2004, **paper II**), suggesting a diminishing investment into traits related to reproduction with advancing age. This is further supported by the fact that females measured twice over a ten-year period showed an individual decrease in wing patch size except for the birds first measured at a younger age. No such relationship was found in males. So the observed concave relationship could be caused either by a selective disappearance of senescent individuals with large wing patches as predicted by classical life-history theory or a within-individual decrease with age in accordance with the condition-based life-history theory, with the latter having more support. Altogether, the results indicate, that white wing patches in conjunction with black wing tips do serve as an ornament indicating Zahavian fitness in the common gull. Since the size of these ornaments is age-dependent, it seems that the common gull shows diminishing investment into reproduction, instead opting to maximize its remaining life expectancy.

3.4. Uropygial gland size as an age-dependent quality indicator (IV)

Studies of senescence patterns in the wild have mostly concentrated on traits most proximate to fitness (e.g. survival and fecundity). Although efforts to study senescence in other phenotypic traits (e.g. body mass, secondary sexual characters, parental investment) and relevant physiological processes (e.g. endocrine function, sarcopenia, oxidative stress, telomere length) are rapidly rising (Nussey et al. 2013), traits related to self-maintenance remain understudied in the context of aging.

Uropygial or preen gland is a holocrine gland, exclusive to birds, located in the integument the posterior free caudal vertebrae. It secretes an oily substance that has been hypothesised to have several nonexclusive functions including plumage maintenance (Giraudeau et al. 2010), water repellence (Moreno-Rueda 2017) and defence against bacteria (Shawkey et al. 2003), ectoparasites (Moreno-Rueda 2010) and fungi (Jacob et al. 1997). Uropygial gland size has been shown to positively correlate with residual body mass and immune function and is therefore related to bird health (Moreno-Rueda 2010). Since preen oil also has water repellent properties, maintaining gland functioning would be especially crucial for a seabird such as the common gull.

Given that preen oil has numerous functions, it is reasonable to assume that maintaining uropygial gland function would have major fitness benefits for an individual. However to my best knowledge, there are no studies investigating the senescence of uropygial gland as a mechanism of self-maintenance. I measured, in a cross-sectional manner, preen gland size, a trait closely related to preen gland functioning (Martin-Vivaldi et al. 2009), of male and female birds aged from 2 to 28 years. The purpose of this study was to test, if there is an association between uropygial gland size and age, if it correlates with other condition-dependant traits (the extent of wing tip abrasion and breeding onset) or feather ornaments and if any of those links are expressed in a sex-specific manner.

As previously established in the studied population (Rattiste 2004, **paper II**, **paper III**), laying date and the size of white wing patches showed a concave relationship with bird age for both sexes. Uropygial gland increased with advancing age for both males and females. Since the study was conducted in a cross-sectional manner, the possibility of selective disappearance of birds with smaller glands cannot be ruled out. Nevertheless the observed pattern is consistent with the idea of common gulls investing more into somatic maintenance than into reproduction in old age, regardless of their sex.

The study failed to detect any sex-specific relationships between uropygial gland size, wing ornamentation, age, and laying date. This is in contrast with numerous examples in literature indicating differences in ageing rates between the sexes and asynchrony among phenotypic traits within sexes in the way they change with age in later life (reviewed by Nussey et al., 2013). As well as previous studies revealing sex-specific temporal patterns in preen gland size, with females reaching a maximum gland size later in the breeding season than males (Golüke and Caspers, 2017, but see also Pap et al. 2010 for the contrary). An explanation for this lack of sex-specific aging patterns in the species could be that although natural selection usually favours a ‘live fast, die young’ strategy for males, numerous exceptions to this rule still exist (Bonduriansky et al. 2008). One of those exceptions could be the common gull, for whom mutual mate choice exists.

Altogether these results suggest, that common gulls with larger glands will reach an older age and start breeding earlier in the season, while reproductive senescence is still evident in older age classes. This supports previous findings in the colony showing an increased investment into soma and a decreased reproductive investment in older age classes (**paper I**, **paper II**, **paper III**).

3.5. Age-specific maternal investment in reproduction through egg quality (V)

From previous studies of the population it has become apparent, that as a long-lived species, the common gull tends to prioritize self-maintenance (**paper I**, **paper II**, **paper IV**) over traits associated to reproduction (Rattiste 2004, **paper III**, **paper IV**). This notion is in accordance with life-history theory, predicting, that reproductive investment in a given event correlates negatively with the expected lifespan of an individual (Williams 1966), further exemplified by empirical evidence from long-lived species showing larger variation in reproductive investment, than adult survival (Hamel et al. 2010, Griesser et al. 2017). Although a decline in reproductive success (Rattiste 2004) and -effort (Griesser et al. 2017) with age has been well-documented in long-lived species, it is unclear, how maternal allocation of resources to offspring in the embryonal stage is affected by senescence. Long-lived birds are the perfect organisms to study this problem, for their reproductive senescence has been proven to be more rapid than somatic senescence (**papers I–III**, Holmes and Ottinger 2003) and their embryo development takes place in a sealed system, so reproductive investment in that stage can be easily measured.

To test age-specific variation in maternal resource allocation I measured in a cross-sectional manner yolk testosterone, carotenoid and vitamin A and E levels of 30 mothers aged 4–23 years. Yolk testosterone content has been shown to positively correlate with offspring growth rate (Pilz et al. 2004) although that positive effect is counterbalanced by a reduction in immune response (Groothuis et al. 2005). Carotenoids are a diverse group of fat-soluble pigments that influence antioxidant status and immunity (Johnson-Dahl et al. 2017) and have also been shown to positively predict chick growth (Saino et al. 2008). The methodology used for carotenoid measurements enabled distinction between different types of carotenoids, so a potential reduction in one carotenoid type was not eclipsed by compensatory upregulation of other carotenoids. Carotenoids and testosterone are suggested to be part of a compensatory mechanism, where the rise of OS by testosterone is neutralised by carotenoids (Giraudeau et al. 2017). Vitamins A and E have also been shown to promote offspring growth across species (Deeming and Pike 2013). It is reasonable to expect, that yolk antioxidant and androgen levels should correlate with maternal age in a concave manner, with middle-aged birds investing the most into offspring, in accordance with previous studies of the colony (Rattiste 2004, **paper II**).

From the eight carotenoids identified in the study, only one, lutein, was affected by maternal age, with eggs from middle-aged mothers having higher lutein content, than the eggs of young or old mothers. This is in line with the theoretical framework (Williams 1966) and various studies, including our own study system, showing a reduced reproductive investment with advancing age (Rattiste 2004, **paper II**, Lewis et al. 2006, Elliott et al. 2014). Yolk vitamin A and E levels showed a similar, though marginally nonsignificant, concave

relationship with age. None of the other dietary antioxidants nor yolk testosterone however correlated with age.

For lutein deposition into egg yolks to be age-dependent, lutein must have an important function in adults and traded off against deposition to the yolk and/or be differently available for birds of different ages. Those two hypotheses are not mutually exclusive and provide a possible link between maternal age and chick quality. Lutein has been shown to be important for both human (Eisenhauer et al. 2017) and possibly avian retinal health (Toomey et al. 2010). Furthermore, lutein is also positively associated with chick growth (Saino et al. 2008) and survival (Romano et al. 2008) in yellow-legged gulls. However for that species and some other gull species, lutein is also the most abundant carotenoid in egg yolk (Blount et al. 2002, Saino et al. 2008), which is not the case for the common gull. Canthaxanthin was the most abundant yolk carotenoid in our study. It is easily absorbed from the diet and effectively deposited into eggs (Surai 2012), while other carotenoid types, like lutein, might be more limiting. So the observed difference in carotenoid and vitamin concentrations between age classes might be due to greater foraging ability, more efficient processing or different allocation of carotenoids and vitamins in middle-aged birds.

Yolk testosterone content did not show any covariation with age. It has been previously suggested, that yolk testosterone and antioxidants are co-regulated to limit the potential effect of prenatal testosterone on OS (Giraudeau and Ducatez 2016). Therefore, it is intriguing to hypothesise, that lower reproductive success of younger and older birds arises from their inability to combat a testosterone-mediated rise in OS in their eggs with sufficient carotenoid deposition.

In conclusion, as predicted by the life-history theory (McNamara et al. 2009) there is an age-associated reduction in maternal investment into embryos in the common gull, expressed through differences in yolk lutein content and therefore potentially undetectable by only measuring overall carotenoid content.

CONCLUSIONS

Neither erythrocyte telomere length nor skin pentosidine concentration correlated with age (**paper I**) nor did any of the multitude of oxidative state and nutrition markers show a senescent decline (**paper II**), while uropygial gland, an important defence mechanism against ectoparasites, fungi and feather degrading bacteria, actually increased with age in a cross sectional sample (**paper IV**). Hence, it is probable that as a long-lived bird species, the common gull has "private" mechanisms of combating physiological deterioration with age, as previously suggested (Holmes and Martin 2009). Determining these mechanisms could be a fruitful path for future studies. The main conclusions of the thesis are visualised on Figure 2.

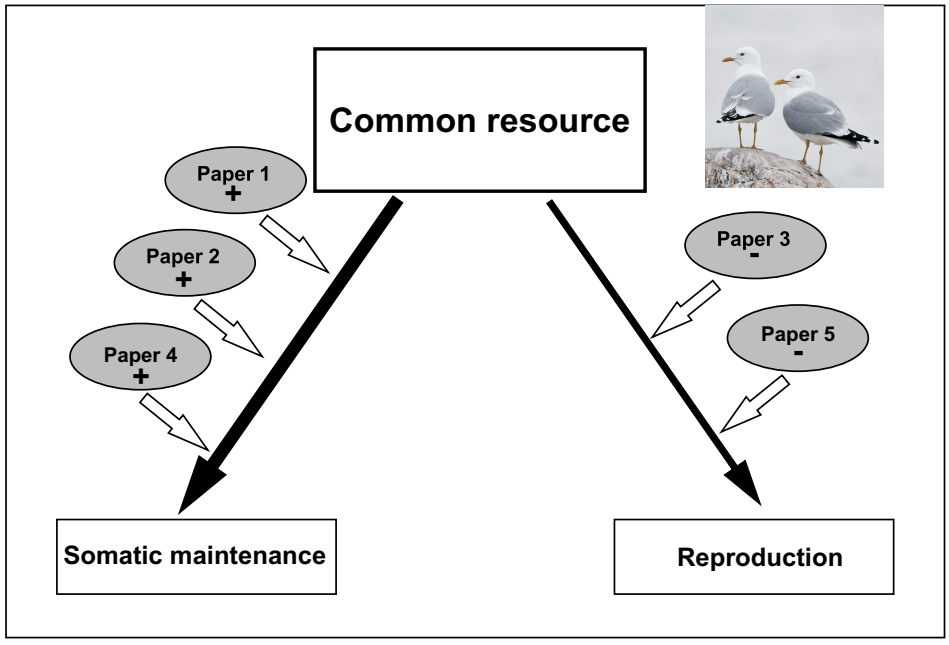

Figure 2. The main conclusions of the thesis visualised. Black arrow sizes indicate the amount of resource allocated for the function. Grey bubbles indicate research papers from the thesis supporting such a resource allocation, +/– in the bubbles indicate either a positive or a negative common resource allocation with advancing age. (Photo: Lauri Saks)

Somewhat paradoxically, there was a clear senescent pattern in both ornamental traits (**paper III**) and investment into progeny (**paper V**). Classical life-history theory predicts that as an organism ages, its residual reproductive value decreases, prompting in many cases an increase in reproductive investment with advancing age. This pattern could easily obscure the observed senescence in those reproductive traits. Since reproductive senescence was clear-cut however,

it offers support for the condition-dependent life-history theory (McNamara et al. 2009), suggesting that animals (especially long-lived species) regulate their reproductive investment in order to optimise their condition and with that, maximise their chances of surviving until next reproduction.

Altogether, the conducted studies indicated, that there is indeed a senescent decline in reproductive traits in the common gull (**paper III**, **paper V**), while senescence in physiological markers associated with maintaining somatic condition could not be detected (**paper I**, **paper II, paper IV**). The results are in line with life-history theory, predicting an increased investment into somatic maintenance compared to reproduction in long-lived species with low extrinsic mortality rates (Williams 1966). Another way to interpret the results would be through the cost of flying. Flying is a very costly physiological function (Wikelski et al. 2003b), and since any reduction in flying ability could be potentially fatal,there is a reason to expect that even short-lived bird species have a more pronounced reproductive than somatic senescence. Indeed, some of them even experience a post-reproductive lifespan (reviewed by Holmes et al. 2003, but see also Sanz and Moreno 2000 for the contrary). For instance, the barn swallow (*Hirundo rustica*), a short-lived species, showed a concave pattern in reproductive success (Balbontin et al. 2012), consistent with reproductive aging (Rattiste 2004). Furthermore, early reproduction had no effect on that species lifespan (Balbontin and Moller 2015), suggesting that even short-lived birds do not trade life-span for increased reproductive success.

All in all, birds and especially seabirds are intriguing model organisms for unravelling the trade-offs that shape senescence in natural populations. However the investigated senescent patterns and underlying trade-offs cannot be considered conclusive. Future studies are needed to investigate the effect senescence has on every possible facet of the common gull's physiology and reproduction. Possible future studies should include aspects like immunosenescence, longitudinal studies of telomere dynamics, the existence of possible anti-cancer mechanisms, investment into antioxidant protection on the level of gene transcription and age dependent differences in offspring quality. Only then can we fully understand the diverse trade-offs shaping that organism's senescence.

SUMMARY

There is a persistent fallacy in biology, that natural populations do not senesce. Furthermore, aging research has traditionally been focused on short-lived laboratory organisms, which have different life-histories and underlying physiological mechanisms, than long-lived ones. Although more and more aging research is being conducted on long-lived natural animal populations, there is still a lack of comprehensive studies analysing both physiological and reproductive senescence in the same system in order to reveal the potential trade-offs shaping senescence in long-lived species. The present thesis aimed to assess the different aspects of senescence in a long-lived seabird and to reveal the potential trade-offs leading to senescence in a long-lived wild bird species.

Firstly, in order to determine if there is physiological senescence in the study system, I aimed to validate two commonly used physiological aging markers (erythrocyte telomere length and skin pentosidine concentration) on male common gulls of known ages. Since both of those markers are also potentially indicative of oxidative damage, the same setup allowed to test if oxidative stress (OS) affected senescence. Since neither erythrocyte telomere length nor skin pentosidine concentration correlated with chronological age of the birds, it would seem, that physiological senescence might be negligible in the study system. Of course, since it was a cross-sectional study, the senescent effect might have been eclipsed by the cohort effect. Since skin pentosidine concentration and erythrocyte telomere length also did not correlate with each other, it is unlikely, that there is a common physiological factor, such as OS, affecting senescence rates in the population.

Aging has long been functionally linked to OS. However most early studies suffered from methodological difficulties. I aimed to investigate if OS caused senescence and if older individuals were more prone to oxidative damage, measuring multiple markers of oxidative damage, antioxidant defence and nutritional state in a three year period. Since none of the measured markers correlated with age, it would seem that aging does not make an organism more prone to OS, at least in the case of the common gull. It is also possible, that the mechanisms responsible for maintaining redox balance in long-lived species like the common gull do not senesce. This is further supported by the fact, that in the same system neither erythrocyte telomere length nor skin pentosidine concentration were affected by age.

Ornamental traits are among the most thoroughly studied subjects in animal ecology. However, there is no consensus on how they should indicate individual quality as an individual senesces. To address that problem, I measured white wingtip patterns of both male and female common gulls and investigated, if they were sexually dimorphic and prone to senescence. As the size of the white wing patches showed a concave relationship with age for both male and female gulls, with middle-aged birds having the largest patches, it would seem that common gulls invest less into reproduction with advancing age.

Aging research in the wild has traditionally been focused on traits most proximate to fitness, like survival or fecundity, while research of aging in self-maintenance mechanisms is lagging behind. I measured in a cross-sectional manner preen glands of differently aged male and female common gulls to see if this trait, closely related to self-maintenance was prone to senescence and if these patterns differed between sexes. As expected from previous results from the same study system, there was no senescent decline in uropygial gland size, a trait associated with somatic maintenance, on the contrary, preen gland seemed to increase with age in both sexes. This offers further support for the notion that common gulls as long-lived species invest more into soma with advancing age.

To further investigate, how reproductive effort depended upon age, I measured yolk testosterone and carotenoid content from eggs of differently aged mothers. Both testosterone and carotenoids have previously been shown to positively affect offspring quality. From the measured carotenoids lutein showed a concave relationship with age, suggesting a reduced reproductive effort with advancing age. Yolk testosterone content was not affected by maternal age. Since testosterone could be immunosuppressive and raise OS levels, senescent mothers could jeopardize offspring quality by limiting yolk carotenoids, known *in vitro* antioxidants.

In conclusion it seems that as a long-lived species, the common gull invests more into somatic maintenance than into reproductive effort, as senescence progresses. This conclusion is supported by life-history theory, suggesting increased somatic investment from species with low extrinsic mortality such as seabirds. However to conclusively prove the existence of the perceived pattern, every aspect of that organism's senescence should be investigated with future studies concentrating on aspects like immunosenescence, longitudinal studies of telomere dynamics, the existence of possible anti-cancer mechanisms, investment into antioxidant protection on the level of gene transcription and age dependent differences in offspring quality.

SUMMARY IN ESTONIAN

Pikaealise merelinnu vananemise mitmetahulisus

Bioloogias on levinud väärarusaam, et looduslikes populatsioonides vananemist ei toimu. Seepärast on vananemise bioloogilised uuringud olnud traditsiooniliselt keskendunud lühiealistele laboriorganismidele. Sellistel mudelorganismidel on aga pikaealistest liikidest erinevad elukäigud ja neid elukäike iseloomustavad füsioloogilised lõivsuhted. Kuigi aina enam on hakatud läbi viima ka uuringuid pikaealistel looduslikel loomaasurkondadel, on siiski vähe uuringuid, mis käsitleksid nii füsioloogilist kui reproduktiivset vananemist samas uurimissüsteemis, avaldamaks potentsiaalseid lõivsuhteid mis pikaealiste liikide vananemise mustreid kujundab. Käesoleva doktoritöö eesmärgiks oli hinnata pikaealise merelinnu – kalakajaka (Larus canus) vananemise erinevaid aspekte ning avastada potentsiaalseid lõivsuhteid, mis pikaealiste liikide vananemist vormivad.

Esiteks mõõtsin ma teada oleva vanusega isastel kalakajakatel kahte laialdaselt kasutatavat füsioloogilist vananemismarkerit (erütrotsüütide telomeeripikkust ja naha pentosidiini sisaldust), et teada saada, kas uuritavas asurkonnas esineb füsioloogilist vananemist. Kuna mõlemad markeritest on ka tundlikud oksüdatiivsele stressile (OS), võimaldas see uurimus ka kindlaks teha, kas vananemine on oksüdatiive stressiga seotud. Kuna ei erütrotsüütide telomeeripikkus ega ka naha pentosidiini sisaldus ei korreleerunud vanusega, võib järeldada, et käesolevas uurimissüsteemis füsioloogilist vananemist tõenäoliselt ei eksisteeri. Kuigi, kuna tegemist oli läbilõikelise uuringuga, võis vanuse mõju markeritele olla varjatud ka kohordi efekti poolt. Samas, kuna naha pentosidiini kontsentratsioon ei korreleerunud ka erütrotsüütide telomeeripikkusega, on ebatõenäoline, et need markerid on mõjutatud ühise füsioloogilise faktori, nagu oksüdatiivse stressi, poolt.

Biogerontoloogilistes uurimistöödes on vananemist traditsiooniliselt seostatud oksüdatiivse stressiga (OS). Enamus uuringuid selles vallas on aga kannatanud metodoloogiliste raskuste all. Püüdsin selgitada, kas vananemine on seotud OS-iga ning, kas vanemad indiviidid on oksüdatiivsete kahjustuste suhtes haavatavamad, mõõtes kolmel järjestikkusel aastal mitmeid oksüdatiivsete kahjustuste, antioksüdantkaitse ja toitumusliku seisundi markereid. Kuna ükski mõõdetud markeritest ei korreleerunud lindude kronoloogilise vanusega, näib, et vananemine ei muuda kalakajakaid OS-ile vastuvõtlikumaks. On ka võimalik, et kalakajakataoliste pikaealiste liikide redokstasakaalu eest vastutavad mehhanismid ei vanane. Seda järeldust toetab ka seik, et eelnevas uuringus ei olnud sama liigi erütrotsüütide telomeeripikkus ning naha pentosidiini sisaldus samuti linnu vanusega seotud.

Sulestikuornamendid on ühed enim uuritud nähtustest loomaökoloogias. Samas pole üksmeelt, kuidas sellised ornamendid peaksid indiviidi vananedes tema kvaliteediga seotud olema. Selle probleemi uurimiseks mõõtsin ma nii emaste kui isaste kalakajakate tiivalaike ning vaatasin, kuidas nende suurus

linnu soost ja vanusest sõltub. Nii emaste kui isaste kalakajakate puhul oli märgatav mittelineaarne seos tiivalaigu suuruse ning linnu vanuse vahel, nii, et keskealistel lindudel olid suurimad tiivalaigud. Seega tundub, et kalakajakad investeerivad vanuse kasvades vähem ornamentidesse.

Vananemise uuringud looduslikes asurkondades on tavaliselt keskendunud otseselt kohasusega seotud tunnustele nagu elumus või sigimine ning teenimatult vähe on pööratud tähelepanu enesehooldusmehhanismide vananemisele. Vähe tähelepanu on pälvinud ka soolised erinevused selliste tunnuste vananemises. Mõõtsin läbilõikeliselt erinevas vanuses emaste ja isaste kalakajakate päranipunääret, tunnust, mis on seotud enesehooldusega, ning uurisin, kas see tunnus vananeb ning kas see vananemine sooti erineb. Kooskõlas eelnevate sama asurkonna uuringutega ei vähenenud päranipunäärme suurus vanuse kasvades, vaid kasvas mõlema soo puhul vanusega lineaarselt. See pakub kinnitust hüpoteesile, et pikaealise liigina investeerib kalakajakas vanuse kasvades rohkem ressursse tervisesäilitamisfunktsioonide toetamisse.

Mõistmaks, kuidas sigimispingutus vanusest sõltub, mõõtsin ma erinevates vanustes emaste lindude munarebu testosterooni ja karotenoidide tasemeid. Nii testosterooni kui karotenoidide puhul on näidatud nende positiivset mõju linnupoegade kvaliteedile, seega peaksid individuaalsed erinevused nende ainete rebusse paigutamises peegeldama erinevusi sigimispingutuses. Mõõdetud karotenoididest vähenes vanusega luteiini tase munades, mis annab tunnistust vanusega vähenenud sigimispingutusest ja/või võimest vastavaid karotenoide munadesse deponeerida. Rebu testosterooni tase aga ei sõltunud vanusest. Kuna testosteroon võib olla immunnsupressiivne ning tõsta ka oksüdatiivse stressi taset ning kuna karotenoididel on *in vitro* antioksüdatiivseid omadusi, on võimalik, et vanemad emad võivad rebu karotenoide vähendades oma järglased oksüdatiivsele stressile haavatavamaks muuta.

Kokkuvõttes tundub, et kalakajakas investeerib pikaealise liigina vanuse kasvades rohkem keha eest hoolitsemisesse kui sigimispingutusse. Selline järeldus on ka kooskõlas elukäigu teooriaga, mis soosib kalakajakataolisel pikaealistel vähese välise suremusega liikidel ressursside eelistatud investeerimist elus püsimisse (võrreldes sigimisinvesteeringutega igal konkreetsel sigimiskorral). Vaadeldud lõivsuhete lõplikuks tõestamiseks tuleb aga uurida kalakajaka vananemise igat aspekti. Tulevased uurimused võiksid seega keskenduda sellistele vananemise tahkudele nagu immuunsüsteemi vananemine, telomeeridünaamika, vähivastaste mehhanismide olemasolu ja vananemine, antioksüdantkaitse geenitraskriptsiooni tasemel ning vanusest tingitud erinevused järglaste kvaliteedis.

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Conference presentations:

Urvik, J; "Live slow, die old", J Monod Conference "Comparative biology of aging", Roscoff, France, October 2015, Oral presentation

Urvik, J; Rattiste, K; Klandorf, H; Sepp, T; Asghar, M; Hasselquist, D; Cooey, M; Hõrak, P; "Telomeres and aging in a long-lived seabird" International conference on Understanding Diversity in Telomere Dynamics, Edinburgh, Scotland, November 2016, Oral presentation

Urvik, J; Sepp, T; Giraudeau, M; Noreikene, K; Rattiste, K; Meitern, R; Hõrak, P; "Do younger mothers produce healthier offspring? Associations between maternal age and offspring telomere loss in a long lived seabird" International conference on Understanding Diversity in Telomere Dynamics, Edinburgh, Scotland, November 2017, Oral presentation

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