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Telomere dynamics and migration patterns in a long-distance migrant, the Arctic skua

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MASTER THESIS

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PREFACE

This Master's thesis was written at the Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim, in collaboration with the Norwegian Institute of Nature Research (NINA) and the BirdMap project. I have been supervised by Claus Bech, Institute of Biology, and by Børge Moe and Sveinn Are Hanssen (NINA). I participated in the field season of 2016 (June-July) in Kongsfjorden, Svalbard, and conducted the telomere analyses at Centre d'Etudes Biologiques de Chizé (CEBC), France, in October-November 2016. I received funding from the Artic Field Grant of the Norwegian Research Council for the field work, and the telomere analyses were funded by King Haakon VII's Education fun and Sparebanken Midt-Norges Gift Fund for students at NTNU. Permissions to conduct the field work and sampling were granted by the Governor of Svalbard (Sysselmannen) and the Norwegian Food Safety Authority (Mattilsynet).

I wish to thank my main supervisor Claus Bech for always showing me new perspectives, patience, and insights into how the world works. You have given me more feedback and help throughout these two years than one could ask for, and our conversations and meetings have always been joyful, inspiring and motivating. Børge Moe, thank you for your tremendous help with the statistics and interpretation of my results. Thank you both for believing in me. Without Børge and Sveinn Are, this thesis could not have been written and I thank you for letting me participate in the BirdMap project. I also wish to thank you for an incredible field work experience, together with Eline Rypdal we made a great team handling the angry birds of the Arctic. I thank Frederic Angeliér for his thorough guidance through the lab work and scientific discussions, and to the people at CEBC for making my stay enjoyable. Thanks to Rob van Bemmelen for the GLS analyses, and to Bernt Rønning and Bart Peeters who helped me work out the "regression to the mean"-issue. I am forever grateful to my friends and family for their much appreciated love and support throughout these two years. Finally, to Erlend Søby, thank you for keeping my motivation high.

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Liv Monica Trondrud

SAMMENDRAG

Telomerer er beskyttende deler på enden av kromosomer som forkortes både under celledeling og av oksidativt stress. Hos langtlevende fugler og pattedyr forkortes telomererene saktere enn hos kortlevde arter og innad en art vil individer med lavest forkortningsrate leve lengst. Fysiologiske belastninger som reproduksjon, furasjering og migrasjon har alle vist å ha en sammenheng med telomerendring. Studier i nyere tid har vist at forskjellige deler av migrasjon, herunder overvintringsområder, tidsbruk og frekvens kan også spille en viktig rolle for telomerdynamikk. I dette studiet ble forholdet mellom telomerdynamikk og individuelle, konsekvente, migrasjonsmønstre til en langtlevende sjøfugl, tyvjoen, studert. Voksne individier ble fanget og tatt prøver av i løpet av hekkesesongen og deres migrasjonsmønstre ble sporet ved hjelp av lysloggere. Både varigheten av migrasjon og overvintringsperioden, hvilket område et invdivid overvintret i, samt den totale avstand flydd, ble utforsket. Det ble funnet et negativt forhold mellom den totale avstanden flydd i løpet av perioden utenfor hekkesesongen og endring i telomerer. En mulig forklaring for dette kan være økt oksidativt stress som en konsekvens av høyere energiforbruk hos fuglene som flydde lengst. Verken tidsbruk under migrasjon og overvintring eller vinterområdet som sådan var relatert til endring i telomerlengde. Tid tilbrakt i vinterområdet var derimot positivt korrelert med kroppskondisjon ved gjenfangst, som kan bety at et individs Fitness kan bedres ved å tilbringe lenger tid i vinterområdet. Et uventet, positivt forhold mellom hodestørrelse, som ble antatt å reflektere kroppstørrelse, og telomerendring ble også funnet. En mulig forklaring på dette kan være at små individer har både høyere vektspesifikk metabolisme og høyere minumum transportkostnader, som begge kan øke risikoen for oksidativt stress. Endel positive endringer i telomerlengde var også observert, men dette antas å reflektere vedlikehold fremfor forlengelse av telomerene. Den totale avstanden flydd i perioden mellom to etterfølgende år har blitt identifisert som en potensiell stressende faktor i migrasjonsmønsteret til tyvjoen. Dette studiet bidrar til å øke kunnskapen om hvilke deler av den årlige syklusen til en art som kan påvirke telomerdynamikk og således overlevelse.

SUMMARY

Telomeres are protective caps at the end of chromosomes that shorten with each cell cycle as well as by oxidative stress. In birds and mammals, telomeres shorten more slowly in long-lived species and, within species, those with a slower shortening are expected to live longer. Physiological burdens such as reproductive effort, foraging and migration patterns have all been linked to greater telomere loss in several avian species. Recent studies have shown that different aspects of migration such as overwintering habitat, timing and frequency may also play an important role in governing telomere dynamics. In the present study the relationship between telomere dynamics and individual, highly consistent, migration patterns of the Arctic skua were investigated. Adult individuals were caught and sampled during the breeding season and their migration patterns were tracked using light-level geolocation devices. The duration of migration and time spent in the wintering area, which region an individual overwintered in, and the total distances travelled, were investigated. A negative relationship between the total distances covered throughout the non-breeding season and change in telomere length was found. Increased oxidative stress as a consequence of higher overall energy expenditure is a possible explanation for this. Neither timing of migration, nor the wintering area itself, showed significant relationships with the change in telomere length. The time spent in the wintering area was positively correlated with body condition upon recapture, however, suggesting that an individuals' fitness may increase by staying in the wintering grounds for longer periods of time. An unexpected, positive relationship between skull length, considered to reflect structural body size, and change in telomere length was also found. A potential explanation for this can be higher weight-specific metabolic rate and higher minimum cost of movement of smaller individuals. Positive changes in telomere length were also observed, but is suggested to reflect high maintenance of the telomeres rather than rapid elongation. The total distances flown by an individual has been identified as a potential stressor outside of the breeding season of the Arctic skua. The present study contributes to fill the knowledge gap on which aspects of the annual cycle of a species that may impact telomere dynamics and consequently survival.

ABBREVIATIONS

ANOVA	Analysis of variance
AICc	Akaike's Information Criteria corrected for small sample size
B	Brensholmen
BC	Body condition
BM	Body mass
CA	Caribbean
CC	Canary current
CEBC	Centre d'Etudes Biologiques de Chizé
DNA	Deoxyribonucleic acid
FC	Falkland current
GG	Gulf of Guinea
GLS	Global location sensor
GPS	Global positioning system
HSD	Honest significant difference
K	Kongsfjorden
kbp	Kilo base pairs
ME	Mediterranean
RBC	Red blood cell
ROS	Reactive oxygen species
RTM	Regression to the mean
TL	Telomere length
TRF	Terminal restriction fragment

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1. INTRODUCTION

1.1 Migration and long-distance flight

Avian migration is the geographical movement which often depends on seasonal variation in resource availability and climate (Newton 2007). It is controlled by a combination of endogenic and exogenic factors (Berthold 1996), and the selection of suitable habitats for overwintering and timing of migration should be optimized to match peaks in resources and favorable environmental conditions (Alerstam and Lindström 1990). Individuals that succeed in matching these factors most often experience higher fecundity, survival and hence overall fitness (Fryxell and Sinclair 1988). In general terms, high quality habitats are considered those that provide low intra- or inter-specific competition, higher food quality or availability, and/or lower pathogen exposure and predation risk (Newton 2007; Piersma and van Gils 2010). The Arctic region provides favourable breeding habitats for a range of birds during the short but highly productive summer. Faced by the cold and resource-depleted winters, however, almost all birds that breed in this region migrate south in the non-breeding season (Johnson and Herter 1990).

While energy expenditure *per se* is a necessary cost of any physical movement, the duration and type of movement can lead to physiological challenges as the energy demands increase. Aerobic metabolism produces oxidative by-products, particularly reactive oxygen species (ROS), and exhausting exercise increases the production of these by-products (Alessio 1993). If not consumed by endogenous or exogenous anti-oxidants, ROS can attack and damage molecules within a cell, or circulate and damage tissues and molecules at other locations. Without sufficient protection and repair from oxidative damage, the physiological function of the targeted components will decrease, and accumulated oxidative damage will eventually lead to cellular senescence. Additionally, the accumulation of oxidative damage can lead to chronic oxidative stress and have detrimental effects on organ- and whole-body physiology (Finkel and Holbrook 2000). In ecology, oxidative stress can thus be considered as the physical or functional consequence of oxidative damage (Skrip and McWilliams 2016).

Long distance flight is a costly activity, and is often carried out over longer periods without concurrent energy intake. It necessitates the breakdown of energy reserves and in the most extreme situations, shrinkage of organs (Piersma 1990; Piersma et al. 1999), and long-distance migrants are inevitably faced with the need to rest, refuel and recover to avoid any long-term physiological damage (Skrip and McWilliams 2016). During long flights, lipids becomes the primary fuel source, and the breakdown of fatty acids produces more oxidative by-products than that of proteins and carbohydrates (McWilliams and Karasov 2004). Increased oxidative stress in relation to long flights and migration have, to my knowledge, only demonstrated in homing pigeons (*Columbia livia*; Costantini et al. 2008) and European robins (*Erithacus rubecula*; Jenni-Eiermann et al. 2014). Recovery from migration during stopovers may help to decrease the degree of damage exerted by oxidative by-products, either by repairing or removing damaged molecules or by increasing antioxidant production and/or consumption (Skrip et al. 2015; Skrip and McWilliams 2016). Spending sufficient time in stopovers, but also in a wintering area, should be beneficial for migratory birds as it enables the recovery from the high energy demands of reproduction and long-distance flight.

Some of the most astonishing examples of extreme migrations come from birds, such as Arctic terns (*Sterna paradisaea*), which travel the distance from the northern hemisphere to Antarctica twice a year, thus having the longest known animal migration route (Egevang et al. 2010; Salomonsen 1967). Another example is the bar-tailed godwit (*Limosa lapponica*) that can cover up to 11 000 km, undertaking the longest non-stop flights ever recorded (Gill et al. 2005). Despite the risk involved and physiological costs of long-distance flight, these migrants are most often rewarded for their effort by either breeding, overwintering, or both, in high-quality habitats that short-distance migrants might not reach (Aharon-Rotman et al. 2016; Alerstam and Lindström 1990).

While much is known about physiological burdens that seabirds face during their relatively stationary periods of breeding, less is known about burdens experienced during the migratory and wintering periods, as they are difficult to track in real-time. Advances in technology of biologging devices have expanded the possibilities for tracking animals across large scales, when they would otherwise be inaccessible to researchers. Global location sensors (GLS), which are small, lightweight devices that record time and ambient light, enable us to follow birds outside

of the breeding season where they disperse across large regions of oceans and coastlines. The bio-logging revolution has led to a substantial increase in the amount of information available on bird migration, and it is still increasing (Wilmers et al. 2015). Tracking the movements of individuals from one breeding season to the next, along with physiological measurements in each breeding season, allow for investigations of the physiological impacts of migration patterns in birds. This integration of movement biology and physiology enable the identification of stressors during the non-breeding season, and evaluate their potential long-term effects on individual quality and fitness.

1.2 Telomeres

Telomeres may both reflect individual quality and physiological stress, and the measurement of changes in telomeres is a method increasingly used also in ecology (Haussmann and Marchetto 2010; Monaghan 2010). Telomeres are non-coding deoxyribose nucleic acid (DNA) repeats on the ends of linear chromosomes, that serve as protective caps for the genetic material during replication (Blackburn and Szostak 1984). In vertebrates they consist of tens to thousands of repetitions of the base sequence $5' - \mathrm{TTAGGG} - 3'$, ending in a guanine-rich single strand that forms a loop structure ("g-strand loop") by tucking back into the double strand. This enables the cells' repair machinery to distinguish between double-stranded breaks and true chromosome ends, preventing intact chromosomes from being joined together (Blackburn and Szostak 1984).

Telomeres shorten with each cell cycle and consequently with age. This shortening is partly due to the "end replication problem", which arises as DNA polymerase is unable to replicate the lagging strand completely, which, without telomeres at the end, would shorten the daughter chromosomes instead. The g-strand loop is thus the product of the leading strand being longer than that of the lagging strand, and by this mechanism telomeres provide essential protection of the genetic material (Olovnikov 1973). Another major cause of telomere shortening is through oxidative damage, the cellular balance between ROS and antioxidant defence (von Zglinicki 2002). This is a consequence of the relatively poor repair mechanism of oxidative damage in telomeric DNA compared to that of non-telomeric DNA, and the guanine-rich sequence of telomeres being more easily targeted by ROS (Oikawa and Kawanishi 1999). In somatic cells, telomeres are usually not elongated and once they reach a critical length most cells will enter

replicative senescence (Monaghan 2010). Telomere shortening has also been shown to increase the probability of mutations occurring in the DNA (von Zglinicki 2002), and a lack of telomeric ends will leave the chromosome unstable (Blackburn 1991).

Telomere lengthening occurs primarily through the enzyme telomerase, a ribonucleoprotein that can synthesize telomeres *de novo* (Greider and Blackburn 1985). In humans, somatic cells normally do not display telomerase activity while proliferative tissues such as bone marrow, gonads and intestines, do (Forsyth et al. 2002). A lack of telomerase activity is associated with replicative senescence as the telomeres shorten, but this also prevents immortal growth of transformed cells and protects against tumor formation (Wright and Shay 2001). In birds, telomerase expression varies between different age classes within a species, generally being higher during early-life and growth, and downregulated when reaching adulthood. However, long-lived species have been shown to have higher levels of bone marrow telomerase throughout their lives compared to short-lived species, which is thought to be linked to the longevity of these species (Haussmann et al. 2004, 2007).

Telomere length (TL) and attrition rates are often greater in larger individuals, where higher loss of telomeres have been associated with growth rates during early life and high cell turnover rates. A study on fledgling house sparrows (*Passer domesticus*) found that structurally larger individuals had shorter telomeres than those of smaller size (Ringsby et al. 2015). In two long-lived seabirds, the European shag (*Phalacrocorax aristotelis*) and the wandering albatross (*Diomedea exulans*), high relative mass gain during the chick stage was associated with increased telomere loss (Hall et al. 2004). In addition to mass gain, chicks of the latter species that hatched late in the season experienced greater attrition rates than those who hatched earlier in the breeding season. While telomere attrition is often greatest during early life and become relatively stable in the adult life, the differences in telomere attrition between adult individuals are often consequences of experienced stress such as physiological burdens and environmental perturbations (Monaghan 2014). Telomere dynamics are therefore a useful tool to investigate differences between adult individuals that may experience different stressors throughout the year.

1.3 Telomeres as biomarkers of stress

Several studies have demonstrated that oxidative stress accelerates telomere loss both *in vitro* (von Zglinicki 2002), and *in vivo* (Tarry-Adkins et al. 2006). Experienced stress has been shown to accelerate telomere shortening in humans (Epel et al. 2004), and telomeres are considered as useful biomarkers of chronic oxidative stress in both humans and in free-living animals (Houben et al. 2008). While stressful events and physiological burdens can lead to oxidative stress, the consequences on telomere length will depend on the individuals' ability to maintain telomeres through protection via anti-oxidants and restoration via telomerase (Blackburn 1990). Longer telomeres at a given age has been associated with survival in tree swallows (*Tachycineta bicolor*; Haussmann et al. 2005) and higher individual quality, reflected as longevity in sand martins (*Riparia riparia*) and as lifetime reproductive success in dunlins (*Calidris alpina*; Pauliny et al. 2006). Telomere length and dynamics have shown to be better predictors of individual life expectancy than chronological age in Alpine swifts (*Apus melba*; Bize et al. 2009), Seychelles warblers (*Acrocephalus sechellensis*; Barrett et al. 2013) and Jackdaws (*Corvus monedula*; Salomons et al. 2009). Furthermore, long-lived species often have slower shortening rates than short-lived ones (Haussmann et al. 2003; Sudyka, Arct, et al. 2016). These findings have lead to the investigations of factors that may govern telomere dynamics such as reproductive effort and increased stress during breeding (Bauch et al. 2013; Beaulieu et al. 2011; Sudyka, Casasole, et al. 2016), and studies of telomere dynamics in relation to migration and other factors during the non-breeding season are increasing.

Shorter duration of migration, and increased frequency of reproduction has shown to shorten the telomeres more rapidly in in female leatherback turtles (*Dermochelys coriacea*; Plot et al. 2012). A similar result was found in black-legged kittiwakes (*Rissa tridactyla*) breeding in the Arctic, where the time spent in the wintering grounds also reduced the degree of telomere loss, and elevated stress during breeding lead to increased loss of telomeres (Schultner et al. 2014). In both of the abovementioned studies, time spent in the winter area or on migration was considered to reflect recovery from the physiological burdens of reproduction. In a study on male American redstarts (*Setophaga ruticilla*), Angelier et al. (2013) observed that those who overwintered in poor-quality habitats lost more telomeres than those overwintering in higher-quality habitats, and that individuals with longer telomeres showed higher return rates

regardless of habitat quality. The habitats in this study varied in food availability, which had previously been associated with body condition and survival of these birds (Marra and Holmes 2001). Furthermore, Young et al. (2017) recently demonstrated that neither wintering home range nor the distance from the breeding colony were related to telomere dynamics in the thick-billed murre (*Uria lomvia*), but foraging intensity in the non-breeding season strongly predicted telomere loss. This was argued to be an indicator of the foraging efficiency of individuals, and that less efficient foragers had higher physiological costs which had a negative consequence on telomeres. Finally, in a passerine species that has both migratory and sedentary subspecies, the dark-eyed junco (*Junco hyemalis*), Bauer et al. (2016) demonstrated that a migratory life-history strategy itself was associated with greater telomere attrition. The authors suggested that the differences in telomere attrition could be a consequence of higher energetic costs experienced by migratory versus non-migratory individuals.

The amount of studies that have investigated telomere dynamics in free-living animals are increasing, but very few have addressed the potential link between migration and telomere dynamics. The studies presented above are, to the best of my knowledge, so far the only ones that have investigated the effect of migration patterns on telomere dynamics in migratory birds and reptiles. They have illustrated that the link between migration stragies and telomere dynamics may be related to the life-history strategies of the species, as different aspects of the non-breeding season also have different influences on telomere dynamics. The present study will contribute to filling this knowledge gap by investigating the effect of highly individual migration patterns in a long-distance migrant, the Arctic skua (*Stercorarius parasiticus*). These birds travel from their Arctic breeding grounds to regions close to the equator every year, thereby covering great distances during migration. A strong site fidelity to both breeding grounds and wintering grounds, the latter ranging from the Caribbean to the Mediterranean, has already been demonstrated for the species (Skottene 2015). This site fidelity may be beneficial if the wintering regions are productive and competition is lower, but may also become a challenge if the productivity of the region decreases. The aim of the present study is thus to identify factors of the migration pattern, including wintering areas, that may affect the telomere dynamics in the Arctic skua during the non-breeding season. This can contribute to the understanding of what challenges these birds face throughout the year and to what degree the consequences can become detrimental.

1.4 Study species

The Arctic skua is a relatively small, migratory seabird with a life expectancy of about 12 years (Furness 1987). They have a circumpolar breeding distribution in the Arctic and sub-Arctic, and overwinter in more southernly regions relatively close to the equator. Arctic skuas are both predatory and kleptoparasitic and similarly with other birds of prey, skuas (family *Stercorariidae*) show reversed sexual dimorphism in size (Furness 1987). A study on Arctic skuas from two breeding populations in the Arctic, in Svalbard and Northern Norway, has provided substantial information about the migration strategies of this species. There is a large variation between individuals in their choice of wintering areas, and those from the same colony, even of the same pair, can overwinter on opposite sides of the Atlantic ocean as well as in the Mediterranean sea. The high consistency and strong individuality of migration strategies along with repeated sampling over two or more years, allow for the investigation of the effect of migration patterns on telomere dynamics in this migratory seabird.

1.5 Objective and predictions

The specific objective of the present study is to investigate the change in telomere length (ΔTL) in Arctic skuas from two populations breeding in the Arctic. The effect on ΔTL will be evaluated in relation to migration patterns in terms of wintering areas, the distances covered during the non-breeding season, and time spent on migration and staging as well as in the overwintering area. The following predictions are made:

I. Individuals who fly greater distances in total will most likely have higher overall energetic costs and are expected to lose more telomeres, due to overall increased oxidative damage, than those who cover shorter distances.

II. Time spent in the wintering areas is expected to excert a positive effect on ΔTL. Consequently, time spent on migration and in staging areas, will have the opposite effect.

III. Individuals overwintering in different areas of the Atlantic ocean and the Mediterranean Sea are expected to, because of their high site-fidelity, be characterized by different ΔTL rates.

2. METHODS

2.1 Capture and sampling

In the present study samples from Arctic skuas breeding in Kongsfjorden, Svalbard (78° N, 11° E) and Brensholmen, Northern Norway (69° N, 18° E) will be used. Individuals of the populations in these two areas have been sampled during the breeding seasons and tracked with GLS devices over the non-breeding season since 2009 and 2011, respectively. I participated in the field work from mid-June to mid-July 2016 in Kongsfjorden, Svalbard (figure 2.1a). During this season, only six out of the 22 Arctic skuas that were caught were recaptured individuals, with two having lost their loggers, and only one had data from the previous year which satisfied the criteria for analyses in this project. The rest of the samples were therefore from previous field seasons in Kongsfjorden dating back to 2010 (n = 19). Additional samples from the population in Brensholmen (figure 2.1b; n = 9), collected between 2011 and 2015, are included. This makes a total sample size of 29 individuals that have been captured in at least two breeding seasons. The same researchers have been leading the field work in both colonies each season, with interchanging field assistants, and the methods of capture and handling have therefore been consistent both between years and colonies.

Nesting sites were located by exploring previously registered global positioning system (GPS; Garmin) locations. New individuals and/or nests were located based on observations of unmarked birds, or the likelihood of a given area being a skua territory. Breeding adult Arctic skuas were caught either on the nest using a remote controlled nest trap, or in the air using a handheld net gun (Super Talon Net Launcher). After capture the birds were taken away from the nest, and the following biometric measurements were recorded: skull (tip of beak to back of skull; vernier caliper, $\pm$ 2 mm), tarsus (vernier caliper, $\pm$ 2 mm), left wing (metal ruler, $\pm$ 5 mm), body mass (BM; hand-held scale, $\pm$ 1 g) and inner and outer tail feathers (metal ruler, $\pm$ 5 mm). Biometric measurements that were used in the present study are presented in appendix A. By staying away from the nest during sampling the partner would be allowed to return to incubate. This reduced the time the eggs were left unprotected and simultaneously allowed for the capture attempt of the second parent with the nest trap. The birds were fitted with one of

five different types of geolocators depending on sampling year (Mk18h, weight 1.9g, Mk9 and Mk15, weight 2.5g, British Antarctic Survey, Cambridge, UK; Mk3006, weight 2.5g, Biotrack, Cambridge, UK; c250, weight 2.5g, Migrate Technology, Cambridge, UK). The total weight of the leg mount (logger, plastic ring, self-amalgating tape, cable tie and glue) amounted to less than 5 g for all types loggers, which is between 0.8 and 1.3 % (for the heaviest and lightest birds, respectively) of the total mass of the Arctic skuas in the present study. This is well within the criteria for weight of equipment mounted on birds, which should preferably be less than 5% of body mass (Hawkins 2004). In the following season the birds were located again and captured by the same procedure, the geolocators were retrieved and new ones deployed. About 2 mL of blood was taken from the brachial vein and stored at -20°C upon returning from the field each day. Before freezing, approximately 0.5 mL of blood was centrifuged (7000 rpm, 10 minutes) and plasma and red blood cells (RBCs) were separated. The RBCs were used in analyses of telomere length. A small sample was preserved in ethanol for molecular sexing which was conducted at Centre d'Etudes Biologiques de Chizé (CEBC) in France and followed the method by Fridolfsson and Ellegren (1999).

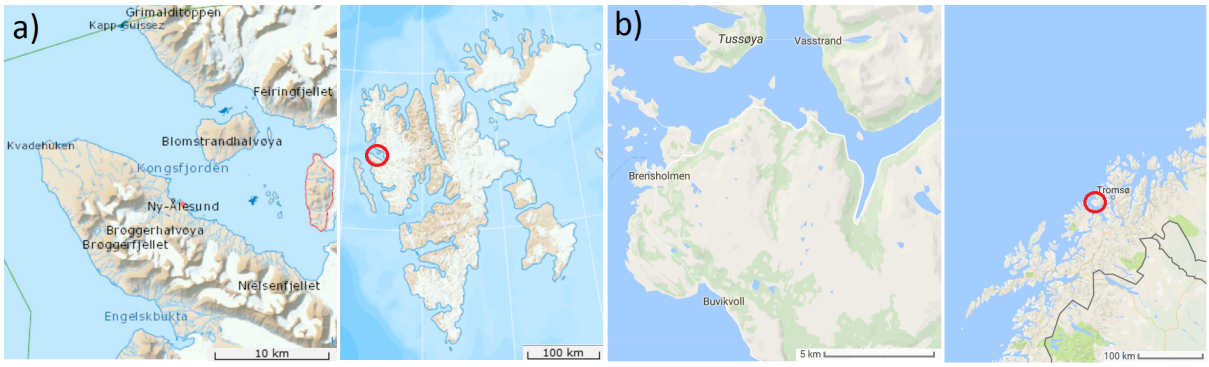

Figure 2.1: Maps of study areas: a) Kongsfjorden and Svalbard, ©Norwegian Polar Insititute, 2017. b) Brensholmen and parts of Northern Norway, Map Data ©Google, 2017.

2.2 Telomere length measurements

The length of terminal restriction fragments (TRF), the non-coding repeats on the end of the chromosome, were determined at CEBC by Southern blotting using the TeloTAGGG Telomere Length Assay kit (Roche, Mannheim, Germany) in October and November 2016. This method is considered the "golden standard" of TL measurements and is particularly useful in analyses of species that are not well studied as it does not require genetic markers or primers (Nussey et al. 2014). A detailed description of the method is presented in appendix B. In summary, TRFs are obtained in five main steps, the first being the preparatory extraction of DNA from RBCs. In contrast to mammalian erythrocytes, those of birds are nucleated (Sturkie and Griminger 1976) and thus allow for DNA extraction. Additionally, using somatic cells which have a short turnover rate, telomere dynamics that reflect physiological output are more correctly adressed than from cells with low turnover rates, such as germ cells.

The second step involved digestion of DNA with restriction enzymes that break the coding regions into smaller molecular weight fragments, while preserving telomeric and sub-telomeric DNA. The third step was separation by gel electrophoresis. After that, the DNA was denatured through southern blotting and hybridized with labeled oligonucleotides complementary to the telomeric sequence. Lastly, the TRF smears were analysed with imaging techniques and the length of the TRFs were derived by comparing to a known DNA length standard provided in the kit. All samples were run on a total of three gels. Samples from the same individual were always run on the same gel to avoid the effect of gel differences on comparing telomere lengths. Three samples from the first gel were selected based on signal strength (high, medium and low; figure 2.2) and used as controls on the subsequent gels to measure and correct for inter-gel variation, which was estimated to 4.8%.

TL was measured in two or more blood samples from the same individual, with either one year between sampling (n = 22), two years (n = 4) or during three subsequent years (n = 3). See table C1 in appendix B for a detailed overview. ΔTL was calculated simply as the difference in TL between the second and the first sampling ($\Delta\mathrm{TL} = \mathrm{TL}_2 - \mathrm{TL}_1$), and divided by 2 if there were two years between sampling times. For the four individuals with three TL measurements, mean rate of change across all years were calculated (ΔTLavg) as well.

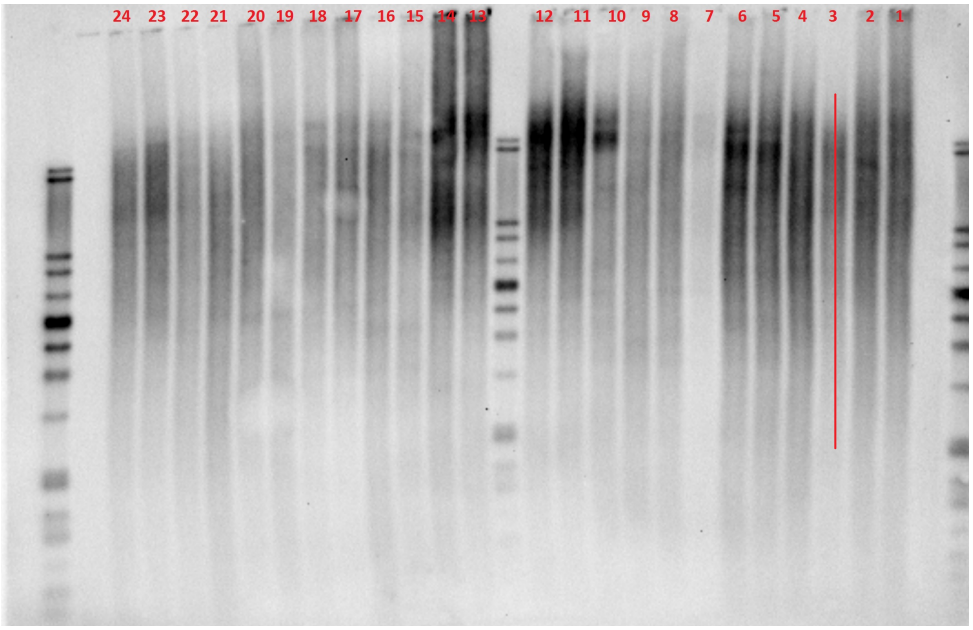

Figure 2.2: ChemiDoc image of the first gel, run on October 28th 2016. A red line is drawn to illustrate how the smear is marked in ImageJ. Samples 7, 9 and 14 were run on all three gels, representing respectively low, medium and high signal strengths and used to correct for inter-gel variations.

2.3 GLS and migration pattern analyses

Estimates of migration data from GLS loggers followed that of van Bemmelen et al. (2017) and were analyzed by van Bemmelen, University of Wageningen, The Netherlands. Briefly, the data was processed in R 3.4.0 (R Development Core Team 2017) using the GeoLight (v 2.0) package (Lisovski and Hahn 2012). Sunset and sunrise were calculated from light measurements using the function 'twilightCalc' with specific light tresholds for each logger model. Two of the recaptured individuals had lost their loggers and migration data was not available for that year, but wintering home ranges had been determined using data from previous years. Typical errors of light-based position estimates are $\pm$ 185 km, and become even greater during spring and autumn equinox (March 20th and September 22nd, respectively; Hill 1994; Phillips et al. 2004). All positions between September 4th to October 9th, and March 1st to April 6th were therefore removed, meaning that two months of the logger year are not available. Nonetheless, comparisons between the migrations patterns are still possible as this is the case for all individuals. The rest of the positions were estimated using a 3-day smoothing equation from Gilg et al. (2013); first, the two positions for each day were averaged, then those of three subsequent days were averaged. This produces a 3-day running mean with one location per day. Total distance

travelled throughout the logger year, and the maximum distance from the colony were estimated based on smoothed positions, and smoothed tracks are shown in figure 2.3.

The time spent in different regions were based on the date of first position below, or above a threshold latitude. The latitude of the breeding areas were defined as being above 65° N; crossing this latitude was considered as a movement out of, or in to, the breeding home range. Wintering home ranges were defined as being south of 35° N for CA, CC, GG and ME as shown in figure 2.4. The latitude of the Mediterranean area is somewhat above 35° N, so the date of arrival in this region was used as the date for crossing 35° N to simplify the analyses. For individuals overwintering in FC, wintering areas were defined as being south of 20° S (figure 2.4). Wintering home ranges were determined using a 75% Kernel density distribution and Lambert azimuthal equal-area projection, also by Rob van Bemmelen. This method maps a sphere to a flat disk, thereby accurately representing area in all regions of the sphere (Steinwand et al. 1995). Migration data is presented in tables D1 (dates) and D2 (time/days and distances) in appendix D. Table 2.1 shows the number of individuals from each colony in each wintering area. There was a strong correlation between wintering area and colony (r_S = -0.72, p = 0.0001), as only individuals from Kongsfjorden overwintered in the Caribbean, and only those from Brensholmen overwintered in the Falkland current region.

Table 2.1: Sample size (N) for each wintering area for the entire data set, separated by colonies: Kongsfjorden (K) and Brensholmen (B).

Abbreviation	Area	N_K	N_B
CA	Caribbean	10	
CC	Canary current	4	1
GG	Gulf of Guinea	5	1
ME	Mediterranean sea	1	1
FC	Falkland current		6
	Total	20	9

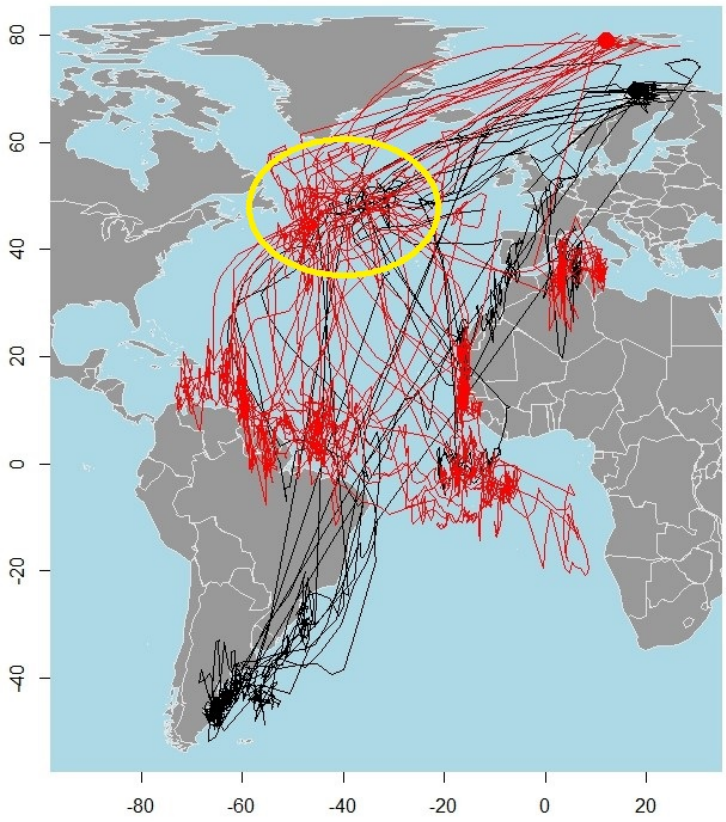

Figure 2.3: Track records of all individuals with migration data from at least one year (n = 27). Several individuals were recaptured twice, so a total of 34 tracks are shown. Red lines represents individuals breeding in Kongsfjorden and black lines those that breed in Brensholmen. The yellow circle mark a common staging area, off the Grand Banks of Newfoundland, where most birds from both colonies staged during both autumn and spring migration.

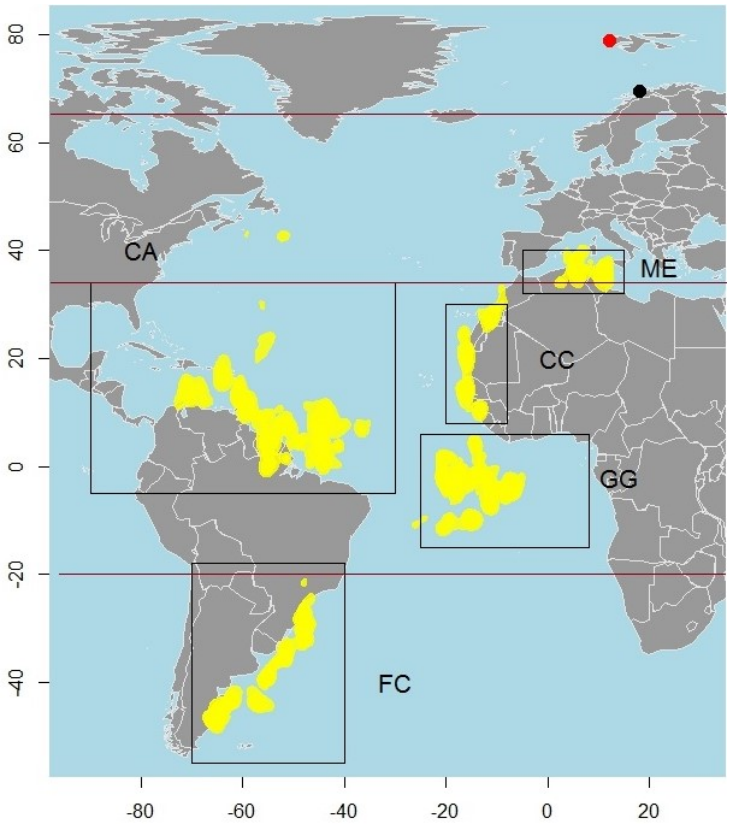

Figure 2.4: Map of the different winter areas of Arctic skuas breeding in Kongsfjorden (red circle) and Brensholmen (black circle). The x- and y-axes show degrees longitude and latitude, respectively. The yellow regions represent the 75% kernel distribution of individuals in each wintering area (n = 27) based on positions between December 1st and February 15th the following year. Red lines mark the latitudes used to separate breeding, staging/migration and wintering areas: 65° N, 35° N and 20° S. See table 2.1 for the winter area abbreviations.

2.4 Statistical analyses

All statistical analyses were conducted in R version 3.4.0 (R Development Core Team 2017). Body condition (BC) was calculated as residuals from a linear regression of BM against the length of the skull for each sex separately. All comparisons between groups were assessed with either Welch's two-sample t-test, presented with a 95% confidence interval, or *post hoc* using Tukey's honest significant difference (HSD). Linear models were created using the "lm" function in R with ΔTL as the response variable. Normality was assessed using q-q plots and the assumptions of linear regression were fulfilled. Correlations between explanatory variables used in the models were tested using Spearman's rank correlation rho (r_S) and presented in appendix E. Results were considered statistically significant if a 95% confidence interval did not overlap zero or if the p-value of a test was lower than 0.05, and approaching significance if $0.05 < \text{p} \leq 0.1$. Analysis of variance (ANOVA) was used to evaluate the difference in ΔTL between individuals of different wintering areas. For all model analyses, model selection using Akaike Information Criteria corrected for small sample sizes (AICc) were conducted with the 'MuMIn' package (Barton 2016). Models with $\Delta\text{AICc} < 2$ (difference between model AICc and the lowest AICc) were considered as best fitting (Burnham and Anderson 2002).

Information on timing, i.e. arrival and departure for any given area, were in a DD-MM-YYYY format. Time spent in a given area or during migration were calculated as the difference in days between departure and arrival, or vice versa. Eight individuals were excluded from the migration analyses, because of incomplete logger data (n = 4) or because there were two years between the telomere samples (n = 4) and thus one-year change in TL that matched with migration data was not available. Therefore, only telomere change across one year was used for evaluation of the effect of migration patterns, making a sample size of 21.

For the analyses of ΔTL and migration patterns, separate linear models were created with the following explanatory variables: total distance travelled in the period between depature from breeding grounds and return in the following year (km; hereafter total distance), days spent between 65° N and 35° N in autumn and spring, reflecting the time spent on migration as well as in staging areas (hereafter autumn/spring migration), days spent in wintering area (hereafter winter duration; CA, CC, GG and ME: south of 35° N; FC: south of 20° S). Additional models

with variables likely to affect ΔTL were included: colony, BM, skull length, BC in recapture year, sex, and change in body mass (ΔBM). To evaluate interaction effects, a model with the interaction term 'total distance travelled $\times$ body mass' was included.

For analyses of ΔTL between individuals in different wintering areas, as well as comparisons of sex and body size, an expanded data set with 29 individuals was used. Here, averaged body mass over all sampling years was used. Averaged ΔTL across two years were used when available, and ΔTL across one year if not. Using the expanded data set, the following variables were tested in separate models: wintering area, sex, colony, average BM, skull length, and averaged BC.

Change in telomere length is in most cases dependent on initial length (TL_1; Nordfjäll et al. 2009), and because of the strong relationship between TL_1 and ΔTL in the present data, ΔTL values were corrected following the procedure by Kelly and Price (2005). Linear models containing the initial length, year between samples, and an interaction term '$TL_1 \times$ sex' were analysed for both corrected and uncorrected ΔTL values. A detailed description of the correction procedure is presented in appendix F.

3. RESULTS

3.1 Telomere length and change

Telomere lengths in the Arctic skuas ranged from 9.3 to 12.5 kilo base pairs (kbp) across all sampling years. There were no differences in TL between males and females for all pooled samples (Welch' t-test, 95% conf. int.: -0.44, 0.25), but there was a significant difference between males and females in TL_1 (95% conf. int.: -0.91, -0.04). Averaged change in telomere length of each individual ranged from -0.90 to 1.39 kbp/year. Individuals with the longest and the shortest initial lengths also had the highest loss and highest increase, respectively, at the second measurement (TL_2). This means that change in telomere length was not independent of the initial length ($r_S = -0.65$, $p = 0.0002$; figure 3.2a). This strong correlation was most likely due to the statistical phenomenon known as regression to the mean (RTM) in repeated measurements; individuals that have values above the population or group mean in the first measurement will tend to have values closer to the mean in the second. Similarly, individuals with values lower than the mean in the first measurement will regress towards the mean in the second measurement (Barnett et al. 2005; Berry et al. 1984; Kelly and Price 2005). The most extreme example of this was of an individual for which four TL measurements were available: between the first and second sample there was no change in TL, and from the second to the third measurement, with two years between sampling, a negative change was apparent. From the third to the fourth TL measurement, however, telomere length increased from 9.6 to 12.5 kbp over a single year (ID #6217934; figure 3.1; table C1). The number of individuals with more than two TL measurements was too low for any statistical analyses of the annual differences in ΔTL within and between individuals, and are only presented as raw data (figure 3.1; table C1).

Following equation (6) in Kelly and Price (2005) the change in telomere length was corrected for RTM (see appendix F for further explanation). Once corrected, values for averaged ΔTL and intital TL were no longer correlated ($r_S = -0.1$, $p = 0.6$; figure 3.2b), and corrected ΔTL ranged from -0.62 to 1.14 kbp/year. The model that included TL_1 and an interaction term with sex and TL_1, with uncorrected ΔTL as the response variable, explained almost half (44 %) of

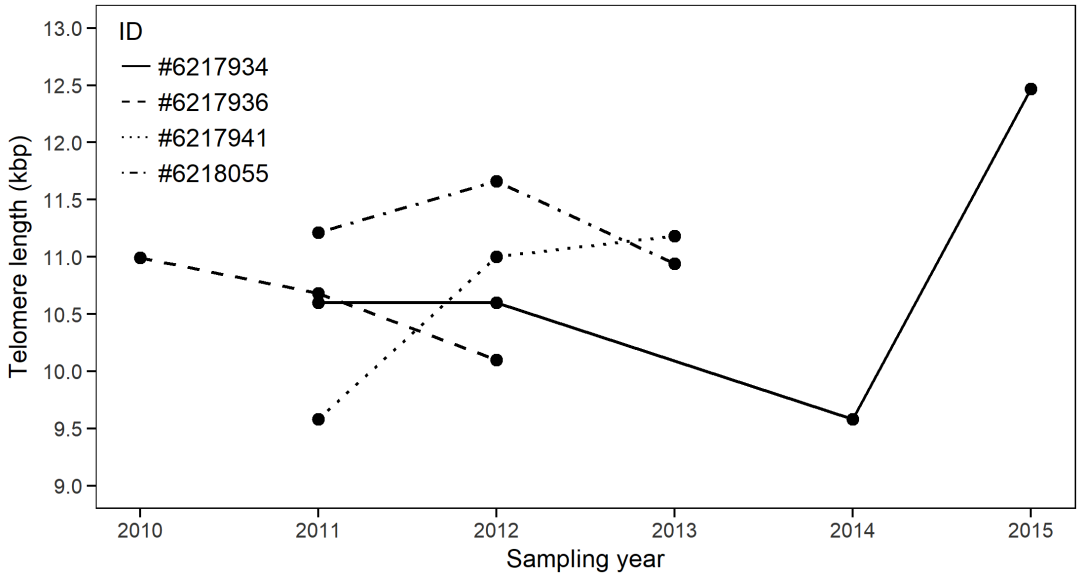

Figure 3.1: Telomere length measurements in individuals for which there where three (n = 3) or four (n = 1) TL measurements. Individual #6217936 (male) was the only one with negative ΔTL across both years. #6217941 (female) had increases in length across both years, while #6218055 (female) had opposite ΔTL rates, resulting in a seemingly small, negative change across two years. The most extreme change was observed in individual #6217934 (female), with no apparent change across the first year, steady decline across the next two years (not sampled in 2013), then a steep increase in the last year.

the variation (Adj. R^2: 0.44, p = 0.0005; figure 3.2a) in the data. After correcting for RTM, the same model only explained 7% of the variation (Adj. R^2: 0.067, p = 0.198; figure 3.2b). While the variable estimate of TL_1 was only significant for the uncorrected values, both sex and the interaction between sex and TL_1 was significant in uncorrected as well as corrected ΔTL values (table F1, appendix F). Investigating this further showed that the correlation between uncorrected ΔTL and TL_1 was actually only significant for females in the first place (r_S = -0.80, p = 0.0002), and not in males (r_S = 0.032, p = 0.9). In any case, the corrected values were used in the following analyses as they were considered to be more representative of the true change in ΔTL within an individual. From here on, references to ΔTL only refer to the RTM-corrected values.

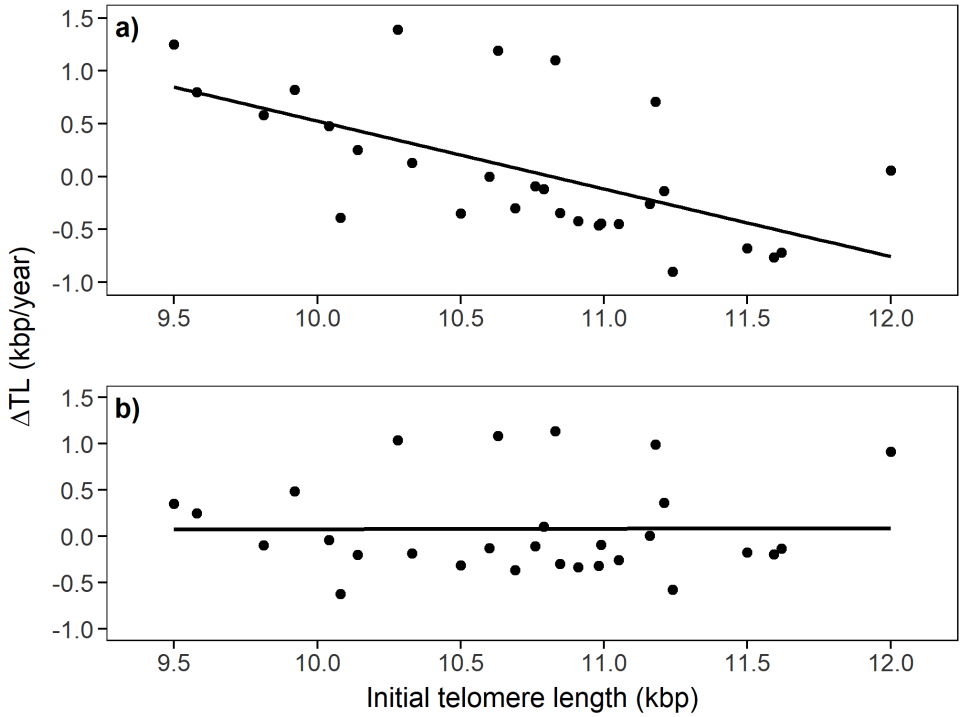

Figure 3.2: Linear regression of ΔTL on initial telomere length. (a) uncorrected values (adjusted R^2: 0.32, p = 0.0007). (b) corrected values (adjusted R^2: -0.04, p = 0.97).

3.2 Migration patterns

The effects of migration variables on ΔTL across one year (from one breeding season to the next) were analysed with linear regression models, and compared by AICc ranking. Presented here are the results with corrected ΔTL values. Three models had ΔAICc values lower than 2: the total distance travelled, skull length, and colony (table 3.1; the lower ranked models are presented in table G1 in appendix G). The total distance travelled, which was the best ranked model, showed a slightly negative but significant effect on ΔTL (figure 3.3). Individuals that flew longer throughout the non-breeding season lost more telomeres than those that flew shorter distances. There was no significant effect on ΔTL of the interaction between body mass and the distances travelled, nor were any of the models including timing variables important (table G1). There was a positive correlation with the time spent in wintering areas and body condition in the second year (r_S = 0.55, p = 0.0001), however, and the correlation between ΔBM and time spent in the wintering area was slightly significant (r_S = 0.43, p = 0.053). These correlations indicate that the longer individuals stayed in the wintering area, the higher was their body condition in

the recapure year. Furthermore, the correlation between total distance travelled and the time spent in wintering areas was almost significant (r_S = -0.37, p = 0.095), so was that with ΔBM (r_S = -0.37, p = 0.101). All correlations are shown in table E2 in the appendix.

Table 3.1: Top ranked models after selection with AICc. Presented are the models and parameters, estimates, 95% confidence intervals, ΔAICc value and weight (w) of each model.

Model	Estimate	2.5%	97.5%	ΔAICc	w
Total distance				0.0	0.28
Intercept	**2.243**	**0.269**	**4.216**		
Variable	**-4.684**$\times 10^{-5}$	**-9.096**$\times 10^{-5}$	**-2.712**$\times 10^{-5}$		
Skull length				0.8	0.19
Intercept	-9.826	-20.270	0.617		
Variable	0.128	-0.006	0.262		
Colony				1.3	0.15
Intercept	-0.188	-0.651	0.275		
Variable: K	0.492	-0.056	1.040		
Null				2.1	0.10
Intercept	0.164	-0.098	0.425		

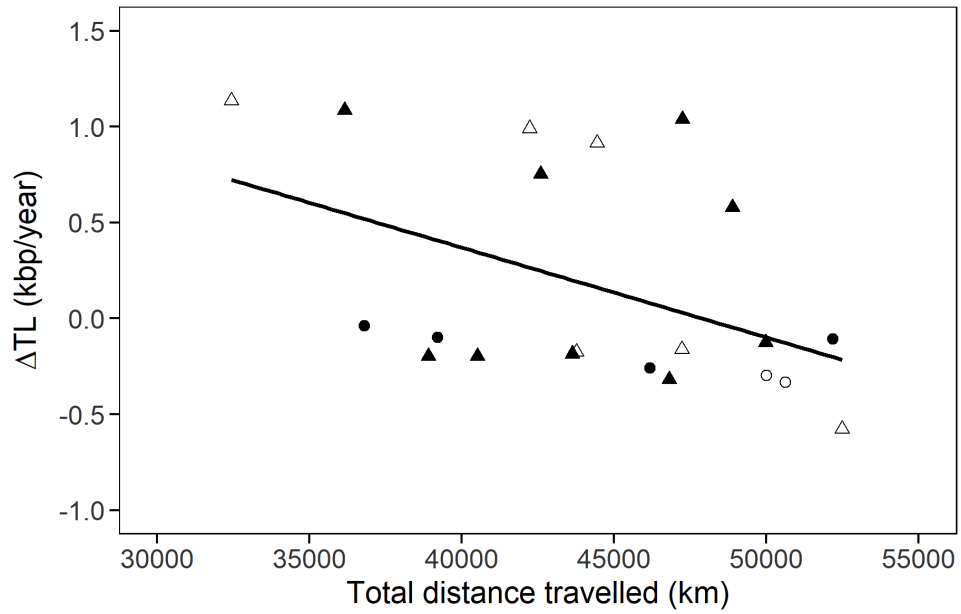

Figure 3.3: Relationship between ΔTL and the total distance travelled throughout a logger year for males (open symbols), and females (closed symbols) of both Brensholmen (circles) and Kongsfjorden (triangles). Linear regression (solid line; Adj. R^2: 0.16, p = 0.04) is based on all individuals.

3.3 Wintering areas

Winter home ranges for individuals overwintering in each region are shown in figure 2.4. Mean ΔTL between individuals overwintering in different regions were tested with an ANOVA and Tukey's HSD. There were no significant difference in ΔTL of individuals overwintering in the different regions (figure 3.4). Complete test results are shown in table G3.

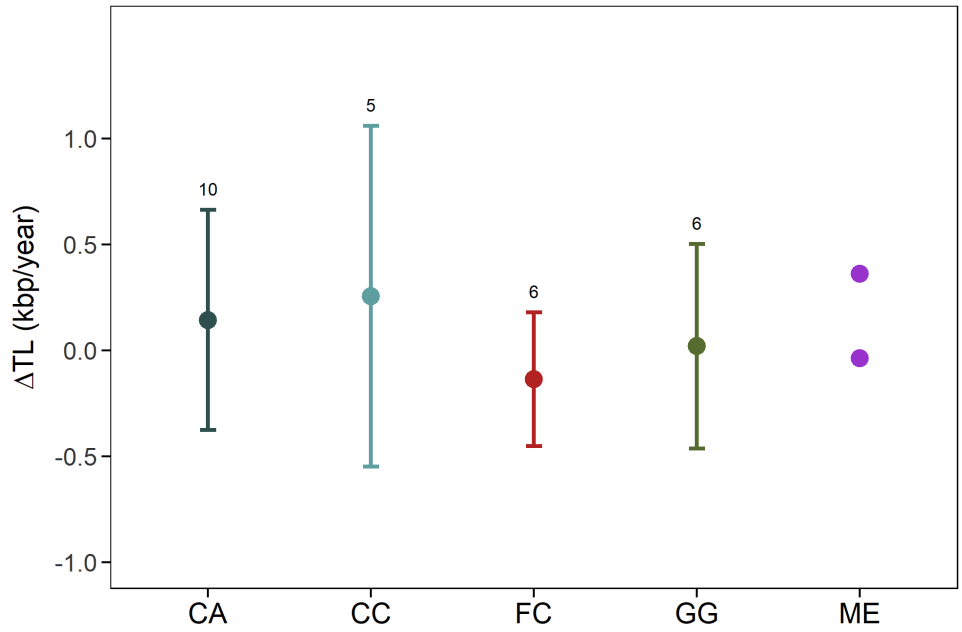

Figure 3.4: Mean ΔTL $\pm$ s.d. of all individuals in each wintering area. Numbers above error bars indicate the sample sizes. There were only two individuals overwintering in ME. CA = Caribbean, CC = Canary current, GG = Gulf of Guinea, ME = Mediterranean, FC = Falkland current.

3.4 Body size

The models including skull length and colony were analysed with the expanded data set which included average ΔTL in order to increase the sample size (from n = 21 to n = 29). The model including skull length and that including colony were again best ranked by AICc, but only that with skull length was significant (table 3.2). Individuals with larger skulls, which was considered to represent structural body size, had more positive ΔTL than individuals with smaller skulls. The relationship between skull length and ΔTL is illustrated in figure 3.5a. Skull length and the total distance travelled in the non-breeding season showed opposite relationships with ΔTL, but these were not significantly correlated (r_S = -0.37, p = 0.1), meaning that smaller individuals did not necessarily travel further than larger ones. Individuals from Kongsfjorden had slightly more positive ΔTL than those of Brensholmen, but this relationship was only approaching significance (table 3.2, figure 3.5b), and since individuals from Kongsfjorden were signifcantly larger than those of Brensholmen (95% conf. int.: -3.32, -1.39) the difference in ΔTL between the colonies could be a consequence of differences in body size.

Table 3.2: Top ranked models after selection with AICc. Presented are the models and parameters, estimates, 95% confidence intervals, ΔAICc value and weight (w) of each model.

Model	Estimate	2.5%	97.5%	ΔAICc	w
Skull length				0.0	0.37
Intercept	**-8.275**	**-16.275**	**-0.276**		
Variable	**0.107**	**0.005**	**0.210**		
Colony				1.0	0.23
Intercept	-0.175	-0.508	0.159		
Variable: K	0.369	-0.033	0.771		
Null				2.1	0.13
Intercept	0.080	-0.114	0.274		

In the Arctic skua, females are the larger sex, and there was a significant difference in body mass between males and females (Welch's t-test, 95% conf. int.: 48.2, 99.4; figure A1, appendix A). Body mass and skull length were strongly correlated (r_S = 0.65, p = 0.0001), but the difference in skull length between males and females was only approaching significance (95% conf. int.: -0.04, 2.54; table 3.3). This means that females were significantly heavier than males, but not necessarily much larger in size. Subsequently, there was no difference in ΔTL between males and females (95% conf. int.: -0.32, 0.50; figure 3.5c), and the model containing body mass was not important (table G2).

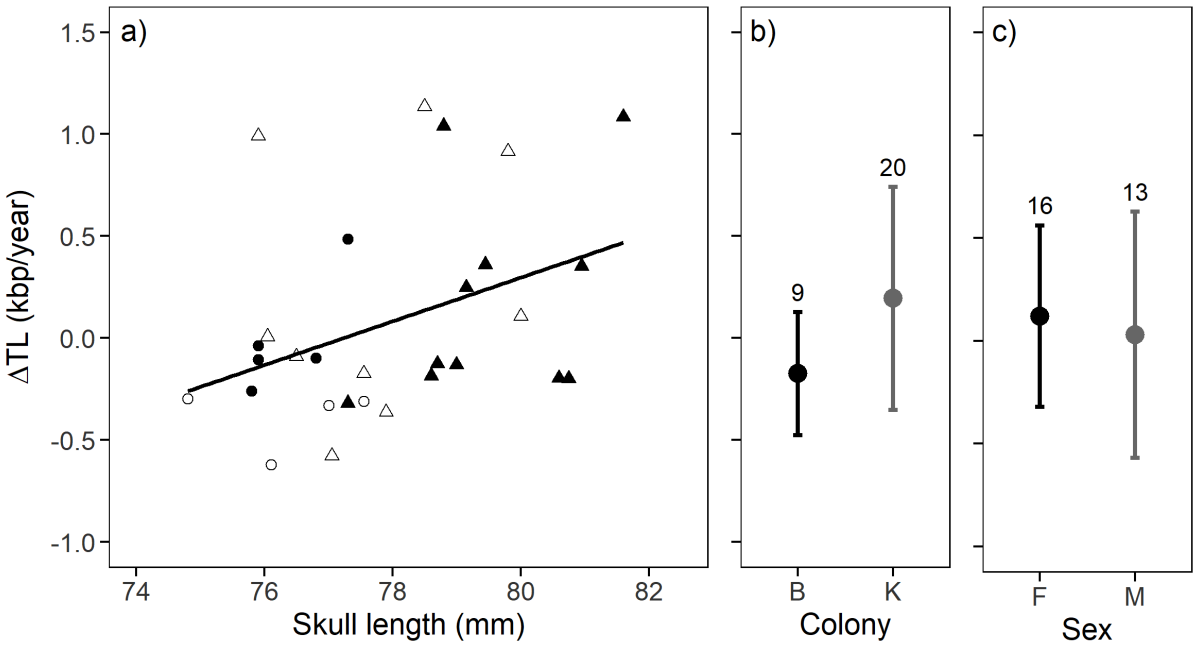

Figure 3.5: (a) Relationship between ΔTL and skull length (mm; adj. R^2: 0.11, p: 0.04); males are shown as open symbols and females as closed symbols. Circles represent individuals from Brensholmen and triangles those of Kongsfjorden. (b) Mean ΔTL $\pm$ s.d. of individuals from Brensholmen (B) and Kongsfjorden (K). (c) Mean ΔTL $\pm$ s.d. for females and males. Numbers above the error bars indicate the sample size of the groups in plots b and c.

Table 3.3: Mean skull length (mm) $\pm$ s.d. of males and females breeding in Kongsfjorden and Brensholmen.

Sex	Kongsfjorden	Brensholmen
F	79.5 $\pm$ 1.28	76.34 $\pm$ 0.67
M	77.7 $\pm$ 1.51	76.36 $\pm$ 1.20

4. DISCUSSION

The aim of the present study was to investigate the potential effect of migration patterns on telomere dynamics in adult Arctic skuas by measuring the distances travelled, wintering areas and the timing of migration. Additional analyses on the potential effect of body size, condition and mass on telomere dynamics were included. As expected, there was a significant, negative association between the total distance travelled and change in telomere length; individuals that covered the longest distances throughout the non-breeding season also lost more telomeres across that period. To the best of my knowledge, this is the first study that has demonstrated a negative relationship between long-distance flight and telomere shortening in a long-lived migratory species. None of the timing variables had any significant effects on change in telomere length, and ΔTL rates between individuals in different wintering areas were not significant, either. Furthermore, a significant and positive relationship between ΔTL and skull length was present; individuals with larger skulls had, on average, more positive ΔTL values than smaller individuals. Lastly, a strong relationship with change in telomere length and intitial length was found, which was considered to be an effect of "regression to the mean". The importance of addressing such statistical problems and the challenges with making assumptions based on this type of data is highlighted.

4.1 Migration patterns

Increased oxidative damage due to long flights has previously been documented in homing pigeons, where individuals that flew longer-than-normal distances had a poorer oxidative status than those that flew shorter distances (Costantini et al. 2008). Also in European robins, the oxidative status was considerably higher in birds caught in-flight than those resting at stop-over sites (Jenni-Eiermann et al. 2014). These studies have shown that oxidative stress can increase during long-distance flight, and that resting between flights is important to recover and reduce potential impacts of oxidative damage. In the present study, individuals that covered the greatest distances in total lost more telomeres than those who covered shorter distances throughout the non-breeding season. This could have been a consequence of increased oxidative damage in individuals that flew greater distances, due to higher overall energy expenditure. The bal-

ance between oxidative damage and anti-oxidants most likely plays an important role during migration, and a study by Costantini et al. (2007) demonstrated that in two migratory species, the garden warbler (*Sylvia borin*) and the barn swallow (*Hirundo rustica*), individuals of higher body condition also had a better oxidative balance after a sustained, oversea flight during spring migration. In the present study, however, there was no significant relationship between body condition and telomere dynamics or the total distance travelled. Since oxidative status was not measured, it is not known whether oxidative damage mediates the link between telomere dynamics and long-distance flight in Arctic skuas. Nonetheless, the result presented here suggests that long-distance flight can lead to increased loss in telomeres. Since these individuals cover similar distances every year, a potential consequence could be lowered fitness and survival through telomere shortening.

Young et al. (2017) found that for the thick-billed murre, out of the migration pattern and aspects of the overwintering area, the only important factor influencing telomere dynamics was foraging effort in the winter area. This could have reflected either local food abundance or individual foraging efficiency. In either case, foraging effort would increase if less food is obtained per foraging trip. Thick-billed murres are diving seabirds, which means that their energy expenditure can be closely linked to diving patterns and foraging effort (Gaston and Jones 1998). Skuas on the other hand do not dive, but spend most of their time on open waters, feeding from the surface or by kleptoparasitism (Furness 1987). There is a possibility that the individuals that flew the longest distances did so as a consequence having to travel further to find sufficient food. The resolution of the data from GLS loggers does not enable the distinction between foraging and migration flight, however, but the distances measured can still reflect movement and overall energy expenditure in relation to flight costs. The results of the present study, together with that of Young et al. (2017), show that movement patterns and consequently overall energy expenditure, can play an important role in telomere dynamics of long-lived seabirds.

Rest during migration, as well as recovery from it, should help buffer against energy depletion and subsequent physiological damage. In the present study, no significant relationship was found between change in telomere length and the time spent in the wintering area. Furthermore, neither the time spent during migration and staging in the autumn nor in the spring was correlated with ΔTL. There was no distinction between the time spent in staging areas and that spent

on migrating, so the lack of any significant effect on ΔTL is not surprising. In black-legged kittiwakes, Schultner et al. (2014) demonstrated that spending more time in the wintering area reduced telomere shortening. This was considered to reflect a better recovery from autumn migration and the previous breeding season. Although the change in telomere length was not linked to migration timing in the Arctic skua, there was a positive relationship between the time spent in the wintering area and body condition in the second year. This indicates that staying in the wintering area for a longer period of time was nonetheless beneficial as individuals that did so returned in a better body condition. Additionally, there was a tendency for individuals that flew greater distances throughout the year to also spent less time in the winter area. Although not a significant correlation, this tendency could suggest that these individuals moved more outside of their wintering area, perhaps due to less available resources in the current year. The change in body mass was almost, negatively, correlated with the total distance travelled as well, supporting the notion that flying longer distances can lead to higher energy depletion. There was no relationship between body condition or change in bodymass and ΔTL, however, suggesting that not energy balance, but energy expenditure as such can be an important factor influencing telomere dynamics in Arctic skuas.

No differences in ΔTL between Arctic skuas overwintering in different regions were observed. There are several possible explanations for this lack of significant differences in ΔTL. First of all, there might not be any large differences in these wintering areas, or such differences might have been too small to detect by looking at change in telomere length. Annual variation in both environmental conditions and food abundance within a wintering area can mask the effect on telomere dynamics, however, as individuals were sampled in different years. While the resource availability in a region might vary between years, regions of consistently poor quality or unsuitable habitats would most likely be selected against; individuals in such areas could face greater challenges of finding sufficient food and be of overall poorer conditions. In the present study, there were no differences in body condition between individuals in different wintering areas. These regions might therefore be sufficient in terms of resource availability and/or habitat quality. Large-scale climate variation has been shown to influence seabird population dynamics. A long-term study on different populations of common eiders (*Somateria mollissima*) showed that climate fluctuations were strongly linked to heterogeneity in survival of these populations (Guéry et al. 2017). For the Arctic skuas, a strong site fidelity to their wintering areas could

potentially lead to selection pressures in the population, should any of the specific regions be of lower quality in terms of resource availability and/or environmental conditions.

Long-lived seabirds are relatively robust to environmental perturbations and should have the capacity to cope in less optimal, short-term conditions (Schreiber and Burger 2001). Only eight individuals had telomere measurements that spanned across two years or more. These ΔTL values were considered to be closer to an averaged situation, but still represent a relatively short period of the lifespan of Arctic skuas. Long-term effects of the environmental conditions in a given area might therefore not have been detected. Detecting any annual variation of a given wintering area, and variation between areas, would necessitate both larger sample sizes and repeated measurements of individuals across greater time scales. This would enable an investigation of whether overwintering in different wintering areas can cause differential survival in Arctic skuas and selection pressures within the populations.

What is not evident in the present study is the degree of repeatability in the timing of migration and the distances flown throughout a given year. The only migration variable that was correlated with the wintering area itself, was the duration of the autumn migration- and staging period. Although several years of information is available for most of the individuals in this study system, migration patterns were only investigated across one year that matched with the telomere measurements. There is a possibility that individuals are more flexible in the timing of migration and distances flown than in the choice of winter area itself. This kind of flexibility has been demonstrated in a close relative of the Arctic skua, the long-tailed skua (*Stercorarius longicaudus*). In this species, individuals had a high site fidelity to their wintering grounds, but were flexible in the timing of migration when facing local environmental conditions in a given year (van Bemmelen et al. 2017). Since Arctic skuas also have a high site fidelity, being able to adjust the timing of migration as well as a particular route should help to buffer against annual differences in resource availability and environmental conditions. Considering this, the distance an individual covers in one year might not be the same in the next. Investigating migration patterns and telomere dynamics across several years could therefore reveal the level of flexibility of Arctic skuas, to what degree environmental conditions govern migration patterns and, in turn, telomere dynamics.

4.2 Body size and change in telomere length

Studies on telomere dynamics and structural size have most often found a negative association between telomere attrition and growth rate (Ringsby et al. 2015). It is also well documented in both birds and in humans that telomeres shorten more rapidly during growth, which is considered to be a consequence of the high cell turnover rates associated with the growth stages (Angelier et al. 2017). In the present study, a positive relationship between body size and change in telomere length was found. It is not clear why smaller individuals had more negative ΔTL than larger ones. One possible explanation could be that there are differences in energetics of small- and large-bodied animals. Small animals have higher weight-specific metabolic rate, which could lead to higher production of ROS. Additionally, larger individuals have lower minimum weight-specific movement costs due to more efficient muscles. Since smaller individuals have both higher weight-specific metabolism and higher minimum cost of transport, these individuals might experience greater physiological burdens which could increase the rate of telomere attrition. There were no significant effects of the interaction between body mass and the distances travelled on ΔTL, nor of body mass *per se*. Lower weight-specific energetic costs could nonetheless be a part of the explanation as to why larger individuals had more positive ΔTL and why the smallest individuals had negative ΔTL.

Although female Arctic skuas are heavier than males, they are not much larger in structural size. While larger-bodied individuals had more positive ΔTL than smaller ones in the present study, there were no significant differences in ΔTL between the sexes. Most studies on sex differences in telomere dynamics within a species have demonstrated slower telomere attrition in females compared to males. Higher attrition rates for females of species displaying reversed sexual size-dimorphism has not yet been documented (reviewed in Barrett and Richardson 2011). However, a few studies on avian species with either size monomorphism or males being slightly larger, have found either more rapid telomere shortening (Barnacle geese *Branta leucopsis;* Pauliny et al. 2012), or shorter telomeres in females compared to males of the same age group (Thick-billed murre; Young et al. 2013).

Lastly, although not significant, a small difference in ΔTL between individuals of the different colonies was observed, and increases in telomere length were more common in individuals breeding in Kongsfjorden than in those breeding in Brensholmen. Differences in telomere dynamics between colonies with either declining or stable populations have been demonstrated in the thick-billed murre, where greater telomere attrition occured in individuals breeding in a declining population (Young et al. 2013). In any case, since the body size of Arctic skuas differed both between the colonies and the sexes, whether there is a true effect of size on ΔTL, or whether it is a consequence of sex- or colony differences, remains to be investigated.

4.3 Telomere lengthening

A substantial amount of increases in telomere length was observed in the present study. Telomeres also seemed highly dynamic within an individual as the rate of change was not necessarily constant nor in the same direction every year (figre 3.1). Telomerase, which synthesize telomeres *de novo*, is not usually expressed in somatic cells post-growth. Upregulation of telomerase in bone marrow in adult seabirds has been demonstrated (Haussmann et al. 2007), however. Great increases in telomere length are nonetheless unlikely to persist over longer periods of time. A large number of correlative studies have shown that telomere dynamics can vary between habitats, sites, and years (Angelier et al. 2017), which also seem to be the case in the present study. Additionally, most studies on telomere dynamics throughout the life span of a species have shown that telomere shortening is more rapid in the early life stages, and stabilize in the adult life stage (reviewed in Angelier et al. 2017). The large variation in TL within an individual observed in the present study can be due to the relatively short time between sampling (1 or 2 years). Considering that the Arctic skuas are long-lived seabirds, telomere attrition is most likely more stable over time scales longer than what has been investigated here (Pauliny et al. 2012). Therefore, the apparent lengthening in the present study is considered to reflect efficient maintenance of the telomeres.

The variation in length and the high amount of positive ΔTL values could also have been a consequence of measurement errors (Steenstrup et al. 2013). Verhulst et al. (2013) demonstrated that the effect of regression to the mean has a tendency to increase strongly with increasing stochastic variation, which is often due to measurement error. A challenge with correcting for

RTM is that in cases where the initial values and subsequent changes are not following the regression pattern, correcting for it may cause the results to become skewed compared to the original values. On the other hand, by not correcting for RTM in the cases where there is a strong effect, statistical analyses based on uncorrected values can be biased by the regression effect. In the present study, the effect of RTM was stronger in females than in males. The analyses of uncorrected values showed a significant difference in ΔTL between males and females. After correcting for RTM, some of the ΔTL values for males changed from being close to zero to become either more positive, or more negative than the uncorrected values, most likely because the relationship between TL_1 and ΔTL was weaker in the first place. Due to the relatively high amount of variation in ΔTL explained by initial telomere length and the sex difference in this initial length, differences in the response using uncorrected values might not reflect the actual biological effect of the variables in question. Conversely, the effect of regression to the mean might be of biological significance if there are differences between the sexes in this response. The present study demonstrated a stronger effect of RTM in females whereas a study on human leukocyte telomeres demonstrated a stronger effect of RTM in men than in women (Verhulst et al. 2013). The underlying cause of these differences is not evident, but a possible explanation could be of opposite heterogamy of the sex chromosomes, as mammalian males are the heterogametic sex (XY), while in birds, females are the heterogametic sex (ZY). Studies concerning differences in changes of a value based on the initial value must consider the effect of regression to the mean in repeated measurements, as it can create results that are skewed by regression effect. The present study illustrates this importance as results based on uncorrected values could have been masked by either differences in initial length or between the sexes. If RTM is not corrected for, initial measurements should be included in the analyses to account for the regression effect.

5. CONCLUSION AND FUTURE WORK

Although the link between mortality, life expectancy and telomere dynamics is widely recognized, very few studies have attempted to link telomere dynamics to life-history strategies throughout the annual cycle of a species, and only a handful of studies have investigated the effect of migration patterns on telomeres. In the present study, a negative relationship between the total distance covered by an individual and change in telomere length across a single year has been documented, which suggests that flying long distances can lead to higher physiological burdens in the Arctic skua. No other factors of the migration pattern were correlated with telomere dynamics. If telomeres truly shorten as a consequence of increased energy expenditures in relation to long-distance flight, individuals that cover these great distances might face lowered survival in subsequent years. The non-breeding season comprises a large part of the annual cycle of migratory seabirds, and stressfull experiences during this period can lead to carry-over effects into the subsequent breeding season. Long-distance flight appears to be a potential stressor of migration in the Arctic skua. There were no direct relationships with body condition and telomere dynamics, but body condition did increase with the time spent in the wintering area, which may improve individual fitness. The large amount of positive changes in telomere length suggest that Arctic skuas have a high degree of telomere protection and/or maintenance, and relatively low shortening rates. In order to evaluate how telomere dynamics are related to overall fitness, studies of longer time scales are necessary, and determining a causal relationship between telomere dynamics, migrations patterns and stress warrants experimental research. Nonetheless, telomere dynamics may provide useful insights into which environmental and biological factors that impact survival and fitness, and the present study highlights the role of flight distances in telomere dynamics of a long-lived, migratory seabird.

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APPENDICES

A Biometrics

Table A1: Sex, winter area and biometrics for all individuals. Presented are the individual metal ID, sex, body mass (g; BM), skull length (mm) and calculated body condition (BC) presented. The numbers following BM and BC indicate when the measurement was taken, i.e. in the first, second or third sampling year.

ID	Sex	Colony	Winter area	BM1	BM2	BM3	Skull length	BC1	BC2	BC3
6217935	F	K	GG	482	481		77.3	-3.5	-4.5	
6217936	M	K	GG	460	458	462	76.5	45.9	43.9	45.7
5184806	M	K	GG	428	447		77.6	0.3	19.3	
6179829	F	K	CA	497	493		78.8	-6.1	-10.1	
6217934	F	K	CA	516	490	497	78.7	14.1	-11.9	-4.7
6217938	M	K	CA	416	427		77.9	-16.2	-5.2	
6217941	F	K	CA	555	543	485	79.2	47.8	35.8	-22.0
6218055	F	K	ME	505	593	533	79.5	-5.7	82.3	22.5
6218801	F	B	FC	498	438		75.8	30.0	-30.0	
6218802	F	B	ME	505	480		75.9	35.9	10.9	
6218805	F	B	FC	488	425		75.9	18.9	-44.1	
6218807	M	B	CC	385		400	76.1	-23.9		-8.9
6218808	M	B	FC	390	405		74.8	-2.0	13.0	
6218809	F	B	FC	450		415	77.3	-35.5		-70.5
6223841	M	K	CA	457		445	80.0	-2.5		-14.5
5184803	F	K	GG	577	490		80.6	52.8	-34.2	
6217919	M	K	CA	508	468		79.8	51.1	11.1	
6217924	F	K	CC	542	530		79.0	36.5	24.5	
6218810	M	B	FC	395		400	77.6	-32.7		-27.7
6223846	F	K	CA	557	500		81.0	28.7	-28.3	
5184807	F	K	CC	515	515		81.6	-20.9	-20.9	
5184808	M	K	CA	425	442		76.1	16.8	33.8	
6218811	F	B	GG	435	525		76.8	-44.7	45.3	
6218813	M	B	FC	375	331		77.0	-45.6	-89.6	
6223849	F	K	CC	491	499		80.8	-35.0	-27.5	
6228552	M	K	GG	417	399		75.9	10.7	-7.8	
6228571	M	K	CC	447	439		78.5	7.0	-1.5	
6228574	M	K	CA	472	396		77.1	50.8	-25.7	
6228558	F	K	CA	484	488		78.6	-17.3	-12.8	

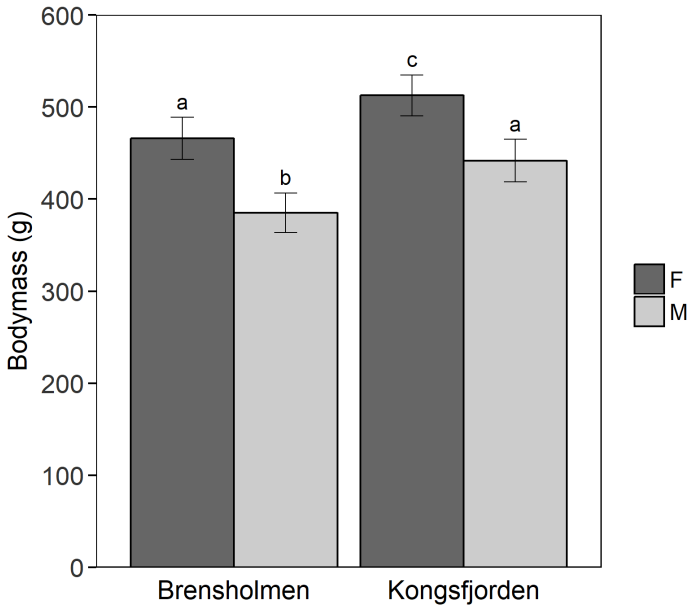

Figure A1: Mean body mass of females and males of Kongsfjorden and Brensholmen, with error bars representing ± s.d. The labels above the bars indicate differences between the groups, with the same letters indicating no difference and unique letters indicate significant difference ($p<0.05$). Differences across all groups were evaluated with Tukey's HSD.

B Telomere analysis

Extraction and digestion

Red blood cells were digested with Proteinase K and DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen), following the method for extraction of nucleated blood cells. The quality (absorbance) and concentration (ng/μL) of DNA in each sample was measured with optical density spectrophotometry (NanoDrop ND-1000). Absorbance values were measured as ratios for 260/280 nm and 230/260 nm. Samples with critically low quality (ng/μL) were re-extracted. If the quality did not improve, the samples were not run. This was the case for two samples in total, for the same individual (total sample size would have been 30, not 29). The volume needed to obtain 1μg of DNA was calculated for each sample and subsequently digested with the restriction enzymes HinfI and RsaI at 37°C for approximately 19 hours. The samples were stored at 4 °C until digestion was stopped with a 5X loading buffer the following afternoon. Digested DNA was then separated with a pulse-field gel electrophoresis (BioRad) on a 0.8% agarose gel at 3.0 V/cm with an initial switch time of 0.5 s to a final switch time of 7 s for 14 hours overnight.

Southern blotting, detection and chemiluminescence

On the following day the gel was first depurinated (with 0.1 M HCl), then denaturated (with a 0.5 M NaOH, 1.5 M NaCl solution) and finally neutralized (with 0.5M Tris-HCl, 3M NaCl solution). The DNA was transferred to a nitrocellulose membrane by southern blot (Hybond N+, Amersham LifeScience, Amersham, UK) overnight. Finally, DNA was fixed by incubating the membrane at 120 °C for 25 minutes. After incubation, the membrane was prepared for hybridation by incubation in digoxigenin at 42 °C for 1 hour, then hybridized with a digoxigenin-labeled telomere specific probe (42 °C, 3 hrs). After hybridation, the membrane was washed several times with buffer solutions, then incubated with anti-digoxigenin antibodies and finally with a detection buffer. The fragments were then visualized by chemiluminescence with a Chemidoc(BioRad). An example of the image produced is shown figure 2.2.

Measuring TL

Telomere lengths were analyzed with ImageJ to extract telomere smear densities. The chemiluminescence signals were corrected for background noise and the intensity of the signal for each sample was calculated by comparing the smears with a known standard provided in the kit. The following figures are chemiluminescence images of the three agarose gels run. To the left, center and right, ladders with known standards (exact number of base pairs) are shown. Samples from the same individual were placed next to each other or as close as possible, and were numbered after the order in which the DNA was extracted. In the software ImageJ, lines were drawn manually to mark the start and end of the smear (i.e. the length) and the optical density for each section of the smear was extracted by the program.

The length of telomere restriction fragments were derived by using the formula 1:

$$T\bar{R}F = \frac{\sum OD_i}{\sum (OD_i)/L_i} \quad (1)$$

Where OD_i is the optical density at position i and L_i is the molecular weight at position i. The formula provides an average telomere length for the entire sample.

C Telomere lengths

Table C1: Telomere length measurements. Presented are individual ID, sex, year (Y) at which sampling occured (for sample 1, 2, 3 and 4), the telomere length (kilo base pairs, kpb; TL) of each sample, and change in telomere length (ΔTL), averaged ΔTL for more than two samples, and RTM-corrected values. Numbers following Y and TL indicate which sampling year the measurement belongs to.

ID	Sex	Y1	Y2	Y3	Y4	TL1	TL2	TL3	TL4	ΔTL	ΔTL avg	ΔTL corr.	ΔTL avg corr.
6217935	F	2010	2011			11.0	10.5			-0.46		-0.32	
6217936	M	2010	2011	2012		11.0	10.7	10.1		-0.31	-0.45	-0.16	-0.09
5184806	M	2011	2012			11.5	10.8			-0.68		-0.17	
6179829	F	2011	2012			10.3	11.7			1.39		1.04	
6217934	F	2011	2012	2014	2015	10.6	10.6	9.6	12.5	0.00	0.47	-0.13	
6217938	M	2011	2012			10.7	10.4			-0.30		-0.36	
6217941	F	2011	2012	2013		9.6	11.0	11.2		1.42	0.80	0.58	0.25
6218055	F	2011	2012	2013		11.2	11.7	10.9		0.45	-0.14	0.75	0.36
6218801	F	2011	2012			11.1	10.6			-0.45		-0.26	
6218802	F	2011	2012			10.0	10.5			0.48		-0.04	
6218805	F	2011	2012			10.8	10.7			-0.09		-0.11	
6218807	M	2011		2013		10.1		9.3			-0.39		-0.62
6218808	M	2011	2012			10.8	10.5			-0.34		-0.30	
6218809	F	2011		2013		9.9		11.6			0.82		0.49
6223841	M	2011		2013		10.8		10.6			-0.12		0.11
5184803	F	2012	2013			11.6	10.8			-0.77		-0.20	
6217919	M	2012	2013			12.0	12.1			0.06		0.92	
6217924	F	2012	2013			11.6	10.9			-0.72		-0.13	
6218810	M	2012		2014		10.5		9.8			-0.35		-0.31
6223846	F	2012	2013			9.5	10.8			1.25		0.35	
5184807	F	2013	2014			10.6	11.8			1.19		1.09	
5184808	M	2013	2014			11.2	10.9			-0.26		0.01	
6218811	F	2013	2014			9.8	10.4			0.58		-0.10	
6218813	M	2014	2015			10.9	10.5			-0.42		-0.33	
6223849	F	2014	2015			10.1	10.4			0.25		-0.20	
6228552	M	2014	2015			11.2	11.9			0.71		0.99	
6228571	M	2014	2015			10.8	11.9			1.10		1.14	
6228574	M	2014	2015			11.2	10.3			-0.90		-0.58	
6228558	F	2015	2016			10.3	10.5			0.13		-0.18	

Table C2: Mean TL ± s.d. at the time of deployment and first recapture and mean ΔTL ± s.d., both RTM-corrected and uncorrected values, for male (M) and female (F) Arctic skuas.

	TL	ΔTL (corrected)	ΔTL (uncorrected)
F	10.74 ± 0.68	0.12 ± 0.44	0.26 ± 0.7
M	10.83 ± 0.67	0.03 ± 0.59	-0.18 ± 0.5
All	10.77 ± 0.67	0.08 ± 0.51	0.06 ± 0.7

D Migration data

Below are explanations for each column in tables D1 and D2 given.

Year tracked	Year of deployment and year of retrieval of GLS logger.
Winter area (WA)	Winter area code. CA: Caribbean, CC: Canary Current, GG: Gulf of Guinea, ME: Mediterranean, FC: Falkland current.
Lay date	Date of egg laying in recapture year.
Last position	Last position recorded before entering region with 24h daylight.
65°N autumn	Date of crossing 65°N during autumn migration.
35°N autumn	Date of crossing 35°N during autumn migration.
35°N spring	Date of depature from wintering area in spring.
65°N spring	Crossing 65 °N during spring migration, i.e. arrival in breeding region.
20°S autumn	Date of arrival in wintering area for those overwintering in FC.
20°S spring	Depature from FC wintering area.
Autumn migr.	Days spent between 65°N and 35°N during autumn migration.
Spring migr.	Days spent 35°N and 65°N during spring migration.
South of 65°N	Days spent below 65°N throughout the logger year.
South of 35°N	Days spent below 35°N throughout the logger year. This represents time spent in wintering area for individuals over-wintering in CA, CC, GG and ME.
South of 20°S	Days spent below 20°S, representing time spent in wintering area for individuals in FC.
Tot. dist.	The total distance travelled (in kilometers) over the entire logger year.
Max. dist.	The maximum distance from the colony that was recorded over the entire logger year.

Table D1: Calendar dates of arrival and departure in areas along the migration route and in the wintering area. See details of each column in D.

Metal ID	Year tracked	Winter area	Lay date	Last position	65°N autumn	35°N autumn	35°N spring	65 °N spring	20°S autumn	20°S spring
5184803	2012-13	GG	12.06.2013	25.05.2013	01.10.2012	14.11.2012	16.05.2013	25.05.2013		
5184806	2011-12	GG	29.06.2012	27.05.2012	03.10.2011	11.10.2011	15.05.2012	29.05.2012		
5184807	2013-14	CC	22.06.2014	28.05.2014	13.10.2013	04.11.2013	23.05.2014	30.05.2014		
6179829	2011-12	CA	14.06.2012	25.05.2012	04.10.2011	03.11.2011	19.05.2012	27.05.2012		
6217919	2012-13	CA	12.06.2013	21.05.2013	07.10.2012	19.11.2012	13.05.2013	21.05.2013		
6217934	2011-12	CA	16.06.2012	29.05.2012	08.10.2011	02.12.2011	18.05.2012	29.05.2012		
6217935	2010-11	GG	12.06.2011	29.05.2011	16.10.2010	26.10.2010	17.05.2011	30.05.2011		
6217936	2010-11	GG	12.06.2011	29.05.2011	14.10.2010	28.10.2010	20.05.2011	29.05.2011		
6217941	2011-12	CA	14.06.2012	31.05.2012	27.09.2011	30.09.2011	14.05.2012	02.06.2012		
6218055	2011-12	ME	20.06.2012	19.05.2012	24.09.2011	14.10.2011	10.05.2012	21.05.2012		
6218801	2011-12	FC	01.06.2012	12.05.2012	31.08.2011	12.09.2011	30.04.2012	11.05.2012	07.10.2011	27.03.2012
6218802	2011-12	ME	08.06.2012	13.05.2012	18.08.2011	22.09.2011	27.04.2012	11.05.2012		
6218805	2011-12	FC	04.06.2012	12.05.2012	06.09.2011	11.09.2011	28.04.2012	13.05.2012	14.10.2011	07.04.2012
6218808	2011-12	FC	04.06.2012	13.05.2012	12.09.2011	19.09.2011	28.04.2012	13.05.2012	17.10.2011	17.04.2012
6218811	2013-14	GG	08.06.2014		22.08.2013	05.09.2013	13.04.2014	03.05.2014		
6218813	2014-15	FC	03.06.2015	16.05.2015	30.08.2014	27.09.2014	11.05.2015	18.05.2015	23.10.2014	20.04.2015
6223849	2014-15	CC	22.06.2015	22.05.2015	20.09.2014	13.10.2014	17.05.2015	24.05.2015		
6228552	2014-15	GG	27.06.2015	15.05.2015	08.10.2014	15.10.2014	02.05.2015	17.05.2015		
6228558	2015-16	CA	14.06.2016	23.05.2016	27.09.2015	29.11.2015	14.05.2016	25.05.2016		
6228571	2014-15	CC	17.06.2015	20.05.2015	25.09.2014	11.10.2014	13.05.2015	22.05.2015		
6228574	2014-15	CA	22.06.2015	01.06.2015	01.10.2014	30.10.2014	24.05.2015	03.06.2015		

Table D2: Number of days spent in each stage along the migration route and in the wintering area, the total distance travelled throughout the year and the maximum distance from colony. See further details in D.

ID	Year tracked	WA	Autumn migr.	Spring migr.	South of 65°N	South of 35°N	South of 20°S	Tot. dist. (km)	Max. dist. (km)
5184803	2012_13	GG	44	9	236	183		40517	10319
5184806	2011_12	GG	8	14	239	217		43767	10540
5184807	2013_14	CC	22	7	229	200		36153	7829
6179829	2011_12	CA	30	8	236	198		47247	10377
6217919	2012_13	CA	43	8	226	175		44440	8838
6217934	2011_12	CA	55	11	234	168		49973	9732
6217935	2010_11	GG	10	13	226	203		46811	11101
6217936	2010_11	GG	14	9	227	204		47224	10809
6217941	2011_12	CA	3	19	249	227		48879	9707
6218055	2011_12	ME	20	11	240	209		42592	6040
6218801	2011_12	FC	12	11	254	231	172	46177	14758
6218802	2011_12	ME	35	14	267	218		36799	5644
6218805	2011_12	FC	5	15	250	230	176	52176	14834
6218808	2011_12	FC	7	15	244	222	183	49997	14847
6218811	2013_14	GG	14	20	254	220		39207	8876
6218813	2014_15	FC	28	7	261	226	179	50609	14443
6223849	2014_15	CC	23	7	246	216		38911	8494
6228552	2014_15	GG	7	15	221	199		42227	9836
6228558	2015_16	CA	63	11	241	167		43630	9476
6228571	2014_15	CC	16	9	239	214		32430	8179
6228574	2014_15	CA	29	10	245	206		52480	9501

E Correlations of explanatory variables

Table E1: Correlation values for all explanatory variables used in the expanded dataset n = 29). Significant correlations (p < 0.05) are highlighted in bold, those approaching significance (p < 0.1) in italics.

	Colony	Winter area	Sex	BM	Skull	Tarsus
Winter area	**0.72**					
Sex	-0.01	-0.06				
BM	**-0.48**	-0.30	**-0.75**			
Skull	**-0.63**	**-0.52**	-0.31	**0.65**		
Tarsus	**-0.65**	**-0.64**	-0.20	**0.57**	**0.56**	
BC	**-0.38**	-0.22	0.07	*0.32*	-0.06	*0.34*

Table E2: Correlation values for all explanatory variables for the migration data set (n = 21). Year represents the year of logger deployment. Significant correlations ($p < 0.05$) are highlighted in bold, those approaching significance ($p < 0.1$) in italics.

	Year	Colony	Winter area	Sex	Aut. migr.	Spring migr.	Winter dur.	BM1	BM2	Skull	Total dist.	Max dist.	BC1	BC2
Colony	-0.10													
Winter area	-0.29	**0.73**												
Sex	0.20	-0.06	0.06											
Aut. migr.	0.35	-0.24	**-0.44**	-0.17										
Spring migr.	-0.31	0.35	0.27	-0.11	**-0.59**									
Winter dur.	-0.08	-0.10	-0.03	0.01	-0.35	0.26								
BM1	-0.22	-0.30	*-0.37*	**-0.65**	*0.38*	-0.18	-0.10							
BM2	-0.16	-0.35	*-0.38*	**-0.71**	0.19	-0.02	0.34	**0.63**						
Skull	0.23	**-0.70**	**-0.63**	-0.31	**0.46**	**-0.56**	0.08	**0.61**	**0.67**					
Total dist.	-0.31	0.19	0.05	0.21	-0.18	0.14	*-0.37*	-0.17	*-0.42*	-0.34				
Max dist.	*-0.39*	0.35	**0.44**	0.21	**-0.44**	0.21	**-0.43**	*-0.39*	**-0.54**	**-0.54**	**0.69**			
BC1	-0.29	-0.16	-0.13	0.18	0.01	0.15	-0.06	*0.41*	-0.22	-0.09	0.21	0.08		
BC2	*-0.39*	-0.16	-0.08	0.16	-0.29	**0.43**	**0.55**	-0.10	0.36	-0.04	-0.19	-0.28	0.04	
ΔBM	-0.08	-0.12	0.00	-0.08	-0.22	0.19	**0.43**	-0.29	**0.47**	0.13	*-0.37*	-0.25	**-0.65**	**0.64**

F Regression to the mean

Regression to the mean is a statistical phenomenon that occurs in repeated-measures analyses when the correlation between the measurements at different times is weak. In ecology, this often occurs when the same attribute in an individual or population is measured at two different times (Berry et al. 1984; Kelly and Price 2005). In the present study, telomere length (individual level) was measured either twice or three times, with one or two years between sampling. Here, individuals that had short telomeres (>10 kbp) always had longer telomeres in the second measurement. The individuals with relatively long telomeres (<11 kbp) *almost* always had shorter telomeres in the second measurement, with a few exemptions (see table C1.) When studying subsets of a population on the basis of their initial measurements, regression to the mean can complicate the analyses. This is because any resulting effect on the change in state using measurements that are strongly affected by RTM could be uninformative as any real biological effect may be clouded by the regression effect.

When adjusting for regression to the mean, the distribution of measurements 1 and 2 (representing TL_1 and TL_2, hereafter X_1 and X_2), are expected to have bivariate normal distributions with means μ_1 and μ_2, and variances σ_1^2 and σ_2^2. Telomere length in the Arctic skuas in this study fulfilled this criterion. To assess if there is a 'regression effect', the correlation ρ between the X_1 and X_2 should be relatively low and/or insignificant, while the difference D between X_1 and X_2 ($X_1 - X_2$), should be strongly correlated with X_1. In this data set, the correlation between X_1 and X_2 was low ($r_S : 0.29$, $p > 0.1$) and the correlation between D and X_2 was high ($r_S : -0.62$, $p < 0.001$). A difference between the two means (Δ = μ_1-μ_2) will be an additive effect, which represents a difference in the measurements beyond that of the regression effect. If a differential effect is evident, the will be a difference in the variance between the two measurement, i.e. $\sigma_1^2 \neq \sigma_2^2$. In this data set, the ratio between the variances did not equal one (F-test, ratio: 1.23, $p = 0.6$) which means that there was a differential effect; individuals above and below the mean decrease or increase in a manner that is expected from the regression effect.

When correcting for this, the differential effect is removed and the remaining difference $\hat{D}$ is that expected from an additive effect (eq. 6 in Kelly and Price 2005):

$$\hat{D} = \hat{\rho}(X_1 - \bar{X_1}) - (X_2 - \bar{X_2}) + \Delta \tag{2}$$

where $\hat{\rho}$ is the correlation between X_1 and X_2, $\bar{X_1}$, $\bar{X_2}$ are the sample means of X_1 and X_2, and Δ is considered the additive effect, i.e. the difference between $\bar{X_1}$ and $\bar{X_2}$. In this data set, there were 21 individuals that only had measurements with one year between sampling (TL_1 and TL_2), four individuals with only two years between (TL_1 and TL_3), and three individuals had both (TL_1, TL_2 and TL_3). When correcting for the regression effect, only samples measured across the same time period where used. Therefore, two calculations were performed, one between TL_1 and TL_2 (n = 25), and one between TL_1 and TL_3 (n = 7). For those individuals with three TL measurements, two different $\hat{D}$ values were calculated. In the expanded data set,

both differences between one year and between two years have been used. These are referred to as ΔTL and ΔTL avg., respectively, and the averaged values are used when possible (i.e. for seven individuals). In the migration data set, only one year changes in TL are used, so only $\hat{D}$ between X_1 and X_2 are compared.

Table F1: Statistical analyses (linear models) of change in telomere length in relation to initial length (TL_1), time interval (years; 1 or 2) between successive samples, sex, and an interaction between sex and TL_1. Presented are variable estimates with standard error and the probability of the estimate in both uncorrected, and corrected ΔTL values.

Variable	Uncorrected values		Corrected values	
	Estimate (s.e.)	Pr($> \|t\|$)	Estimate (s.e.)	Pr($> \|t\|$)
Intercept	9.08 (2.01)	0.0001	2.02 (2.00)	0.32
Sex (M)	-9.04 (4.15)	0.0400	-9.12 (4.14)	0.04
TL1	-0.85 (0.20)	0.0002	-0.17 (0.20)	0.40
Interval (years)	-0.12 (0.24)	0.6000	0.11 (0.24)	0.65
TL1 $\times$ sex(M)	0.82 (0.38)	0.0400	0.83 (0.38)	0.04

G Model selection and ANOVA

Migration data

Table G1: Models with ΔAICc values above 2, using the migration data set and RTM-corrected ΔTL values. Presented are the models and variables, intercept and variable estimates, 95% confidence intervals, and ΔAICc value and weight (w) of each model.

Model	Estimate	2.5%	97.5%	ΔAICc	w
Total distance + BC2				2.2	0.080
Intercept	2.070	0.026	4.108		
Total distance	-4.277×10^{-5}	-8.844×10^{-5}	2.898×10^{-6}		
BC2	2.848×10^{-3}	-4.080×10^{-3}	9.776×10^{-3}		
Body mass				2.6	0.077
Intercept	1.323	-3.450	0.804		
Variable	0.003	-0.001	0.008		
Body condition				3.2	0.05
Intercept	0.176	-0.085	0.436		
Variable	0.004	-0.003	0.012		
Total distance × BM				3.6	0.04
Intercept	14.330	-6.003	34.664		
Total distance	-3.252×10^{-4}	-7.604×10^{-4}	1.099×10^{-4}		
Body mass	-2.687×10^{-2}	-7.053×10^{-2}	1.678×10^{-2}		
Interaction	6.218×10^{-7}	-3.215×10^{-7}	1.565×10^{-6}		
ΔBM				3.8	0.040
Intercept	0.197	-0.075	0.469		
Variable	0.002	-0.003	0.008		
Winter duration				4.0	0.037
Intercept	1.045	-3.853	1.763		
Variable	0.006	-0.008	0.020		
Spring 'migration'				4.4	0.027
Intercept	0.413	-0.463	1.290		
Variable	-0.022	-0.094	0.051		
Autumn 'migration'				4.7	0.023
Intercept	0.231	0.234	0.695		
Variable	-0.003	-0.019	0.013		
Sex				4.8	0.022
Intercept	0.148	-0.193	0.491		
Variable: Sex(M)	0.039	-0.516	0.594		

Expanded data set

Table G2: Models with ΔAICc values above 2, using the expanded data set and RTM-corrected ΔTL values. Presented are the models and variables, intercept and variable estimates, 95% confidence intervals, ΔAICc value and weight (w) of each model.

Model	Estimate	2.5%	97.5%	ΔAICc	w
Body mass				2.34	0.123
Intercept	-1.221	-3.055	0.613		
Body mass	0.003	-0.001	0.007		
Sex				4.33	0.046
Intercept	0.120	-0.145	0.385		
Sex: M	-0.090	-0.486	0.306		
$TL_1 \times$ Sex				4.90	0.034
Intercept	2.030	-2.103	6.163		
Variable: TL_1	-0.182	-0.575	0.211		
Variable: Sex(M)	-8.520	-16.505	-0.535		
$TL_1 \times$ sex(M)	0.776	0.040	1.511		

Tukey HSD of winter area differences

Table G3: Tukey HSD of ANOVA with ΔTL as the response and winter areas as explanatory variables. The columns show the difference between the means for each area, lower and upper values for a 95% confidence interval, and the adjusted p value of the test.

Winter areas	diff	2.5%	97.5%	p adj
CC - CA	0.08	-1.02	1.18	1.00
GG - CA	-0.38	-1.42	0.66	0.81
ME - CA	-0.03	-1.59	1.52	1.00
FC - CA	-0.34	-1.38	0.69	0.86
GG - CC	-0.46	-1.68	0.75	0.79
ME - CC	-0.11	-1.79	1.57	1.00
FC - CC	-0.43	-1.64	0.79	0.84
ME - GG	0.35	-1.29	1.99	0.97
FC - GG	0.04	-1.12	1.20	1.00
FC - ME	-0.31	-1.95	1.33	0.98