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Causes and consequences of individual variation in migratory behaviour of Cory's Shearwaters

" Documento Definitivo"

Doutoramento em Biologia e Ecologia das Alterações Globais
Especialidade de Biologia e Ecologia Marinha

Marie Claire Gatt

Tese orientada por:
José Pedro Granadeiro
Paulo Catry

Documento especialmente elaborado para a obtenção do grau de doutor

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CONTENTS

RESUMO

A migração diferencial pode dar origem a diferente *fitness* experienciado para os indivíduos de uma população devido a efeitos de *carry-over, i.e.* o efeito das condições experienciadas durante uma época nas fases seguintes do ciclo de vida. A maioria das populações de animais migradores correspondem a migradores parciais, nas quais uma parte da população migra e a outra permanece residente durante todo o ano. Apesar de ser uma estratégia muito observada e importante na compreensão da evolução e das consequências ecológicas das decisões migratórias, a migração parcial tem sido pouco estudada. As espécies de aves pertencentes à ordem Procellariiformes são um modelo interessante para o estudo da migração. Estas aves marinhas distinguem-se de outros taxa de aves classicamente estudados, uma vez que o seu voo planado dinâmico reduz o custo do voo e têm a capacidade de se alimentar em viagem ativa (sem necessidade de efectuar paragens).

O objetivo desta tese de doutoramento é investigar as causas e consequências da migração diferencial, particularmente a migração parcial, em cagarras *Calonectris diomedea*. As cagarras passam o período não reprodutor em várias zonas oceânicas distintas no Oceano Atlântico e no Oceano Índico, sendo que uma proporção de machos permanece na Corrente das Canárias durante todo o ano. Esta tese combina o seguimento remoto em grande escala, durante dois anos, com uma abordagem multidisciplinar, respondendo às seguintes questões:

i. A estratégia migratória correlaciona-se com a personalidade nas cagarras?
ii. A estratégia migratória e o sucesso reprodutor têm consequências na época reprodutora seguinte em termos de condição física?
iii. As cagarras migradoras e residentes estão diferencialmente expostas a poluentes durante o período não reprodutor?

Foram realizados testes comportamentais para avaliar o comportamento exploratório e a reação à extração do ninho em machos de cagarras, e testámos correlações com a estratégia migratória. Embora o comportamento exploratório não tenha estado relacionado com a estratégia migratória, os machos residentes mostraram maior probabilidade de não reagir à extração do ninho.

A estratégia migratória e o insucesso reprodutor precoce tiveram implicações na imunidade inata, stress e massa corporal residual de machos no regresso à colónia no ano seguinte. As aves residentes mostraram uma menor intensidade das malformações nas penas caudais (*fault bars* - um indicador de stress durante o período não reprodutor), em comparação com as migradoras. A estratégia migratória e o esforço reprodutivo não tiveram outros impactos na condição fisiológica.

Neste estudo, foi quantificada a extensão da exposição ao mercúrio durante o período não reprodutor em indivíduos que passaram esse período em diferentes áreas, e relacionado com a posição trófica. As cagarras que permaneceram residentes na Corrente das Canárias tiveram concentrações de mercúrio nas penas mais baixas do que as migradoras, como resultado da posição trófica significativamente mais baixa ocupada pelos residentes durante o período não reprodutor. As diferenças na reação comportamental e fisiológica ao stress entre machos migradores e residentes sugerem que os primeiros podem-se distinguir pelo limiar de stress. Demonstrado que a manutenção somática após eventos de ciclos anuais com elevado custo seja priorizada e que a migração tenha um custo baixo nas cagarras. Mesmo assim, é claro que os subgrupos da população experienciam diferentes condições ao longo da época não reprodutora, evidenciada na posição trófica e exposição ao mercúrio. Estas diferenças no ambiente experienciado, em conjunto com as diferenças endógenas da personalidade (*coping style*), sugerem que a migração diferencial tem o potencial de gerar mudanças demográficas nesta população. Este estudo sublinha a importância das variações interindividuais na ecologia e evolução populacional.

Palavras-chave

Migração, personalidade, fisiologia, ecotoxicologia, variações interindividuais

ABSTRACT

Differential migration can result in subgroups of a population experiencing differential fitness as a result of carry-over effects, where conditions experienced over one season influence future life-history stages. Most migratory populations across the animal kingdom display partial migration, whereby a portion of the population migrates while the other remains resident year-round. Despite its ubiquity and its utility in understanding the evolution and ecological consequences of migratory decisions, partial migration has been under-studied. Tubenoses (Order Procellariiformes) are interesting model species for migration research; they differ from other avian taxa classically studied since dynamic soaring reduces the cost of flight, and they are able to feed while on the move.

The aim of this doctoral thesis is to investigate aspects surrounding the causes and consequences of differential migration, particularly partial migration, in Cory's Shearwater *Calonectris diomedea*. Cory's Shearwaters spend the non-breeding season in several distinct oceanic zones across the Atlantic and into the Indian Ocean, while a proportion of males remain resident in the Canary Current year-round. Specifically, this thesis merges large-scale year-round remote tracking over two years with a multidisciplinary approach to ask:

i. Does migratory strategy correlate with personality traits in the Cory's Shearwater?
ii. Do migration and reproductive success carry-over to the following breeding season in terms of physical condition?
iii. Are migrant and resident Cory's Shearwaters differentially exposed to pollutants over the non-breeding period?

We carried out behavioural tests evaluating exploratory behaviour and reaction to extraction from the nest in male Cory's Shearwaters, and tested for correlations with migratory strategy. While exploratory behaviour was unrelated to migratory strategy, males that were resident were more likely to be unreactive towards extraction from the nest.

We investigated whether migratory strategy and early reproductive failure had implications on innate immunity, stress, and residual body mass of males on return to the colony the following year. Residents had a lower tail feather fault bar intensity, an indicator of stress during the non-breeding period, compared to migrants. Migratory strategy and reproductive effort had no impact on physiological condition otherwise.

We quantified the extent of over-winter mercury exposure in individuals spending the non-breeding period in different areas, and related it to trophic position. Cory's Shearwaters that remained resident in the Canary Current had lower feather mercury concentrations than migrants, as a result of the significantly lower trophic position residents occupied during the non-breeding season studied.

The differences in behavioural and physiological stress response between migratory and resident males suggest that they may be distinguishable by their stress threshold. While we show that somatic maintenance after costly annual cycle events is prioritised and that migration is cheap in the Cory's Shearwater, it is clear that sub-groups of the population experience different conditions over the non-breeding season, evidenced in trophic position and mercury exposure. Both this and endogenous differences in coping style suggest that differential migration has the potential to drive demographic changes in this population. This collection of studies continues to bring to the forefront the importance of inter-individual variations in population ecology and evolution.

Keywords

Migration ecology, personality, physiology, ecotoxicology, inter-individual variation

CHAPTER 1

GENERAL INTRODUCTION

Migration is a phenomenon observed in animal species across all major taxonomic groups and which has captured our fascination and curiosity since time immemorial. Annual migrations involve seasonal, round-trip movements, generally between breeding grounds and non-breeding areas, driven by the search of resources. In order to correctly understand and interpret its evolution migration must be defined as a property of the individual on which selection can act and shape population outcomes (Dingle & Alistair Drake, 2007). As a result, studying the causes and consequences of inter-individual variability in migratory strategy becomes pivotal in shaping our understanding of the evolution of migration.

DIFFERENTIAL MIGRATION

Differential migration can take the form of variability in migratory phenology, routes, or destinations, and can range between microhabitat scales to trans-continental differences in non-breeding areas, commonly among individuals of different age groups, social dominance status, or sex (Briedis & Bauer, 2018). One of the various forms of differential migration is partial migration, whereby a portion of the population migrates while the other remains resident year-round (Chapman, Brönmark, Nilsson, & Hansson, 2011; Lack, 1944). Differential migration reduces migratory connectivity – the extent to which populations are spatio-temporally connected throughout the annual cycle. As a result, subgroups of a population may experience different environmental conditions, possibly leading to differential fitness (Briedis & Bauer, 2018). Therefore, the persistence of differential migration depends on either an evolutionary stable state, whereby either strategy has equivalent fitness returns, or on a conditional strategy in which changing individual states or environmental conditions create an overall balance in the benefits of the two strategies (Chapman et al., 2011; Lundberg, 1988). While weak migratory connectivity is often associated with greater population resistance due to the buffering of negative impacts on the population as a whole (Gilroy, Gill, Butchart, Jones, & Franco, 2016), impacts on population dynamics could rather be amplified, depending on which demographic group experiences negative effects, particularly in the case of sexual segregation (Briedis & Bauer, 2018).

The mechanism by which conditions experienced over one season influence future life-history stages are termed carry-over effects (Harrison, Blount, Inger, Norris, & Bearhop, 2011; Norris & Marra, 2007). There is abundant evidence that the non-breeding season affects future reproductive attempts in well-studied avian systems, where diet and habitat quality during the non-breeding period have impacts on arrival time, condition at breeding grounds and the probability of reproductive success (Bearhop, Hilton, Votler, & Waldron, 2004; Hoye, Hahn, Nolet, & Klaassen, 2012; Marra, Hobson, & Holmes, 1998; Norris, Marra, Kyser, Sherry, & Ratcliffe, 2004; Shoji et al., 2015). Conversely, how conditions experienced during the reproductive period may influence subsequent annual cycle stages as a result of the costs of reproduction has been generally less explored (Harrison et al., 2011, but see below). At a broader timescale, differential migration can directly influence the persistence of populations or species, as explained above. A recent multi-taxa meta-analysis of the fitness balance between different migratory strategies has found that residents show higher survival probability than migrants, with a smaller improvement in breeding success (Buchan, Gilroy, Catry, & Franco, 2020), in line with expectations that environmental change, particularly milder climatic conditions, may favour residency (Reid et al., 2018).

Most migratory populations across the animal kingdom display partial migration (Chapman et al., 2011). Despite its ubiquity and its utility in understanding the evolution and ecological consequences of migratory decisions, partial migration has been under-studied, but has been attracting increasing attention with the improved applicability of remote tracking technologies to determine the movements of individuals over the annual cycle (Buchan et al., 2020; Chapman et al., 2011; McKinnon & Love, 2018; Reid et al., 2018). Until recently, there has been a lack of studies comparing different migratory strategies within the same population, which leaves many unanswered questions on what demographic consequences inter-individual variation in migratory decisions can actually have (Reid et al., 2018). At the base of these questions is the theory of a migratory syndrome, whereby there are common physiological and behavioural traits that separate migrants from residents (Dingle, 2006). However, these are likely phylogenetically constrained, meaning that the causes and consequences of migratory decisions would be different in different taxa (Piersma, Pérez-Tris, Mouritsen, Bauchinger, & Bairlein, 2005).

SEABIRDS AS MODEL SPECIES

Seabirds are marine top-predators in all the world’s oceans. They have been the target of ecological research for decades as a result of the conspicuousness of their spectacular large breeding colonies. During the breeding period, they act as central-place foragers, constrained by the need to return to their egg or chick at the breeding colony. However, once the breeding period ends they are free to exploit more favourable habitats elsewhere, resulting in inter-seasonal changes in their foraging ecology and diet (R. Phillips, Lewis, González-Solís, & Daunt, 2017; Schreiber & Burger, 2002). Their migratory behaviour has until recently been poorly understood due to the remoteness of non-breeding areas out at sea (Burger & Shaffer, 2008). Some of the longest and fastest migrations are carried out by seabird species (Croxall, 2005; Egevang et al., 2010; Shaffer et al., 2006). Tubenoses (Order Procellariiformes) in particular are interesting model species for migration research since they differ from other taxa classically studied (such as passerines and waders) in that the cost of flight can often be very low as a result of dynamic soaring, and they are able to feed while on the move (Dias, Granadeiro, & Catry, 2012).

Among these long-distance migrants are several examples of differential migration (Davis, Maftei, & Mallory, 2016; González-Solís, Croxall, Oro, & Ruiz, 2007; Perez, Granadeiro, Dias, Alonso, & Catry, 2014; R. A. Phillips, Silk, Croxall, Afanasyev, & Bennett, 2005; Shaffer et al., 2006). Reproductive outcome has been found to influence migratory decisions in seabirds in terms of timing of outward migration and duration of the non-breeding period (Bogdanova et al., 2017; Catry, Dias, Phillips, & Granadeiro, 2013; Fayet et al., 2016; R. A. Phillips et al., 2005), and non-breeding spatial distribution (Bogdanova et al., 2011; Jaeger et al., 2014). Sexual segregation in diet or distribution appears to be common in dimorphic seabird species, both during the breeding (R. A. Phillips, McGill, Dawson, & Bearhop, 2011) and non-breeding seasons (R. A. Phillips, Bearhop, McGill, & Dawson, 2009).

Seabirds are often used as sentinels of ocean health in terms of productivity and pollutants as they integrate the effects of changes along the food chain. Moreover, as a result of the wide-ranging movements of more pelagic species, they also respond to change at different spatial scales (Piatt, Sydeman, & Wiese, 2007). At the same time, seabirds are one of the most threatened avian groups in the world, with almost half of all species listed as threatened or near threatened in the International Union for Conservation of Nature (IUCN) Red List (BirdLife International, 2018). Invasive alien predators at the colony, fisheries bycatch, and climate change together affect two-thirds of seabird species (Dias et al., 2019). Their life histories – long-lived with delayed sexual maturity, high reproductive investment into few offspring, and philopatry – make them particularly vulnerable to suffering population declines (Brooke, 2004; Dias et al., 2019). As a result, understanding the consequences of their differential movements is also of conservation concern.

THE CORY'S SHEARWATER STUDY SYSTEM

The Cory's Shearwater, *Calonectis borealis*, is a medium-sized procellariiforme seabird with a breeding distribution in the North East Atlantic and near Mediterranean. Individuals are highly faithful to their breeding colonies – predominantly insular sites in the North East Atlantic and near Mediterranean – and generally migrate long distances to winter across the Atlantic Ocean (BirdLife International, 2017). Like other procellariiformes, they are also largely faithful to their reproductive partners, and invest in a prolonged breeding season during which they lay a single egg without the possibility of replacing it (Brooke, 2004).

The species' largest breeding colony is on the Portuguese island of Selvagem Grande in the Madeiran archipelago (Lat 30.140556, Long -015.868889), home to around 30 000 breeding pairs (Granadeiro, Dias, Rebelo, Santos, & Catry, 2006). Over 450 regularly occupied nest cavities within rubble walls are the subject of long-term research, with annual monitoring of nest occupancy and breeding output resulting in hundreds of ringed individuals, many of known or approximated ages (Catry, Granadeiro, Ramos, Phillips, & Oliveira, 2011; Dias, Granadeiro, Phillips, Alonso, & Catry, 2011). Year-round tracking of a number of these individuals has uncovered detailed information on the variability of their migratory strategy at both the population and the individual level. At the population scale, Cory's Shearwaters spend the non-breeding season in several distinct oceanic zones across the Atlantic and into the Indian Ocean – the majority migrate to the Benguela Current, a smaller proportion continues into the Indian Ocean to the Agulhas Current, and smaller numbers migrate to the Brazilian Current, the central South Atlantic, or to the North West Atlantic (Dias et al., 2011). However, a proportion of adults, almost exclusively males, remain resident in the Canary Current year-round, a strategy thought to confer reproductive advantages the following year as a result of earlier return to the breeding colony where competition for nest sites is harsh (Perez et al., 2014).

Down at the individual level, repeated tracking of individuals over several years has revealed how individuals exhibit high non-breeding destination fidelity over their tracking history. Nevertheless, they demonstrate the ability to occasionally migrate to different wintering sites, switching between the northern and southern hemisphere, between West and East Atlantic, or between the Atlantic and Indian Oceans (Dias, Granadeiro, & Catry, 2013; Dias et al., 2011). Age, sex, individual quality, or site quality did not explain the tendency to change non-breeding destinations between years (Dias et al., 2011). Individual choice of non-breeding destination is also unrelated to age, and sex only determines residency (male biased) or migration into the Agulhas Current (female biased) (Perez et al., 2014). Many of the potential causes behind such different movement decisions remain unexplored. Moreover, the spatially separated wintering grounds and common breeding site allow for the exploration of differing carry-over effects from separate non-breeding areas and environmental monitoring across a whole ocean using a single species (Alves et al., 2016; Costantini et al., 2014).

This thesis provides novel outlooks into the causes and consequences of individual variation in migratory behaviour, with a special relevance to pelagic marine birds, by investigating how personality, physiology, and heavy metal exposure relate to migratory strategy in the Cory's Shearwater.

OBJECTIVES OF THIS THESIS

The aim of this doctoral thesis is to investigate aspects surrounding the causes and consequences of differential migration, particularly partial migration, in the Cory's Shearwater. Specifically, we asked:

i. Does migratory strategy correlate with personality traits in the Cory's Shearwater?
ii. Do migration and reproductive success carry-over to the following breeding season in terms of physical condition?
iii. Are migrant and resident Cory's Shearwaters differentially exposed to mercury pollution over the non-breeding period?

The investigations into these research questions are compiled here, as manuscripts of scientific articles, as follows:

Chapter 2: Migrants and residents of a long-lived seabird differ in their behavioural response to a stressor

Inter-individual differences in behaviour have been recognised as a major driver of population ecology, but correlations between animal personality and migratory strategy have rarely been carried out. We investigated whether male Cory's Shearwaters show consistent behavioural differences in their exploratory behaviour and in their reaction to extraction from the nest, and correlated their behavioural traits with migratory strategy over a two year period.

Chapter 3: Reproduction and migration do not carry costs in physical condition between years in a long-lived seabird

We looked into trade-offs between annual cycle activities and somatic self-maintenance, reflected in innate immune indices, stress markers, and residual body mass. The physiological carry-over effects of migration have rarely been investigated by comparing migratory strategies within the same population.

Chapter 4: Generalist seabirds as biomonitors of ocean mercury: the importance of accurate trophic position assignment

We compared mercury exposure and trophic position during the non-breeding period in Cory’s Shearwaters of different migratory strategies. This chapter makes use of compound specific isotope analysis of amino acids (CSIA-AA) in order to reliably compare trophic positions over distant geographic areas with different nitrogen baseline isoscapes.

Chapter 5: Untangling causes of variation in mercury concentration between flight feathers

This chapter supports the interpretation of feather mercury from a late-moulted flight feather in the previous chapter by investigating the mechanisms controlling the deposition of mercury into feathers.

Moreover, two further manuscripts on topics related to the subject of this thesis which I co-authored during the course of this PhD are enclosed as appendices:

Appendix 1: Sex-specific telomere length and dynamics in relation to age and reproductive success in Cory’s shearwaters

We measured telomere length and dynamics – a biomarker for ageing – in Cory's Shearwaters to assess their relationship to age, sex and reproductive success, both from one year to the next and using long-term data.

Appendix 2: Global monitoring of mercury in the mesopelagic domain using body feathers of Bulwer's petrel as a bioindicator

Feather mercury and CSIA-derived trophic position were assessed in Bulwer’s Petrels (*Bulweria bulwerii*) from breeding colonies in both the Atlantic and Pacific oceans. Unlike

Cory’s Shearwaters, which are generalists, Bulwer’s Petrels are specialised predators of mesopelagic prey.

REFERENCES

Alves, L. M. F., Nunes, M., Marchand, P., Le Bizec, B., Mendes, S., Correia, J. P. S., … Novais, S. C. (2016). Blue sharks (*Prionace glauca*) as bioindicators of pollution and health in the Atlantic Ocean: Contamination levels and biochemical stress responses. *Science of The Total Environment*, *563–564*, 282–292. https://doi.org/10.1016/j.scitotenv.2016.04.085

Bearhop, S., Hilton, G. M., Votler, S. C., & Waldron, S. (2004). Stable isotope ratios indicate that body condition in migrating passerines is influenced by winter habitat. *Proceedings of the Royal Society B: Biological Sciences*, *271*(SUPPL. 4), 215–218. https://doi.org/10.1098/rsbl.2003.0129

BirdLife International. (2017). *Species factsheet: Calonectris borealis*. Retrieved from http://datazone.birdlife.org/species/factsheet/corys-shearwater-calonectris-borealis/text

BirdLife International. (2018). *State of the world’s birds: taking the pulse of the planet*. Cambridge, UK: BirdLife International.

Bogdanova, M. I., Butler, A., Wanless, S., Moe, B., Anker-Nilssen, T., Frederiksen, M., … Daunt, F. (2017). Multi-colony tracking reveals spatio-temporal variation in carry-over effects between breeding success and winter movements in a pelagic seabird. *Marine Ecology Progress Series*, *578*, 167–181. https://doi.org/10.3354/meps12096

Bogdanova, M. I., Daunt, F., Newell, M., Phillips, R. A., Harris, M. P., & Wanless, S. (2011). Seasonal interactions in the Black-legged Kittiwake, *Rissa tridactyla*: links between breeding performance and winter distribution. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1717), 2412–2418. https://doi.org/10.1098/rspb.2010.2601

Briedis, M., & Bauer, S. (2018). Migratory connectivity in the context of differential migration. *Biology Letters*, *14*(12), 20180679. https://doi.org/10.1098/rsbl.2018.0679

Brooke, M. (2004). *Albatrosses and petrels across the world*. Oxford University Press, Oxford.

Buchan, C., Gilroy, J. J., Catry, I., & Franco, A. M. A. (2020). Fitness consequences of different migratory strategies in partially migratory populations: A multi-taxa meta-analysis. *Journal of Animal Ecology*, *89*(3), 678–690. https://doi.org/10.1111/1365-2656.13155

Burger, A. E., & Shaffer, S. A. (2008). Application of tracking and data-logging technology in research and conservation of seabirds. *The Auk*, *125*(2), 253–264. https://doi.org/10.1525/auk.2008.1408

Catry, P., Dias, M. P., Phillips, R. A., & Granadeiro, J. P. (2013). Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: an experimental demonstration. *Ecology*, *94*(6), 1230–1235. https://doi.org/10.1890/12-2177.1

Catry, P., Granadeiro, J. P., Ramos, J., Phillips, R. A., & Oliveira, P. (2011). Either taking it easy or feeling too tired: Old Cory's Shearwaters display reduced activity levels while at sea. *Journal of Ornithology*, *152*(3), 549–555. https://doi.org/10.1007/s10336-010-0616-7

Chapman, B. B., Brönmark, C., Nilsson, J.-Å., & Hansson, L.-A. (2011). The ecology and evolution of partial migration. *Oikos*, *120*(12), 1764–1775. https://doi.org/10.1111/j.1600-0706.2011.20131.x

Costantini, D., Meillère, A., Carravieri, A., Lecomte, V., Sorci, G., Faivre, B., … Chastel, O. (2014). Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. *Oecologia*, *175*, 1107–1116. https://doi.org/10.1007/s00442-014-2975-x

Croxall, J. P. (2005). Global Circumnavigations: Tracking Year-Round Ranges of Nonbreeding Albatrosses. *Science*, *307*(5707), 249–250. https://doi.org/10.1126/science.1106042

Davis, S. E., Maftei, M., & Mallory, M. L. (2016). Migratory connectivity at high latitudes: Sabine's Gulls (*Xema sabini*) from a colony in the Canadian High Arctic migrate to different oceans. *PLOS ONE*, *11*(12), e0166043. https://doi.org/10.1371/journal.pone.0166043

Dias, M. P., Granadeiro, J. P., & Catry, P. (2012). Do seabirds differ from other migrants in their travel arrangements? On route strategies of Cory's Shearwater during its trans-equatorial journey. *PLoS ONE*, *7*(11), e49376. https://doi.org/10.1371/journal.pone.0049376

Dias, M. P., Granadeiro, J. P., & Catry, P. (2013). Individual variability in the migratory path and stopovers of a long-distance pelagic migrant. *Animal Behaviour*, *86*(2). https://doi.org/10.1016/j.anbehav.2013.05.026

Dias, M. P., Granadeiro, J. P., Phillips, R. A., Alonso, H., & Catry, P. (2011). Breaking the routine: individual Cory's shearwaters shift winter destinations between hemispheres and across ocean basins. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1713), 1786–1793. https://doi.org/10.1098/rspb.2010.2114

Dias, M. P., Martin, R., Pearmain, E. J., Burfield, I. J., Small, C., Phillips, R. A., … Croxall, J. P. (2019). Threats to seabirds: A global assessment. *Biological Conservation*, *237*(June), 525–537. https://doi.org/10.1016/j.biocon.2019.06.033

Dingle, H. (2006). Animal migration: is there a common migratory syndrome? *Journal of Ornithology*, *147*(2), 212–220. https://doi.org/10.1007/s10336-005-0052-2

Dingle, H., & Alistair Drake, V. (2007). What is migration? *BioScience*, *57*(2), 113–121. https://doi.org/10.1641/B570206

Egevang, C., Stenhouse, I. J., Phillips, R. A., Petersen, A., Fox, J. W., & Silk, J. R. D. (2010). Tracking of Arctic terns *Sterna paradisaea* reveals longest animal migration. *PNAS*, *107*(5), 2078–2081. https://doi.org/10.1073/pnas.0909493107

Fayet, A. L., Freeman, R., Shoji, A., Kirk, H. L., Padget, O., Perrins, C. M., & Guilford, T. (2016). Carry-over effects on the annual cycle of a migratory seabird: an experimental study. *Journal of Animal Ecology*, *85*(6), 1516–1527. https://doi.org/10.1111/1365-2656.12580

Gilroy, J. J., Gill, J. A., Butchart, S. H. M., Jones, V. R., & Franco, A. M. A. (2016). Migratory diversity predicts population declines in birds. *Ecology Letters*, *19*(3), 308–317. https://doi.org/10.1111/ele.12569

González-Solís, J., Croxall, J. P., Oro, D., & Ruiz, X. (2007). Trans-equatorial migration and mixing in the wintering areas of a pelagic seabird. *Frontiers in Ecology and the*

Environment, *5*(6), 297–301. https://doi.org/10.1890/1540-9295(2007)5[297:TMAMIT]2.0.CO;2

Granadeiro, J. P., Dias, M. P., Rebelo, R., Santos, C. D., & Catry, P. (2006). Numbers and population trends of Cory’s Shearwater *Calonectris diomedea* at Selvagem Grande, Northeast Atlantic. *Waterbirds*, *29*(1), 56–60. https://doi.org/10.1675/1524-4695(2006)29[56:naptoc]2.0.co;2

Harrison, X. A., Blount, J. D., Inger, R., Norris, D. R., & Bearhop, S. (2011). Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology*, *80*(1), 4–18. https://doi.org/10.1111/j.1365-2656.2010.01740.x

Hoye, B. J., Hahn, S., Nolet, B. A., & Klaassen, M. (2012). Habitat use throughout migration: Linking individual consistency, prior breeding success and future breeding potential. *Journal of Animal Ecology*, *81*(3), 657–666. https://doi.org/10.1111/j.1365-2656.2011.01948.x

Jaeger, A., Goutte, A., Lecomte, V. J., Richard, P., Chastel, O., Barbraud, C., … Cherel, Y. (2014). Age, sex, and breeding status shape a complex foraging pattern in an extremely long-lived seabird. *Ecology*, *95*(8), 2324–2333. https://doi.org/10.1890/13-1376.1

Lack, D. (1944). The problem of partial migration. *British Birds*, *37*(122–130).

Lundberg, P. (1988). The evolution of partial migration in Birds. *Trends in Ecology and Evolution*, *3*(7), 172–175. https://doi.org/10.1016/0169-5347(88)90035-3

Marra, P. P., Hobson, K. A., & Holmes, R. T. (1998). Linking winter and summer events in a migratory bird by using stable- carbon isotopes. *Science*, *282*(5395), 1884–1886. https://doi.org/10.1126/science.282.5395.1884

McKinnon, E. A., & Love, O. P. (2018). Ten years tracking the migrations of small landbirds: Lessons learned in the golden age of bio-logging. *The Auk*, *135*(4), 834–856. https://doi.org/10.1642/AUK-17-202.1

Norris, D. R., & Marra, P. P. (2007). Seasonal interactions, habitat quality, and population dynamics in migratory birds. *The Condor*, *109*(3), 535. https://doi.org/10.1650/8350.1

Norris, D. R., Marra, P. P., Kyser, T. K., Sherry, T. W., & Ratcliffe, L. M. (2004). Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1534), 59–64. https://doi.org/10.1098/rspb.2003.2569

Perez, C., Granadeiro, J. P., Dias, M. P., Alonso, H., & Catry, P. (2014). When males are more inclined to stay at home: insights into the partial migration of a pelagic seabird provided by geolocators and isotopes. *Behavioral Ecology*, *25*(2), 313–319. https://doi.org/10.1093/beheco/art119

Phillips, R. A., Bearhop, S., McGill, R. A. R., & Dawson, D. A. (2009). Stable isotopes reveal individual variation in migration strategies and habitat preferences in a suite of seabirds during the nonbreeding period. *Oecologia*, *160*(4), 795–806. https://doi.org/10.1007/s00442-009-1342-9

Phillips, R. A., McGill, R. A. R., Dawson, D. A., & Bearhop, S. (2011). Sexual segregation in distribution, diet and trophic level of seabirds: Insights from stable isotope analysis. *Marine Biology*, *158*(10), 2199–2208. https://doi.org/10.1007/s00227-011-1725-4

Phillips, R. A., Silk, J. R. D., Croxall, J. P., Afanasyev, V., & Bennett, V. J. (2005). Summer distribution and migration of nonbreeding albatrosses: Individual consistencies and implications for conservation. *Ecology*, *86*(9), 2386–2396. https://doi.org/10.1890/04-1885

Phillips, R., Lewis, S., González-Solís, J., & Daunt, F. (2017). Causes and consequences of individual variability and specialization in foraging and migration strategies of seabirds. *Marine Ecology Progress Series*, *578*, 117–150. https://doi.org/10.3354/meps12217

Piatt, J. F., Sydeman, W. J., & Wiese, F. (2007). Introduction: A modern role for seabirds as indicators. *Marine Ecology Progress Series*, *352*, 199–204. https://doi.org/10.3354/meps07070

Piersma, T., Pérez-Tris, J., Mouritsen, H., Bauchinger, U., & Bairlein, F. (2005). Is There a “Migratory Syndrome” Common to All Migrant Birds? *Annals of the New York Academy of Sciences*, *1046*(1), 282–293. https://doi.org/10.1196/annals.1343.026

Reid, J. M., Travis, J. M. J., Daunt, F., Burthe, S. J., Wanless, S., & Dytham, C. (2018). Population and evolutionary dynamics in spatially structured seasonally varying environments. *Biological Reviews*, *93*(3), 1578–1603. https://doi.org/10.1111/brv.12409

Schreiber, E. A., & Burger, J. (2002). *Biology of marine birds*. CRC Press.

Shaffer, S. A., Tremblay, Y., Weimerskirch, H., Scott, D., Thompson, D. R., Sagar, P. M., … Costa, D. P. (2006). Migratory shearwaters integrate oceanic resources across the Pacific Ocean in an endless summer. *Proceedings of the National Academy of Sciences of the United States of America, 103*(34), 12799–12802. https://doi.org/10.1073/pnas.0603715103

Shoji, A., Aris-Brosou, S., Culina, A., Fayet, A., Kirk, H., Padget, O., … Guilford, T. (2015). Breeding phenology and winter activity predict subsequent breeding success in a trans-global migratory seabird. *Biology Letters, 11*(10), 20150671. https://doi.org/10.1098/rsbl.2015.0671

CHAPTER 2

MIGRANTS AND RESIDENTS OF A LONG-LIVED SEABIRD DIFFER IN THEIR BEHAVIOURAL RESPONSE TO A STRESSOR

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ABSTRACT

Inter-individual variation in behaviour has been recognised as a major driver of population ecology, but its relationship to migratory strategy has been ill-explored. Here, we investigated whether male migrant and resident Cory's Shearwaters (*Calonectris borealis*), a long-lived partially migratory seabird, are distinguishable by their temperament at the colony. We tracked a large number of individuals over two winters using GLS devices and assessed whether exploratory behaviour and reaction to extraction from the nest corresponded to migratory strategy over this period. While exploratory behaviour was unrelated to migratory strategy, birds that were resident were more likely to be unreactive towards extraction from the nest. This difference in behavioural stress response, together with previous findings that migrants display higher physiological stress over winter, suggests that migrants and residents may be distinguishable by their stress threshold. Insights into how temperament is related to the causes and consequences of differential migration, such as this study, may help us understand possible variability in resilience to environmental change within species and populations.

Keywords: behaviour, *Calonectris borealis*, partial migration, personality, seabird, stress response

INTRODUCTION

Inter-individual variation in behaviour has been recognised as a major driver of population ecology; individual responses in exploratory behaviour, boldness, and aggression, among others, have been related to each other, to survival and fitness, and explained in terms of evolutionary stability (Chapman, Hulthén, et al., 2011; Dingemanse & Réale, 2005; A. L. K. Nilsson, Nilsson, Alerstam, & Bäckman, 2010; Réale, Reader, Sol, Mcdougall, & Dingemanse, 2007; Wolf & Weissing, 2012). Studies of individual behavioural responses to stimuli, or temperament, have found long-lived individual consistencies constituting personality traits, and correlations of personality traits within populations, creating behavioural syndromes (Carter, Feeney, Marshall, Cowlishaw, & Heinsohn, 2013). Despite the ubiquity of partial migration in the animal kingdom (Chapman, Brönmark, Nilsson, &

Hansson, 2011) and our increasing ability to track animal movements, investigations into temperamental correlates of migratory tendency are few and the subject remains ill-explored (Chapman, Hulthén, et al., 2011; Found & St. Clair, 2017; A. L. K. Nilsson et al., 2010; Odermatt, Frommen, & Menz, 2017).

The Cory's Shearwater (*Calonectris borealis*) is a long-lived, monogamous, migratory seabird in which males are partial migrants - in the population breeding on Selvagem Grande, Madeira, a small proportion of males remain resident in the upwelling Canary Current surrounding the breeding colony, while the rest of the population undertakes trans-equatorial migrations to non-breeding areas off southern Africa and in the south-west Atlantic (Perez, Granadeiro, Dias, Alonso, & Catry, 2014). Despite an apparent flexibility in over-winter movements and destinations, individuals are largely faithful to a particular non-breeding area (Dias, Granadeiro, Phillips, Alonso, & Catry, 2011). Migratory males return to the colony showing signs of higher overwinter stress levels than residents, as assessed by feather corticosterone (Pérez, Granadeiro, Dias, & Catry, 2016) and tail feather fault bar intensity (own unpublished data). This difference could either be attributed to harsher environmental conditions experienced at distant non-breeding areas, although evidence of carry-over effects from this is absent (own unpublished data), or to an intrinsic difference in stress responses between migrants and residents (J.-Å. Nilsson, Brönmark, Hansson, & Chapman, 2014).

To date, there has been some research relating personality to foraging movements in seabirds (Kittiwake *Rissa tridactyla*: Harris et al., 2019; Cory's Shearwater *Calonectris borealis*: Krüger, Pereira, Paiva, & Ramos, 2019; Black-browed Albatross *Thalassarche melanophris*: Patrick & Weimerskirch, 2014). The general indication is that bold individuals are more superficial explorers, less flexible in their foraging movements, and fare better in conditions with high competition and high environmental predictability. These "proactive" personality types are often associated with low physiological stress responses, as opposed to "reactive" personalities on the other end of the spectrum, which elicit high physiological stress responses and are associated with cautious, fearful, and slow exploring personalities (Cockrem, 2007; Koolhaas et al., 1999). While proactive personality traits have been seen in migratory individuals of Roach (*Rutilus rutilus*) and Blue Tits (*Cyanistes caeruleus*) (Chapman, Hulthén, et al., 2011; A. L. K. Nilsson et al., 2010), it is not yet known whether this trend persists in partially migratory seabirds.

Here we explored whether migratory strategy in male Cory's Shearwaters is linked to two behavioural traits assessed during the pre-laying and incubation periods: (i) exploration of a novel environment and (ii) response to extraction from the nest. The same males were tracked overwinter using GLS loggers. Based on the current scientific knowledge, and given the predominant medium-term, individual persistence in migratory strategy (Dias, Granadeiro, & Catry, 2013; Perez et al., 2014), we expected migrant and resident males to differ in their behaviour, reflecting evolutionary correlates between migratory strategy and the behavioural adaptations required in the different environmental and biotic pressures experienced. We also investigated the potential confounding effect of age due to the effect prior experience or pace-of-life could have on behaviour (Hall et al., 2015; A. L. K. Nilsson, Nilsson, & Mettke-Hofmann, 2016).

Male Cory’s Shearwaters that remain resident return to the colony earlier the following breeding season compared to migrants, which increases the probability of securing a nest and successfully reproducing (Perez et al., 2014). In order to disentangle possible effects of previous reproductive success/effort from those of migratory strategy on temperament, we compared the behaviour of individuals in which reproductive failure was experimentally induced against that of control individuals that successfully reproduced.

METHODS

STUDY SYSTEM AND GLS TRACKING

Selvagem Grande (Lat 30.140556, Long -015.868889) is an island nature reserve that forms part of the Madeiran Archipelago, Portugal. Around 30 000 pairs of Cory’s Shearwaters breed on the island, arriving at their nest burrows between mid-February and early April (hereafter, the pre-laying period) to undertake a long period of nest defence and mating before laying a single egg in early June, which they incubate for around two months. Over 450 regularly occupied nest cavities within rubble walls are the subject of long-term research, with annual monitoring of nest occupancy and breeding output resulting in hundreds of ringed individuals, many of known or approximated ages (Catry, Granadeiro, Ramos, Phillips, & Oliveira, 2011; Dias et al., 2011).

Cory’s Shearwaters occupy distinct oceanic currents during the non-breeding period. The majority migrate to the Benguela and Agulhas Currents, while smaller numbers migrate to currents in the southwest and northwest Atlantic. Resident males remain largely in the Canary Current. These movements have been extensively studied in this population and non-breeding destinations described in several publications (Catry, Dias, Phillips, & Granadeiro, 2011; Dias, Granadeiro, & Catry, 2012; Dias et al., 2011; Perez et al., 2014). In 2016 and 2017 we deployed a large number of geolocation loggers (GLS, Migrate Technology model C330, 3.3g, recording mode 9; 122 in 2016, 104 in 2017) to track individuals' migratory movements over winter. GLS light level data was analysed using IntiProc (version 1.03, Migrate Technology Ltd.). Sun elevation angle was calculated from device calibration data, setting a linear elevation angle when both pre- and post-calibration data was available. The (main) non-breeding area was assigned from the mapped tracking data, revealing the migratory strategy of 110 males (98 overwinter movements 2016-2017 and 83 overwinter movements 2017-2018). GLS were leg mounted and weighed less than 1% of the birds' body weight (male Cory's Shearwaters typically weigh over 700g, own data), which, given the attachment method, can be assumed to bear minimal effects on survival and fitness (Bodey et al., 2017). The resighting rate of birds fitted with GLS is consistent with the rate of return of breeding birds from one year to the next, at ca. 85% (Mougin, Jouanin, & Roux, 1997).

As part of a different study assessing carry-over effects of reproductive success, during the breeding season of 2017, 25 nests were selected for egg removal to experimentally reduce parental effort by inducing early breeding failure (Catry, Dias, Phillips, & Granadeiro, 2013), with another 25 males selected as controls. Each group contained males of a variety of ages and previous migratory strategies. The eggs of these "experimental birds" were removed between the end of June and early July, corresponding to around one month after laying.

BEHAVIOURAL TESTS

We carried out behavioural tests over three breeding seasons between 2016 and 2018.

We assessed exploration in an emergence/novel environment test. Birds were extracted from their nest, placed into a small, black carrier box, and transported to a small room (approximately 2 × 3 m) which served as the novel environment. The bird was then transferred into another black box with a removable flap door situated in a corner of the room. Over a 10 minute time period, birds could leave the open box into the empty room while being filmed. The whole process lasted approximately 20 minutes, keeping the time between capture at the nest and the start of the test constant. Behavioural tests were carried out during the incubation period of 2016 and during both pre-laying and incubation periods of

2017 (see Table 1 for number of birds tested per field season). In total, 114 males were tested; of these, 27 were tested twice over the two years, and five were tested three times. From this test we extracted two scores; whether or not the bird left the box within the stipulated time (binary "emergence" score) and, if it did, the latency (in seconds) for the bird to leave it.

Table 1 The number of individual male Cory's Shearwaters tested for exploration of a novel environment and reaction on extraction from the nest in each field season over the period of study. (Note: Two individuals were tested twice for exploration behaviour during the pre-laying period of 2017)

	2016	**2017**		**2018**	
	Incubation	Pre-laying	Incubation	Pre-laying	Incubation
Exploration	32	69	43	-	-
Extraction scores	-	38	78	91	58

In 2017 and 2018 we tested the reaction on extraction from the nest in male Cory's Shearwaters when retrieving GLS in the pre-laying period and/or as part of annual nest monitoring during incubation. A binary "extraction score" was assigned to classify individuals as either "reactive" (1) or "unreactive" (0) depending on their behaviour throughout the process of extraction from the nest, namely their reaction to an approaching hand, reaction on being caught, and resistance to being pulled out of the nest (Table 2). Combinations of behaviours that could not be classified into either of the two classes according to this classification were not considered. Between 55-60% of all assessments resulted in a classification of either "reactive" or "unreactive". All assessments were performed by the same person to minimise subjectivity, and were only carried out at nests where the target individuals were the sole occupants of the nest at the time of testing, were facing towards the nest entrance, and could be reached easily without the use of a noose – this is the scenario for the vast majority of nests in the study site. Extraction scores were obtained during pre-laying and incubation periods of 2017 and 2018 (see Table 1 for number of birds tested per field season). In total, 153 males were scored; of these, 43 males were tested twice over the two years, 17 three times, and 11 more than three times.

Table 2 Scoring system used to classify Cory's Shearwaters' reaction on extraction from the nest ("extraction score"). An individual had to satisfy all the conditions described in order to be classified as either reactive or unreactive.

Reactive (1)	**The bird bites the approaching hand.** **The bird bites hard and/or attacks and releases continuously.** **The bird struggles on being pulled out and resists extraction.**
Unreactive (0)	The bird does not react to the entering hand, does not try to escape. The head or beak of the bird can be grabbed with little to no reaction from the bird. The bird does not resist extraction.
NA	The bird cannot be scored since it does not satisfy the initial assumptions and/or the conditions for classification.

STATISTICAL ANALYSIS

Statistical analyses were performed on R Statistical Package, version 3.5.1 (R Core Team, 2019).

In 2018, extraction scores were obtained from 20 males from the "experimental" group and 20 control males which successfully reproduced in 2017. We assessed whether previous breeding success had any impact on extraction response the following year by comparing the extraction scores of experimental and control individuals by means of a chi-squared test.

We estimated individual repeatability in exploration (emergence) and extraction over the entire period of study to ensure that individuals were consistent in their behaviour over this timeframe, and that therefore temperament could be related to migratory strategy over the two years. Repeatability was calculated as the intraclass correlation coefficient with a binomial error distribution (package rptR, Nakagawa & Schielzeth, 2010) using bootstrapping without randomisation (1,000 iterations) to estimate confidence intervals. The low number of individuals that left the box in repeated assessments precluded the ability to assess repeatability in latency.

To investigate links between individual temperament and migratory strategy (migratory/resident) we ran generalised linear (mixed-effect) models (function "glm", package *stats* or function “glmer”, package *lme4*) (Bates, Maechler, Bolker, & Walker, 2015) relating latency (quasi-poisson error structure), emergence score, and extraction score (both with a binomial error structure) as dependent variables in separate models to migratory strategy (migrant vs resident) in successfully tracked individuals, with individual as a random intercept in the last two models due to the many repeated assessments on the same individuals. Age and annual cycle stage (pre-laying vs incubation) were added as potentially confounding fixed effects. Testing for an effect of age on the extraction score also tests for habituation to handling, since individuals used in this study experienced being extracted from their nest annually or almost annually throughout their adult life, with older birds having been handled more often. Not all individuals whose behaviour was assessed were successfully tracked, and a small proportion were of unknown age (model sample sizes in Table 3).

Linear regression estimates (“Est”) are presented with their standard error.

RESULTS

Male Cory’s Shearwaters were consistent in their migratory strategy during the two tracked years – of the 75 males tracked in both years, none changed strategy. In total, 19 males remained predominantly in the Canary Current over the non-breeding season, 89 migrated to the south Atlantic, and another two overwintered in the north-west Atlantic.

EXPLORATION

Emergence was not repeatable over the whole dataset (R = 0.103, CI = 0 – 0.282, p = 0.212), nor when analysing only data collected during incubation (R = 0.08, CI = 0 – 0.229, p = 0.304, n = 75 observations of 61 individuals).

Mixed effect model results suggest that annual cycle stage significantly influenced emergence into the novel environment – while 59% and 51% of individuals left the box when tested during incubation periods in 2016 and 2017 respectively, only 28% did during pre-laying 2017 (Table 3). Emergence was not related to age or migratory strategy.

Among those instances where individuals did emerge, their latency to leave the box was determined both by age, with an increase in latency in older birds, and annual cycle stage (Figure 1), but was unrelated to migratory strategy (Table 3).

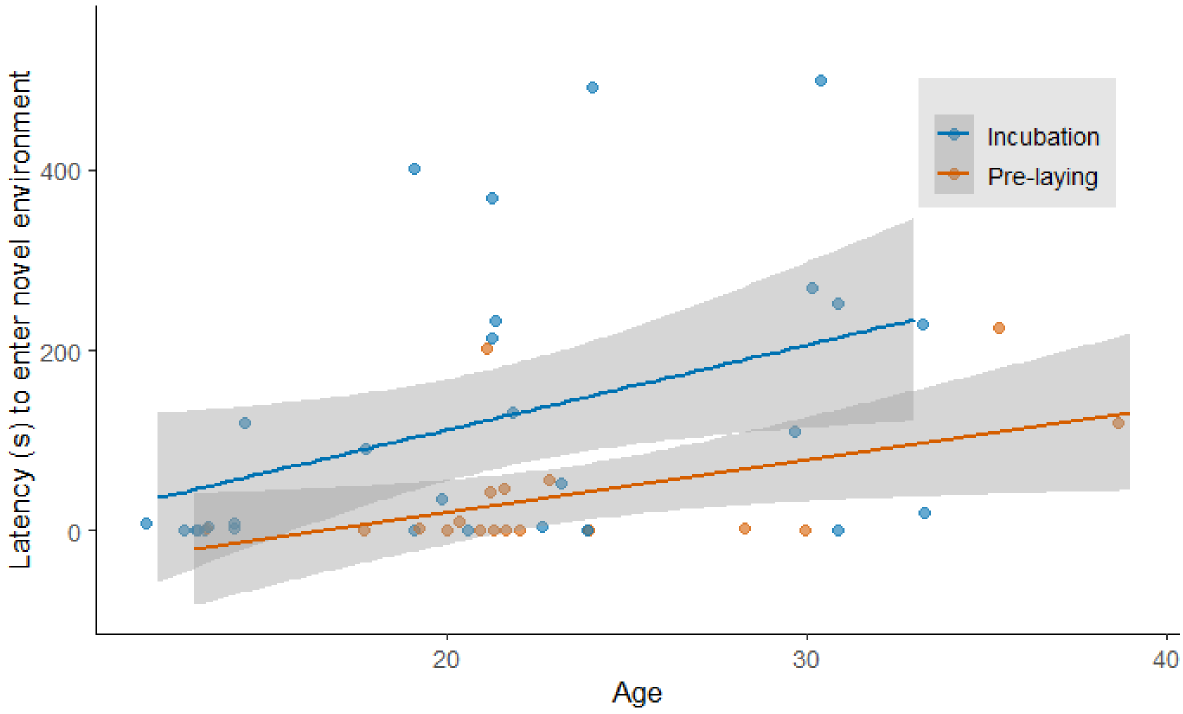

Figure 1 The latency (s) for Cory's Shearwaters to enter a novel environment increased with age. The plot only contains data of individuals that left the box and entered the novel environment during the 10 minute test period. This corresponds to 29 of 50 individuals tested during incubation (blue), but only 18 of 58 individuals tested during the pre-laying period (orange). Shaded areas represent the 95% confidence interval.

Table 3 Results of generalised linear (mixed) models testing the effects of Cory's Shearwater migratory strategy on emergence into a novel environment, the latency for individuals to leave a box into a novel environment, and the behavioural score on extraction from the nest, with age and annual cycle stage (pre-laying vs incubation) as fixed effects. Reference for migratory strategy is resident and for stage is incubation. Significant results (α = 0.05) are in italic.

	Emergence score (n = 102 observations of 81 individuals)			**Latency (n = 43)**			**Extraction score (n = 140 observations of 67 individuals)**		
	Est (± SE)	**z**	**p**	**Est (± SE)**	**t**	**p**	**Est (± SE)**	**z**	**p**
Migratory strategy	-0.32 ± 0.76	-0.42	0.676	0.18 ± 0.51	0.36	0.721	*2.67 ± 1.28*	*2.09*	*0.037*
Age	0.05 ±0.04	1.18	0.240	*0.08 ± 0.03*	*2.48*	*0.018*	-0.05 ± 0.07	-0.72	0.471
Stage	*-1.46 ± 0.70*	*-2.09*	*0.037*	*-1.30 ± 0.52*	*-2.49*	*0.017*	-0.23 ± 0.57	-0.40	0.692

EXTRACTION SCORE

Previous breeding success, experimentally manipulated, had no impact on extraction score the following year ($\chi2 = 1.64$, df = 1, $p = 0.201$, $n = 40$, Table 4). Therefore, we can exclude that differences in extraction score between migrants and residents are a result of possible differences in reproductive success between the two groups. Moreover, while none of the 75 repeatedly tracked males changed migratory strategy over the two years, 33 experienced a change in their reproductive outcome.

Table 4 The number of Cory's Shearwaters from the experimental group (failed breeders) and control group (successful breeders) that fell within either of the two classifications of reaction from extraction from the nest (unreactive vs reactive).

	Extraction score	
	Unreactive	Reactive
Experimental group – failed breeders	14	6
Control group - successful breeders	9	11

Repeatability estimates suggest that Cory's Shearwaters are consistent in their response on extraction, rarely switching from "reactive" to "unreactive" responses over the course of the two years ($R = 0.646$, CI = 0.253 – 0.711, $p < 0.0001$). While R is high compared to those reported in the literature (Bell, Hankison, & Laskowski, 2009), it may be inflated due to the omission of behaviours that did not classify under the two extremes.

The GLMM results suggest that annual cycle stage and age had no influence on extraction score. The lack of an effect of age suggests that there is no indication that birds habituate to extraction from the nest. We found that individuals' response to extraction from the nest was correlated with their migratory strategy; Migratory males ($n = 56$) were more likely to have been reactive towards extraction from the nest than were resident males ($n = 11$) (Figure 2, Table 3).

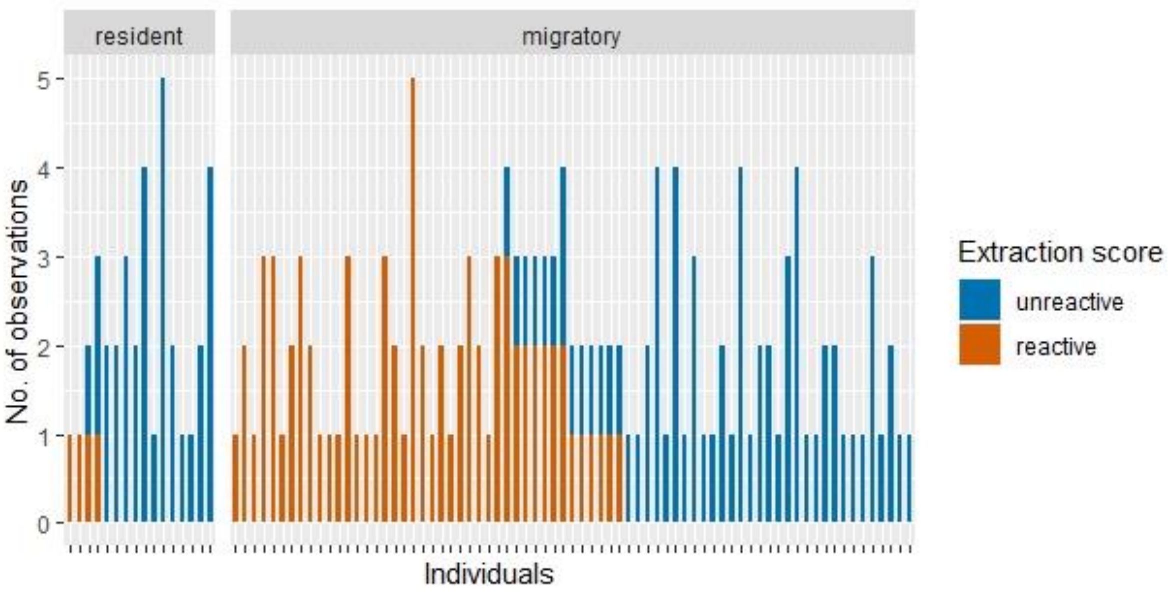

Figure 2 Cory’s Shearwaters’ behavioural reaction to extraction from the nest is correlated with their migratory strategy; resident males are more likely to be unreactive in response to extraction than migrant males. Bars represent the outcomes of assessments of extraction score, with unreactive responses in blue and reactive responses in orange.

DISCUSSION

Our results demonstrate a link between Cory’s Shearwater migratory strategy and reaction on extraction from the nest, and propose a new potential behavioural test that can be further investigated for wider use in similar biological systems. Links between migratory strategy and behavioural traits have so far only been explored in a handful of studies in very few taxa across the animal kingdom, and only one bird species (Roach *Rutilus rutilus*: Chapman, Hulthén, et al., 2011; Wild Elk *Cervus canadensis*: Found & St. Clair, 2017; Blue Tit: A. L. K. Nilsson et al., 2010; Hoverfly *Episyrphus* sps.: Odermatt et al., 2017). This study provides valuable insights into the correlating behavioural features of partial migrants from a perspective that is not very well explored, making within-population comparisons with a large sample size of simultaneously tracked individuals.

Resident male Cory’s Shearwaters were found to be more likely to be unreactive to extraction from the nest than were migrants. Within the same population, migrants and residents have also been found to differ in their physiological stress responses over the non-breeding period, with residents showing lower feather corticosterone concentrations and fewer tail feather fault bars (Pérez et al., 2016; own unpublished data). Tail feather fault bar intensity reported in Gatt et al. (in print; Chapter 3) reflected experienced acute stress, such as antagonistic inter-individual interactions and inclement weather (Jovani & Rohwer, 2016), over the period of tail feather growth between 2017 and 2018, which coincides with the period of behavioural assessments presented here. While we do not exclude that extrinsic factors at the non-breeding areas may be acting, our current finding, that migrant and resident males differ in their behavioural reaction to a standardised stressor at their common breeding colony, suggests that the differences in stress responses reported within this population may be, at least partly, a result of a personality trait within the population with variation in an endogenous threshold to a stress response between males of different migratory strategies.

Our observations appear to contradict the previously reported relationships between physiological stress responses and personality type if the greater reactivity on extraction from the nest is interpreted as aggressiveness forming part of a “proactive” personality (Cockrem, 2007). However, we have observed that the more reactive individuals are more likely to attempt to escape the nest after handling and show more visible stress responses, such as panting. We therefore argue that it is rather low reactivity to extraction from the nest that reflects greater boldness, similar to interpretations of Kittiwakes at the nest presented with a novel object (Harris et al., 2019). Under this interpretation, higher reactivity towards extraction would be a sign of heightened stress response and self-defence, rather than nest defence, making the correlation with higher activity of the parasympathetic system recorded over winter in agreement with previous literature. The understanding of the physiological basis of these behaviours would benefit from the analysis of blood corticosterone alongside behavioural assessments in future studies.

Coping style appears to determine individual spatial distribution and survival under different scenarios (Cockrem, 2012; Dingemanse, Both, Drent, & Tinbergen, 2004; Koolhaas et al., 1999). “Reactive” personalities are more successful in unpredictable conditions due to their higher plasticity in behaviour and more thorough exploration. “Proactive” personalities are less neophobic but explore new environments more superficially and are more repetitive in their behaviour (Benus, Bohus, Koolhaas, & van Oortmerssen, 1991; Cockrem, 2007). These correlated suites of characteristics have already been seen in the foraging behaviour of Cory’s Shearwaters (Krüger et al., 2019). Resilience to competition in bold individuals is also hypothesised to be an important factor determining foraging movements in seabirds, as suggested by their closer foraging distributions relative to the colony (Harris et al., 2019;

Krüger et al., 2019; Patrick & Weimerskirch, 2014). If these trends hold for the determination of migratory strategy in Cory’s Shearwater, we could expect that male Cory’s Shearwaters remaining resident at the Canary Current experience a more predictable environment, similar to that experienced during the breeding season, but perhaps also greater competition than migrants experience.

The novel environment test appears to be assessing different behavioural traits that are not strongly linked to the patterns discussed above and that it is not effective in measuring individuals’ boldness in this system. The low individual repeatability across contexts suggests that the behaviours exhibited in the novel environment test do not reflect a personality trait. Cory’s Shearwater’s exploratory behaviour shows phenotypic plasticity, particularly across annual cycle stages. The low likelihood to enter an unfamiliar environment during the pre-laying season may have fitness consequences in accordance with parental investment theory and nest defence intensity hypotheses (Montgomerie & Weatherhead, 1988). In the pre-laying period, Cory’s Shearwaters invest highly in nest defence and intense fights between individuals are often observed at dense colonies such as on Selvagem Grande. Such fights can result in injury, and occasionally even death (pers. obs.). Entering unfamiliar environments may increase the risks of unnecessary fights resulting in high energy costs or physical damage. Additionally, during incubation birds may experience a perceived urgency to return to the nest due to increased motivation to provide parental care.

The novel environment test also exposed how young Cory’s Shearwaters, which recruited as breeding birds during or in the years directly before this study, emerged into the novel environment faster than older birds. One hypothesis in the literature that could explain this is that stress responses are suppressed in experienced breeders at ages at which the potential for current reproductive success are high, but are not suppressed in young, inexperienced birds which give greater value to their immediate survival than their reproductive attempt (Elliott et al., 2014; Montgomerie & Weatherhead, 1988).

We acknowledge that the timeframe of the analysis presented here is not representative of the longevity of shearwaters and advise caution when interpreting the permanence of behavioural type, which may change over the long term, similar to individual quality (Catry et al., 1999) and migratory strategy (Perez et al., 2014). We encourage more long term individual observations of long-lived species to improve our understanding of the role of temperament in population ecology. The assessment of behaviour on extraction from the nest has potential to be a simple, straightforward and informative measure of stress response for burrow nesting seabirds. This may be a particularly useful tool in regularly monitored populations in which individuals need to be handled for other purposes, avoiding the need to expose the animals to an additional stressor. However, the interpretation of birds' responses requires further investigation in order to be able to extract more information. In particular, among the assessments which are not considered here, some birds retreated as a reaction to an approaching hand rather than staying put or attacking, which resulted in some confusion as to where such a response would lie in an ordinal list of categories of increasing reactivity (Patrick, Charmantier, & Weimerskirch, 2013). An ability to classify more reactions could help describe a greater proportion of a population. Moreover, the extraction test can be further validated as a measurement of behavioural stress response by quantifying blood corticosterone levels in assessed birds.

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Permits

Procedures were approved by ISPA's Ethical Committee for Animal Welfare and were carried out under licenses issued by the Direção Geral de Alimentação e Veterinária, Portugal, license number DGAV 0421/2017, and by the Instituto das Florestas e da Conservação da Natureza, Portugal, IFCN licence 1/S (2017).

REFERENCES

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/doi:10.18637/jss.v067.i01

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, *77*(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022.Bell

Benus, R. F., Bohus, B., Koolhaas, J. M., & van Oortmerssen, G. A. (1991). Heritable variation for aggression as a reflection of individual coping strategies. *Experientia*, *47*(10), 1008–1019. https://doi.org/10.1007/BF01923336

Bodey, T. W., Cleasby, I. R., Bell, F., Parr, N., Schultz, A., Votier, S. C., & Bearhop, S. (2017). A phylogenetically controlled meta-analysis of biologging device effects on birds: Deleterious effects and a call for more standardized reporting of study data. *Methods in Ecology and Evolution*, *9*(4), 946–955. https://doi.org/10.1111/ijlh.12426

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biological Reviews*, *88*, 465–475. https://doi.org/10.1111/brv.12007

Catry, P., Dias, M. P., Phillips, R. A., & Granadeiro, J. P. (2011). Different means to the same end: Long-distance migrant seabirds from two colonies differ in behaviour, despite common wintering grounds. *PLoS ONE*, *6*(10), 4–9. https://doi.org/10.1371/journal.pone.0026079

Catry, P., Dias, M. P., Phillips, R. A., & Granadeiro, J. P. (2013). Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: an experimental demonstration. *Ecology*, *94*(6), 1230–1235. https://doi.org/10.1890/12-2177.1

Catry, P., Granadeiro, J. P., Ramos, J., Phillips, R. A., & Oliveira, P. (2011). Either taking it easy or feeling too tired: Old Cory's Shearwaters display reduced activity levels while at sea. *Journal of Ornithology*, *152*(3), 549–555. https://doi.org/10.1007/s10336-010-0616-7

Catry, P., Ruxton, G. D., Ratcliffe, N., Hamer, K. C., Furness, R. W., & Mar, N. (1999). Short-lived repeatabilities in long-lived Great Skuas: Implications for the study of individual quality. *Oikos*, *84*(3), 473–479. https://doi.org/10.2307/3546426

Chapman, B. B., Brönmark, C., Nilsson, J.-Å., & Hansson, L.-A. (2011). The ecology and evolution of partial migration. *Oikos*, *120*(12), 1764–1775. https://doi.org/10.1111/j.1600-0706.2011.20131.x

Chapman, B. B., Hulthén, K., Blomqvist, D. R., Hansson, L.-A., Nilsson, J.-Å., Brodersen, J., … Brönmark, C. (2011). To boldly go: individual differences in boldness influence migratory tendency. *Ecology Letters*, *14*(9), 871–876. https://doi.org/10.1111/j.1461-0248.2011.01648.x

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*(SUPLL. 2), 169–178. https://doi.org/10.1007/s10336-007-0175-8

Cockrem, J. F. (2012). Corticosterone responses and personality in birds: Individual variation and the ability to cope with environmental changes due to climate change. *General and Comparative Endocrinology*, *190*, 156–163. https://doi.org/10.1016/j.ygcen.2013.02.021

Dias, M. P., Granadeiro, J. P., & Catry, P. (2012). Do seabirds differ from other migrants in their travel arrangements? On route strategies of Cory’s Shearwater during its trans-equatorial journey. *PLoS ONE*, *7*(11), e49376. https://doi.org/10.1371/journal.pone.0049376

Dias, M. P., Granadeiro, J. P., & Catry, P. (2013). Individual variability in the migratory path and stopovers of a long-distance pelagic migrant. *Animal Behaviour*, *86*(2). https://doi.org/10.1016/j.anbehav.2013.05.026

Dias, M. P., Granadeiro, J. P., Phillips, R. A., Alonso, H., & Catry, P. (2011). Breaking the routine: individual Cory’s shearwaters shift winter destinations between hemispheres and across ocean basins. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1713), 1786–1793. https://doi.org/10.1098/rspb.2010.2114

Dingemanse, N. J., Both, C., Drent, P. J., & Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1541), 847–852. https://doi.org/10.1098/rspb.2004.2680

Dingemanse, N. J., & Réale, D. (2005). Natural selection and animal personality. *Behaviour*, *142*, 1159–1184.

Elliott, K. H., Reilly, K. M. O., Hatch, S. A., Gaston, A. J., Hare, J. F., & Anderson, W. G. (2014). The prudent parent meets old age : A high stress response in very old seabirds supports the terminal restraint hypothesis. *Hormones and Behavior*, *66*(5), 828–837. https://doi.org/10.1016/j.yhbeh.2014.11.001

Found, R., & St. Clair, C. C. (2017). Ambidextrous ungulates have more flexible behaviour, bolder personalities and migrate less. *Royal Society Open Science*, *4*(2), 160958. https://doi.org/10.1098/rsos.160958

Hall, M. L., van Asten, T., Katsis, A. C., Dingemanse, N. J., Magrath, M. J. L., & Mulder, R. A. (2015). Animal personality and pace-of-life syndromes: do fast-exploring fairy-wrens die young? *Frontiers in Ecology and Evolution*, *3*, 28. https://doi.org/10.3389/fevo.2015.00028

Harris, S. M., Descamps, S., Sneddon, L. U., Bertrand, P., Chastel, O., & Patrick, S. C. (2019). Personality predicts foraging site fidelity and trip repeatability in a marine predator. *Journal of Animal Ecology*, *1*, 1365-2656.13106. https://doi.org/10.1111/1365-2656.13106

Jovani, R., & Rohwer, S. (2016). Fault bars in bird feathers: mechanisms, and ecological and evolutionary causes and consequences. *Biological Reviews*, *92*(2), 1113–1127. https://doi.org/10.1111/brv.12273

Koolhaas, J. ., Korte, S. ., De Boer, S. ., Van Der Vegt, B. ., Van Reenen, C. ., Hopster, H., … Blokhuis, H. . (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, *23*(7), 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Krüger, L., Pereira, J. M., Paiva, V. H., & Ramos, J. A. (2019). Personality influences foraging of a seabird under contrasting environmental conditions. *Journal of Experimental Marine Biology and Ecology*, *516*(September 2018), 123–131. https://doi.org/10.1016/j.jembe.2019.04.003

Montgomerie, R. D., & Weatherhead, P. J. (1988). Risks and rewards of nest defence by parent birds. *The Quarterly Review of Biology*, *63*(2), 167–187. Retrieved from https://www.jstor.org/stable/2830999

Mougin, J.-L., Jouanin, C., & Roux, F. (1997). Intermittent breeding in Cory's Shearwater *Calonectris diomedea* of Selvagem Grande, North Atlantic. *Ibis*, *139*(1), 40–44. https://doi.org/10.1111/j.1474-919X.1997.tb04502.x

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews*, *85*(4), 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x

Nilsson, A. L. K., Nilsson, J.-Å., Alerstam, T., & Bäckman, J. (2010). Migratory and resident blue tits *Cyanistes caeruleus* differ in their reaction to a novel object. *Naturwissenschaften*, *97*(11), 981–985. https://doi.org/10.1007/s00114-010-0714-7

Nilsson, A. L. K., Nilsson, J.-Å., & Mettke-Hofmann, C. (2016). Energy reserves, information need and a pinch of personality determine decision-making on route in partially migratory blue tits. *PLOS ONE*, *11*(10), e0163213. https://doi.org/10.1371/journal.pone.0163213

Nilsson, J.-Å., Brönmark, C., Hansson, L.-A., & Chapman, B. B. (2014). Individuality in movement: the role of animal personality. In Lars-Anders Hansson & Susanne Akesson (Eds.), *Animal Movement Across Scales* (pp. 90–109). Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199677184.003.0006

Odermatt, J., Frommen, J. G., & Menz, M. H. M. (2017). Consistent behavioural differences between migratory and resident hoverflies. *Animal Behaviour*, *127*, 187–195. https://doi.org/10.1016/j.anbehav.2017.03.015

Patrick, S. C., Charmantier, A., & Weimerskirch, H. (2013). Differences in boldness are repeatable and heritable in a long-lived marine predator. *Ecology and Evolution*, *3*(13), 4291–4299. https://doi.org/10.1002/ece3.748

Patrick, S. C., & Weimerskirch, H. (2014). Personality, foraging and fitness consequences in a long lived seabird. *PLoS ONE*, *9*(2), e87269. https://doi.org/10.1371/journal.pone.0087269

Perez, C., Granadeiro, J. P., Dias, M. P., Alonso, H., & Catry, P. (2014). When males are more inclined to stay at home: insights into the partial migration of a pelagic seabird provided by geolocators and isotopes. *Behavioral Ecology*, *25*(2), 313–319. https://doi.org/10.1093/beheco/art119

Pérez, C., Granadeiro, J. P., Dias, M. P., & Catry, P. (2016). Sex and migratory strategy influence corticosterone levels in winter-grown feathers, with positive breeding effects in a migratory pelagic seabird. *Oecologia*, *181*(4), 1025–1033. https://doi.org/10.1007/s00442-016-3625-2

R Core Team. (2019). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Réale, D., Reader, S. M., Sol, D., Mcdougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82,* 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology and Evolution*, *27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

CHAPTER 3

REPRODUCTION AND MIGRATION DO NOT CARRY COSTS IN PHYSICAL CONDITION BETWEEN YEARS IN A LONG-LIVED SEABIRD

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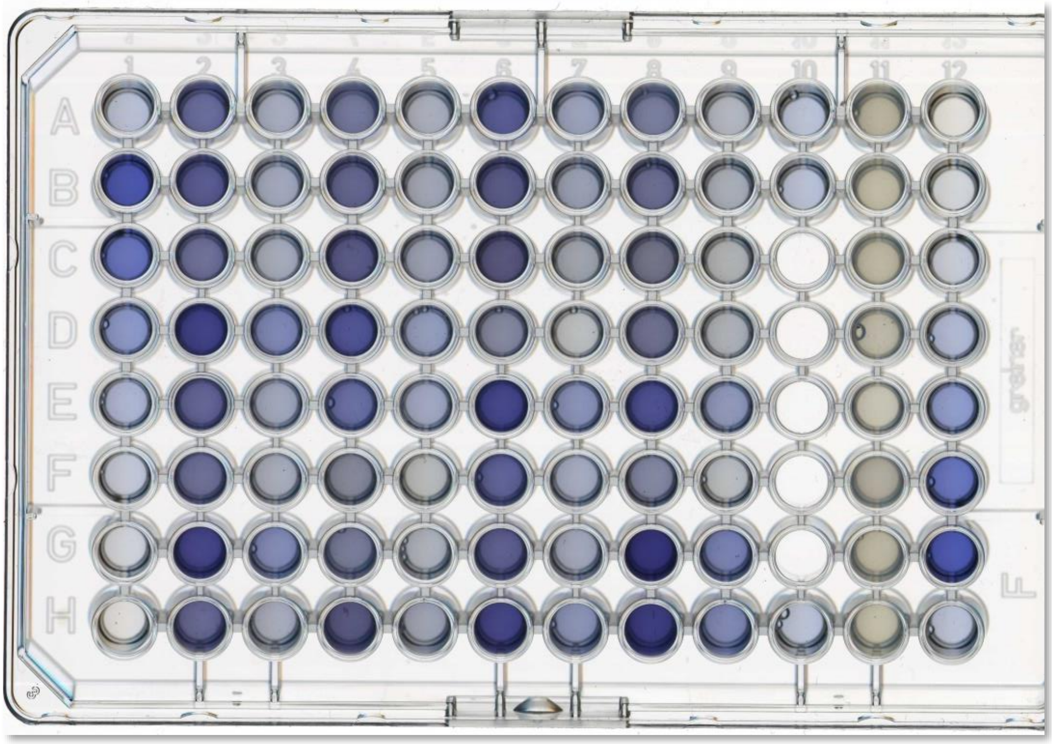

ABSTRACT

Life history theory suggests a trade-off between costly activities such as breeding and migration and somatic self-maintenance. However, how the short-term cost of parental effort is expressed in species with a slow pace-of-life is not well understood. Also, investigating carry-over effects of migration is most meaningful when comparing migratory strategies within the same population, but this has rarely been done. We explore this hypothesis in a long-lived, pelagic seabird, the Cory's Shearwater, *Calonectris borealis*, where males display partial migration. By manipulating reproductive effort and taking advantage of the natural variation in migratory strategy, we investigate whether early reproductive failure and migratory strategy had implications on the physical condition of males on return to the colony the following year. We experimentally induced breeding failure from mid-incubation, tracked the over-winter movements of these males and of males that invested in parental effort, and assessed innate immunity, stress, and residual body mass the following year. Early breeding failure resulted in earlier return to the colony among all males, associated with greater probability of reproductive success. Residents had a lower tail feather fault bar intensity, an indicator of stress during the non-breeding period, compared to migrants. Reproductive effort and migratory strategy had no impact on physiological condition otherwise. Our results provide evidence that in species with a slow-pace of life, such as the Cory's Shearwater, somatic maintenance is prioritised, with the costs of reproduction and migration paid in delayed arrival date.

Keywords: carry-over effects, fault bars, life history, migration, physiology

INTRODUCTION

Life history theory states that under conditions of limited resources organisms face a trade-off between energetically expensive activities. One such trade-off reflects the relative importance of current reproductive output against survival and future reproduction. It is often expected that reproductive effort comes at the cost of somatic self-maintenance (Stearns, 1992). Since migratory decisions can have energetic consequences, these decisions may also be under energetic trade-offs, within which body condition and immune function may play a mediatory or causative role (Buehler & Piersma, 2008; Møller & Szép, 2011). The observation of carry-over effects is evidence for the existence of such trade-offs, where the conditions experienced in one season have effects on fitness and survival in a following season (Harrison, Blount, Inger, Norris, & Bearhop, 2011).

Recently, the physiological mechanisms that mediate carry-over effects have entered into focus, with hormones, oxidative damage, immune function, and metabolic activity among the better studied aspects (Hegemann, Marra, & Tieleman, 2015; Hegemann, Matson, Flinks, & Tieleman, 2013; Selman, Blount, Nussey, & Speakman, 2012). Results on the physiological basis of carry-over effects across species, however, have been mixed both in terms of the significance and direction of relationships (Cohen, Mauck, Wheelwright, Huntington, & McGraw, 2009; Lee, 2006). One potential explanation for this is the effect that life-history strategy has on the nature of trade-offs due to the differing priorities given to current reproductive effort and somatic maintenance by species on different ends of the pace-of-life spectrum (Cohen et al., 2009; Lee, 2006; Ricklefs & Wikelski, 2002). Seabird families are classically described as having a slow pace-of-life. Studies on carry-over effects in various migratory seabird species have previously found that breeding failure impacts phenology of post-breeding migration (Bogdanova et al., 2011; Catry, Dias, Phillips, & Granadeiro, 2013) and have had mixed results regarding the persistence of these effects into the following year (Bogdanova et al., 2011; Catry et al., 2013; Fayet et al., 2016; R. Ramos, Llabrés, Monclús, López-Béjar, & González-Solís, 2018). How environmental conditions outside the breeding period modulate carry-over effects on future fitness in seabirds is not well established.

Partial migration may have consequences on population dynamics since its members are exposed to different conditions in the non-breeding period (Chapman, Brönmark, Nilsson, & Hansson, 2011). For instance, arrival body condition and the date of arrival at the breeding grounds influence reproductive output in migratory avian species (Rowe, Ludwig, & Schluter, 1994), and both have been reported to carry-over from conditions in the non-breeding area (Harrison et al., 2011; Inger et al., 2010). Moreover, members of a partially migratory population may demonstrate different physiological adaptations to cope with the environments they are routinely exposed to (Hegemann et al., 2015). The non-specific innate immune system acts as a first line of defence against physiological stress, inflammation and infection and is tightly linked to acquired immunity (Berzins, Gilchrist, Matson, & Burness, 2011). It appears to play a role in energetic trade-offs with migration as it has been found to differ between migrants and residents (Hegemann et al., 2015). Clearly, comparisons of carry-over effects of, and/or physiological adaptations to, different migratory strategies are most meaningful when it is possible to investigate migratory strategies within the same population. However, such investigations, particularly in species which experience long distance movements within the migratory portion of the population, are not common.

The Cory’s Shearwater (*Calonectris borealis*) is a long-lived, partially migratory seabird. In common with other Procellariiformes, both members of a pair participate in the incubation of a single egg and the rearing of the chick during a prolonged breeding season, without the possibility of a second clutch in case of early breeding failure. The majority of the population breeding on Selvagem Grande, Madeira, leaves the breeding colony between October and November and spends the non-breeding period in the Benguela Current in the south-east Atlantic and the adjacent Agulhas Current in the Indian Ocean or other oceanic currents in the southwest and northwest Atlantic, while around a fifth of males remain predominantly resident year round, although some visit secondary wintering areas away from the Canary Current for shorter periods (Dias, Granadeiro, Phillips, Alonso, & Catry, 2011). Repeated over-winter tracking of individuals reveal that Cory’s Shearwaters are largely faithful to a particular non-breeding area, but occasionally utilise different non-breeding areas during their lifetime (Dias, Granadeiro, & Catry, 2013; Dias et al., 2011). Residency results in earlier arrival at the colony at the start of the breeding season, which positively affects the probability of a successful breeding attempt and is thought to be the evolutionary driver for overwinter residency in males (Catry et al., 2013; Perez, Granadeiro, Dias, Alonso, & Catry, 2014). Individuals that remain resident have previously been recorded to experience lower corticosterone levels over winter compared to migrants (Pérez, Granadeiro, Dias, & Catry, 2016). However, whether this difference is a reflection of underlying body condition is not known.

Here, we investigate the role of physical condition in the carry-over effects of reproductive effort and migratory strategy in a population of Cory’s Shearwater breeding on Selvagem Grande. We experimentally manipulated reproductive effort in a group of males, the partially migratory sex, by inducing breeding failure during incubation and subsequently tracked these and males that invested in parental effort over winter using light-level geolocation (GLS). We then assessed size-corrected body mass, nitric oxide concentration (NOx), plasma concentration of haptoglobin, natural antibody and complement activity (hemagglutination-hemolysis), and tail feather fault bar intensity as proxies of physical condition on arrival to the colony after return migration. Using these data, we investigated whether early (experimentally induced) reproductive failure and migratory strategy had implications on physical condition on return.

We expect that the early relief of breeding duties, earlier than in previous similar experimental set-ups (Catry et al., 2013), should influence the energy budget available to Cory's Shearwaters during the breeding period due to reduced parental effort. The maintenance of the non-specific innate immune system (haptoglobin, hemagglutination-hemolysis), long-term stress (fault bar intensity) and inflammation (NOx, haptoglobin) markers, size-corrected body mass and/or date of return to the colony the following year should differ between experimental and control groups if the costs of reproductive effort persist over the non-breeding season as carry-over effects in body condition (Buehler, Tieleman, & Piersma, 2010; Owen-Ashley & Wingfield, 2007). These indices of body condition should also reflect the migratory strategy of Cory's Shearwaters if habitat quality, exposure to infection, and/or intrinsic adaptations (in the case of innate immunity) differ substantially between migrants and residents over the non-breeding season (Hegemann, Matson, Both, & Tieleman, 2012; Nwaogu, Cresswell, Versteegh, & Tieleman, 2019).

MATERIAL AND METHODS

STUDY SITE AND SAMPLE COLLECTION

Selvagem Grande (Lat 30.140 N , Long 15.869 W) is an island nature reserve located ca. 300 km south of the Madeiran Archipelago, Portugal. Around 30 000 pairs of Cory's Shearwaters breed on the island. A sub-population is the subject of long-term research, with the vast majority of individuals ringed, of known or approximated age, known sex, and measured (culmen length, bill height at base, bill height at gonys, and wing length) (Granadeiro, Dias, Rebelo, Santos, & Catry, 2006). Cory's Shearwaters return to their nest burrows after the non-breeding season between mid-February and early April to undertake a long period of nest defence and mating before laying a single egg in early June, which they incubate for around two months (Zino, Zino, Maul, & Biscoito, 1987). The chick rearing season lasts until around November, with adults leaving the colony to spend the non-breeding season at sea in discrete non-breeding areas (Catry, Dias, Phillips, & Granadeiro, 2011). Individual reproductive success is recorded annually in this population, with observations carried out early in the incubation period and in the late chick-rearing period.

Between the end of June and early July 2017, around one month after laying and one month before hatching, 25 nests were selected for egg removal to experimentally induce breeding failure during incubation. These nests were selected to represent males of a variety of ages and both successful and unsuccessful breeders from the previous breeding season. These males (the “experimental group”) were fitted with leg-mounted geolocation devices (GLS, Migrate Technology model C330) to record their overwinter movements. The total weight of the deployment was well below 1% of the birds’ body weight and can be assumed to bear minimal or no effects on survival and fitness (Bodey et al., 2017). This method resulted in an 88% retrieval rate in 2018, consistent with the rate of return of breeding birds from one year to the next (Mougin, Jouanin, & Roux, 1997). From a pool of 80 males also GLS-tracked over the same winter as part of a different investigation, we selected 24 which successfully reproduced in 2017, with an age distribution similar to the males in the experimental group, to serve as the “parental effort” group for comparisons of carry-over effects of reproductive effort.

In 2018 nest attendance was checked daily between mid-February and mid-April to encounter GLS-equipped birds on their first day after return migration. GLS data confirms that Cory’s Shearwaters fly directly to the colony and their nest on arrival from migration. Males that formed part of the experimental and parental effort groups were extracted from their nest burrows by hand, were weighed (to the nearest 10g) using a spring balance and between 1ml and 1.5ml of blood were collected from the brachial vein using a syringe flushed with heparin prior to use. The duration of manipulation lasted a maximum of 15 minutes. The collected whole blood was centrifuged and the resulting plasma frozen at -20°C until analysed. These procedures were carried out within a few hours of blood collection. Non-breeding destination was assigned from the GLS positional data, comparing to core utilisation distributions described for this population (Catry et al., 2011; Dias, Granadeiro, & Catry, 2012a; Dias et al., 2011; Perez et al., 2014) (Figure 1).

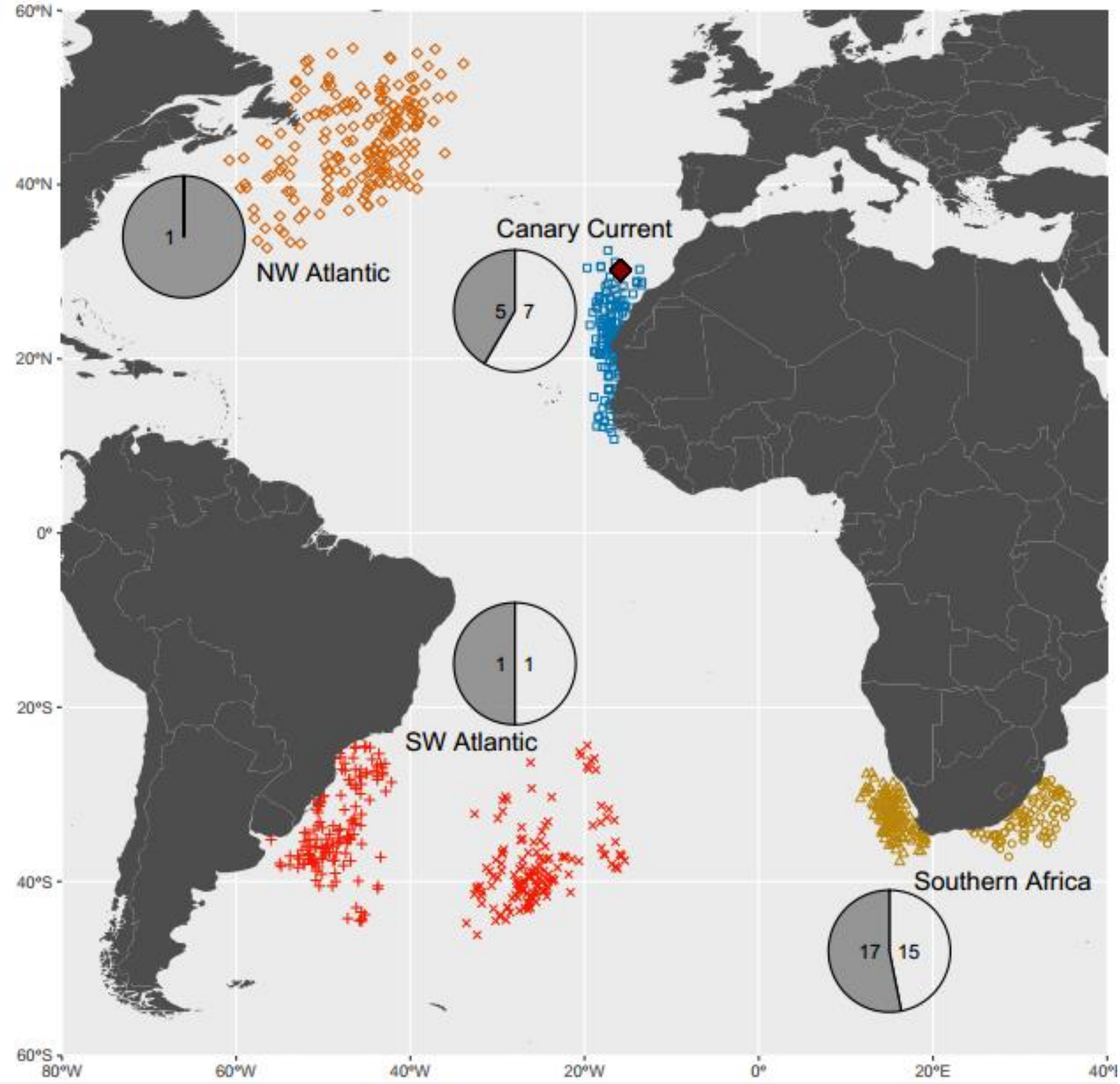

Figure 1 Cory's Shearwaters spend the non-breeding season in discrete ocean currents, either remaining resident in the Canary Current (blue), or migrating to currents off southern Africa (yellow), to the southwest Atlantic (red) or the northwest Atlantic (orange). Pie charts represent the number of males wintering in each location that either had their previous reproductive effort experimentally reduced (experimental group, empty segments) or were naturally successful (parental effort group, filled segments). The colony location is denoted by the solid diamond. Different shapes represent the positional data (GLS) of six different individuals between December and February.

During the incubation period of 2018, the same birds were reencountered as part of annual nest monitoring and tail feather fault bar intensity was assessed. Fault bars are translucent bands along the breadth of a feather that are produced by low keratin deposition during feather growth as a result of acute stress events, such as inclement weather and negative inter-individual interactions. These can range in severity from faint bars to extreme keratin depletion resulting in feather breakage (Jovani & Rohwer, 2016). Tail feathers experience lower canalisation against fault bars compared to more functionally important feathers to flight, such as remiges, therefore are better suited to reflect experienced stress (Boonekamp, Dijkstra, Dijkstra, & Verhulst, 2017; Bortolotti, Dawson, & Murza, 2002). In Cory's Shearwaters, tail feathers start moulting at the end of the breeding season and most of the population completes tail moult by their return to the colony (Alonso, Matias, Granadeiro, & Catry, 2009). Therefore, fault bars integrate stress mostly experienced over the non-breeding period and are a cheap, minimally invasive method to obtain data on long-term stress. Due to the presence of feathers broken at fault bars, the number of affected tail feathers, rather than the total number of fault bars, is considered here as fault bar intensity (Bortolotti et al., 2002). Characterising the severity of fault bars has not been shown to provide additional information (Boonekamp et al., 2017).

PHYSICAL CONDITION INDICES

Residual body mass (body mass corrected for body size) is often used as an index of nutritional body condition shown to correlate with health status and reproductive performance (Gómez-Díaz, Navarro, & González-Solís, 2008; Green, 2001). Body size was estimated by inputting biometric measurements (culmen length, bill height at base, bill height at gonys, and wing length) into a principal component analysis. Residual body mass was obtained by extracting the residuals from a regression between mass and the resulting first principle component (Bortolotti et al., 2002).

The plasma samples were analysed for haptoglobin concentration, hemolysis and hemagglutination scores, and nitric oxide concentration at the Groningen Institute for Evolutionary Life Sciences, University of Groningen, The Netherlands. Haptoglobin is an acute phase protein that responds to acute infection, inflammation, or trauma with a marked and rapid increase in concentration in the blood (Matson, Horrocks, Versteegh, & Tieleman, 2012). We quantified haptoglobin (mg ml^{-1}) using the manual method of a commercially-available colorimetric functional assay (TP801; Tri-Delta Diagnostics, NJ, USA), with adjustments described in Matson et al. (2012), measuring absorbances at two wave-lengths: a scan at 450nm, taken before the last reagent was added, to measure the redness of the plasma samples, and a scan at 630nm representing the concentration of haptoglobin in the plasma. A hemolysis/hemagglutination assay, as described in Matson et al (2005), assesses nonspecific natural antibodies (agglutination score) and natural antibody-mediated complement activation (lysis score) to monitor responses of innate immune system (Matson et al., 2005). The plasma concentration of nitric oxide, a multifunctional signalling molecule with roles in the neural system and the inflammation process of the non-specific immune system (Sild & Hõrak, 2009), was obtained by quantifying total plasma nitrite and nitrate concentration (NOx, µM) following the methods described in Sild & Horak (2009).

STATISTICAL ANALYSIS

Statistical analyses were performed with R Statistical Package, version 3.5.1 (R Core Team, 2019).

We first tested whether male arrival date at the colony in 2018 was related to subsequent probability of securing a nest and incubating an egg (‘laying success’; 1 = egg present during the incubation period of 2018, 0 = no egg recorded in nest), considering the males from the parental effort group, the experimentally manipulated males, and an additional 30 males that were also tracked overwinter and whose age was known (n = 70) to increase statistical power. We ran a generalised linear model (function “glm”) with a binomial error structure relating laying success against the date of return to the colony and age.

We ran a chi-squared test in order to confirm that experimental and parental effort groups did not differ significantly in the migratory strategies represented by each.

We assessed the effects of previous reproductive effort and migratory strategy on (i) subsequent date of return to the colony in male Cory’s Shearwaters, (ii) haptoglobin concentration, (iii) lysis and (iv) agglutination scores, (v) NOx, (vi) residual body mass, and (vii) fault bar intensity separately. We compared Akaike’s Information Criterion adjusted for small sample sizes (AICc) and Akaike weights (Burnham & Anderson, 2002) for all possible (generalised) linear models (functions “lm”, “glm”; function “dredge”, package *MuMIn*) for each dependent variable, including main effects reproductive effort and migratory strategy and the interaction term between them. In the analysis of haptoglobin, redness as measured at 450nm was kept in all models as a covariate, and statistics are not reported. Migratory strategy was treated as a binary value differentiating between individuals that spent the non-breeding period largely resident in the Canary Current and those that migrated to the

Benguela and Agulhas Currents off South Africa, the SW Atlantic, or the NW Atlantic for the entirety of the non-breeding period. In models investigating date of return to the colony we used a more conservative definition of migratory strategy where we differentiated between individuals that remained resident year-round from those that migrated or carried out shorter large-scale movements during the non-breeding period (see below) due to the expected effects even shorter movements have on arrival date. Age was not included in these models since the experimental and parental effort groups contained males of similar age groups and its inclusion in a linear model was not found to improve the model predictability (but see Appendix S1 of the Supplementary Material). Haptoglobin concentration, lysis score, and NOx were modelled with a gamma error distribution, while fault bar intensity was modelled with a Poisson error distribution. The best supported model was considered to be that with the lowest AICc, with models with a difference in AICc (ΔAICc) smaller than 2 considered competitive. In these cases, a model averaging approach was carried out to obtain model-averaged parameter estimates (function “model.avg”, package *MuMIn*) (Burnham & Anderson, 2002). Sample sizes are in Table 1.

In order to control for any possible effect of individual quality, we repeated the analyses of the effects of migratory strategy and reproductive effort comparing males from the experimental group with a control group of males of similar ages selected at the same time as the egg-removal, containing both naturally successful and naturally failed breeders. This is presented in Appendix S2 of the Supplementary Material.

Of the 12 males that did not leave the Canary Current after the breeding season, four spent between 8 and 16 days at a secondary wintering area in the South Atlantic and one carried out a large loop flight to the South Atlantic prior to returning to the colony. For this reason, it is not possible to isolate differences in body condition that result from migratory flight due to the small number of individuals that did not undertake long-distance movements.

Statistical estimates are represented with their standard error (Est ± SE).

Table 1 The classification of male Cory's Shearwaters in terms of reproductive effort (experimental vs the naturally successful parental effort group) and migratory strategy (resident or migratory) for each of the models assessing the carry-over effects of reproductive effort and migratory strategy on physical condition and date of return to the colony. The sample size in each model is denoted by *n*.

	Experimental		**Successful**	
	Resident	Migratory	Resident	Migratory
Haptoglobin (n = 45)	7	16	5	17
Lysis (n = 47)	7	16	5	19
Agglutination (n = 47)	7	16	5	19
NOx (n = 38)	7	12	5	14
Residual body mass (n = 45)	7	15	5	17
Fault bar intensity (n = 41)	7	12	5	17
Arrival at nest (n = 47)	3	16	4	19

RESULTS

None of the males tracked between 2017 and 2018 for which overwinter movements of the previous year are known (unpublished data) changed migration destination, including 20 males whose reproductive effort was experimentally reduced. This suggests that the manipulation of reproductive effort did not influence migratory decision. This allows us to disentangle the potential concurrent effects of reproductive effort and migratory strategy. The non-breeding destinations of males of both experimentally induced reproductive failure and natural reproductive success are displayed in Figure 1. The distribution of migratory destinations between the two groups does not differ significantly ($\chi^2 = 1.44$, df = 3, p = 0.697).

Early arrival at the colony in 2018 in males was significantly related to an increased probability of securing a nest and incubating an egg within our sample of 70 males (GLM: Est = -0.07 ± 0.03, z = -2.83, p = 0.005). Age had no significant effect on laying success (GLM: Est = -0.02 ± 0.05, z = -0.45, p = 0.653).

Male Cory's Shearwaters whose reproductive effort was experimentally reduced returned to the colony earlier the following year compared to males investing in parental effort (Est = 6.72 ± 2.96 days; Figure 2). As has been previously reported (Perez et al., 2014), residents returned to the colony earlier than migrants (Est = 16.48 ± 3.95 days). There was no evidence for an interactive effect between reproductive effort and migratory strategy (Table 2).

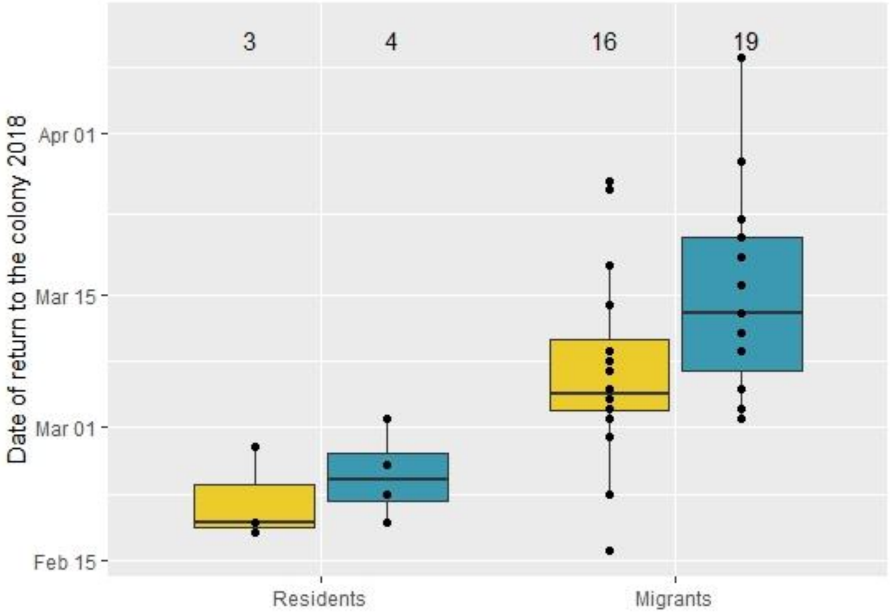

Figure 2 Male Cory's Shearwaters that were relieved of breeding duties during incubation in 2017 (experimental group, yellow) returned earlier to the colony on Selvagem Grande the following spring compared to males from the parental effort group (blue) that raised a chick. Males that spent the non-breeding season resident in the Canary Current returned earlier than migrants. Sample sizes are indicated above each box.

Null models, including no explanatory variables, were the best supported models for haptoglobin concentration, lysis and agglutination scores, and NOx (Table 2). Migratory strategy and previous reproductive effort featured in models explaining haptoglobin concentration and lysis and agglutination scores, which had a ΔAICc < 2. However, the model averaged estimates and standard errors of these variables suggested that their effect on the immune indices was likely negligible (Table 2, Table 3). The mean values of indices of physical condition found in this population are presented in Appendix S3 of the Supplementary Material.

The null model was also the best supported model explaining residual body mass, although all possible models returned a ΔAICc < 2 and were considered competitive models (Table 2; see also ESM). However, the estimates and standard errors of the explanatory variables suggest that none of them had a strong effect on residual body mass on return to the colony (Table 3, Figure 3)

Males that migrated had more tail feathers affected by fault bars than those that remained resident (Table 3, Figure 4).

Using a control group of males of mixed reproductive outcomes in 2017 instead of a group of only (naturally) successful males did not change the model results (see Appendix S2 of the Supplementary Material).

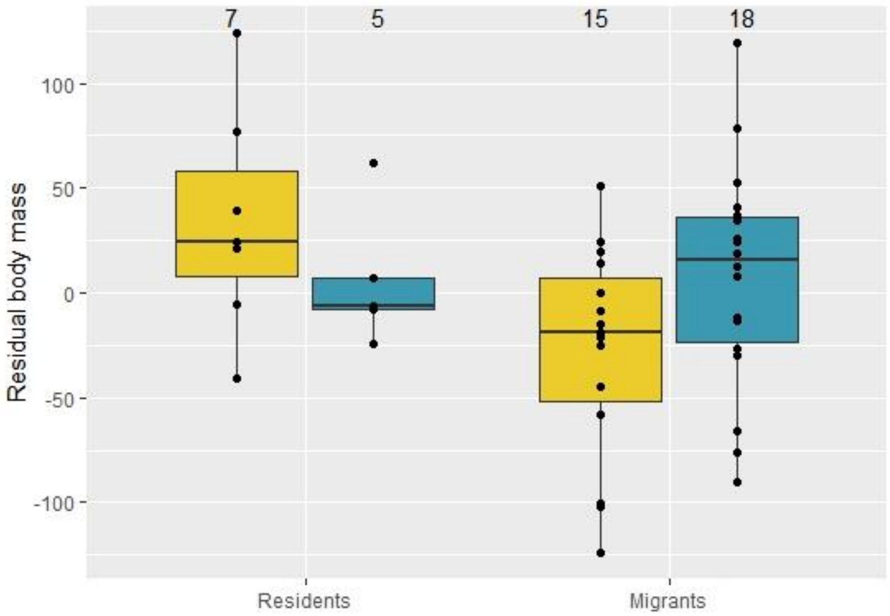

Figure 3 Residual body mass of male Cory's Shearwaters on return to the colony as classified by migratory strategy and by previous reproductive effort (experimental group, yellow, vs parental effort group, blue). Sample sizes are indicated above each box.

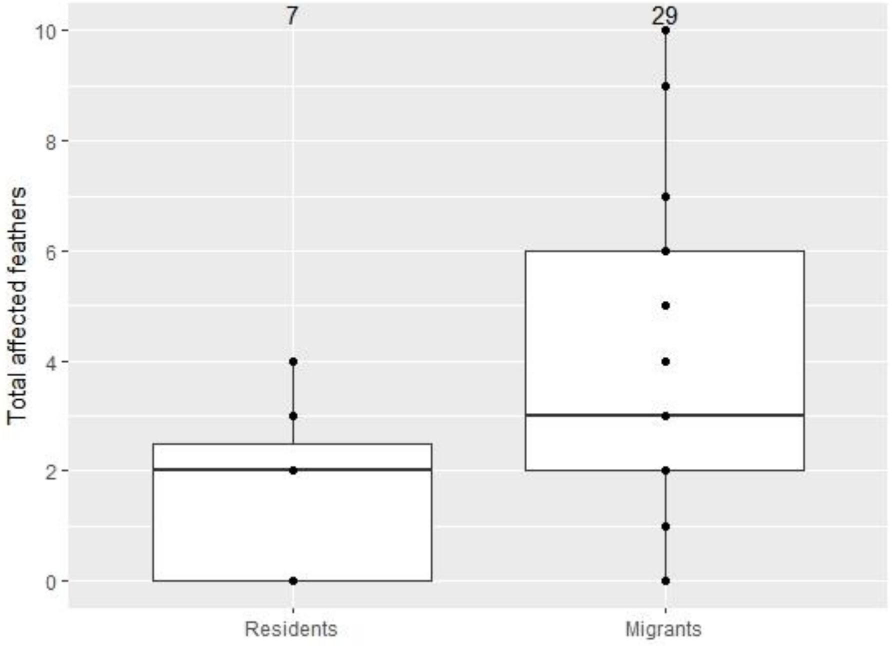

Figure 4 The number of tail feathers affected by fault bars in Cory's Shearwaters was significantly lower in males that remained resident in the Canary Current than in males that migrated to the Benguela and Agulhas currents. Sample sizes are indicated above each box.

Table 2 AICc values and AICc weights for the selection of (generalised) linear models assessing the effects of reproductive effort and/or migratory strategy on date of return to the colony and physical condition indices in male Cory's Shearwaters. Models in bold are equally supported by the data (ΔAICc <2). K = number of estimable parameters including the intercept.

Dependent variable		model	K	AICc	ΔAICc	AICc weights
(i)	**Arrival at nest**	**Rep. + Mig. Strat.**	**3**	**314.6**	**0**	**0.63**
		Rep. * Mig. Strat.	4	316.7	2.18	0.211
		Mig. Strat.	2	317.3	2.77	0.157
		Rep.	2	327.6	13.06	0.001
		Null model	1	328.7	14.14	0.001
(ii)	**Haptoglobin**	**Null model**	**2**	**-65**	**0**	**0.488**
		Mig. Strat.	**3**	**-63.3**	**1.74**	**0.205**
		Rep.	3	-63	2.05	0.175

		Rep. + Mig. Strat.	4	-61.2	3.82	0.072
		Rep. * Mig. Strat.	5	-60.8	4.22	0.059
(iii)	**Lysis**	**Null model**	**1**	**48.4**	**0**	**0.511**
		Rep.	**2**	**50.2**	**1.81**	**0.207**
		Mig. Strat.	2	50.5	2.17	0.173
		Rep. + Mig. Strat.	3	52.4	4.03	0.068
		Rep. * Mig. Strat.	4	53.4	5.04	0.041
(iv)	**Agglutination**	**Null model**	**1**	**118.2**	**0**	**0.463**
		Rep.	**2**	**119.5**	**1.34**	**0.237**
		Mig. Strat.	**2**	**120.1**	**1.89**	**0.18**
		Rep. + Mig. Strat.	3	121.6	3.45	0.082
		Rep. * Mig. Strat.	4	123.2	5.01	0.038
(v)	**NOx**	**Null model**	**1**	**-348.2**	**0**	**0.551**
		Rep.	2	-346	2.16	0.187
		Mig. Strat.	2	-346	2.19	0.184
		Rep. + Mig. Strat.	3	-343.8	4.44	0.06
		Rep. * Mig. Strat.	4	-341.4	6.85	0.018
(vi)	**Residual body mass**	**Null model**	**1**	**349.8**	**0**	**0.296**
		Mig. Strat.	**2**	**350.4**	**0.52**	**0.228**
		Rep. + Mig. Strat.	**3**	**351**	**1.14**	**0.168**
		Rep.	**2**	**351.1**	**1.28**	**0.156**
		Rep. * Mig. Strat.	**4**	**351.2**	**1.34**	**0.152**
(vii)	**Fault bar intensity**	**Mig. Strat.**	**2**	**187.8**	**0**	**0.497**
		Rep. + Mig. Strat.	**3**	**189.1**	**1.25**	**0.266**
		Rep. * Mig. Strat.	**4**	**189.5**	**1.71**	**0.212**
		Null model	1	195.1	7.27	0.013
		Rep.	2	195.1	7.28	0.013

Table 3 Model-averaged parameter estimates, their standard errors (±SE), and 95% confidence intervals (CI), calculated across models considered competitive (ΔAICc < 2). See Table 2 and methods for more details.

Dependent variable	Independent variables	Est ± SE	95% CI	
			Lower	Upper
Haptoglobin	Mig. Strat	0.36 ± 0.44	-0.53	1.25
Lysis	Rep.	0.04 ± 0.06	-0.08	0.15
Agglutination	Mig. Strat	-0.17 ± 0.28	-0.73	0.39
	Rep.	-0.23 ± 0.24	-0.71	0.25
Residual body mass	Mig. Strat	-40.85 ± 28.62	-98.64	16.94
	Rep.	10.29 ± 32.03	-54.24	74.81
	Mig. Strat * Rep.	63.89 ± 41.29	-20.69	148.48
Fault bar intensity	Mig. Strat	0.57 ± 0.28	0.02	1.12
	Rep.	-0.06 ± 0.42	-0.89	0.77
	Mig. Strat * Rep.	0.66 ± 0.48	-0.31	1.63

DISCUSSION

Our results illustrate the ways in which a long-lived seabird with a slow pace of life balances the costs of annual activities. We show how Cory’s Shearwaters with different previous reproductive effort and migratory strategy return to the colony in similar physiological condition, as reflected by a broad selection of parameters, and that costs are paid in phenological differences while somatic maintenance is prioritised.

LIFE HISTORY AND CARRY-OVER EFFECTS FROM REPRODUCTION

The results from our experimental manipulation of reproductive effort demonstrate that the cost of such effort incurred in Cory’s Shearwaters is largely reflected in their arrival date rather than their physiological state on return from migration. The primary explanation for these observations is that somatic maintenance is prioritised in K-strategists as future survival and reproductive potential are favoured over current reproductive output (Stearns, 1992). Analysis of telomere shortening, a biomarker for ageing, within the same experimental set-up also support these observations as manipulated reproductive effort had no significant impact on telomere length from one year to the next (Bauch, Gatt, Granadeiro, Verhulst, & Catry, 2020). The cost for self-maintenance appears to be paid by delaying arrival date, as reflected by the earlier arrival of males whose reproductive effort was experimentally reduced, irrespective of migratory strategy. Despite the lower sample sizes available for this comparison in arrival date, the results obtained are in agreement with previous observations in this colony (Catry et al., 2013). In this population, the date of arrival at the colony can influence reproductive success (Catry et al., 2013; Perez et al., 2014, this paper). Therefore, in some years and under certain environmental conditions a delayed arrival may result in a missed breeding attempt.

The implication of not carrying the somatic costs of reproduction into the following year is that Cory’s Shearwaters “settle the bill” over the non-breeding period. Previous phenological analyses have shown that the delayed return to the colony of successful breeders is a result of spending a longer time in the wintering area (Catry et al., 2013). Cory’s Shearwaters spend large proportions of time resting on the water in the non-breeding season compared to the breeding season (R. Ramos et al., 2018). While this has been associated with low flight activity during moult in some seabirds (Cherel, Quillfeldt, Delord, & Weimerskirch, 2016), it may also suggest that there is a low energy requirement associated with spending the non-breeding period in highly productive waters (Péron et al., 2010). This could explain the delay in successful breeders to return to the colony, as they spend more time in the non-breeding area where somatic maintenance can take place.

A lack of detectable carry-over effects of reproductive effort on body condition has been found in other seabirds (Great Skua *Stercorarius skua*: Bourgeon et al., 2014; Manx Shearwater *Puffinus puffinus*: Fayet et al., 2016; Leach’s Storm-petrel *Oceanodroma leucorhoa*: Gilmour et al., 2014). Interestingly, while experimental manipulations decreasing parental effort in seabirds often do not have consequences on physical condition and/or future reproductive success, those increasing provisioning duration of chicks (Fayet et al., 2016) or clutch size (Berzins et al., 2011) did, and the negative effects of increased reproductive effort have also been seen in other avian groups (Santos & Nakagawa, 2012). This suggests that one could expect carry-over effects from the cost of reproduction to manifest in years with adverse environmental conditions which elevate the usual cost of successfully completing a breeding attempt.

Our results on the effects of relaxation of parental duties on physical condition conform to previous studies on the same colony of Cory's Shearwaters on Selvagem Grande (Catry et al., 2013; Perez et al., 2014) as well as to aspects from a similar study on Cory's Shearwater from Gran Canaria (R. Ramos et al., 2018), but also highlight strong year effects or possible colony-specific differences. Data from two colonies suggest that reproductive success in one year does not influence reproductive success in the following year (Catry et al., 2013; R. Ramos et al., 2018). However, the relationships of reproductive success with arrival date and over-winter stress levels may be colony-specific. While inducing breeding failure during either incubation (this study) or chick rearing (Catry et al., 2013) resulted in earlier return to the Selvagem Grande colony the following year, this was not the case for birds breeding on Gran Canaria (R. Ramos et al., 2018). The impact of arrival date on reproductive success has been established in the Selvagem Grande population and probably stems from the very large size of the colony, causing high intra-specific competition for good quality nesting sites which in turn results in common fights between males on arrival to the colony (Catry et al., 2013; pers. observ. J. A. Ramos, Monteiro, Sola, & Moniz, 1997), which could be less intense on Gran Canaria. Additionally, Cory's Shearwaters from the Gran Canaria colony experienced increased stress levels during the non-breeding period after experiencing higher reproductive effort (R. Ramos et al., 2018), but this was not the case in birds breeding on Selvagem Grande as investigated over two separate years using different stress markers (Catry et al., 2013; this study). Ramos et al. (2018) suggest that corticosterone may mediate the impacts of reproductive effort on traits such as haematological health state. However, we found no evidence for this in the current study. The lack of a detectable relationship of reproductive success between years despite consequences in arrival date in the system on Selvagem Grande is probably due to the greater complexity of the system and the additional role of other confounding factors which cannot be controlled or easily accounted for.

COSTS OF MIGRATORY STRATEGY

Migratory strategy significantly determines overwinter stress levels for birds breeding on Selvagem Grande, reflected in both feather corticosterone levels (Perez et al., 2014) as well as fault bar intensity (this study), two markers known to correlate within a feather (Bortolotti, Marchant, Blas, & Cabezas, 2009). This difference in experienced stress between non-breeding areas was not seen in birds breeding on Gran Canaria (R. Ramos et al., 2018). The lower stress levels recorded in residents compared to migrant males could allude to residents experiencing better conditions overwinter. However, such differences do not appear to translate into long-term physiological changes, as evidenced by the similarity between residents and migrants in haematological measurements, again suggesting a great ability at somatic maintenance. An alternative explanation could be that within the population on Selvagem Grande resident males have a lower propensity to stress compared to migrants due to a separation in coping styles (Koolhaas et al., 1999). Investigations of behavioural differences between migrants and residents during the breeding season suggest that this may indeed be the case (M. Gatt et al., unpublished data).

Innate immunity, while strongly related to acquired immunity, is genetically mediated (Matson et al., 2005) and may vary seasonally in relation to the environmental changes to which a population is adapted (Hegemann et al., 2012; Versteegh, Helm, Kleynhans, Gwinner, & Tieleman, 2014). Differences in innate immune parameters have been found between migrants and residents from one year to the next in Skylarks which, like Cory’s Shearwaters, show individual flexibility in their migratory strategy (Hegemann et al., 2015). The similarity reported here between Cory's Shearwaters of different migratory strategies could suggest that the larger proportion of the annual cycle spent in the common breeding ground (Catry et al., 2011) may have a greater influence on innate immune function, both in terms of genetic adaptation and of infection and inflammation risk, than the period spent in separate non-breeding areas.

The long-distance movements undertaken by a number of predominantly resident birds preceding their return to the colony complicates an analysis of the cost of long-distance migratory flight in this system. Dynamic soaring flight performed by shearwaters and their ability to feed *en route* would suggest that migratory flight should not be very costly (Dias, Granadeiro, & Catry, 2012b). Indeed, perhaps the observation of Cory’s Shearwaters undertaking trans-oceanic flights over a few days just before returning to the breeding ground could also be evidence to this.

In conclusion, our data suggest that demanding annual cycle events in Cory’s Shearwaters do not result in depleted physical condition carrying over into the following breeding season. Instead, delayed arrival at the colony perhaps serves to continue processes of somatic maintenance during the less energy expensive non-breeding period, which secures survival but can potentially have negative consequences on future breeding potential. While the causes of differences in stress response with migratory strategy are yet to be uncovered, these also do not appear to integrate into lasting physiological effects.

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REFERENCES

Alonso, H., Matias, R., Granadeiro, J. P., & Catry, P. (2009). Moult strategies of Cory's Shearwaters *Calonectris diomedea borealis*: the influence of colony location, sex and individual breeding status. *Journal of Ornithology*, *150*(2), 329–337. https://doi.org/10.1007/s10336-008-0354-2

Bauch, C., Gatt, M. C., Granadeiro, J. P., Verhulst, S., & Catry, P. (2020). Sex-specific telomere length and dynamics in relation to age and reproductive success in Cory's shearwaters. *Molecular Ecology*, *29*(7), 1344–1357. https://doi.org/10.1111/mec.15399

Berzins, L. L., Gilchrist, H. G., Matson, K. D., & Burness, G. (2011). Sex-specific effects of increased incubation demand on innate immunity in Black Guillemots. *Physiological and Biochemical Zoology*, *84*(2), 222–229. https://doi.org/10.1086/658373

Bodey, T. W., Cleasby, I. R., Bell, F., Parr, N., Schultz, A., Votier, S. C., & Bearhop, S. (2017). A phylogenetically controlled meta-analysis of biologging device effects on birds: Deleterious effects and a call for more standardized reporting of study data. *Methods in Ecology and Evolution*, *9*(4), 946–955. https://doi.org/10.1111/ijlh.12426

Bogdanova, M. I., Daunt, F., Newell, M., Phillips, R. A., Harris, M. P., & Wanless, S. (2011). Seasonal interactions in the Black-legged Kittiwake, *Rissa tridactyla*: links between breeding performance and winter distribution. *Proceedings of the Royal Society B: Biological Sciences, 278*(1717), 2412–2418. https://doi.org/10.1098/rspb.2010.2601

Boonekamp, J. J., Dijkstra, R., Dijkstra, C., & Verhulst, S. (2017). Canalization of development reduces the utility of traits as fitness biomarkers: feather fault bars in nestling birds. *Functional Ecology, 31*(3), 719–727. https://doi.org/10.1111/1365-2435.12765

Bortolotti, G. R., Dawson, R. D., & Murza, G. L. (2002). Stress during feather development predicts fitness potential. *Journal of Animal Ecology, 71*(2), 333–342. https://doi.org/10.1046/j.1365-2656.2002.00602.x

Bortolotti, G. R., Marchant, T., Blas, J., & Cabezas, S. (2009). Tracking stress: Localisation, deposition and stability of corticosterone in feathers. *Journal of Experimental Biology, 212*(10), 1477–1482. https://doi.org/10.1242/jeb.022152

Bourgeon, S., Leat, E. H. K., Magnusdóttir, E., Furness, R. W., Strøm, H., Petersen, A., … Bustnes, J. O. (2014). Feather corticosterone levels on wintering grounds have no carry-over effects on breeding among three populations of Great Skuas (*Stercorarius skua*). *PLoS ONE, 9*(6), 1–6. https://doi.org/10.1371/journal.pone.0100439

Buehler, D. M., & Piersma, T. (2008). Travelling on a budget: Predictions and ecological evidence for bottlenecks in the annual cycle of long-distance migrants. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1490), 247–266. https://doi.org/10.1098/rstb.2007.2138

Buehler, D. M., Tieleman, B. I., & Piersma, T. (2010). How do migratory species stay healthy over the annual cycle? A conceptual model for immune function and for resistance to disease. *Integrative and Comparative Biology, 50*(3), 346–357. https://doi.org/10.1093/icb/icq055

Burnham, K. P., & Anderson, D. R. (2002). Model selection and multimodel inference: A practical information-theoretic approach (2nd ed.). New York: Springer New York.

Catry, P., Dias, M. P., Phillips, R. A., & Granadeiro, J. P. (2011). Different means to the same end: Long-distance migrant seabirds from two colonies differ in behaviour, despite common wintering grounds. *PLoS ONE*, *6*(10), 4–9. https://doi.org/10.1371/journal.pone.0026079

Catry, P., Dias, M. P., Phillips, R. A., & Granadeiro, J. P. (2013). Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: an experimental demonstration. *Ecology*, *94*(6), 1230–1235. https://doi.org/10.1890/12-2177.1

Chapman, B. B., Brönmark, C., Nilsson, J.-Å., & Hansson, L.-A. (2011). The ecology and evolution of partial migration. *Oikos*, *120*(12), 1764–1775. https://doi.org/10.1111/j.1600-0706.2011.20131.x

Cherel, Y., Quillfeldt, P., Delord, K., & Weimerskirch, H. (2016). Combination of at-sea activity, aeolocation and feather stable isotopes documents where and when seabirds molt. *Frontiers in Ecology and Evolution*, *4*. https://doi.org/10.3389/fevo.2016.00003

Cohen, A. A., Mauck, R. A., Wheelwright, N. T., Huntington, C. E., & McGraw, K. J. (2009). Complexity in relationships between antioxidants and individual life-history parameters in a seabird and a songbird. *Oikos*, *118*(12), 1854–1861. https://doi.org/10.1111/j.1600-0706.2009.17785.x

Dias, M. P., Granadeiro, J. P., & Catry, P. (2012a). Do seabirds differ from other migrants in their travel arrangements? On route strategies of Cory’s Shearwater during its trans-equatorial journey. *PLoS ONE*, *7*(11), e49376. https://doi.org/10.1371/journal.pone.0049376

Dias, M. P., Granadeiro, J. P., & Catry, P. (2012b). Working the day or the night shift? Foraging schedules of Cory’s shearwaters vary according to marine habitat. *Marine Ecology Progress Series*, *467*, 245–252. https://doi.org/10.3354/meps09966

Dias, M. P., Granadeiro, J. P., & Catry, P. (2013). Individual variability in the migratory path and stopovers of a long-distance pelagic migrant. *Animal Behaviour*, *86*(2). https://doi.org/10.1016/j.anbehav.2013.05.026

Dias, M. P., Granadeiro, J. P., Phillips, R. A., Alonso, H., & Catry, P. (2011). Breaking the routine: individual Cory’s shearwaters shift winter destinations between hemispheres and across ocean basins. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1713), 1786–1793. https://doi.org/10.1098/rspb.2010.2114

Fayet, A. L., Freeman, R., Shoji, A., Kirk, H. L., Padget, O., Perrins, C. M., & Guilford, T. (2016). Carry-over effects on the annual cycle of a migratory seabird: an experimental study. *Journal of Animal Ecology*, *85*(6), 1516–1527. https://doi.org/10.1111/1365-2656.12580

Gilmour, M. E., Lattin, C. R., Romero, L. M., Haussmann, M. F., Mauck, R. A., & Dearborn, D. C. (2014). Finding the best predictor of reproductive performance of Leach's Storm-Petrels. *The Auk*, *132*(1), 191–205. https://doi.org/10.1642/auk-14-21.1

Gómez-Díaz, E., Navarro, J., & González-Solís, J. (2008). Ectoparasite community structure on three closely related seabird hosts: A multiscale approach combining ecological and genetic data. *Ecography*, *31*(4), 477–489. https://doi.org/10.1111/j.0906-7590.2008.05330.x

Granadeiro, J. P., Dias, M. P., Rebelo, R., Santos, C. D., & Catry, P. (2006). Numbers and population trends of Cory's Shearwater *Calonectris diomedea* at Selvagem Grande, Northeast Atlantic. *Waterbirds*, *29*(1), 56–60. https://doi.org/10.1675/1524-4695(2006)29[56:naptoc]2.0.co;2

Green, A. J. (2001). Mass/length residuals: Measures of body condition or generators of spurious results? *Ecology*, *82*(5), 1473–1483. https://doi.org/https://doi.org/10.1890/0012-9658(2001)082[1473:MLRMOB]2.0.CO;2

Harrison, X. A., Blount, J. D., Inger, R., Norris, D. R., & Bearhop, S. (2011). Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology*, *80*(1), 4–18. https://doi.org/10.1111/j.1365-2656.2010.01740.x

Hegemann, A., Marra, P. P., & Tieleman, B. I. (2015). Causes and consequences of partial migration in a passerine bird. *The American Naturalist*, *186*(4), 531–546. https://doi.org/10.1086/682667

Hegemann, A., Matson, K. D., Both, C., & Tieleman, B. I. (2012). Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years. *Oecologia*, *170*(3), 605–618. https://doi.org/10.1007/s00442-012-2339-3

Hegemann, A., Matson, K. D., Flinks, H., & Tieleman, B. I. (2013). Offspring pay sooner, parents pay later: Experimental manipulation of body mass reveals trade-offs between immune function, reproduction and survival. *Frontiers in Zoology*, *10*(1). https://doi.org/10.1186/1742-9994-10-77

Inger, R., Harrison, X. A., Ruxton, G. D., Newton, J., Colhoun, K., Gudmundsson, G. A., … Bearhop, S. (2010). Carry-over effects reveal reproductive costs in a long-distance migrant. *Journal of Animal Ecology*, *79*(5), 974–982. https://doi.org/10.1111/j.1365-2656.2010.01712.x

Jovani, R., & Rohwer, S. (2016). Fault bars in bird feathers: mechanisms, and ecological and evolutionary causes and consequences. *Biological Reviews*, *92*(2), 1113–1127. https://doi.org/10.1111/brv.12273

Koolhaas, J. ., Korte, S. ., De Boer, S. ., Van Der Vegt, B. ., Van Reenen, C. ., Hopster, H., … Blokhuis, H. . (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, *23*(7), 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Lee, K. A. (2006). Linking immune defenses and life history at the levels of the individual and the species. *Integrative and Comparative Biology*, *46*(6), 1000–1015. https://doi.org/10.1093/icb/icl049

Matson, K. D., Horrocks, N. P. C., Versteegh, M. A., & Tieleman, B. I. (2012). Baseline haptoglobin concentrations are repeatable and predictive of certain aspects of a subsequent experimentally-induced inflammatory response. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *162*(1), 7–15. https://doi.org/10.1016/j.cbpa.2012.01.010

Matson, K. D., Ricklefs, R. E., & Klasing, K. C. (2005). A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental and Comparative Immunology*, *29*(3), 275–286. https://doi.org/10.1016/j.dci.2004.07.006

Møller, A. P., & Szép, T. (2011). The role of parasites in ecology and evolution of migration and migratory connectivity. *Journal of Ornithology*, *152*, 1–10. https://doi.org/10.1007/s10336-010-0621-x

Mougin, J.-L., Jouanin, C., & Roux, F. (1997). Intermittent breeding in Cory's Shearwater *Calonectris diomedea* of Selvagem Grande, North Atlantic. *Ibis*, *139*(1), 40–44. https://doi.org/10.1111/j.1474-919X.1997.tb04502.x

Nwaogu, C. J., Cresswell, W., Versteegh, M. A., & Tieleman, B. I. (2019). Seasonal differences in baseline innate immune function are better explained by environment than annual cycle stage in a year-round breeding tropical songbird. *Journal of Animal Ecology*, *88*(4), 537–553. https://doi.org/10.1111/1365-2656.12948

Owen-Ashley, N. T., & Wingfield, J. C. (2007). Acute phase responses of passerine birds: Characterization and seasonal variation. *Journal of Ornithology*, *148*(SUPLL. 2), 583–591. https://doi.org/10.1007/s10336-007-0197-2

Perez, C., Granadeiro, J. P., Dias, M. P., Alonso, H., & Catry, P. (2014). When males are more inclined to stay at home: insights into the partial migration of a pelagic seabird provided by geolocators and isotopes. *Behavioral Ecology*, *25*(2), 313–319. https://doi.org/10.1093/beheco/art119

Pérez, C., Granadeiro, J. P., Dias, M. P., & Catry, P. (2016). Sex and migratory strategy influence corticosterone levels in winter-grown feathers, with positive breeding effects in a migratory pelagic seabird. *Oecologia*, *181*(4), 1025–1033. https://doi.org/10.1007/s00442-016-3625-2

Péron, C., Delord, K., Phillips, R. A., Charbonnier, Y., Marteau, C., Louzao, M., & Weimerskirch, H. (2010). Seasonal variation in oceanographic habitat and behaviour of white-chinned petrels *Procellaria aequinoctialis* from Kerguelen Island. *Marine Ecology Progress Series*, *416*, 267–284. https://doi.org/10.3354/meps08785

R Core Team. (2019). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Ramos, J. A., Monteiro, L. R., Sola, E., & Moniz, Z. (1997). Characteristics and competition for nest cavities in burrowing Procellariiformes. *The Condor*, *99*(3), 634–641. https://doi.org/10.2307/1370475

Ramos, R., Llabrés, V., Monclús, L., López-Béjar, M., & González-Solís, J. (2018). Costs of breeding are rapidly buffered and do not affect migratory behavior in a long-lived bird species. *Ecology*, *99*(9), 2010–2024. https://doi.org/10.1002/ecy.2435

Ricklefs, R. E., & Wikelski, M. (2002). The physiology/life-history nexus. *Trends in Ecology & Evolution*, *17*(10), 462–468. https://doi.org/10.1016/S0169-5347(02)02578-8

Rowe, L., Ludwig, D., & Schluter, D. (1994). Time, condition, and the seasonal decline of avian clutch size. *The American Naturalist*, *143*(4), 698–722. https://doi.org/10.2307/2462907

Santos, E. S. A., & Nakagawa, S. (2012). The costs of parental care: A meta-analysis of the trade-off between parental effort and survival in birds. *Journal of Evolutionary Biology*, *25*(9), 1911–1917. https://doi.org/10.1111/j.1420-9101.2012.02569.x

Selman, C., Blount, J. D., Nussey, D. H., & Speakman, J. R. (2012). Oxidative damage, ageing, and life-history evolution: Where now? *Trends in Ecology and Evolution*, *27*(10), 570–577. https://doi.org/10.1016/j.tree.2012.06.006

Sild, E., & Hõrak, P. (2009). Nitric oxide production: An easily measurable condition index for vertebrates. *Behavioral Ecology and Sociobiology*, *63*(6), 959–966. https://doi.org/10.1007/s00265-009-0710-0

Stearns, S. C. (1992). *The Evolution of Life Histories*. London: Oxford Univeristy Press.

Versteegh, M. A., Helm, B., Kleynhans, E. J., Gwinner, E., & Tieleman, B. I. (2014). Genetic and phenotypically flexible components of seasonal variation in immune function. *Journal of Experimental Biology*, *217*(9), 1510–1518. https://doi.org/10.1242/jeb.097105

Zino, P. A., Zino, F., Maul, T., & Biscoito, J. M. (1987). The laying, incubation and fledging periods of Cory’s Shearwater Calonectris diomedea borealis on Selvagem Grande in 1984. *Ibis*, *129*(2), 393–398. https://doi.org/10.1111/j.1474-919X.1987.tb03185.x

SUPPLEMENTARY MATERIAL

APPENDIX S1. HAPTOGLOBIN AND AGE

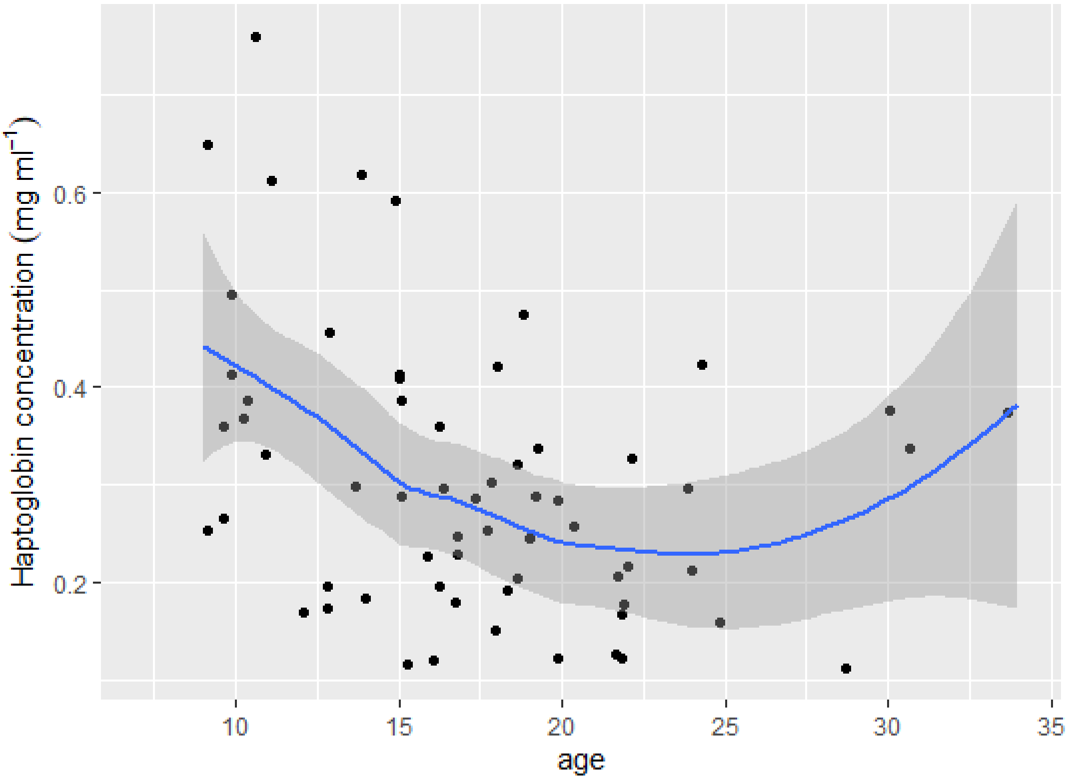

Figure S1 Haptoglobin concentration varies with age in adult Cory's Shearwaters in a cross-sectional observation of males between 9 and 34 years of age (n = 62). Shaded area around LOESS line corresponds to the 95% confidence interval.

APPENDIX S2. MODEL RESULTS COMPARING EXPERIMENTALLY INDUCED REPRODUCTIVE FAILURE USING A CONTROL GROUP OF MIXED REPRODUCTIVE OUTCOME

Of 24 control males, 18 bred successfully in 2017 while 6 failed to hatch their egg or raise a chick to fledging age. Their migratory destinations are presented in Table S1. The model comparison methods are described in the methods of the manuscript. AICc values for model selection are in Table S2.

Male Cory's Shearwaters in which reproductive failure was induced returned to the colony slightly earlier than control males. Migrants returned later than residents (Table S3).

Migratory strategy featured in models explaining haptoglobin concentration, agglutination and lysis scores, and NOx which had a ΔAICc < 2. However, the model averaged estimates and standard errors suggested that migratory strategy had a negligible effect on the immune indices (Table S2, Table S3).

While migratory strategy and reproductive failure alone had no detectable effect on residual body mass, the interaction between reproductive failure and migratory strategy had a weak effect, in that control males arrived heavier than males from the experimental group among migrants (Table S3).

Migrants had a higher fault bar intensity than residents (Table S3).

Table S1 Non-breeding destinations of male Cory's Shearwaters forming part of the experimental group with induced reproductive failure and the control group. The number of control individuals from each non-breeding destination that were not successful in their reproductive attempt in 2017 is shown in brackets.

	Non-breeding destination			
	Canary Current	**North Atlantic**	**South Africa**	**South America**
Experimental group	7	0	15	1
Control group	5 *(1)*	2 *(1)*	16 *(4)*	1 *(0)*

Table S2 AICc values and AICc weights for the selection of (generalised) linear models assessing the effects of reproductive effort and/or migratory strategy on date of return to the colony and physical condition indices in male Cory's Shearwaters (using a control group of mixed reproductive success). Models in bold are equally supported by the data (ΔAICc <2). K = number of estimable parameters including the intercept.

Dependent variable	**model**	**K**	**AICc**	**ΔAICc**	**AICc weights**
Arrival at nest	**Rep. + Mig. Strat.**	**3**	**307.9**	**0**	**0.501**
	Mig. Strat.	**2**	**308.7**	**0.73**	**0.347**
	Rep. * Mig. Strat.	4	310.4	2.52	0.142
	Rep.	2	316.9	9.01	0.006

	Null model	1	317.3	9.42	0.005
Haptoglobin	**Null model**	**2**	**-64.3**	**0**	**0.448**
	Mig. Strat.	**3**	**-63.2**	**1.16**	**0.251**
	Rep.	3	-61.9	2.41	0.134
	Rep. * Mig. Strat.	5	-61.2	3.07	0.096
	Rep. + Mig. Strat.	4	-60.6	3.69	0.071
Lysis	**Null model**	**1**	**55.3**	**0**	**0.533**
	Mig. Strat.	**2**	**57.2**	**1.83**	**0.213**
	Rep.	2	57.6	2.29	0.17
	Rep. + Mig. Strat.	3	59.6	4.22	0.065
	Rep. * Mig. Strat.	4	62.1	6.72	0.019
Agglutination	**Null model**	**1**	**108.7**	**0**	**0.435**
	Mig. Strat.	**2**	**109.5**	**0.79**	**0.293**
	Rep.	2	110.8	2.11	0.151
	Rep. + Mig. Strat.	3	111.8	3.1	0.092
	Rep. * Mig. Strat.	4	114.1	5.45	0.029
NOx	**Null model**	**1**	**-336.4**	**0**	**0.514**

	Mig. Strat.	**2**	**-334.5**	**1.88**	**0.201**
	Rep.	2	-334.1	2.27	0.165
	Rep. + Mig. Strat.	3	-332.2	4.23	0.062
	Rep. * Mig. Strat.	4	-332	4.38	0.058
Residual body mass	**Null model**	**1**	**348.9**	**0**	**0.417**
	Mig. Strat.	**2**	**350.2**	**1.3**	**0.218**
	Rep. * Mig. Strat.	**4**	**350.9**	**1.95**	**0.158**
	Rep.	2	351.1	2.21	0.139
	Rep. + Mig. Strat.	3	352.5	3.6	0.069
Fault bar intensity	**Mig. Strat.**	**2**	**182.9**	**0**	**0.575**
	Rep. + Mig. Strat.	**3**	**184.7**	**1.86**	**0.227**
	Rep. * Mig. Strat.	4	185.6	2.74	0.147
	Null model	1	188.7	5.81	0.032
	Rep.	2	189.7	6.81	0.019

Table S3 Model-averaged parameter estimates, their standard errors (±SE), and 95% confidence intervals (CI), calculated across models considered competitive (ΔAICc < 2). See Table S2 and methods in manuscript for more details.

Dependent variable	**Independent variables**	**Est ± SE**	**95% CI**	
			Lower	**Upper**
Arrival at nest	Mig. Strat.	14.98 ± 4.30	6.29	23.67
	Rep.	-5.29 ± 3.01	-11.38	81.24
Haptoglobin	Mig. Strat.	0.53 ± 0.47	-0.42	1.47
Lysis	Mig. Strat.	0.04 ± 0.07	-0.09	0.17
Agglutination	Mig. Strat.	-0.30 ± 0.25	-0.80	0.20
NOx	Mig. Strat.	19.80 ± 31.09	-43.26	82.85
Residual body mass	Mig. Strat.	-3.82 ± 30.77	-65.68	58.03
	Rep.	41.30 ± 31.07	-22.34	104.95
	Mig. Strat * Rep.	-78.62 ± 38.32	-157.10	-0.13
Fault bar intensity	Mig. Strat.	0.60 ± 0.23	0.14	1.06
	Rep.	-0.12 ± 0.18	-0.49	0.24

APPENDIX S3. *MEAN VALUES OF PHYSICAL CONDITION INDICES IN ADULT MALE CORY'S SHEARWATERS FROM THE SELVAGEM GRANDE BREEDING POPULATION.*

Table S4 Means, standard deviations (std. dev), ranges, and sample sizes (n) for indices of physical condition measured in adult male Cory's Shearwaters from the Selvagem Grande colony.

Condition index	**mean**	**std. dev.**	**range**	**n**
Haptoglobin (mg ml^{-1})	0.294	0.120	0.111 - 0.612	45
Lysis	0.5	0.4	0 – 1.8	47
Agglutination	4.9	0.8	3.0 – 6.3	47
NOx (µM)	0.006	0.003	0.002 – 0.015	38
Fault bar intensity (no. of affected tail feathers)	3.3	2.6	0 - 10	41

CHAPTER 4

GENERALIST SEABIRDS AS BIOMONITORS OF OCEAN MERCURY: THE IMPORTANCE OF ACCURATE TROPHIC POSITION ASSIGNMENT

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ABSTRACT

Monitoring mercury concentration in the marine environment is pivotal due to the risks that mercury intake poses to the ecosystem and human health. It is therefore of interest to make reliable, comparative measurements over large geographic areas. Here, the utility of wide-ranging generalist seabirds as mercury biomonitors at an ocean basin scale was assessed, using the Cory’s Shearwater as a model species. The mercury concentration in flight feathers moulted at distant non-breeding areas of geolocator-tracked birds was quantified, reflecting contamination in various geographic areas. Compound-specific isotope analysis of amino acids was used to obtain comparable trophic positionestimates controlled for baseline isoscape. Birds that remained resident in the Canary Current integrated less mercury into their feathers than those that migrated to either the Benguela or Agulhas currents. Residents also occupied a significantly lower trophic position during the non-breeding season than migrants, largely explaining the difference in mercury exposure. Both mercury concentration and trophic position were similar in individuals spending the non-breeding period in the Benguela and Agulhas currents. This paper highlights the importance of accurate trophic position calculation in order to understand mercury exposure in wide-ranging predators and for meaningful spatial comparisons.

Keywords: biomagnification, biomonitoring, CSIA-AA, mercury, seabird, trophic position

INTRODUCTION

Mercury is a trace metal which can cause severe negative impacts on the nervous system at elevated doses. Since marine top predators have mercury values that are comparable to those which humans may be exposed to (Monteiro & Furness, 1995), researchers have used fish, marine mammals, and several species of seabirds as biomonitors of mercury contamination. The foraging and movement ecology of many seabird populations are well-known, allowing the quantification of mercury exposure integrated over known geographical and temporal scales (Monteiro & Furness, 1995). As with other birds, plumage is one of the major sinks for mercury excretion, where mercury is deposited during feather growth and is therefore cut off from body circulation once the feather is devascularised (Monteiro & Furness, 2001). The sampling of discrete feathers for which the timing of moult is known could therefore allow higher temporal and spatial resolution of mercury exposure at the time of feather growth

(Hobson, 1999). Additionally, since feather sampling is only minimally invasive, many individuals can be assessed with little or no negative effects (Monteiro & Furness, 1995).

Mercury biomagnifies, and its concentration in the body is dependent on the trophic position (TP) and diet composition of the studied organism (Lavoie, Jardine, Chumchal, Kidd, & Campbell, 2013; Thompson, Furness, & Monteiro, 1998). Stable isotope analysis (SIA) of nitrogen (δ^{15}N) is routinely used to assign trophic status due to the differential assimilation of nitrogen isotopes up the food chain (Hobson, Piatt, & Pitocchelli, 1994). However, interpretation of bulk SIA over large oceanic areas is complicated by the lack of a well-defined and stable isoscape at the regional level in the marine environment (Graham, Koch, Newsome, McMahon, & Aurioles, 2010). Consequently, interpreting how much of the stable isotope ratio variation is a product of TP rather than spatial variation in baseline levels is usually difficult. A developing method that can overcome this is compound-specific stable isotope analysis of amino acids (CSIA-AA). CSIA-AA can measure δ^{15}N in individual “source” and “trophic” amino acids which differ in the extent of ^{15}N-fractionationalong the food chain. The combination of the stable isotope signature of amino acids from these two classes is reliably used to reflect TP (Ohkouchi et al., 2017; Quillfeldt et al., 2017).

Here, the utility of wide-ranging generalist seabirds as mercury biomonitors at an ocean basin scale was assessed, using the Cory’s Shearwater (*Calonectris borealis*) as a model species. The majority of Cory’s Shearwaters breeding on Selvagem Grande, Madeira, spends the non-breeding period in the Benguela Current in the south-east Atlantic and the adjacent Agulhas Current in the Indian Ocean, while around a fifth of males remain predominantly resident year round in the Canary Current, and a smaller number of individuals use non-breeding areas in the northwest and southwest Atlantic (Dias, Granadeiro, Phillips, Alonso, & Catry, 2011). By sampling flight feathers grown during the non-breeding period from birds that were simultaneously tracked using light-level geolocators (GLS), feather mercury concentration and CSIA-derived TP in males was quantified in order to (1) investigate whether Cory’s Shearwaters spending the non-breeding period in geographically separated areas are exposed to different environmental mercury concentrations, and (2) assess whether differences in mercury concentration are a result of different non-breeding TP. Whether (3) the sexes were differentially exposed to environmental mercury in their common non-breeding areas was also assessed by analysing feather mercury in females. Cory’s Shearwaters are largely epipelagic predators of fish and cephalopods, but can also incorporate mesopelagic species in their diet in small amounts (Alonso et al., 2014). While differences in environmental mercury contamination are expected in such geographically distinct areas, using CSIA-AA one might also expect to see TP differences from diet flexibility towards predominant prey, which would also expose individuals to different mercury concentrations. If males and females differed in their mercury exposure in shared non-breeding areas, it would also suggest trophic segregation in the sexes over the non-breeding season.

METHODS

FIELD WORK

Selvagem Grande (Lat 30.140556, Long -015.868889) is an island nature reserve ca. 300km south of the Madeiran Archipelago, Portugal. Between June and July2016 GLS (Migrate Technology model C330) were deployed on 182 incubating Cory's Shearwaters. GLS were leg mounted and constituted less than 1% of the birds' body weight, which, given the attachment method, can be assumed to bear minimal or no effects on survival and fitness (Bodey et al., 2017). In 2017, the GLS were retrieved and flight feathers were sampled. Secondary 8 (S8) is postulated to moult late in the non-breeding area after many other flight feathers (Ramos, Militão, González-Solís, & Ruiz, 2009). Therefore, 2cm of the tip of both S8 feathers were sampled to quantify mercury exposure in the non-breeding area, together with the corresponding isotope signature in a subsample of individuals. The resighting rate (~90%) of birds fitted with GLS is consistent with the rate of return of breeding birds from one year to the next (Mougin, Jouanin, & Roux, 1997).

POSITIONAL DATA ANALYSIS

GLS light level data was analysed using IntiProc (version 1.03, Migrate Technology Ltd.). Sun elevation angle was calculated from device calibration data. Cory's Shearwaters occupy distinct oceanic currents during the non-breeding period(Dias et al., 2011), and these non-breeding areas can be assigned from the mapped tracking data (Figure 1).

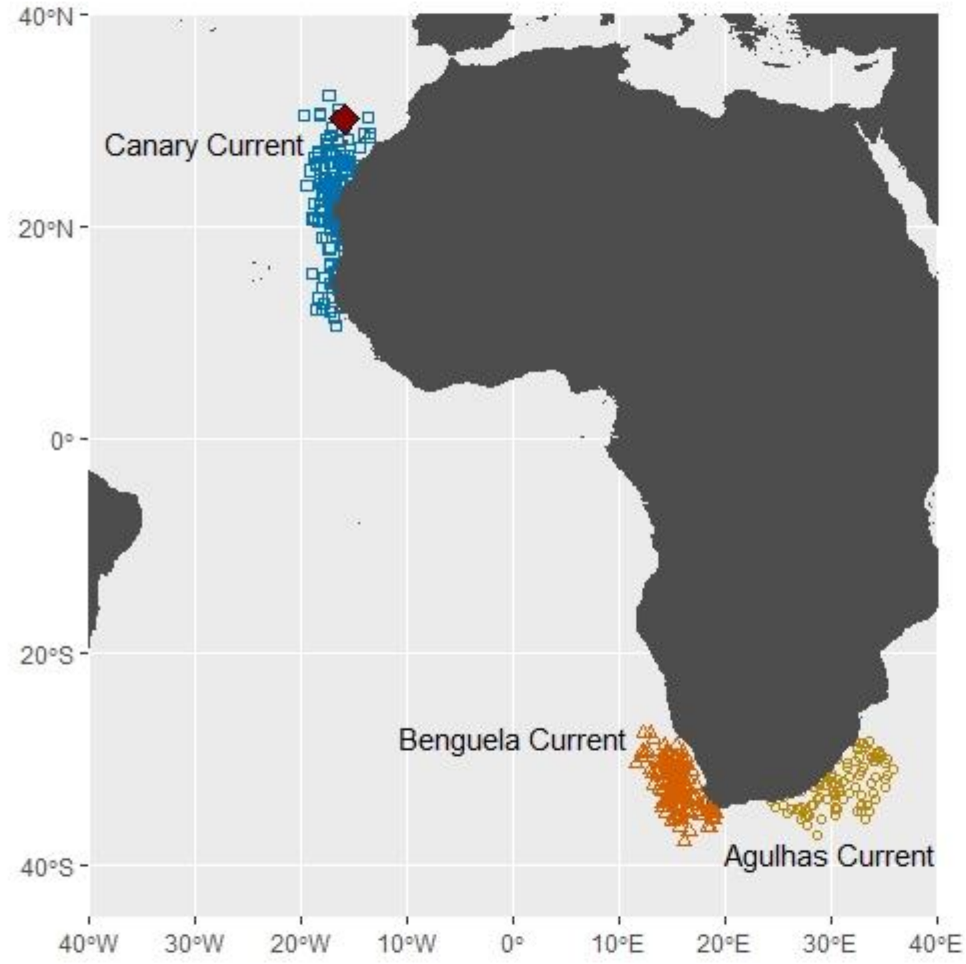

Figure 1 The GLS positional data of three Cory's Shearwaters (circles, triangles, and squares) during the non-breeding period (December 2016 to February 2017). The colony location (Selvagem Grande) is denoted by the solid diamond in the Canary Current. While residents, exclusively males, remain largely in the Canary Current region during this period (squares), most migrants traverse the Atlantic towards southern Africa to spend the non-breeding period in the Benguela Current (triangles) or the Agulhas Current (circles) in the Indian Ocean.

MERCURY QUANTIFICATION AND CSIA-AA

Mercury concentration in homogenised feather samples was quantified by thermal decomposition atomic absorption spectrometry with gold amalgamation using an AMA-254 spectrophotometer (LECO, Czech Republic), as described by Costley et al. (2000). This procedure does not require sample pre-treatment or sample pre-concentration. Accuracy and precision were assured by regular analysis of certified reference material SRM2976, obtained from the National Research Council of Canada, throughout each day of analyses. The mercury recovery rates ranged between 80% and 100% and sample mercury concentrations were corrected for the daily recovery percentage of the reference material. The lower limit of detection was 0.2 mgkg^{-1} (dry weight). All samples were measured in duplicate, or until obtaining two readings with a coefficient of variation under 10%, and their average used in all statistical tests.

CSIA-AA analysis of $\delta^{15}N$ was carried out on S8 samples from seven males from each of the three major non-breeding areas – Canary Current, Benguela Current, and Agulhas Current. Males were prioritised over females due to financial constraints, and since males show a greater variety in their non-breeding destinations than females. These were selected at random after excluding individuals with outlying feather mercury concentrations or which visited secondary non-breeding areas during the non-breeding period. Around 4mg of feather was homogenised and analysed at the UC Davis Stable Isotope Facility. Gas chromatography combustion isotope ratio mass spectrometry was performed following Walsh et al. (2014) and Yarnes and Herszage (2017).

DATA ANALYSIS

All statistical analyses were carried out on R version 3.6.2 (R Core Team, 2019).

Feather mercury concentrations were obtained from 120 Cory’s Shearwaters of known non-breeding destinations. Of these, 14 spent the non-breeding period in areas of the northwest and southwest Atlantic – these were not considered in the statistical analysis due to the small sample sizes in each non-breeding area.

A linear model (‘lm’, package *stats*) was run to investigate the effect of sex on feather mercury among individuals that migrated to the Agulhas and Benguela currents, including the interaction between sex and non-breeding area. Resident individuals were excluded from this model since these are exclusively males.

The effect of non-breeding area on feather mercury concentration was assessed by running an ANOVA and post-hoc Tukey test (‘aov’, ‘TuckeyHSD’, package *stats*) considering only males spending the non-breeding season in the three major non-breeding areas (Canary Current, Benguela Current, and Agulhas Current).

TP was calculated from $\delta^{15}N$ values of glutamic acid (glu) and phenylalanine (phe). Including multiple trophic discrimination factors (TDF) in the estimation of TP to integrate the span of the trophic web produces more robust results, calculated as follows:

EQUATION 1

$$TL = 2 + \frac{glu - phe - 7.6‰ - 3.4‰}{3.5‰}$$

where 7.6 is the TDF for TP at the base of the aquatic food chain (*Δ_{herbivore}*), 3.5 is the TDF for seabird feathers (*Δ_{carnivore})*, and 3.4 is the difference in δ¹⁵N between glu and phe in primary producers (*β)* (McMahon & McCarthy, 2016; Ohkouchi et al., 2017; Quillfeldt et al., 2017).Uncertainty in TP was calculated by propagation of errors to take into account both analytical and ecological variation using the analytical solution of differentiation (Ohkouchi et al., 2017):

EQUATION 2

$$\sigma_{TP}^2 = \left(\frac{1}{\Delta_{carnivore}}\right)^2 \sigma_{\delta 15N(glu)}^2 + \left(\frac{-1}{\Delta_{carnivore}}\right)^2 \sigma_{\delta 15N(phe)}^2 + \left(\frac{1}{\Delta_{carnivore}}\right)^2 \sigma_{\beta}^2 + \left(\frac{-1}{\Delta_{carnivore}}\right)^2 \sigma_{\Delta_{carnivore}}^2 + \left\{\frac{-1}{\Delta_{carnivore}^2}\left(\delta^{15}N_{glu} - \delta^{15}N_{phe} + \beta - \Delta_{herbivore}\right)\right\}^2 \sigma_{herbivore}^2$$

where $\sigma_{\Delta carnivore}$ and $\sigma_{\Delta herbivore}$ are estimated at 0.4‰(McMahon, Polito, Abel, Mccarthy, & Thorrold, 2015) and 1.4‰ (Chikaraishi, Kashiyama, Ogawa, Kitazato, & Ohkouchi, 2007) respectively, and $\sigma_{\beta}$is 0.9‰ (Chikaraishi et al., 2009).

An ANOVA and post-hoc Tukey test were carried out to compare the TP of males in the three non-breeding areas, and ran a linear model relating CSIA-AA-derived TP with feather mercury concentration in the 21 males for which TP was calculated.

The trophic magnification factor (TMF) indicates the level of biomagnification of a pollutant up a food chain. This was calculated as the antilog of the slope in the linear regression between the $\log_{10}$-transformed feather mercury concentration and CSIA-AA-derived TP (Borgå et al., 2012).

RESULTS

The mean (± std. dev.) feather mercury concentration across the population was 5.49 ± 1.78 mg kg-1. Males (n = 58) harboured significantly higher feather mercury concentrations than females (n = 30) (Table 1, Figure 2). However, non-breeding area significantly affected mercury concentration in S8 feathers (ANOVA F2, 106 = 8.01, p = 0.0007) – male Cory's Shearwaters wintering in the Canary Current had significantly lower feather mercury concentrations (4.23 ± 1.38 mg kg-1, n = 18) than males that migrated to either Agulhas (6.45 ± 1.56 mg kg-1, n = 8) or Benguela Currents (5.91 ± 1.73 mg kg-1, n = 50) (Table 1, Figure 2).

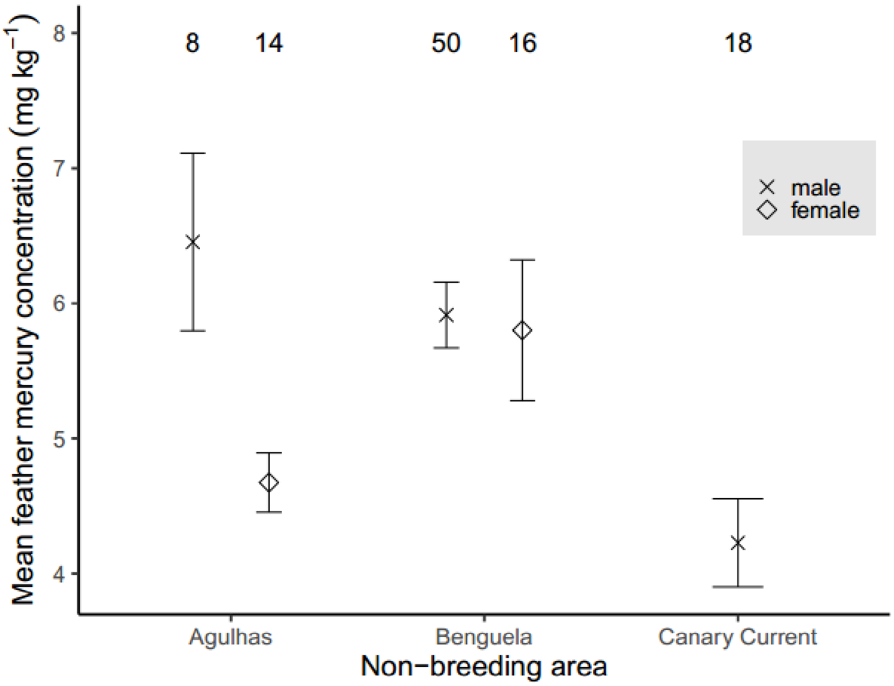

Figure 2 Mean (± standard error) mercury concentrations of male and female Cory's Shearwater S8 feathers grown in the non-breeding areas. Only males overwinter in the Canary Current. Sample sizes are indicated above each point.

Table 1 (A) Linear model results comparing feather mercury concentration between sexes, taking only migrants into consideration. (B) Tukey comparisons of feather mercury concentrations from males spending the non-breeding season in the Agulhas, Benguela, or Canary Currents. Significant results are in italics.

(A)	Estimate ± SE	t	p
Sex	*-1.78 ± 0.76*	*-2.36*	*0.021*
Non-breeding area	-2.21 ± 1.44	-1.53	0.129
*Sex * Non-breeding area*	*1.67 ± 0.90*	*1.85*	*0.067*
(B)	Tukey multiple comparisons of means		
Agulhas - Benguela	p = 0.671		
Agulhas – Canary Current	*p = 0.007*		
Benguela – Canary Current	*p = 0.001*		

Propagated errors associated with TP, calculated by Equation2, were all less than 0.5 (mean = 0.35), indicating the precision of this technique. Resident male Cory's Shearwaters occupied a significantly lower TP than migratory males (ANOVA: $F_{2, 21} = 28.5$, $p < 0.0001$, Tukey post-hoc tests: Benguela – Agulhas: p = 0.60, Canary Current – Agulhas: p<0.0001, Canary Current – Benguela: p<0.0001). TP was strongly correlated with log-transformed feather mercury concentration (Est = 0.30 (±0.07), t = 4.29, p = 0.0004, $R^2 = 0.465$), suggesting that non-breeding diet differences had a significant contribution to mercury exposure in the different non-breeding areas (Figure 3). The TMF for this system, calculated as the exponent of the slope of this relationship ($10^{0.30}$), was 1.98.

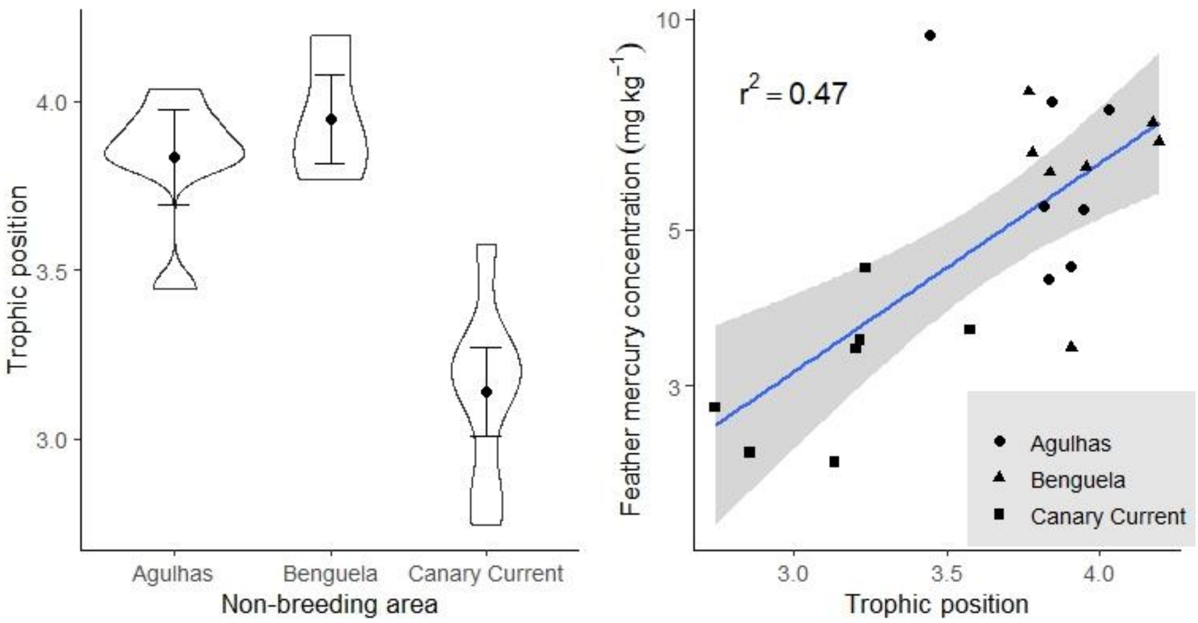

Figure 3 Left: Violin plots showing how non-breeding trophic position (TP), as calculated from the results of compound specific isotope analysis of feather amino acids, is significantly lower in males remaining resident than in those that migrate. Points and error bars represent average TP ± uncertainty of the average calculated from propagated error (n = 7 for each non-breeding area). **Right:** The relationship between log10 feather mercury concentration and TP as measured in secondary 8 (n = 21). The shaded area represents the 95% confidence interval.

DISCUSSION

Our study shows how CSIA-AA-derived TP can help untangle the causes of variation in mercury concentrations among spatially separated groups of individuals, improving the understanding of mercury exposure in generalist top predators often used as biomonitors, and validating single-species studies for the monitoring of mercury over oceanic scales. Our results also suggest that the levels of contamination in the Agulhas and Benguela currents are similar.

Given the importance of plumage as a mercury sink, it is plausible that mercury intake during the breeding season shapes feather mercury deposition during moult (Furness, Muirhead, & Woodburn, 1986). Previous studies pooling migrants travelling to various non-breeding areas reported an uncoupling between mercury concentration and isotope ratios inS8 feathers grown in the non-breeding season, suggesting the possibility of a limited contemporaneous origin of mercury in these feathers and a larger role of the inter-moult body burden

(*Calonectris* shearwaters: Ramos, González-Solís, et al., 2009; Northern Fulmars *Fulmarus glacialis*, Great Skuas *Catharacta skua*: Thompson, Bearhop, Speakman, & Furness, 1998). However, these studies were limited by the use of bulk SIA, as opposed to CSIA-AA, which, in the absence of baseline values, is less accurate in estimating TP for comparisons between geographically distinct areas (Ohkouchi et al., 2017). The methodological advantage of CSIA-AA, coupled with the large differences observed between groups, enabled the detection of a relationship between TP and mercury concentration in a late-moulted feather in the non-breeding period, suggesting an effect of contemporaneous diet on mercury concentrations.

The TMF of mercury calculated using the CSIA-AA-derived TP of Cory's Shearwaters ($10^{0.30}$) is higher than the mean TMF value often quoted for marine ecosystems ($10^{0.20}$), which could suggest factors other than TP are responsible for the differences in mercury concentration. However, the TMF is not uniform across different elements of a community (Lavoie et al., 2013). Moreover, diet composition may influence mercury exposure even without a change in TP, such as the case of the higher mercury burden in mesopelagic compared to epipelagic prey, both present in the diet of generalist predators (Thompson, Furness, et al., 1998). Therefore, while a strong influence of TP on feather mercury concentration is clear in spatially separated Cory's Shearwaters, the contributions of differences in environmental contaminant levels or foraging strategy to the observed mercury values cannot be excluded. The causes of this large TP difference between these two sites merit further research.

While differences in feather mercury concentrations are often reported between males and females, our results largely arise from the greater sex-specific difference seen in birds spending the non-breeding period in the Agulhas Current, explaining the near-significant interaction of non-breeding area and sex. While sex-specific foraging differences are mostly documented in the breeding grounds (Alonso et al., 2014) there is some evidence that males and females differ in their ecological niche during the non-breeding period (De Felipe et al., 2019).In the absence of sexual spatial segregation within common non-breeding areas, the cause of sex-differences in mercury exposure in the Agulhas Current is likely a result of different foraging behaviour or diet composition. However, the drivers for this segregation do not appear to be present in the Benguela Current, where both sexes co-occur as well.

CONCLUSION

Comparisons of mercury concentrations over time and space using generalist predators are likely to remain a challenge despite methodological improvements. Nevertheless, seabirds represent an important sentinel group of flagship species that can be used in the communication of data to policy makers and the public (Lescroël et al., 2016). The utility of feathers as discrete sampling units of large temporal and spatial scales is possibly unparalleled. Coupling limited CSIA-AA results with bulk SIA from a larger sample of individuals has started to be used to increase the sample size of investigations (Dolgova et al., 2018). The greater understanding of flexible foraging and mercury exposure afforded improves our ability to use migratory generalist predators in environmental monitoring. Furthermore, our data supports the idea that sex differences over the non-breeding period may occur under certain conditions, meriting further research.

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Author contributions

PC and JPG conceptualised the study and acquired funding, MCG planned the methodology and drafted the MS, MCG and BR carried out the fieldwork and analysed the data, BR carried out the mercury quantification under EP's supervision. All authors were involved in the reviewing and editing of the MS.

REFERENCES

Alonso, H., Granadeiro, J. P., Waap, S., Xavier, J., Symondson, W. O. C., Ramos, J. A., & Catry, P. (2014). An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. *Molecular Ecology*, *23*(15), 3719–3733. https://doi.org/10.1111/mec.12785

Bodey, T. W., Cleasby, I. R., Bell, F., Parr, N., Schultz, A., Votier, S. C., & Bearhop, S. (2017). A phylogenetically controlled meta-analysis of biologging device effects on birds: Deleterious effects and a call for more standardized reporting of study data. *Methods in Ecology and Evolution*, *9*(4), 946–955. https://doi.org/10.1111/ijlh.12426

Borgå, K., Kidd, K. A., Muir, D. C. G., Berglund, O., Conder, J. M., Gobas, F. A. P. C., … Powellkk, D. E. (2012). Trophic magnification factors: Considerations of ecology, ecosystems, and study design. *Integrated Environmental Assessment and Management*, *8*(1), 64–84. https://doi.org/10.1002/ieam.244

Chikaraishi, Y., Kashiyama, Y., Ogawa, N. O., Kitazato, H., & Ohkouchi, N. (2007). Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: Implications for aquatic food web studies. *Marine Ecology Progress Series*, *342*(2003), 85–90. https://doi.org/10.3354/meps342085

Chikaraishi, Y., Ogawa, N. O., Kashiyama, Y., Takano, Y., Suga, H., Tomitani, A., … Ohkouchi, N. (2009). Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnology and Oceanography: Methods*, *7*(11), 740–750. https://doi.org/10.4319/lom.2009.7.740

Costley, C. T., Mossop, K. F., Dean, J. R., Garden, L. M., Marshall, J., & Carroll, J. (2000). Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. *Analytica Chimica Acta*, *405*(1–2), 179–183. https://doi.org/10.1016/S0003-2670(99)00742-4

De Felipe, F., Reyes-González, J. M., Militão, T., Neves, V. C., Bried, J., Oro, D., … González-Solís, J. (2019). Does sexual segregation occur during the non-breeding period? A comparative analysis in spatial and feeding ecology of three *Calonectris* shearwaters. *Ecology and Evolution*, *in press*(June), 1–18. https://doi.org/10.1002/ece3.5501

Dias, M. P., Granadeiro, J. P., Phillips, R. A., Alonso, H., & Catry, P. (2011). Breaking the routine: individual Cory's shearwaters shift winter destinations between hemispheres and across ocean basins. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1713), 1786–1793. https://doi.org/10.1098/rspb.2010.2114

Dolgova, S., Popp, B. N., Courtoreille, K., Espie, R. H. M., Maclean, B., McMaster, M., … Hebert, C. E. (2018). Spatial trends in a biomagnifying contaminant: Application of amino acid compound-specific stable nitrogen isotope analysis to the interpretation of bird mercury levels. *Environmental Toxicology and Chemistry*, *37*(5), 1466–1475. https://doi.org/10.1002/etc.4113

Furness, R. W., Muirhead, S. J., & Woodburn, M. (1986). Using bird feathers to measure mercury in the environment: Relationships between mercury content and moult. *Marine Pollution Bulletin*, *17*(1), 27–30.

Graham, B. S., Koch, P. L., Newsome, S. D., McMahon, K. W., & Aurioles, D. (2010). Using Isoscapes to Trace the Movements and Foraging Behavior of Top Predators in Oceanic Ecosystems. In J. West, G. Bowen, T. Dawson, & K. Tu (Eds.), *Isoscapes* (pp. 299–318). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-90-481-3354-3_14

Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, *120*(3), 314–326. https://doi.org/10.1007/s004420050865

Hobson, K. A., Piatt, J. F., & Pitocchelli, J. (1994). Using stable isotopes to determine seabird trophic relationships. *The Journal of Animal Ecology*, *63*(4), 786. https://doi.org/10.2307/5256

Lavoie, R. A., Jardine, T. D., Chumchal, M. M., Kidd, K. A., & Campbell, L. M. (2013). Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. *Environmental Science and Technology*, *47*(23), 13385–13394. https://doi.org/10.1021/es403103t

Lescroël, A., Mathevet, R., Péron, C., Authier, M., Provost, P., Takahashi, A., & Grémillet, D. (2016). Seeing the ocean through the eyes of seabirds: A new path for marine conservation? *Marine Policy*, *68*, 212–220. https://doi.org/10.1016/j.marpol.2016.02.015

McMahon, K. W., & McCarthy, M. D. (2016). Embracing variability in amino acid δ 15 N fractionation: mechanisms, implications, and applications for trophic ecology. *Ecosphere*, *7*(12), 1–26. https://doi.org/10.1002/ecs2.1511

McMahon, K. W., Polito, M. J., Abel, S., Mccarthy, M. D., & Thorrold, S. R. (2015). Carbon and nitrogen isotope fractionation of amino acids in an avian marine predator, the gentoo penguin (*Pygoscelis papua*). *Ecology and Evolution*, *5*(6), 1278–1290. https://doi.org/10.1002/ece3.1437

Monteiro, L. R., & Furness, R. W. (1995). Seabirds as monitors of mercury in the marine environment. *Water, Air, and Soil Pollution*, *80*(1–4), 851–870. https://doi.org/10.1007/BF01189736

Monteiro, L. R., & Furness, R. W. (2001). Kinetics, dose–response, and excretion of methylmercury in free-living adult Cory’s Shearwaters. *Environmental Science & Technology*, *35*(4), 739–746. https://doi.org/10.1021/ES000114A

Mougin, J.-L., Jouanin, C., & Roux, F. (1997). Intermittent breeding in Cory’s Shearwater *Calonectris diomedea* of Selvagem Grande, North Atlantic. *Ibis*, *139*(1), 40–44. https://doi.org/10.1111/j.1474-919X.1997.tb04502.x

Ohkouchi, N., Chikaraishi, Y., Close, H. G., Fry, B., Larsen, T., Madigan, D. J., … Yokoyama, Y. (2017). Advances in the application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies. *Organic Geochemistry*, *113*, 150–174. https://doi.org/10.1016/j.orggeochem.2017.07.009

Quillfeldt, P., Thorn, S., Richter, B., Nabte, M., Coria, N., Masello, J. F., … Libertelli, M. (2017). Testing the usefulness of hydrogen and compound-specific stable isotope analyses in seabird feathers: a case study in two sympatric Antarctic storm-petrels. *Marine Biology*, *164*(9), 192. https://doi.org/10.1007/s00227-017-3224-8

R Core Team. (2019). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Ramos, R., González-Solís, J., Forero, M. G., Moreno, R., Gómez-Díaz, E., Ruiz, X., & Hobson, K. A. (2009). The influence of breeding colony and sex on mercury, selenium and lead levels and carbon and nitrogen stable isotope signatures in summer and winter feathers of *Calonectris* shearwaters. *Oecologia*, *159*(2), 345–354. https://doi.org/10.1007/s00442-008-1215-7

Ramos, R., Militão, T., González-Solís, J., & Ruiz, X. (2009). Moulting strategies of a long-distance migratory seabird, the Mediterranean Cory's Shearwater *Calonectris diomedea* diomedea. *Ibis, 151*(1), 151–159. https://doi.org/10.1111/j.1474-919X.2008.00877.x

Thompson, D. R., Bearhop, S., Speakman, J. R., & Furness, R. W. (1998). Feathers as a means of monitoring mercury in seabirds: Insights from stable isotope analysis. *Environmental Pollution*, *101*(2), 193–200. https://doi.org/10.1016/S0269-7491(98)00078-5

Thompson, D. R., Furness, R. W., & Monteiro, L. R. (1998). Seabirds as biomonitors of mercury inputs to epipelagic and mesopelagic marine food chains. *Science of the Total Environment*, *213*(1–3), 299–305. https://doi.org/10.1016/S0048-9697(98)00103-X

Walsh, R. G., He, S., & Yarnes, C. T. (2014). Compound-specific δ13C and δ15N analysis of amino acids: A rapid, chloroformate-based method for ecological studies. *Rapid Communications in Mass Spectrometry, 28*(1), 96–108. https://doi.org/10.1002/rcm.6761

Yarnes, C. T., & Herszage, J. (2017). The relative influence of derivatization and normalization procedures on the compound-specific stable isotope analysis of nitrogen in amino acids. *Rapid Communications in Mass Spectrometry*, *31*(8), 693–704. https://doi.org/10.1002/rcm.7832

CHAPTER 5

UNTANGLING CAUSES OF VARIATION IN MERCURY CONCENTRATION BETWEEN FLIGHT FEATHERS

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ABSTRACT

Bird feathers are one of the most widely used animal tissue in mercury biomonitoring, owing to the ease of collection and storage. They are also the principal excretory pathway of mercury in birds. However, limitations in our understanding of the physiology of mercury deposition in feathers has placed doubt on the interpretation of feather mercury concentratoins. Throughout the literature, moult sequence and the depletion of the body mercury pool have been taken to explain patterns such as the decrease in feather mercury from the innermost (P1) to the outermost primary feather (P10) of the wing. However, it has been suggested that this pattern is rather a measurement artefact as a result of the increased feather mass to length ratio along the primaries, resulting in a dilution effect in heavier feathers. Here, we attempt to untangle the causes of variation in feather mercury concentrations by quantifying the mercury concentration as μg of mercury (i) per gram of feather, (ii) per millimetre of feather, and (iii) per day of feather growth in the primary feathers of Bulwer's Petrel *Bulweria bulwerii* chicks, effectively controlling for some of the axes of variation that may be acting in adults, and monitoring the growth rate of primary feathers in chicks. The mercury concentration in Bulwer's petrel chicks' primaries increased from the innermost to the outermost primary for all three concentration measures, following the order of feather emergence. These observations confirm that the pattern of mercury concentration across primary feathers is not an artefact of the measure of concentration, but is likely an effect of the order of feather growth, whereby the earlier grown feathers are exposed to higher blood mercury concentrations than are later moulted feathers as a result of blood mercury depletion.

Keywords: biomonitoring, mercury, feathers, pollution, seabirds

INTRODUCTION

The use of wildlife as biomonitors of mercury is routine in order to quantify mercury pollution and the associated bioaccumulation risks to the health of humans and other animals (Chételat, Ackerman, Eagles-Smith, & Hebert, 2020). Several tissues, particularly of top predators in both the marine and terrestrial ecosystems, have been targeted for mercury quantification, but one of the most practical and widely used is probably bird feathers (Albert, Renedo, Bustamante, & Fort, 2019; Appelquist, Asbirk, & Drabæk, 1984; Burger, 1994; Furness, Muirhead, & Woodburn, 1986; Thompson, Bearhop, Speakman, & Furness, 1998).

Feathers are complex, inert keratin structures (Crewther, Fraser, Lennox, & Lindley, 1965) which are disconnected from the bird's blood circulation once fully grown. For this reason, and since they are moulted seasonally, feathers can be sampled from live birds relatively non-intrusively and non-destructively. They are the most important excretory pathway of mercury in birds, which is deposited in the form of methyl mercury during feather growth (Braune & Gaskin, 1987; Lewis & Furness, 1991; Monteiro & Furness, 2001). Mercury bound within feathers is then stable with respect to environmental exposure (Appelquist et al., 1984). As a result, feathers do not require specific storage conditions, making them favourable over blood and tissue samples in remote field conditions (Appelquist et al., 1984).

Knowledge of the moult sequence of feathers, coupled with information on the movement ecology of a species, has been used to determine mercury exposure at specific temporal and spatial scales (Gatt, Reis, Granadeiro, Pereira, & Catry, 2020; Mallory et al., 2015; Ramos, González-Solís, et al., 2009; Watanuki et al., 2015). However, the physiological mechanisms controlling feather mercury deposition are not well known (Bortolotti, 2010), and this may have important consequences on data interpretation. Across avian taxa, mercury concentration in primary feathers is often seen to decrease from the innermost (P1) to the outermost primary (P10) (Furness et al., 1986; Martínez, Crespo, Fernández, Aboal, & Carballeira, 2012; Peterson, Ackerman, Toney, & Herzog, 2019). Throughout the literature, this pattern has been explained as a result of moult sequence and the depletion of the body mercury pool (Braune & Gaskin, 1987; Carravieri, Bustamante, Churlaud, Fromant, & Cherel, 2014; Dauwe, Bervoets, Pinxten, Blust, & Eens, 2003; Furness et al., 1986; Thompson et al., 1998). However, it has been suggested that this pattern is rather a measurement artefact as a result of the increased feather mass to length ratio along the primaries, resulting in a dilution effect in heavier feathers (Bortolotti, 2010). The latter is based on the argument that mercury deposition into feathers is dependent on the amount of time that the growing feather is exposed to the blood circulation (time-dependent deposition), and is not bound to the feather in a mass-dependent fashion. Without a better understanding of the mechanisms controlling mercury deposition into feathers, interpretation of feather mercury may be unreliable or spurious (Bond, 2010).

Here, we attempt to untangle the causes of variation in feather mercury concentrations by quantifying the mercury concentration in the primary feathers of seabird chicks, effectively controlling for some of the axes of variation that may be acting in adults. Bulwer's Petrels (*Bulweria bulwerii*) are small, nocturnal, mesopelagic predators, which breed in large numbers on Deserta Grande, Madeira, in the east Atlantic Ocean (Waap et al., 2017). They experience a fledging period of 61 days on average and a fledging success usually above 80% (Nunes, 2000). Adult Bulwer's Petrels act as central place foragers during the breeding period, which spans between April and September (Nunes & Vicente, 1998). As a result, chicks are exposed to mercury contamination from the parents' provisioning trips within a restricted geographical range around the colony (Chaurand & Weimerskirch, 1994; Shoji et al., 2015; Wischnewski et al., 2019).

Chicks grow their flight feathers simultaneously at the nest (Nunes & Vicente, 1998). If moult sequence determines feather mercury concentration in adults, we would expect chick primary feathers to have similar mercury concentrations, reflecting their simultaneous growth. If inter-feather differences persist in simultaneously grown feathers, other factors must be at play.

Bortolotti (2010) proposed that measuring the concentration of mercury as μg of mercury per gram of feather induces artefacts as a result of the variation in the length-to-weight ratio of feathers of various sizes and shapes and the time-dependent deposition of mercury into growing feathers, suggesting mercury concentration as μg of mercury per millimetre of feather as an alternative to control for this. Here, we designed an analysis protocol that allows for the quantification of the mass of feather mercury per millimetre of feather to test this hypothesis. If any pattern seen in mercury concentration expressed as μg of mercury per gram of feather persists under this new concentration measurement then the trend in mercury concentration across primaries is not a direct artefact of this phenomenon.

Although the rate of wing growth has been assessed for Bulwer's petrels (Nunes & Vicente, 1998), individual flight feather growth rate has not. In addition to collecting primary feathers from Bulwer's chicks found dead, we also measured the growth rate of primary feathers in live chicks to be able to calculate the mass of mercury deposited per day of feather growth in each feather, which should be a more accurate representation of the time-dependent deposition of mercury in flight feathers (Roque et al., 2016).

METHODS

FIELDWORK

Fieldwork was carried out on the island nature reserve of Deserta Grande, Madeira, Portugal (32°30'48"N, 16°30'33"W). Deserta Grande hosts one of the largest Bulwer's Petrel populations of the Atlantic (Catry et al., 2015), with a large number of accessible nests on the site of the field station.

During the chick-rearing period of 2018, a large number of nests were monitored regularly as part of another study. Nine dead chicks with advanced primary feather growth were encountered and the primary feathers from their right wing were collected whole.

Primary feather growth rates were measured in live chicks. The primary feathers of the right wing of 14 chicks were measured every four days using a pin-ruler from 28 days of age until 60 days of age.

SAMPLE PREPARATION AND ANALYSIS

The sampled whole primary feathers were weighed and measured (total length and vane length). In order to be able to calculate the concentration of mercury expressed per gram of feather as well as per millimetre of feather length (Bortolotti, 2010), five consecutive 8mm-long segments from each primary feather (P1 – P10) were cut perpendicularly to the rachis (Figure 1). These segments were analysed whole for total mercury, after being weighed, in order to obtain an average value per feather.

Total mercury was quantified using thermal decomposition atomic absorption spectrometry with gold amalgamation on a LECO AMA-254. Certified reference material (TORT-3) was analysed daily to ensure measurement accuracy and precision. The recovery efficiency was 87.91 ± 13.41% (n = 50). Segments were analysed to obtain at least two concordant readings from which an average could be calculated, tolerating a coefficient of variation (CV) up to 15% since the larger than standard sample weights required in the analytical method resulted in greater intra-feather variation in mercury concentration.

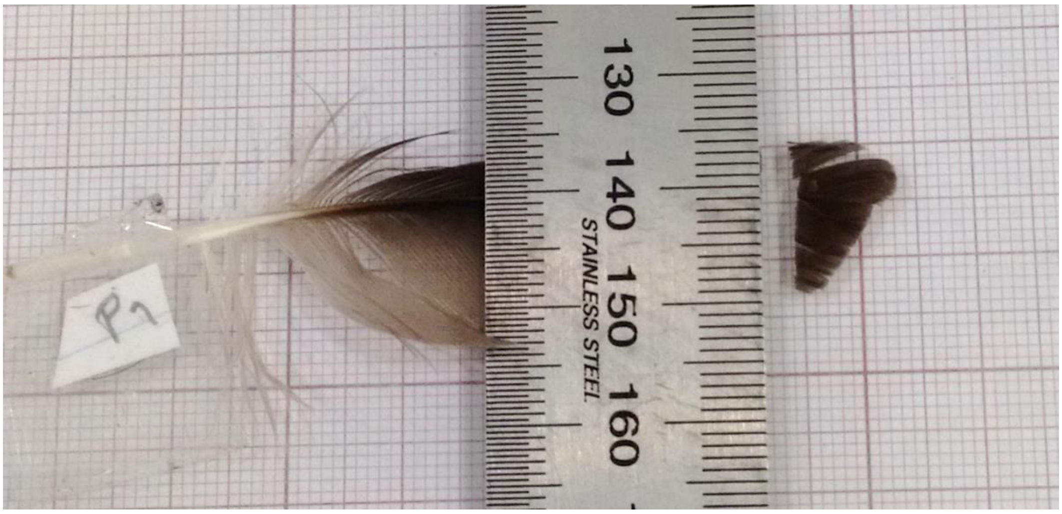

Figure 1 The line of dissection of the outermost segment (8mm of tip) on the innermost primary feather (P01) of a Bulwer's Petrel chick.

STATISTICAL ANALYSIS

All statistical analysis was carried out using the R statistical software (R Core Team, 2019).

The growth rate for each primary feather was taken as the coefficient of a regression of feather length against chick age (in days) over the first 45 days of age, during which time feather growth is almost linear (Figure 2). The length of feather analysed for mercury in chicks also corresponds to this growth period. The mass of mercury deposited per day of feather growth was calculated by multiplying the mercury concentration expressed per millimetre of each feather with the growth rate for the corresponding feather type.

Feather mercury concentrations (expressed as μg of mercury per gram of feather, per millimetre of feather, and per day of feather growth) were calculated from the averaged concordant mercury readings of segments for each feather. These were then transformed to relative feather mercury, calculated as the deviation (%) in a feather's mercury concentration from the mean feather mercury concentration over all analysed primaries for each individual.

We analysed the pattern of mercury concentration along the primaries using one-way ANOVA (function *aov*, package "stats"), relating relative feather mercury concentration to primary feather position. This was done for all three concentration measures of mercury in chick primaries.

We also assessed the pattern of feather mercury along the length of the feathers by comparing the mercury concentrations (expressed as µg of mercury per gram of feather and transformed to relative deviation from the mean mercury concentration across all primaries for each individual) of the consecutive segments analysed. The effect of feather segment on relative mercury was tested in a two-way ANOVA, including primary feather as an explanatory variable.

RESULTS

CHICK PRIMARY GROWTH RATE

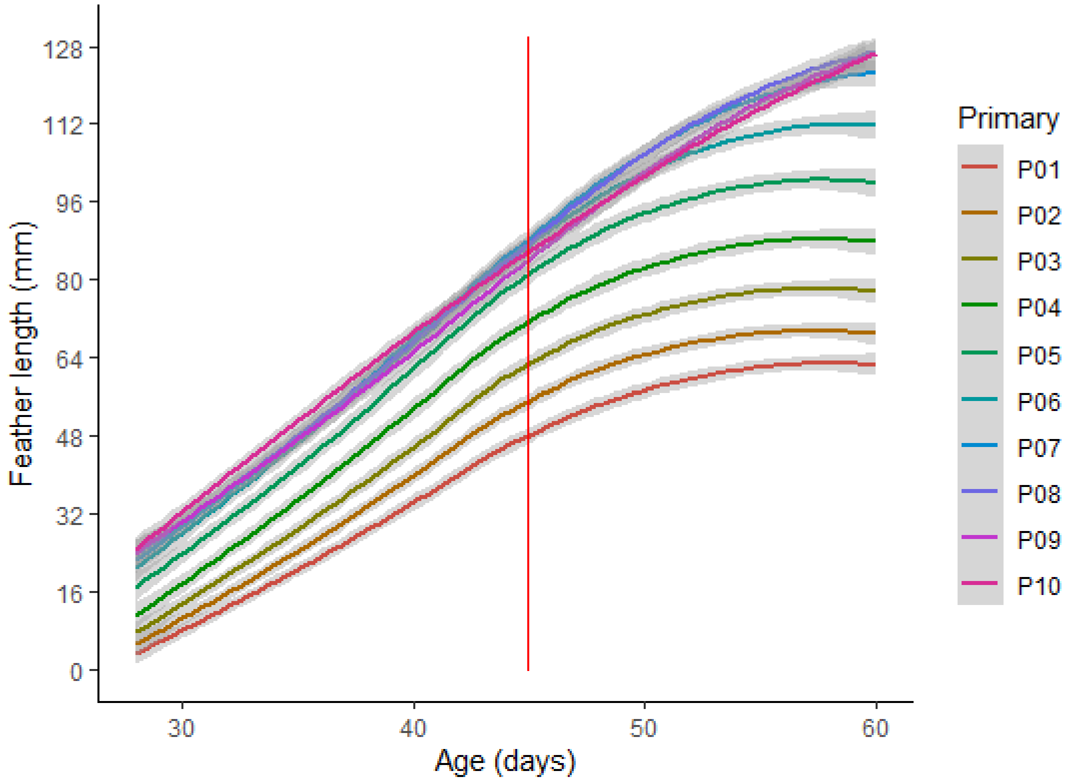

Figure 2 The growth of primary feathers of juvenile Bulwer's Petrels (n = 14) between 28 and 60 days since hatching on Deserta Grande. The growth rate used in this study was calculated over the period between 28 and 45 days since hatching (red line). Shading indicates the confidence interval.

Primary feather growth rates were calculated from measurements of 14 live chicks – all chicks survived to fledging. Growth rates differed between feathers and were similar between individuals (standard error of growth coefficients ranged between 0.08 – 0.11) (Figure 3). Notably, outer feathers started growing before inner feathers, as apparent from the feather length at the start of observations (Figure 2). The ratio of feather weight to vane length increases from the inner to outer primaries, as expected (Figure 3).

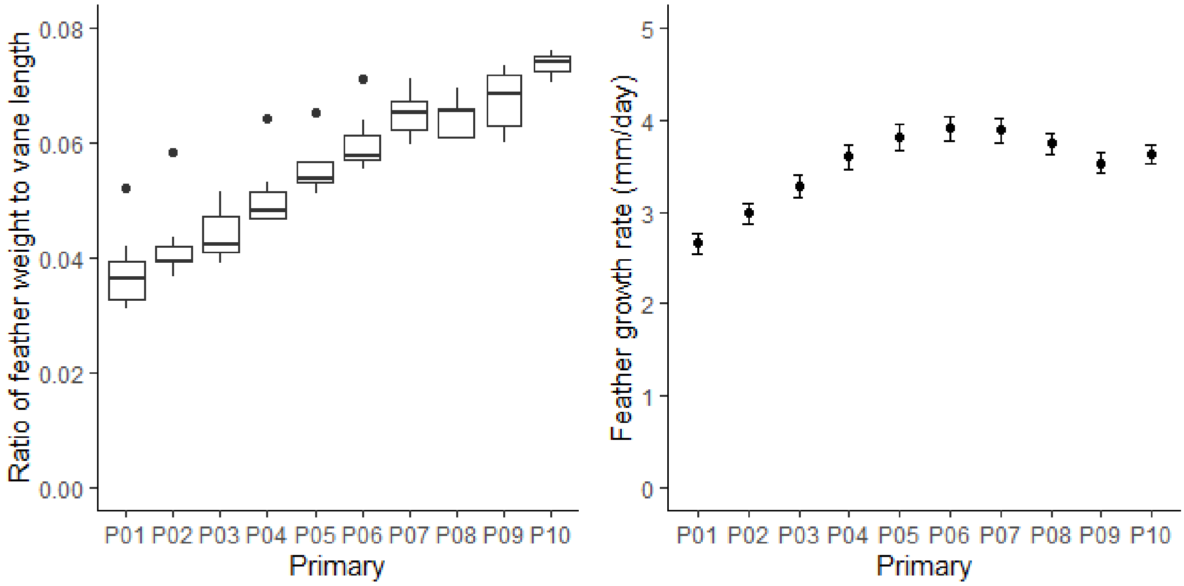

Figure 3 (Left) The ratio of feather weight to vane length is higher in the outer primaries, compared to inner primaries, in juvenile Bulwer's Petrels. **(Right)** The growth rate (calculated between 28 and 45 days since hatching) differs among primary feathers. Whiskers represent standard errors.

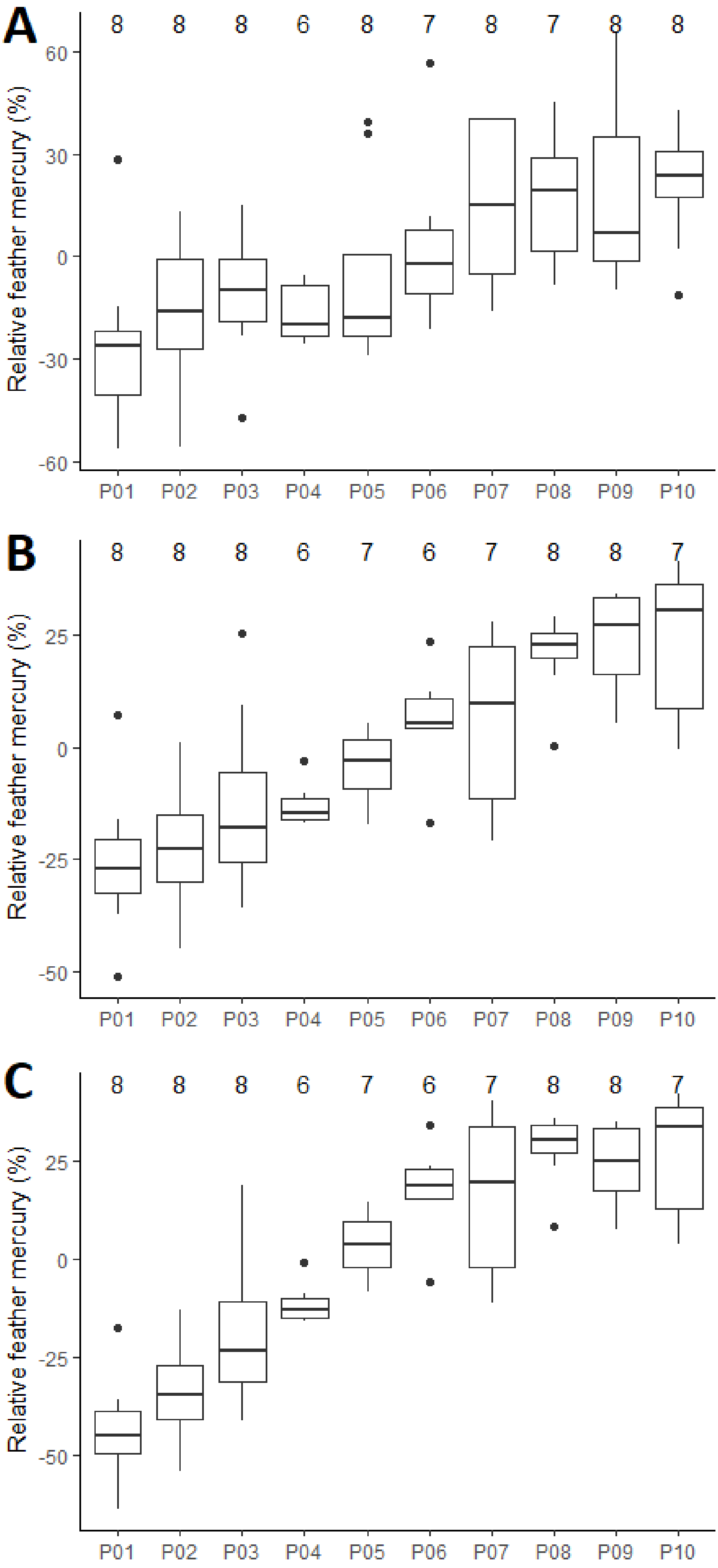
A
8 8 8 6 8 7 8 7 8 8
60
30
0
-30
-60
Relative feather mercury (%)
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10
B
8 8 8 6 7 6 7 8 8 7
25
0
-25
-50
Relative feather mercury (%)
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10
C
8 8 8 6 7 6 7 8 8 7
25
0
-25
-50
Relative feather mercury (%)
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10

Figure 4 The patterns of deviation in feather mercury concentration expressed as µg of mercury (A) per gram of feather, (B) per millimetre of feather, and (C) per day of feather growth increase from the innermost (P01) to the outermost (P10) primary similarly across all three measures in Bulwer's Petrel chicks. Relative mercury concentrations are presented as the deviation (%) in a feather's mercury concentration from the mean feather mercury concentration of all analysed primaries in an individual. Sample sizes are presented above each box.

PRIMARY FEATHER MERCURY CONCENTRATION

We obtained the mercury concentrations of between eight and ten primary feathers from eight Bulwer's Petrel chicks. The average mercury concentration across all samples was 1.79 ± 0.64 µg/g. Feather mercury concentration was significantly different among primary feathers (ANOVA results comparing mercury concentration expressed as µg of mercury (i) per gram of feather: $F = 4.29$, $p < 0.001$, (ii) per millimetre of feather; $F = 12.05$, $p < 0.001$, (iii) per day of feather growth: $F = 26.71$, $p < 0.001$). The mercury concentration of chicks' primary feathers appears to increase from the innermost (P01) to the outermost (P10), with primary type having a significant effect on feather mercury concentration. This is opposite to what is generally seen in other birds, where mercury concentration is highest in the innermost primaries, which are moulted first in many avian species. The differences between feathers became more distinct when using measures that better reflected time-dependent deposition of mercury (Figure 4).

Apart from the effect primary feather had on mercury concentration, mercury content also decreased along each feather ($F = 65.62$, $p < 0.001$); the outermost segments of the earlier grown outer feathers have the highest mercury concentration, with the proximal segments of these feathers having similar mercury loads to the distal segments of the inner primaries (Figure 5), which would be growing at around the same time (Figure 2).

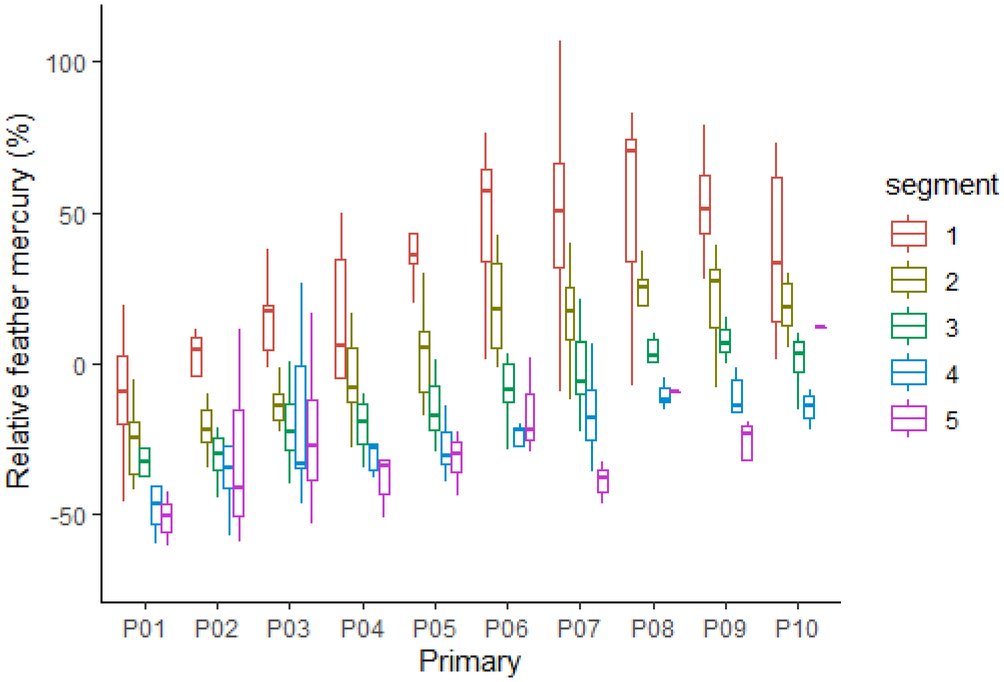

Figure 5 Mercury concentration decreases from the feather tip (the first to be formed) to the proximal end of the feather in the primaries of Bulwer's Petrel chicks. The proximal segments of outer primaries have similar mercury concentrations to distal segments of inner primaries, which are growing at the same time. Feather segments are numbered from the most distal (1) to the most proximal (5) sampled position of the feather.

DISCUSSION

Using data from chicks in which we monitored feather growth, we provide evidence that the pattern of mercury concentration across primary feathers is not an artefact of the measure of concentration, but is likely an effect of the order of feather growth, whereby the earlier grown feathers are exposed to higher blood mercury concentrations than are later moulted feathers as a result of blood mercury depletion.

In adult birds across diverse taxa, mercury concentration decreases from the innermost to the outermost primaries following the order of descendent primary moult (Accipitiformes: Sparrowhawk *Accipiter nisus*, Northern Goshawk *Accipiter gentilis*, Common Buzzard *Buteo buteo*; Strigiformes: Little Owl *Athene noctua*; Charadriiformes: Bonaparte's gulls *Larus philadelphia*, Great Skua *Catharacta skua*, Kittiwake *Rissa tridactyla*; Procellariiformes: Fulmar *Fulmarus glacialis*, Manx Shearwater *Puffinus puffinus*, Atlantic Petrel *Pterodroma incerta*, Soft-plumaged Petrel *Pterodroma mollis*, Kerguelen Petrel *Aphrodroma brevirostris*, Great Shearwater *Ardenna gravis)* (Braune & Gaskin, 1987; Dauwe et al., 2003; Furness et al., 1986; Martínez et al., 2012). Feather mercury concentration follows the chronology of feather moult even in the Peregrine Falcon (*Falco peregrinus*), in which primary moult is divergent starting with P4 (Lindberg & Odsjö, 1983), but no obvious pattern is detected in the Barn Owl (*Tyto alba*, Dauwe et al., 2003; Roque et al., 2016) or Tawny Owl (*Strix aluco*, Varela, García-Seoane, Fernández, Carballeira, & Aboal, 2016) in which primary moult is arrested and irregular (Cramp & Simmons, 1985).

The mercury concentration in the primary feathers of Bulwer's Petrel chicks decreases from the outermost to the innermost primaries, along the order of feather growth. While the primaries of nestlings are growing simultaneously, they do not start growing at exactly the same time, with primary emergence occurring in descending order from P10 to P1. As a result, blood mercury would have already been deposited in the growing outer primaries before the inner primaries start to grow. This is further corroborated by the similar mercury loads of the outermost segment of P1 and the third or fourth segments of distal feathers which were growing at the same time.

Our results unambiguously refute the idea that the pattern in primary feather mercury is only an artefact of the unit of concentration used (Bortolotti, 2010). Taking a time-dependent measure of feather mercury concentration in Bulwer's Petrel feathers to address the theory proposed by Bortolotti (2010), either by measuring mercury concentration expressed as μg of mercury per millimetre of feather or by transforming it into a rate of mercury deposition, does not account for the variation in mercury in primaries, which persisted. Rather, it seems to have made the pattern clearer, which supports the idea that mercury does indeed enter the feather structure in a time-dependent manner (Bortolotti, 2010).

Similar conclusions were reached by Carravieri et al. (2014) in comparisons of mercury concentrations between synchronously growing body feathers of juvenile White-chinned Petrels *Procellaria aequinoctialis* and adult King Penguins *Aptenodytes patagonicus* and body feathers of adult Antarctic Prions *Pachyptila desolata*, which moult sequentially over a prolonged period of time. The higher inter-feather variation in mercury concentration seen in adult Antarctic Prion body feathers suggested that the timing of feather growth does indeed have consequences on feather mercury deposition. Our results build up on this as they evidently show this effect in feathers of a known growth order in juveniles which have no geographical between-feather variation. Previous attempts to characterise mercury deposition in juvenile flight feathers were inconclusive in this regard and did not directly test for the time-dependent deposition of mercury (Roque et al., 2016).

Very little is known on the moulting order in small petrels, except that primary moult occurs in the non-breeding area and is possibly arrested during migration (Bridge, 2006; Monteiro, Ramos, Furness, & del Nevo, 1996). However, primary feather isotope data from adult Bulwer's Petrels generally support the notion that the order of primary moult is descendent (Cruz-Flores, Militão, Ramos, & Gonzalez-Solis, 2018), as in Shearwaters (Ramos, Militão, González-Solís, & Ruiz, 2009). Their results also suggest that outer secondaries and outer rectrices are moulted later than primaries (Cruz-Flores et al., 2018). While many moult patterns appear to be similar across avian taxa, species-specific differences in moult chronology could alter the importance of the body load of mercury in shaping a given feather’s mercury concentration.

CONCLUSION

Our findings confirm that primary mercury concentrations do reflect mercury accumulated prior to feather growth (Ramos, González-Solís, et al., 2009; Thompson et al., 1998), more so in early grown or moulted feathers, such as proximal primaries in the adults of most bird species which carry the highest mercury loads. As a result, studies monitoring mercury exposure, particularly those interested in assessing mercury intake in discrete life history stages and/or in particular geographical areas in the case of migratory species, are advised to avoid feathers that are among the first to be moulted.

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Author contributions

MCG and PC conceptualised the study and planned the methodology, RF and DL carried out the fieldwork, RF, and MCG carried out the sample preparation and supervised the sample analysis, MCG analysed the data and led the writing of the manuscript, PC, JPG, and EP supervised the study, all authors were involved in the reviewing and editing of the manuscript.

REFERENCES

Albert, C., Renedo, M., Bustamante, P., & Fort, J. (2019). Using blood and feathers to investigate large-scale Hg contamination in Arctic seabirds: A review. *Environmental Research*, *177*(July), 108588. https://doi.org/10.1016/j.envres.2019.108588

Appelquist, H., Asbirk, S., & Drabæk, I. (1984). Mercury monitoring: Mercury stability in bird feathers. *Marine Pollution Bulletin*, *15*(1), 22–24. https://doi.org/10.1016/0025-326X(84)90419-3

Bond, A. L. (2010). Relationships between stable isotopes and metal contaminants in feathers are spurious and biologically uninformative. *Environmental Pollution*, *158*(5), 1182–1184. https://doi.org/10.1016/j.envpol.2010.01.004

Bortolotti, G. R. (2010). Flaws and pitfalls in the chemical analysis of feathers : bad news — good news for avian chemoecology and toxicology. *Ecological Applications*, *20*(6), 1766–1774. https://doi.org/10.1890/09-1473.1

Braune, B., & Gaskin, D. (1987). Mercury levels in Bonaparte’s gulls (*Larus philadelphia*) during autumn molt in the Quoddy region, New Brunswick, Canada. *Archives of Environmental Contamination and Toxicology*, *549*, 539–549. https://doi.org/10.1007/BF01055810

Bridge, E. S. (2006). Influences of morphology and behavior on wing-molt strategies in seabirds. *Marine Ornithology*, *34*(1), 7–19.

Burger, J. (1994). Metals in avian feathers: bioindicators of environmental pollution. In E. Hodgson (Ed.), *Reviews in Environmental Toxicology and Applied Pharmacology* (pp. 5:203–311).

Carravieri, A., Bustamante, P., Churlaud, C., Fromant, A., & Cherel, Y. (2014). Moulting patterns drive within-individual variations of stable isotopes and mercury in seabird body feathers: Implications for monitoring of the marine environment. *Marine Biology*, *161*(4), 963–968. https://doi.org/10.1007/s00227-014-2394-x

Catry, P., Dias, M. P., Catry, T., Pedro, P., Tenreiro, P., & Menezes, D. (2015). Bulwer's petrels breeding numbers on the Desertas Islands (Madeira): improved estimates indicate the NE Atlantic population to be much larger than previously thought. *Airo*, *23*, 10–14.

Chaurand, T., & Weimerskirch, H. (1994). The regular alternation of short and long foraging trips in the Blue Petrel *Halobaena caerulea*: A previously undescribed strategy of food provisioning in a pelagic seabird. *The Journal of Animal Ecology*, *63*(2), 275. https://doi.org/10.2307/5546

Chételat, J., Ackerman, J. T., Eagles-Smith, C. A., & Hebert, C. E. (2020). Methylmercury exposure in wildlife: A review of the ecological and physiological processes affecting contaminant concentrations and their interpretation. *Science of the Total Environment*, *711*. https://doi.org/10.1016/j.scitotenv.2019.135117

Cramp, S., & Simmons, K. E. L. (1985). Handbook of the Birds of Europe, the Middle East and North Africa: the Birds of the Western Palearctic. Vol. IV Terns to Woodpeckers. Oxford, UK: Oxford Univeristy Press.

Crewther, W. G., Fraser, R. D. B., Lennox, F. G., & Lindley, H. (1965). The chemistry of keratins. *Advances in Protein Chemistry*, *20*(C), 191–346. https://doi.org/10.1016/S0065-3233(08)60390-3

Cruz-Flores, M., Militão, T., Ramos, R., & Gonzalez-Solis, J. (2018). Using marine isoscapes to infer movements of oceanic migrants: The case of Bulwer's petrel, *Bulweria bulwerii*, in the Atlantic Ocean. *PLoS ONE*, *13*(6), 1–13. https://doi.org/10.1371/journal.pone.0198667

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R., & Eens, M. (2003). Variation of heavy metals within and among feathers of birds of prey: Effects of molt and external contamination. *Environmental Pollution*, *124*(3), 429–436. https://doi.org/10.1016/S0269-7491(03)00044-7

Furness, R. W., Muirhead, S. J., & Woodburn, M. (1986). Using bird feathers to measure mercury in the environment: Relationships between mercury content and moult. *Marine Pollution Bulletin*, *17*(1), 27–30.

Gatt, M. C., Reis, B., Granadeiro, J. P., Pereira, E., & Catry, P. (2020). Generalist seabirds as biomonitors of ocean mercury: The importance of accurate trophic position assignment. *Science of The Total Environment*, *740*, 140159. https://doi.org/10.1016/j.scitotenv.2020.140159

Lewis, S. A., & Furness, R. W. (1991). Mercury accumulation and excretion in laboratory reared black-headed gull *Larus ridibundus* chicks. *Archives of Environmental Contamination and Toxicology*, *21*(2), 316–320. https://doi.org/10.1007/BF01055352

Lindberg, P., & Odsjö, T. (1983). Mercury levels in feathers of peregrine falcon Falco peregrinus compared with total mercury content in some of its prey species in Sweden. *Environmental Pollution. Series B, Chemical and Physical*, *5*(4), 297–318. https://doi.org/10.1016/0143-148X(83)90023-X

Mallory, M. L., Braune, B. M., Provencher, J. F., Callaghan, D. B., Gilchrist, H. G., Edmonds, S. T., … O’Driscoll, N. J. (2015). Mercury concentrations in feathers of marine birds in Arctic Canada. *Marine Pollution Bulletin*, *98*(1–2), 308–313. https://doi.org/10.1016/j.marpolbul.2015.06.043

Martínez, A., Crespo, D., Fernández, J. Á., Aboal, J. R., & Carballeira, A. (2012). Selection of flight feathers from Buteo buteo and Accipiter gentilis for use in biomonitoring heavy metal contamination. *Science of the Total Environment*, *425*, 254–261. https://doi.org/10.1016/j.scitotenv.2012.03.017

Monteiro, L. R., & Furness, R. W. (2001). Kinetics, dose‒response, and excretion of methylmercury in free-living adult Cory’s Shearwaters. *Environmental Science & Technology*, *35*(4), 739–746. https://doi.org/10.1021/ES000114A

Monteiro, L. R., Ramos, J. A., Furness, R. W., & del Nevo, A. J. (1996). Movements, morphology, breeding, molt, diet and feeding of seabirds in the Azores. *Colonial Waterbirds*, *19*(1), 82–97.

Nunes, M. (2000). New data on the Bulwer’s Petrel breeding biology in the Desertas Islands (Madeira Archipelago). *Arquipelago. Life and Marine Sciences, Supplement*, 167–173.

Nunes, M., & Vicente, L. (1998). Breeding cycle and nestling growth of Bulwer’s Petrel on the Desertas Islands, Portugal. *Colonial Waterbirds, 21*(2), 198–204.

Peterson, S. H., Ackerman, J. T., Toney, M., & Herzog, M. P. (2019). Mercury concentrations vary within and among individual bird feathers: A critical evaluation and guidelines for feather use in mercury monitoring programs. *Environmental Toxicology and Chemistry, 38*(6), 1164–1187. https://doi.org/10.1002/etc.4430

R Core Team. (2019). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Ramos, R., González-Solís, J., Forero, M. G., Moreno, R., Gómez-Díaz, E., Ruiz, X., & Hobson, K. A. (2009). The influence of breeding colony and sex on mercury, selenium and lead levels and carbon and nitrogen stable isotope signatures in summer and winter feathers of *Calonectris* shearwaters. *Oecologia*, *159*(2), 345–354. https://doi.org/10.1007/s00442-008-1215-7

Ramos, R., Militão, T., González-Solís, J., & Ruiz, X. (2009). Moulting strategies of a long-distance migratory seabird, the Mediterranean Cory’s Shearwater *Calonectris diomedea* diomedea. *Ibis, 151*(1), 151–159. https://doi.org/10.1111/j.1474-919X.2008.00877.x

Roque, I., Lourenço, R., Marques, A., Coelho, J. P., Coelho, C., Pereira, E., … Roulin, A. (2016). Barn owl feathers as biomonitors of mercury: sources of variation in sampling procedures. *Ecotoxicology, 25*(3), 469–480. https://doi.org/10.1007/s10646-015-1604-8

Shoji, A., Aris-Brosou, S., Fayet, A., Padget, O., Perrins, C., & Guilford, T. (2015). Dual foraging and pair coordination during chick provisioning by Manx shearwaters: empirical evidence supported by a simple model. *Journal of Experimental Biology*, *218*(13), 2116–2123. https://doi.org/10.1242/jeb.120626

Thompson, D. R., Bearhop, S., Speakman, J. R., & Furness, R. W. (1998). Feathers as a means of monitoring mercury in seabirds: Insights from stable isotope analysis. *Environmental Pollution*, *101*(2), 193–200. https://doi.org/10.1016/S0269-7491(98)00078-5

Varela, Z., García-Seoane, R., Fernández, J. A., Carballeira, A., & Aboal, J. R. (2016). Study of temporal trends in mercury concentrations in the primary flight feathers of *Strix aluco*.

Ecotoxicology and Environmental Safety, *130*, 199–206. https://doi.org/10.1016/j.ecoenv.2016.04.006

Waap, S., Symondson, W. O. C., Granadeiro, J. P., Alonso, H., Serra-Gonçalves, C., Dias, M. P., & Catry, P. (2017). The diet of a nocturnal pelagic predator, the Bulwer's petrel, across the lunar cycle. *Scientific Reports*, *7*(1), 1384. https://doi.org/10.1038/s41598-017-01312-3

Watanuki, Y., Yamamoto, T., Yamashita, A., Ishii, C., Ikenaka, Y., Nakayama, S. M. M., … Phillips, R. A. (2015). Mercury concentrations in primary feathers reflect pollutant exposure in discrete non-breeding grounds used by Short-tailed Shearwaters. *Journal of Ornithology*, *156*(3), 847–850. https://doi.org/10.1007/s10336-015-1205-6

Wischnewski, S., Arneill, G. E., Bennison, A. W., Dillane, E., Poupart, T. A., Hinde, C. A., … Quinn, J. L. (2019). Variation in foraging strategies over a large spatial scale reduces parent–offspring conflict in Manx Shearwaters. *Animal Behaviour*, *151*, 165–176. https://doi.org/10.1016/j.anbehav.2019.03.014

CHAPTER 6

GENERAL DISCUSSION

This thesis builds on the growing knowledge base on the causes and consequences of differential migration of populations. We are as yet only scratching the surface of a complex, but intriguing, topic. Nevertheless, the collection of studies enclosed in this thesis brings to the forefront the importance of inter-individual variations in population ecology and evolution (Wolf & Weissing, 2012). Chapter 2 presents the first investigation linking individual behaviour types to migratory strategy in seabirds, and only the second avian species in a small handful of studies on the subject. Chapter 3 assesses physical condition of migrants and residents from the same population immediately after return to the colony from non-breeding areas, an approach not easily carried out in many other taxa. Chapter 4 uses novel isotopic techniques in order to meaningfully compare mercury exposure over large geographical scales, while Chapter 5 provides empirical evidence confirming the role of feather growth order in determining feather mercury concentration.

A COMMON COPING STYLE AMONG RESIDENT CORY'S SHEARWATERS

Chapters 2 and 3 assess behavioural stress responses and physiological stress markers within our study population of Cory's Shearwaters over the same time period. Our results reveal two things: (i) resident males are less likely to have reactive behavioural responses to a stressor, and (ii) resident males also experience fewer acute stress responses during tail feather growth which result in fault bars (Jovani & Rohwer, 2016) compared to migratory males. The correlation of behavioural and endocrine responses to stress constitutes coping style, described in many taxa of both wild and domesticated animals (Koolhaas et al., 1999). The major axis of this variation runs from active/bold/aggressive personalities which exhibit low physiological stress responses, to reactive/shy/docile personalities with high physiological stress responses (Cockrem, 2007). The differences we find in behavioural and physiological stress response between migratory and resident male Cory's Shearwaters suggest that individuals with different migratory strategies may be distinguishable by their stress threshold.

In birds, there is evidence that coping styles, and, broadly, individual personality, determine how individuals react to each other and the environment around them. The consequence of this is that migrants and residents may have different adaptations and responses to conditions experienced (Cockrem, 2012). Published correlations between personality and movement ecology have largely been restricted to foraging ecology, with substantial work having been carried out on this topic in seabirds. What is often reported is that seabirds with active personalities are more consistent in their foraging behaviour (Harris et al., 2019; Krüger, Pereira, Paiva, & Ramos, 2019) and more (superficial) explorers than reactive personalities (Patrick, Pinaud, & Weimerskirch, 2017; Patrick & Weimerskirch, 2014). Moreover, personality types have already been identified as factors influencing how foraging seabirds react to changing environmental conditions between years (Krüger et al., 2019), sometimes to the extent of rendering different fitness consequences (Patrick & Weimerskirch, 2014).

It is difficult to tell cause from consequence, but the distinction in coping style between migrant and resident male Cory's suggests that individuals must exhibit certain traits in order to choose residency over distant, but possibly more productive and/or less competitive, non-breeding areas (Wolf, Van Doorn, & Weissing, 2008). Therefore, there is the potential for differential behavioural responses to the environment within the population of Cory’s Shearwaters on which selection can act on.

CONSEQUENCES OF DIFFERENTIAL MIGRATION

Despite the fact that somatic maintenance after costly annual cycle events is prioritised and that migration is cheap in the Cory’s Shearwater (Chapter 3), this thesis demonstrates that sub-groups of the population are experiencing different conditions over the non-breeding season, evidenced in trophic position and mercury exposure (Chapter 4), and possibly suggested by the difference in feather fault bar intensity (Chapter 3).

Foraging within widely separated areas exposes individuals to different foraging conditions and chemical pollutants. Top predators assimilate chemical signatures from their environment, bioaccumulating compounds which are not easily excreted and which may pose physiological stress, such as heavy metals (Costantini et al., 2014; Van der Oost, Beyer, & Vermeulen, 2003). While our results in Chapter 5 advise caution in the interpretation of flight feather mercury loads due to the effect of moult sequence in the depletion of previously ingested mercury (Furness, Muirhead, & Woodburn, 1986), the selection of late moulted flight feathers for analysis should ensure that most of the body burden of mercury would have been excreted into the primaries and earlier-moulted secondaries (Alonso, Matias, Granadeiro, & Catry, 2009; R. Ramos, Militão, González-Solís, & Ruiz, 2009). The strong correlation reported in this thesis between mercury concentration and isotope-derived trophic position supports the idea that the mercury concentrations reported here reflect the situation during the non-breeding period. It is unclear what the cause of the diet difference between migrants to the Benguela and Agulhas currents and residents was over the analysed non-breeding period of 2016 to 2017. However, diet analysis of Cory's Shearwaters at the breeding grounds during the chick-rearing periods of 2016 through 2018 found a striking dietary shift from shoaling pelagic fish, such as Chub Mackerel (*Scomber colias*), and squid to Longspine Snipefish *Macroramphosus scolopax* in 2017 and 2018 (J. Romero, unpublished data). Such regional differences in prey abundances seem to shape the foraging ecology of a generalist predator, such as the Cory's Shearwater.

Both these experienced environmental differences and endogenous differences in coping style suggest that differential migration has the potential to drive population changes in this study system. The low migratory connectivity in Cory's Shearwaters has a strong aspect of sexual segregation to it, whereby only males remain resident and females disproportionately migrate to the Agulhas current (Perez, Granadeiro, Dias, Alonso, & Catry, 2014), the causes and consequences of the latter remaining relatively unexplored (but see Chapter 4 for comparisons of feather mercury). This weak migratory connectivity has the potential to ether buffer negative impacts on the population as a whole or, conversely, amplify them if an important demographic is disproportionately affected (Briedis & Bauer, 2018; Gilroy, Gill, Butchart, Jones, & Franco, 2016).

The stability of the system depends on the nature of the fluctuations in the environmental factors shaping individual fitness (Dingemanse, Both, Drent, & Tinbergen, 2004), and how these may change in direction and frequency as a result of human-induced global change. While the consequences of climate change are complex and not very well understood (Rodríguez et al., 2019), ocean warming and acidification will likely change the trophic structure and prey availability (Burrows et al., 2011), and the increased frequency of extreme weather events are expected to have negative consequences on seabird survival where they occur (Jentsch, Kreyling, & Beierkuhnlein, 2007). Conservation actions are unlikely to mitigate the direct actions of climate change. However, measures to improve regional conditions where risks are identified may help increase population resilience (Rodríguez et al., 2019). While threats at the colony are well documented and quantifiable, those at sea and, particularly, during the non-breeding period, are more obscure (Dias et al., 2019; Rodríguez et al., 2019). *Calonectris* shearwaters are impacted by fisheries bycatch, which can drive survival differences between colonies as a result of differential exposure (Genovart et al., 2018). Individual personality has also already been shown to impact susceptibility to bycatch (Patrick & Weimerskirch, 2014). Identifying overlaps with fisheries during the non-breeding period could help form conservation action (Savoca et al., 2020). Additionally, it would be beneficial to collect more evidence on the impacts of overfishing and increased foraging competition (Rodríguez et al., 2019). As is the case in all migratory taxa, it is increasingly clear that species conservation relies on international collaboration in order to protect highly mobile species across their annual cycle range (Dunn et al., 2019).

WHAT NEXT IN THE STUDY OF DIFFERENTIAL MIGRATION

As is typically the case, this body of works opens up further questions.

Behavioural assessments of animals have been largely limited to observations over a few years at most (Bell, Hankison, & Laskowski, 2009). In seabirds and other long-lived animals, this is not representative of their longevity. Studies into the permanence of behavioural type could uncover whether individuals, which may experience differences in their quality and stress responses over time and/or with age (Catry et al., 1999; Elliott et al., 2014), change in their personality type, and how this links to their movement ecology.

Much of this thesis focuses on differences among males in comparisons of migration strategy. However, there still remains potential in exploring the extents of sexual segregation in the non-breeding period and its consequences (De Felipe et al., 2019; Perez et al., 2014). The differences in the roles of the two sexes during the pre-breeding period – males invest more in nest defence (Perez et al., 2014; J. A. Ramos, Monteiro, Sola, & Moniz, 1997), while females eventually invest in the production of a nutritionally expensive egg (Mallory, Forbes, Ankney, & Alisauskas, 2008; Ricklefs, 1974) – suggest that they may be under different energetic pressures. This could drive segregation in the non-breeding period even in sexually monomorphic species, the consequences of which could influence future reproductive success (Fayet, Shoji, Freeman, Perrins, & Guilford, 2017; Hedd, Montevecchi, Phillips, & Fifield, 2014).

The hereditary basis for migratory decisions in long-lived species in which migratory strategy shows plasticity would be another pivotal question to address. The Cory's Shearwater study system on Selvagem Grande is starting to reach the capacity to investigate this, as birds ringed as chicks from known parents are recruiting into the breeding population and can be tracked over the non-breeding period, as can their parents which are still active breeders. A few similar long-term datasets exist, such as those for Eurasian Spoonbills (*Platalea leucorodia*) in the Netherlands (Lok, Veldhoen, Overdijk, Tinbergen, & Piersma, 2017), for European Shags (*Phalacrocorax aristotelis*) in Scotland (Grist et al., 2014), and for Black-legged Kittiwakes (*Rissa tridactyla*) in Alaska (Elliott et al., 2014).

REFERENCES

Alonso, H., Matias, R., Granadeiro, J. P., & Catry, P. (2009). Moult strategies of Cory's Shearwaters *Calonectris diomedea borealis*: the influence of colony location, sex and individual breeding status. *Journal of Ornithology*, *150*(2), 329–337. https://doi.org/10.1007/s10336-008-0354-2

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, *77*(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022.Bell

Briedis, M., & Bauer, S. (2018). Migratory connectivity in the context of differential migration. *Biology Letters*, *14*(12), 20180679. https://doi.org/10.1098/rsbl.2018.0679

Burrows, M. T., Schoeman, D. S., Buckley, L. B., Moore, P., Poloczanska, E. S., Brander, K. M., … Richardson, A. J. (2011). The pace of shifting climate in marine and terrestrial ecosystems. *Science*, *334*(6056), 652–655. https://doi.org/10.1126/science.1210288

Catry, P., Ruxton, G. D., Ratcliffe, N., Hamer, K. C., Furness, R. W., & Mar, N. (1999). Short-lived repeatabilities in long-lived Great Skuas: Implications for the study of individual quality. *Oikos*, *84*(3), 473–479. https://doi.org/10.2307/3546426

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*(SUPLL. 2), 169–178. https://doi.org/10.1007/s10336-007-0175-8

Cockrem, J. F. (2012). Corticosterone responses and personality in birds: Individual variation and the ability to cope with environmental changes due to climate change. *General and Comparative Endocrinology, 190*, 156–163. https://doi.org/10.1016/j.ygcen.2013.02.021

Costantini, D., Meillère, A., Carravieri, A., Lecomte, V., Sorci, G., Faivre, B., … Chastel, O. (2014). Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. *Oecologia*, *175*, 1107–1116. https://doi.org/10.1007/s00442-014-2975-x

De Felipe, F., Reyes-González, J. M., Militão, T., Neves, V. C., Bried, J., Oro, D., … González-Solís, J. (2019). Does sexual segregation occur during the non-breeding period? A comparative analysis in spatial and feeding ecology of three *Calonectris* shearwaters. *Ecology and Evolution, in press*(June), 1–18. https://doi.org/10.1002/ece3.5501

Dias, M. P., Martin, R., Pearmain, E. J., Burfield, I. J., Small, C., Phillips, R. A., … Croxall, J. P. (2019). Threats to seabirds: A global assessment. *Biological Conservation*, *237*(June), 525–537. https://doi.org/10.1016/j.biocon.2019.06.033

Dingemanse, N. J., Both, C., Drent, P. J., & Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(1541), 847–852. https://doi.org/10.1098/rspb.2004.2680

Dunn, D. C., Harrison, A. L., Curtice, C., DeLand, S., Donnelly, B., Fujioka, E., … Halpin, P. N. (2019). The importance of migratory connectivity for global ocean policy. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1911). https://doi.org/10.1098/rspb.2019.1472

Elliott, K. H., Reilly, K. M. O., Hatch, S. A., Gaston, A. J., Hare, J. F., & Anderson, W. G. (2014). The prudent parent meets old age : A high stress response in very old seabirds supports the terminal restraint hypothesis. *Hormones and Behavior*, *66*(5), 828–837. https://doi.org/10.1016/j.yhbeh.2014.11.001

Fayet, A. L., Shoji, A., Freeman, R., Perrins, C. M., & Guilford, T. (2017). Within-pair similarity in migration route and female winter foraging effort predict pair breeding performance in a monogamous seabird. *Marine Ecology Progress Series*, *569*, 243–252. https://doi.org/10.3354/meps12083

Furness, R. W., Muirhead, S. J., & Woodburn, M. (1986). Using bird feathers to measure mercury in the environment: Relationships between mercury content and moult. *Marine Pollution Bulletin*, *17*(1), 27–30.

Genovart, M., Bécares, J., Igual, J. M., Martínez-Abraín, A., Escandell, R., Sánchez, A., … Oro, D. (2018). Differential adult survival at close seabird colonies: The importance of spatial foraging segregation and bycatch risk during the breeding season. *Global Change Biology*, *24*(3), 1279–1290. https://doi.org/10.1111/gcb.13997

Gilroy, J. J., Gill, J. A., Butchart, S. H. M., Jones, V. R., & Franco, A. M. A. (2016). Migratory diversity predicts population declines in birds. *Ecology Letters*, *19*(3), 308–317. https://doi.org/10.1111/ele.12569

Grist, H., Daunt, F., Wanless, S., Nelson, E. J., Harris, M. P., Newell, M., … Reid, J. M. (2014). Site fidelity and individual variation in winter location in partially migratory European shags. *PLoS ONE*, *9*(6). https://doi.org/10.1371/journal.pone.0098562

Harris, S. M., Descamps, S., Sneddon, L. U., Bertrand, P., Chastel, O., & Patrick, S. C. (2019). Personality predicts foraging site fidelity and trip repeatability in a marine predator. *Journal of Animal Ecology*, *1*, 1365-2656.13106. https://doi.org/10.1111/1365-2656.13106

Hedd, A., Montevecchi, W. A., Phillips, R. A., & Fifield, D. A. (2014). Seasonal sexual segregation by monomorphic Sooty Shearwaters *Puffinus griseus* reflects different reproductive roles during the pre-laying period. *PloS One*, *9*(1), e85572. https://doi.org/10.1371/journal.pone.0085572

Jentsch, A., Kreyling, J., & Beierkuhnlein, C. (2007). A new generation of climate change experiments: events, not trends. *Frontiers in Ecology and the Environment*, *5*(7), 365–374.

Jovani, R., & Rohwer, S. (2016). Fault bars in bird feathers: mechanisms, and ecological and evolutionary causes and consequences. *Biological Reviews*, *92*(2), 1113–1127. https://doi.org/10.1111/brv.12273

Koolhaas, J. ., Korte, S. ., De Boer, S. ., Van Der Vegt, B. ., Van Reenen, C. ., Hopster, H., … Blokhuis, H. . (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, *23*(7), 925–935. https://doi.org/10.1016/S0149-7634(99)00026-3

Krüger, L., Pereira, J. M., Paiva, V. H., & Ramos, J. A. (2019). Personality influences foraging of a seabird under contrasting environmental conditions. *Journal of Experimental Marine Biology and Ecology*, *516*(September 2018), 123–131. https://doi.org/10.1016/j.jembe.2019.04.003

Lok, T., Veldhoen, L., Overdijk, O., Tinbergen, J. M., & Piersma, T. (2017). An age-dependent fitness cost of migration? Old trans-Saharan migrating spoonbills breed later than those staying in Europe, and late breeders have lower recruitment. *Journal of Animal Ecology*, *86*(5), 998–1009. https://doi.org/10.1111/1365-2656.12706

Mallory, M. L., Forbes, M. R., Ankney, C. D., & Alisauskas, R. T. (2008). Nutrient dynamics and constraints on the pre-laying exodus of High Arctic Northern Fulmars. *Aquatic Biology*, *4*, 211–223. https://doi.org/10.3354/ab00113

Patrick, S. C., Pinaud, D., & Weimerskirch, H. (2017). Boldness predicts an individual's position along an exploration-exploitation foraging trade-off. *Journal of Animal Ecology*, *86*(5), 1257–1268. https://doi.org/10.1111/1365-2656.12724

Patrick, S. C., & Weimerskirch, H. (2014). Personality, foraging and fitness consequences in a long lived seabird. *PLoS ONE*, *9*(2), e87269. https://doi.org/10.1371/journal.pone.0087269

Perez, C., Granadeiro, J. P., Dias, M. P., Alonso, H., & Catry, P. (2014). When males are more inclined to stay at home: insights into the partial migration of a pelagic seabird provided by geolocators and isotopes. *Behavioral Ecology*, *25*(2), 313–319. https://doi.org/10.1093/beheco/art119

Ramos, J. A., Monteiro, L. R., Sola, E., & Moniz, Z. (1997). Characteristics and competition for nest cavities in burrowing Procellariiformes. *The Condor*, *99*(3), 634–641. https://doi.org/10.2307/1370475

Ramos, R., Militão, T., González-Solís, J., & Ruiz, X. (2009). Moulting strategies of a long-distance migratory seabird, the Mediterranean Cory's Shearwater *Calonectris diomedea* diomedea. *Ibis*, *151*(1), 151–159. https://doi.org/10.1111/j.1474-919X.2008.00877.x

Ricklefs, R. E. (1974). Energetics of reproduction in birds. In R. A. Paynter (Ed.), *Avian energetics* (No. 15, pp. 152–292). Nuttall Ornithol Club, Cambridge.

Rodríguez, A., Arcos, J. M., Bretagnolle, V., Dias, M. P., Holmes, N. D., Louzao, M., … Chiaradia, A. (2019). Future directions in conservation research on petrels and shearwaters. *Frontiers in Marine Science*, *6*(March), 1–27. https://doi.org/10.3389/fmars.2019.00094

Savoca, M. S., Brodie, S., Welch, H., Hoover, A., Benaka, L. R., Bograd, S. J., & Hazen, E. L. (2020). Comprehensive bycatch assessment in US fisheries for prioritizing management. *Nature Sustainability*, *3*(6), 472–480. https://doi.org/10.1038/s41893-020-0506-9

Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology*, *13*(2), 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6

Wolf, M., Van Doorn, G. S., & Weissing, F. J. (2008). Evolutionary emergence of responsive and unresponsive personalities. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(41), 15825–15830. https://doi.org/10.1073/pnas.0805473105

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology and Evolution*, *27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

APPENDIX A1

SEX-SPECIFIC TELOMERE LENGTH AND DYNAMICS IN RELATION TO AGE AND REPRODUCTIVE SUCCESS IN CORY'S SHEARWATERS

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ABSTRACT

Individuals in free-living animal populations generally differ substantially in reproductive success, lifespan and other fitness-related traits and the molecular mechanisms underlying this variation are poorly understood. Telomere length and dynamics are candidate traits explaining this variation, as long telomeres predict a higher survival probability and telomere loss has been shown to reflect experienced "life stress". However, telomere dynamics among very long-lived species are unresolved. Additionally, it is generally not well understood how telomeres relate with reproductive success or sex. We measured telomere length and dynamics in erythrocytes to assess their relation to age, sex and reproduction in Cory's Shearwaters (*Calonectris borealis*), a long-lived seabird, in the context of a long-term study. Adult males had on average 231 bp longer telomeres than females independent of age. In females, telomere length changed relatively little with age, whereas male telomere length declined significantly. Telomere shortening within males from one year to the next was three times higher than the inter-annual shortening rate based on cross-sectional data of males. Past long-term reproductive success was sex-specifically reflected in age-corrected telomere length: males with on average high fledgling production were characterised by shorter telomeres, whereas successful females had longer telomeres and we discuss hypotheses that may explain this contrast. In conclusion, telomere length and dynamics in relation to age and reproduction are sex dependent in Cory’s Shearwaters and these findings contribute to our understanding of what characterises individual variation in fitness.

Keywords: Ageing, life-history, reproduction, biomarker, fitness, survival

INTRODUCTION

In wild populations, large differences exist in fitness-related life-history traits between individuals (e.g. Fay, Barbraud, Delord & Weimerskirch, 2018; Hamel, Côté, Gaillard & Festa-Bianchet, 2009). To understand this life-history diversity, which ultimately affects population demography and dynamics (Coulson *et al.*, 2006; Hamel *et al.*, 2018; Pelletier, Clutton-Brock, Pemberton, Tuljapurkar & Coulson, 2007), it is necessary to investigate the mechanisms that mediate this diversity. In recent years, telomere length (TL) has emerged as a biomarker of ageing and individual state (Young, 2018). Telomeres are evolutionarily conserved DNA sequence repeats, which form the ends of chromosomes together with specific proteins and contribute to genome stability (O’Sullivan & Karlseder, 2010). TL varies considerably between individuals from very early life onwards (Sabharwal *et al.*, 2017) due to inheritance and variation in telomere dynamics over life (Bauch, Boonekamp, Korsten, Mulder & Verhulst, 2019; Dugdale & Richardson, 2018). Short TL is a biomarker of reduced health and survival probability in many organisms (Opresko & Shay, 2017; Boonekamp, Simons, Hemerik & Verhulst, 2013; Joeng, Song, Kong-Joo & Lee, 2004; Wilbourn *et al.*, 2018). Telomeres shorten due to incomplete replication during cell division, which can be accelerated by DNA- and protein damaging factors and attenuated or counter-acted by maintenance processes (Chan & Blackburn, 2004). Telomere shortening rate has been shown to differ between individuals, for example in relation to exposure to stress, resource-based life-history trade-offs or environmental conditions and it has thus been suggested to reflect somatic costs of exposure to these challenges (Angelier, Costantini, Blévin & Chastel, 2018; Monaghan, 2014; Young, 2018).

Meta-analyses investigating the cross-sectional relationship between TL and age in bird and mammal species showed a higher telomere decline in short lived species (Haussmann *et al.*, 2003; Tricola *et al.*, 2018), which was confirmed by a meta-analysis on the few available longitudinal studies (Sudyka, Arct, Drobniak, Gustafsson & Cichoń, 2016). However, the pattern is inconsistent among the longest-lived species of birds (and even less is known for wild mammals), with the cross-sectional relationship between TL and age found to be positive (Oystercatcher *Haematopus ostralegus,* Leach's Storm Petrel *Oceanodroma leucorhoa*: Tricola *et al.,* 2018), insignificant both when tested longitudinally (Adélie Penguin *Pygoscelis adeliae*: Beaulieu, Reichert, Le Maho, Ancel & Criscuolo, 2011; Magellanic Penguin *Spheniscus magellanicus*: Cerchiara *et al.,* 2017) or cross-sectionally (Black-browed Albatross *Thalassarche melanophrys*: Angelier, Weimerskirch, Barbraud & Chastel, 2019; Magellanic Penguin: Cerchiara *et al.,* 2017), or negative only when including data from chicks (Southern Giant Petrel *Macronectes giganteus*: Foote *et al.,* 2011a; European Shag *Phalacrocorax aristotelis,* Wandering Albatross *Diomedea exulans*: Hall *et al.,* 2004; Northern Fulmar *Fulmarus glacialis,* Tricola *et al.,* 2018). Potential and non-mutually exclusive explanations for the diverse relationships found in these very long-lived species are i) telomere lengthening or ii) telomere maintenance during adulthood and telomere shortening restricted to early life or iii) telomere shortening and selective disappearance of individuals with short telomeres (Haussmann & Mauck, 2008). At the population level, the observed cross-sectional relationship can differ from the effect within individuals to the point of showing the opposite trend (van de Pol & Verhulst, 2006). This highlights the need to carry-out longitudinal investigations of telomere dynamics in long-lived species to establish within individual changes with age. Long-lived species are particularly interesting to study telomere dynamics, as costs of telomere erosion, or the processes of senescence it reflects, may be more likely to become apparent in species where extrinsic mortality is low.

Differences in senescence patterns or lifespan between the sexes have been found within some species (e.g. invertebrates: Jemielity et al. 2007; vertebrates: Beirne, Delahay & Young, 2015; Steenstrup *et al.,* 2017). A meta-analysis in humans, for example, has shown that females, which generally live longer, have longer telomeres than males (Gardner *et al.,* 2014). This has raised the question whether on the cellular level telomere length and/or shortening are involved in the mechanism underlying the sex difference in longevity, but the relationship between sex, telomeres and lifespan in other taxa is not clear (Barrett & Richardson, 2011). Differences in telomere shortening between the sexes have been explained by sex-specific patterns of resource allocation, due to differences in requirements. For example, telomere loss has been associated with different body size of the sexes in

Southern Giant Petrels, potentially reflecting a trade-off between growth and somatic maintenance (Foote *et al.,* 2011a) and has been related to different roles of the sexes during reproduction in some species (Bauch, Becker & Verhulst, 2013; Bauch, Riechert, Verhulst & Becker, 2016; Ryan *et al.* 2018), but not in others (Young *et al.,* 2013).

The relationship between TL and reproductive success has been studied across taxa and shown to be positive in several studies (Angelier *et al.,* 2019; Atema, 2017; Le Vaillant *et al.,* 2015; Parolini *et al.,* 2017; Pauliny, Wagner, Augustin, Szép & Blomqvist, 2006), which has been interpreted as positive correlations between traits in natural populations based on heterogeneity of individual quality (Angelier *et al.* 2019). Thus, individuals of high quality potentially have enough resources to invest into reproduction and self-maintenance as opposed to lower quality individuals. However, in other studies the relationship between TL and reproductive success was negative (Bauch *et al.,* 2013; Plot, Criscuolo, Zahn & Georges, 2012; Ryan *et al.,* 2018), suggesting that when high reproductive success is due to high reproductive effort this is achieved at the expense of TL (Bauch *et al.,* 2016; Sudyka, Arct, Drobniak, Gustafsson & Cichoń, 2019). An increased reproductive effort could lead to elevated oxidative stress or lower mitochondrial efficiency and consequently enhance telomere loss (Haussmann & Marchetto, 2010; Stier, Reichert, Criscuolo & Bize, 2015). When tested experimentally, telomere loss reflected reproductive effort in studies that manipulated the possibility to reproduce (Heidinger *et al.,* 2012; Kotrschal, Ilmonen & Penn, 2007), manipulated brood size (Reichert *et al.,* 2014; Sudyka *et al.,* 2014) or increased stress or activity via experimental treatment with corticosterone (Schultner, Moe, Chastel, Bech & Kitaysky, 2014), but not in studies that manipulated foraging effort or parental provisioning by increasing workload (Atema, 2017; Beaulieu *et al.,* 2011). Better knowledge of the relationship between telomere dynamics and reproduction will increase our understanding of within- and between individual differences in reproductive success and how this varies between species.

We measured TL using telomere restriction fragment (TRF) analysis in erythrocytes of Cory's Shearwaters *Calonectris borealis*, a long-lived seabird of the order Procellariiformes, sampled in two consecutive years. Our study individuals are part of a long-term population study and were of known sex, (estimated) age and long-term past reproductive success (Campioni, Granadeiro & Catry, 2016). Our aims were (1) to test the relationship between TL and age in the population of this long-lived seabird species, based on individuals with ages ranging from 7 to 36 years and, (2) to assess telomere dynamics within individuals between years, to be able to disentangle patterns on the population level from within-individual effects. To investigate the relationship between telomeres and reproduction, we analysed (3) past average long-term reproductive success and TL cross-sectionally. If TL is a biomarker of individual quality, individuals with higher reproductive success would be characterised by longer telomeres. However, shorter telomeres could be a sign that higher reproductive success was achieved by higher reproductive effort, which in turn led to higher telomere loss and consequently shorter telomeres (as suggested in Bauch *et al.*, 2013). Therefore, we tested (4) reproductive success and simultaneous and subsequent telomere dynamics within a year (longitudinally) and (5) manipulated reproduction in a subset of individuals by removing their single egg clutch and related it to subsequent telomere dynamics. Thus, if reproductive effort comes at the expense of somatic maintenance, one could expect a comparatively higher telomere loss in individuals raising chicks to fledging compared to individuals that lose their egg or chick and hence undertake lower parental effort. However, if heterogeneity in phenotypic quality masks such effect, we would expect to detect a reduced telomere loss only in an experimental setup, in individuals freed from parental care. (6) Furthermore, we tested for sex differences in telomere length and dynamics throughout the study as Cory's Shearwater males and females differ in the following traits: (a) Life-history theory predicts a resource allocation trade-off between growth and self-maintenance (Stearns, 1992). If this is reflected in TL, males would be expected to have shorter telomeres than females as they are the larger sex in this species (Granadeiro, 1993). (b) Males provide more parental care than females in this species (Granadeiro, Burns & Furness, 1998), which may be reflected in higher telomere loss in males. (c) Males have lower survival rates than females (Mougin, Jouanin & Roux, 2000a), which may be reflected in their telomeres, either by shorter TL in males or in higher telomere loss if the latter better reflects senescence.

MATERIALS AND METHODS

STUDY SPECIES

We studied Cory's Shearwaters (*Calonectris borealis*) breeding on Selvagem Grande (30°09'N, 15°52'W), a 4 km^2 island nature reserve located ca. 300 km south of the Madeiran archipelago of Portugal. This population is subject to a long-term study, where birds were ringed since 1978 (with a reduced intensity only between 2000-2003) and reproductive success (fledging success) in ~500 nests has been monitored annually since 2004 (Campioni *et al.*, 2016; Mougin *et al.*, 2000a). There is no terrestrial predation on the island. Our dataset contains breeding birds aged between 7 and 36 years (females: n=79, mean±SD: 17.49±4.91 years; males: n=101, mean±SD: 17.14±5.93 years). 52 of the 180 birds included in this study were marked with numbered metal rings as chicks for lifelong identification, and hence their exact age is known (mean age±SD: 16.35±8.12 years). 128 birds were ringed as adults and presumed to be recruits when first captured, as breeding birds are highly philopatric (Mougin, Granadeiro, Jouanin & Roux, 1999) and birds that occupy nests (successful and unsuccessful breeding attempts) have been identified regularly in our study area. Therefore, an age of 9 years was assigned to these birds at ringing, as 8.9 (±1.7) years is the average (±SD) age of recruitment in this colony for both sexes (Mougin *et al.*, 2000a). The mean age±SD of those birds during our study was 17.7±4.0 years. The sexes are dimorphic with males being on average larger in all available morphological characteristics (Granadeiro, 1993). Study birds were sexed with high accuracy (>99%) using a combination of three methods: a discriminant function based on bill measurements (Granadeiro, 1993), vocalisations (Thibault, Bregatgnolle & Rabuñal, 1997) and a cross-validation of the sexes of breeding partners.

The studied birds bred in individually marked artificial nest cavities in stone walls on the island plateau. Like other members of the Procellariiformes, they lay a single egg per breeding attempt and there are no replacement clutches (Warham, 1990). Birds return from their wintering areas between February and April, with males arriving on average earlier than females to secure and defend nest cavities, frequently resulting in intra-specific fights, while females attend the nest site less often and are absent from the colony for the period of egg formation prior to laying (Catry, Dias, Phillips & Granadeiro, 2013; Granadeiro *et al.*, 1998; Ramos, Monteiro, Sola & Moniz, 1997). Egg laying in the colony occurs from late May to early June and the incubation period lasts ca. 54 days (Mougin, Jouanin, Roux & Zino, 2000b). Chicks fledge after ca. 97 days. The parents equally share incubation, but fathers visit the nest more frequently during pre-laying and chick rearing (Granadeiro *et al.*, 1998).

MANIPULATION OF REPRODUCTION

In 2017, reproduction was experimentally manipulated in 25 randomly chosen ringed breeding pairs in the study nests by removing the clutch between late June and early July, thus inducing reproduction failure and freeing birds from subsequent parental effort. Individuals of similar ages (23 males and 39 females) breeding at the same time were assigned as control group, i.e. without manipulation.

24 males (96%) and 22 females (88%) from the 50 manipulated individuals and 20 males (87%) and 33 females (85%) from the control group were recorded and resampled in 2018.

BLOOD SAMPLE COLLECTION AND TELOMERE ANALYSIS

Adult birds were caught at their nest sites and blood sampled by puncturing the *vena brachialis* between June and July 2017 during incubation and again directly after returning from the wintering grounds between February and April 2018. Samples were first stored in 2% EDTA buffer at 4-7°C and then snap frozen in 40% glycerol buffer for permanent storage at -80°C within 4 weeks of collection. We measured terminally located TLs using telomere restriction fragment analysis without DNA denaturation (modified from Salomons *et al.*, 2009). First, we removed the glycerol buffer, washed the cells and isolated DNA from 5µl of erythrocytes using CHEF Genomic DNA Plug kit for preparation of intact, chromosome-sized DNA (Bio-Rad, Hercules, CA, USA). Cells in the agarose plugs were digested overnight with Proteinase K at 50°C. Isolated DNA (half of the plug per sample) was restricted overnight simultaneously with *Hind*III (60U), *Hinf*I (30U) and *Msp*I (60U) in NEB2 buffer (New England Biolabs Inc., Beverly, MA, USA) at 37°C. Subsequently, the restricted DNA was separated by pulsed-field gel electrophoresis in a 0.8% agarose gel (Pulsed Field Certified Agarose, Bio-Rad) at 14°C for 24h at 3.5V/cm, initial switch time 0.5s, final switch time 7.0s. For size calibration ^{32}P-labelled size markers (1kb DNA ladder, New England Biolabs Inc.; DNA Molecular Weight Marker XV, Roche Diagnostics, Basel, Switzerland) were added. Subsequently, gels were dried (gel dryer, Bio-Rad, model 538) at room temperature and hybridised overnight at 37°C with ^{32}P-labelled oligonucleotides (5'-CCCTAA-3')$_4$ that bind to the single-strand overhang of telomeres of non-denatured DNA. Unbound oligonucleotides were removed by washing the gel for 30min at 37°C with 0.25x saline-sodium citrate buffer. The radioactive signal of the sample specific TL distribution was detected by a phosphor screen (MS, Perkin-Elmer Inc., Waltham, MA, USA), exposed for ~20h, and visualised using a phosphor imager (Cyclone Storage Phosphor System, Perkin-Elmer Inc.). TL per sample was calculated using IMAGEJ (v. 1.38x) as described by Salomons *et al.* (2009). For each sample the limits for the telomere distribution was lane-specifically set at the point of the lowest signal (i.e. background intensity) and the individual average of the TL distribution was used for further analysis. Samples of low quality that produced low signals were excluded (9%). Samples were run on 14 gels. Repeated samples of the same individuals were on the same gels, whereas sexes, ages and treatment groups were randomised over all gels. The coefficient of variation of one control sample of one randomly chosen Cory's Shearwater run on all gels was 2.49%. Within-individual repeatability for TL of five individuals sampled repeatedly in 2018 (sampling interval between 21 and 38 days) and analysed on the same gel was 89.9% (calculated following Lessels & Boag, 1987). Both the coefficient of variation as well as the repeatability values (as compared to other studies) indicate a high quality of the TL data.

STATISTICAL ANALYSES

We analysed TL variation using linear mixed effects models with restricted maximum-likelihood estimates in R (version 3.5.1, R Core Team) using packages lme4 (Bates, Mächler, Bolker & Walker, 2015) and lmerTest (Kuznetsova, Brockhoff & Christensen, 2017). We selected models to test specific predictions in line with our study design. Model fit for linear mixed effects models was calculated as conditional R^2 using the package MuMIn (Bartoń, 2019).

To disentangle within- and between-individual telomere shortening we used the method of within-subject centring (van de Pol & Wright, 2009). To this end we used the mean age per individual and the deviation from the mean (delta age) per sample (taking into account the exact sampling interval in days). The estimate 'mean age' in the model stands for between individual differences, the cross-sectional slope of the relationship between TL and age. The estimate 'delta age' represents the within-individual effect of telomere shortening, the slope based on longitudinal data. To test for differences in TL between the sexes, we included sex as a factor. As random effects we included bird ID to account for repeated TL data of the same individuals and gel ID to control for between gel differences. Subsequently, we added the interactions sex*mean age and sex*delta age to test for potential sex differences in the relationship between TL and age (based on cross-sectional analysis: mean age) and for differences in within-individual telomere dynamics (longitudinally: delta age). Based on these findings we then ran the model for the sexes separately. The dataset for these analyses included all telomere data except for follow-up measurements of manipulated individuals because of potential experimental effects.

To test if the slopes of between- (mean age) and within-individual (delta age) telomere shortening in males were significantly different, we ran the model with mean age and exact age as two covariates. The exact age includes between- and within-individual effects and the estimate for mean age in this model represents the difference between the between- and within-individual effects (van de Pol & Wright, 2009). If mean age in this model is significant, slopes between- and within-individuals are significantly different, supporting a selective (dis-)appearance of individuals in the population.

As estimate of past long-term reproductive success, we used the average number of fledglings over the past up to 13 years, which allows us to include a maximum of available data on reproduction and the most long-lived individuals, and estimated its association with TL in 2017 including sex as factor and the interaction between sex and past reproductive success. Age was included as covariate and gel ID as random effect in the models. In a second analysis we re-ran the model and gave the data points of individuals different weights depending on the number of years or reproductive events each individual contributed. As a weighing factor we used the square root of the number of years (Sokal & Rohlf, 1995). Additionally, we ran the same models with average hatching success instead of fledging success. As results with and without weighing factor are qualitatively identical, we only report the model without weighing factor.

We compared TLs of breeding partners using a linear model with male TL as dependent variable and female TL as covariate as well as male age to correct for telomere change with age in males. Further, we tested if telomere shortening from 2017 to 2018 was correlated among breeding partners using a Pearson correlation. As partners were not necessarily blood sampled the same day, telomere shortening was corrected for sampling interval.

To test for a longitudinal relationship between reproductive success and TL (unmanipulated birds only), we ran a model including TL as dependent variable, mean age, delta age and reproductive success (fledgling in 2017 yes or no) as covariates and sex as factor.

We investigated potential effects of experimental manipulation of reproduction on telomere shortening in a model with experiment group as factor (coded 0 for birds whose egg was removed and 1 for control birds) and an interaction between experiment group and delta age. Bird ID was added as random effect for repeated telomere data between years. For the model that included both sexes, nest ID was added as a random effect, but did not explain additional variance and hence is not reported.

RESULTS

AGE AND SEX EFFECTS

TL declined significantly with age, both cross-sectionally, comparing individuals that differ in age (‘mean age’, Table 1A), and longitudinally, within individuals at different ages (‘delta age’, Table 1A). TL in samples collected on the same individuals in different years were highly correlated in both sexes (Fig.1; males: r=0.90, n=47, p<0.001; females: r=0.97, n=35, p<0.001). Telomeres of males were on average 231±94 bp longer than telomeres of females (Table 1A). Adding the interaction between ‘mean age’ and ‘sex’ to this model showed this to be significant (Table 1B), and we therefore repeated the analysis for the sexes separately. This revealed that among males, older individuals had shorter telomeres, and the slope of this cross-sectional effect was -34 bp per year (‘mean age’, Table 1C, Fig. 2A). Longitudinal telomere shortening was approximately three times higher, at -111 bp per year (‘delta age’, Table 1C). However, the difference in slope between- and within-individuals did not reach statistical significance (t=1.33, n=145 samples of 98 birds, p=0.19). In females there was no significant telomere shortening with age, neither cross-sectionally (Table 1D, Fig. 2B) nor longitudinally (Table 1D, Fig. 1B, 1C), and both effects were markedly weaker in females compared to males (Figs. 1 & 2).

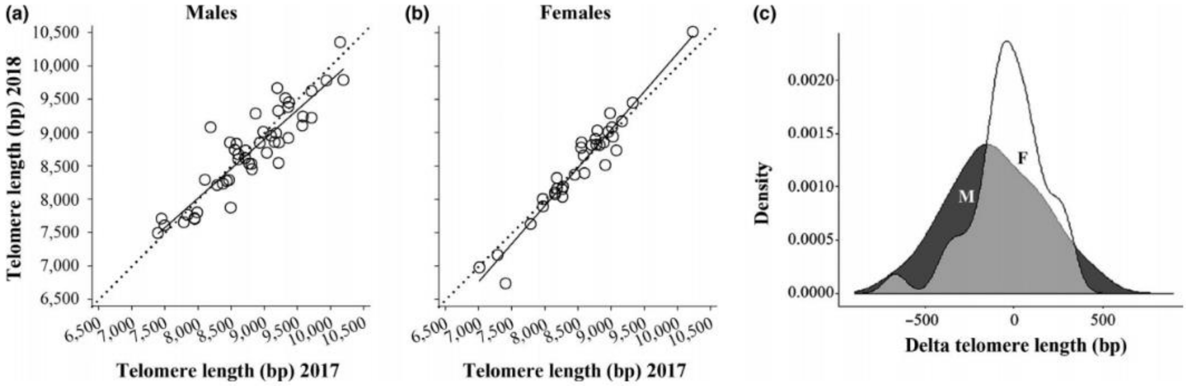

Fig. 1. Longitudinal telomere length dynamics (unmanipulated birds only). Correlation of telomere length within **(a)** males and **(b)** females measured in 2017 and 2018. The dotted line represents y=x. Solid lines are regression lines for males r=0.90 and females r=0.97. **(c)** Density plot of telomere length change (delta) within individuals between the two sampling years, with negative values showing telomere shortening. Males = black, females = white. For statistics see Table 1.

We know the exact age of part of the sampled individuals (52/180), and know an estimated age for the remainder of the birds, which could potentially bias the findings described above that are based on cross-sectional data. We tested this by running the analyses with the subset of birds of exactly known ages only. This did not change the findings (table S1). Therefore, we consider it reasonable to assume that data of birds that were assigned an estimated age did not bias our results (for further support see below the results from longitudinal data that are independent of the exact ages of adult birds.)

We tested for a relationship of TL or telomere loss between breeding partners. But TLs of breeding partners were not correlated (*r*=0.35, *n*=40, *p*=0.39), and neither was their telomere shortening rate from 2017 to 2018 (*r*=0.055, *n*=20, *p*=0.82).

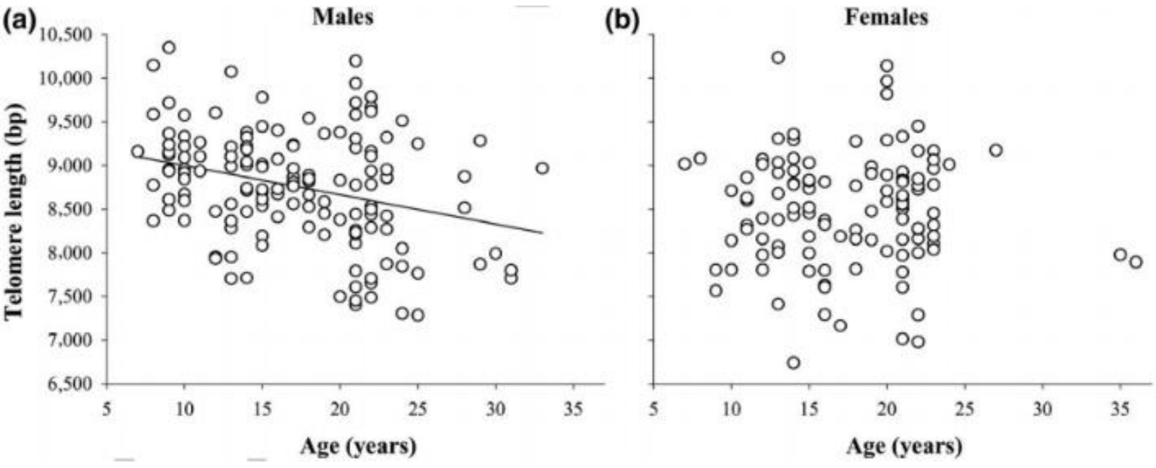

Fig. 2. Telomere length in relation to age in **(a)** males and **(b)** females with regression lines if significant. For statistics see Table 1.

Table 1. Linear mixed effects models testing for telomere shortening (bp / year) in adult male and female Cory's Shearwaters. Using within-subject centring we distinguished between-individual (mean age per bird) and within-individual (delta age, for repeated measures the deviation from the mean) effects. (A) With sex as a fixed effect to test for sex differences in telomere length (estimate is difference in females relative to males). (B) By including interactions assessing differences in telomere dynamics between the sexes. (C) Males only. (D) Females only.

Telomere length	Model terms	Estimate	Std. error	df	t	p
(A) both sexes	intercept	9077.4	170.5	115.9	53.23	<0.001
n=258 samples /	fixed effects:					
176 individuals	mean age	-18.6	8.9	168.4	-2.10	0.037
	delta age	-82.8	39.1	86.9	-2.12	0.037
	sex	-230.7	94.0	172.4	-2.46	0.015
		σ^2				
	random effects:					
	bird ID	0.828				
	gel ID	0.089				
	residual	0.084				
(B) both sexes	intercept	9314.4	196.9	147.4	47.30	<0.001
n=258 samples /	fixed effects:					
176 individuals	mean age	-32.6	10.6	174.8	-3.07	0.002
	delta age	-114.2	51.4	86.3	-2.22	0.029
	sex	-923.4	315.6	172.5	-2.93	0.004
	sex*mean age	40.2	17.4	170.0	2.31	0.022
	sex*delta age	68.5	79.2	86.5	0.87	0.390
		σ^2				
	random effects:					
	bird ID	0.825				
	gel ID	0.089				
	residual	0.086				
(C) males	intercept	9329.9	190.8	78.0	48.89	<0.001
n=145 samples /	fixed effects:					

98 individuals	mean age	-33.7	10.2	98.1	-3.31	0.001
	delta age	-111.5	57.9	51.3	-1.93	0.0598
		σ^2				
	random effects:					
	bird ID	0.772				
	gel ID	0.109				
	residual	0.119				
(D) females	intercept	8392.1	285.4	62.7	29.41	<0.001
n=113 samples /	fixed effects:					
78 individuals	mean age	7.97	15.4	74.3	0.52	0.607
	delta age	-46.7	47.4	35.4	-0.97	0.331
		σ^2				
	random effects:					
	bird ID	0.866				
	gel ID	0.087				
	residual	0.048				

Model fit (conditional R^2): (A) R^2=0.921, (B) R^2=0.921, (C) R^2=0.892, (D) R^2=0.953

NATURAL VARIATION IN REPRODUCTIVE SUCCESS

TL was correlated with past reproductive success (average fledgling production over up to 13 years including 2017), but this pattern differed significantly between the sexes (Table 2A). More successful males were characterised by shorter age-corrected TL (Fig.3A), while more successful females had longer age-corrected TL (Fig.3B). Patterns in both sexes were statistically significant when tested separately (Tables 2B & 2C) and also apparent when relating TL to past hatching success (average hatchling production over up to 13 years including 2017; table S2). The analysis including only birds of exactly known ages supports the finding of the relationship between reproductive success and TL (Table S3). As older birds contribute data of more years to the individual average reproductive success, which could bias the results, we re-ran the models with weighted data of reproductive success. The results did not change our findings (compare Table S4 and Table 2).

Longitudinal analysis revealed the change in TL from 2017 to 2018 to be independent of natural variation in reproductive success in 2017 in both sexes (Table 3).

Table 2. Linear mixed effects model testing for effects of long-term reproductive success (average fledgling production over the past up to 13 years) on telomere length. **(A)** Both sexes (estimate for females relative to males), **(B)** males, **(C)** females.

telomere length	model terms	estimate	std. error	df	t	p
(A)	intercept	9667.1	224.0	129.3	43.2	<0.001
n=150 birds	fixed effects:					
	age	-17.1	9.4	143.8	-1.82	0.071
	reprod. success	-886.7	253.7	142.1	-3.50	0.001
	sex	-1317.6	255.2	143.1	-5.16	<0.001
	reprod. success * sex	1656.1	360.1	141.5	4.60	<0.001
		σ^2				
	random effects:					
	gel ID	0.063				
	residual	0.937				
(B)	intercept	9719.1	230.0		42.3	<0.001
n=82 males	fixed effects:					
	age	-20.0	11.3		-1.78	0.079
	reprod. success	-898.4	248.8		-3.61	<0.001
(C)	intercept	8251.3	281.9		29.27	<0.001
n=68 females	fixed effects:					
	age	-11.4	16.2		-0.70	0.484
	reprod. success	762.9	296.8		2.57	0.013

Model fit (conditional R^2): (A) R^2=0.220, (B) R^2=0.202, (C) R^2=0.093

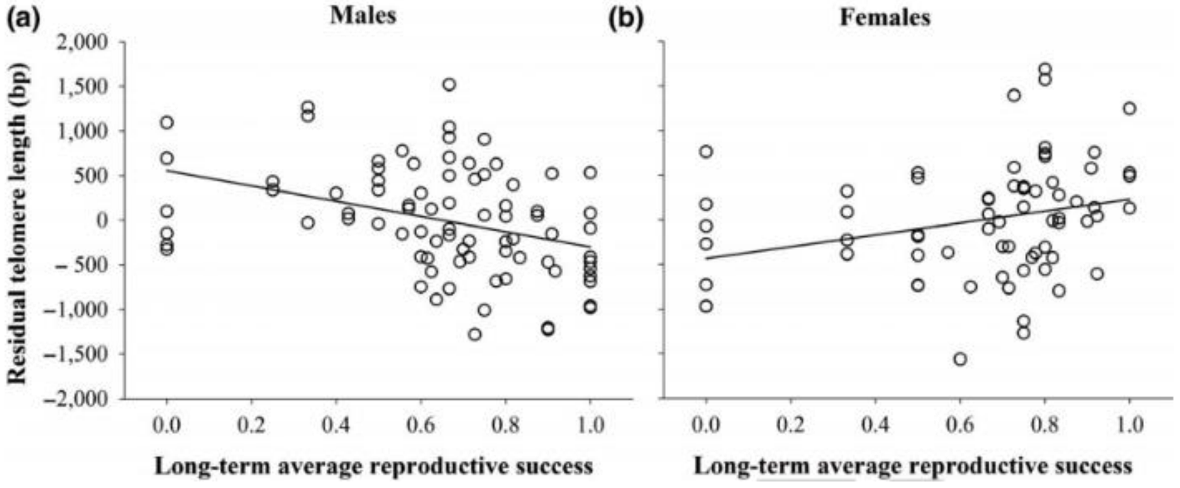

Fig. 3. Residual telomere length (age-corrected) in relation to average long-term reproductive success (average fledgling production over the last up to 13 years) in **(a)** males and **(b)** females, incl. regression lines. For statistics see Table 2.

Table 3. Linear mixed effects model testing for the relationship between telomere length and loss in relation to reproductive success in 2017 in unmanipulated birds. (A) Both sexes, full model, (B) both sexes, reduced model, (C) males, (D) females. Bird ID included as random effect as telomere length has been measured longitudinally.

telomere length	model terms	estimate	std. error	df	t	p
(A)	intercept	9476.5	312.6	69.2	30.3	<0.001
n=164 samples /	fixed effects:					
82 birds	mean age	-25.6	14.2	76.5	-1.80	0.076
	delta age	-223.2	110.9	78.0	-2.01	0.048
	sex	-725.3	299.2	77.0	-2.42	0.018
	fledgling	-365.5	225.2	76.0	-1.62	0.109
	fledgling*delta age	138.2	126.0	78.0	1.10	0.276
	sex*delta age	127.2	166.8	78.0	0.76	0.448
	sex*fledgling	603.5	343.2	76.1	1.76	0.083
	sex*fledgling*delta age	-72.1	190.9	78.	-0.38	0.707
		σ^2				
	random effects:					
	bird ID	0.900				

	gel ID	0.022				
	residual	0.078				
(B)	intercept	9271.9	309.9	71.6	29.9	<0.001
n=164 samples /	fixed effects:					
82 birds	mean age	-31.9	14.4	78.6	-2.22	0.030
	delta age	-167.0	82.3	80.0	-2.03	0.046
	fledgling	-100.7	172.8	76.9	-0.58	0.562
	fledgling*delta age	104.8	94.0	80.0	1.11	0.268
		σ^2				
	random effects:					
	bird ID	0.887				
	gel ID	0.040				
	residual	0.073				
(C)	intercept	9578.8	347.7	44.0	27.55	<0.001
n=94 samples	fixed effects:					
47 males	mean age	-31.9	17.3	44.0	-1.85	0.072
	delta age	-223.2	125.7	45.0	-1.78	0.083
	fledgling	-360.5	218.6	44.0	-1.65	0.106
	fledgling*delta age	138.2	142.8	45.0	0.97	0.338
		σ^2				
	random effects:					
	bird ID	0.895				
	residual	0.105				
(D)	intercept	8471.9	555.0	32.0	15.3	<0.001
n=70 samples	fixed effects:					
35 females	mean age	-12.6	24.6	32.0	-0.51	0.613
	delta age	-96.0	97.6	33.0	-0.98	0.332
	fledgling	291.6	280.4	32.0	1.04	0.306
	fledgling*delta age	66.1	112.3	33.0	0.59	0.560
		σ^2				
	random effects:					
	bird ID	0.956				
	residual	0.044				

Model fit (conditional R^2): (A) $R^2=0.932$, (B) $R^2=0.932$, (C) $R^2=0.907$, (D) $R^2=0.958$

MANIPULATION OF REPRODUCTIVE EFFORT

Pre-manipulation TL, age and sampling interval did not differ between manipulated and control birds in either sex (Table 4). The reduction of parental effort achieved through experimental egg removal did not result in a reduced telomere loss of manipulated birds compared to controls (sexes combined, table 5A). In males, there was a trend in the opposite direction, with manipulated males losing more telomere base pairs than control males (Table 5B). In females, telomere attrition was not affected by the egg removal experiment (Table 5C).

Table 4. Descriptive information (mean ± S.D.) on the individuals sampled in 2017 according to their sex and status with respect to reproduction in 2017. 'Additional' birds are unmanipulated but not part of the control group. Sampling interval refers to the time elapsed between the samples taken in 2017 and 2018.

	Manipulated		Control		Additional	
	males	females	males	females	males	females
n	23	24	21	38	59	17
age (years)	17.9±5.1	15.6±5.3	17.2±4.3	17.2±4.9	15.9±6.7	18.5±4.2
TL (bp)	8910±526	8452±611	8679±469	8597±496	8794±732	8656±976
sampling interval (days)	263±10	253±9	263±14	263±11	269±10	264±15

Table 5. Linear mixed effects model testing for effects of experimentally manipulated reproductive effort on telomere dynamics. **(A)** Both sexes (estimate for females relative to males), **(B)** males, **(C)** females. Bird ID included as random effect as telomere length has been measured longitudinally.

Telomere length	Model terms	Estimate	Std. error	df	t	p
(A)	intercept	8674.5	256.8	69.0	33.8	<0.001
n=148 samples /	fixed effects:					
74 birds	mean age	1.5	11.8	69.0	0.13	0.901
	delta age	-255.4	90.4	70.0	-2.83	0.006
	sex	-312.3	179.1	69.0	-1.74	0.086
	experiment group	-26.8	181.1	69.0	-0.15	0.883
	experiment group*delta age	229.5	128.3	70.0	1.79	0.078
	sex*delta age	241.7	127.3	70.0	1.90	0.062
	sex*experiment group	108.5	242.7	69.0	0.45	0.656
	sex*experiment*delta age	-293.7	173.3	70.0	-1.70	0.095
		σ^2				
	random effects:					
	bird ID	0.876				
	residual	0.124				
(B)	intercept	8966.5	333.9	29.0	26.86	<0.001
n= 64 samples /	fixed effects:					
32 males	mean age	-14.0	16.6	29.0	-0.84	0.407
	delta age	-255.5	89.0	30.0	-2.87	0.007
	experiment group	-38.5	162.1	29.0	-0.24	0.814
	experiment group*delta age	229.5	126.3	30.0	1.82	0.079
		σ^2				
	random effects:					
	bird ID	0.852				
	residual	0.148				
(C)	intercept	8195.8	291.0	39.0	28.17	<0.001

n= 84 samples /	fixed effects:					
42 females	mean age	11.9	16.4	39.0	0.73	0.471
	delta age	-13.7	90.7	40.0	-0.15	0.880
	experiment group	64.8	172.7	39.0	0.38	0.709
	experiment group*delta age	-64.2	117.9	40.0	-0.55	0.589
		σ^2				
	random effects:					
	bird ID	0.890				
	residual	0.111				

Model fit (conditional R^2): (A) R^2=0.884, (B) R^2=0.857, (C) R^2=0.891

DISCUSSION

It is a general observation that vertebrate TL declines with age (Haussmann *et al.,* 2003; Tricola *et al.,* 2018), but whether this also holds true in very long-lived species of birds - among them Procellariifomes, an order of pelagic seabirds - has not previously been confirmed within individuals. Hence our results, based on high precision TL data, provide the first evidence of telomere shortening in a Procellariiform during adulthood, both cross-sectionally and longitudinally, i.e. within individuals. Furthermore, we show that TL is sex-specifically associated with past reproductive success, positively in females and negatively in males.

AGE AND SEX EFFECTS

While there was a significant decline in TL in our dataset when the sexes were pooled, further analysis showed this to differ between the sexes, with TL declining faster with age in males (cross-sectionally and longitudinally) compared to females, in which the observed decline was not statistically significant. The extent to which telomere shortening rates differ between the sexes and how and why such differences arise is not well known (Barrett & Richardson, 2011). It may be that females are generally better at maintaining their telomeres, e.g. sex hormones may play a role by promoting antioxidant defence and/or telomerase activity (Aviv, 2002). Another and not mutually exclusive possibility is that the sex difference in shortening rate may reflect the division of labour during the breeding period, with males doing a larger share of nest defence and parental care in Cory’s Shearwaters

(Granadeiro *et al.,* 1998). Stress or higher activity during reproduction, as induced or reflected by higher corticosterone levels, have been shown to relate to higher telomere shortening (Angelier *et al.,* 2018; Schultner *et al.,* 2014), potentially via increased oxidative stress levels or inefficient mitochondrial efficiency (Haussmann & Marchetto, 2010; Stier *et al.,* 2015). Furthermore, a stronger competition for reproduction in males, as suggested by frequently observed serious fights for nesting sites in our study population, could lead to higher investment in reproduction at the cost of a lower investment into self-maintenance (reflected in higher telomere loss) and the observed lower survival probability in males (Mougin *et al.,* 2000a). Higher rates of senescence in the sex under higher competition for reproduction has also been found in European badgers *Meles meles* (Beirne *et al.,* 2015). Telomeres of adult males shortened about three times faster within individuals, compared to the cross-sectional age effect. The difference between the slopes did not reach statistical significance, which may in part be due to the fact that the within-individual estimate was based on age differences of less than a year. However, the difference was in the expected direction (telomere loss: within > between), in line with males with shorter telomeres being more likely to disappear from the breeding population (i.e. die), as in other studies on wild vertebrates (Wilbourn *et al.,* 2018).

Males had on average longer telomeres than females (controlling for age), while telomere shortening was faster in adult males. This means that male TL must already be longer than female TL early in life, when breeding for the first time. Whether this sex difference is already present in the zygote, arises between zygote stage and first breeding due to differential telomere attrition rates, or is due to sex-specific differential TL-dependent selection of breeding birds, remains to be investigated. As females are the smaller sex in Cory's Shearwaters, shorter telomeres in females cannot be explained by a trade-off between growth and telomere maintenance as suggested for Southern Giant Petrels, where the larger sex, males, had shorter telomeres (Foote *et al.,* 2011a). In lesser Black-backed Gulls *Larus fuscus* males tended to have longer telomeres than females as hatchlings (Foote, Gault, Nasir & Monaghan, 2011b), providing support for the existence of sex differences already early in life. On the other hand, results from a study on Thick-billed Murres *Uria lomvia* support that sex differences arise during life due to differences in telomere shortening, as TL did not yet differ early in life (Young *et al.,* 2013). In humans, females have longer telomeres than males. This sex difference in TL is already present at birth and persists over life (Factor-Litvak *et al.,* 2016). Nevertheless, telomere dynamics differed between the sexes. Telomere shortening in females related to the reproductive period in that the rate of telomere loss slowed down after menopause (thus at older ages), while in males telomere shortening tended to increase with age (Dalgård *et al.*, 2015).

Sex differences in lifespan are known to occur in many species (e.g. Liker & Szekely, 2005), but the underlying mechanisms are not well understood (Austad & Fischer, 2016). Given that survival is associated with TL in many species, the question has been raised whether sex differences in TL and/or telomere shortening contribute to sex differences in lifespan (e.g. Barrett & Richardson, 2011). Our results are interesting in this respect, because in Cory's Shearwaters the females enjoy a higher survival rate (Mougin *et al.*, 2000a), while they have shorter telomeres, which is in contrast to what would be expected if TL contributed to the sex difference in lifespan. On the other hand, telomere shortening was faster in the shorter-lived sex (males) in both, Cory's Shearwaters (this study) and in humans, at least later in life (Dalgård *et al.*, 2015). These comparisons suggest telomere shortening rate to be more relevant for explaining sex differences in lifespan than absolute TL, as also found in a comparative analysis between species (Tricola *et al.*, 2018), but more studies are needed before more definitive conclusions can be drawn with respect to sex differences in telomere dynamics.

TELOMERE LENGTH AND REPRODUCTIVE SUCCESS

Given that longer age-corrected telomeres are usually assumed to reflect higher phenotypic quality, because of the positive association with health and survival, one could expect to also find a positive association between TL and reproductive success. While this was confirmed in some wild populations (Angelier *et al.*, 2019; Atema, 2017; Le Vaillant *et al.*, 2015; Pauliny *et al.*, 2006), this pattern is not universal. For example, in another seabird species, the Common Tern *Sterna hirundo*, more successful individuals had shorter telomeres than less successful individuals (Bauch *et al.*, 2013; Bichet *et al.*, 2020). The pattern in Cory's Shearwaters is clearly more complex, with past reproductive success (average fledgling production over up to 13 years) being sex-specifically reflected in TL, with on average more successfully reproducing males being characterised by shorter telomeres, whereas the opposite was true for females. This pattern was already apparent in the hatching success (average hatchling production over up to 13 years) that birds achieved over the years.

A possible scenario that explains the sex differences in TL is that there is a compensation of parental provisioning between the sexes (Wright & Cuthill, 1989). Thus, if females are paired with males that provision their chick at a high rate, resulting in high reproductive success, the females themselves could in turn have to work less hard, which results in slower telomere shortening and, hence, longer age-dependent telomeres in females. Alternatively, females with longer telomeres may obtain more successful males than females with shorter telomeres. However, neither TLs of breeding partners nor their telomere dynamics were correlated. Thus, our data did not support either of these two hypotheses, but statistical power for these tests was limited and a larger data set is required to test these predictions with sufficient power. We consider it likely that heterogeneity in phenotypic quality is expressed as a positive covariance between TL and reproductive success in females, in line with findings in black-browed albatrosses (Angelier *et al.*, 2019). In this species, there was a positive association between TL and reproductive success in both sexes, which is consistent with chick provisioning rates being similar in the sexes (Huin, Prince & Briggs, 2000). A stronger intra-sexual reproductive competition in males than females, as mentioned previously, could lead to a negative relationship between long-term reproductive success and TL in Cory's Shearwater males. That sexual differences in telomere selection exist in the wild has also been found in Sand Lizards *Lacerta agilis* (Olsson *et al.*, 2011).

TELOMERE DYNAMICS AND REPRODUCTIVE EFFORT/REPRODUCTIVE SUCCESS

TL at any point in life is the result of TL at the start of life and subsequent telomere dynamics. Based on life-history theory, a trade-off between investment in reproduction and self-maintenance, with TL as biomarker, sex-dependent reproductive effort may have contributed to the observed sex differences in TL. Cross-sectional data cannot resolve this question and we therefore examined longitudinal associations between TL and reproductive success. For example, Common Terns with higher reproductive output showed higher telomere loss, with the exception of the most successful individuals (Bauch *et al.*, 2013). We did not detect such longitudinal effect in our current data set in either sex, but statistical power was modest. Due to heterogeneity in individual quality, reproductive success (longer parental care) is a rather crude measure of reproductive effort. Experimental manipulations of reproductive effort could shed light on the way the observed associations between reproductive success and TL have arisen. To this end, we removed the single egg from pairs, to test experimentally for an effect of reproductive effort on telomere dynamics. The experimental reduction of parental effort in 2017 did not significantly affect telomere dynamics in either sex.

The absence of a longitudinal effect of reproductive success (natural or experimental) on TL may be explained by the relatively short sampling interval for a long-lived species like Cory's Shearwater, where slow telomere attrition can be expected (as shown in an inter-species comparison, Sudyka *et al.,* 2016). Alternatively, only an experimental increase in reproductive effort may have had an effect on telomere dynamics as shown in zebra finches *Taeniopygia guttata* (Reichert *et al.,* 2014) or as shown more generally with an effect on survival across species (Santos & Nakagawa, 2012). In the case of our study species, however, a brood size increase would have been outside the natural range and thus an unnatural increase of reproductive effort as Cory's shearwater lay only one egg per breeding attempt. Further, such effect on telomere dynamics may only become apparent under less favourable environmental conditions, as shown for example in chicks of a different Procellariiform species (European Storm Petrel *Hydrobates pelagicus*) in differing years (Watson, Bolton & Monaghan, 2015) or adult Thick-billed Murres in differing colonies (Young *et al.*, 2013). Nevertheless, and along with the sex-specific TL dependent selection hypothesis, we consider that reproductive effort could have mediated the negative relationship between TL and long-term reproductive success in males.

In conclusion, we show clear associations between TL and life histories of Cory's Shearwater and these associations differed in direction between the sexes. Telomeres thereby provide a window on the physiological and molecular causes of individual and sex-specific variation in ageing and reproductive success.

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Data Accessibility

The data that support the findings of this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.j0zpc869z (Bauch, Gatt, Granadeiro, Verhulst, & Catry, 2020).

Author Contributions

PC, JPG and SV planned the project, MCG performed the manipulation and collected samples & data in 2017 and in 2018 together with CB, CB analysed telomeres, CB performed data analyses, CB wrote the manuscript together with SV, all authors commented on the manuscript.

REFERENCES

Angelier, F., Costantini, D., Blévin, P., & Chastel, O. (2018) Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. *General and Comparative Endocrinology*, 256, 99-111. doi: 10.1016/j.ygcen.2017.07.007

Angelier, F., Weimerskirch, H., Barbraud, C., & Chastel, O. (2019) Is telomere length a molecular marker of individual quality? Insights from a long-lived bird. *Functional Ecology*, 33, 1076-1087. doi: 10.1111/1365-2435.13307

Atema, E. (2017) Telomeres, workload and life-history in great tits. PhD thesis, University of Groningen.

Austad, S. N., & Fischer, K. E. (2016) Sex differences in lifespan. *Cell Metabolism*, 23, 1022-1033. doi: 10.1016/j.cmet.2016.05.019

Aviv, A. (2002) Telomeres, sex, reactive oxygen species, and human cardiovascular aging. *Journal of Molecular Medicine*, 80, 689-695. doi: 10.1007/s00109-002-0377-8

Barrett, E. L. B., & Richardson, D. S. (2011) Sex differences in telomeres and lifespan. *Aging Cell*, 10, 913-921. doi: 10.1111/j.1474-9726.2011.00741.x

Bartoń, K. (2019) MuMIn: Multi-Model Inference. R package version 1.43.6. https://cran.r-project.org/web/packages/MuMIn/

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67, 1-48. Version 1.1-21. doi: 10.18637/jss.v067.i01

Bauch, C., Becker, P. H., & Verhulst, S. (2013) Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society of London B: Biological Sciences*, 280, 20122540. doi: 10.1098/rspb.2012.2540

Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2019) Epigenetic inheritance of telomere length in wild birds. *PLoS Genetics*, 15, e1007827. doi: 10.1371/journal.pgen.1007827

Bauch, C., Gatt, M. C., Granadeiro, J. P., Verhulst, S., & Catry, P. (2020) Data from: Sex-specific telomere length and dynamics in relation to age and reproductive success in Cory's shearwaters. *Dryad Digital Repository*, doi: 10.5061/dryad.j0zpc869z

Bauch, C., Riechert, J., Verhulst, S., & Becker, P. H. (2016) Telomere length reflects reproductive effort indicated by corticosterone levels in a long-lived seabird. *Molecular Ecology*, 25, 5785-5794. doi: 10.1111/mec.13874

Beaulieu, M., Reichert, S., Le Maho, Y., Ancel, A., & Criscuolo, F. (2011) Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. *Functional Ecology*, 25, 577-585. doi: 10.1111/j.1365-2435.2010.01825.x

Beirne, C., Delahay, R., & Young, A. (2015) Sex differences in senescence: the role of intra-sexual competition in early adulthood. *Proceedings of the Royal Society of London B*, 282, 20151086. doi: 10.1098/rspb.2015.1086

Bichet, C., Bouwhuis, S., Bauch, C., Verhulst, S., Becker, P. H., & Vedder, O. (2020) Telomere length is repeatable, shortens with age and reproductive success, and predicts remaining lifespan in a long-lived seabird. *Molecular Ecology*, 29, 429-441. doi: 10.1111/mec.15331

Boonekamp, J. J., Simons, M. J. P., Hemerik, L., & Verhulst, S. (2013) Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging Cell*, 12, 330–332. doi: 10.1111/acel.12050

Campioni, L., Granadeiro, J. P., & Catry, P. (2016) Niche segregation between immature and adult seabirds: does progressive maturation play a role? *Behavioural Ecology* 27, 426-433. doi: 10.1093/beheco/arv167

Catry, P., Dias, M. P., Phillips, R. A., & Granadeiro, J. P. (2013) Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: an experimental demonstration. *Ecology*, 94, 1230-1235. doi: 10.1890/12-2177.1

Cerchiara, J. A., Risques, R. A., Prunkard, D., Smith, J. R., Kane, O. J., & Boersma, P. D. (2017) Magellanic penguin telomeres do not shorten with age with increased reproductive effort, investment, and basal corticosterone. *Ecology and Evolution*, 7, 5682-5691. doi: 10.1002/ece3.3128

Chan, S. R. W. L., & Blackburn, E. H. (2004) Telomeres and Telomerase. *Philosophical Transactions of the Royal Society London B*, 359, 109-121. doi: 10.1098/rstb.2003.1370

Coulson, T., Benton, T. G., Lundberg, P., Dall, S. R. X., Kendall, B. E., & Gaillard, J.-M. (2006) Estimating individual contributions to population growth: evolutionary fitness in ecological time. *Proceedings of the Royal Society B*, 273, 547–555. doi: 10.1098/rspb.2005.3357

Dalgård, C., Benetos, A., Verhulst, S., Labat, C., Kark, J. D., Christensen, K., ... Aviv, A. (2015) Leukocyte telomere length dynamics in women and men: menopause vs age effects. *International Journal of Epidemiology*, 44, 1688-1695. doi: 10.1093/ije/dyv165

Dugdale, H. L., & Richardson, D. S. (2018) Heritability of telomere variation: it is all about the environment. *Philosophical Transactions of the Royal Society B*, 373, 20160450. doi: 10.1098/rstb.2016.0450

Factor-Litvak, P., Susser, E., Kezios, K., McKeague, I., Kark, J. D., Hoffman, M., ... Aviv, A. (2016) Leukocyte Telomere Length in Newborns: Implications for the Role of Telomeres in Human Disease. *Pediatrics*, 137, e20153927. doi: 10.1542/peds.2015-3927

Fay, R., Barbraud, C., Delord, K., & Weimerskirch, H. (2018) From early life to senescence: individual heterogeneity in a long-lived seabird. *Ecological Monographs,* 88, 60–73. doi 10.1002/ecm.1275

Foote, C. G., Daunt, F., González-Solís, J., Nasir, L., Phillips, R. A., & Monaghan, P. (2011a) Individual state and survival prospects: age, sex, and telomere length in a long-lived seabird. *Behavioral Ecology,* 22, 156-161. doi: 10.1093/beheco/arq178

Foote, C. G., Gault, E. A., Nasir, L., & Monaghan, P. (2011b) Telomere dynamics in relation to early growth conditions in the wild in the lesser black-backed gull. *Journal of Zoology,* 283, 203-209. doi: 10.1111/j.1469-7998.2010.00774.x

Gardner, M., Bann, D., Wiley, L., Cooper, R., Hardy, R., Nitsch, D., … Ben-Shlomo, Y., and Halcyon study team (2014) Gender and telomere length: systematic review and meta-analysis. *Experimental Gerontology,* 51,15–27. doi: 10.1016/j.exger.2013.12.004

Granadeiro, J. P. (1993) Variation in measurements of Cory's shearwater between populations and sexing by discriminant analysis. *Ringing & Migration,* 14, 103-112. doi: 10.1080/03078698.1993.9674051

Granadeiro, J. P., Burns, M. D., & Furness, R. W. (1998) Patterns of activity and burrow attendance in Cory’s Shearwater *Calonectris diomedea* as revealed by a novel logging technique. *Ibis,* 140, 458-466. doi: 10.1111/j.1474-919X.1998.tb04607.x

Hall, M. E., Nasir, L., Daunt, F., Gault, E. A., Croxall, J. P., Wanless, S., & Monaghan, P. (2004) Telomere loss in relation to age and early environment in long-lived birds. *Proceedings of the Royal Society of London B: Biological Sciences*, 271, 1571–1576. doi: 10.1098/rspb.2004.2768

Hamel, S., Côté, S. D., Gaillard, J.-M., & Festa-Bianchet, M. (2009) Individual variation in reproductive costs of reproduction: high-quality females always do better. *Journal of Animal Ecology,* 78, 143–151. doi: 10.1111/j.1365-2656.2008.01459.x

Hamel, S., Gaillard, J.-M., Douhard, M., Festa-Bianchet, M., Pelletier, F., & Yoccoz, N. G. (2018) Quantifying individual heterogeneity and its influence on life-history trajectories: different methods for different questions and contexts. *Oikos*, 127, 687–704. doi: 10.1111/oik.04725

Haussmann, M. F., & Marchetto, N. M. (2010) Telomeres: Linking stress and survival, ecology and evolution. *Current Zoology*, 56, 714-727.

Haussmann, M. F., & Mauck, R. A. (2008) Telomeres and Longevity: Testing an Evolutionary Hypothesis. *Molecular Biology and Evolution*, 25, 220–228. doi: 10.1093/molbev/msm244

Haussmann, M. F., Winkler, D. W., O'Reilly, K. M., Huntington, C. E., Nisbet, I. C. T., & Vleck, C. M. (2003) Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings of the Royal Society of London B: Biological Sciences*, 270, 1387-1392. doi: 10.1098/rspb.2003.2385

Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012) Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences of the U.S.A.*, 109, 1743-1748. doi: 10.1073/pnas.1113306109

Huin, N., Prince, P. A., & Briggs, D. R. (2000) Chick provisioning rates and growth in Black-browed Albatross *Diomedea melanophris* and Grey-headed Albatross *D. chrysostoma* at Bird Island, South Georgia. *Ibis*, 142, 550-565. doi: 10.1111/j.1474-919X.2000.tb04456.x

Jemielity, S., Kimura, M., Parker, K. M., Parker, J. D., Cao, X., Aviv, A., & Keller, L. (2007) Short telomeres in short-lived males: what are the molecular and evolutionary causes? *Aging Cell*, 6, 225–233. doi: 10.1111/j.1474-9726.2007.00279.x

Joeng, K. S., Song, E. J., Kong-Joo, L., & Lee, J. (2004) Long lifespan in worms with long telomeric DNA. *Nature Genetics*, 36, 607-611. doi: 10.1038/ng1356

Kotrschal, A., Ilmonen, P., & Penn, D. J. (2007) Stress impacts telomere dynamics. *Biology Letters*, 3, 128-130. doi: 10.1098/rsbl.2006.0594

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82, 1–26. Version 3.1-0. doi: 10.18637/jss.v082.i13

Lessells, C. M., & Boag, P. T. (1987) Unrepeatable repeatabilites: a common mistake. *The Auk*, 104, 116-121. doi: 10.2307/4087240

Le Vaillant, M., Viblanc, V. A., Saraux, C., Le Bohec, C., Le Maho, Y., Kato, A., Criscuolo, F., & Ropert-Coudert, Y. (2015) Telomere length reflects individual quality in free-living adult king penguins. *Polar Biology,* 38, 2059-2067. doi: 10.1007/s00300-015-1766-0

Liker, A., & Székely, T. (2005) Mortality Costs of Sexual Selection and Parental Care in Natural Populations of Birds. *Evolution*, 59, 890-897.

Monaghan, P. (2014) Organismal stress, telomeres and life histories. *Journal of Experimental Biology*, 217, 57–66. doi: 10.1242/jeb.090043

Mougin, J.-L., Granadeiro, J. P., Jouanin, C., & Roux, F. (1999) Philopatry and faithfulness to nest site in Cory's Shearwaters *Calonectris diomedea* at Selvagem Grande. *Ostrich*, 70, 229-232. doi: 10.1080/00306525.1999.9634242

Mougin, J.-L., Jouanin, C., & Roux, F (2000a) Démographie du puffin cendré *Calonectris diomedea* de Selvagem Grande. *Revue d'Écologie (La terre et la vie),* 55, 275–290.

Mougin, J.-L., Jouanin, C., Roux, F., & Zino, F. (2000b) Fledging weight and juvenile survival of Cory's Shearwaters *Calonectris diomedea* on Selvagem Grande. *Ringing & Migration*, 20, 107-110. doi: 10.1080/03078698.2000.9674231

O'Sullivan, R. J., & Karlseder, J. (2010) Telomeres: protecting chromosomes against genome instability. *Nature Rev: Mol Cell Biol*, 11, 171-181. doi: 10.1038/nrm2848

Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., Miller, E., & Blomqvist, D. (2011) Sexual differences in telomere selection in the wild. *Molecular Ecology*, 20, 2085-2099. doi: 10.1111/j.1365-294X.2011.05085.x

Opresko, P. L., & Shay, J. W. (2017) Telomere-associated aging disorders. *Ageing Research Reviews*, 33, 52-66. doi: 10.1016/j.arr.2016.05.009

Parolini, M., Romano, A., Costanzo, A., Khoriauli, L., Santagostino, M., Nergadze, S. G., … Saino, N. (2017) Telomere length is reflected by plumage coloration and predicts seasonal reproductive success in the barn swallow. *Molecular Ecology*, 26, 6100-6109. doi: 10.1111/mec.14340

Pauliny, A., Wagner, R., Augustin, J., Szép, T., & Blomqvist, D. (2006) Age-independent telomere length predicts fitness in two bird species. *Molecular Ecology*, 15, 1681-1687. doi: 10.1111/j.1365-294X.2006.02862.x

Pelletier, F., Clutton-Brock, T., Pemberton, J., Tuljapurkar, S., & Coulson, T. (2007) The Evolutionary Demography of Ecological Change: Linking Trait Variation and Population Growth. *Science*, 315, 1571-1574. doi: 10.1126/science.1139024

Plot, V., Criscuolo, F., Zahn, S., & Georges, J.-Y. (2012) Telomeres, Age and Reproduction in a Long-Lived Reptile. *PLoS ONE*, 7, e40855. doi: 10.1371/journal.pone.0040855

R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2017.

Ramos, J. A., Monteiro, L. R., Sola, E., & Moniz, Z. (1997) Characteristics and competition for nest cavities in burrowing procellariiformes. *The Condor*, 99, 634-641. doi: 10.2307/1370475

Reichert, S., Stier, A., Zahn, S., Arrivé, M., Bize, P., Massemin, S., & Criscuolo, F. (2014) Increased brood size leads to persistent eroded telomeres. *Frontiers in Ecology and Evolution*, 2, 9. doi: 10.3389/fevo.2014.00009

Ryan, C. P., Hayes, M. G., Lee, N. R., McDade, T. W., Jones, M. J., Kobor, M. S., ... Eisenberg, D. T. A. (2018) Reproduction predicts shorter telomeres and epigenetic age acceleration among young adult women. *Scientific Reports*, 8, 11100. doi: 10.1038/s41598-018-29486-4

Sabharwal, S., Verhulst, S., Guirguis, G., Kark, J. D., Labat, C., Roche, N. E., … Aviv, A. (2017). The Blood-and-Muscle Telomere Model in Early Life. *The FASEB Journal*, 32, 529-534. doi: 10.1096/fj.201700630R

Salomons, H. M., Mulder, G. A., van de Zande, L., Haussmann, M. F., Linskens, M. H. K., & Verhulst, S. (2009) Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society of London B: Biological Sciences*, 276, 3157–3165. doi: 10.1098/rspb.2009.0517

Santos, E. S. A., & Nakagawa S. (2012) The cost of parental care: a meta-analysis of the trade-off between parental effort and survival in birds. *Journal of Evolutionary Biology*, 25, 1911-1917. doi: 10.1111/j.1420-9101.2012.02569.x

Schultner, J., Moe, B., Chastel, O., Bech, C., & Kitaysky, A. S. (2014) Migration and stress during reproduction govern telomere dynamics in a seabird. *Biology Letters*, 10: 20130889. doi: 10.1098/rsbl.2013.0889

Sokal, R. R., & Rohlf, F. J. (1995) Biometry: the principles and practice of statistics in biological research. 3rd ed., New York, NY: *Freeman WH & Company.*

Stearns, S. C. (1992) The Evolution of Life Histories. Oxford, UK: *Oxford University Press*.

Steenstrup, T., Kark, J. D., Verhulst, S., Thinggaard, M., Hjelmborg, J. V. B., Dalgård, C., ... Aviv, A. (2017) Telomeres and the natural lifespan limit in humans. *Aging*, 9, 1130-1140.

Stier, A., Reichert, S., Criscuolo, F., & Bize P. (2015) Red blood cells open promising avenues for longitudinal studies of ageing in laboratory, non-model and wild animals. *Experimental Gerontology*, 71, 118-134. doi: 10.1016/j.exger.2015.09.001

Sudyka, J., Arct, A., Drobniak, S., Dubiec, A., Gustafsson, L., & Cichoń, M. (2014) Experimentally increased reproductive effort alters telomere length in the blue tit (*Cyanistes caeruleus*). *Journal of Evolutionary Biology*, 27, 2258–2264. doi: 10.1111/jeb.12479

Sudyka, J., Arct, A., Drobniak, S., Gustafsson, L., & Cichoń, M. (2016) Longitudinal studies confirm faster telomere erosion in short-lived bird species. *Journal of Ornithology*, 157, 373-375. doi: 10.1007/s10336-015-1304-4

Sudyka, J., Arct, A., Drobniak, S., Gustafsson, L., & Cichoń, M. (2019) Birds with high lifetime reproductive success experience increased telomere loss. *Biology Letters*, 15, 20180637. doi: 10.1098/rsbl.2018.0637

Thibault, J. C., Bregatgnolle, V., & Rabuñal, J. L. (1997) *Calonectris diomedea* Cory's Shearwater. *BWP Update*, 1, 75-98.

Tricola, G. M., Simons, M. J. P., Atema, E., Boughton, R. K., Brown, J. L., Dearborn, D. C., … Haussmann, M. F. (2018) The rate of telomere loss is related to maximum lifespan in birds. *Philosophical Transactions of the Royal Society B*, 373, 20160445. doi: 10.1098/rstb.2016.0445 van de Pol, M., & Verhulst, S. (2006) Age-Dependent Traits: A New Statistical Model to Separate Within- and Between-Individual Effects. *The American Naturalist*, 167, 766-773. doi: 10.1086/503331

van de Pol, M., & Wright, J. (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. Animal Behaviour, 77, 753–758. doi: 10.1016/j.anbehav.2008.11.006

Warham, J. (1990) The Petrels: Their Ecology and Breeding Systems. Academic Press, London.

Watson, H., Bolton, M., & Monaghan, P. (2015) Variation in early-life telomere dynamics in a long-lived bird: links to environmental conditions and survival. *The Journal of Experimental Biology*, 218, 668-674. doi: 10.1242/jeb.104265

Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., & Boonekamp, J. J. (2018) The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philosophical Transactions of the Royal Society B*, 373, 20160447. doi: 10.1098/rstb.2016.0447

Wright, J., & Cuthill, I. (1989) Manipulation of sex differences in parental care. *Behavioral Ecology and Sociobiology*, 25,171-181. doi: 10.1007/BF00302916

Young, A. J. (2018) The role of telomere in the mechanisms and evolution of life-history trade-offs and ageing. *Philosophical Transactions of the Royal Society B*, 373, 20160452. doi: 10.1098/rstb.2016.0452

Young, R. C., Kitaysky, A. S., Haussmann, M. F., Descamps, S., Orben, R. A., Elliott, K. H., & Gaston, A. J. (2013) Age, Sex, and Telomere Dynamics in a Long-Lived Seabird with Male-Biased Parental Care. *PLoS ONE*, 8, e74931. doi: 10.1371/journal.pone.0074931

APPENDIX A2

GLOBAL MONITORING OF MERCURY IN THE MESOPELAGIC DOMAIN USING BODY FEATHERS OF BULWER'S PETREL AS A BIOINDICATOR

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ABSTRACT

Mercury pollution is rapidly growing and is a source of major concern. Mercury contamination is particularly prevalent in biota of the mesopelagic layers of the open ocean, but these realms are little studied, and we lack a large scale picture of contamination in living organisms of this region. The Bulwer's petrel *Bulweria bulwerii*, a species of migratory seabird, is a highly specialised predator of mesopelagic fish and squid, and therefore can be used as a bioindicator for the mesopelagic domain. Mercury accumulated by the Bulwer's petrel through diet is excreted through body feathers during the moulting process in adults and feather growth in chicks, reflecting contamination in the non-breeding and breeding periods, respectively, and hence the influence of different, largely non-overlapping breeding and non-breeding ranges. We studied mercury in feathers (adults and chicks) and the trophic position of chicks sampled in two colonies from Atlantic Ocean (Portugal and Cape Verde) and two colonies from Pacific Ocean (Japan and Hawaii). Mercury levels in Bulwer's petrel decreased by ca. 24 % from 1992-1994 to 2018 on colonies of the North Atlantic Ocean, probably reflecting a decline in atmospheric inputs of mercury. We found significantly lower levels of mercury in adult and chick samples from Pacific Ocean comparing with samples from Atlantic Ocean. In contrast, we did not detect differences in trophic position of chicks amongst colonies and oceans, suggesting that differences in mercury measured in feathers reflect levels of environmental contamination, rather than differences in the structure of the trophic chain in different oceans. We conclude that despite a reduction in mercury levels in the Atlantic Ocean in recent decades, mesopelagic organisms in this ocean remain more heavily contaminated than in the Pacific at tropical and subtropical latitudes. We suggest that Bulwer's petrel is a highly suitable species to monitor the global contamination of mercury in the mesopelagic domain.

Keywords: Mercury, mesopelagic specialists, foraging, biomonitoring, compound-specific stable isotope analysis

INTRODUCTION

While mercury occurs naturally in the environment from geological sources, the vast majority of mercury currently present in the marine environment is of anthropogenic origin, from activities including gold extraction, waste incineration and the use of fossil fuels (Driscoll et al., 2013; Esdaile and Chalker, 2018; Gworek et al., 2016). Mercury is widely transported in the atmosphere and is distributed through oceanic circulation (Driscoll et al., 2013). For pelagic ocean zones, the dominant source of mercury is atmospheric deposition (an exception is the Arctic Ocean where coastal erosion is likely the dominant source) (Obris et al., 2018). This elemental mercury sinks adsorbed to particles and is reduced to methylmercury by biogeochemical processes in low oxygen environments, such as the mesopelagic zone (200 – 1000 m below the ocean surface) (Choy et al., 2009; Lamborg et al., 2014; Mason and Fitzgerald, 1991; Sunderland et al., 2009). It is in this organic form that it is biomagnified up the food chain, with top predators such as seabirds exhibiting elevated concentrations of mercury (Lavoie et al., 2013). In fact, pelagic seabirds are one of the most exposed vertebrate groups to persistent and toxic mercury (e.g. Carravieri et al., 2018, 2020; Furtado et al., 2019, 2020; Monteiro and Furness, 1995, 1997; Kim et al., 1996).

Mesopelagic fauna provide a trophic link between surface and deep waters as a result of their diel vertical migration (Kelly et al., 2019). Consequently, they also transport methylmercury into the epipelagic domain (Madigan et al., 2018; Motta el al., 2019; Thompson et al., 1998). Some seabirds, such as the Bulwer’s petrel (*Bulweria bulwerii*), are specialised predators of mesopelagic prey (Harrison et al., 1983; Spear et al., 2007; Waap et al., 2017), making them ideal candidates to monitor oceanic mercury and its transport between mesopelagic and surface waters. The Bulwer's petrel has a large distribution in the tropical and subtropical waters of the world's oceans (Brooke, 2004; Dias et al., 2015, Ramos et al., 2015). In the Pacific Ocean, it breeds on Japan, the Hawaiian Islands, eastern China and French Polynesia, while in the Atlantic it breeds on the Macaronesian archipelagos of the Azores, Madeira, Canaries and Cape Verde (Brooke, 2004).

Apart from geographical differences in mercury contamination, mercury exposure is dependent on trophic position (Lavoie et al., 2013; Monteiro et al., 1998). Recently, compound-specific stable isotope analysis of amino acids (CSIA-AA) has been used to determine trophic position of seabirds robustly (e.g. Gagne et al., 2018; Gatt et al., 2020; McMahon et al., 2015; Quillfeldt et al., 2017; Quillfeldt and Masello, 2020). By comparing the relative enrichment of ^{15}N in "source" and "trophic" amino acids, typically phenylalanine and glutamic acid respectively, CSIA-AA effectively overcomes the limitations in interpreting bulk isotope ratios in the ocean as a result of a poorly-defined baseline isoscape (Graham et al., 2010). Phenylalanine represents the isotope ratio of primary producers at the base of the food chain, effectively providing the isotopic baseline needed to calculate the trophic position. In contrast, glutamic acid is increasingly enriched with ^{15}N as it undergoes nitrogen fractionation up the food chain (Ohkouchi et al., 2017).

Here, we investigate geographical differences in mercury concentration in feathers of adults and chicks of Bulwer's petrels from two Atlantic and two Pacific colonies, which should reflect contamination levels in the mesopelagic domain. Furthermore, we quantified CSIA-derived trophic position in chicks to determine whether any differences in mercury exposure may be a result of trophic position. Feathers are the major sink for mercury excretion in birds, where mercury is deposited during feather growth, reflecting accumulation through diet over this period (Monteiro and Furness, 2001). As a result, quantifying mercury in feathers provides temporal and spatial contexts (Hobson, 1999; Monteiro and Furness, 1995). We tested whether (a) mercury exposure in adults and chicks was significantly influenced by geographical area, and (b) whether trophic position differed significantly among chicks from different colonies and oceans.

During the breeding season, seabirds act as central place foragers, restricting provisioning trips to waters within the colony surroundings to be able to regularly feed the chick (Chaurand

and Weimerskirch, 1994; Granadeiro et al, 1998; Shoji et al., 2015; Wischnewski et al., 2019). GPS tracking data from Raso, Cape Verde, place the mean maximum displacement from the colony of chick-rearing Bulwer's petrels at 335 ± 159 km (V. H. Paiva, unpublished data). Assuming similar foraging strategies across the four colonies, chick trophic position and mercury concentration therefore reflect the scenario in the broad neighbourhood of the colony location. The mercury values obtained from adult feathers, on the other hand, are expected to largely reflect mercury exposure during the non-breeding period, when most body feathers are moulted (Furness et al., 1986; Monteiro et al., 1996; Monteiro and Furness, 2001; own unpublished data). Bulwer's petrels are mid- to long-distance migrants, moving into tropical deep, open oceanic areas in the non-breeding season in the Atlantic (Dias et al., 2015; Ramos et al., 2015), and probably migrating from Pacific breeding colonies to central and eastern tropical Pacific waters and to the Indian Ocean west of the Maldives (Brooke, 2004; Harrison, 1990). So, while in the breeding season, Bulwer's petrels can be seen as biological samplers of a defined area (of low hundreds of thousands km^2) around the breeding colonies. Outside the breeding season, the wide-ranging and scarcely overlapping movements of different individuals (Dias et al., 2015) mean that they are sampling at an ocean-basin scale (millions of km^2). Given that Bulwer's petrels are known to be specialist predators of small mesopelagic fishes and squids (Spear et al., 2007; Waap et al., 2017), we could expect trophic position not to differ between colonies. If this proves to be the case, variability in chick and mercury concentrations would reflect geographical variation in mercury pollution at medium to large geographical scales.

METHODS

STUDY SITE AND SAMPLING PROCEDURE

Bulwer's petrels are small (ca. 100g) highly pelagic seabirds which nest in cavities in oceanic islands. We collected Bulwer's petrels feather samples during the 2018 breeding season from two colonies in the Atlantic Ocean – Deserta Grande (32°30′N 16°30′W) of the Madeiran archipelago, Portugal, and Raso Islet (16°37′N 24°35′W) in Cape Verde and from two colonies in the Pacific Ocean – Nihoa Island (23°03′N; 161°55′W) in Hawaii, USA, and Minami-jima Island (27°02′N; 142°10′E) in Japan (Fig. 1). Eight to ten body feathers were collected from incubating adult Bulwer's petrels (n = 71 in total, 15 – 20 individuals per colony) and from chicks towards the end of the chick-rearing period (n = 75 in total, 15 – 20 individuals per colony) (Table 1) and stored in polyethylene bags.

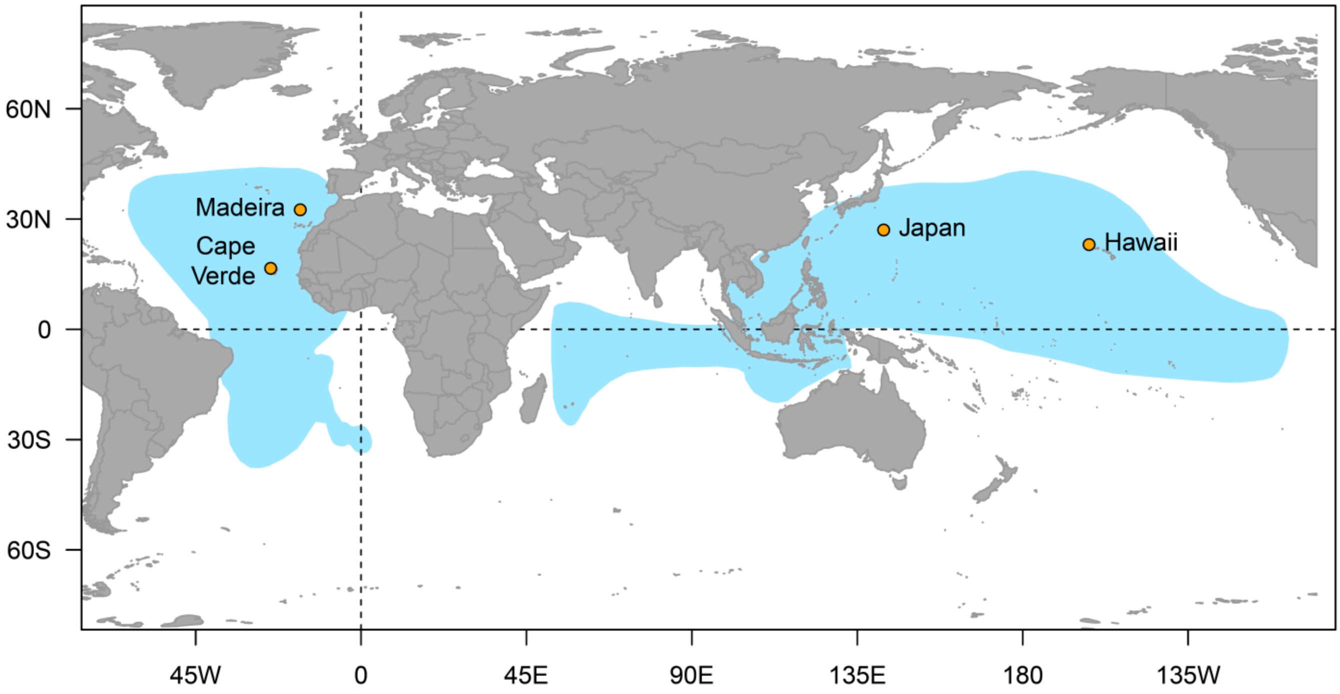

Fig. 1 Location of the four breeding colonies where Bulwer's petrels were sampled, and the species distribution during non-breeding season (blue), adapted from Brooke (2014), Dias et al. (2015) and Ramos et al. (2015).

MERCURY ANALYSES

The feathers were cut into fine pieces to produce a homogeneous sample. We used thermal decomposition atomic absorption spectrometry with gold amalgamation in LECO AMA-254 equipment, to determine the total concentration of mercury in the body feathers. The mass of samples varied between 0.26 mg and 1.02 mg (0.51 ± 0.14 mg). Accuracy and precision were assured by the daily analysis of a certified reference material (CRM) of similar matrix to the samples (TORT-3). The results for the TORT-3 were always within the certified value (0.292 ± 0.022 mg kg^{-1}) that gave recovery efficiencies of 82.72 ± 3.38 %, n = 17. We set a maximum coefficient of variation of 10% in a minimum of three mercury readings.

COMPOUND-SPECIFIC ISOTOPE ANALYSIS OF AMINO ACIDS

Collected feather samples were homogenized and sent to the Stable Isotope Facility at the University of California, Davis, for CSIA-AA of ^{15}N following calibration techniques detailed in Walsh et al. (2014) and Yarnes and Herszage (2017). Amino acids first underwent acid hydrolysis (6M HCl, 70 min, 150 ºC under a N_2 headspace) before derivatization as *N*-acetyl methyl esters. These derivatives were injected at 260 ºC (splitless, 1 min) and separated on a polar gas chromatography column (Agilent DB-35) and combusted at a constant flow rate of 2 mL/min under the following temperature program: 70 °C (hold 2 min); 140 °C (15 °C min^{-1}, hold 4 min); 240 °C (12 °C min^{-1}, hold 5 min); and 255 °C (8 °C min^{-1}, hold 35 min). GC-C-IRMS was performed on a Thermo Trace GC 1310 gas chromatograph linked to a Thermo Scientific Delta V Advantage isotope-ratio mass spectrometer via a GC IsoLink II combustion interface. The combustion reactor is a NiO tube containing CuO and NiO wires maintained at 1000 °C. Water is subsequently removed through a Nafion dryer before the analyte gases are transferred to the IRMS. During ^{15}N analysis, CO_2 is removed from the post-combustion carrier stream through the use of a liquid nitrogen trap to prevent isobaric interferences within the ion source. Samples were analysed in duplicate, and triplicate measurements were recorded when average standard deviation exceeded ± 1‰. Final quality assessment was based on the accuracy and precision of unbiased quality control materials, which included a calibrate amino acid mixture, UCD AA3, and multiple natural materials.

CALCULATING TROPHIC POSITIONS

Trophic position of chicks (Table 2) was calculated from δ^{15}N values of glutamic acid (Glx) and phenylalanine (Phe). Including multiple trophic discrimination factors (TDF) in the estimation of trophic position to integrate the span of the trophic chain produces more robust results, calculated as follows:

$$TP = 2 + \frac{Glx - Phe - 3.5‰ - 3.4‰}{6.2‰} \quad (a)$$

where 6.2‰ is the TDF for trophic position at the base of the aquatic food chain ($\Delta_{\text{herbivore}}$) (McMahon and McCarthy, 2016), 3.5‰ is the trophic discrimination factor for seabird feathers ($\Delta_{\text{carnivore}}$), and 3.4‰ is the difference in δ^{15}N between glutamic acid and phenylalanine in primary producers (β) (McMahon and McCarthy, 2016; Ohkouchi et al., 2017; Quillfeldt and Masello, 2020).

To take into account both analytical and ecological variation, the uncertainty in trophic position was calculated by propagation of errors (Ohkouchi et al., 2017):

$$\begin{aligned}\sigma_{\mathrm{TP}}^{2} = {} & \left(\frac{1}{\Delta_{\mathrm{carnivore}}}\right)^{2}\sigma_{\delta 15\mathrm{N(Glx)}}^{2} + \left(\frac{-1}{\Delta_{\mathrm{carnivore}}}\right)^{2}\sigma_{\delta 15\mathrm{N(Phe)}}^{2} + \left(\frac{1}{\Delta_{\mathrm{carnivore}}}\right)^{2}\sigma_{\beta}^{2} \\ & + \left(\frac{-1}{\Delta_{\mathrm{carnivore}}}\right)^{2}\sigma_{\Delta_{\mathrm{carnivore}}}^{2} \\ & + \left\{\frac{-1}{\Delta_{\mathrm{carnivore}}^{2}}\left(\delta^{15}\mathrm{N}_{\mathrm{Glx}} - \delta^{15}\mathrm{N}_{\mathrm{Phe}} + \beta - \Delta_{\mathrm{herbivore}}\right)\right\}^{2}\sigma_{\mathrm{herbivore}}^{2} \qquad (b)\end{aligned}$$

where $\sigma_{\Delta\mathrm{carnivore}}$ and $\sigma_{\Delta\mathrm{herbivore}}$ are estimated at 0.4‰ (McMahon et al., 2015) and 1.4‰ (Chikaraishi et al., 2007) respectively, and σ_{β} is 0.9‰ (Chikaraishi et al., 2009).

STATISTICAL ANALYSIS

All statistical analyses were carried out with R statistical software (R Core Team, 2020). The means of mercury concentration are presented with standard deviations. To compare the mean feather mercury concentrations among adults and geographical areas, we used ANOVA, followed by Tukey post hoc tests and another one for chicks. We used analysis of variance (one-way ANOVA) to compare the trophic position of chicks in the different breeding areas, after checking for data normality. We also use Welch's t-tests for simple comparisons where appropriate.

RESULTS

We analysed body feather mercury concentrations for 146 Bulwer’s petrels from four colonies over two oceans, comprising 71 adults and 75 chicks (Table 1).

We found significant differences in mercury levels between breeding colonies of Bulwer’s petrel for adults (ANOVA: $F_{3,67} = 29.22$, $p < 0.001$) and chicks (ANOVA: $F_{3,71} = 20.79$, $p < 0.001$). Post-hoc Tukey tests indicated that Bulwer’s petrels from the Atlantic Ocean had higher feather mercury levels than those from the Pacific Ocean (adults – Atlantic Ocean: 16.07 ± 1.88 mg kg^{-1} and Pacific Ocean: 10.56 ± 3.08 mg kg^{-1}; chicks – Atlantic Ocean : 4.70 ± 1.73 mg kg^{-1} and Pacific Ocean: 2.52 ± 0.75 mg kg^{-1}). Furthermore, Post-hoc Tukey tests indicated that adults and chicks from different colonies in the same ocean basin had the same mean concentrations of mercury.

Table 1 Mercury concentration in feathers of adults and chicks of Bulwer's petrel (mean ±SD and range, mg kg^{-1} dry wt).

	[Hg] mg kg^{-1}		Number of samples
	Mean ± SD	Range	
Chicks			
Cape Verde	5.11 ± 1.76	2.70 – 9.96	15
Madeira	4.38 ± 1.69	2.94 – 8.25	20
Japan	2.13 ± 0.38	1.44 – 3.17	20
Hawaii	2.90 ± 0.84	1.81 – 5.21	20
Adults			
Cape Verde	15.65 ± 2.22	10.61 – 19.09	20
Madeira	16.49 ± 1.39	14.60 – 18.76	20
Japan	10.27 ± 1.11	8.74 – 12.39	15
Hawaii	10.83 ± 4.21	3.80 – 23.61	16

There were no significant differences in the trophic position of chicks among colonies (Table 2) (ANOVA: $F_{3,20} = 0.81$, $p = 0.50$). Propagated errors associated with trophic position, determined by equation (b), were all < 0.45 (mean = 0.32), indicating the precision of this method.

The last study on mercury in feathers of adult Bulwer's Petrels from the Madeiran archipelago, with values from 1992-1994, reports a concentration of 21.6 ± 0.7 mg kg^{-1} (range 12.20 – 33.80 mg kg^{-1}, n = 55) (Monteiro and Furness, 1997). These are significantly higher than the values obtained in this study for the colony of Deserta Grande, Madeira (Welch's t-test: t = 15.74, df = 22.60, $p < 0.0001$) (see Table 1).

Table 2 CSIA-AA-derived trophic position analysed from body feathers of chicks of Bulwer's petrel.

Ocean	Colony	Trophic Position		Number of samples
		Mean ± propagated error	Range	
Atlantic Ocean	Cape Verde	3.37 ± 0.33	3.24 – 3.58	6
	Madeira	3.35 ± 0.33	3.19 – 3.47	6
Pacific Ocean	Japan	3.36 ± 0.33	3.22 – 3.60	6
	Hawaii	3.45 ± 0.30	3.32 – 3.56	6

DISCUSSION

We evaluated the concentration of mercury in Bulwer's petrels from four colonies across the Atlantic and Pacific Oceans. To our knowledge, this is the first single-species study to assess oceanic mercury in biota of the mesopelagic domain at the tropics and sub-tropics on a global scale.

From diet analysis carried out in the Atlantic and throughout the Pacific, Bulwer's petrels are known to be specialists, foraging almost exclusively on mesopelagic fish (mainly Myctophidae, Gonostomatidae, Phosichthyidae, Sternoptychidae, Centriscidae, Melamphaidae, Macrouridae and Melanonidae) and squid (mainly Ommastrephidae, Histioteuthidae, Mastigoteuthidae, Chiroteuthidae and Cranchiidae) (Harrison et al., 1983; Spear et al., 2007; Waap et al., 2017). The similarity in trophic position of chicks from different colonies across two oceans reported here strongly suggests that the trophic niche and foraging strategies in Bulwer's petrel are highly conserved across geographies. Moreover, the similarity in mercury concentrations in adult Bulwer's petrels from the same ocean continues to reinforce the idea that Bulwer's petrels are specialist predators year-round, with similar diet and habitat in the non-breeding season irrespective of colony. Together, these results identify the Bulwer's petrel as an ideal monitor of mercury bioavailability in different geographic areas in the oceans (Gatt et al., 2020; Walsh, 1990). Similarities in mercury exposure in adults from the Japanese and Hawaiian colonies could also suggest that they spend the non-breeding period broadly overlapping in oceanic areas, as do Bulwer's petrels from different breeding ranges in the Atlantic (Ramos et al., 2015).

Both adult and chick feathers of Bulwer's petrels from Atlantic colonies had significantly higher (ca. 52 % in adults and ca. 86% in chicks) mercury concentrations than those from the Pacific Ocean. Given that trophic differences are not expected, the best explanation for this is higher mercury levels in mesopelagic fish and squid in the Atlantic compared to the Pacific, resulting from different bioavailability of methylmercury (e.g. Becker et al., 2016; Carravieri et al., 2014). Such disparate results may arise from a complex interplay of factors, which include amongst others, variation in atmospheric deposition, variation in productivity and microbial activity and differences in plankton communities, as different types of phytoplankton display highly distinct bioaccumulation rates (Zhang et al., 2020). Our finding is in accordance with previous investigations finding higher mercury concentrations in the mesopelagic domain in the Central South Atlantic than in the central and eastern Pacific (Gill and Fitzgerald, 1988, Bowman et al., 2020).

Mercury concentrations in the North Atlantic waters appear to have decreased during the last several decades, likely due to reduced atmospheric deposition (Bowman et al., 2015; Cossa et al., 2020; Obris et al., 2018), reflected in temporal comparisons of mercury contamination in Bluefish (*Pomatomus saltatrix*; Cross et al., 2015*)* and Atlantic bluefin tuna (*Thunnus thynnus*; Lee et al., 2016) in the northwest Atlantic, and in Striped dolphins (*Stenella coeruleoalba*) in the Mediterranean (Borrell et al., 2014). Our results are in accordance, suggesting a significant decrease in mercury concentration from 1992-94 to 2018 (Monteiro and Furness, 1997, this study). Data from the Pacific show an increase in mercury bioavailability (Drevnick et al., 2015). However, in many cases the temporal trends in marine biota do not faithfully parallel changes in atmospheric inputs (Wang et al., 2019), due the slow transport of mercury into lower ocean levels where it is transformed to methylmercury and assimilated by biota (Driscoll et al., 2013).

Studies on broad spatial and temporal trends in oceanic mercury concentrations often compare data from tuna species (Drevnick et al., 2015; Houssard et al., 2019; Lee et al., 2016; Manhães et al., 2020). However, given that tuna are widely recognized as opportunistic generalist top predators, feeding facultatively on both epipelagic and mesopelagic prey (Duffy et al., 2017; Olafsdotti et al., 2016), their mercury exposure may reflect the confounding effects of environmental contaminant levels, feeding compartment (epi- or mesopelagic) and trophic position (Gatt et al., 2020). Bulwer's petrel feathers could complement the current monitoring in intermediate waters without the difficulties of quantifying and interpreting the influence of trophic position.

Our observation that chicks bore lower mercury concentration than adults is in agreement with similar comparisons in other seabird taxa (Becker et al., 2002; Tavares et al., 2013). This is attributed to the shorter period of time during which mercury is accumulated in the body through the diet in chicks before they are able to excrete it into growing feathers (Bustamante et al., 2016; Furness et al., 1986; Thompson et al., 1998).

Mercury concentrations in body feathers of adult Bulwer's petrels are higher than those in many other seabirds (e.g. Anderson et al., 2009; Carravieri et al., 2014; Furtado et al., 2019; Tavares et al., 2013), comparable to concentrations found in large albatross species (Carravieri et al., 2014). Such high concentrations reflect the Bulwer's petrel's dependency on mesopelagic prey. Mercury concentrations between 5 and 40 mg kg^{-1} in feathers of waterbirds have been reported by some studies to carry negative impacts on reproductive parameters or survival (Scheuhammer et al., 2007; Whitney and Cristol, 2017; Wolfe et al., 1998;). However, most research suggests that seabirds exhibit extraordinary resistance to mercury contamination (Carravieri et al., 2018, 2020; Gilmour et al., 2019; Wolfe et al., 1998), with the highest concentrations recorded at 95 mg kg^{-1} in an adult male Wandering Albatross (*Diomedea exulans*) with no obvious impacts to its fitness (Bustamante et al., 2016).

CONCLUSIONS

Our results suggest that, given its similarity in trophic position across the two oceans, and its wide raging behaviour, the Bulwer's petrel is a reliable indicator of mercury contamination in the tropical and sub-tropical mesopelagic domain at a global scale. Results of mercury concentrations suggest that birds in the Atlantic Ocean are currently exposed to higher mercury concentrations than those in the Pacific Ocean, reflecting higher contamination in fish and squid of the mesopelagic compartment of the former ocean. Results also indicate that mercury levels in the high seas of the tropical/subtropical Atlantic Ocean have been declining over the past 2-3 decades. The use of body feathers provides an accessible and non-invasive method for such monitoring. The high concentration of mercury in Bulwer's petrels warrants further research to determine if it alters behaviour, reproductive success or survival.

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Author contributions

PC and JPG conceptualised the study and acquired funding, RF planned the methodology and drafted the manuscript, RR, KH, VHP, DM collected the samples. RF, JPG and MCG, compiled and analyzed the data. RF, JPG, MCG and PC led the writing of the manuscript. PC, JPG and EP supervised the work. All authors contributed critically to revisions. All authors have read the submitted version of the manuscript and approve its submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

Anderson, O.R.J., Phillips, R.A., McDonald, R.A., Shore, R.F., McGill, R.A.R., Bearhop, S., 2009. Influence of trophic position and foraging range on mercury levels within a seabird community. Mar. Ecol. Prog. Ser. 375, 277–288. https://doi.org/10.3354/meps07784.

Becker, P.H., González-Solís, J., Behrends, B., Croxall, J.P., 2002. Feather mercury levels in seabirds at South Georgia: influences of trophic position, sex and age. Mar. Ecol. Prog. Ser. 243, 261–269. https://doi.org/10.3354/meps243261.

Becker, P.H., Goutner, V., Ryan, P.G., González-Solís, J., 2016. Feather mercury concentrations in Southern Ocean seabirds: Variation by species, site and time. Environ. Pollut. 216, 253–263. https://doi.org/10.1016/j.envpol.2016.05.061.

Borrell, A., Aguilar, A., Tornero, V., Drago, M., 2014. Concentrations of mercury in tissues of striped dolphins suggest decline of pollution in Mediterranean open waters. Chemosphere 107, 319–323. https://doi.org/10.1016/j.chemosphere.2013.12.076.

Bowman, K.L., Hammerschmidt, C.R., Lamborg, C.H., Swarr, G., 2015. Mercury in the North Atlantic Ocean: The U.S. GEOTRACES zonal and meridional sections. Deep. Res. Part II Top. Stud. Oceanogr. 116, 251–261. https://doi.org/10.1016/j.dsr2.2014.07.004.

Bowman, K.L., Lamborg, C.H., Agather, A.M., 2020. A global perspective on mercury cycling in the ocean. Sci. Total Environ. 710, 136166. https://doi.org/10.1016/j.scitotenv.2019.136166.

Brooke, M. Albatrosses and petrels across the world. Oxford: Oxford University Press, 2004.

Bustamante, P., Carravieri, A., Goutte, A., Barbraud, C., Delord, K., Chastel, O., Weimerskirch, H., Cherel, Y., 2016. High feather mercury concentrations in the wandering albatross are related to sex, breeding status and trophic ecology with no demographic consequences. Environ. Res. 144, 1–10. https://doi.org/10.1016/j.envres.2015.10.024.

Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Chastel, O., Cherel, Y., 2020. Trace elements and persistent organic pollutants in chicks of 13 seabird species from

Antarctica to the subtropics. Environ. Int. 134. https://doi.org/10.1016/j.envint.2019.105225.

Carravieri, A., Cherel, Y., Blévin, P., Brault-favrou, M., Chastel, O., Bustamante, P., 2014. Mercury exposure in a large subantarctic avian community. Environ. Pollut. 190, 51–57. https://doi.org/10.1016/j.envpol.2014.03.017.

Carravieri, A., Fort, J., Tarroux, A., Cherel, Y., Love, O.P., Prieur, S., Brault-Favrou, M., Bustamante, P., Descamps, S., 2018. Mercury exposure and short-term consequences on physiology and reproduction in Antarctic petrels. Environ. Pollut. https://doi.org/10.1016/j.envpol.2017.11.004.

Chaurand, T., Weimerskirch, H., 1994. The regular alternation of short and long foraging trips in the blue petrel *Halobaena caerulea*: A previously undescribed strategy of food provisioning in a pelagic seabird. J. Anim. Ecol. 63, 275. https://doi.org/10.2307/5546.

Chikaraishi, Y., Kashiyama, Y., Ogawa, N.O., Kitazato, H., Ohkouchi, N., 2007. Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: implications for aquatic food web studies. Mar. Ecol. Prog. Ser. 342, 85–90. https://doi.org/10.3354/meps342085.

Chikaraishi, Y., Ogawa, N.O., Kashiyama, Y., Takano, Y., Suga, H., Tomitani, A.,Miyashita, H., Kitazato, H., Ohkouchi, N., 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. Limnol. Oceanogr. Methods 7, 740–750. https://doi.org/10.4319/lom.2009.7.740.

Choy, C., Popp, B., Kaneko, J., Drazen, J., 2009. The influence of depth of mercury levels in pelagic fishes and their prey. Proc Natl Acad Sci 106, 13865–13869.

Cossa, D., Knoery, J., Boye, M., Marusczak, N., Thomas, B., Courau, P., Sprovieri, F., 2020. Oceanic mercury concentrations on both sides of the Strait of Gibraltar decreased between 1989 and 2012. Anthropocene 29. https://doi.org/10.1016/j.ancene.2019.100230.

Cross, F.A., Evans, D.W., Barber, R.T., 2015. Decadal declines of mercury in adult bluefish (1972-2011) from the mid-Atlantic coast of the U.S.A. Environ. Sci. Technol. 49, 9064–9072. https://doi.org/10.1021/acs.est.5b01953.

Dias, M.P., Alho, M., Granadeiro, J.P., Catry, P., 2015. Wanderer of the deepest seas: Migratory behaviour and distribution of the highly pelagic Bulwer's petrel. J. Ornithol. 156, 955–962. https://doi.org/10.1007/s10336-015-1210-9.

Drevnick, P.E., Lamborg, C.H., Horgan, M.J., 2015. Increase in mercury in Pacific yellowfin tuna. Environ. Toxicol. Chem. 34, 931–934. https://doi.org/10.1002/etc.2883.

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a global pollutant: Sources, pathways, and effects. Environ. Sci. Technol. 47, 4967–4983. https://doi.org/10.1021/es305071v.

Duffy, L.M., Kuhnert, P.M., Pethybridge, H.R., Young, J.W., Olson, R.J., Logan, J.M., Goñi, N., Romanov, E., Allain, V., Staudinger, M.D., Abecassis, M., Choy, C.A., Hobday, A.J., Simier, M., Galván-Magaña, F., Potier, M., Ménard, F., 2017. Global trophic ecology of yellowfin, bigeye, and albacore tunas: Understanding predation on micronekton communities at ocean-basin scales. Deep. Res. Part II Top. Stud. Oceanogr. 140, 55–73. https://doi.org/10.1016/j.dsr2.2017.03.003.

Esdaile, L.J., Chalker, J.M., 2018. The mercury problem in artisanal and small-scale gold mining. Chem Eur J. 24, 6905–6916. https://doi.org/10.1002/chem.201704840.

Furness, R.W., Muirhead, S.J., Woodburn, M., 1986. Using bird feathers to measure mercury in the environment: relationships between mercury content and moult. Mar. Pollur Bull. 17, 27–30.

Furtado, R., Granadeiro, J.P., Campioni, L., Silva, M., Pereira, E., Catry, P., 2020. Trace elements' reference levels in blood of breeding black-browed albatrosses *Thalassarche melanophris* from the Falkland Islands. Environ. Sci. Pollut. Res. 1–9. https://doi.org/10.1007/s11356-020-09928-1.

Furtado, R., Pereira, M.E., Granadeiro, J.P., Catry, P., 2019. Body feather mercury and arsenic concentrations in five species of seabirds from the Falkland Islands. Mar. Pollut. Bull. 149. https://doi.org/10.1016/j.marpolbul.2019.110574.

Gagne, T.O., David Hyrenbach, K., Hagemann, M.E., Van Houtan, K.S., 2018. Trophic signatures of seabirds suggest shifts in oceanic ecosystems. Sci. Adv. 4, eaao3946. https://doi.org/10.1126/sciadv.aao3946.

Gatt, M.C., Reis, B., Granadeiro, J.P., Pereira, E., Catry, P., 2020. Generalist seabirds as biomonitors of ocean mercury: The importance of accurate trophic position assignment. Sci. Total Environ. 740, 140159. https://doi.org/10.1016/j.scitotenv.2020.140159.

Gill, G.A., Fitzgerald, W.F., 1988. Vertical mercury distributions in the oceans. Geochim. Cosmochim. Acta 52, 1719–1728. https://doi.org/10.1016/0016-7037(88)90240-2.

Gilmour, M.E., Lavers, J.L., Lamborg, C., Chastel, O., Kania, S.A., Shaffer, S.A., 2019. Mercury as an indicator of foraging ecology but not the breeding hormone prolactin in seabirds. Ecol. Indic. 103, 248–259. https://doi.org/10.1016/j.ecolind.2019.04.016.

Graham, B.S., Koch, P.L., Newsome, S.D., McMahon, K.W., Aurioles, D. Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In: West, J., Bowen, G.J., Dawson, T.E., Tu, K.P. (Eds.), Isoscapes: Understanding movement, pattern and process on earth through isotope mapping. Springer, New York, 2009, 299–318 pp.

Granadeiro, J.P., Nunes, M., Silva, M.C., Furness, R.W., 1998. Flexible foraging strategy of Cory’s shearwater, *Calonectris diomedea*, during the chick-rearing period. Anim. Behav. 56, 1169–1176. https://doi.org/10.1006/anbe.1998.0827.

Gworek, B., Bemowska-Kałabun, O., Kijeńska, M., Wrzosek-Jakubowska, J., 2016. Mercury in Marine and Oceanic Waters – a Review. Water. Air. Soil Pollut. 227. https://doi.org/10.1007/s11270-016-3060-3.

Harrison, C.S. Seabirds of Hawaii: Natural history and conservation. Ithaca, Cornell University Press, 1990.

Harrison, C.S., Hida, T.S., Seki, M.P., 1983. Hawaiian seabird feeding ecology. Wildl. Monogr. 47, 3–71.

Hobson KA., 1999. Tracing origins and migration of wildlife using stable isotopes: A review. Oecologia. 120, 314-326.

Houssard, P., Point, D., Tremblay-Boyer, L., Allain, V., Pethybridge, H., Masbou, J., Ferriss, B.E., Baya, P.A., Lagane, C., Menkes, C.E., Letourneur, Y., Lorrain, A., 2019. A model of mercury distribution in tuna from the western and central Pacific Ocean: Influence of physiology, ecology and environmental factors. Environ. Sci. Technol. 53, 1422–1431. https://doi.org/10.1021/acs.est.8b06058.

Kelly, T.B., Davison, P.C., Goericke, R., Landry, M.R., Ohman, M.D., Stukel, M.R., 2019. The importance of mesozooplankton diel vertical migration for sustaining a mesopelagic food web. Front. Mar. Sci. 6, 1–18. https://doi.org/10.3389/fmars.2019.00508.

Kim, E.Y., Murakami, T., Saeki, K., Tatsukawa, R., 1996. Mercury levels and its chemical form in tissues and organs of seabirds. Arch. Environ. Contam. Toxicol. 30, 259–266. https://doi.org/10.1007/s002449900035.

Lamborg, C.H., Hammerschmidt, C.R., Bowman, K.L., Swarr, G.J., Munson, K.M., Ohnemus, D.C., Lam, P.J., Heimbürger, L.E., Rijkenberg, M.J.A., Saito, M.A., 2014. A global ocean inventory of anthropogenic mercury based on water column measurements. Nature 512, 65–68. https://doi.org/10.1038/nature13563.

Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. Environ. Sci. Technol. 47, 13385–13394. https://doi.org/10.1021/es403103t.

Lee, C.S., Lutcavage, M.E., Chandler, E., Madigan, D.J., Cerrato, R.M., Fisher, N.S., 2016. Declining mercury concentrations in bluefin tuna reflect reduced emissions to the North Atlantic Ocean. Environ. Sci. Technol. 50, 12825–12830. https://doi.org/10.1021/acs.est.6b04328.

Madigan, D.J., Li, M., Yin, R., Baumann, H., Snodgrass, O.E., Dewar, H., Krabbenhoft, D.P., Baumann, Z., Fisher, N.S., Balcom, P., Sunderland, E.M., 2018. Mercury stable isotopes reveal influence of foraging depth on mercury concentrations and growth in Pacific bluefin tuna. Environ. Sci. Technol. 52, 6256–6264. https://doi.org/10.1021/acs.est.7b06429.

Manhães, B.M.R., Picaluga, A. de S., Bisi, T.L., Azevedo, A.F., Torres, J.P.M., Malm, O., Lailson-Brito, J., 2020. Tracking mercury in the southwestern Atlantic Ocean: the use of tuna and tuna-like species as indicators of bioavailability. Environ. Sci. Pollut. Res. 27, 6813–6823. https://doi.org/10.1007/s11356-019-07275-4.

Mason, R.P., Fitzgerald, W.F., 1991. Mercury speciation in open Ocean waters. Water, Air, Soil Pollut. 56 56, 779–789.

McMahon, K.W., McCarthy, M.D., 2016. Embracing variability in amino acid δ^{15}N fractionation: Mechanisms, implications, and applications for trophic ecology. Ecosphere 7, 1–26. https://doi.org/10.1002/ecs2.1511.

McMahon, K.W., Polito, M.J., Abel, S., Mccarthy, M.D., Thorrold, S.R., 2015. Carbon and nitrogen isotope fractionation of amino acids in an avian marine predator, the gentoo penguin (*Pygoscelis papua*). Ecol. Evol. 5, 1278–1290. https://doi.org/10.1002/ece3.1437.

Monteiro, L.R., Furness, R.W., 2001. Kinetics, dose-response, and excretion of methylmercury in free-living adult Cory’s shearwaters. Environ. Sci. Technol. 35, 739–746. https://doi.org/10.1021/es000114a.

Monteiro, L.R., Furness, R.W., 1997. Accelerated increase in mercury contamination in North Atlantic mesopelagic food chains as indicated by time series of seabird feathers. Environ. Toxicol. Chem. 16, 2489–2493. https://doi.org/10.1002/etc.5620161208.

Monteiro, L.R., Furness, R.W., 1995. Seabirds as monitors of mercury in the marine environment. Water. Air. Soil Pollut. 80, 851–870.

Monteiro, L.R., Granadeiro, J.P., Furness, R.W., 1998. Relationship between mercury levels and diet in Azores seabirds. Mar. Ecol. Prog. Ser. 166, 259–265. https://doi.org/10.3354/meps166259.

Monteiro, L.R., Ramos, J.A., Furness, R.W., Del Nevo, A.J., 1996. Movements, morphology, breeding, molt, diet and feeding of seabirds in the Azores. Waterbirds 19, 82–97. https://doi.org/10.2307/1521810.

Motta, L.C., Blum, J.D., Johnson, M.W., Umhau, B.P., Popp, B.N., Washburn, S.J., Drazen, J.C., Benitez-Nelson, C.R., Hannides, C.C.S., Close, H.G., Lamborg, C.H., 2019. Mercury cycling in the North Pacific Subtropical Gyre as revealed by mercury stable isotope ratios. Global Biogeochem. Cycles 33, 777–794. https://doi.org/10.1029/2018GB006057.

Obrist, D., Kirk, J.L., Zhang, L., Sunderland, E.M., Jiskra, M., Selin, N.E., 2018. A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. Ambio 47, 116–140. https://doi.org/10.1007/s13280-017-1004-9.

Ohkouchi, N., Chikaraishi, Y., Close, H.G., Fry, B., Larsen, T., Madigan, D.J., McCarthy, M.D., McMahon, K.W., Nagata, T., Naito, Y.I., Ogawa, N.O., Popp, B.N., Steffan, S., Takano, Y., Tayasu, I., Wyatt, A.S.J., Yamaguchi, Y.T., Yokoyama, Y., 2017. Advances in the application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies. Org. Geochem. 113, 150–174. https://doi.org/10.1016/j.orggeochem.2017.07.009.

Olafsdottir, D., MacKenzie, B.R., Chosson-P, V., Ingimundardottir, T., 2016. Dietary evidence of mesopelagic and pelagic foraging by atlantic bluefin tuna (*Thunnus thynnus* L.) during autumn migrations to the Iceland Basin. Front. Mar. Sci. 3. https://doi.org/10.3389/fmars.2016.00108.

Quillfeldt, P., Masello, J.F., 2020. Compound-specific stable isotope analyses in Falkland Islands seabirds reveal seasonal changes in trophic positions. BMC Ecol. 20:21, 1–12. https://doi.org/10.1186/s12898-020-00288-5.

Quillfeldt, P., Thorn, S., Richter, B., Nabte, M., Coria, N., Masello, J.F., Massaro, M., Neves, V.C., Libertelli, M., 2017. Testing the usefulness of hydrogen and compound-specific stable isotope analyses in seabird feathers: a case study in two sympatric Antarctic storm-petrels. Mar. Biol. 164, 1–7. https://doi.org/10.1007/s00227-017-3224-8.

R Core Team., 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Ramos, R., Sanz, V., Militão, T., Bried, J., Neves, V.C., Biscoito, M., Phillips, R.A., Zino, F., González-Solís, J., 2015. Leapfrog migration and habitat preferences of a small oceanic seabird, Bulwer's petrel (*Bulweria bulwerii*). J. Biogeogr. 42, 1651–1664. https://doi.org/10.1111/jbi.12541.

Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B., Murray, M.W., 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36, 12–18. https://doi.org/10.1579/0044-7447(2007)36[12:EOEMOT]2.0.CO;2.

Shoji, A., Aris-Brosou, S., Fayet, A., Padget, O., Perrins, C., Guilford, T., 2015. Dual foraging and pair coordination during chick provisioning by Manx shearwaters: Empirical evidence supported by a simple model. J. Exp. Biol. 218, 2116–2123. https://doi.org/10.1242/jeb.120626.

Spear, L.B., Ainley, D.G., Walker, W.A., 2007. Foraging dynamics of seabirds in the eastern tropical Pacific Ocean. Stud. Avian Biol. 1–99.

Sunderland, E.M., Krabbenhoft, D.P., Moreau, J.W., Strode, S.A., Landing, W.M., 2009. Mercury sources, distribution, and bioavailability in the North Pacific Ocean: Insights from data and models. Global Biogeochem. Cycles 23, 1–14. https://doi.org/10.1029/2008GB003425.

Tavares, S., Xavier, J.C., Phillips, R.A., Pereira, M.E., Pardal, M.A., 2013. Influence of age, sex and breeding status on mercury accumulation patterns in the wandering albatross *Diomedea exulans*. Environ. Pollut. 181, 315–320. https://doi.org/10.1016/j.envpol.2013.06.032.

Thompson, D., Furness, R., Monteiro, L., 1998. Seabirds as biomonitors of mercury inputs to epipelagic and mesopelagic marine food chains. Sci Total Env. 213, 299–305.

Waap, S., Symondson, W.O.C., Granadeiro, J.P., Alonso, H., Serra-Goncalves, C., DIas, M.P., Catry, P., 2017. The diet of a nocturnal pelagic predator, the Bulwer's petrel, across the lunar cycle. Sci. Rep. 7, 1–10. https://doi.org/10.1038/s41598-017-01312-3.

Walsh, R.G., He, S., Yarnes, C.T., 2014. Compound-specific δ^{13}C and δ^{15}N analysis of amino acids: A rapid, chloroformate-based method for ecological studies. Rapid Commun. Mass Spectrom. 28, 96–108. https://doi.org/10.1002/rcm.6761.

Walsh, P. M. The use of seabirds as monitors of heavy metals in the marine environment. In: Furness RW, Rainbow PS (eds) Heavy metals in the marine environment. CRC Press, New. CRC Press, New York, 1990, 183-204 pp.

Wang, F., Outridge, P., Xinbin, F., Bo, M., Heimbürger, L.-E., Mason, R., 2019. How closely do mercury trends in fish and other aquatic wildlife track those in the atmosphere? – Implications for evaluating the effectiveness of the Minamata Convention. Sci. Total Environ. 674, 58–70.

Whitney, M.C., Cristol, D.A., 2017. Impacts of sublethal mercury exposure on birds: A detailed review. Rev. Environ. Contam. Toxicol. 244, 113–163. https://doi.org/10.1007/398.

Wischnewski, S., Arneill, G.E., Bennison, A.W., Dillane, E., Poupart, T.A., Hinde, C.A., Jessopp, M.J., Quinn, J.L., 2019. Variation in foraging strategies over a large spatial scale reduces parent–offspring conflict in Manx shearwaters. Anim. Behav. 151, 165–176. https://doi.org/10.1016/j.anbehav.2019.03.014.

Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A., 1998. Effects of mercury on wildlife: A comprehensive review. Environ. Toxicol. Chem. 17, 146–160.

Yarnes, C.T., Herszage, J., 2017. The relative influence of derivatization and normalization procedures on the compound-specific stable isotope analysis of nitrogen in amino acids. Rapid Commun. Mass Spectrom. 31, 693–704. https://doi.org/10.1002/rcm.7832.

Zhang, Y., Soerensen, A.L., Schartup, A.T., Sunderland, E.M., 2020. A global model for methylmercury formation and uptake at the base of marine food webs. Global Biogeochem. Cycles 34, 0–3. https://doi.org/10.1029/2019GB006348