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HEAVY METALS IN ATLANTIC SEABIRDS: CURRENT LEVELS AND POSSIBLE IMPACTS IN FITNESS AND BEHAVIOUR OF SELECTED SPECIES.

Ricardo Miranda Furtado Graça

This thesis is submitted in partial fulfilment for the degree of PhD in
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“Produzem-se anualmente centenas de milhões de toneladas de resíduos, muitos deles não biodegradáveis: resíduos domésticos e comerciais, detritos de demolições, resíduos clínicos, eletrónicos e industriais, resíduos altamente tóxicos e radioativos.”

“Os oceanos contêm não só a maior parte da água do planeta, mas também a maior parte da vasta variedade dos seres vivos, muitos deles ainda desconhecidos para nós e ameaçados por diversas causas (…)”

“O problema crescente dos resíduos marinhos e da proteção das áreas marinhas para além das fronteiras nacionais continua a representar um desafio especial.”

“Em definitivo, precisamos de um acordo sobre os regimes de governação para toda a gama dos chamados bens comuns globais.”

Carta Encíclica “*Laudato Si*, sobre o Cuidado da Casa Comum”, do Santo Padre Francisco, Roma, 24 de Maio de 2015.

DEDICATÓRIA

Crescer a amar uma pessoa é o mesmo
que passar uma *vida*
inteira a admirá-la.

Dedico este trabalho
aos meus avós maternos
e avô paterno, “In Memorian”

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Por fim, ao sol, ao mar à vida.

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Comportamento alimentar; Comportamento no ninho; mercúrio; oligoelementos; aves marinhas; oceano; biomonitorização; nível trófico; aptidão física

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Foraging behaviour; behaviour in the nest; mercury; trace elements; seabirds; ocean; biomonitoring; trophic level; fitness

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RESUMO

As aves marinhas são omnipresentes, predadores de topo no oceano, e estão sujeitas a concentrações elevadas de metilmercúrio, mas as relações entre o mercúrio e o comportamento continuam a ser mal compreendidas. O foco central desta tese é determinar as concentrações de mercúrio e outros metais pesados em penas e sangue de aves marinhas e avaliar os efeitos da presença de mercúrio sobre o comportamento e a aptidão de algumas espécies de aves marinhas.

1. Os efeitos do mercúrio no comportamento das aves foram revistos no Capítulo 2. Os relatos de efeitos detectáveis do mercúrio no comportamento das aves são, de facto, poucos. Os efeitos detectáveis sobre o comportamento das aves são, na sua maioria, descritos em indivíduos expostos a concentrações subletais em laboratórios. Os efeitos, quando observados na natureza, são muitas vezes difíceis de descrever e quantificar. O efeito do mercúrio é mais amplamente estudado em aves adultas do que em aves jovens. Tanto quanto podemos verificar, o efeito do mercúrio no comportamento dos pintos das aves marinhas nunca foi estudado.

2. No Capítulo 3 foi feita uma análise dos efeitos do mercúrio na sobrevivência das aves marinhas. Apesar das provas de níveis elevados de mercúrio em aves marinhas, há muito poucos estudos robustos que reportem uma relação deletéria entre as concentrações de mercúrio em indivíduos e a sua aptidão. A sobrevivência da prole não parece ser afectada no ninho, embora alguns estudos prevejam taxas reduzidas de pulgas. A exposição ao mercúrio não parece causar alterações significativas na longevidade dos adultos, embora os estudos a longo prazo que incorporam dados sobre o mercúrio sejam limitados. É possível que as aves marinhas estejam adaptadas às concentrações relativamente elevadas de mercúrio presentes no ecossistema marinho.

3. A concentração de mercúrio e arsénio nas penas do corpo de cinco espécies de aves marinhas das Ilhas Falkland foi estudada no Capítulo 4. Neste estudo foram amostradas diferentes espécies e localizações nas Malvinas, para avaliar a variabilidade inter-espécies e espacial dentro desta região. Os pinguins roqueiros da Ilha Beauchene, tinham concentrações mais elevadas de mercúrio e arsénico do que os de outras colónias. A concentração de mercúrio em plumas de albatroz-de-sobrancelha-negra *Thalassarche melanophris* aumentou desde 1986. Os resultados sugerem que o arsénico não se biomagnifica da mesma forma que o mercúrio ao longo das teias alimentares.

4. Também estudámos (Capítulo 5) a concentração de elementos vestigiais no sangue de albatrozes de raça negra das Ilhas Falklands, que aqui mostramos, através do rastreio com geolocadores, forragens sobre a maior parte da Plataforma Patagónica. As concentrações de elementos vestigiais no sangue não foram significativamente diferentes entre ilhas, o que é consistente com as observações de comportamentos de forragens revelando que as aves de ambas as ilhas forragearam em geral as mesmas áreas nos meses que antecederam a amostragem. As concentrações de arsénio e selénio nas fêmeas eram mais elevadas do que nos machos. As diferenças relacionadas com o sexo na concentração destes elementos podem estar relacionadas com ligeiras diferenças desconhecidas na dieta ou com diferenças na assimilação entre os sexos.

5. A alma-negra *Bulweria bulwerii* (predadores aviários altamente especializados de presas mesopelágicas) foram utilizados como biomonitores dos níveis de mercúrio no domínio mesopelágico dos oceanos Pacífico e Atlântico no Capítulo 6. As colónias atlânticas mostraram concentrações mais elevadas de mercúrio do que as do Pacífico. Os níveis tróficos derivados da análise isotópica de compostos específicos para pintos eram semelhantes entre as colónias, sugerindo que as diferenças entre os locais não se deviam a diferenças no nível trófico dessas populações. Os níveis de mercúrio das penas registados foram inferiores aos registados em 1992 para o Atlântico.

6. Os possíveis efeitos adversos do mercúrio em pintos de alma-negra *Bulweria bulwerii* foram avaliados no Capítulo 7. A taxa de crescimento dos pintos foi negativamente correlacionada com a contaminação por mercúrio. Os pintos com coeficientes de taxa de crescimento reduzidos para a massa corporal apresentavam um crescimento retardado do comprimento das asas. Altas concentrações de mercúrio foram associadas ao atraso no crescimento dos pintos de alma-negra *Bulweria bulwerii*. O comportamento dos pintos no ninho não foi influenciado pelos níveis de mercúrio.

7. Os pintos de alma-negra *Bulweria bulwerii* têm algumas das mais elevadas concentrações de mercúrio no sangue relatadas entre as aves marinhas. Medimos o mercúrio no sangue de cada animal adulto de alma-negra *Bulweria bulwerii* e avaliámos o seu desempenho em termos de forragens. Não houve relação entre a contaminação por mercúrio e o desempenho de forragem, medido através da duração da viagem de forragem e do ganho diário de massa durante uma viagem de forragem. A alma-negra *Bulweria bulwerii* parece ser altamente resistente à contaminação por mercúrio.

Os resultados desta tese fornecem uma linha de referência para estudos de bioindicação utilizando penas e sangue de espécies seleccionadas de aves marinhas no Oceano Atlântico e Pacífico. Apesar dos níveis excepcionalmente elevados de mercúrio acumulado pelas aves marinhas, o seu comportamento parece em grande parte não ser afectado pelas concentrações de mercúrio em toda a gama aqui medida.

ABSTRACT

The effect of mercury is more widely studied in adult birds than in young birds. Seabirds are ubiquitous, top predators in ocean, and are subjected to elevated methylmercury concentrations, but relationships between mercury and behaviour remain poorly understood. The central focus of this thesis is to determine the concentrations of mercury and other heavy metals in feathers and blood of seabirds and to evaluate the effects of the presence of mercury on the behaviour and fitness of some seabird species.

1. The effects of mercury in the behaviour of birds was reviewed in Chapter 2. Reports of detectable effects of mercury on avian behaviour are, in fact, few. Detectable effects on avian behaviour are mostly described in individuals exposed to sub-lethal concentrations in laboratories. The effects, when observed in nature, are often difficult to describe and quantify. As far as we can ascertain, the effect of mercury on the behaviour of seabird chicks has never been studied.

2. A review of the effects of mercury on survival of seabirds was undertaken in Chapter 3. Despite evidence of elevated mercury levels in seabirds, there are very few robust studies reporting a deleterious relationship between mercury concentrations in individuals and their fitness. Survival of offspring does not appear to be affected in the nest, although some studies predict reduced fledging rates. Mercury exposure does not appear to cause significant changes in adult longevity, although long-term studies incorporating mercury data are limited. It is possible that seabirds are adapted to the relatively high concentrations of mercury present in the marine ecosystem.

3. The concentration of mercury and arsenic in body feathers of five species of seabirds of Falkland Islands was studied in Chapter 4. In this study we sampled different species and locations in the Falklands, to evaluate the inter-species and spatial variability within this region. Rockhopper penguins from Beauchene Island, had higher concentrations of mercury and arsenic than those from other colonies. The concentration of mercury in feathers of black-browed albatrosses increased since 1986. Results suggest that arsenic does not biomagnify the same way as mercury does along food webs.

4. We also studied (Chapter 5) the concentration of trace elements in blood of black-browed albatrosses *Thalassarche melanophris* from the Falklands Islands, which we here show, by tracking with geolocators, forage over most of the Patagonian Shelf. Blood concentrations of trace elements were not significantly different between islands, which is consistent with observations from foraging behaviour revealing that birds from both

islands foraged in broadly the same areas in the months before sampling. Arsenic and selenium concentrations in females were higher than in males. Sex-related differences in the concentration of these elements may be related to unknown slight differences in diet or to differences in assimilation between sexes.

5. Bulwer's petrels *Bulweria bulwerii* (highly specialised avian predators of mesopelagic prey) were used as biomonitors of mercury levels in the mesopelagic domain of the Pacific and Atlantic oceans in Chapter 6. Atlantic colonies showed higher mercury concentrations than those from the Pacific. CSIA-AA-derived trophic levels for chicks were similar among colonies, suggesting that differences between locations were not due to differences in the trophic level of those populations. Feather mercury levels recorded were lower than those reported in 1992 for the Atlantic.

6. Possible adverse effects of mercury in chicks of Bulwer’s petrels *Bulweria bulwerii* were evaluated in Chapter 7. Chick growth rate was negatively correlated to mercury contamination. Chicks with reduced growth rate coefficients for body mass exhibited retarded growth of wing length. High mercury concentrations were associated with delayed fledging in Bulwer’s petrel *Bulweria bulwerii* chicks. The behaviour of chicks in the nest was not influenced by mercury levels.

7. Bulwer’s petrel have some of the highest reported blood mercury concentrations amongst seabirds. We measured mercury in the blood of individual adult Bulwer’s petrel and assessed their foraging performance. There was no relationship between mercury contamination and foraging performance, as measured through foraging trip duration and daily mass gain during a foraging trip. Bulwer’s petrels *Bulweria bulwerii* seem to be highly resilient to mercury contamination.

The results of this thesis provide a reference line for bioindication studies using feathers and blood from selected seabird species on the Atlantic and Pacific Ocean. Despite the exceptionally high levels of mercury accumulated by seabirds, their behaviour seems largely unaffected by mercury concentrations across the range measured here.

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CHAPTER 1

GENERAL INTRODUCTION

The surface of planet Earth is mostly composed of water, with more than 70 % of it covered by the ocean (Visbeck, 2018). The oceans are the heart of Planet Earth and play a central role in the environment and the future of humanity. In the past it was the ocean that allowed life to exist on Earth, and it is still the ocean that allows the Earth to be habitable. The ocean in very important for life of humans, in the Earth, because their resources, biodiversity and fundamentally, because contributes to the regulation of global processes such as climate.

The oceans support a large biodiversity, which has been progressively affected by anthropic activity (e.g., Wolfe et al., 1998; McCauley et al., 2015). Currently, marine pollution, originating from heavy metals is one of the major environmental problems of our Earth, despite the efforts made to reduce the emission of pollutants. In 1958, when the issue of marine pollution was first addressed at the First Conference on Ocean Laws, few papers had been written on this issue and those that had been written were especially focused on the spillage and release of oil into the sea (Leous & Parry, 2005).

1.1. TRACE ELEMENTS IN MARINE ECOSYSTEMS

Trace elements occur naturally in the geosphere, atmosphere, hydrosphere and biosphere and mainly include metals and metalloids (Senesi et al., 1999), for example mercury, arsenic and selenium. Metals are usually identified as elements that are good conductors of electricity, and generally enter into chemical reactions as cations (Walker et al., 2012). Metalloids have characteristics of both non-metals and metals (Walker et al., 2012), and arsenic is commonly classified as such.

Trace elements are usually present in the environment due to natural sources, but can also be considered contaminants when they come from human activities. Natural sources mainly include soil erosion, volcanic emissions (e.g., Driscoll et al., 2013; Esdaile and Chalker, 2018). On the other hand, various anthropogenic sources (sewage effluents, agricultural runoff, oil extraction, transport and mining) also contribute to the availability of metals and metalloids in the ocean, including water and sediments (e.g., Gaiero et al. 2002).

1.1.1. DISTRIBUTION

The distribution of trace elements in marine waters is complex (Boye et al. 2012). Trace elements are not uniformly distributed in the ocean, and their concentration varies between areas as well as vertically within the water column (Cutter and Cutter 1995; de Villiers 1999; Bown et al. 2011; Cossa et al. 2011; Boye et al. 2012; Heller and Croot 2015; Schlesinger et al. 2017). In the ocean, currents and mixing, including upwelling zones, contribute to the resuspension of sediments and organic particles, resulting in a natural redistribution of trace elements, notably those that behave as nutrients (e.g., copper and selenium) (Orren and Monteiro 1985; Cutter and Cutter 1995; Zhang et al. 2014; Gworek et al. 2016). Elements such as mercury are massively transported through the global atmospheric circulation, and others such as cadmium, cobalt, lead and manganese are distributed through the oceanic circulation.

1.1.2. THE TOXICITY OF THE ELEMENTS

Several trace elements (cobalt, copper, manganese, selenium and vanadium) are essential components of physiological and biochemical processes in organisms (e.g. vertebrates), but beyond certain concentration these elements also become toxic (Abdulla and Chmielnicka 1990; Senesi et al. 1999; Bansal and Asthama 2018). Others, such as arsenic, cadmium, mercury and strontium, are poorly tolerated by organisms and are generally considered as elements not essential for life and are known to be toxic even at low concentration levels (Naidu et al. 1999; Senesi et al. 1999; Bansal and Asthama 2018). Mercury and arsenic are considered as some of the most highly toxic and carcinogenic elements by the US Environmental Protection Agency (EPA) (ATSDR 2022). The toxicity of trace elements depends not only on their concentrations in the body, but also on their chemical forms (Yokel et al. 2006). For example, methylmercury and arsenite behave differently from the elemental forms and are currently considered highly toxic (Singh et al., 2011). Mercury is currently one of the most bioavailable toxic elements in the oceans, and a reason for concerns for the scientific community.

1.2. WHAT DO WE KNOW ABOUT MERCURY?

Mercury exposure may be a potential risk factor not only for parameters of growth, but also for a range of nervous system disorders such as behaviour disorders and poor cognitive outcome. In vertebrates, a number of different behavioural domains can be affected by exposure to mercury. Mercury, once present in the body, can lead to the development of deleterious/negative, and sometimes irreversible, effects that have been noted in human populations (Doi et al., 1984). Among the mercury compounds, the effects of mercury vapor or methylmercury compounds on essential behaviours have been well recognized in both human intoxication cases and other vertebrates (e.g., Evans et al., 1975). Alterations in reflex, motor coordination, learning, and various kinds of behaviours have been recognized as the neurological signs of experimental methylmercury intoxication (Cristol and Evers, 2020; Fimreite and Karstad, 1971; Onishchenko et al., 2007).

1.2.1. DISTRIBUTION IN THE OCEAN

Mercury is widely transported in the atmosphere and is also distributed in marine ecosystems 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) (Obrist et al., 2018). This elemental mercury sinks to deep waters when adsorbed to particles, where it is transformed 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).

The mesopelagic domain is one of the most methylmercury contaminated areas in the world’s oceans (Cossa et al., 2018, 2020; Sunderland et al., 2009). Currently, there is a global ocean inventory of mercury based on water column measurements and water bodies that report the differences across the oceans (Driscoll et al., 2013; Lamborg et al., 2014). In the ocean, mercury occurs in lower concentrations at the surface (< 100 m) than in deeper waters (Cutter and Cutter 1995; Cutter et al., 2001; Cossa et al., 2018, 2020). Lamborg et al (2014) estimated that the ocean contains 1,390 Mmol of dissolved total mercury, with 22 Mmol in the surface ocean (0 –100 m) , 292 Mmol in the intermediate depths (100 – 1000 m) and 1260 Mmol in waters deeper than 1000 m. Mercury concentrations in Northwest Atlantic Ocean are elevated compared to the central North Pacific Ocean at similar depths (c.a. 200 – 2000 m) (Gill and Fitzgerald, 1988). The same occurs in mesopelagic domain of South Atlantic when compared with Central South Pacific (see review 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; Obrist et al., 2018).

Methylmercury is known to biomagnify in marine food web and hence top predators have elevated levels of mercury (Monteiro et al., 1998; Santos et al., 2006; Lavoie et al. 2013). In the ocean, mesopelagic fauna plays an important role in oceans providing a vital trophic link between surface and deep waters during diel vertical migration (Davison et al., 2015; Kelly et al., 2019). Mesopelagic organisms are generally found in deep oceanic layers (> 200 m) and are involved in mercury transport to the surface (Motta et al., 2019). Consequently, they also transport great concentrations of methylmercury into the epipelagic domain (Madigan et al., 2018; Motta et al., 2019; Thompson et al., 1998).

1.3. SEABIRDS AS ENVIRONMENTAL INDICATORS

Seabirds are predators, known to accumulate a variety of trace elements, such as mercury and arsenic, present in the marine environment (Anderson et al., 2009, 2010). In recent years, concern about the long-term effects of environmental contaminants has increased. Hence, the need for monitoring the levels and effects of environmental contaminants has increased markedly in recent decades with the manifestation of serious toxicological phenomena directly associated with anthropogenic emissions of pollutants into the environment.

One way to assess the impacts of mercury pollution in the ocean is the use of seabirds as bioindicator organisms. Currently, most species of seabirds are easy to monitor at relatively low cost, and there is a thorough knowledge of their biology and ecology (Straube, et al., 2010). A bioindicator organism is understood as "which belongs to a particular species or group of species or even biological communities whose presence, abundance and abiotic conditions serve to correlate one or more anthropic or natural factors with potential impact" (Maia et al., 2001; Sydeman, et al., 2006). The responses provided by these organisms allow for quantifications, assessments and predictions to be made (Maia et al., 2001). Seabirds have been widely used as bioindicators of metal contamination, due to their vulnerability, wide distribution worldwide, having a relatively long life cycle, and occupying top positions in the trophic chain (Burger 1993; Kim et al. 1996; Perez-Lopez et al. 2006).

The dynamics of mercury in seabirds can be viewed as a several-factor model involving ingestion from diet, uptake in the intestine, transportation in blood, accumulation in internal organs (e.g., liver, kidney, muscle) with redistribution to the feathers during feather growth, and elimination in eggs and excretions (Monteiro and Furness, 1995). Essentially all (ca. > 82%) mercury found in feathers and egg components of seabirds is methylmercury (Bond and Diamond, 2009).

1.4. LINKING ECOTOXICOLOGY AND BEHAVIOUR

Understanding different foraging patterns is important to understanding the pattern and risk of uptake of contaminants. Like other animals, different species of seabirds have different foraging behaviours and eat different types of preys (e.g., Monteiro and Furness, 1997). A fine understanding of the spatial ecology, foraging locations and trophic ecology are necessary conditions to interpret and understand the concentrations of contaminants measured in the tissues of seabirds.

On the other hand, contaminants such as mercury are likely to affect the behaviour of wild species, with potential impacts on their demography and conservation, but these aspects have been seldom studies with seabirds as models and in the natural environment.

Seabirds, with their visibility and charisma, combined with their accessibility at the nesting colonies, are interesting species that can function as flagship species, facilitating the communication of complex and important scientific ideas and measurements to the general public and decision makers.

1.5. THESIS OVERVIEW

This dissertation has a broad approach. The main objective of my PhD work is to make a general assessment of environmental contamination by mercury and other trace elements in different colonies of various seabird species in the Atlantic and Pacific Oceans, using seabirds as monitors, and to evaluate the consequences of mercury assimilation in seabirds. This dissertation provides further insights into the contamination levels in seabirds and undertakes an initial assessment of the trace elements contamination in some globally important colonies of the world. A large part of this thesis has focussed particularly on mercury, because of its distinctive attributes as a persistent and global toxicant in the ocean that bioaccumulates and biomagnifies in marine food webs.

This thesis includes in the first two chapters the review of the topics investigated and discussed in the following chapters. In Chapters 2-3 existing information and studies on the effects of mercury on bird behaviour in general and seabird survival in particular are summarised, encompassing the results of different research works. In Chapters 4 - 6, selected seabird species were used to portray the spatial and/or temporal trends of environmental contamination in the Atlantic Ocean and the Pacific Ocean. Finally, Chapters 7-8 evaluated the relationships between mercury, growth and behaviour of adult and young seabirds in a seabird species with one of the highest mercury concentrations in the order Procellariformes. In the last chapter I make a general discussion about the findings, where I elaborate the main conclusions of the thesis. Moreover, one further manuscript on topics related to the subject of this thesis which I co-authored during the course of this PhD is enclosed as appendices.

Chapter 2 – A SYSTEMATIC REVIEW OF THE IMPACT OF MERCURY EXPOSURE ON AVIAN BEHAVIOUR

As neurological processes underlie not only simple motor behaviour such as walking and eating, but also all complex cognitive and behavioural acts, it is expected that heavy exposure to mercury in the wild will cause not only motor, but also behavioural and cognitive changes in birds. In this chapter we review the reported effects of mercury on bird behaviour in general.

Chapter 3 – A REVIEW OF THE EFFECT OF MERCURY CONCENTRATION ON THE SURVIVAL OF SEABIRDS

There is little solid evidence to show that current levels of mercury in the ocean have a negative impact on seabird survival. In this chapter we have collated and summarised the existing information. Although studies have reported relatively high concentrations in seabirds, there has been no evidence of a relationship between mercury and survival of individuals.

Chapter 4 – BODY FEATHER MERCURY AND ARSENIC CONCENTRATIONS IN FIVE SPECIES OF SEABIRDS FROM THE FALKLAND ISLANDS

In this chapter we describe for the first time the concentration of mercury and arsenic in feathers of one species of cormorant (Phalacrocoracidae): imperial Shag *Phalacrocorax atriceps*; two species of penguins (Sphenisciformes): gentoo *Pygoscelis papua* and rockhopper penguin *Eudyptes chrysocome*; and two species of petrels (Procellariiformes):

black-browed albatross *Thalassarche melanophris* and thin-billed prion *Pachyptila belcheri*, in various breeding sites of the Falkland Islands. Despite several similar studies performed in other parts of the Southern Ocean there was virtually no information regarding the Falkland Islands and the Southern Patagonian Shelf. This chapter establishes baseline levels for mercury and arsenic in seabirds of the Southern Patagonian shelf that will be useful for future monitoring.

Chapter 5 – TRACE ELEMENTS' REFERENCE LEVELS IN BLOOD OF BREEDING BLACK-BROWED ALBATROSSES *THALASSARCHE MELANOPHRIS* FROM THE FALKLAND ISLANDS

A comparative perspective of trace elements concentrations in blood of black-browed albatross *Thalassarche melanophris* is presented in this chapter. A comprehensive analysis of trace elements between sexes in two different locations throughout the Falkland Islands enables an overall view of the levels presented by black-browed albatross sampled during the breeding season. In this chapter we describe for the first time the levels for nine trace elements in black-browed albatross from the Falkland Islands that will be useful for future monitoring under a scenario of potential rapid changes in the ocean biogeochemistry.

Chapter 6 – MONITORING OF MERCURY IN THE MESOPELAGIC DOMAIN OF THE PACIFIC AND ATLANTIC OCEANS USING BODY FEATHERS OF BULWER'S PETREL AS A BIOINDICATOR

In this chapter we investigate geographical differences in mercury concentration in feathers of adults and chicks of Bulwer's petrels *Bulweria bulwerii* from two Atlantic and two Pacific colonies, which reflect contamination levels in the mesopelagic domain. To our knowledge, this is the first single-species study to assess mercury in birds mostly relying on the mesopelagic domain at the tropics and sub-tropics on a scale of multiple ocean basins.

Chapter 7 – EFFECTS OF MERCURY ON GROWTH, BEHAVIOUR AND FLEDGING AGE OF BULWER'S PETREL CHICKS

In this chapter we explored the relationship between mercury concentration in plumage and growth rate of body mass, growth rate of wing length and age of fledging in Bulwer's petrel *Bulweria bulwerii*. Furthermore, we evaluated effects of mercury accumulation in the behaviour of chicks in the nest.

Chapter 8 – BULWER’S PETRELS ACCUMULATE HIGH MERCURY CONCENTRATIONS WITHOUT NOTICEABLE EFFECTS ON FORAGING BEHAVIOUR.

In this chapter we measured the Bulwer’s petrel *Bulweria bulwerii* trip duration and daily mass gains during foraging and examined their relationship with blood mercury concentration during the incubation period.

Appendix 1: UNTANGLING CAUSES OF VARIATION IN MERCURY CONCENTRATION BETWEEN FLIGHT FEATHERS

CHAPTER 2

A SYSTEMATIC REVIEW OF THE IMPACT OF MERCURY EXPOSURE ON AVIAN BEHAVIOUR

Ricardo Furtado

2.1. ABSTRACT

Planet Earth is under increasing pressure from anthropogenic activities, including significant and rapidly increasing inputs of mercury pollution, although mercury is naturally present in the environment. The conversion of inorganic mercury by anaerobic microorganisms produces methylmercury, which bioaccumulates at successive trophic levels in the food web. In animal systems, methylmercury has been incriminated in a wide spectrum of toxic effects and it is considered one of the persistent ubiquitous heavy metals. Researchers have long suspected that mercury can be a potent toxicant for birds. However, the effects of mercury accumulated in the body of birds are complex and are not yet fully understood, principally on the behaviour. Available data on mercury contamination of birds was synthesized and a literature review was conducted to describe the effects of mercury on bird behaviours. Mercury treatment altered the architecture of brains and changed the behaviour of birds. Some studies with captive birds exposed to mercury have documented ataxia, lethargy, reduced appetite, and aberrant reproductive behaviours. However, there is limited evidence to show that current levels of mercury in wild birds have a negative impact on them. This is particularly true for seabirds. Most of the research done to date has been focused on waterbirds and songbirds, leaving the assessment of mercury's threats to the behaviour of seabirds incomplete. From the present review, it is concluded that reports of detectable effects of mercury on avian behaviour are, in fact, sparse. Detectable effects on avian behaviours are mostly described in individuals exposed to sub-lethal concentrations in laboratory; when observed in nature, they are often difficult to describe; mercury exposure does not appear to cause significant changes in behaviours of birds` chicks and as far as we know, the effect of mercury on the behaviours of seabird chicks has never been studied. We conclude that mercury is a potential toxicant for birds, but more research is needed to understand the doses and exposure times necessary to trigger damage to the behaviour of birds.

2.2. INTRODUCTION

Concern about human and wildlife exposure to mercury has increased in recent decades (Driscoll et al., 2013). Much of this concern is driven by a growing public awareness of the toxic and sometimes irreversible effects of mercury that have been observed in human populations and in laboratory animals (Hunter et al., 1940; Takeuchi et al., 1962).

Since ancient times, metals have been linked to human technological evolution. Early man first used metals to build tools and weapons and as our understanding of metallurgy has advanced, metals have played an essential role in civilisation, allowing the way to today's modern society (Damas et al., 2014). Throughout history, humans have exploited mineral resources, releasing waste into the soil, air and water (Obrist et al., 2018). Although humans are naturally exposed to metals such as mercury through weathering of rocks, soil erosion, volcanic emissions and dissolution of soluble salts in water, the main exposure is promoted through anthropogenic activities (e.g., gold mining) (Driscoll et al., 2013).

In ancient times, humans thought mercury was very special because it was the only metal to be liquid at room temperature. This characteristic fascinated peoples and cultures such as the Egyptians, Romans, Chinese, Hindus, Arabs and Europeans (Damas et al., 2014). Mercury was for centuries considered to promote human health and life (Hirschhorn et al., 2001). For centuries, humans drank mixtures containing mercury intended to cure diseases and extend the length of their lives (Hirschhorn et al., 2001; Damas et al., 2014). These mixtures, once ingested, often caused traumatic bodily experiences, and even death, yet strangely, they continued to be ingested for centuries (Hirschhorn et al., 2001).

The first recorded account of animal experiments on mercury toxicity comes from the 9th century in Persia (Abdel-Halim, 2011). To understand whether mercury was toxic to Humans, Al-Razi (Rhazes) fed monkeys diets containing mercury (Abdel-Halim, 2011). After the experiment, the alchemist recommended only using mercury as an external medicine, which was ignored until the late 19th century (Thomann, 2015).

Concern about the exposure of humans and wildlife to metals, especially mercury began in the late 19th century following the discovery of organic mercury compounds (Barrett, 2010). Early reports of methylmercury toxicity in humans described a distinct set of symptoms, including sensory changes (e.g., vision, hearing), motor changes (e.g., loss of coordination, impaired speech), and death (Hunter et al., 1940).

After the Industrial Revolution, various industries increased the demand for and use of metals, such as mercury sulphide, releasing large concentrations of methylmercury-containing wastes into the environment. The biggest environmental disaster caused by mercury poisoning occurred in the middle of the next century in Japan. The offspring of the inhabitants living off Minamata Bay began to be born with severe disabilities (mental impairments and impaired motor development) due to mercury poisoning (Takeuchi et al., 1962). An estimated 27 tonnes of methylmercury were dumped into Minamata Bay between 1932 and 1968 by the chemical industry (e.g., production of acetylene, acetaldehyde, acetic acid, vinyl chloride and octanol), using the acetaldehyde for the production of plastic material (Mitra, 2019). Takeuchi et al. (1962), found that all the physical, behavioural and cognitive disorders, which characterised Minamata disease, resulted from impairment of brain function caused by the consumption of fish or shellfish with high levels of mercury (about 100 mg/kg). The mercury content in the hair of coastal inhabitants of the Shiranui Sea was detected at levels never before found in humans (max. 705 mg/kg) (Harada, 1995).

Before the onset of symptoms in humans, birds were already showing symptoms of what would come to be called Minamata disease (Baker, 2008). Some seabirds, which some authors describe as gulls of unidentified species that consumed large quantities of fish in this bay, showed changes in their physical condition, such as frequent falls during flight, uncontrolled movements and paralysis (Takeuchi et al., 1957; Harada, 1994). Takeuchi et al. (1957) found neurological changes in these birds, such as degeneration of the spinal cord, changes in the cerebellum and cerebral cortex, characteristic of Minamata disease, but did not measure mercury levels in their tissues. Only a few years later, Doi et al. (1984) reported the mercury content in feathers of slaty-backed gull (*Larus schistisagus*) feeding in the Bay, which did not exceed 5.2 mg/kg in specimens collected in 1969. To our knowledge, this is the first report of the negative effects of mercury ingestion, in birds, in the wild.

Following the Minamata disaster and the discovery in the 1960s of the bioaccumulation and biomagnification of mercury in food chains (Barrett, 2010), researchers began to look more closely at the impact of mercury pollution on vertebrates. Since birds in many aspects of physiology are not very different from humans (Scanes, 2020), they began to be used as laboratory models.

Toxic effects of mercury on the nervous system in birds are revealed by overt neurological signs that are clearly observable both histologically and behaviourally. These effects fall within the range of motor and sensory effects and are typically reflected in operant conditioning phenomena (loss of ability to walk, fly). Captive Great egrets *Ardea alba* (Spalding et al., 2000), Red-tailed hawks *Buteo jamaicensis* (Fimreite and Karstad, 1971), Rock Pigeons *Columbia livia* (Laties and Evans, 1980; Evans et al, 1982), Mallards *Anas platyrhynchos* (Heinz and Locke, 1976), Zebra Finches *Taeniopygia guttata* (Scheuhammer, 1988; Wolf et al., 2017; Scoville et al., 2020), Double-crested cormorant *Phalacrocorax auritus* (Loerzel et al, 1999), American kestrel *Falco sparverius* (Bennett et al., 2009) and Common pheasant *Phasianus colchicus* (Borg et al., 1969) dosed with mercury/methylmercury concentrations between 0.5 and 7.8 ppm via the diet for weeks suffered severe tissue damage to the nervous system, resulting in loss of the ability to fly, walk and stand, even though dosage levels exceeded those typically observed in nature for these species.

Recent studies show that levels of methylmercury present in nature, can affect some neurological functions in birds, such as neurotransmitter function and neural migration, specifically in juvenile saltmarsh sparrow *Ammospiza caudacuta* with concentrations of 3.4 mg/kg in the first primary feather and 2.9 mg/kg mercury in the other flight feathers (Scoville and Lane, 2013). Scheuhammer et al. (2008) also report significant relationships between mercury and neurotransmitter function in Bald eagles *Haliaeetus leucocephalus* with liver mercury concentrations between 0.5 and 670 mg/kg wet wt., and in Common loon *Gavia immer* with liver mercury concentrations between 0.5 and 104 mg/kg wet wt. In another sample of Common loon *Gavia immer*, Hamilton et al. (2011) observed no correlation between the birds' liver mercury levels and neurotransmitter function, however these individuals had much lower liver mercury values (around 22.8 mg/kg wet wt.). Despite the differences in methodology used in each of these studies, these results imply that mercury present in nature may indeed adversely affect the neurological system of birds.

As neurological processes underlie not only simple motor behaviour such as walking and eating, but also all complex cognitive and behavioural acts, it is expected that heavy exposure to mercury in the wild will cause not only motor, but also behavioural and cognitive changes in birds, which may threaten their survival. Furthermore, given the increase in mercury pollution in some areas of the Earth, and the observation that mercury levels in nature cause neurological changes (e.g., Takeuchi et al., 1957; Scoville and Lane, 2013), it is necessary to understand whether mercury is also affecting bird behaviour.

Bringing together all the existing work to date is essential to understand what is known about the effects of mercury on the behaviour of birds. Here, we summarise the reported effects of mercury on bird behaviour in general.

2.3. METHODS

To identify relevant literature, the search terms "behaviour" and "mercury" were combined with "birds" or "seabirds" and with "effects" in Google Scholar, and then used the "snowball" approach, exhaustively searching both the literature cited in these articles and the articles in which they were cited.

We then examined the abstracts of each article and rejected articles that were not related to the effects of mercury on bird behaviour. We reviewed a wide range of scientific publications, published in international scientific journals. Few studies (n = 38) focused exclusively on the effects of mercury on behaviour, with most of the studies reviewed having another topic as the central theme of the study. The literature search was conducted until March 2022. The date range of the articles consulted was between 1969 and 2022.

For each study, the following information was extracted: age, behaviours studied, bird species, tissue type, mean and range mercury concentration, units of measurement (including whether data were reported in wet weight or dry weight). When average mercury concentrations could only be obtained from figures, and not as values in a table or in the text, the mercury concentration was visually approximated within the figure. Within the same study, average mercury concentrations were kept separate for each species and behaviour studied.

To make mercury data equivalent across bird tissues, published equations of tissue mercury correlations were used to convert all mercury concentrations into blood-equivalent mercury levels. To convert mercury concentrations in bird tissues into mercury concentrations in blood (wet weight), we used the following equations from Eagles-Smith et al. (2008):

(a) $\ln Blood\ (Wet\ Weight) = 0.673 * \ln Feather\ (Dry\ Weight) - 1.673$

(b) $\ln Female\ Blood\ (Wet\ Weight) = 1.0734 * \ln Eggs\ (fww) + 0.8149$

Mercury concentrations in some studies was presented in mg/kg dry weight (dw). Because blood is also measured and reported on a wet-weight basis, the formula (Soldatini et al., 2020):

(c) $Blood\ (Wet\ Weight) = Blood\ (Dry\ weight) * 0.21$

can be used to convert dry-weight values to wet-weight values (e.g., assuming an average 79% of moisture) as previously done (Ackerman et al., 2016), to allow comparison of mercury in blood among different species.

2.3. RESULTS/DISCUSSION

The effects of mercury concentrations on bird behaviour can be assessed using different techniques and methods. A total of 38 studies investigating the effects of mercury levels on bird behaviour were reviewed, and data on mercury concentrations in different bird tissues are presented in Tables 1 - 4. The literature review showed that blood has been the most analysed matrix to assess the effect of mercury on individuals, followed by feathers and eggs.

Previous studies on the effects of mercury on birds have been limited to the general aspects of physiology, survival and reproduction. The effects of mercury on behaviour during the non-breeding season and the breeding season have been described and grouped. The studies presented investigated the effects of mercury on behaviour in adult and young birds.

2.3.1. BEHAVIOUR OF ADULTS

Exposure to mercury can alter adult behaviour during breeding and non-breeding season in a number of ways. Several researchers have examined the impact of mercury on the timing of breeding, courtship behaviours, incubation behaviour, parental behaviours during chick rearing, migration behaviours, foraging behaviours and self-maintenance behaviours.

2.3.1.1. TIMING OF BREEDING

Provencher et al. (2016) explored whether mercury exposure influenced the timing of arrival at breeding colonies in common eiders *Somateria mollissima*. In their observations they found no influence of mercury levels on the date of arrival of individuals at the colony (levels of mercury in blood: 0.4 - 0.5 mg/kg w wet.). Similarly, Mills et al., 2020 found no relationship between mercury levels in feathers of grey-headed albatross *Thalassarche chrysostoma* and date of arrival (levels of mercury in feathers: 2.0 - 31.5 mg/kg dw).

2.3.1.2 COURTSHIP BEHAVIOURS

Yu et al. (2016, 2017), when exposing, zebra finches *Taeniopygia guttata* in the laboratory to different concentrations of mercury (0.2 and 3.2 mg/kg), observed no impact on the mating behaviour of this species (levels of mercury in blood: ca. 0.01 - 0.1 mg/kg w wet.). Mercury injection into the egg also had no impact on the song quality of individuals (Yu et al. 2017). Similarly, Greene et al. (2018), reported that song attractiveness and quality in zebra finches males, exposed to 1.2 mg/kg mercury via diet, also did not differ significantly compared to the control group (levels of mercury in blood: unexposed males: Mean ± SD: 0.007 ± 0.001 mg/kg w wet., and exposed males Mean ± SD: 15.9 ± 0.07 mg/kg w wet.).

In nature, Hallinger et al. (2010), reported changes in song frequencies in carolina wrens *Thryothorus ludovicianus* (levels of mercury in blood: ca. 0.2 - 5.9 mg/kg w wet.), house wrens *Troglodytes aedon* (levels of mercury in blood: ca. 0.1 - 8.4 mg/kg w wet.), and song sparrows *Melospiza melodia* (levels of mercury in blood: ca. 0.1- 4.9 mg/kg w wet.) as mercury levels increased. The authors, however, assume the need for further studies to verify if the changes found affect the reproductive success of individuals. McKay and Maher (2012) when studying the songs of males of nelson's sparrows *Ammodramus nelsoni*, observed a significant relationship between high tonal frequency and blood mercury levels (levels of mercury in blood 0.9 - 6.4 mg/kg w wet.). However, they found no significant relationship between blood mercury levels, and other song characteristics (bouts per minute and gap duration). However, both studies did not study whether the differences found directly affected mating.

On the other hand, Frederick and Jayasena (2011), when dosing white ibises *Eudocimus albus* (exposure concentration: 0.05, 0.1, 0.3 mg/kg) reported a significant decrease in courtship behaviours in the dosed individuals, and a significant increase in the number of unproductive nests in relation to the control group (levels of mercury in feathers: 0.47-51.3 mg/kg; levels of mercury in blood: 0.7 - 4.0 mg/kg w wet.). Previously, Heath and Frederick (2005), suggested that the high abandonment rates of white ibises in the wild were due to the concentrations of mercury present in individuals, although they did not test the significance of the observed relationship (levels of mercury in feathers: 0.3 - 20.0 mg/kg).

2.3.1.3 INCUBATION BEHAVIOUR

Tartu et al. (2015), when examining incubation behaviour in snow petrels *Pagodroma nivea*, reported that males with higher levels of mercury (around ca. 1.9 mg/kg dw) in blood were more likely to neglect their egg, yet the mechanisms affected are not understood. Similarly, Jackson et al. (2011) observing the same behaviour in carolina wren *Thryothorus ludovicianus*, also reported that individuals with higher concentrations of mercury were more susceptible to abandoning their nests, than individuals from sites with lower levels of mercury in blood (levels of mercury in blood: 0.6 - 8.4 mg/kg wet wt.). Jackson et al. (2011), assume that they have not statistically tested the relationship between incubation time and mercury concentrations, highlighting the need for further future research.

In nature, Hartman et al. (2019) reported that overall, wild female tree swallows *Tachycineta bicolor* that laid eggs with high mercury and have high concentration in blood spent less time incubating their eggs than females that laid eggs with lower mercury concentration (levels of mercury in blood: 0.30 - 1.74 mg/kg w wet.; levels of mercury in eggs: 0.07 - 0.53 mg/kg wet wt.). Albers et al. (2007) dosing american kestrels *Falco sparverius* with mercury (exposure concentration: 0.6, 1.7, 2.8, 3.9, 5.0 mg/kg dw) reported that exposed individuals spent less time incubating eggs (levels of mercury in eggs: 2.0 - 19.1 mg/kg w wet.) than control individuals. Similarly, Evers et al. (2008) observing common loon *Gavia immer* populations in the wild, found that individuals with the highest levels of mercury in blood (levels of mercury in blood: 0.13 - 11.80 mg/kg w wet.) spent less time incubating their eggs. However, Evers et al. (2008), Albers et al. (2007) and Hartman et al. (2019), due to the method of data collection or insufficient sample sizes, did not test the statistical significance of the relationships found, so the alleged effects of mercury on behaviour can be viewed as hypotheses for future confirmation rather than results already found.

However, the researchers determined that parental nesting behaviours involved in egg incubation, which plays a significant role in embryonic development, were not altered for mercury in tern eggs (mean ± SD: 1.47 ± 0.76 mg/kg fww) in a population of forster's terns *Sterna forsteri* (Taylor et al., 2018). Blévin et al. (2020) reported an unclear relationship between mercury in blood (males: mean ± SD: 2.00 ± 0.59 mg/kg dw; females: mean ± SD: 1.43 ± 0.38 mg/kg dw) and incubation behaviour for arctic black-legged *kittiwakes Rissa tridactyla*. Similarly, Chin et al. (2017), when observing behaviour during reproduction in zebra finches *Taeniopygia guttata*, dosed with 1.2 mg/kg mercury and in unexposed individuals, found no significant difference between groups on nest attendance or in incubation period (levels of mercury in blood: control birds: Mean ± SE: 0.005 ± 0.0004 mg/kg w wet., mercury-exposed birds: Mean ± SE: 16.06 ± 0.38 mg/kg w wet.). However, eggs with a lower mercury content tended to have significantly shorter incubation periods (levels of mercury in eggs: ca. 0.02 - 3.35 mg/kg w wet.). Recently, Smith et al. (2022) studying the relationship between mercury concentration in eggs and the incubation behaviour of females in common eiders *Somateria mollissima*, found a marginally significant correlation between mercury concentrations and interruptions during incubation.

2.3.1.4. PARENTAL BEHAVIOURS DURING CHICK-REARING

Merril et al. (2005) observed a decrease in provisioning effort, during chick rearing in common loon *Gavia immer*, with increasing levels of mercury in blood of the parents (levels of mercury in blood: ca. 0.2 - ca. 5.8 mg/kg w wet.). However, in their conclusions, the authors do not emphasise the relationship found, since it was not statistically tested.

On the other hand, Fallacara et al. (2011) when dosing male American kestrels *Falco sparverius* with mercury (exposure concentration: 0.6, 3.9 mg/kg dw), reported a possible increase in cases of parental offspring cannibalism in individuals dosed with 3.9 mg/kg mercury than in the other groups. However, they observed cases in the other groups (control group and those dosed with 0.6 mg/kg mercury), assuming that they did not test for a relationship between mercury in blood and cannibalism (levels of mercury in blood 0.1 - 9.1 mg/kg wet wt). In their conclusions they assume that further studies are needed to verify that mercury levels do indeed potentiate cannibalism in the offspring.

2.3.1.5. FORAGING AND SELF MAINTENANCE BEHAVIOUR

Armstrong et al. (1963), when dosing pigeons *Columba livia domestica* with mercury vapour (17 mg/m^3), observed that exposed individuals showed behavioural changes, usually evidenced by tremors, involving the head, neck and wings. They also observed that the tremors were pronounced during preening, foraging and when the individuals walked. In their conclusions, the authors point out that the individuals returned to display a normal behavioural pattern after the end of exposure.

On the other hand, Olsen et al. (2000), observing the feeding behaviour of common loon adults, in situ, found that individuals with more than 4 mg/kg of mercury in blood dipped significantly more, than individuals with less than 4 mg/kg of mercury in blood. Despite the significant difference, the authors assume that to obtain a solid conclusion more studies should be done, since the correlation found was not very strong and the number of individuals observed was rather small. Evers et al. (2008) observed a larger number of adults of this species and concluded that individuals spent less time in high-energy behaviours (decreased levels of foraging activity) as mercury levels increased (levels of mercury in blood: 0.13 - 11.80 mg/kg w wet.; levels of mercury in secondary feathers: 1.4 - 75.7 mg/kg). However, Evers et al. (2008) did not specify which high-energy behaviours were affected (e.g., swimming and foraging), nor did they statistically test the relationship found. Contrarily, Merrill et al. (2005) when observing the feeding behaviour also of this species, concluded that, capture success was not related to levels of mercury in blood (levels of mercury in blood: ca. 0.2 - ca. 5.8 mg/kg w wet.).

Bouton et al. (1999), dosing great egrets *Ardea alba* (exposure concentration: 0.5, 5.0 mg/kg), reported that mercury significantly affected holding behaviour, but not foraging efficiency. The authors found significant differences between dosed and control individuals, specifically, in activity levels (e.g., preening, head down, vocalization), in tendency to seek shade, and in motivation to hunt prey. They also point out that most of the individuals dosed with 5 mg/kg mercury died before the start of the trials.

Adams and Frederick (2008) evaluating feeding performance in white ibises *Eudocimus albus* dosed with mercury (exposure concentration: 0.05, 0.1, 0.3 mg/kg) did not find a cohesive link between mercury exposure and feeding behaviour. The authors first emphasised a significant relationship between loss of foraging efficiency and mercury in individuals dosed with 0.1 mg/kg mercury, but then assumed it to be inconclusive, as they found no significant relationship in individuals exposed to higher mercury concentrations (0.3 mg/kg).

On the other hand, Kobiela et al. 2015, when studying the behaviour of zebra finches in the face of predators, dosed with 1.2 mg/kg mercury, reported that the dosed individuals react differently to the presence of predators compared to the control group, waiting significantly longer to feed after observing a predator, especially in the high-risk exposure scenario (levels of mercury in blood: unexposed birds: Mean ± SD: 0.07 ± 0.09 mg/kg w wet., and exposed birds: Mean ± SD: 13.93 ± 3.60 mg/kg w wet). The authors in their conclusions, point out that this type of response by the mercury-exposed group could lead to an increased risk of starvation in these individuals, effectively evidencing no negative relationship from mercury exposure.

Finally, Seewagen (2013), assessing the impact of mercury concentrations in northern Waterthrushes *Parkesia noveboracensis*, found that the levels present in the blood of individuals does not seem to affect foraging efficiency (levels of mercury in blood: 0.1 - 2.1 mg/kg ww).

2.3.1.6. FLIGHT AND MIGRATION BEHAVIOUR

Ma et al. (2018) when studying the effects of mercury (levels of mercury in blood: ca. 0.001 - 20 mg/kg ww.) in yellow-rumped warbler *Setophaga coronata*, exposed to different mercury concentrations (exposure concentrations: 0.5 and 1.0 mg/kg), also reported some behavioural differences, in some dosed groups. The individuals dosed, for 14 days and exposed to 0.5 mg/kg mercury, showed significantly shorter flight duration and longer strike duration, without changes in vertical take-off performance (Ma et al., 2018). The authors emphasize the differences found; however, they did not evaluate the performance of the group exposed to 1.0 mg/kg of mercury, justifying that the concentrations caused by this exposure, in the body of these individuals, were higher than those found in wild populations. Although several trials were carried out, the number of individuals tested was small (n = 4), which does not allow the extrapolation of solid conclusions. On the other hand, Seewagen et al. (2019) when assessing the impact of mercury exposure (exposure concentrations: 0.5 mg/kg) on migratory behaviour, of the same species, found no evidence that mercury (levels of mercury in blood of dosed birds: 5.63 - 7.63 mg/kg w wet.) altered flight dynamics and orientation during migration. However, they observed a delay in departure for migration, which they did not test statistically due to the small number of individuals (control n = 7; dosed n = 3). Despite the difference found, which they assume is due to mercury exposure, the authors point out that they cannot draw strong conclusions due to the small number of individuals studied.

Carlson et al. (2014) when dosing european starlings *Sturnus vulgaris* with mercury (0.75, or 1.5 mg/kg), reported that individuals exposed to the highest mercury concentrations, expended significantly less energy during take-off flight and consequently performed less well in escape (take-off) flight.

2.3.2. BEHAVIOUR OF CHICKS

Chicks are different from adult birds in several respects, including differences in metabolism, patterns of behaviour and changes of organ systems and functions (e.g., D'Amico et al., 2014; Sprague and Breuner, 2010), which led the researchers to also assess the reaction of chicks to mercury.

Heinz (1975), was one of the first researchers to study the impact of mercury on bird chicks, using as a laboratory model Mallard ducklings *Anas platyrhynchos* that have early or nestlings. Heinz (1975) fed ducks with diets containing between 0.5 and 3 mg/kg and evaluated the effect on the behaviour of the offspring. When evaluating the behaviour of the offspring (levels of mercury in eggs: 0.5 - 9.2 mg/kg w wet.), he observed that responses to maternal calling and predators were not altered by mercury exposure. The following year, Heinz (1976a) again evaluated the behaviour of new offspring (mating offspring of adult individuals exposed to mercury in Heinz (1975)), but also observed no differences in behaviour between mercury-exposed and control offspring (levels of mercury in eggs: 0.8 - 7.4 mg/kg w wet.; levels of mercury in primary feathers: 11.2 - 68.7 mg/kg). However, Heinz (1975) and Heinz (1976a), report a hyper-response, with individuals showing significant hypertrophied, impulsive and disorganised motor behaviour in the offspring of parents exposed to 3 mg/kg mercury relative to the offspring of parents exposed to 0.5 mg/kg mercury and control. Many investigations assume this to be an obvious deleterious effect of mercury on offspring behaviour, however this hyper-response does not actually impair escape aptitude to predators or response to parental calls.

Similarly, Heinz (1976b), also found no significant differences in response to maternal calls and avoidance of predators in first generation offspring (levels of mercury in eggs: Mean ± SE: 0.9 ± 0.098 mg/kg w wet.; levels of mercury in primary feathers: Mean ± SE: 9.0 ± 0.396 mg/kg), resulting from reproduction between individuals previously studied by Heinz (1976a) and exposed to concentrations of 0.5 mg/kg mercury.

Finally, Heinz (1979), when evaluating the behaviour of third generation of chicks, resulting from reproduction between individuals previously studied by Heinz (1976b) and exposed to mercury concentrations of 0.5 mg/kg, also found no relationship between chicks behaviour and mercury levels. However, when pooling data from three generations, authors reported a small significant relationship between response to maternal calls and mercury levels (levels of mercury in eggs: 0.8 - 0.9 mg/kg; levels of mercury in primary feathers: 11.2 - 68.7 mg/kg). Although, the author assumes that the relationship between the variables under study is marginal, the study is cited in over 50 publications as evidence of a significant effect of mercury in birds, which in fact is only marginal and results from the aggregation of data from three generations and not verified in each generation of offspring.

Years later, Kenow and his team used Heinz's methodology to study the impact of mercury on common loon *Gavia immer* chicks. In their first study, Kenow et al. (2003) observed no differences in feeding behaviour in common loon chicks exposed to different doses of mercury (exposure concentration: 0.1, 0.5 and 1.5 mg/kg) and with blood mercury levels between 0.1 - 20.0 mg/kg. In subsequent investigations, Kenow and his team, by injecting mercury (exposure concentration: 0.5, 1.3 and 2.9 mg/kg) into common loons eggs (levels of mercury in blood: 1.7 - 12.0 mg/kg; levels of mercury in eggs: 0.6 - 4.2 mg/kg) (Kenow et al, 2011), or by subjecting the offspring (levels of mercury in blood: 0.1 - 15.0 mg/kg) to mercury concentrations of 0.08, 0.4 and 1.2 mg/kg through the diet (Kenow et al., 2010), observed that surviving chicks did not show substantial and significant differences in their response to startle stimuli, parental call and ability (e.g., walk, influenced the probability that the chick would right itself within 60 s during indoor trials, locomotion across a platform). In addition, they also showed no significant differences in other behaviours, such as preening, peering, locomotion, agonistic behaviour or resting (Kenow et al., 2010). However, Kenow et al. (2010) emphasize in their findings a small significant difference in the righting test, found only by grouping variables, stating that older chicks exposed to 0.4 and 1.2 mg/kg mercury were less likely to complete the righting test. Kenow et al. (2011) report only differences in the behaviour of chicks excluded from eggs injected with 2.9 mg/kg mercury (n = 2) (including crossing a platform faster, spending more time on platforms and in sunlight). From the outset, we consider that the concentrations resulting from the injection of 2.9 mg/kg into eggs are not concentrations normally found in wild populations of this species. Besides, these two individuals would be close to have lethal concentrations in their bodies, and naturally they would have to present physiological and behavioural differences. Finally, some researchers have also begun to assess the relationship between mercury and behaviour, in situ, in this species. Nocera and Taylor (1998), observing the behaviour of Common loon chicks found that the chicks spent significantly more time preening and less time back-riding (brooding by riding on a parent's back) as mercury levels in blood increased (levels of mercury in blood: 0.2 - 1.3 mg/kg w wet.), although they did not change their swimming or diving habits.

Recently, Morran et al. (2018), when exposing zebra finches to different concentrations of mercury (exposure concentrations: 0.0315 mg/kg bw/day and 0.075 kg/mg bw/day), reported that no significant differences occurred in age of fledging, age of self feeding, latency to feed and latency to resume feeding after removal of the neophobia (blood mercury, control birds: Mean ± SD: 0.006 ± 0.006 mg/kg w wet, low MeHg treatment group birds and exposed Mean ± SD: 0.297 ± 0.067 mg/kg w wet., and for high MeHg treatment group birds Mean ± SD: 0.734 ± 0.163 mg/kg w wet.). On the other hand, Swaddle et al. (2017) when dosing zebra finches with 1.2 mg/kg mercury, reported significant changes in cognition in dosed individuals (levels of mercury in blood: control birds: Mean ± SE: 0.011 ± 0.002 mg/kg w wet., mercury-exposed birds: Mean ± SE: 17.79 ± 0.927 mg/kg w wet.), which included impaired spatial memory, but not inhibitory control or ability to associate colour with food. The same colony of exposed zebra finches exhibited behavioural changes, including significantly increased activity (hyperactivity) and subordination to individuals not exposed to mercury. However, they showed no significant differences in latency to approach feeder, latency to approach perch closest to feeder, number of visits to feeder and number of visits to perch closest to feeder.

On the other hand, when mercury was injected into white leghorn chicken eggs (exposure concentration: 0.2, 0.6, 2.0, 3.2, 6.4, mg/kg), the surviving chicks did not differ in their response to frightening stimuli (Rutkiewicz et al. 2013).

Finally, Moye et al. (2016) when evaluating the behaviour of broods of homing Pigeons *Columba livia* from individuals exposed to mercury concentrations of 1.0 mg/kg, reported that broods of birds exposed to methylmercury exhibit visual differences and learning disabilities compared to the control group, however these differences were neither quantified nor tested. In their conclusions, the authors assume that there are differences in flight times between groups, but that these are not consistent to be related to mercury exposure.

2.4. CONCLUSION

The increase in global emissions of pollutant gases, such as mercury, has occurred in recent centuries due to the growth in demand for products for the daily life of human beings. Environmental contamination by mercury threatens not only human populations but all wildlife. Although reducing emissions is a global concern, emissions continue to increase in many parts of the world, particularly in developing countries (Streets et al., 2019, 2011). Birds are clearly vulnerable organisms to mercury contamination (see reviews Ackerman et al., 2016; Whitney and Cristol, 2018; Cristol and Evers, 2020; Seewagen, 2010, 2020), yet, despite numerous claims that mercury affects wild bird behaviour, there are indeed very few reports showing this in a rigorous and convincing manner. Papers often quoted as evidence for an effect of mercury in birds provide very little evidence supported by adequate observation and experimental protocols and corresponding statistical analyses.

Our review indicates that currently, behaviour is still an unreliable parameter of the sublethal effects of mercury in wild birds. We also highlight that there is a substantial knowledge gap on the effect of mercury on the behaviour of species inhabiting more remote environments, such as seabirds. We also found that the associations of mercury to behaviour appear to be clearer under controlled conditions and further investigations are needed. We conclude that the effects of mercury on behaviour vary between bird groups, species, and depend on the levels assimilated, but assume that a direct comparison between studies is not possible. We stress that our results further indicate the need for more studies both in the laboratory and in the field on the effects of mercury on bird behaviour and on larger samples of individuals and species.

We draw attention to historical and recent findings on some effects of mercury pollution on bird behaviour, which as described by researchers (see review Whitney and Cristol

2018), should be analysed carefully and updated to provide guidance to all stakeholders concerned with the effects of mercury on birds.

In the Future, further research into the impact of mercury on bird behaviour is likely to benefit conservation efforts. It is clearly useful to draw attention to possible links between mercury pollution and changing bird behaviour. However, the lack of any link between mercury and behaviour in recent studies in the wild that nonetheless show high mercury concentrations emphasises the need for rigorous methods that examine mercury accumulation and behaviour on an individual basis.

Table 2.1. Summary of toxicity benchmarks for the effects of methylmercury in blood on Timing of breeding and Courtship behaviours of adults bird**.**

Behaviours	Common name / Scientific name	Mercury exposure	Original tissue Hg concentration						Blood Hg concentration (ug/g, ww)		Hg toxic effect	Impairment	Study
			Blood			Feathers							
			Mean	Range	Units	Mean	Range	Units	Mean	Range			
Timing of breeding	Common eiders *Somateria mollissima*	Natural diet	0.21	0.08 - 0.43	ug/g, ww	-	-	-	0.21	0.08 - 0.43	Undetected	-	Provencher et al., 2016
	Grey-headed albatrosses *Thalassarche chrysostoma*		1.1	0.3 – 2.1	ug/g, ww	-	-	-	1.1	0.3 – 2.1	Detected	-	Mills et al., 2020
Courtship behaviours	Zebra finches *Taeniopygia guttata*	Egg injection	-	0.01 – 0.1	ug/g, ww	-	-	-	-	0.01 – 0.1	Undetected	-	Yu et al., 2016
			-	-	ug/g, ww	-	-	-	-	-	Undetected	-	Yu et al., 2017
		Altered diet	15.9	-	ug/g, ww	-	-	-	15.9	-	Undetected	-	Greene et al., 2018
	Carolina wrens *Thryothorus ludovicianus*	Natural diet	-	0.2 – 5.9	ug/g, ww	-	-	-	-	0.2 – 5.9	Partially detected	Changes in songs	Hallinger et al., 2010
	House wrens *Troglodytes aedon*		-	0.1 – 8.4	ug/g, ww	-	-	-	-	0.1 – 8.4			
	Song sparrows *Melospiza melodia*		-	0.1 – 4.9	ug/g, ww	-	-	-	-	0.1 – 4.9			
	Nelson’s sparrows *Ammodramus nelsoni*		-	0.9 – 6.4	ug/g, ww	-	-	-	-	0.9 – 6.4	Partially detected	Changes in songs	McKay and Maher, 2012
	White ibises *Eudocimus albus*	Altered diet	-	0.7 – 4.0	ug/g, ww	-	-	-	-	0.7 – 4.0	Partially detected	Homosexual couples	Frederick and Jayasena, 2011
		Natural diet	-	-	-	-	0.3 – 20.0	ug/g, dw	-	0.1 – 1.4	Undetected	-	Heath and Frederick, 2005

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Table 2.2. Summary of toxicity benchmarks for the effects of methylmercury exposure on incubation behaviour and parental behaviours during chick-rearing.

Behaviours	Common name / Scientific name	Form of mercury exposure	Original tissue Hg concentration							Blood-equivalent Hg (ug/g, ww)		Hg toxic effect	Impairment	Study
			Blood		Feathers		Eggs		Units					
			Mean	Range	Mean	Range	Mean	Range		Mean	Range			
Incubation behaviour	Snow petrels *Pagodroma nivea*	Natural diet	1.9	0.9 – 4.0	-	-	-	-	ug/g, dw	0.4	0.2 – 0.8	Detected	Increased egg neglect for males	Tartu et al., 2015
	Carolina wren *Thryothorus ludovicianus*		-	0.6 - 8.4	-	-	-	-	ug/g, ww	-	0.6 – 8.4	Partially detected	Eggs neglect	Jackson et al., 2011
	Tree swallows *Tachycineta bicolor*		0.7	0.3 – 1.7	-	-	0.2	0.1 – 0.5	ug/g, ww	0.7	0.3 – 1.7	Partially detected	Changes in incubation period	Hartman et al., 2019
	Common loon *Gavia immer*		1.7	0.1 – 11.8	-	-	1.6	0.2 – 9.0	ug/g, ww	1.7	0.1 – 11.8	Partially detected	Changes in incubation period	Evers et al., 2008
	Forster's terns *Sterna forsteri*		-	-	-	-	1.5	-	ug/g, ww		-	Undetected	-	Taylor et al., 2018
	Arctic black-legged kittiwakes *Rissa tridactyla*		1.7	-	-	-	-	-	ug/g, dw	0.4	-	Undetected	-	Blévin et al., 2020
	Common eiders *Somateria mollissima*		-	-	-	-	-	0.3 – 2.7	ug/g, dw	-	0.6 – 6.6	Partially detected	Changes in incubation behaviour	Smith et al., 2022
	Zebra finches *Taeniopygia guttata*	Altered diet	16.1	-	-	-	3.4	-	ug/g, ww	16.1	-	Partially detected	Changes in incubation period	Chin et al., 2017
	American kestrels *Falco sparverius*		-	-	-	-	-	2.0 – 19.1	ug/g, ww	-	4.8 – 53.6	Partially detected	Changes in incubation period	Albers et al., 2007
Parental behaviours during chick-rearing	Common loon *Gavia immer*	Natural diet	0.3	0.2 - 5.8	-	-	-	-	ug/g, ww	0.3	0.2 - 5.8	Undetected	-	Merrill et al., 2005
	American kestrels *Falco sparverius*	Altered diet	-	0.1 – 9.1	-	-	-	-	ug/g, ww	-	0.1 – 9.1	Partially detected	Cases of offspring cannibalism	Fallacara et al., 2011

Table 2.3. Summary of toxicity benchmarks for the effects of methylmercury exposure on Foraging, Self maintenance behaviour, flight and migration behaviour of adults bird.

Behaviours	Common name / Scientific name	Mercury exposure	Original Blood Hg concentration			Blood Hg concentration (ug/g, ww)		Hg toxic effect	Impairment	Study
			Mean	Range	Units	Mean	Range			
Foraging and Self maintenance behaviour	Pigeons *Columba livia domestica*	Inhalation	-	-	-	-	-	Detected	Tremors	Armstrong et al., 1963
	Mallard ducklings *Anas platyrhynchos*	Natural diet	-	-	ug/g, ww	-	-	Partially detected	Decreased diving activity	Olsen et al., 2000
			1.7	0.1 – 11.8	ug/g, ww	1.7	0.1 - 11.8	Partially detected	Decreased activity	Evers et al., 2008
			-	0.2 – 5.8	ug/g, ww	-	0.2 – 5.8	Undetected	-	Merrill et al., 2005
	Great egret *Ardea alba*	Altered diet	-	-	ug/g, ww	-	-	Detected	Decreased Self maintenance activity	Bouton et al., 1999
	White ibis *Eudocimus albus*		-	-	ug/g, ww	-	-	Undetected	-	Adams and Frederick, 2008
	Zebra finch *Taeniopygia guttata*		13.9	8.0 – 24.8	ug/g, ww	13.9	8.0 - 24.8	Partially detected	Increased time to forage	Kobiela et al. 2015
	Northern Waterthrushes *Parkesia noveboracensis*	Natural diet	0.4	0.1 – 2.1	ug/g, ww	0.4	0.1 – 2.1	0.4	-	Seewagen, 2013
Flight and migration behaviour	Yellow-rumped warbler *Setophaga coronata*	Altered diet	13.5	-	ug/g, dw	2.8	-	Undetected	-	Ma et al., 2018
			6.6	5.6 –7.6	ug/g, ww	6.6	5.6 –7.6	Detected	Delayed departure for migration	Seewagen et al., 2019
	European starlings *Sturnus vulgaris*		-	3.2 – 17.4	ug/g, ww	-	3.2 – 17.4	Detected	Reduced performance in escape flight	Carlson et al., 2014

Table 2.4. Summary of toxicity benchmarks for the effects of methylmercury exposure on behaviour of chicks bird during chick rearing

Behaviours	Common name / Scientific name	Form of mercury exposure	Original tissue Hg concentration							Blood-equivalent Hg (ug/g, ww)		Hg toxic effect	Impairment	Study
			Blood		Primary Feathers		Eggs		Units					
			Mean	Range	Mean	Range	Mean	Range		Mean	Range			
behaviours of chicks bird during chick-rearing	Mallard ducklings *Anas platyrhynchos*	Altered Diet	-	-	-	-	-	0.5 – 9.2	ug/g, ww	-	1.1 – 25.5	Undetected	-	Heinz, 1975
			-	-	40.0	-	4.9	-	ug/g, ww	12.4	-	Undetected	-	Heinz, 1976a
			-	-	9.0	-	0.9	-	ug/g, ww	2.0	-	Undetected	-	Heinz, 1976b
			-	-	-	9.0 – 11.2	-	0.8 – 0.9	ug/g, ww	-	1.8 – 2.0	Undetected	-	Heinz, 1979
	Common loon *Gavia immer*		-	0.1 – 20.0	-	-	-	-	ug/g, ww	-	0.1 – 20.0	Undetected	-	Kenow et al., 2003
			-	0.1 – 15.0	-	-	-	-	ug/g, ww	-	0.1 – 15.0	Undetected	-	Kenow et al., 2010
		Egg injection	-	1.7 – 12.0	-	-	-	0.6 – 4.2	ug/g, ww	-	1.7 – 12.0	Partially detected	Poisoning	Kenow et al., 2011
		Natural diet	-	0.2 – 1.3	-	-	-	-	ug/g, ww	-	0.2 – 1.3	Detected	Time of preening and back-riding	Nocera and Taylor, 1998
	Zebra finch *Taeniopygia guttata*	Altered Diet	0.5	0.1 – 1.1	-	-	-	-	ug/g, ww	0.5	0.1 – 1.1	Undetected	-	Morran et al., 2018
			17.9	-	-	-	-	-	ug/g, ww	17.9	-	Detectected	Cognition	Swaddle et al., 2017
	Leghorn chicken *Gallus gallus domesticus*	Egg injection	-	-	-	-	-	-	-	-	-	Undetected	-	Rutkiewicz et al., 2013
	Homing Pigeons *Columba livia*	Altered diet	-	-	-	-	-	-	-	-	-	Undetected	-	Moye et al., 2016

CHAPTER 3

A REVIEW OF THE EFFECT OF MERCURY CONCENTRATION ON THE SURVIVAL OF SEABIRDS

Ricardo Furtado

3.1. ABSTRACT

Mercury comes from anthropogenic and natural sources, but human activities have considerably increased the global amount of mercury circulating worldwide. Seabirds are vulnerable organisms to mercury contamination. Researchers have long suspected that mercury may be a potent toxicant to seabirds. As a neurotoxin, mercury may impair birds' navigation, flight performance, and foraging ability. Thus, it is reasonable to assume that mercury exposure may contribute to impaired survival of individuals. However, there is little solid evidence to show that current levels of mercury in the ocean negatively impact seabird survival. Here we group and summarise the existing information. Although studies have reported relatively high concentrations in seabirds, there has been no evidence of a relationship between mercury and survival of individuals. We further conclude that there are few studies on the subject. Furthermore, we highlight that the understanding of the threats of mercury to seabird survival remains very incomplete.

3.2. INTRODUCTION

Aggressive chemical stimulation (e.g., from toxic pollutants) can cause a behavioural and/or biological response without causing immediate lethal effects (Depledge et al., 1995; Zala and Penn, 2004). Mercury is a toxic, global metal that has a broad impact on neurological processes in vertebrates, studied in laboratory settings (e.g., Wolfe et al., 1998; Driscoll et al., 2013). Strikingly, however, very little is known about its effects on animal survival in the wild.

On the planet, mercury concentrations are highest near geologically active areas (e.g., regions of high volcanic activity, hydrovents), in areas with extensive industrial activity (e.g., in gold mining zones,z onas of waste incineration and fossil fuel burning and extraction sites), near coastal environments (due to elevated atmospheric deposition and sediment elevation due to coastal upwelling) (Driscoll et al, 2013; Esdaile and Chalker, 2018), and in the deep ocean (Cossa et al., 2018).

In the ocean, mercury is most toxic when it is transformed to methylmercury (Wolfe et al., 1998), a process that occurs naturally in the mesopelagic oceanic domain due to low oxygen concentrations at depths (ca. 200 - 1500 m) (Bowman et al., 2015; Cossa et al., 2018). In the 1960s, advances in analytical equipment allowed the chemical analysis of various forms of mercury in environmental samples (Barreto, 2010), resulting in the discovery that organic mercury biomagnifies along food chains and the identification of environmental methylation of mercury in the deep ocean (e.g., Santos 2006).

In the eyes of researchers, by the early 21st century methylmercury had become an ocean-scale problem, not simply a local problem (Zhang et al., 2014). By bioaccumulating and biomagnifying along the oceanic food web, the highest mercury values are found in top predators. Thus, top predator species such as seabirds, which are associated with the open and deep ocean, are at greater risk of accumulating high amounts of methylmercury (e.g., Gatt et al., 2020; Furtado et al., 2020, 2021).

Mercury levels in seabirds have been widely recorded worldwide (e.g., Becker et al., 2016; Furtado et al., 2020, 2021; Shoji et al., 2021), although some attempts have been made to quantify the risks (e.g., Chastel et al., 2022), however, the negative relationship between mercury and seabird survival has yet to be found. As a neurotoxin, methylmercury has been linked to negative and lethal outcomes in birds (e.g., Frederick and Jayasena, 2011; Sepúlveda et al. 1999). However, while decreased survival due to mercury pollution of offspring in the nest has already been described, mainly due to effects via contaminated eggs or via poor parental care (e.g., Albers et al. 2007; Kenow et al. 2011), reduced longevity after leaving the nest is very poorly documented (e.g., Cristol and Evers, 2020).

Some studies report a decline in hatched eggs due to mercury exposure (egg injection, concentrations between 3.2 and 90.0 ppm) in zebra finches *Taeniopygia guttata* (Yu et al., 2016; Heddle et al., 2020), mallard ducklings *Anas platyrhynchos* (Hoffman and Moore, 1979; Heinz et al., 2011; Klimstra et al., 2012) and common loon *Gavia immer* (Kenow et al., 2011). These laboratory studies, report high mortality rates in eggs exposed to the highest concentrations of mercury, which are actually considered lethal, so these negative results are not very relevant.

On the other hand, several authors dosing females, prior to egg laying, (concentrations between 0.03 and 5.2 ppm) of mallard ducklings *Anas platyrhynchos* (Heinz, 1979), common loon *Gavia immer* (Barr 1986), American black duck *Anas rubripes* (Finley and Stendell, 1978), American kestrels *Falco sparverius* (Albers et al, 2007) and in white ibis *Eudocimus albus* (Frederick and Jayasena 2010), reported a reduction in the number of chicks hatched.

In nature, for example Weech et al. (2006) reported no correlation between environmental mercury exposure (mercury concentration in blood ca. 1.6 - 9.4 mg/kg wet weight) and the number of chicks hatched in Bald eagle *Haliaeetus leucocephalus*. Similarly, brood survival of tree swallow *Tachycineta bicolor* (Custer et al., 2012), black-crowned night-heron *Nycticorax nycticorax* (Henny et al., 2002), Acadian flycatchers *Empidonax virescens* (Rowse et al., 2014), and eastern bluebirds *Sialia sialis* (McCullagh et al. 2015), were also unrelated to mercury exposure (blood mercury concentration between ca. 0.1 - 5.9 mg/kg wet weight). However, Brasso and Cristol (2008) and Taylor and Cristol (2015), found a negative relationship between mercury levels (blood mercury concentration between 2.2 - 3.7 mg/kg wet weight) of broods and number of surviving offspring.

In adult birds in the wild, mercury present in the environment does not seem to be directly affecting the longevity of individuals. Several researchers have found no significant relationships between mercury levels (blood mercury concentration between ca. 0.1 - 7.4 mg/kg wet weight) and survival in snowy egrets *Egretta thula* (Henny et al., 2017), free-living common loons (Mitro et al., 2008) and common eiders *Somateria mollissima* (Provencher et al., 2017). Furthermore, Meyer et al. (1998) also failed to find a relationship between the annual adult return rate of common loons and mercury levels. On the other hand, the low recapture probability (individuals with more mercury were less recaptured) in white-winged scoters *Melanitta deglandi* was related to blood mercury concentration (ca. 0.2 mg/kg wet weight) in individuals (Wayland et al. 2008). However, there was no correlation between capture probability and levels of king eiders *Somateria spectabilis*, in individuals with the same mean mercury concentrations (Wayland et al. 2008).

In the wild, in the mid-20th century, some unidentified species of gulls living around Minamata Bay in Japan, which consumed large quantities of fish in this bay, developed mysterious neurological diseases. These animals developed symptoms such as instability, sudden death during flight (paralysis and frequent falling in flight) (Takeuchi et al., 1957; Harada, 1994). Content, although this was the largest and most impactful case of mercury poisoning in the natural environment in the world, there are almost no public records/studies of the time. Although the relationship between mercury poisoning and mortality exists in seabirds, little is known about the effects on their survival produced by current mercury levels.

According to the mercury concentrations found in some seabird species, researchers have predicted obvious deleterious effects (e.g., Anderson et al., 2009, 2010; Carravieri et al., 2022). However, several studies have found no relationship between mercury levels and survival (e.g., Thompson et al., 1991; Pollet et al., 2017), which may be explained by the development of various behavioural and/or physiological strategies.

Seabirds exhibit various behavioural strategies to overcome the challenges that the environment provides them (e.g., Cheng et al., 2019). To survive, they often make decisions that may favour or hinder their propensity to survive (e.g., Cheng et al., 2019; Rotics et al., 2021). Such behavioural differences between individuals, such as in decisions made during migration (e.g., timing, destination) and/or during feeding (movement activity), or in the face of one-off situations occurring in the environment (e.g., cyclones) may influence the survival of individuals (Bracey et al., 2021; Weimerskirch and Prudor, 2019; Ventura et al., 2021; Cheng et al., 2019).

The long-term survival of the species and/or individuals depends on the ability to learn, and respond in exceptional ways to a potential or actual threatening stimulus, such as pollution or cyclones (e.g., Lempidakis et al., 2022; Roman et al., 2020). Thus, variation in individual behaviour may translate into variations in survival probability (e.g., Roman et al., 2020; Weimerskirch and Prudor, 2019). On the other hand, the ability to interact with challenging environments requires the coordination of sensory, motor and physiological systems that underpin adequate strategies for survival (e.g., Lempidakis et al., 2022; Sato et al., 2009). Such strategies should therefore account for much of the variation in individual survival.

Given the increase in mercury pollution in some areas of the planet and ocean, and the realisation that mercury can affect vertebrate survival, a review of what is known about the effects of mercury on brood, juvenile and adult survival in seabirds is essential. Here, we summarize research that has undertaken to study the relationship between mercury levels and seabird survival.

3.3. METHODOS

To identify relevant literature, the search terms "seabirds" and "mercury" were combined with "survival" or "reproduction" and with "success" in Google Scholar, thoroughly searching both the research output, the literature cited in these articles and the articles in which they were cited. I then examined the abstracts of each article and rejected articles that were not related to the topic. Additional material such as books, and book chapters were also used.

We reviewed a wide range of publications to gather available data on the relationship between mercury and seabird survival. Although many studies measure mercury concentrations in seabirds, few focus on the relationship between mercury and survival.

For each study, the following information was extracted: age, seabird species, tissue type, mean and range mercury concentrations, units of measurement (including whether data were reported in wet weight or dry weight). Within the same study, mercury concentrations were kept separate for each species. To convert mercury concentrations in feathers of seabirds into mercury levels in blood (wet weight), the following equations from Eagles-Smith et al. (2008):

(a) $\ln Blood\ (Wet\ Weight) = 0.673 * \ln Feather\ (Dry\ Weight) - 1.673$

Mercury concentrations in some studies was presented in mg/kg dry weight (dw). Because blood is also measured and reported on a wet-weight basis, the formula (Soldatini et al., 2020):

(b) $Blood\ (Wet\ Weight) = Blood\ (Dry\ weight) * 0.21$

can be used to convert dry-weight values to wet-weight values (e.g., assuming an average 79% of moisture) as previously done (Ackerman et al., 2016), to allow comparison of mercury in blood among different species.

3.4. RESULTS / DISCUSSION

The effects of mercury concentrations on avian survival can be assessed using different techniques and methods. In total, 12 studies investigating the effects of mercury concentrations on chick and adult survival in seabirds were reviewed, and data on mercury concentrations in feathers and blood of individuals are presented in Tables 3.1 and 3.2.

The literature review showed that blood has been the most analysed matrix to assess the effect of mercury on individuals. The effects of mercury on survival during the non-breeding season and the breeding season were described and grouped. The studies presented investigated the effects of mercury on survival in adult individuals and fry.

3.4.1. SURVIVAL OF ADULT BIRDS

Mercury levels in feathers and blood of seabirds (Table 3.1) does not appear to have a direct effect on survival. Thompson et al. (1991) found no effect of mercury concentration in feathers (mean ± SD: 7.0 ± 5.1; range: 1.2 – 32.4 mg/kg) on the return rates of Great Skuas *Catharacta skua* to their breeding colony the following year. Similarly, Pollet et al. (2017) reported that the presence of mercury in blood (mean ± SD: 0.82 ± 0.45 mg/kg wet wt.; range: 0.24 to 2.33 mg/kg wet wt.) had not altered the return rate to the breeding colony (defined as the percent of previously banded adults seen in any subsequent season) in Leach’s storm-petrels *Oceanodroma leucorhoa*. Black legged kittiwakes *Rissa tridactyla* with mercury concentrations in blood around 2 mg/kg dw also had not jeopardized survival rate (Goutte et al., 2015). Equally, the survival rate was unrelated with mercury in blood in south polar skua *Catharacta maccormicki* (Mean ± SE: 2.1 ± 0.2 mg/kg dw) and in brown skua *Stercorarius antarcticus* (Mean ± SE: 8.2 ± 0.2 mg/kg dw) (Goutte et al., 2014b). Although wandering albatrosses *Diomedea exulans* displayed some of the highest mercury concentrations in feathers reported for seabirds, ranging from 5.9 to 95 mg/kg dw, their survival rate had no relationship with mercury (Bustamante et al., 2016). Likewise, the survival rate was unrelated with mercury in feathers in little auk *Alle alle*, even though the body condition declines with increased mercury concentration (Amélineau et al., 2019). Finally, no connection was either found between mercury ranging from 2.0 to 18.7 mg/kg dw in blood and the survival rate in wandering albatrosses (Goutte et al., 2014a). So, Mercury has not been associated with reduction in annual adult return rate (which in highly philopatric seabirds generally equates to survival rate).

Recently, Bauch et al. (2022), reported that Cory's Shearwater *Calonectris borealis* individuals with higher mercury concentrations (mercury in feathers: ca. 1.5 - 12.00 mg/kg dw) had smaller telomeres, without any influence for the time being on the survival of individuals. However, they point out that changes in genetic material can have serious consequences on the survival of individuals and ultimately on the fate of populations.

3.4.2. SURVIVAL OF CHICKS BIRDS

Few studies have investigated the effects of mercury contamination on offspring survival in seabirds. As in adults, chicks are exposed to high concentrations of mercury. Mercury concentrations are summarised by species and tissues in Table 3.2.

There was a nonsignificant trend between survival in the nest of nestling Leach's storm-petrels (Pollet et al., 2017) and wandering albatrosses (Bustamante et al., 2016) and levels of mercury in parents (values quoted table 1). No significant relationship was also observed between fledging success of great skuas` chicks (Thompson et al., 1991) and mercury in feathers of chicks (Mean ± SD: 1.3 ± 0.4 mg/kg Range: 0.7 – 2.4 mg/kg fresh weight). Post-fledging survival and mercury in blood of chicks (Mean ± SD: 0.3 ± 0.01 mg/ kg wet wt.) and in feathers (6.44 ± 0.28 mg/kg dw) were also not related in Forster's terns *Sterna forsteri* (Ackerman et al., 2008). Similarly, survival of chicks were not related to maternal mercury in Antarctic petrels *Thalassoica antarctica* (red blood cell mercury concentrations: 1.38 ± 0.43 mg/kg dw) (Carravieri et al., 2021) and was unrelated to parental mercury levels in the same species (red blood cell mercury concentrations: 0.84 ± 0.25 (range 0.42 - 2.71) mg/kg dw) (Carravieri et al., 2018). Similarly, Ibañez et al. (2022) the survival of chicks was unrelated with mercury in blood of breeders (around ca. 0.8 mg/kg dw) of brown skua *Stercorarius antarcticus*, suggesting that mercury contamination is currently not a major conservation concern for this population.

Finally, the only such studies reporting probability of reduced fledging success has been reported on wandering albatrosses (Goutte et al., 2014a) and in grey-headed albatrosses *Thalassarche chrysostoma* (Mills et al., 2020). Goutte et al. (2014a), reported that levels of mercury in blood of wandering albatrosses parents were related with a decreased probability of fledging. Mills et al. (2020), reported that chicks from males of grey-headed albatrosses with higher levels of mercury in feathers (14.96 ± 9.05 mg/kg dw) were more likely to die. However, the authors assume the need for more studies in the future.

There is a growing awareness that resistance to methylmercury toxicity is closely linked also to various physiological mechanisms/reactions, such as the interaction between mercury and selenium (Berry and Ralston, 2008). Selenium is a nutritionally essential element with particularly important roles in brain and endocrine tissues (Berry and

Ralston, 2008). Recently, advances in the application of high-resolution energy X-ray absorption spectroscopy (HR-XANES) in wildlife and seabirds have identified that methylmercury is detoxified to non-toxic mercury selenide (Manceau et al., 2021a). Manceau et al. (2021b), reported that this mechanism may be more efficient in seabirds than in land birds because selenium is naturally abundant in seawater, so individuals probably never fall below metabolic requirements for selenium, which is essential for mercury detoxification.

3.5. CONCLUSION

Historical and recent findings concerning mercury pollution in seabirds, as described by researchers, need to be further explored to provide more data and sound conclusions. Mercury pollution was partially recognised as a major risk to human and wildlife health in the 1980s of the 20th century (Doi et al., 1984). However, mercury pollution has been side lined by public opinion and is currently not a source of major concern for policy makers.

Seabirds are top predators in oceanic food chains and are therefore subject to high concentrations of methylmercury. However, the relationship between mercury pollution and seabird health and fitness remains poorly understood. We conclude that seabird survival is not currently strongly affected by mercury, and consequently neither are their population dynamics. However, few studies address or study the long-term effects of mercury on populations.

Thus, future sampling should consider other seabird colonies that have high body mercury content, and that can contribute to understanding the impact of mercury on their survival.

Table 3.1. Summary of toxicity benchmarks for the effects of methylmercury exposure on survival of adult seabirds.

Common name /Scientific name	Original tissue Hg concentration					Blood-equivalent Hg (ug/g, ww)		Hg toxic effect	Study
	Body Feathers		Blood		Units	Mean	Range		
	Mean	Range	Mean	Range					
great skuas *Catharacta skua*	7.0	1.2 – 32.4	-	-	ug/g, fresh weight	0.7	0.2 – 2.0	Not detected	Thompson et al., 1991
leach’s storm-petrels *Oceanodroma leucorhoa*	-	-	0.8	0.2 – 2.3	ug/g, ww	0.8	0.2 – 2.3	Not detected	Pollet et al., 2017
wandering albatross *Diomedea exulans*	24.0	6.0 – 95.0	-	-	ug/g, dw	1.6	0.6 – 4.0	Not detected	Bustamante et al., 2016
brown skuas *Stercorarius antarcticus*	-	-	8.2	-	ug/g, dw	1.7	-	Not detected	Goutte et al., 2014b
south polar skuas *Catharacta maccormicki*	-	-	2.1	-	ug/g, dw	0.4	-	Not detected	
little auks *Alle alle*	-	1.0 – 1.8	-	-	ug/g, dw	-	0.2 – 0.3	Not detected	Amélineau et al., 2019
cory's Shearwaters *Calonectris borealis*	5.3	ca. 1.5- 12.0	-	-	ug/g, dw	0.6	-	Not detected	Bauch et al., 2022

Table 3.2. Summary of toxicity benchmarks for the effects of methylmercury exposure on survival of chick seabirds.

Common name /Scientific name	Age Sampled	Original tissue Hg concentration						Blood-equivalent Hg (ug/g, ww)		Hg toxic effect	Study
		Body Feathers			Blood			Mean	Range		
		Mean	Range	Units	Mean	Range	Units				
great skuas *Catharacta skua*	Chicks	1.3	0.7 – 2.4	ug/g, fw	-	-	-	0.2	0.1 – 0.3	Not detected	Thompson et al., 1991
wandering albatross *Diomedea exulans*	Breeders	-	-	-	-	2.0 – 18.7	ug/g, dw	-	0.4 – 3.9	Parcially detected	Goutte et al., 2014a
forster's terns *Sterna forsteri*	Chicks	6.4	ca. 3.0 – 17.0	ug/g, fw	0.3	-	ug/g, ww	0.3	-	Not detected	Ackerman et al., 2008
grey-headed albatrosses *Thalassarche chrysostoma*	Breeders	13.0	2.0 − 35.1	ug/g, dw	-	-	-	1.1	0.3 – 2.1	Parcially detected	Mills et al., 2020
Antarctic petrels *Thalassoica antarctica*	Breeders	-	-	-	0.8	0.4 – 2.7	ug/g, dw	0.2	0.1 – 0.6	Not detected	Carravieri et al., 2018
	Breeders	2.4	1.2 – 4.1	ug/g, dw	1.4	0.7 – 3.1	ug/g, dw	0.3	0.1 – 0.7	Not detected	Carravieri et al., 2021
brown skuas *Stercorarius antarcticus*	Breeders	-	-	-	0.8	0.4 – 2.3	ug/g, dw	0.2	0.1 – 0.5	Not detected	Ibañez et al., 2022

CHAPTER 4

BODY FEATHER MERCURY AND ARSENIC CONCENTRATIONS IN FIVE SPECIES OF SEABIRDS FROM THE FALKLAND ISLANDS.

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4.1. ABSTRACT

Several pollutants, including heavy metals, magnify along the food chain, and top predators such as seabirds can be used to monitor their trends in the marine environment. We studied mercury and arsenic contamination in body feathers in penguins, petrels and cormorants in three islands of the Falklands Islands. There were significant differences among species and sites in the concentration of trace elements in feathers. Black-browed albatrosses and gentoo penguins had consistently high mercury concentrations on New Island, while Rockhopper penguins and imperial shags presented considerably higher concentrations at Beauchene Island. Mercury levels in black-browed albatrosses increased since 1986 on one of the islands, probably reflecting world-wide emission trends. Rockhopper penguins exhibited high arsenic levels, but levels were less variable among species, and were not correlated with mercury levels, suggesting low biomagnification. These results provide a reference line for bioindication studies using feathers from species on the Falkland Islands.

4.2. INTRODUCTION

The levels of bioaccumulation of mercury and arsenic are important indicators of environmental quality and animal health (Metcheva et al., 2011; Scheuhammer et al., 2007). Mercury is a toxic metal (Kim et al., 1996) whereas arsenic is a metalloid, which has both properties of non-metals and of metals, and it is usually considered as a non-essential element, occupying top positions in lists of toxicants (Sánchez-Virosta et al., 2015).

Mercury is commonly present in the environment because of natural sources but can be also be regarded as a contaminant when originating from human activities (including gold extraction, waste incineration and use of fossil fuels) (Driscoll et al., 2013; Esdaile and Chalker 2018). Mercury concentrations vary spatially across the oceans (Lamborg et al., 2014). Currently, there is a global ocean inventory of anthropogenic mercury based on water column measurements that report the increase of mercury concentration in oceanic waters, compared to pre-anthropogenic conditions (Driscoll et al., 2013; Lamborg et al., 2014). The remote Antarctic waters exhibit some of the highest methylated mercury species concentrations observed in open ocean, notably within the Antarctic Zone, and Southern Zone (Cossa et al., 2011). The methylated mercury species concentrations tend to be higher in the Antarctic Bottom Water compared to the Antarctic Intermediate Water (Cossa et al., 2011). Mercury is a persistent and bioaccumulative metal in wildlife (Kim et al., 1996) and its concentrations are magnified through the food web, thus levels in marine top predators are often much higher magnitude than those in the water (Santos et al., 2006).

Arsenic is a trace element that can be toxic, depending on its chemical form and concentration (Rahman et al., 2012). The main sources of pollution by this metal are mining-related activities, pesticides, coal burning and wood-preserving arsenicals (Garelick et al., 2008; Kubota et al., 2003). Non-ferrous metal production, particularly copper smelting processes, is the most important anthropogenic contributor to arsenic emissions (Pacyna and Pacyna, 2001). The concentration of inorganic forms of arsenic appears to be higher in the Antarctic waters than in the other areas from the oceans (Cutter et al., 2001; Santosa et al., 1994). Arsenic concentration in Southern Ocean exhibits typical values 0.88 to 1.03 ug L^{-1} for the uncontaminated offshore waters (Grotti and Frache, 2007).

Recently, Trevizani et al (2018) demonstrated a negative correlation between trophic level and concentration of arsenic in the benthic trophic web of Antarctica. Arsenic can have negative effects on a diversity of organisms, especially with lower trophic levels because of the incidence of biodilution processes along the food webs (Hayase et al., 2010; Huang, 2016). However, reports of arsenic biomagnification and bioaccumulation, are not consistent in the literature (Majer et al., 2014; Rahman et al., 2012).

Seabirds are among the animals that display higher concentrations of mercury and arsenic in their bodies. Since they occupy high trophic positions, they are more susceptible to elevated trace elements concentrations, compared to other groups within the marine food web (Diamond and Devlin, 2003). They ingest methylmercury and arsenic through their food, and eliminate part of that through feathers during their growth, and also through excreta and eggs (Monteiro and Furness 1995; Thompson and Furness, 1989).

Feathers of seabirds have been used as bioindicators of diverse contaminants in marine environment (Carravieri et al., 2013, 2016; Cherel et al., 2018; Furness and Camphuysen 1997). During the feather growth (2 or 3 weeks), metals incorporate in the keratin structure (reflecting levels in blood during the period of feather growth) (Bearhop et al., 2000). Mercury has an affinity to the sulphydryl groups and arsenic compounds binds strongly for the thiol groups of keration in feathers (Appelquist et al., 1984; Murphy et al., 1990).

The consequences of trace elements contamination are difficult to assess for wild seabirds. Presence of mercury (1 – 40 mg/kg) in waterbirds tissues have been shown in some studies to be associated with negative consequences, including reduced reproductive success (reduced hatchability due to increases in early mortality of embryos, reduced clutch size, embryonic deformity), flight feather asymmetry and increased numbers of nematode parasites (Dietz et al., 2013; Evers et al., 2008; Wayland et al., 2001; Wolfe et al., 1998). The presence of mercury has also been reported to have negative effects in some species of seabirds, resulting in low body condition index and decreased breeding output (later hatching date, lower hatching success and chick survival) (Bond et al., 2015; Dietz et al., 2013; Finkelstein et al., 2007; Goutte et al., 2014; Tartu et al., 2015). However, recent studies provide conflicting evidence by showing that the presence of mercury in feathers, blood and eggs (> 1 mg/kg) did not affect reproductive parameters (e.g., hatching date, return rate), body condition and short-term reproductive output (Braune et al., 2016; Bustamante et al., 2016; Carravieri et al., 2017; Goutte et al., 2015; Pollet et al., 2017).

In relation to arsenic, field observations and experimental studies in passerines have revealed behavioural changes at elevated arsenic concentrations, such as increased aggressiveness during the incubation period, smaller song repertoire sizes and lower singing activity, reduced food consumption, increased resting time and negative effects on reproduction including decreased clutch and brood size, reduced hatching and reduced overall breeding success (see review Sánchez-Virosta et al., 2015). Nevertheless, the effects of arsenic in seabirds is not documented, but there are some studies about arsenic concentration levels in tissues (Anderson et al., 2010; Jerez et al., 2013).

The Patagonian Shelf Large Marine Ecosystem is one of the most productive areas in the world's oceans, with important human fisheries and very significant gatherings of resident and migratory marine predators of conservation concern (Augé et al., 2018; Croxall and Wood 2002; White et al., 2002). There are few studies of toxic metals concentrations in marine predators in this region (but see Becker et al., 2016; Brasso et al., 2015; Carravieri et al., 2017). Analysis of mercury concentrations in feather samples collected before 50's of the past century and after this date showed slight, but significant, increases in ca. 30% of sub-Antarctic procellariiform species (n = 10 species, Thompson et al., 1993). Data on mercury levels in seabirds from the Falkland Islands are generally scarce and relates to black-browed albatrosses at a single location (Thompson et al. 1993).

The goal of this paper was to determine mercury and arsenic concentrations in body feathers in five species of seabirds of Falkland Islands, filling a gap in the knowledge about current contamination in the Patagonian shelf large marine ecosystem and providing a baseline for future comparisons. In this study we sampled different species and locations in the Falklands, to evaluate the inter-species and spatial variability within this region.

4.3. METHODS

Feather sampling was carried out in the Falkland Islands (51°S 59°W) in the Southwest Atlantic (see supplementary material Fig. B1) during December 2016 and January 2017. We sampled adult birds during the period of egg hatching, when no feather moult takes place. Body feathers (ca. 10) were collected from five species on three islands (Fig. 4.1) of the archipelago (New Island, Beauchene Island and Kidney Island). Feathers were sampled from one species of cormorant (Phalacrocoracidae): imperial Shag *Phalacrocorax atriceps*; two species of penguins (Sphenisciformes): gentoo *Pygoscelis papua* and rockhopper penguin *Eudyptes chrysocome*; and two species of petrels (Procellariiformes): black-browed albatross *Thalassarche melanophris* and thin-billed prion *Pachyptila belcheri*. Sample sizes used for mercury analyses are given in Table 4. 1.

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 1.00 mg and 4.71 mg. We set a maximum coefficient of variation of 10% in a minimum of 3 mercury reading (see supplementary material Fig. B2). Precision of the method were supported on the analysis of certified reference material: SRM - 2976 (muscle tissue; certified value = 0.061 ± 0.0036 mg/kg) that gave recovery efficiencies of 85.4 ± 3.1%, n = 30.

The evaluation of arsenic in feathers involved a microwave-assisted acid digestion in high pressure Teflon closed vessels (XP-1500), of ca. 200 mg of finely cut feathers. Quality control of each digestion was assessed by measuring a blank sample (reagent mixture) and a certified reference material (CRM – TORT-II).

The digestion occurred in a two-step process: 1) addition of 1 mL of HNO_3, 1 mL of H_2O and 1 mL of H_2O_2, followed by a waiting period of 15 minutes. The vial was then cold and submitted to the 1st digestion cycle; 2) after cooling down to room temperature, addition of 1 mL H_2O_2 and promptly close the vessels to run the 2nd digestion cycle. Both microwave (CEM MARS 5) cycles followed the same temperature ramp program: 1) rise Temperature to 135 ºC in 4 min; 2) rise T to 195 ºC in 5 min; 3) hold T at 195 ºC for 4 min.

The liquid sampled resultant from the acid digestion was collected for a final volume of 25 mL in ultra-pure water (MQ filtration system) and directly analyzed by ICP-MS (Thermo X Series). The experimental conditions for ICP-MS analysis were as follows: forward power 1400 W, peak jumping mode, 150 sweeps per replicate, dwell time 10 minutes and dead time 30 minutes. The polyatomic and isobaric interferences were minimized by setting the ratios $^{137}Ba^{2+}/^{137}Ba$ and $^{140}Ce^{16}O/^{140}Ce$ to 0.02, under routine operating conditions. The internal standard was ^{115}In. Analysis quality assurance procedures included a 7-point calibration curve and the verification of procedural blank samples at each batch of 10 samples, which accounted for less than 1% of the element's concentrations. The limits of quantification (LOQ) for arsenic was calculated by analyzing repeated blanks and the same procedure used for samples, and by subtracting the mean by the standard deviation (SD) of the values multiplied by three.

All statistical analyses were carried out with the R package. Means are presented with standard deviations. Differences in levels of mercury and arsenic among seabird species on New Island were evaluated using analysis of variance (One-way ANOVA). Islands differences in elements were evaluated by Two-way ANOVA followed by Tukey post hoc tests. To test the biomagnification behaviour of As, we tested the correlation between this element and mercury concentrations in body feathers (since this trace element is known to biomagnify across the food web).

L4.4. RESULTS

There were significant differences in the mercury concentration in body feathers among the five seabird species on New Island (Table 4.1, ANOVA: $F_{4,98} = 38.51$, $p < 0.001$). On New Island, black-browed albatross and gentoo penguins presented the highest concentrations of mercury, which differed significantly from those of prions and imperial shag, showing the lowest mean mercury values. There were also significant differences in the mercury concentration among some species on different islands (Two-way ANOVA: $F_{3,151} = 14.88$, $p < 0.001$, island effect) (Fig. 4.2). Post hoc Tukey tests indicated that mercury concentrations for rockhopper penguins and imperial shags were higher on Beauchene Island, but the same did not apply for black-browed albatrosses and gentoo penguins.

In case of arsenic, there were significant differences in concentration in body feathers of different species on New Island (ANOVA: $F_{4,84} = 35.30$, $p < 0.001$). Prions and imperial shags had the highest concentrations of arsenic on New Island. There were also significant differences in the arsenic concentration between species of different islands (Two-way ANOVA: $F_{3,105} = 32.67$, $p < 0.001$, island effect) (Fig. 4.3). Post hoc Tukey tests indicated that arsenic concentration for rockhopper penguins was higher on Beauchene Island. Imperial shag, black-browed albatrosses and gentoo penguins had the same mean concentrations of arsenic on both places.

After accounting for the effects of species and study site, we did not find any correlation between arsenic and mercury concentrations (ANCOVA: $F_{1,139} = 1.18$, $p = 0.28$).

4.5. DISCUSSION

In this study we evaluated the concentration of mercury and arsenic in five species in three distinct sites of the Falkland Islands (Table 4.1). The mean mercury concentration decreased on New Island in the order black-browed albatross > gentoo penguins > rockhopper penguins > imperial shags > prions. The mean arsenic concentration decreased on New Island in the order imperial shags > prions > rockhopper penguins > black-browed albatross > gentoo penguins. Ours is the first study to evaluate the concentration of arsenic in body feathers of rockhopper penguins and imperial shags.

Increasingly studies are linking sublethal concentrations of mercury to negative effects in a wide range of birds (Whitney and Cristol, 2018). Some studies have reported that mercury concentrations in feathers of waterbirds between 5 and 40 mg/kg can have negative impacts, expressed by changes in reproduction, by neurodevelopmental or behavioural deficits (e.g., Evers et al., 2008; Scheuhammer et al., 2007; Wolfe et al., 1998). Overall, mercury levels in feathers of seabirds analyzed in this study are below the 5 mg/kg threshold in 96.0% of the individuals sampled (n = 206), and the highest value recorded in our sample was 6.65 mg/kg (in a black-browed albatross). Few studies investigating relationships between mercury and breeding, or survival have found a suggestion for an impact of contamination in seabirds, which may suggest that seabirds display a high resistance to mercury contamination (Carravieri et al., 2017; Goutte et al., 2015; Pollet et al., 2017; Thompson et al., 1991). Nevertheless, the are reports of possible negative impacts (Bond et al., 2015; Braune et al., 2006; Dietz et al., 2013; Finkelstein et al., 2007; Goutte et al., 2014a, b; Tartu et al., 2013, 2014, 2015) and the issue clearly needs more study.

The only previous study on mercury in feathers of Falkland seabirds dates back to 1986 and reports a concentration of mean 2.68 ± 1.20 mg/kg (range 1.00 – 6.64 mg/kg) in black-browed albatrosses (n = 30) (Thompson et al., 1993). Comparing with the values obtained in this study for this species (3.38 ± 1.24 mg/kg, n = 39), there is a significant increase in mercury concentration in 30 years (t = 2.367, df = 63, $p = 0.021$). The causes of temporal differences in mercury contamination are difficult to determine due to a lack of long-term data related to black-browed albatross diet and trophic levels. Other studies with seabirds in the Southern Ocean have similarly found an increase in mercury levels, which might indicate a temporal increase in mercury bioavailability in this region (Becker et al., 2016; Carravieri et al., 2017).

Mercury is known to biomagnify in marine food chains and hence seabirds at higher trophic levels should display higher mercury concentrations. Studies on New Island based on stable isotopes indicated that gentoo penguins and black-browed albatross have a higher trophic level, rockhopper penguins have intermediate trophic level and thin-billed prions have a lower trophic (Granadeiro et al., 2014; Weiss et al., 2009), which is consistent with the high and low levels of mercury concentrations measured in those seabird species by our study. However, imperial shags present a relatively high trophic level (Weiss et al., 2009) and nevertheless show low mercury contamination.

In Falkland archipelago, different colonies of seabirds exhibited different levels of trace elements contamination. All seabird species examined start moulting their body feathers more or less in the same period (end of the breeding season, starting in January/February (Bernstein and Maxson, 1981; Brooke, 1985; Catry et al., 2013b; McMahon et al., 2015; Quillfeldt et al., 2015). Therefore, differences in trace elements contamination of seabird species among islands can result from differences in the local bioavailability of mercury, differences in foraging areas or differences in food preferences (type of prey) at various breeding sites (Becker et al., 2016; Carravieri et al., 2013).

Rockhopper penguins and imperial shags from Beauchene had significantly higher mercury levels than at New Island. During the breeding season, rockhopper penguins feed around the colonies (c.a 10 – 100km) (Boersma et al., 2002; Ludynia et al., 2013; Pütz et al., 2003). The non-breeding distribution of rockhopper penguins (from New and Kidney Island) was similar to southern rockhopper penguins from Steeple Jason Island (Ratcliffe et al., 2014; Pütz et al., 2002), with birds mainly wintering along Patagonian Shelf in the Subtropical Zone. Contrary, rockhopper penguins of Beauchene Island mostly occur in the Subantarctic Zone and Polar Frontal Zone, in deeper waters (Ratcliffe et al., 2014). Furthermore, rockhopper penguins from Beauchene, during the breeding season feed on mesopelagic preys (Clausen and Pütz, 2002; Croxall et al., 1985). Because mesopelagic organisms generally have higher concentrations of mercury, it is likely that the high levels of mercury found on Beauchene reflect the use that rockhopper penguins there make of deep ocean environments and associated prey.

The same could to some extent apply to imperial shags. During the breeding season imperial shags feed around the nesting sites (Weiss et al., 2009), making trips up to the maximum distances of 30 km (Quillfeldt et al., 2011). Beauchene Island is very near to the outer edge of the Patagonian shelf (ca. 18 Km). This implies that imperial shags from

Beauchene can feed in deeper waters of over the *Patagonian slope* in contrast with those from New Island, which are limited to shelf waters. Hence, imperial shags on Beauchene may have access to prey from deeper water food chains which may accumulate more mercury.

Black-browed albatrosses from Beauchene and New Island extensively overlap their foraging areas over the Southern Patagonian shelf, namely around Staten Island, and hence it is expectable that mercury levels are similar in the two colonies, as found here (Catry et al., 2013a; and own unpublished data).

Arsenic exists in the ocean as arsenate and arsenite forms, and arsenite has been considered to be more toxic when compared with arsenate (Singh et al., 2011). Arsenate is the predominant form of arsenic in sea-water (Santosa et al., 1994). Arsenic biomagnification has been reported mainly in the form of lipid-soluble arsenic (Hayase et al., 2010; Huang, 2016). Arsenolipids are assumed to be finish products of arsenate, involving reduction and oxidative methylation and adenosylation (Dembitsky and Levitsky, 2004). Our results indicate that, in contrast to mercury, the species with high arsenic concentrations are the ones known to feed as low trophic levels on the Patagonian shelf, which suggests that there is little or no arsenic biomagnification along this food web (Weiss et al., 2009)

Rockhopper penguins were the only species to show differences in arsenic concentration between locations, with higher levels at Beauchene Island than at New Island and Kidney Island. One possible explanation for this difference may be in the increase of arsenic pollution in offshore waters, as rockhopper penguins from Beauchene forage during part of the year over deep-sea waters of the Subantarctic Zone and Polar Frontal Zone (Ratcliffe et al., 2014). Arsenic present in waters (arsenate and arsenite) in South Atlantic Ocean is depleted in the surface waters and increases to relatively constant deep-water concentrations (Cutter et al., 2001; Cutter and Cutter, 1995;). In the deeper waters (Antarctic Bottom Water, ca.5000 m), arsenite and arsenate displays constant and high concentrations (19.3 ± 0.3 nmol/l) (Cutter et al., 2001). The concentration of arsenite in the Antarctic Intermediate Water and Upper Circumpolar Deep Water (ca. 750–1500 m) is found to be comparatively higher than in the other areas of ocean (Cutter et al., 2001; Santosa et al., 1994).

There have been previous studies of mercury in the species treated here in the Southern Ocean (Anderson et al., 2009; Becker et al., 2002, 2016; Brasso et al., 2014, 2015; Calle et al., 2015; Carravieri et al., 2014, 2016; Pedro et al., 2015). The values obtained in this study in comparison with other studies are intermediate (see supplementary material Table B1). There are few studies documenting arsenic on feathers of seabirds (Anderson et al., 2010; Fromant et al., 2016; Jerez et al., 2011, 2013; Metcheva et al., 2006) (see supplementary material Table B2), and our research contributes to start filling this important gap.

This study establishes baseline levels for mercury and arsenic in seabirds of the Southern Patagonian shelf that will be useful for future monitoring. High concentrations of these metals were found in rockhopper penguins populations considered here, particularly at Beauchene Island. This warrant further research to determine if such levels are impacting reproduction or survival and to determine the mechanisms responsible for such bioaccumulation. Global mercury emissions are predicted to keep increasing in the next decades (Streets et al., 2009), and the observed increase contamination detected in black-browed albatrosses may reflect such patterns in these birds, which occupy the highest trophic levels in this area.

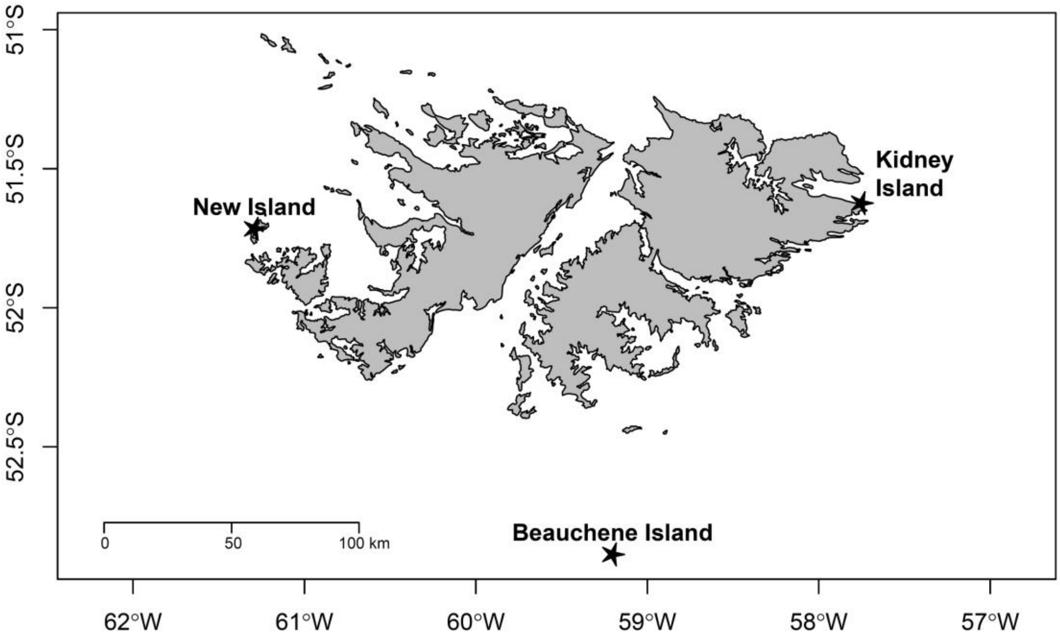

Fig. 4.1 Geographical location of sampling sites at the Falkland Islands in the *South Atlantic* Ocean.

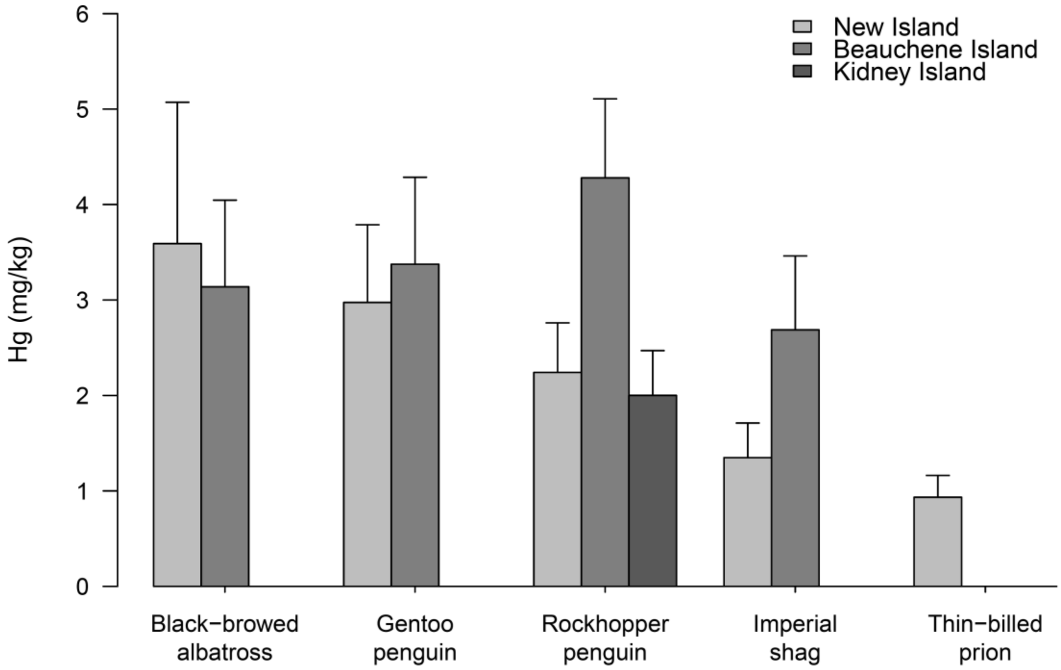

Fig. 4.2 Body feather mercury concentrations (mean ± SD) in 5 species from Falkland Islands.

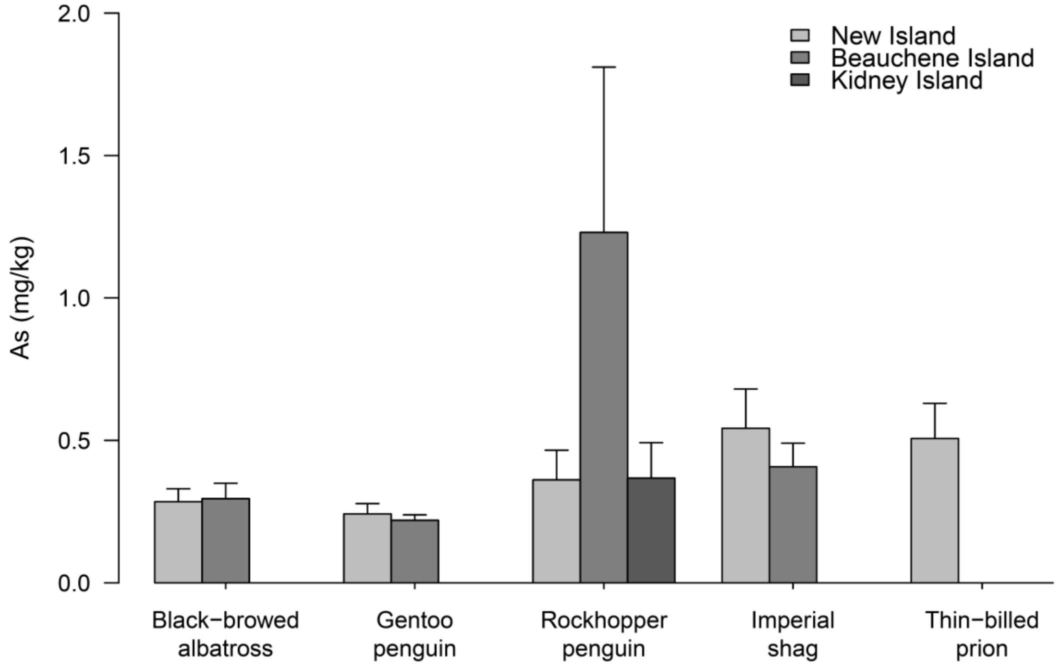

Fig. 4.3 Body feather arsenic concentrations (mean ± SD) in 5 species from Falkland Islands.

Table 4.1 Total mercury and arsenic levels (mg kg^{-1}) in body feathers of seabirds from the Falkland Islands.

		[Hg] (mg kg^{-1})			[As] (mg kg^{-1})		
Species	Location	Sample size	Mean ± SD	Range	Sample size	Mean ± SD	Range
Gentoo penguin *Pygoscelis papua*	New Island	20	2.97 ± 0.81	1.77 – 4.56	20	0.24 ± 0.04	0.19 – 0.31
	Beauchene Island	20	3.37 ± 0.91	1.99 – 4.96	2	0.22 ± 0.01	0.21 – 0.23
Rockhopper penguin *Eudyptes chrysocome*	New Island	20	2.24 ± 0.52	1.43 – 3.18	15	0.36 ± 0.10	0.24 – 0.63
	Beauchene Island	20	4.28 ± 0.83	2.67 – 5.79	13	1.23 ± 0.56	0.98 – 2.90
Imperial shag *Phalacrocorax atriceps*	Kidney Island	25	2.00 ± 0.47	1.21 – 3.37	19	0.37 ± 0.12	0.23 – 0.78
	New Island	20	1.35 ± 0.36	0.83 – 2.07	20	0.54 ± 0.13	0.34 – 0.99
	Beauchene Island	20	2.69 ± 0.77	1.36 – 4.36	20	0.41 ± 0.08	0.29 – 0.62
Black-browed albatross *Thalassarche melanophris*	New Island	21	3.59 ± 1.48	1.80 – 6.65	19	0.28 ± 0.04	0.20 – 0.36
	Beauchene Island	18	3.14 ± 0.91	1.75 – 4.56	4	0.30 ± 0.05	0.25 – 0.36
Thin-billed prion *Pachyptila belcheri*	New Island	22	0.93 ± 0.23	0.65 – 1.47	15	0.51 ± 0.12	0.37 – 0.85

CHAPTER 5

TRACE ELEMENTS’ REFERENCE LEVELS IN BLOOD OF BREEDING BLACK-BROWED ALBATROSSES *THALASSARCHE MELANOPHRIS* FROM THE FALKLAND ISLANDS

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5.1. ABSTRACT

Trace elements’ concentration in the ocean is fast growing and is a source of major concern. Being charismatic and at the top of food chains, seabirds are often used as biological monitors of contaminants. We studied the concentration of trace elements in blood of black-browed albatross from the Falklands Islands, which we here show, by tracking with Geolocators, forage over most of the Patagonian Shelf. Levels of trace elements were measured in males and females from two different islands. Blood concentrations of trace elements were not significantly different between islands, which is consistent with observations from foraging behaviour revealing that birds from both islands foraged in broadly the same areas in the months before sampling. Arsenic and selenium concentrations in females were higher than in males. Sex-related differences in the concentration of these elements may be related to unknown slight differences in diet or to differences in assimilation between sexes. These results provide reference values for monitoring elemental contamination in the Patagonian Shelf Marine Ecosystem using black-browed albatrosses, one of the most abundant top predators and a suitable sentinel for the region’s environmental health.

5.2. INTRODUCTION

Contamination by trace elements is growing at a fast pace and is an issue of major concern for the health of the environment and organisms (Scheuhammer 1987; Sánchez-Virosta et al. 2015; Evers 2018; Preeti et al. 2018). Plants and animals often assimilate and bioaccumulate trace elements in a predictable manner and therefore some taxa have been successfully used as biological monitors of elemental pollution in marine and terrestrial ecosystems (Anderson et al. 2010; Yildirim and Sasmaz 2017; Furtado et al. 2019).

Several trace elements (cobalt, copper, manganese, selenium, and vanadium) are essential components of physiological and biochemical processes of organisms (e.g. vertebrates), but beyond certain levels of concentration these elements also become toxic (Abdulla and Chmielnicka 1990; Senesi et al. 1999; Bansal and Asthama 2018) Others, such as arsenic, cadmium, mercury, and strontium , are poorly tolerated by the organisms and are usually considered as a non-essential elements for life and are known to be toxic even at low concentration levels (Naidu et al. 1999; Senesi et al. 1999; Bansal and Asthama 2018). Arsenic is considered as one of the most highly toxic and carcinogenic elements by the US Environmental Protection Agency (EPA) (ATSDR 2020).

The Patagonian Shelf Large Marine Ecosystem, in the Southern Ocean, is one of the most productive marine areas worldwide (Croxall and Wood 2002). Here currents and mixing, including localised upwelling, contribute to resuspension of sediments and organic particles, resulting in a natural redistribution of trace elements, notably those behaving like nutrients (e.g. copper and selenium) (Orren and Monteiro 1985; Cutter and Cutter 1995; Zhang et al. 2014; Gworek et al. 2016). Elements such as mercury are massively transported by the global atmospheric circulation and other such as cadmium, cobalt, lead and manganese, are distributed through oceanic circulation. Such inflows to what is present in the Southern Ocean determine the concentration of elements in this marine ecosystem (Chester 1990; Gaiero et al. 2003; Bargagli 2008; Cossa et al. 2011). Moreover, several anthropogenic sources (sewage effluents, agricultural runoff, oil extraction and transportation and mining) contribute to contamination by metals and metalloids in the ocean, including water and sediments (Gaiero et al. 2002).

The distribution of trace elements in marine waters is complex (Boye et al. 2012). Trace elements are not distributed uniformly in the ocean, and their concentration vary among areas as well as vertically within the water column (Cutter and Cutter 1995; de Villiers 1999; Bown et al. 2011; Cossa et al. 2011; Boye et al. 2012; Heller and Croot 2015; Schlesinger et al. 2017). Such variability in the distribution of trace elements causes different assimilation patterns within food webs (Santos et al. 2006). Moreover, elements such as cadmium, cobalt, copper, manganese, strontium and vanadium are accumulated by organisms of different trophic levels, but do not biomagnify (even in top predators, such as seabirds) because biodilution processes increase significantly with trophic position (Campbell et al. 2005; Nfon et al. 2009; Konovalenko et al. 2016; Liu et al. 2016, 2017, 2019; Signa et al. 2019). Conversely, the extent of arsenic and selenium biomagnification through the food web is a complex issue and is still a matter of scientific debate (Campbell et al. 2005; Mathews and Fisher 2008; Nfon et al. 2009; Stewart et al. 2010; Huang 2016; Furtado et al 2019). Selenium is transferred in food chains, and biomagnification does occur (Rainbow 2018). Stewart et al (2010) proposed a "trophic transfer function" to describe increased selenium concentrations in marine food webs, because transfer from prey to predator is dependent on concentration in a non-linear manner. Arsenic, usually in its inorganic forms, decrease with increasing trophic levels, but organic arsenic, especially arsenobetaine, increases (see review Huang 2016). The behaviour of mercury is well-known, and it biomagnifies through the food chains, thus top predators generally show higher mercury levels than primary producers (Santos et al. 2006).

Seabirds are top marine predators known to accumulate a variety of trace elements present in marine environment (Anderson et al. 2010; Tavares et al. 2013; Carravieri et al. 2014, 2018). Those are accumulated in different tissues such as blood, feathers, liver, kidney and muscle (Anderson et al. 2009; Aazami et al. 2011). Blood has been widely used to assess medium- to short-term variations in element concentrations of trace elements in the environment, reflecting accumulation through diet over approximately one to two months preceding sampling in seabirds (Hobson and Clark 1992, 1993; Carleton and Martínez del Rio 2005; Zanden et al. 2015). This period vary among taxa according to metabolism and it can be higher in some other vertebrates such as reptiles (Rosenblatt and Heithaus 2013).

The Falkland Islands are located in the Southwest Atlantic Ocean, where the level of trace elements bioavailability is poorly documented (Furtado et al. 2019). Black-browed albatrosses *Thalassarche melanophris* from the Falkland Islands mostly forage in the Subantarctic Zone, over the rich waters of the Patagonian Shelf (Granadeiro et al. 2017; Ponchon et al. 2019). Furthermore, birds are relatively sedentary, remaining in the Patagonian shelf and shelf-break also during the non-breeding season (Ponchon et al.

2019). Globally, the species is currently classified as Least Concern according to the IUCN (IUCN, 2020).

The levels of bioaccumulation of trace elements are important indicators of environmental quality and health of seabirds (Nicholson and Osborn 1983; Anderson et al. 2010; Sánchez-Virosta et al. 2015; Evers 2018). Seabirds are generally abundant at their nesting colonies, forage over wide oceanic areas, they are often easy to capture and can be sampled with minimal disturbance. Blood is a good tissue to monitor levels of several trace elements over the Patagonian Shelf Large Marine Ecosystems during the breeding season. An estimated 67.0% - 70.0% of the global population of this albatross species nests in the Falkland Island (ACAP 2009), with a total population in excess of 475,500 pairs (Wolfaardt 2013). Being well-known charismatic top predators in Patagonian Shelf ecosystem, black-browed albatrosses have the potential to be used as biological monitors and to communicate environmental issues to decision-makers and the general public.

The goal of this research was to establish baseline levels of trace elements in blood of black-browed albatrosses, as samplers of the Patagonian Shelf Large Marine Ecosystem, during the breeding period . We determined trace elements concentrations in blood samples of birds from Beauchene and New Island, two islands located in the South and West of the Falkland Islands, respectively, roughly reflecting accumulation between October and December 2016. We also assessed the spatial distribution of birds from both islands during this period. Finally, we tested whether there were differences in the accumulation of trace elements among sexes, as found in other waterbirds and in albatrosses for cadmium (e.g. Carravieri et al. 2014), mercury (e.g. Tavares et al. 2013) and selenium (e.g. Wilson et al. 2004).

5.3. METHODS

5.3.1. STUDY SITES AND TRACE ELEMENTS CONCENTRATION SURVEY

Blood sampling was carried out during December 2016 and early January 2017. We collected a small amount of blood (< 2.0 ml) from 20 adult black-browed albatrosses breeding on New Island (51°43'S, 61°19'W) and 18 birds from Beauchene Island (52°50' S, 59°10' W). We took a small aliquot of each sample into a different container for sex determination using molecular methods. The sampling procedure took less than 5 minutes to complete, and all birds resumed their incubation and brooding duties after being released at the nest.

Samples for elements determination were lyophilized during 48 h and ground to powder for homogenization, before digestion. All samples were digested following an adaptation of the EPA method 3051A (Link et al. 1998). Blood samples were digested using a CEM MARS 5, model 240/50 microwave digestion system, with continuous temperature and pressure monitoring in a tightly closed vessel. Closed vessels also minimize potential losses of analytes due to volatilization (Link et al. 1998). Previously, PFA vessels were decontaminated in the microwave system using 3.0 ml of HNO_3, and then rinsed with ultrapure water and air-dried. Approximately 50.0 mg (overall range: 4.1 – 190.3 mg) of sample were weighted in PFA digestion vessels and subsequently we added 0.4 mL of nitric acid (HNO_3). The microwave was programmed for 5 minutes, at 1600 W and 165 ºC, followed by 5 minutes at 1600 W and 190 ºC. The PFA vessels were then placed in a plate heater, and the contents evaporated to near dryness at 150 ºC. Finally, the residue was dissolved in 0.4 mL of hydrogen peroxide (H_2O_2), and the solutions heated for 30 minutes, at 150 ºC. After cooling at room temperature, the solutions were transferred into 10.0 mL volumetric flasks and then filled with ultrapure water (MQ filtration system) to the 10.0 mL and then analysed by high resolution ICP-MS (Thermo Elemental, X-Series). The experimental conditions for ICP-MS analysis were as follows: forward power 1400 W, peak jumping mode, 150 sweeps per replicate, dwell time 10 minutes and dead time 30 minutes. The polyatomic and isobaric interferences were minimized by setting the ratios $^{137}Ba^{2+}/^{137}Ba$ and $^{140}Ce^{16}O/^{140}Ce$ to 0.02, under routine operating conditions. The internal standard was ^{115}In. For quality control, the certified reference material NIST-SRM 2976 (mussel tissue) and TORT-2 (Lobster Hepatopancreas) were analysed simultaneously (see results in Supplementary Table C1).

Among trace elements, arsenic, mercury, selenium, and strontium were quantified in all individuals, while cadmium, cobalt, manganese and vanadium could only be detected in a few black-browed albatrosses. The limit of quantification (LoQ), was calculated as ten times the standard deviation (SD) of the blank divided by the slope of the analytical curve, respectively (Taleuzzaman 2018), expressed in concentration (ug/L). The limit of quantification was converted to mg/kg dry weight per sample (Supplementary Table C2 and Supplementary Table C3).

5.3.2. SEX DETERMINATION

Black-browed albatrosses exhibit a clear sexual dimorphism in some behaviours and in the present work, 12 birds were sexed by direct observation of the pair during the pre-laying period. Whenever this was not possible, we used the molecular-based method described in Fridolfsson and Ellegren (1999) using DNA extracted from blood samples (n = 26).

5.3.3. GEOLOCATION DATA

In order to obtain information on the areas used by albatrosses prior to the collection of our samples, we deployed leg-mounted Mk19 light-level geolocators (GLS) on 20 adult black-browed albatrosses on Beauchene Island in December 2015, which were then recovered in January 2017. Of these, 12 loggers delivered usable data. Positions were obtained from light information analyzed following the method employed by Dias et al. (2011) and Phillips et al. (2004). GLS data of black-browed albatross from New Island were not available for 2016, and therefore we used GLS information from 7 adult birds collected in 2013. GLS positions obtained between October and December (e.g., 3 months prior to sample collection) were examined using Kernel Density Estimate (KDE) analysis, which were used to depict 50.0%, 75.0% and 90.0% of density contour areas (the estimated foraging range). All calculations were carried out with the adehabitatHR package (Calenge 2006) running in R (R Core Team 2019).

5.4.4. DATA ANALYSIS

Trace elements levels with concentrations lower than limit of quantification in more than 50% of the individuals, which included cadmium, cobalt, manganese and vanadium, were excluded from subsequent statistical analyses (e.g., Anderson et al. 2010). The effects of island and sex in levels of trace elements were evaluated by two-way ANOVAs, after checking for data normality. Covariance models (ANCOVA) were used to test the effect of sex in the correlations between trace elements concentrations. Means are presented with standard deviations.

5.4. RESULTS

5.4.1. CONCENTRATION OF TRACE ELEMENTS IN BLOOD

Levels of arsenic, cadmium, cobalt, copper, manganese, mercury, selenium, strontium and vanadium in blood of black-browed albatross are shown in Table 5.1.

We did not find any differences between islands in any of the trace elements analyzed. Conversely, there were significant differences between sexes (Fig. 5.2) in the concentrations of arsenic (Two-way ANOVA, effect of sex: $F_{1,34} = 7.56$, $p = 0.009$) and selenium ($F_{1,34} = 8.83$, $p = 0.005$) , with males showing lower concentrations than females (arsenic: females: 3.02 ± 0.97 mg/kg, males: 2.37 ± 0.49 mg/kg; selenium: females: 52.50 ± 24.13 mg/kg, males: 33.94 ± 13.43 mg/kg).

The analysis of covariance showed a significant correlation between the levels of selenium and arsenic ($F_{1,35} = 99.5$, $p < 0.001$), but there was no effect of sex ($F_{1,35} = 1.46$, $p = 0.234$). The same was observed in the relationship between copper and strontium ($F_{1,34} = 61.4$, $p < 0.001$, no effect of sex, $F_{1,34} = 0.48$, $p = 0.496$) and selenium and copper ($F_{1,34} = 4.67$, $p = 0.038$, no effect of sex, $F_{1,34} = 0.03$, $p = 0.864$).

The analysis of covariance revealed differences among sexes in the correlation between arsenic and copper ($F_{1,34} = 4.40$, $p = 0.043$), with concentrations of arsenic in females being ca. 0.6 mg/kg higher than in males, for the same values of copper ($F_{1,34} = 6.81$, $p = 0.013$). The same was observed for the relationship between selenium and mercury ($F_{1,35} = 5.62$, $p = 0.023$), with concentrations of selenium in females being ca. 18.6 mg/kg higher than in males for the same values of mercury($F_{1,35} = 10.52$, $p = 0.002$).

5.5. DISCUSSION

Birds tracked in this study carried out foraging trips within the Patagonian shelf. More specifically, kernel density distributions of black-browed albatrosses from New Island and Beauchene Island (islands located 200 km apart) revealed no major differences in the areas of the Patagonian Shelf used between October and December (Fig. 5.1). Despite the high overlap between the kernels (ca. 62.0%), birds from New Island also seem to be using an area slightly more to the north than the one used by those from Beauchene Island. The levels of trace elements measured in black-browed albatross blood in those two different islands should be indicative of contamination in this broad Patagonian Shelf region (Table 1). The mean concentration of trace elements decreased in the following order: selenium > arsenic > copper > mercury > strontium > vanadium > manganese > cadmium > cobalt. Levels of contaminants in the Falkland Islands were generally low when compared with data from other species and locations in the Southern Ocean. Arsenic was the exception, as birds from the Falkland Islands showed comparatively high concentrations of this element (Supplementary Table C4).

We did not find any difference between islands in the levels of arsenic, copper, mercury, selenium and strontium in the blood of albatrosses (Table 5.1), which fits well with the large overlap in foraging areas of birds from the two colonies. The diet of albatross from New Island are broadly composed of fish (mostly *Sprattus fuegensis* and *Patagonotothen* sp.), Crustacea (mostly lobster krill *Munida gregaria*) and Scyphozoa (e.g., jellyfish) (McInnes et al. 2017a, b) and Thompson (1992), reported the same groups of prey for albatrosses from Beauchene Island, which again concurs with a high overlap in feeding areas and similar levels of contamination. Furtado et al. (2019) reported no significant differences in mercury levels in feathers of black-browed albatross between New Island and Beauchene Island.

In the ocean, arsenic, cadmium, copper, mercury, selenium and strontium occur in lower concentrations at the surface (< 100 m) than in deeper waters (Westerlund and Ghman 1991; Cutter and Cutter 1995; de Villiers 1999; Cutter et al. 2001; Heller and Croot 2015). Methylmercury concentration increases with depth to an intermediate maximum in the mesopelagic domain (Cossa et al. 2011). The concentration of copper, mercury and strontium appears to be higher in the Antarctic waters than in the other oceanic areas (Cossa et al., 2011; de Villiers, 1999; Heller and Croot, 2015). Furthermore, Antarctic Intermediate Waters exhibit some of the highest arsenic concentration observed in the ocean (Santosa et al. 1994; Cutter et al. 2001). For example, *Euphausia superba*, a commonly found prey in the diet of black-browed albatross from South Georgia, has 5.5 ± 1.1 mg/kg of selenium and 0.01 ± 0.01 mg/kg of mercury (Anderson et al. 2009, 2010). Recently Sontag et al. (2019), reported concentration of methylmercury between 0.00074 to 0.00294 mg/kg and 0.00026 to 0.00161 mg/kg in juvenile and adult *Euphausia superba* respectively. Besides contrasting levels of background contamination, differences in the food web structure may help explain the higher concentrations of several trace elements in black-browed albatrosses in South Georgia, compared to the Falkland Islands (Supplementary Table A4). In the former region, albatrosses forage mostly over deep oceanic waters (e.g., Wakefield et al. 2012) and will have a different exposure compared to those foraging around the Falkland Islands, where the birds forage almost exclusively over shallower environments on the shelf (e.g., Granadeiro et al. 2017; Ponchon et al. 2019).

The concentration of arsenic and selenium were significantly higher in females than in males (Fig. 5.2, Table 5.1). Males and females black-browed albatrosses from the Falklands show similar foraging areas year-round, and particularly during October-December (Ponchon et al. 2019; own unpublished data). Furthermore, they seem to have similar diets as assessed by nitrogen and carbon stable isotopes (Campioni et al. 2016). Hence, one could expect that element levels would be lower for females than males, because females have the possibility to eliminate some contaminants through excretion in eggs (Monteiro and Furness 1995; Kubota et al. 2002; Ackerman et al. 2016). However, arsenic and selenium levels were higher in females than in males, suggesting that maternal transfer of these elements to the egg may be limited in this species. Metabolic differences between sexes and/or different assimilation rates may explain the lower levels of arsenic and selenium found in males. There have been previous studies that reported lower levels of arsenic, mercury and selenium in males than females (Becker et al. 2002; González-Solís et al. 2002; Wilson et al. 2004; Taggart et al. 2006; Lucia et al. 2010; Carvalho et al. 2013; Tavares et al. 2013; Carravieri et al. 2014; Ackerman et al. 2016). However, this pattern is dependent on the species of birds and/or location. Bustamante et al. (2016), have reported a difference in mercury, between female and male wandering albatrosses *Diomedea exulans* from South Georgia and suggested that maternal transfer of mercury to the egg was limited in this species. Pon et al. (2011) and Tavares et al. (2013) have reported no effect of sex in cadmium, cooper and mercury in feathers of albatrosses. However, the significance of the presence of elements such as cadmium in feathers is more difficult to assess, as cadmium does not seem to integrate this biological matrix in response to exposure levels.

We found a positive correlation between levels of mercury and of selenium in blood of black-browed albatrosses. This relationship is important, since selenium interacts with mercury to form inert complexes of high molecular weight in the blood, especially in the erythrocytes, and forms harmless non-diffusible complexes (mercury-selenium) in the liver and kidney and therefore acts as a form of protection against methylmercury toxicity (thus delivering a “detoxifying” effect for methylmercury) (Cuvin-Aralar and Furness 1991; Imura and Naganuma 1991; Yang et al. 2008). Some researchers have reported that mercury concentrations in feathers, liver and kidney of birds between 5 and 40 mg/kg can have negative impacts expressed by changes in reproduction and survival (Wolfe et al. 1998; Evers, 2008; Whitney and Cristol 2018). Overall, mercury levels in blood of black-browed albatross analyzed in this study are below the 5 mg/kg threshold in 97 % of the individuals sampled (n = 38), and the highest value recorded in our sample was 5.19 mg/kg. Most researchers investigating relationships between blood mercury and breeding and/or survival have found no suggestion for an impact of mercury in seabirds, which may indicate that seabirds exhibit extraordinary resistance to contamination (Goutte et al. 2014, 2015; Pollet et al., 2017; Bond et al. 2015; Tartu et al. 2016; Carravieri et al. 2018).

In our albatross samples, copper was correlated with strontium, arsenic and selenium and arsenic with selenium. Recent research suggests selenium antagonizes the toxicity of arsenic mainly through sequestration of this element into biologically inert arsenic - selenium compounds and/or through increasing the arsenic methylation capacity in the body (decreased tissue accumulation of arsenics and its toxic effects) in diverse organs and tissues (see review Zwolak 2020). These correlations between elements suggests an interaction between elements in the blood or, alternatively, an assimilation from a common source of pollution (e.g., foraging area or type of prey), but further research is required to unveil the mechanisms behind such relationships. We also highlight that our study presents the first baseline data on the concentrations of cobalt in blood of black-browed albatross.

5.6. CONCLUSION

This study establishes baseline levels for nine trace elements in black-browed albatross of the Falkland Islands that will be useful for future monitoring under a scenario of rapid changes in the ocean biogeochemistry (Geibert 2018). Albatrosses from Falkland Islands are exposed to low-to-moderate concentrations of trace elements, as compared to other islands and species in the Southern Ocean (Supplementary Table A4). Results of trace element concentrations suggest that birds from different Falkland colonies are exposed to the same background levels, which reflects the large overlap in foraging areas. The concentrations of arsenic and selenium varied with sex, despite the fact that the foraging areas of males and females show almost complete overlap (Ponchon et al. 2019; own unpublished data). Potential differences between sexes in diet and physiology deserve further investigation.

Table 5.1 Trace elements concentrations (number of samples; mean ± SD, mg/kg (Range) in blood of black-browed albatross from the Falkland Islands. Two-way ANOVA were performed to compare trace elements concentrations between sexes and colonies. Significant statistics ($p < 0.05$) are highlighted **in bold**.

Trace elements	Colonies								Two-way ANOVA effect of sex	Two-way ANOVA effect of island
	Beauchene Island				New Island					
	Female		Male		Female		Male			
	Mean ± SD (Range)	Number of samples	Mean ± SD (Range)	Number of samples	Mean ± SD (Range)	Number of samples	Mean ± SD (Range)	Number of samples		
Arsenic (As)	2.8 ± 1.0 (1.9 – 4.8)	6	2.4 ± 0.6 (1.7 – 3.6)	12	3.2 ± 0.9 (2.1 – 4.2)	5	2.4 ± 0.4 (1.6 – 3.0)	15	**$F_{1,34}$ = 7.56, p = 0.009**	$F_{1,34}$ = 0.06, p = 0.81
Cadmium (Cd)		0		0	0.1 ± 0.03 (0.01 – 0.1)	4	0.07 ± 0.1 (0.01 – 0.2)	3		
Cobalt (Co)		0		0		0	0.02 ± 0.02 (0.01 – 0.05)	3		
Cooper (Cu)	1.6 ± 0.4 (1.2 – 2.3)	6	1.5 ± 0.2 (1.3 – 1.8)	11	1.9 ± 0.2 (1.6 – 2.0)	5	1.6 ± 0.4 (1.3 – 2.9)	15	$F_{1,31}$ = 0.69, p = 0.41	$F_{1,34}$ = 1.41, p = 0.24
Manganese (Mg)		0	0.1	1	0.2 ± 0.1 (0.08– 0.4)	4	0.2 ± 0.2 (0.07– 0.9)	14		
Mercury (Hg)	1.3 ± 0.7 (0.4 – 2.2)	6	1.5 ± 1.3 (0.4 – 5.2)	12	1.9 ± 0.8 (1.1 – 3.1)	5	1.6 ± 0.6 (0.5 – 3.0)	15	$F_{1,34}$ = 0.002, p = 0.96	$F_{1,34}$ = 0.78, p = 0.38
Selenium (Se)	54.0 ± 31.5 (28.6 – 111.0)	6	32.3 ± 18.2 (15.1 – 70.7)	12	50.7 ± 14.5 (37.0 – 69.6)	5	35.3 ± 8.4 (19.6 – 47.5)	15	**$F_{1,34}$ = 8.83, p = 0.005**	$F_{1,34}$ = 0.004, p = 0.95
Strontium (Sr)	0.9 ± 0.9 (0.4 – 2.6)	6	0.5 ± 0.3 (0.2 – 1.1)	12	1.2 ± 0.7 (0.6 – 2.2)	5	0.9 ± 0.9 (0.4 – 4.0)	15	$F_{1,34}$ = 2.93, p = 0.10	$F_{1,34}$ = 1.55, p = 0.22
Vanadium (V)		0		0	0.8 ± 0.1 (0.7 – 0.9)	2	0.7	1		

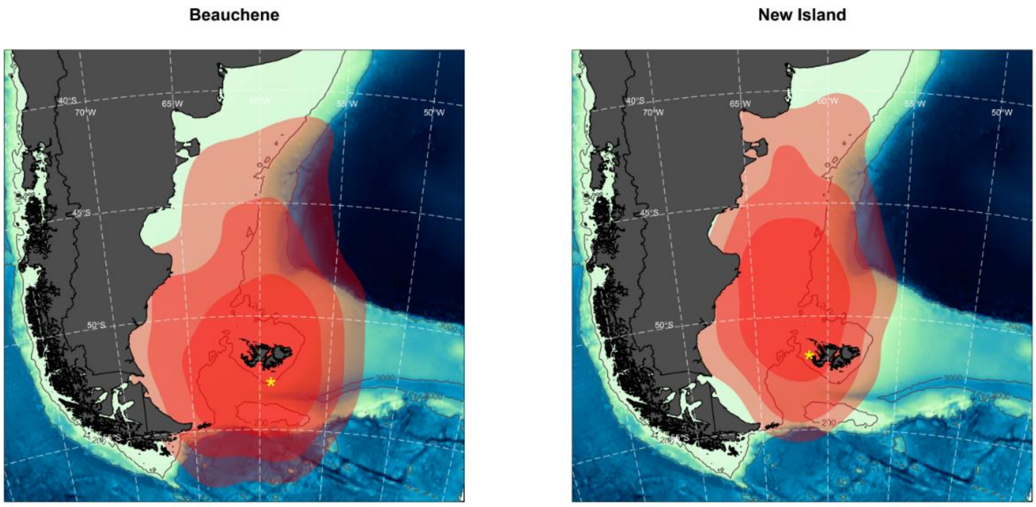

Fig. 5.1. Kernel density estimates of Black-browed albatrosses (BBA) successfully tagged with geolocators at Beauchene (2016, N=12) and New Island (2013, N=7) between October and December (incubation-early chick-rearing). Colors represent 50%, 75% and 90% contours.

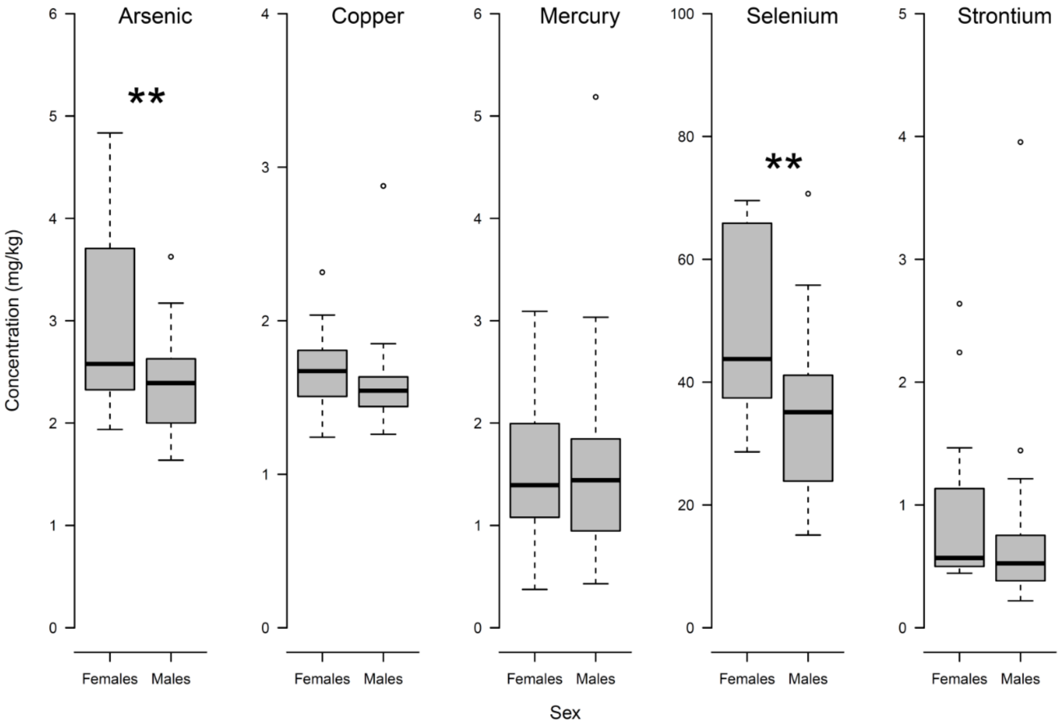

Fig. 5.2. Black-browed albatross blood concentrations (mg/kg) of five trace elements (arsenic, copper, mercury, selenium, and strontium) grouped by sex. Stars indicate statistically significant differences ($p < 0.05$). The box shows the inter-quartile range (25–75th percentile), the bold line is the median, the whiskers represent maximum and minimum, and (when present) dots indicate outliers.

CHAPTER 6

MONITORING OF MERCURY IN THE MESOPELAGIC DOMAIN OF THE PACIFIC AND ATLANTIC OCEANS USING BODY FEATHERS OF BULWER'S PETREL AS A BIOINDICATOR

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6.1. ABSTRACT

Global mercury pollution has markedly and consistently grown over the past 70 years (although with regional variations in trends) 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 birds through diet is excreted into 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 and the trophic position in two colonies from the Atlantic Ocean (Portugal and Cape Verde) and two colonies from the Pacific Ocean (Japan and Hawaii). We found significantly lower levels of mercury in adult and chick samples from the Pacific Ocean compared with samples from the Atlantic Ocean. However, 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 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.

6.2. INTRODUCTION

Global mercury pollution has markedly and consistently grown over the past 70 years (although with regional variations in trends) as a result of anthropogenic activities such as gold extraction, industrial production, waste incineration and the use of fossil fuels (Driscoll et al., 2013; Esdaile and Chalker, 2018; Gworek et al., 2016; Lamborg et al., 2014; Streets et al., 2019, 2011). Mercury is widely transported in the atmosphere and is also distributed in marine ecosystems 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) (Obrist et al., 2018). This elemental mercury sinks adsorbed to particles and is transformed 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, 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 et al., 2019; Thompson et al., 1998).

Top predators, such as pelagic seabirds, particularly those that feed on mesopelagic prey, are highly exposed 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). Because in-situ monitoring of the mesopelagic layer is difficult, bioindicators, as seabirds, are commonly used to monitor these ecosystems. Many biomonitoring efforts report on inter-species comparisons which may introduce other sources of variation, predominantly differences in prey type both at the inter- and intra-species level (e.g., Drevnick et al., 2015; Fleishman et al., 2019; Monteiro and Furness, 1997; Thompson et al., 1998). Some seabirds, such as the Bulwer's petrel (*Bulweria bulwerii*), are specialised predators of mesopelagic prey (Neves et al., 2011; Harrison et al., 1983; Spear et al., 2007; Waap et al., 2017). Their low variability in diet (Spear et al., 2007; Waap et al., 2017) and wide spatial distribution (Dias et al., 2015; Brooke, 2004) makes them ideal candidates to monitor oceanic mercury contamination over multiple ocean basins. The Bulwer's petrel is a small (ca. 100g), highly pelagic seabird with 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).

During the non-breeding season, Bulwer's petrels from Desertas and Cape Verde are mid- to long-distance migrants, moving into tropical deep, open oceanic areas in the Atlantic (Dias et al., 2015; Ramos et al., 2015). Those nesting in Hawaii and Japan migrate to central and eastern tropical Pacific waters and into the Indian Ocean (Brooke, 2004; Harrison, 1990). Outside the breeding season, the wide-ranging movements of birds from colonies from Atlantic and Pacific Ocean (e.g., Dias et al., 2015) mean that populations are sampling at an ocean-basin scale (millions of km2). Mercury values obtained from adult feathers are expected to largely reflect mercury exposure during the non-breeding period, when most body feathers are moulted (ca. mid-September to March) (Furness et al., 1986; Howell, 2012; Monteiro et al., 1996; own unpublished data). On the other hand, during the breeding-season seabirds act as central place foragers, restricting provisioning trips to waters within a range of the colony 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, the chicks of Bulwer's petrels can be seen as biological samplers of a defined area (of tens of thousands of km2) around the breeding colonies.

Apart from geographical differences in mercury contamination, mercury levels in tissue are 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., 2020b; 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 oceanic taxa 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). Given that Bulwer’s petrels are known to be specialist predators of small mesopelagic fishes and squids (Neves et al., 2011; Spear et al., 2007; Waap et al., 2017), we could expect trophic position to be similar between colonies. If trophic positions are similar among individuals from different colonies, then variability in mercury concentrations would reflect geographical variation in mercury contamination at medium to large geographical scales.

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 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). It is known, however, that some mercury is accumulated previous to moult, and the first feathers to be moulted display higher concentrations than the ones moulted later (Gatt et al. 2020a). Avoiding feathers that are moulted earlier (such as the inner primaries) and taking a large number of feathers minimizes and dilutes this problem. Furthermore, this problem does not affect large chicks, for which the contribution of egg mercury is likely very small and virtually all the mercury originates from diet in the well-defined period ranging from hatching to feather growth (Ackerman et al., 2011; Bearhop et al., 2000). 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.

6.3. METHODS

6.3.1. STUDY SITE AND SAMPLING PROCEDURE

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. 6.1). The colonies sampled have similar breeding phenology (April - October) (Chiba, 2020; Cruz-Flores et al., 2018; Kohno et al., 1986; Nunes and Vicente, 1998; Whittow, 1994). Eight to ten body feathers were collected from incubating adult birds (n = 71 in total, 15 – 20 individuals per colony) and growing feathers from chicks towards the end of the chick-rearing period (August – October, before fledging) (n = 75 in total, 15 – 20 individuals per colony) (Table 6.1). Feathers were clipped, in the superior umbilicus of feather, excluding the calamus, and collected from various locations on the body (dorsal and ventral; below the neck and above the lower extremities) and stored in polyethylene bags.

6.3.2. MERCURY ANALYSES

The feathers were cut into fine pieces to produce a homogeneous sample, and an electronic micro-balance (Sartorius M5P, Sartorius AG, Gottingen, with 0.001 mg precision) was used to prepare between 0.26 mg and 1.02 mg (mean = 0.51 ± 0.14 mg) of sample for mercury determination. 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 (Costley et al., 2000). This procedure does not require sample pre-treatment (e.g., wash), and also allows for a small sample mass to be used. Briefly, feather samples were placed in a nickel boat and covered with aluminium oxide to prevent sample dispersion. Subsequently, the boat enters a combustion tube containing a catalyst where the sample is dried at 120°C, followed by decomposition at 850°C. Analyses were performed in triplicate per bird, blanks were analysed at the beginning of each set of samples, and the coefficient of variation between replicates never exceeded 10%. Accuracy and precision were assured by the daily analysis of five readings of a certified reference material (CRM) lobster hepatopancreas TORT-3 (Lobster hepatopancreas from the National Research Council of Canada; certified mercury concentration: 0.292 ± 0.022 mg kg^{-1} dw). The obtained values (mean ± SD) for the TORT-3 analyses ranged from 75 to 90 % (recovery efficiencies of 82.72 ± 3.38 %, n = 17), results were corrected using the daily recovery efficiency of CRM. The mass of TORT-3 used for quality control analyses was adjusted to be within the range of total mercury (in ng) present in the samples, with a maximum coefficient of variation of 10%. The limit of detection for this analytical method is 0.01 ng g^{-1} of total mercury. Mercury concentrations in feathers are presented in mg kg^{-1} fresh weight.

6.3.3. COMPOUND-SPECIFIC ISOTOPE ANALYSIS OF AMINO ACIDS

Collected feather samples were homogenized, as in the analysis of mercury, 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 calibrated amino acid mixture, UCD AA3, and multiple natural materials (fish skin gelatin reference material, whale baleen reference material and shark muscle reference material).

6.3.4. CALCULATING TROPHIC POSITIONS

Trophic position of chicks (Table 6.2) was calculated from the nitrogen stable isotope values (δ^{15}N) of glutamic acid (Glx) and phenylalanine (Phe). Including multiple trophic discrimination factors ($\mathrm{TDF}_{\mathrm{Glx\text{-}Phe}}$) in the estimation of trophic position, to integrate the span of the trophic web, 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 trophic discrimination factor for trophic position at the base of the aquatic food chain ($\Delta_{\mathrm{herbivore}}$) (McMahon and McCarthy, 2016), 3.5‰ is the trophic discrimination factor for seabird feathers ($\Delta_{\mathrm{carnivore}}$), 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):

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

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).

6.3.5. STATISTICAL ANALYSIS

All statistical analyses were carried out with R statistical software (R Core Team, 2020). The means of mercury concentrations are presented with standard deviations. Mercury concentrations in feathers of adults from Hawaii showed a wide range of values, from 3.80 to 23.61 mg kg^{-1}, with the latter value being considered an outlier. We removed this outlier, but note that retaining it does not qualitatively change any results and statistical conclusions. To compare the mean feather mercury concentrations of adults and chicks among geographical areas, we used ANOVA, followed by Tukey post hoc tests. 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.

6.4. RESULTS

We found significant differences in mercury levels among breeding colonies of Bulwer's petrel for adults (ANOVA: $F_{3,67} = 56.6$, $p < 0.001$) 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: 10.12 ± 1.94 mg kg^{-1}; chicks – Atlantic Ocean: 4.70 ± 1.73 mg kg^{-1} and Pacific Ocean: 2.52 ± 0.75 mg kg^{-1}), with no difference between colonies in the same ocean basin (Atlantic Ocean: ANOVA: $F_{1,73} = 0.25$, $p = 0.619$ and Pacific Ocean: ANOVA: $F_{1,68} = 0.11$, $p = 0.745$).

There were no significant differences in the trophic position of chicks among colonies (Table 6.2) (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.

Previous studies of mercury in ventral whole feathers of adult Bulwer's Petrels from the Madeiran archipelago, collected from 1992-1994, report a concentration of 21.6 ± 0.7 mg kg^{-1} (mean ± SE) (range 12.20 – 33.80 mg kg^{-1}, n = 55) (Monteiro and Furness, 1997). These values are significantly higher than those obtained in this study for the colony of Deserta Grande, Madeira (mean ± SE: 16.49 ± 0.31 mg kg^{-1} (range 14.16 – 18.76 mg kg^{-1}, n = 20)) (Welch's t-test: $t = 6.7$, $df = 69$, $p < 0.0001$).

6.5. DISCUSSION

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

The diet of Bulwer's Petrel in the Pacific Ocean has been studied in the north-western Hawaiian Islands (Harrison et al. 1983), analysing induced or spontaneous regurgitates, and in the open sea (eastern tropical Pacific Ocean), through the analysis of stomach contents of birds shot for research purposes (Spear et al., 2007). The diet, in the Atlantic Ocean, was studied in the Azores archipelago, analysing induced regurgitates (Neves et al., 2011) and in the Madeiran Archipelago through DNA barcoding, using regurgitations of chicks (Waap et al., 2017). These studies showed that Bulwer's petrels forage almost exclusively on mesopelagic fish (mainly Myctophidae, Gonostomatidae, Phosichthyidae, Sternoptychidae, Centriscidae, Melamphaidae, Macrouridae and Melanonidae) and squid (mainly Ommastrephidae, Histioteuthidae, Mastigoteuthidae, Chiroteuthidae and Cranchiidae) (Neves et al., 2011; 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 high, but similar across locations, mercury concentrations in adult Bulwer’s petrels from the same oceans reinforce the idea that Bulwer’s petrels are specialist predators year-round, with a diet based on mesopelagic preys in both the non-breeding and the breeding seasons, irrespective of colony location. Furthermore, adult Bulwer’s petrels from Hawaii, sampled in 2010, appear to occupy a similar trophic position (ca. 3.8) to that of chicks of Bulwer’s petrel from Hawaii (ca. 3.5), sampled in this study (Gagne et al. 2018). Together, these results identify the Bulwer’s petrel as an ideal monitor of mercury bioavailability in different geographic areas in the oceans. Similarities in mercury exposure in adults from the Japanese and Hawaiian colonies also suggest that they spend the non-breeding period in broadly overlapping oceanic areas, as do Bulwer’s petrels from different breeding ranges in the Atlantic (Ramos et al., 2015), or that the mesopelagic preys within the Pacific basin have similar mercury concentrations.

Adults and chicks of Bulwer’s petrels from Atlantic colonies had significantly higher (ca. 59 % in adults and ca. 86% in chicks) mercury concentrations than those from the Pacific Ocean. Given that trophic differences are not apparent, the most likely explanation for this is that mesopelagic fish and squid in the Atlantic have higher mercury levels as compared to the Pacific, resulting from different bioavailability of methylmercury in mesopelagic zones (e.g., Becker et al., 2016; Carravieri et al., 2014). Such large variation in mercury concentrations of mesopelagic species between the Atlantic and Pacific oceans 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 findings are in accordance with previous investigations reporting higher mercury concentrations in deep water in the mesopelagic domain in the Central South Atlantic (ca. 1.3 ± 0.62 pM in the South Atlantic) than in the central and eastern Pacific (ca. 0.61 ± 0.19 pM in the Central South Pacific and 0.59 ± 0.25 pM in the Eastern Tropical 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; Obrist et al., 2018). Such reduction in mercury deposition or mercury concentrations in ocean waters are thought to be driving the declines detected in time series 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, when compared to those of Monteiro and Furness (1997) for Bulwer's petrels are perfectly in line with the above findings and further support the existence of a decline from 1992 to 2018. We note that our methodological approach was slightly different from the one adopted by Monteiro and Furness (1997), as they used whole feathers while we excluded the calamus. Given that the calamus has lower mercury concentrations than the rachis and the vane (Peterson et al., 2019), we would expect, all other things being equal, that our mercury measurements would be slightly higher than those in the former study (note that the calamus only represents ca. 11% of the feather mass; Peterson et al., 2019). The fact that more recent samples had lower, not higher, mercury concentrations reinforces our conclusions. However, we recognise that measurements in 2 points in time cannot substitute a complete time-series to detect a temporal trend. 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 organisms (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, layers in the ocean where they feed (epi- or mesopelagic) and trophic position (Gatt et al., 2020b). Bulwer's petrel feathers could complement the current monitoring in intermediate waters without the difficulties of quantifying and interpreting the influence of trophic position, which seems to be similar in both oceans.

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., Furtado et al., 2019; Monteiro et al., 1999), comparable to concentrations found in some large albatross species (Thompson et al., 1993). 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, 2018; 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).

6.6. CONCLUSIONS

Our results suggest that, given the similarity in trophic position across the two oceans, and its wide-ranging foraging behaviour, the Bulwer's petrel provides an integrated measure of mercury contamination in the tropical and sub-tropical mesopelagic domain at the scale of multiple ocean basins. Results 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 Atlantic Ocean. Results also suggest that mercury levels in the oceanic waters of the tropical/subtropical Atlantic Ocean have declined over the past 2-3 decades, but more data are needed to further confirm this trend. The use of body feathers provides an accessible and non-invasive method for such monitoring. The exceptionally high concentration of mercury in Bulwer's petrels warrants further research to determine potential detrimental effects on behaviour, reproductive success or survival.

Table 6.1 Mercury concentration in feathers of adults and chicks of Bulwer's petrel (mean ±SD and range, mg kg^{-1} fresh wt).

Age	Ocean	Colony	[Hg] mg kg^{-1}		Number of samples
			Mean ± SD	Range	
Chicks	Atlantic Ocean	Cape Verde	5.11 ± 1.76	2.70 – 9.96	15
		Madeira	4.38 ± 1.69	2.94 – 8.25	20
	Pacific Ocean	Japan	2.13 ± 0.38	1.44 – 3.17	20
		Hawaii	2.90 ± 0.84	1.81 – 5.21	20
Adults	Atlantic Ocean	Cape Verde	15.65 ± 2.22	10.61 – 19.09	20
		Madeira	16.49 ± 1.39	14.60 – 18.76	20
	Pacific Ocean	Japan	10.27 ± 1.11	8.74 – 12.39	15
		Hawaii	10.00 ± 2.55	3.80 – 13.97*	15

*An outlier was removed (21.8 $mgkg^{-1}$)

Table 6.2 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

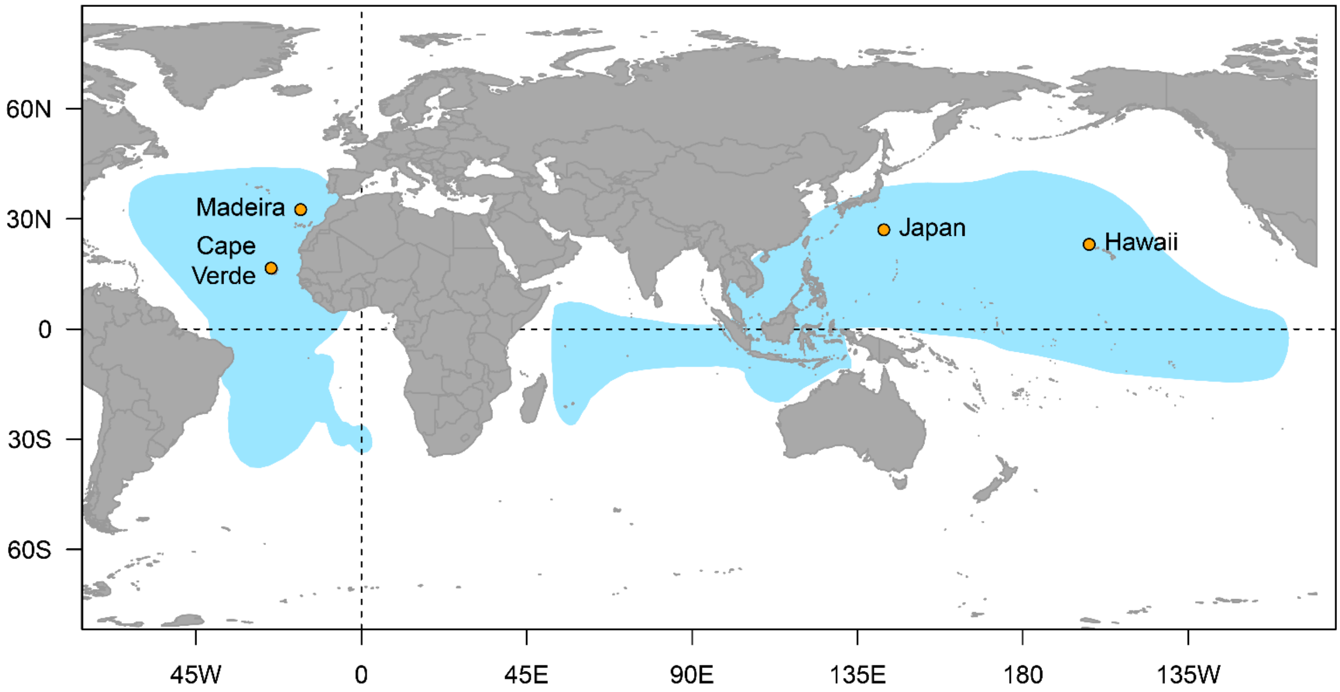

Fig. 6.1 Location of the four breeding colonies where Bulwer's petrels were sampled, and the species distribution during non-breeding season (blue), adapted from Brooke (2014), Dias et al. (2015) and Ramos et al. (2015).

CHAPTER 7

EFFECTS OF MERCURY ON GROWTH, BEHAVIOUR AND FLEDGING AGE OF BULWER'S PETREL CHICKS

Ricardo Furtado

7.1. ABSTRACT

The health effects of mercury exposure represent a significant threat to ecosystems and human welfare worldwide. Environmental mercury is a global toxicant metal that has large-ranging impacts on the organ systems of vertebrates (e.g., central nervous system), but almost nothing is known about how this modifies behaviour and development. Mercury exposure in young stage might be harmful to the physical growth as well as neurodevelopment. Uncontrolled movement and posture have been most widely reported, both in animal experiments and in incidents of human and seabirds exposure. While it is well-known that the toxicity of mercury for birds is related to its bioavailability in the diet and environment in which the birds feed, few studies have addressed mercury effects in growth and behaviour of seabirds. In our first study, to identify possible deleterious effects of mercury exposure in the development of Bulwer’s petrel chicks, we explored the relationship between mercury concentration in plumage and growth rate (body mass and wing length) and age of fledging. The growth rate was observed to vary greatly between chicks. Chicks with reduced growth rate coefficients exhibited high mercury concentration in body feathers. Chicks with reduced growth rate coefficients for body mass exhibited retarded growth of wing length. Furthermore, chicks which fledged at an early age displayed a much faster growth rate and lower mercury concentrations than later fledging chicks. We conclude that high mercury concentrations may decrease growth rate and severely increases a chick’s age of fledging, although alternative possibilities are also discussed. The second part of this study focused on the behaviour in the nest, we measured behaviours typically employed by the procellariforms in the nest. Six behavioural indices were measured, including head turns, body turns, body shake, preening events, wing-stretches, and steps. We found no evidence that mercury influenced nest behaviour.

Together, our results indicate that elevated levels of mercury are associated with negatively affected development (growth and fledging) of chicks but not behaviour.

7.2. INTRODUCTION

Birds may face multiple risks while feeding, such as the risk of exposure to environmental conditions (e.g., assimilation of toxic elements) (e.g., Furtado et al., 2019, 2020). Aggressive chemical stimulation, such as mercury assimilation, can cause a behavioural and/or biological response or lead to exhibiting behavioural changes that can be lethal (Depledge et al., 1995; Zala and Penn, 2004). Mercury is a toxic metal that has a broad impact on physiological processes in vertebrates (e.g., Wolf et al., 1998; Driscoll et al., 2013), leading to healthy individuals, suffering progressive deterioration in fitness (see review, Wolfe et al., 1998).

It has been postulated, although not yet established, that mercury can affect development by damaging cell membranes and disturbing the function of enzymes in various metabolic pathways in mammals (Jan et al., 2015). Some of the earliest animal models of mercury neurotoxicity, as well as post-mortem examination of exposed human fetal brain, implicate disruptions in cell migration and associated derangement of the characteristic layers of the neocortex or cerebellum (Choi et al., 1978). It is also possible that mercury exposure can alter development of cerebellum in birds, part of the brain essential for coordination of movement (e.g., Scoville et al., 2020).

Several laboratory researches that have dosed birds with experimental mercury concentrations have shown that mercury exposure can cause brain damage and demyelination of neurons, retard neurodevelopment and affect physical coordination (Bennett et al.., 2009; Borg et al, 1969; Evans et al., 1982; Fimreite and Karstad, 1971; Heinz and Locke, 1976; Laties and Evans, 1980; Loerzel et al., 1999; Scheuhammer, 1988; Scoville et al., 2020; Spalding et al., 2000; Wolf et al., 2017). In nature, mercury toxicity occurs primarily through the disruption of neurotransmission mechanisms and subsequent impairment of nervous system functions (Scheuhammer et al., 2008; Scoville and Lane, 2013). Since brain processes underlie not only simple motor behaviours, such as walking and eating, but also all complex cognitive and behavioural acts, strong mercury exposure can be expected to cause not only motor, growth, but also behavioural and cognitive changes.

Armstrong et al. (1963), subjected pigeons, *Columba livia domestica*, to mercury vapour and observed that exposed individuals showed behavioural changes, usually evidenced by tremors involving the head, neck and wings. Furthermore, Bouton et al. (1999), dosing adults Great egrets *Ardea alba*, reported that mercury significantly affected maintenance behaviour. The authors found significant differences between individuals dosed with near-lethal concentrations and controls, specifically, in activity levels (e.g., preening, head down, vocalization), in tendency to seek shade, and in motivation to hunt prey. In chicks, Kenow et al. (2011) reported a negative effect of mercury exposure, however, only in a small number of individuals exposed to sub-lethal concentrations of mercury. On the other hand, Heinz, reported that chicks exposed to higher levels of mercury are more likely to have problems associated with hyperactivity (with individuals exhibiting hypertrophied, impulsive and disorganised motor behaviour) (Heinz, 1975, 1976, 1979). In nature, Nocera and Taylor (1998), observing the behaviour of Common loon *Gavia immer* chicks, found that the chicks spent significantly more time preening and less time back-riding (brooding by riding on a parent's back) as mercury levels increased in their blood, although they did not change their swimming or diving habits.

Growth and the development of young birds are important part of their life and can also be affected by mercury. A small number of laboratory investigations, that dosed birds with experimental mercury concentrations, demonstrated that mercury exposure cause changes in growth of young birds (Morran et al., 2018; Kenow et al., 2003; Spalding et al., 2000; Yu et al., 2016). In nature, mercury exposure resulted in some biologically significant changes in size of body components in California clapper rail (*Rallus longirostris obsoletus*) (Ackerman et al. 2012). Similarly, in some species of seabirds growth is affected. Increasing mercury contamination of little auk *Alle alle* environments directly impacts their breeding performances, especially growth rate of chicks (chick growth rates decreased when mercury increased) (Amélineau et al., 2019). Santos et al. (2020), reported that exposure to mercury, due to maternally deposited mercury in eggs, negatively affect the embryonic development in chicks of black-backed gull *Larus fuscus*. On the other hand, the nestling growth rate of Leach’s storm- petrels *Oceanodroma leucorhoa* (Pollet et al., 2017) and body condition of snow petrels *snow petrels* (Tartu et al., 2015) were not related to mercury levels. Pollet et al. (2017) and Tartu et al. (2015) also have not reported any effect of mercury in survival of chicks.

Few studies have been able to detect effects of methylmercury on growth, behaviour or survival of seabirds, exposed with high mercury through their natural diets. In the ocean, mercury is transformed into methylmercury (the most toxic form of mercury), a process that naturally occurs in mesopelagic oceanic domain, due to the low oxygen concentrations (ca. 200 - 1500 m) (Bowman et al., 2015; Cossa et al., 2018). Methylmercury bioaccumulates in tissues of organisms and biomagnifies from low trophic level to the next and, consequently, top predators, such as various seabirds (e.g., Furtado et al., 2021).

Bulwer's petrel is a small seabird abundant in tropical and sub-tropical oceanic waters, occurring in the Atlantic, Indian and Pacific Oceans (Brooke, 2004). During the non-breeding season, Bulwer's petrels are mid- to long-distance migrants, moving into tropical deep, open oceanic areas (e.g., Dias et al., 2015). During incubation period, Bulwer's petrels are specialized in foraging over deep ocean basins, in lower productivity zones, where they exploit scarce and unpredictable oceanic resources at great distances from the colony (Dias et al., 2016; Ventura et al., 2022). During the breeding season, birds consume a large variety of mesopelagic organisms, enriched with methylmercury, particularly fish and squid that occupy surface waters at night as a result of diel vertical migrations (Spear et al., 2007; Neves et al., 2011; Waap et al., 2017; Semeniuk and Dastoor, 2017). Therefore, the daily surfacing of deep scattering organisms makes mercury available in epipelagic layers and food webs (Madigan et al., 2018; Motta et al., 2019; Thompson et al., 1998). Bulwer's petrels and other specialists' predators of mesopelagic prey are particularly susceptible to accumulate large concentrations of mercury and they have some of the highest concentrations of mercury reported in birds (Monteiro et al., 1999; Thompson et al., 1998), making them ideal candidates to monitor the effects of mercury.

This study has two main broad objectives. The first is to assess whether mercury accumulation in chicks correlates with growth rates (body mass and wing length) and development to the point of fledging and therefore also with fledging age. The second broad objective was to assess whether mercury accumulation in the chicks affected their behaviour in the nest. To our knowledge, this is the first study the attempts to assess if mercury concentration in wild birds affects the behaviour of offspring to the point of fledging.

7.3. METHODS

Fieldwork was carried out from June to October 2016 and 2018, in a colony of Bulwer's petrel at the Deserta Grande Island (33°20′N, 17°20′W), Madeira archipelago, Portugal. Birds' arrival to the colony starts in April with prospecting individuals reinforcing pair bonds before egg laying. Bulwer's petrel lay a single egg at the end of May/early June. Both parents contribute to incubation (45 ± 2.5 days; range: 40 – 63 days) and chick rearing, with the chick being continuously guarded for the first 2 – 8 days following hatching (Nunes and Vicente, 1998). Eggs hatch in July and chicks are ready to fledge in late-September and early-October (This study; Nunes and Vicente, 1998).

Nests (2016: n = 95; 2018: n = 85 were marked during incubation (early June) and checked daily during the day (when no adult arrivals or departures were recorded) until the end of the breeding season. To quantify fledging age, nests were visited every day to check for the hatching date and after hatching the presence of chicks.

7.3.1. MEASUREMENTS AND GROWTH

After hatching, chicks were weighed every two days. Two Pesola spring balances of 100g and 300g, accurate to 1g and 2g respectively, were used. Wing length of chicks was measured every four days, beginning six days after hatching, using a ruler accurate to 0.5 mm. Nests were usually visited during the day at intervals of 24 hours. Only chicks that survived to fledge were used in this analysis, which included 63 and 52 chicks from 2016 and 2018, respectively. Curve relating body weight to age (see Supplementary material Fig. D1) was fitted by the "positive-negative Gompertz curves" developed by Huin and Prince (2000):

$$W\ (t) = A\ exp^{[-exp^{-k_1(t-t_1)}-exp^{k_2(t-t_2)}]}$$

Where, A is the asymptotic mass, t_1 the time at which the growth reaches its maximum rate at mass A/e and k_1 being the slope of log(W(t)) at t_1. After an initial period of relative stability, mass loss becomes more important, with the maximum loss rate occurring at t_2 at mass 1/e; at that point t_2, k_2 is the slope of log (W(t)). However, the two parts of the equation (original and negative Gompertz curves) interact with each other in such a way that the asymptotic mass A is never reached, and each inflection points no longer occur at t1 and t2. Peak mass W_{max} (g), age of chick reaches its peak mass (days), average growth rate (g d^{-1}) from day 1 to day of peak mass, maximum growth rate (g d^{-1}), age at which chick reaches its maximum growth rate (days), age at which chick reaches its 90 grams (days), growth rate of chick at 20 days old (g d^{-1}) and body mass of chick at 20 days old (g) were calculated from the growth curve determined for Bulwer's petrel chicks. Curve relating body weight was adjusted for 115 chicks, 3 chicks were excluded due to lacunes of weight measurement, but note that retaining it does not qualitatively change any results and statistical conclusions.

Curve relating wing length to age (see Supplementary material Fig. D2) was fitted by the Richard's equation (Richards, 1959; Myers, 1986):

$$W(t) = \frac{A}{(1 + \ t_1 \exp(-kt))^{\frac{1}{m}}}$$

where, A is the asymptotic (or peak) length of wing, k is the growth rate constant, m is the slope of growth and t_1 is the point of inflection (the age at which the maximum growth rate of wing occurs). Peak length of wing (mm), average growth rate of wing length (mm d^{-1}) from day 1 to day last day the wing was measured, maximum growth rate of wing length (mm d^{-1}), age at which the chick reaches its maximum growth rate of wing length (mm), growth rate of chick at 20 days old (mm d^{-1}) and wing length at 60 days old (mm) were calculated from the growth curve determined for Bulwer's petrel chicks. Curve relating wing length to age was adjusted for 115 chicks.

These equations allow the extraction of key parameters of chick growth in an objective and repeatable manner, instead of relying on raw data which can be highly variable. This is especially true for species with protracted growth, such as Procellariformes, where chicks are fed irregularly.

7.3.2. FLEDGING BEHAVIOUR

We considered fledging to have occurred in chicks measured if the nest was empty, chicks previously fully feathered and in good health, older than 50 days of life and/or during the previous visits the nestling had weighed < 120 g and had a wing chord >175 mm (Nunes, 2000; Nunes and Vicente, 1998). Weight at fledging was recorded on that day or the day before and wing length was recorded on that day or up to four days before. The date of fledging was considered as the last day on which the chick was found in the nest. Fledging age was quantified as the number of days between the egg hatched and the day of the departure, considering the day after hatching as the first day of life. Fledging age was quantified for 112 chicks, fledging age of 3 chicks in 2016 were not registered due to the end of the field season (the chicks in last day of season had 70, 73 and 60 days old).

During the sampling, in 2016, 3 chicks died in the first five days old, five chicks between 10 and 25 days old and 3 chicks have disappeared from the nest between 20 and 30 days old. In 2018, 5 chicks died in the first five days old, 4 chicks between 10 and 25 days old and 1 chicks have disappeared from the nest between 20 and 30 days old.

7.3.3. BEHAVIOUR OF CHICKS

Forty-two chicks of Bulwer's petrel experienced a behavioural test. A behavioural test was used to measure the frequency of nest behaviours during development. We carried out behavioural tests over the 2018 breeding season, when chicks were 41 days old. Birds were extracted from their nest and placed into an arena which served as the novel environment, near the nest, under a sheltered roof. The arena was impermeable on the bottom, had washable fabric on the sides in blue and uncovered on the top (allowing natural light to enter). The arena was 0.4 m long, 0.3 m wide and 0.2 m high, which was an rectangular space. Observation of behaviours was made during the day. Furthermore, observations are made at the same time each day so that observations was made under similar conditions from day to day and between seven and nine o'clock in the morning.

Chicks were tested one at a time. To start a trial, a chick was positioned inside the arena at a predefined 'start' point location of 0.07 m diameter, in the centre of the arena. Following a 30 second acclimation period, we recorded the chick's behaviours for 300 seconds using a Sony Camera. Once observation was completed, chicks were weighed, measured for wing length, and returned to their nest. The ethogram (see Table 7.1) was created during several preliminary observations and based on the behaviours observed by Cunningham et al. (2006) in chicks of blue petrel *Halobaena caerulea*. We identified eight behaviours, but only measured seven. We measured the frequency of head turns, body turns, body shake, preening events, wing stretches and foot movements (see table 7.1) during development and explored their association with the mercury. We did not quantify frequency of beak movements, because the beak was not easily distinguished from the plumage. We measured individual exploratory behaviour and explored their interaction with mercury. Finally, we used the position as an index of exploratory behaviour (‘exploratory score’). When birds had not moved from the initial mark, exploratory score was 0, when birds had move from start mark, exploratory score was 1.

Table 7.1. Description of behaviours (adapted by Cunningham et al., 2006; Zhao et al., 2020)

Behaviour	Explanation of behaviour	Measure
Head turns	Sweep of the head to the right or the left	Frequency
Body turns	Turning of the body at least 45° to the right or the left	Frequency
Preen	Rubbing the beak or head over the feathers	Frequency
Wing stretches	Extension of the wing	Frequency
Shake body	Shaking of the body, head and/or wing	Frequency
Exploratory behaviour	When the chicks move out of the start mark to explore its environment. The chicks lowers its head toward the floor touching its beak as if looking for something.	0 or 1 score
Foot movement	Motions of the paws during locomotion	Frequency

7.3.4. MERCURY ANALYSIS

To quantify mercury levels, we collected 10 body feathers from each of the chicks with forty days old in 2016 and 2018. Feathers were clipped, in the superior umbilicus of feather, excluding the calamus, and collected from the dorsal region below the neck. All samples of feathers were stored in polythene bags. Before mercury analysis, feathers were dried at ambient laboratory temperature.

Total mercury concentration in down plumage and dorsal body feathers was quantified by atomic absorption spectrophotometry with thermal decomposition (Costley et al., 2000) in LECO AMA-254 with a detection limit of 0.01 ng of mercury. Samples for mercury determination were cut into fine pieces to produce a homogeneous sample, before mercury quantification, and an electronic micro-balance (Sartorius M5P, Sartorius AG, Gottingen, with 0.001 mg precision) was used to prepare 0.35 mg and 1.82 mg (0.77 ± 0.25 mg) of feathers. For each sample, analyses were repeated two or three times, until the relative standard deviation for the aliquots was < 10%. Subsequently, the mean of the repeated mercury measurements was used for statistical analysis. All sample measurements were above the equipment's limit of detection. The limit of detection for this analytical method is 0.01 ng g^{-1} of total mercury. Blanks were systematically run between samples (two or three procedural blanks). The mercury concentrations in procedural blanks were always below the detection limit of the equipment. Precision and accuracy of the analytical method was evaluated by analysis of certified reference material (CRM). CRM was analysed at the starting, middle and at the end of the analytical cycle. This included: CRM - lobster hepatopancreas TORT-3; NRC, Canada; reference values were of 0.292 ± 0.022 mg kg^{-1} dry weight (dw), mean measured ± SD = 0.256 ± 0.02 mg kg^{-1} dw (n = 60). Thus, the recovery of the CRM was 88 ± 7 %. Results were corrected using the daily recovery efficiency of CRM.

7.3.5. DATA ANALYSES

We used one-way analysis of variance (ANOVA) to test for differences in mercury concentration and average growth rate across years, after ensuring that assumptions of normality and homogeneity of variance were met.

The relationship between mercury and growth of body mass and wing length was examined using principal component multiple regression analysis (PCA) to avoid the confounding effects of collinearity among independent variables (parameters of growth). PCA produces ordination axes that reduce the total residual sum of squares among all the variables included (e.g., parameters of growth). The primary axis (PC1) represents the greatest proportion of component variation among the samples. The secondary axis (PC2) explains the greatest proportion of any remaining variation that is uncorrelated with PC1. All PCA were performed on standardised values, so that those elements with vastly different ranges could be analysed together. Covariance models (ANCOVA) were then used to examine the significance relationships between mercury concentration and PC scores, with year as a factor. The interactions in the responses were evaluated.

Posteriorly, ANCOVA were used to test the effect of mercury concentrations of Bulwer's petrel chicks on age of fledging, with year as a factor. The interactions in the responses were also evaluated. Equally, to relate fledging age with growth of body mass PC1, we also used the analysis of covariance model, with year as the covariate, also looking at the interaction between these two factors. In addition, the effect of wing length on age of fledging was tested with analysis of covariance model, with year as the covariate. The interactions in the responses were also evaluated.

We then used simple linear regression to examine the relationship between different chicks' behaviours (head turns, body turns, paw movements, wings stretch, preening and shaking) and mercury contamination.

Factors affecting the exploratory behaviour were evaluated using a generalized linear model with a binomial error distribution and logit link function.

All statistical analyses were carried out with R statistical software (R Core Team, 2021). Means are given with standard errors. All statistical tests were considered significant when $p < 0.05$.

7.4. RESULTS

7.4.1. CONCENTRATIONS OF MERCURY

Mercury in body feather of chicks ranged from 3.42 to 17.57 mg/kg (mean ± SD = 9.69 ± 3.25, N = 115) and differed significantly among years ($F_{1,113} = 70.56$, $P < 0.001$), with 2016 showing a higher values of mercury (mean ± SD = 11.52 ± 2.95; range: 5.0 – 17.56 mg/kg, N= 63) when compared to 2018 (mean ± SD = 7.48 ± 2.0; range 3.42 – 12.21 mg/kg, N = 52).

7.4.2. GROWTH OF BODY MASS

Chicks achieved the peak mass at 49 ± 4 days old on average (range: 41 – 58 days old ; N = 62 chicks) and reached the peak mass of 130 ± 15 grams on average (range: 99 – 165 grams; N = 62 chicks) in 2016. For 2018, chicks achieved the peak mass at 43 ± 3 days (range: 37 – 50 days; N = 50 chicks) and reached the peak mass of 142 ± 14 grams on average (range: 116 – 175 grams; N = 50 chicks). Peak mass was significantly different between years (peak mass: $F_{1,110} = 19.84$, $P < 0.001$). Similarly, age of chick when it reaches its peak mass was also significantly different between years (age of chick reaches its peak mass: $F_{1,110} = 75.14$, $P < 0.001$).

Average growth rate of chicks ranged from 1.42 to 3.78 g d^{-1} (mean ± SD = 2.57 ± 0.53, N = 112) and differed significantly among years ($F_{1,110} = 72.40$, $P < 0.001$), with 2016 showing lower values of average growth rate (mean ± SD = 2.27 ± 0.40; range: 1.42 – 3.73 g d^{-1}, N= 62) when compared to 2018 (mean ± SD = 2.94 ± 0.43; range: 2.02 – 3.78 g d^{-1}, N = 50).

7.4.3. RELATIONSHIPS BETWEEN CHICK GROWTH AND MERCURY

For variables related to body mass, the PCA (see Supplementary material Fig. D3) reduced 8 parameters of growth to two PCs that together explained 87.6% of the variation of the original 8 variables. The primary axis (PC1) represents the paraments related with mass and growth rates and explained 71.4 % of the variance. The secondary axis (PC2) represents the paraments related with age and explained 16.2 % (Table 7.2). The analysis of covariance demonstrated that there was a significant and negative association between PC1 and mercury in body feathers ($F_{1,109} = 70.25$, $p < 0.001$), with 2018 showing a higher PC1 when compared to 2016 ($F_{1,109} = 23.29$, $p < 0.001$) (Fig. 2). There was no interaction between the mercury and year ($p = 0.808$). The analysis of covariance containing PC2 demonstrated that there was not a significant association between PC2 and mercury in body feathers ($F_{1,109} = 0.37$, $p = 0.542$) nor any influence of year on PC2 ($F_{1,109} = 0.18$, $p = 0.675$). There was also no interaction between the mercury and year ($p = 0.706$).

For variables related to wing length, the PCA (see electronic Supplementary material Fig. D4) reduced 6 parameters of growth to two PCs that together explained 87.2 % of the variation of the original 6 variables. The primary axis (PC1) represents the paraments related with growth rates and explained 69.2 %. The secondary axis (PC2) represents the paraments related with age and explained 18.0 % (Table 7.2). Similarly, the analysis of covariance showed that there was a negative significant relationship between PC1 and mercury in body feathers ($F_{1,112} = 113.56$, $p < 0.001$), with 2018 showing a higher PC1 than 2016, for the same values of mercury ($F_{1,112} = 39.3$, $p < 0.001$) (Fig. 3). There was no interaction between the mercury and year ($p = 0.469$). The analysis of covariance containing PC2 showed that there was not a significant association between PC2 and mercury in body feathers ($F_{1,112} = 1.34$, $p = 0.250$), but there was effect of year ($F_{1,112} = 5.66$, $p = 0.019$), with 2016 showing a higher PC2 when compared to 2018. There was also no interaction between the mercury and the year ($p = 0.395$).

Table 7.2 Loadings (expressed as correlation coefficient of each single contaminant with PC1 or PC2) of variables onto the first two principal components. The first two principal components explained 87.5 % of body mass (PC1 = 71.4 %, PC2 = 16.1 %) and 87.2 % of wing length (PC1 = 69.2%, PC2 = 18.0%). Positive and negative loadings indicate a positive or a negative correlation between the parameters and the PC1 or the PC2; when the absolute value of correlation coefficients was ± 0.3, they were considered important (**bold font**) in defining the PC.

Body mass			**Wing length**		
Variable	PC1	PC2	**Variable**	PC1	PC2
Peak mass W_{max} (g)	**0.3147978**	**-0.4680269**	Peak length of wing (mm)	0.2492439	**-0.791485**
Age of chick reaches its peak mass (days)	**-0.3526350**	-0.1658435	Wing length at 60 days old (mm)	**0.4695138**	-0.047565
Maximum growth rate (g d^{-1})	**0.3924723**	0.03368502	Maximum growth rate of wing length (mm d^{-1})	**0.3311088**	**0.6080722**
Age at which chick reaches its maximum growth rate (days)	-0.1624564	**-0.77389683**	Age at which the chick reaches its maximum growth rate of wing length (mm)	**-0.4139389**	-0.038972
Average growth rate (g d^{-1})	**0.3986489**	-0.21754446	Growth rate of chick at 20 days old (mm d^{-1})	**0.4637103**	0.0041991
Age at which chick reaches its 90 grams (days)	**-0.3850922**	-0.13807722	Average growth rate of wing length (mm d^{-1})	**0.4705620**	0.0003998
Body mass of chick at 20 days old (g)	**0.3817958**	0.18462765	**% Variance explained**	69.2	18.0
Growth rate of chick at 20 days old (g d^{-1})	**0.3783376**	-0.23003785	**Standard deviation**	2.0375	1.0392
% Variance explained	71.4	16.1			
Standard deviation	2.3904	1.1365			

7.4.4. FLEDGING BEHAVIOUR

Chicks fledged when 66 ± 3 days old (61 – 75 days old ; N = 60 nests) in 2016 and when 61 ± 3 days old (56 – 69 days old; N = 52 nests) in 2018.

Chicks of Bulwer's petrel which fledged at an early age displayed a lower mercury concentration than chicks which fledged at a later age. The analysis of covariance showed a significant positive association between levels of mercury in body feathers and age of fledging ($F_{1,109}$ = 69.13, $p < 0.001$), with age of fledging in 2016 being ca. 4 days old higher than in 2018, for the same values of mercury ($F_{1,109}$ = 24.61, $p < 0.001$) (Fig 7.1). There was no interaction between the mercury and the year ($p = 0.9686$).

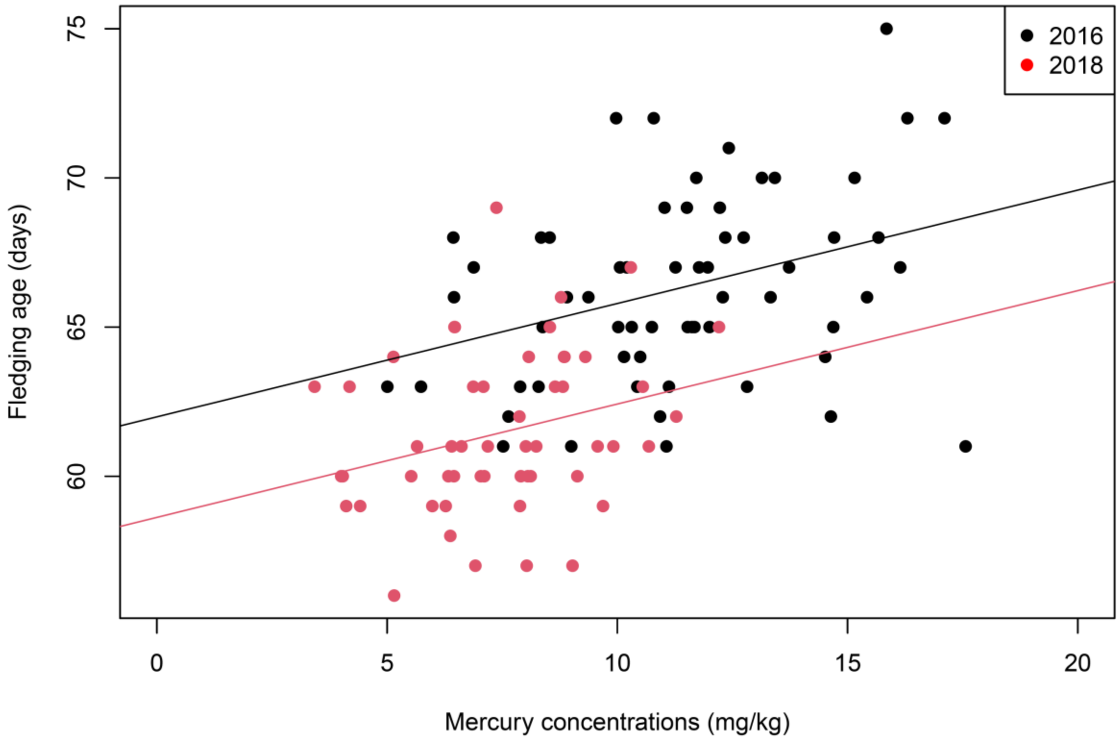

Fig. 7.1 Age of fledging (days) in relation to concentrations of mercury (mg/kg) in body feathers of chicks Bulwer’s petrel at Desertas Islands, Madeira Archipelago, Portugal (n = 112)

Chicks which fledged at an early age grew faster than chicks which fledged at a later age, the analysis of covariance showed that there was a significant negative relationship between body mass PC1 and fledging age ($F_{1,106} = 87.78$, $p < 0.001$) (Fig. 7.2), with age of fledging in 2016 being ca. 3.3 days higher than in 2018, for the same values of body mass PC1 ($F_{1,106} = 24.23$, $p < 0.001$). There was no interaction between mercury and year ($p = 0.408$). This was true not only for growth of body mass but also for wing length.

Similarly, the analysis of covariance showed that there was a significant negative relationship between wing length PC1 and fledging age ($F_{1,109} = 217.60$, $p < 0.001$), with no effect of year ($F_{1,109} = 2.45$, $p = 0.121$) (Fig. 7.3).

When testing which variables predicted the age of fledging in chicks (n= 112), PC1 of body mass (p=0.0013), mercury (p=0.0083) and year (p=0.0005) had a significant predictive power.

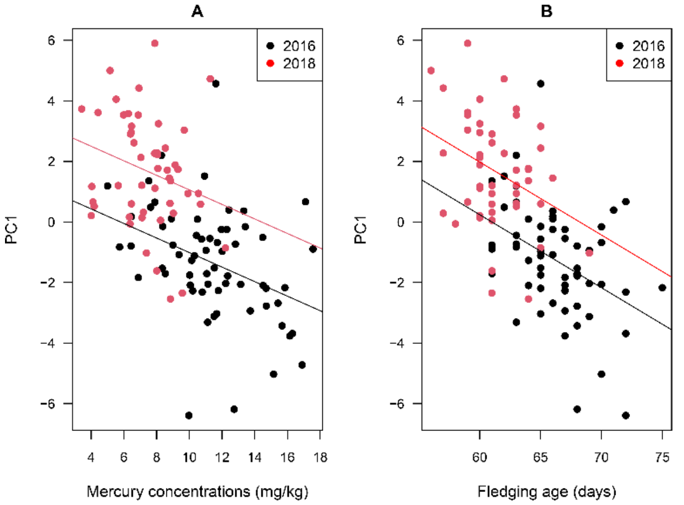

Fig.7. 2 (A) PC1 of body weight in relation to concentrations of mercury (mg/kg) in body feathers (n=112) and (B) PC1 in relation to age of fledging (days) of chicks Bulwer's petrel at Desertas Islands, Madeira Archipelago, Portugal (n = 109).

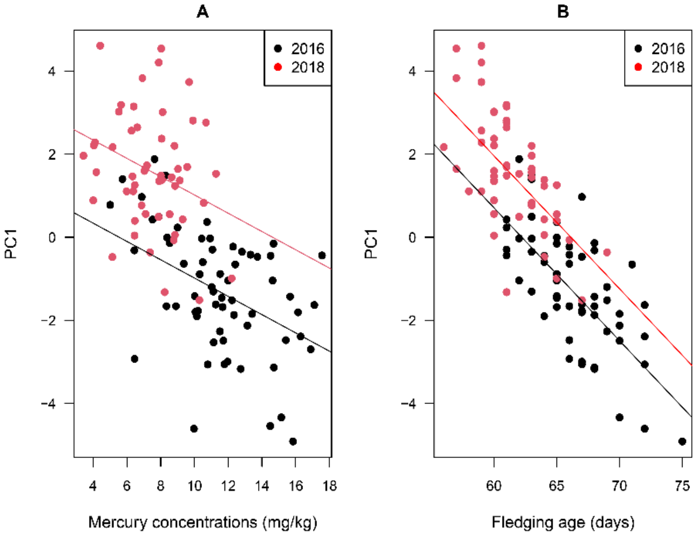

Fig. 7.3 (A) PC1 of wing length in relation to concentrations of mercury (mg/kg) in body feathers (n= 115) and (B) PC1 in relation to age of fledging (days) of chicks Bulwer's petrel at Desertas Islands, Madeira Archipelago, Portugal (n = 112).

7.4.5. RELATIONSHIPS BETWEEN BEHAVIOUR OF CHICKS AND MERCURY

A total of seven individual behaviour traits were quantified (Table 7.1). Fig 7.4 shows the mean ± SD of behaviours during behavioural experiment.

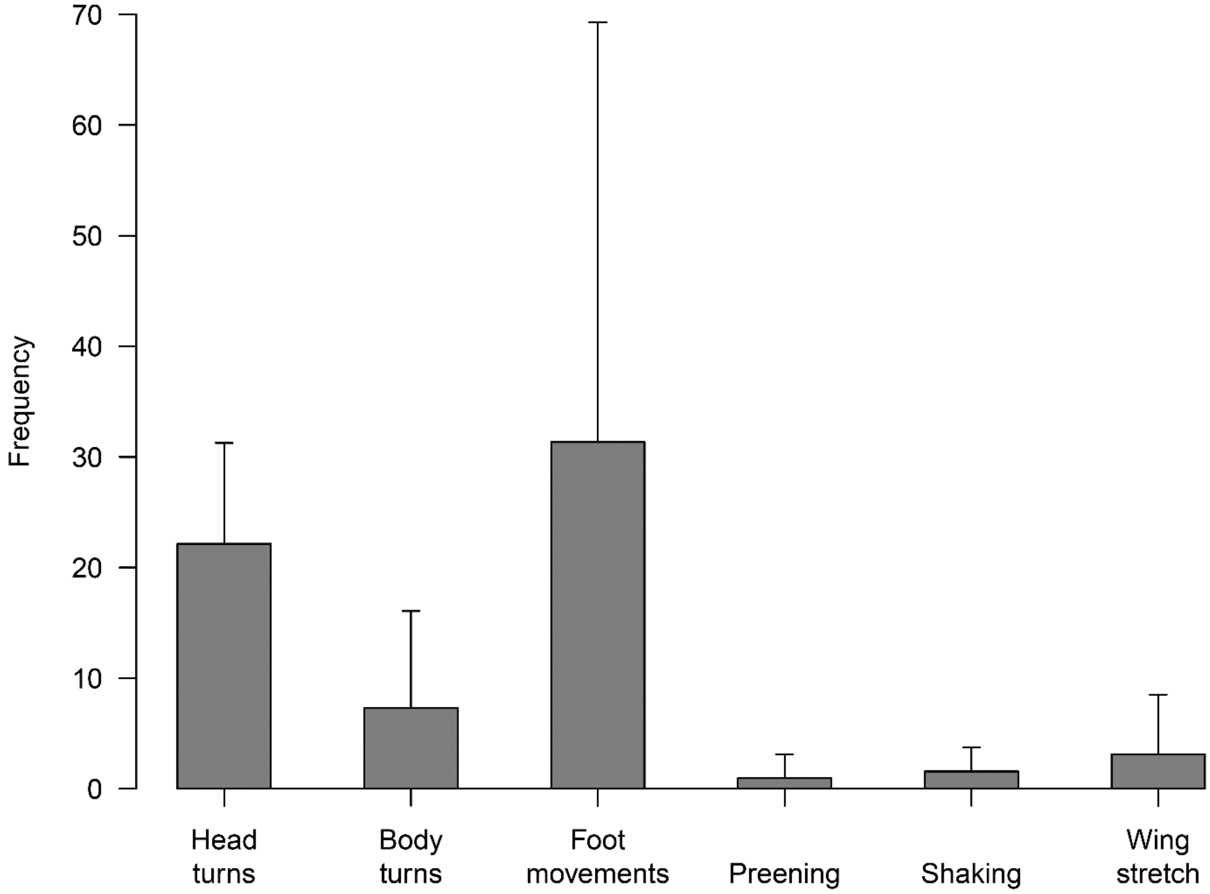

Fig 7.4. Rate of chick movements (mean ± SD) per 300 seconds, Desertas Islands, Madeira, Portugal (N= 42 chicks).

There was no significant relationship between mercury concentration and behaviour of chicks (Table 7.3). Finally, there was a non-significant relationship between exploratory behaviour and mercury concentration (GLM: 0.06 ± 0.14 (SE), z ratio = 0.46, p = 0.647).

Table 7.3. Summary of linear model (LM) outputs used for testing the differences between mercury concentration - independent variable - on frequency of different types of behaviours - dependent variable. Parametric coefficients were also shown (e.g., estimated degrees of freedom ± SE and t test. Significant statistics ($p < 0.05$) are highlighted in italics.

	Estimate ± SE	t test	p value
(a) Head turns			
Intercept	23.66 ± 4.86	4.87	*< 0.001*
Mercury concentration	-0.20 ± 0.60	-0.33	0.746
(b) Body turns			
Intercept	6.12 ± 4.67	1.31	0.198
Mercury concentration	0.16 ± 0.58	0.27	0.791
(c) Foot Movements			
Intercept	16.92 ± 20.10	0.84	0.405
Mercury concentration	1.87 ± 2.49	0.75	0.457
(d) Preening			
Intercept	0.73 ± 1.16	0.63	0.532
Mercury concentration	0.03 ± 0.14	0.20	0.843
(e) Shaking			
Intercept	1.45 ± 1.17	1.24	0.222
Mercury concentration	0.02 ± 0.14	0.11	0.912
(f) Wing stretch			
Intercept	2.64 ± 2.88	0.92	0.364
Mercury concentration	0.06 ± 0.36	0.17	0.864

7.5. DISCUSSION

The results of this study give support for the hypothesis of a negative effect of exposure to mercury in the chicks of seabirds. The observed mercury concentrations in Bulwer’s petrel are high, and their significant association with various parameters of growth of body mass and wing length highlights the possibility that, at these concentrations, mercury can have an effect on physiology and behaviour. A strong variation was revealed in the mercury signatures of plumage of chicks of Bulwer’s petrel, suggesting considerable individual variation in the mercury exposure. Our results indicate that chicks of Bulwer’s petrel can be impaired by exposure to mercuric. The results of this study indicated: (1) that mercury concentrations in Bulwer’s petrel chicks in the Northeast Atlantic varied between years (2) that mercury of body feathers was related with development/growth of chicks (3) that chicks with high mercury levels in body feathers fly at an older age, possibly due to a slower growth and (4) that mercury did not influence behaviour of chicks in the nest.

The levels of mercury measured in body feathers of chick of Bulwer’s petrels may be indicative of contamination in the Atlantic Ocean, during breeding season. We found differences between years in the levels of mercury in feathers. Feathers are one of the major tissues for mercury excretion in young birds, where mercury from blood is deposited during feather growth, reflecting accumulation through diet over this period (Monteiro and Furness 2001). Thus, concentration of mercury is dependent on the trophic position and composition of diet of parents (Monteiro et al., 1998). Therefore, differences in mercury contamination among years can result from differences in the regional bioavailability of mercury, differences in foraging areas or differences in food quality (type of prey). Similarly, there was a significant difference between years in mean growth statistics due probably to environmental conditions. This pattern is consistent with earlier studies which suggested that the chick growth rates is a function of the environmental conditions experienced during growth (Nunes and Vicente, 1998; Pollet et al., 2017).

In this study, the pattern found is typical of Bulwer's petrels and other Procellariformes. Maximum weight data are similar to report from Nunes and Vicente (1998), where chicks achieve maximum weight peak at 40 ± 6.1 days old (28-49 days, N = 30 chicks). Consistent with earlier observations, growth of wing length is slow initially, increase rapidly and then slow again (This study; Nunes and Vicente, 1998). The fast growth of the wing length after about 36 days may increase the mobility of chicks after hatching and thus improve their chances of escaping potential predators (e.g., yellow-legged gulls) while being unable to fly (see Supplementary material Fig. D2). Wing growth is crucial for fledging and may therefore be inflexible.

To the best of our knowledge, this study is one of the first in the world to demonstrate a negative relationship between mercury and the parameters of growth in individual seabirds in the wild. In this study, high levels of mercury were related to a general decline of growth parameters in chicks of Bulwer's petrels. Specifically, our study showed that body mass increased to a maximal value later in chicks with higher levels of mercury than in chicks exposed of lower mercury concentration. These results suggest that environmental mercury has a clear impact on the development of Bulwer's petrels. However, the mechanisms by which mercury may affect growth in early life are unclear.

Our findings are in line with previous research reporting that mercury concentrations in feathers of adults can be associated with slower growth in their chicks. Amélineau et al. (2019) reported that mercury levels of little auk *Alle alle* have directly impacts in breeding performances, especially in growth rate of chicks. Chicks with slower growth rates have parents with higher mercury levels than chicks with faster growth rates. However, Amélineau et al. (2019) not evaluated the concentrations in body of chicks.

The literature on heavy metals exposure suggests that metals may be directly toxic to tissues, such as bones (Rodríguez and Mandalunis, 2018). For example, in vertebrates, lead exposure that has been associated with stunting in the first years of life demonstrate that lead affects growth via toxic effects on bone tissue (e.g., Ronis et al., 2001; Wani et al., 2015). Most recently, a growing body of literature has explored the impact of mercury on a variety of health outcomes in vertebrates. Findings include potential adverse effects on foetal growth and young growth. In Humans, recent evidence raises the possibility of effects of mercury exposure on foetal growth and on infant growth. Cord blood mercury (Grandjean et al. 2003; Kim et al. 2011) and late-pregnancy maternal blood mercury (Kim et al. 2011) have been associated with impaired infant growth of weight within the first two years of life. However, the elevated susceptibility of infants and children to mercury toxicity is yet to be explained. Thus, the potential impact of mercury on growth and development is uncertain, although there is suggestive evidence of an effect in vertebrates.

Mercury levels reported in this study were very high compared to many other seabirds (e.g., Monteiro, 1996), but we did not find any associations with chicks' behaviour in the nest. This similarity between chicks despite considerable variation in mercury levels suggests that, at the present concentrations, mercury does not have a strong impact in simple behavioural performance of chicks. The dose of mercury can be an important determinant of whether or not mercury can change the behaviour. In vertebrates, changes in behaviour are common after acquired brain injury, because brain controls different types of behaviour (Hall et al., 2015). The exposure of mercury that can affect physiology may not be large enough to produce significant brain injury and consequently altered behaviour.

Our results are the first to find a negative relationship between mercury levels and fledging behaviour in young seabirds. The perturbation of mercury can have significant effects on timing of fledging. The behavioural analysis in Bulwer's petrel indicated that high mercury exposure was not enough to produce significant changes on nest behaviour and apparently lesions in the brain, but strong enough to change the age of fledging. Previously, in adults and chicks of other seabird species, there is no evidence of changes in behaviour (e.g., return rate and fledging) in relation to high mercury levels (e.g., Pollet et al., 2017; Thompson et al., 1991).

This is the first study to confirm the association of environmental mercury with changes in parameters of growth and increased age of fledging in chicks of seabirds. Alternatively, it is possible that the nutritional quality of the diet and the quantity of food affect growth and fledging age and mercury levels too. There is a possibility that the birds with the higher mercury content were fed on lower quality prey and/or prey that was scarcer and harder to obtain, which was also prey with higher mercury concentration, leading to an association between mercury levels and growth rates in chicks. Further work will be needed to assess whether mercury directly affected growth or whether this alternative explanation was the cause of the patterns described in this paper.

7.6. CONCLUSION

The approach adopted in this study to relate contamination with growth gave relevant insights and could be replicated in other systems. For Bulwer’s petrel, our results demonstrate that mercury correlates with growth and age of fledging. Chicks with higher levels of mercury in body feathers have significantly slower growth rates of body mass, peak mass later, and fly later compared with lower mercury concentrations chicks. It remains to be determined if mercury affected development directly or whether mercury correlated with diet quality and abundance, with these later factors directly affecting growth.

The results of this study show that there is a high presence of mercury in the body of chicks, during the development. However, no association between behaviours and mercury was observed. For many simple behavioural endpoints, there is still a need to link behaviour with ecological stressors, such as mercury; currently, only a limited number of studies have addressed this issue. We propose that the kind of quantitative, in situ observational methodology that we used could be used in other species of seabirds to evaluate possible impacts of mercury in behaviour.

CHAPTER 8

BULWER'S PETRELS ACCUMULATE HIGH MERCURY CONCENTRATIONS WITHOUT NOTICEABLE EFFECTS ON FORAGING BEHAVIOUR.

Ricardo Furtado

8.1. ABSTRACT

Mercury has always been present in the environment. Both natural events and a vast range of human activities over centuries have mobilized the mercury in ways that pose environmental and human health risks. Several studies have documented alterations in the consumption of preys, reduction of parameters of reproduction, and aberrant behaviours in birds exposed to mercury. Still, it has been focused on songbirds and waterbirds, their habitat types, and only some life history stages (e.g., reproduction), leaving the assessment of mercury's threats to birds incomplete. Seabirds, particularly the order Procellariformes (albatrosses, petrels, and shearwaters) accumulate high concentrations of mercury in their tissues and are little studied. The Bulwer's petrel, *Bulweria bulwerii,* is a highly specialised predator of mesopelagic fish and squid, and therefore is exposed to relatively high mercury concentrations through bioaccumulation and biomagnification. However, the impact of mercury contamination in the behaviour of seabirds has seldom been examined. We measured the Bulwer's petrel trip duration and daily mass gains during foraging and examined their relationship with blood mercury concentration during the incubation period. The concentration of mercury in blood averaged 10.91 ± 1.63 mg kg^{-1} dw and varied from 8.04 to 14.18 mg kg^{-1} dw. Our results indicate that foraging trip duration and mass gained per day of foraging were not correlated to the mercury levels in the blood. Despite the exceptionally high levels of mercury accumulated by Bulwer's petrels, their behaviour seems largely unaffected by mercury concentrations across the range measured here. This methodology did not affect reproduction, because reproductive success was 71 % in birds studied (N= 48 nests) and was 63% in birds not sampled during incubation (N = 52 nests).

8.2. INTRODUCTION

Heavy metals, plastics, pesticides, herbicides, fungicides, food and fuel additives, and numerous environmental contaminants correspond to chemicals to which humans, birds, fish and other organisms are routinely exposed (e.g., Driscoll et al., 2013; Furtado et al., 2016, 2019, 2020; Senesi et al., 1999). Mercury and/or methylmercury is a familiar example of pollutant that has accumulated slowly through time and unexpectedly caused permanent, deleterious effects in humans, birds, and other organisms. There is an urgent need to find out what effects the substances accumulating in our environment may be having on us and wildlife.

Mercury is commonly present in the environment because of natural sources but can also be regarded as a pollutant when originating from human activities (including gold extraction, waste incineration, industrial production and use of fossil fuels) (Driscoll et al., 2013; Esdaile and Chalker, 2018). In the ocean, once converted by microbes to methylmercury in low oxygen environments, such as the mesopelagic zone (200 – 1500 m below the ocean surface), this organic form of mercury bioaccumulate and biomagnify through marine food webs and can be highly toxic to organisms, such as top predators (e.g., humans and seabirds) (Liu et al., 2020; Monteiro et al., 1998; Senesi et al., 1999). As a result, top predators acquire greater body burdens of mercury than the preys they consume.

Exposure and toxicity of environmental mercury to seabirds can be enhanced or lessened by the available source forms of mercury and other species' dependant factors such as trophic status, foraging preferences during either the breeding or non-breeding seasons the type of habitat used during the breeding season and migration behaviour (e.g., Albert et al., 2019; Anderson et al., 2009; Becker et al., 2016; Furtado et al., 2019; Binkowski et al., 2021). Seabirds obtain and assimilate energy and nutrients through diet, during feeding behaviour, that is fundamental to the lives of birds (Kramer et al., 2001; Remsen and Robinson, 1990). Although foraging offers many benefits, it has its pitfalls too. Birds, especially seabirds, face multiple risks whilst foraging, such as the risk of assimilation of mercury (e.g., Gatt et al., 2020; Thompson et al., 1998; Furtado et al., 2019). Seabirds, particularly those that occupy high trophic levels and have a behaviour that allows them to explore deep ocean zones and feed on mesopelagic prey, are highly exposed to persistent and toxic mercury (e.g., Thompson et a., 1998; Furtado et al., 2021), because mesopelagic fauna transport methylmercury into the epipelagic domain (Motta et al., 2019). On the other hand, seabirds that feed around their nesting sites, foraging in areas over the continental shelf and feed on benthic and/or pelagic prey are lowly exposed to mercury (Anderson et al., 2009; Thompson et al., 1998; Furtado et al., 2019). Thus, understanding foraging behaviour is essential to understand the basis of risks of mercury exposure. Bulwer's petrels consume a large variety of mesopelagic organisms, enriched in methylmercury, particularly fish and squid that occupy surface waters at night as a result of diel vertical migrations (Neves et al., 2011; Waap et al., 2017; Spear et al., 2007). Due to this specialization in mesopelagic preys, Bulwer's petrels have some of the highest concentrations of mercury reported in seabirds (Monteiro et al., 1999; Thompson et al., 1998).

Bulwer's petrels also choose where and when to search for food, and how to move between locations and seasons. During the non-breeding season, Bulwer's petrels are mid- to long-distance migrants, moving into tropical deep, open oceanic areas (e.g., Dias et al., 2015). During the incubation period, Bulwer's petrels are specialized in foraging over deep ocean basins, in lower productivity zones, where they exploit scarce and unpredictable oceanic resources at great distances from the colony (Dias et al., 2016). Although mercury assimilation is associated with foraging habitats (Furtado et al., 2021), what remains less well understood are the risks of assimilation to Bulwer's petrels health.

Humans and other animals, such as birds, can use several behavioural and/or physiological strategies or adaptations to reduce the risk/effects of environmental contaminants (e.g., Monteiro et al., 1995). There are behavioural strategies by which humans can avoid or reduce mercury poisoning, such as avoid or reduce mercury intake through changes in the type of food and select a mixed diet to dilute the effect of mercury (Crépet et al., 2005; Selin, 2011). For example, birds have physiological strategies involving storing or internally eliminating mercury when consumed (e.g., excrete mercury through growing feathers) (Bearhop et al., 2000). They can also complexify, degrade, or detoxify the mercury, through internal interaction between mercury and selenium to: form inert complexes of high molecular weight in the blood, especially in the erythrocytes; form harmless non-diffusible complexes (mercury-selenium) in the liver and kidney (Cuvin-Aralar and Furness 1991; Imura and Naganuma 1991; Yang et al.

2008; Manceau et al., 2021); and/or tolerate the mercury once eaten (Thompson et al., 1991). Vertebrates' health risks posed by toxic elements rarely follow a simple linear relationship, because it is impossible to separate changes in behaviour from the external consequences of mercury assimilation, since physiology affect the animals' behaviour (Scheuhammer, 1987; Wolfe et al., 1998).

Assimilation of mercury can have immediate or long-term profound effects on an animal's health (e.g., Wolfe et al., 1998). Mercury in vertebrates can cause death (Scheuhammer 1987, Heinz 1996) and severe pathology in organs' system, especially central nervous system dysfunctions, with associated brain lesions, particularly in the cerebellum and cerebral cortex in man and other vertebrates (Azevedo et al., 2012; Cristol and Evers, 2020; Wolfe et al., 1998; Chang, 1977). In wildlife, the most common acute neurological effects are the loss of motor skills, coordination and reduction in motivation (Wolfe et al., 1998). Among the earliest signs of dysfunction reported in humans exposed to methylmercury are impaired vision, muscle weakness, impacts to their cognitive thinking, memory, attention and language, fine motor skills, physical malformations, and signs of developmental retardation (Azevedo et al., 2012).

The essential behaviours for life of birds, which involve coordinated movements, can also be affected by mercury (Spalding et al., 2000; Fimreite and Karstad, 1971; Laties and Evans, 1980; Evans et al., 1982; Heinz and Locke, 1976); Scheuhammer, 1988; Wolf et al., 2017; Scoville et al., 2020; Loerzel et al., 1999; Bennett et al., 2009). Mercury intoxication can also not affect foraging behaviour in laboratory studies. Adams and Frederick (2008) when evaluating feeding performance in White ibises *Eudocimus albus* dosed with mercury found no cohesive link between mercury exposure and foraging behaviour. On the other hand, Bouton et al. (1999) when dosing Great egrets Ardea alba, reported that mercury not affected foraging efficiency.

In Nature, Olsen et al. (2000), when observing, in situ, the feeding behaviour of Common loon adults *Gavia immer*, found that individuals with more than 4 mg/kg of mercury in blood dipped significantly more, than individuals with less than 4 mg/kg of mercury in blood. In contrast, Merrill et al. (2005), when observing feeding behaviour also of this species, concluded that, capture success was not related to blood mercury levels (levels of mercury in blood: ca. 0.2 - ca. 5.8 mg/kg w wet.). Similarly, Seewagen (2013), in assessing the impact of mercury concentrations in Northern Waterthrushes *Parkesia* *noveboracensis*, found that the levels present in the body of the individuals did not seem to affect foraging behaviour (levels of mercury in blood: 0.1 - 2.1 mg/kg ww). However, the consequences of mercury concentration on foraging behaviour are unclear and poorly studied in nature.

In seabirds, the majority of studies provide evidence that the presence of mercury in blood of adults (> 1 mg / kg dry weight) does not influence seabird's behaviours (e.g., incubation behaviour, survival and parental behaviours) (e.g., Blévin et al., 2018, 2020; Taylor et al., 2016; Pollet et al., 2017; Carravieri et al., 2018; Thompson et al., 1991).

The main goal of this study is to investigate the effects of mercury on foraging behaviour of the Bulwer's petrel. In this study, mercury values were measured in blood, which provide trophic information on a few weeks before sampling (Hobson and Clark 1992, 1993), thus corresponding here to the pre-laying period/incubation period. On Deserta Grande, in the Madeiran archipelago, the incubation season lasts from early June to mid-July during which males and females alternately incubate the egg or forage for a number of consecutive days (Nunes, 2000). To determine whether mercury concentrations in the blood altered foraging and incubation behaviour and fitness, we monitored a colony of Bulwer's petrels, to (a) relate the values for mass gained per day at sea with mercury concentrations in birds during incubation and (b) relate blood mercury concentration with foraging trip duration by investigating nest attendance.

8.3. METHODS

This study was carried out on Deserta Grande (32˚5'N, -16˚5'W), the largest of a group of islands lying ca. 20 km from Madeira, Portugal between 13th June and 20th July 2018. Desertas Islands hosts one of the largest colonies of Bulwer's petrels (Meirinho et al., 2014), crudely estimated at 45.000 breeding pairs (Catry et al., 2015).

8.3.1. BLOOD SAMPLING

To quantify mercury levels in adult birds, we collected blood samples during the incubation period and before mass measured (between 13 and 18 June) from 56 randomly selected adults. Only one member from each breeding pair was sampled. Blood was taken from the brachial vein into capillary tubes (ca. 70 µL) and then transferred to centrifuge tubes that were kept frozen until processing at laboratory.

8.3.2. MONITORING OF MASS AND TRIP DURATION

A number of available nests were selected and individually marked early in the incubation period. One member of the incubating pair was distinguishably marked with a small white paint on the forehead and the tips of the tail feathers, to facilitate its identification within the pair. Sex could not be determined in the field. During incubation, members of the pair alternate between attending the egg or doing a foraging trip that typically lasts several days. The daily attendance of each partner was recorded throughout incubation to determine incubation shift length and foraging trip duration. During the incubation period, the mass of marked parents was measured on the day following its return from a foraging trip, to avoid immediate disturbance after arrival and to allow for meal digestion, using a 300 g Pesola spring balance (Ramos et al., 2009) and every four days until the members exchanged incubating. After we weighed the birds, we returned them immediately to their nests to resume incubation. For birds not weighed on the day preceding a changeover, we estimated mass at departure by using the last mass recorded and the mean rate of mass loss during shift incubation. (Catry et al., 2006; Ramos et al., 2009). Data of foraging trip and body mass changes were successfully obtained for 48 individuals for which Hg concentration had been measured.

8.3.3. MERCURY ANALYSIS

Total mercury concentration in the blood of 48 birds was quantified by atomic absorption spectrophotometry with thermal decomposition (Costley et al., 2000) in LECO AMA-254 with a detection limit of 0.01 ng of mercury. Samples for mercury determination were lyophilized during 48 h and ground to powder for homogenization, before mercury quantification. The electronic micro-balance (Sartorius M5P, Sartorius AG, Gottingen, with 0.001 mg precision) was used to prepare between 0.36 mg and 0.99 mg (mean = 0.51 ± 0.11 mg). For each sample, analyses were repeated two or three times, until the standard deviation for the aliquots was <10%. Subsequently, the mean of the repeated mercury measurements was used for statistical analysis. All sample measurements were above the limit of detection of the equipment. Blanks were systematically run between samples (two or three procedural blanks). The mercury concentrations in procedural blanks were always below the detection limit of the equipment (0.01 ng of mercury). Precision and accuracy of the analytical method was evaluated by analysis of certified reference material (CRM). CRM was analysed at the beginning, middle and at the end of the analytical cycle. This included: CRM - lobster hepatopancreas TORT-3; NRC, Canada; reference values were of 0.292 ± 0.022 mg kg^{-1} dry weight (dw), and the mean determinations ± SD were 0.251 ± 0.007 mg kg^{-1} dw (n = 45). Thus, the recovery of the CRM was 86.0 ± 0.96%. Results were corrected using the daily recovery efficiency of CRM. Mercury concentrations in blood are presented in mg kg $^{-1}$ *dry weight* (dw). Because blood is also measured and reported on a wet-weight basis, the formula (Soldatini et al., 2020):

$$Blood\ (Wet\ \ Weight) = Blood\ (Dry\, Weight) * 0.21$$

can be used to convert dry-weight values to wet-weight values (e.g., assuming an average 79% of moisture) as previously done (Ackerman et al., 2016a), to allow comparison of mercury in blood among different species.

8.3.4. STATISTICAL ANALYSIS

All statistical analyses were carried out with R statistical software (R Core Team, 2020). We used generalized linear regressions to test for associations between blood mercury concentration and foraging trips duration and mass gained per day of foraging. Diagnostic plots and Shapiro normality tests were performed on residuals to test whether the data sufficiently met the assumption of the models. Means are presented with standard deviations unless stated otherwise.

8.4. RESULTS

We obtained data on foraging trips duration and mass gain from 48 individuals during the incubation period. The foraging trips of Bulwer's petrels during the incubation period lasted, on average, 11.4 ± 1.2 days, ranging from 8 to 14 days (n = 48). The mass gained per day of foraging, on average, was 2.09 ± 0.72 g (range: 0.54 - 3.73 $g.day^{-1}$; n = 48).

Adult Bulwer’s petrels’ total mercury in blood ranged from 8.04 to 14.18 mg kg^{-1} dw (mean ± SD: 10.91 ± 1.63 mg kg^{-1} dw). Average mercury concentrations in our study were equivalent to an average of 2.29 ± 0.34, range of 1.69 – 2.98 in wet weight).

Foraging trips duration was not related with blood mercury in adults of Bulwer’s petrel (LM: effect of mercury = 0.12 ± 0.11 (SE), t = 1.07, p = 0.29) (Fig. 8.1). Finally, there was no significant relationship between adult blood mercury and mass gained per day of foraging (LM: effect of mercury = 0.08 ± 0.06 (SE), t = 1.25, p = 0.22) (Fig. 8.2).

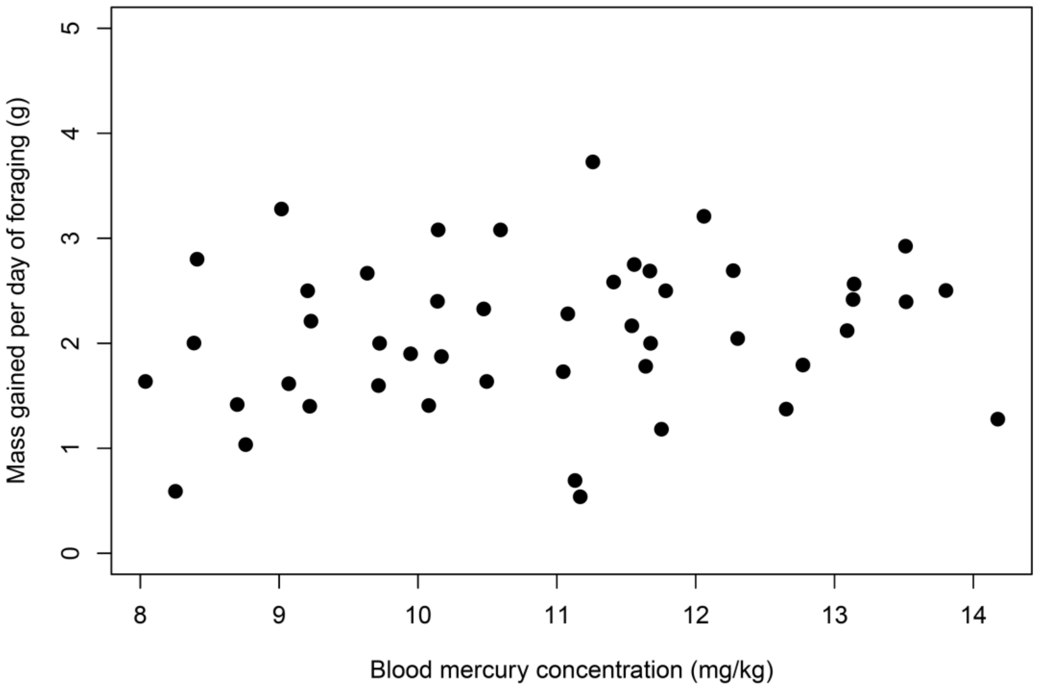

Figure 8.1 Blood mercury concentration (mg/kg, dw) in relation to foraging trip duration (days) in adults Bulwer’s petrel.

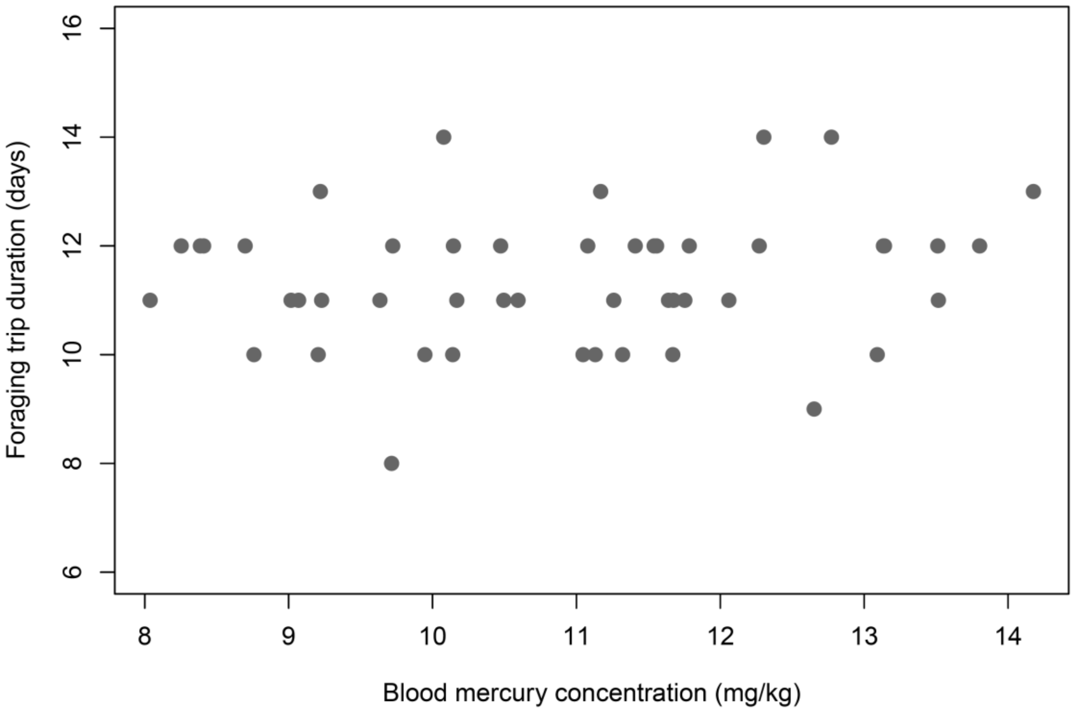

Figure 8.2 Blood mercury concentration (mg/kg, dw) in relation to mass gained per day of foraging (grams) in adults Bulwer’s petrel.

8.5. DISCUSSION

In this study we provide the first record of mercury concentrations in blood of Bulwer's petrel and examined the potential effects of this heavy metal in foraging parameters of this species. Despite the unusually high concentrations and the reasonable variation among individuals in the mercury burden in blood, we found no negative effects of this toxicant in the foraging behaviour of the species. Our results show no differences in behaviour and body-mass gain between birds with lower mercury concentrations and birds with higher mercury concentrations in the blood.

The mercury concentration reported in our results for Bulwer's petrel adults are in a range of values reported for adult Pterodroma petrels, during pre-laying period (e.g., Magenta petrels (*Pterodroma magentae*), mean ± SD: 11.72 ± 3.58 mg kg^{-1} dw) (Thébault et al., 2021). Blood mercury concentrations in the Bulwer's petrel and Pterodroma petrels are amongst the highest ever recorded in seabirds (Cherel et al., 2018; Thébault et al., 2021). Furthermore, the high levels of mercury in the feathers of Bulwer's petrel (mean ± SD: 16.49 ± 1.39 mg kg-1 dw) confirm that adult Bulwer's petrels are also exposed to high concentrations of mercury during the non-breeding season (Furtado et al., 2021). Dietary investigations carried out during the breeding season, indicated that the Bulwer's petrel is a specialist predator of mesopelagic organisms, particularly fish (e.g., Myctophidae, Gonostomatidae, Phosichthyidae) and squid (e.g., Ommastrephidae, Histioteuthidae, Mastigoteuthidae) (Neves et al., 2011; Waap et al., 2017) that occupy surface waters at night as a result of diel vertical migrations (Kelly et al., 2019). The vertical migration of species that form the "deep scattering layer" is an important mechanism linking the mesopelagic and epipelagic domains and their food webs (Motta el al., 2019). A few seabirds, including Bulwer's petrels but also Pterodroma petrels, have specialised in exploring these mesopelagic resources in an efficient way (Wapp et al., 2017). These preys are captured in mesopelagic domains in open ocean area, with high concentrations of methylmercury (Gill and Fitzgerald, 1988; Bowman et al., 2020), often very far from the breeding colonies (e.g., Dias et al., 2015, 2016; Taylor et al., 2020), and are the reason for the exceptionally high concentration found in these seabirds (Monteiro et al., 1996; Bustamante et al., 2006; Thompson et al., 1998).

Although adverse effects of mercury in the blood of Bulwer's petrel have not been determined, the concentrations in the present study were higher than most previously reported threshold concentrations for toxic effects in birds (Ackerman et al., 2016). Ackerman et al. (2016a) proposed that blood methylmercury concentrations between 4.8 mg/kg dw and 14.3 mg/kg dw have a moderate risk to birds, based on a review of the literature. Overall, total mercury levels in the blood of adult Bulwer's petrels analysed in this study are between 4.8 and 14.3 mg/kg dw (n = 48), and the highest value recorded in our sample was 14.18 mg/kg dw. Thus, adult Bulwer's petrels can be considered to have moderate risk to methylmercury toxicity. However, blood mercury at levels measured in our study do not seem to have an influence in the behaviour of the birds during foraging (as reflected in foraging trip duration) or in their food intake (as reflected in daily mass gain during the foraging trip).

Our data agrees with previous research reporting a limited effect of blood mercury in the behaviour, immune function, and reproduction success of seabirds (Goutte et al., 2015, Pollet et al., 2017; Carravieri et al., 2018; Thébault et al., 2021). However, some studies suggested that mercury can cause changes in the immune function, the endocrine system (decreased segregation of LH) (Finkelstein et al., 2007; Tartu et al., 2013) and incubation behaviour (egg neglect) (Tartu et al., 2015), without demonstrating any clear adverse effects in the population dynamics (e.g., increased mortality), suggesting that seabirds exhibit tolerance to mercury contamination.

Thus, it seems that resistance to toxic effects in some species and groups of birds such as seabirds imply a tolerance to levels of mercury. Elucidation of the mechanisms that provide resistance to mercury in seabirds is beyond the reach of this research and would require physiological experiments specially designed for that purpose.

In many vertebrates, including seabirds, high concentrations of mercury may be tackled using different natural defence mechanisms for depurating. Once ingested, mercury is efficiently absorbed and transported to internal tissues, where it can be stored, demethylated or excreted. For example, liver and kidney tissue have the capacity to store inert complexes of mercury (e.g., mercury-selenium), which acts as a form of protection against methylmercury toxicity (Kim et al. 1996; Wolfe et al. 1998; Yang et al., 2008). In addition, a significant part of the mercury burden is stored in feathers, strongly binding to the keratin of feathers in levels proportional to body burden (Gatt et al., 2020). Therefore, a considerable amount of mercury is regularly excreted during moult (Bearhop et al., 2000; Furness et al., 1986; Monteiro and Furness 1995). In females, a lesser fraction is also mobilised during egg production and therefore transferred to chicks (Burgess et al., 2013; Monteiro and Furness 2001; Kubota et al. 2002; Ackerman et al., 2016b).

In conclusion, this research is a significant contribution to our understanding of mercury exposure and accumulation in blood of a mesopelagic seabird from a remote site of the Northeast Atlantic. Our results document that the Bulwer's petrel has an extremely exposure to mercury during the incubation period and possibly year-round, highlighting the extent of mercury availability in the mesopelagic layer of the Northeast Atlantic. It is clearly useful to draw attention to possible links between mercury contamination and foraging behaviour in wild populations. The lack of any association between mercury and foraging behaviour in this study, at a colony which does nonetheless exhibit high mercury concentrations and a chronic exposure, due of their specialization in mesopelagic preys, emphasizes the need for rigorous methods which examine mercury accumulation, physiology, and reproduction.

CHAPTER 9

GENERAL DISCUSSION

Ricardo Furtado

This dissertation focused mainly on mercury, because this element is considered one of the most potentially hazardous pollutants, as it has no known biological function, and is highly toxic, unlike most other trace elements. In addition, it tends to accumulate along the food chain and its concentration can be reliably measured in feathers.

Mercury treatment alter the architecture of brains and change the behaviour of birds. Some studies with captive birds exposed to mercury have documented ataxia, lethargy, reduced appetite, and aberrant reproductive behaviours (see Chapter 2 and 3 for a literature review). However, there is limited evidence to show that current levels of mercury in wild birds have a negative impact on them. Most of the research done to date has been focused on waterbirds and songbirds, leaving the assessment of mercury's threats to the behaviour of seabirds incomplete. Detectable effects on avian behaviours are mostly described in individuals exposed to sub-lethal concentrations in laboratory. On the other hand, when observed in nature, non-natural/abnormal behaviours? are often difficult to identify and? describe. Furthermore, mercury exposure does not appear to cause significant changes in behaviours of birds ‘chicks and as far as we know, the effect of mercury on the behaviours of seabird chicks has never been studied. This is particularly true for seabirds, as was concluded after the research carried out in Chapter 2.

Seabirds are vulnerable organisms to mercury contamination. Researchers have long suspected that mercury may be a potent toxicant to seabirds. As a neurotoxin, mercury may impair birds' navigation, flight performance, and foraging ability. Thus, it is reasonable to assume that mercury exposure may also contribute to impaired survival of individuals. However, as reported in Chapter 3, there is little solid evidence to show that current levels of mercury in the ocean negatively impact seabird survival. Although studies have reported relatively high concentrations in seabirds, there has been no evidence of a relationship between mercury and individual survival, as found in Chapter 3.

Since most information on bird mercury concentrations is available on feathers and blood, here I have chosen to focus on these two tissues for comparisons. Results on feathers indicate that seabirds have a wide range of mercury concentrations, reflecting a large variation in exposure between species, as illustrated by our study in the Falklands Islands. Here, , different colonies of seabirds exhibited different levels of mercury and arsenic contamination in feathers. Notably, black-browed albatrosses and gentoo penguins had consistently high mercury concentrations on New Island, which differed significantly from those of prions, imperial shag and rockhopper penguins, while rockhopper penguins and imperial shags presented considerably higher concentrations at Beauchene Island. The non-breeding distribution of rockhopper penguins (from New and Kidney Island) was similar to those from Steeple Jason Island (Ratcliffe et al., 2014; Pütz et al., 2002), with birds mainly wintering along Patagonian Shelf in the Subtropical Zone. Contrary, rockhopper penguins of Beauchene Island mostly occur in the Subantarctic Zone and Polar Frontal Zone, in deeper waters (Ratcliffe et al., 2014). Furthermore, rockhopper penguins from Beauchene, during the breeding season feed on mesopelagic preys (Clausen and Pütz, 2002; Croxall et al., 1985). Such movements may explain the difference in mercury concentration.

In relation of arsenic, Rockhopper penguins exhibited high arsenic levels, but levels were less variable among species, and were not correlated with mercury levels, suggesting low biomagnification. Imperial shag, black-browed albatrosses and gentoo penguins had the same mean concentrations of arsenic on both places. Differences in trace elements contamination of seabird species among islands can result from differences in the local bioavailability of mercury, differences in foraging areas and/or differences in type of food. The study developed in Chapter 4 establishes baseline levels for mercury and arsenic in seabirds of the Southern Patagonian shelf that will be useful for future monitoring.

Similarly, blood concentrations of trace elements in black-browed albatrosses were not significantly different between islands, which is consistent with observations from foraging behaviour revealing that birds from both islands foraged in broadly the same areas in the months before sampling. Arsenic and selenium concentrations in females were higher than in males. Sex-related differences in the concentration of these elements may be related to unknown slight differences in diet or to differences in assimilation between sexes.

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 also used as a bioindicator for the ocean, specifically of the mesopelagic domain. Bulwer's petrels from the Atlantic Ocean had higher feather mercury levels than those from the Pacific Ocean, with no difference between colonies in the same ocean basin, which is consistent with previous investigations reporting higher mercury concentrations in deep water in the mesopelagic domain in the Central South Atlantic (ca. 1.3± 0.62 pM in the South Atlantic) than in the central and eastern Pacific (ca. 0.61 ± 0.19 pM in the Central South Pacific and 0.59 ± 0.25 pM in the Eastern Tropical Pacific) (Gill and Fitzgerald, 1988; Bowman et al., 2020).

In this thesis we showed that the trophic position of chicks from different colonies in the Atlantic and the Pacific. This strongly suggests that the foraging strategies and therefore the trophic preferences in Bulwer's petrel are highly conserved in these distinct oceans. Moreover, the high mercury concentrations in adult Bulwer's petrels and their similarity among sampled oceans further reinforce the idea that Bulwer's petrels are specialist on mesopelagic preys in both the non-breeding and the breeding seasons, irrespective of colony location, as found in Chapter 6.

Mercury concentrations were high in Bulwer's petrels compared to other species, but does this mean that seabirds may suffer negative effects? In Chapter 7, we examined the relationship between mercury concentration in feathers and growth rate (body mass and wing length), age of fledging and behaviour in Bulwer's petrel chicks. Chicks with lower growth rates had higher mercury concentration in body feathers, and those with lower body mass growth also showed retarded growth of wing length. Furthermore, chicks which fledged at an early age displayed a much faster growth rate and lower mercury concentrations than later fledging chicks. The literature on heavy metals exposure suggests that metals may be directly toxic to tissues, such as bones (Rodríguez and Mandalunis, 2018). For example, in vertebrates, lead exposure that has been associated with stunting in the first years of life demonstrate that lead affects growth via toxic effects on bone tissue (e.g., Ronis et al., 2001; Wani et al., 2015).

No evidence that mercury influences behaviour in the nest was observed. In this thesis we report a noticeable variation in mercury levels, and yet there was a striking similarity of the behaviour of chicks. This seem to suggest that, despite being very high, the current levels of mercury do not have a strong impact in simple behavioural performance. In fact, the absolute concentrations of mercury can be determine whether or not this metal can cause disruption measurable at the behavioural level. In vertebrates, changes in behaviour can follow brain injury, because brain controls different types of behaviour (Hall et al., 2015). Our results are the first to find a negative relationship between mercury levels and fledging behaviour in young seabirds. The perturbation of mercury can have significant effects on timing of fledging

Several studies have documented alterations in the consumption of various prey, reductions of parameters of reproduction, and aberrant behaviours in birds exposed to mercury, as reported in Chapter 2. The Bulwer's petrel, Bulweria bulwerii, is a highly specialised predator of mesopelagic fish and squid, and these prey are known to present very high mercury levels. As other mesopelagic specialists, Bulwer's petrels showed high mercury concentrations resulting from bioaccumulation and biomagnification. Surprisingly, the impact of such contamination in the behaviour of adult seabirds has seldom been examined. Despite the unusually high concentrations and the reasonable variation among individuals in the mercury burden in blood, no negative effects of this toxicant in the foraging behaviour of the species was found by our study. Our results show no differences in behaviour and body-mass gain between birds with lower mercury concentrations and birds with higher mercury concentrations in the blood.

In conclusion, this thesis showed that seabirds can successfully be used to monitor temporal (see Chapter 4, 5 and 6) and spatial variation in mercury presence in ecosystems. The results presented here also suggest that mercury has the potential to affect the physical and behavioural development of seabirds, but that in general seabirds are highly resilient to this pollutant and despite currently accumulating high levels of the toxicant, most of their behaviour, breeding performance, behavioural patterns and survival remain largely unaffected. Nevertheless, more research is needed to further elucidate the effects of mercury on seabirds.

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APPENDIX A

UNTANGLING CAUSES OF VARIATION IN MERCURY CONCENTRATION BETWEEN FLIGHT FEATHERS

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A1. 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 concentrations. 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.

A2. 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).

A3. METHODS

A3.1. 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.

A3.2. 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.

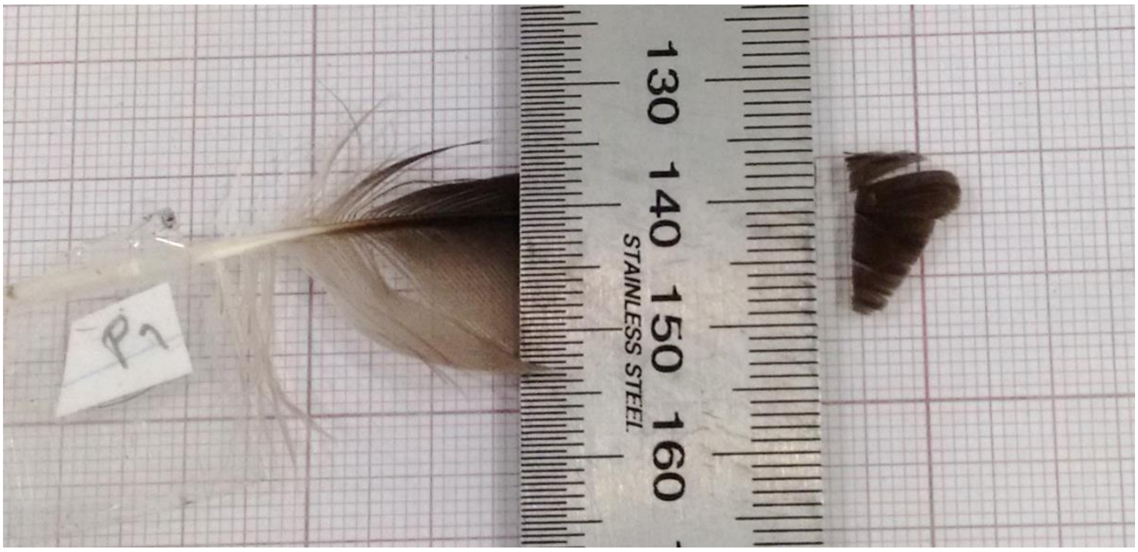

Figure 1 The line of dissection of the outermost segment (8mm of tip) on the innermost primary feather (P01) of a Bulwer's Petrel chick.

A3.3. 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.

A4. RESULTS

A4.1. CHICK PRIMARY GROWTH RATE

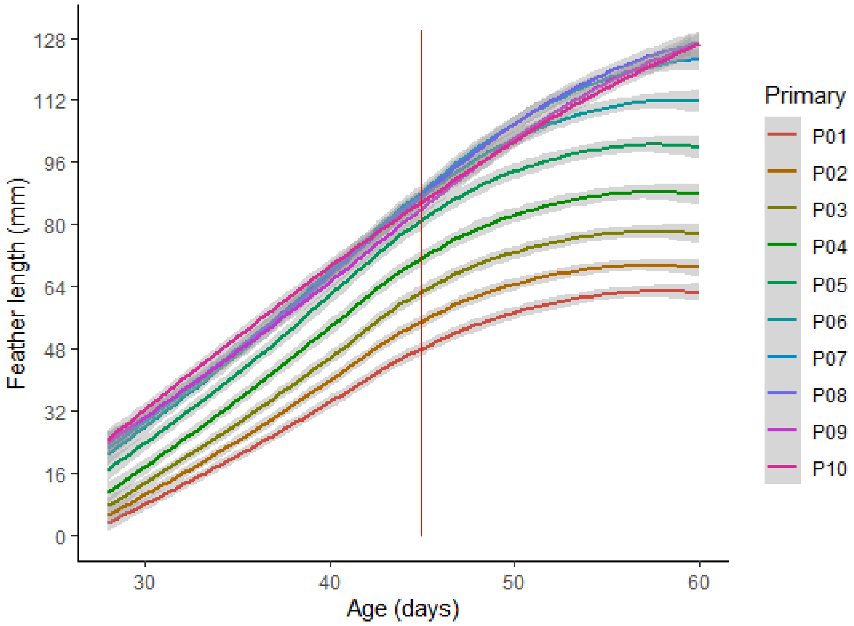

Figure 2 The growth of primary feathers of juvenile Bulwer's Petrels (n = 14) between 28 and 60 days since hatching on Deserta Grande. The growth rate used in this study was

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).

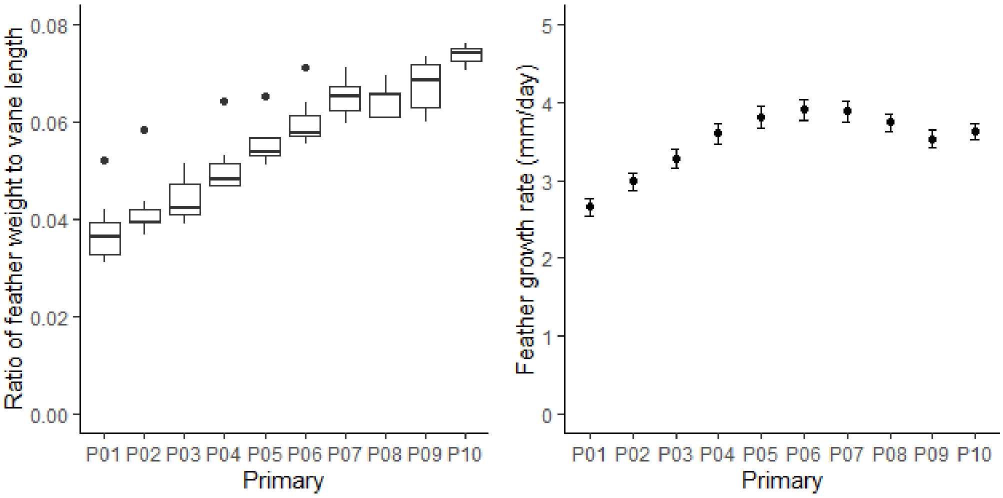

Figure 3 (Left) The ratio of feather weight to vane length is higher in the outer primaries, compared to inner primaries, in juvenile Bulwer's Petrels. **(Right)** The growth rate (calculated between 28 and 45 days since hatching) differs among primary feathers. Whiskers represent standard errors.

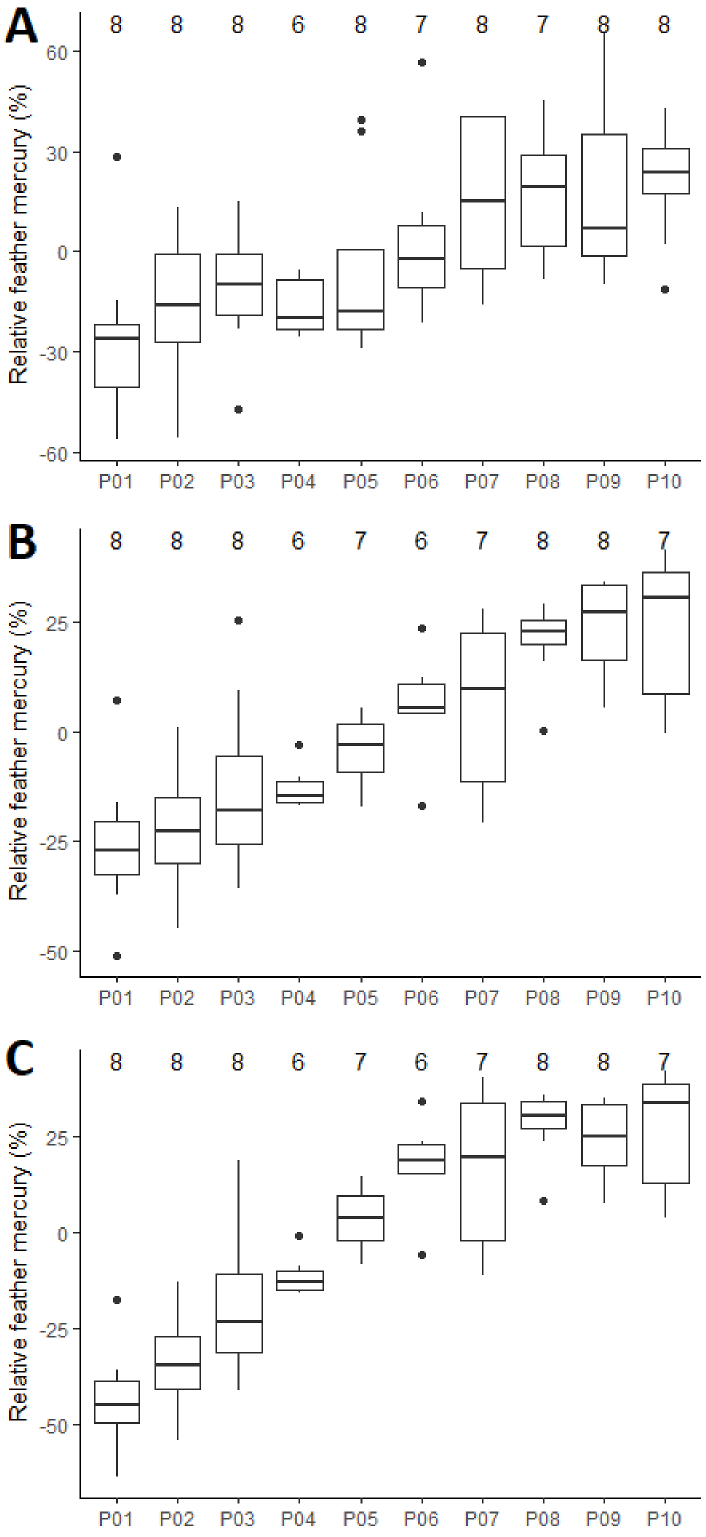

Figure 4 The patterns of deviation in feather mercury concentration expressed as µg of mercury (A) per gram of feather, (B) per millimetre of feather, and (C) per day of feather growth increase from the innermost (P01) to the outermost (P10) primary similarly across all three measures in Bulwer's Petrel 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.

A4.2. 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).

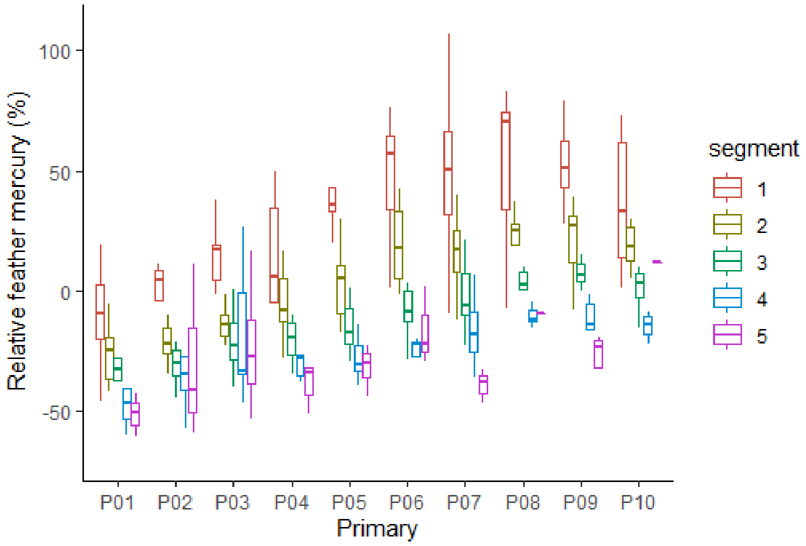

Figure 5 Mercury concentration decreases from the feather tip (the first to be formed) to the proximal end of the feather in the primaries of Bulwer's Petrel chicks. The proximal segments of outer primaries have

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.

A5. 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.

A6. 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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ANNEX B: SUPPLEMENTARY MATERIALS AND METHODS

CHAPTER 4

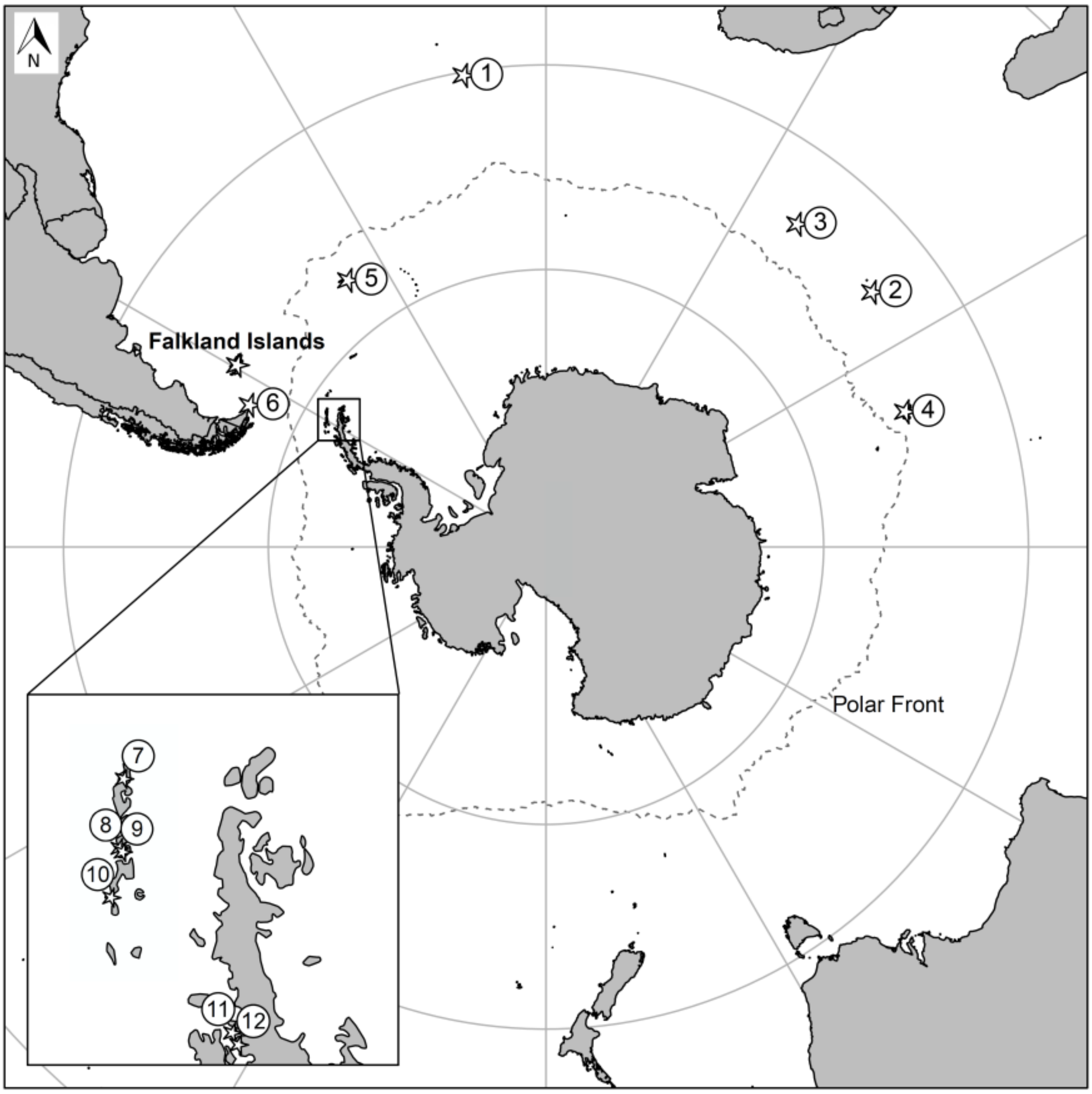

Fig. B1 Location of the breeding island where seabirds were sampled (Falkland Islands). Data from (1) Gough Island; (2) Crozet Islands; (3) Marion Island; (4) Kerguelen Island; (5) South Georgia; (6) Staten Island; (7) King George Island; (8) Barrientos Island; (9) Greenwich Island; (10) Livingston Island; (11) Ronge Island and (12) Paradise Bay are available for comparison.

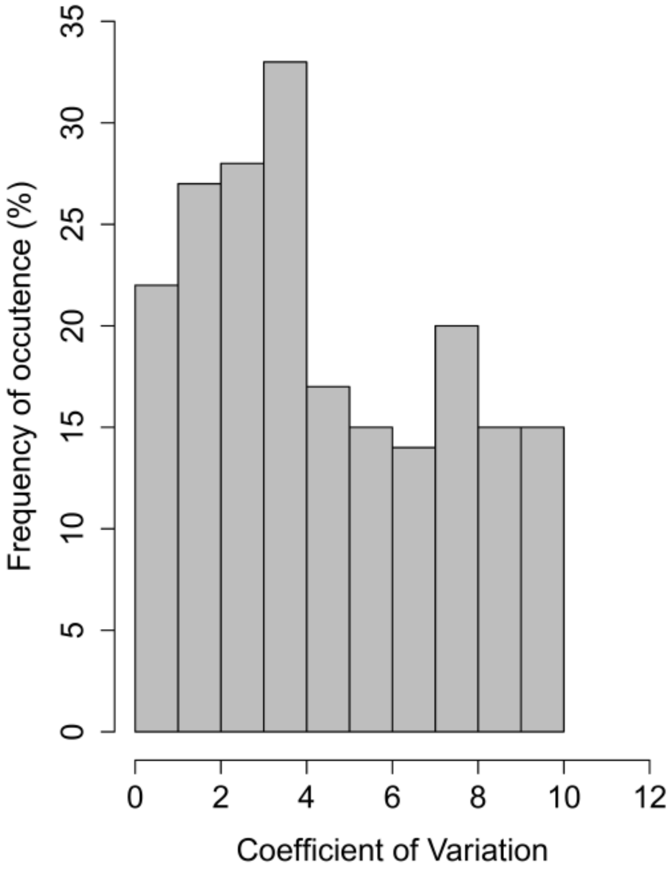

Fig. B2 Frequency of occurrence for the Coefficient of Variation of the mercury analyzed values (more than five feathers per seabirds).

Table B1 Review of mercury concentrations (mg/kg; mean ± SD, (n), reference)) in body feathers of adult seabirds' species from this study (**in bold**) and other studies in the Southern Ocean: 1) Carravieri et al., 2016; 2) Carravieri et al., 2014; 3) Pedro et al., 2015; 4) Brasso et al., 2015; 5) Brasso et al., 2014; 6) Calle et al., 2015; 7) Becker et al., 2016; 8) Becker et al., 2002; 9) Anderson et al., 2009. Data since 1998. All the existing literature was reviewed up to date (December 2018).

Locations	Common name / Scientific name				
	Gentoo penguin *Pygoscelis papua*	Rockhopper penguin *Eudyptes chrysocome*	Shag *Phalacrocorax spp.*	Black-browed albatross *Thalassarche melanophris*	Prion *Pachyptila spp.*
Gough Island					0.75 ± 0.62 (10) 7)
Crozet Islands	5.23 ± 1.12 (12) 1)	1.62 ± 0.35 (12) 1)			
Marion Island		1.80 ± 0.68 (10) 7)			
Kerguelen Island	5.85 ± 3.00 (12) 2)	1.96 ± 0.41 (12) 2)	5.30 ± 2.33 (30) 2)*	4.07 ± 1.78 (30) 2)	0.63 ± 0.55(20) 2)
Falkland Islands	**3.17 ±0.87 (40)**	**2.78 ±1.17 (65)**	**2.02 ±0.89 (40)**	**3.38 ± 1.24 (39)**	**0.93 ± 0.23 (21)**
South Georgia	1.13 ± 0.62 (29) 3) 0.85 ± 0.88 (20) 4)		6.48 ± 1.78 (10) 8)	8.35 ± 2.63 (16) 9)	4.51 ± 1.26 (15) 9)**
Staten Island		5.10 ± 1.46 (61) 4)			
King George Island	0.42 ± 0.41(89) 5)				
Greenwich and Barrientos Islands	0.49 ± 0.20 (18) 6)				
Livingston Island	0.31 ± 0.14 (10) 7)				

* *Phalacrocorax verrucosus* ** *Pachyptila desolata*

Table B2 Review of arsenic concentrations (mg/kg; mean ± SD, (n), reference)) in body feathers of adult seabirds' species from this study (**in bold**) and other studies in the Southern Ocean; 1) Jerez et al., 2013; 2) Jerez et al., 2011; 3) Metcheva et al., 2006; 4) Anderson et al., 2010; 5) Fromant et al., 2016. Data since 2000. All the existing literature was reviewed up to date (December 2018).

Locations	Common name / Scientific name				
	Gentoo penguins *Pygoscelis papua*	Rockhopper penguins *Eudyptes chrysocome chrysocome*	Imperial Shag *Phalacrocorax atriceps albiventer*	Black-browed albatross *Thalassarche melanophris*	Prions *Pachyptila spp.*
Kerguelen Island					0.37 (2) 5)*
Falkland Islands	**0.24 ± 0.03 (22)**	**0.6 ± 0.49 (37)**	**0.47 ± 0.13 (40)**	**0.29 ± 0.04 (23)**	**0.51 ± 0.12 (15)**
South Georgia				0.12 ± 0.04 (16) 4)	0.41 ± 0.23 (16) 4)*
King George Island	0.10 ± 0.05 (2) 1) 0.05 ± 0.04 (20) 2)				
Livingston Island	0.02 ± 0.02 (3) 2) 0.88 ± 0.32 (14) 3)				
Ronge Island	0.04 ± 0.02 (8) 2)				
Paradise Bay	0.07 (1) 2)				

Pachyptila desolata

CHAPTER 5

Table C1 Results for Certified reference material: SRM – 2976 and TORT – 2.

Elements	Certified reference material (CRM)	Certified value (mg/kg; mean ± SD)	Digestion (mg/ kg; mean ± SD, range, (n))	Recovery efficiencies (Percentage; range)
Arsenic	SRM – 2976	13.3 ± 1.8	14.18 ± 0.75 13.62 – 15.04 (3)	104 – 113
	TORT – 2	21.6 ± 1.8	22.71 ± 0.64 21.97 – 23.14 (3)	102 – 107
Cadmium	SRM – 2976	0.82 ± 0.16	0.82 ± 0.04 0.78 – 0.85 (3)	95 – 103
	TORT – 2	26.7 ± 0.6	26.51 ± 0.07 26.43 – 26.58 (3)	99 – 100
Cobalt	SRM – 2976	0.61 ± 0.02	0.63 ± 0.02 0.61 – 0.66 (3)	100 – 106
	TORT – 2	0.51 ± 0.09	0.51 ± 0.01 0.50 – 0.51 (3)	98 – 100
Copper	SRM – 2976	4.02 ± 0.33	3.76 ± 0.05 3.70 – 3.80 (3)	92 – 95
	TORT – 2	106 ± 10	97.27 ± 1.73 95.38 – 98.79 (3)	90 – 93
Mercury	SRM – 2976	0.061 ± 0.0036	0.06 ± 0.01 < LOD – 0.06 (2)	86 – 104
	TORT – 2	0.27 + 0.06	0.29 ± 0.01 0.28 – 0.30 (3)	105 – 112
Manganese	SRM – 2976	33 ± 2	36.62 ± 2.20 34.31 – 38.71 (3)	104 – 117
	TORT – 2	13.6 ± 1.2	13.31 ± 0.19 13.15 – 13.52 (3)	97 – 99
Selenium	SRM – 2976	1.80 ± 0.15	< LOD	-
	TORT – 2	5.63 ± 0.67	6.34 ± 0.33 6.00 – 6.66 (3)	107 – 118
Strontium	SRM – 2976	93 ± 2	68.81 ± 4.35 63.80 – 70.99 (3)	69 – 77
	TORT – 2	45.2 ± 1.9	43.86 ± 4.53 38.63 – 46.49 (3)	86 – 103
Vanadium	TORT – 2	1.64 ± 0.19	1.74 ± 0.02 1.73 – 1.76 (3)	105 – 107

Table C2 Limit of quantification (LoQ) per sample (mg/Kg) and arsenic, cadmium, cobalt, copper and manganese concentrations per sample (mg/kg) in blood of black-browed albatross from Beauchene and New Island.

Island	Sample	Mass (mg)	Limit of Quantification (LoQ) (mg/Kg)					Trace elements concentration (mg/kg)				
			Arsenic	Cadmium	Cobalt	Copper	Manganese	Arsenic	Cadmium	Cobalt	Copper	Manganese
Beauchene	1	40.87	0.23	0.03	0.03	0.16	0.10	1.80	< LoQ	< LoQ	1.26	< LoQ
	2	36.71	0.26	0.03	0.03	0.18	0.11	2.58	< LoQ	< LoQ	1.44	< LoQ
	3	4.11	2.29	0.29	0.29	1.61	1.00	3.03	< LoQ	< LoQ	2.32	< LoQ
	4	23.00	0.41	0.05	0.05	0.29	0.18	2.02	< LoQ	< LoQ	1.82	< LoQ
	5	5.51	1.71	0.22	0.22	1.20	0.74	2.85	< LoQ	< LoQ	< LoQ	< LoQ
	6	45.95	0.20	0.03	0.03	0.14	0.09	1.71	< LoQ	< LoQ	1.40	< LoQ
	7	17.22	0.55	0.07	0.07	0.38	0.24	1.84	< LoQ	< LoQ	1.59	< LoQ
	8	17.88	0.53	0.07	0.07	0.37	0.23	4.83	< LoQ	< LoQ	1.60	< LoQ
	9	8.90	1.06	0.13	0.13	0.74	0.46	2.76	< LoQ	< LoQ	1.63	< LoQ
	10	16.31	0.58	0.07	0.07	0.40	0.25	1.94	< LoQ	< LoQ	1.24	< LoQ
	11	43.32	0.22	0.03	0.03	0.15	0.09	2.30	< LoQ	< LoQ	1.38	< LoQ
	12	32.02	0.29	0.04	0.04	0.21	0.13	1.98	< LoQ	<LoQ	1.45	< LoQ
	13	34.70	0.27	0.03	0.03	0.19	0.12	2.35	< LoQ	< LoQ	1.69	< LoQ
	14	27.81	0.34	0.04	0.04	0.24	0.15	2.15	< LoQ	<LoQ	1.63	< LoQ
	15	44.14	0.21	0.03	0.03	0.15	0.09	3.62	< LoQ	< LoQ	1.51	< LoQ
	16	21.29	0.44	0.06	0.06	0.31	0.19	3.17	< LoQ	< LoQ	1.56	< LoQ
	17	42.59	0.22	0.03	0.03	0.15	0.10	2.03	< LoQ	< LoQ	1.59	0.13
	18	29.10	0.32	0.04	0.04	0.23	0.14	2.54	< LoQ	< LoQ	1.49	< LoQ
New Island	19	82.10	0.11	0.01	0.01	0.08	0.05	2.49	< LoQ	< LoQ	1.43	0.11
	20	73.34	0.13	0.02	0.02	0.09	0.06	2.57	< LoQ	< LoQ	1.53	0.08
	21	7.40	1.27	0.16	0.16	0.89	0.55	2.68	0.24	< LoQ	2.88	0.88
	22	89.60	0.10	0.01	0.01	0.07	0.05	2.39	< LoQ	< LoQ	1.45	0.09
	23	120.66	0.08	0.01	0.01	0.05	0.03	2.02	< LoQ	< LoQ	1.41	0.08
	24	30.51	0.31	0.04	0.04	0.22	0.13	2.57	< LoQ	0.05	1.53	0.16
	25	71.29	0.13	0.02	0.02	0.09	0.06	2.12	< LoQ	< LoQ	1.62	0.09
	26	22.58	0.42	0.05	0.05	0.29	0.18	2.42	< LoQ	< LoQ	1.85	0.23
	27	36.79	0.26	0.03	0.03	0.18	0.11	1.93	< LoQ	< LoQ	1.32	< LoQ
	28	127.72	0.07	0.01	0.01	0.05	0.03	2.96	0.02	0.01	1.64	0.09
	29	47.03	0.20	0.03	0.03	0.14	0.09	2.48	< LoQ	< LoQ	1.60	0.13
	30	50.74	0.19	0.02	0.02	0.13	0.08	3.01	< LoQ	< LoQ	1.67	0.13
	31	52.79	0.18	0.02	0.02	0.13	0.08	2.26	< LoQ	< LoQ	1.44	0.11
	32	17.06	0.55	0.07	0.07	0.39	0.24	3.56	< LoQ	< LoQ	1.93	< LoQ
	33	142.43	0.07	0.01	0.01	0.05	0.03	2.35	0.01	< LoQ	1.67	0.08
	34	24.89	0.38	0.05	0.05	0.27	0.16	3.86	< LoQ	< LoQ	1.57	0.21
	35	120.47	0.08	0.01	0.01	0.05	0.03	1.93	0.02	< LoQ	1.83	0.08
	36	190.28	0.05	0.01	0.01	0.03	0.02	1.64	0.01	0.01	1.40	0.07
	37	37.81	0.25	0.03	0.03	0.17	0.11	4.24	0.05	< LoQ	2.04	0.20
	38	41.68	0.23	0.03	0.03	0.16	0.10	2.14	0.07	< LoQ	1.69	0.43

Table C3 Limit of quantification (LoQ) per sample (mg/Kg) and mercury, selenium, strontium and vanadium concentrations per sample (mg/kg) in blood of black-browed albatross from Beauchene and New Island.

Island	Sample	Mass (mg)	Limit of Quantification (LoQ) (mg/Kg)				Trace elements concentration (mg/kg)			
			Mercury	Selenium	Strontium	Vanadium	Mercury	Selenium	Strontium	Vanadium
Beauchene	1	40.87	0.09	0.61	0.07	0.45	0.57	15.51	0.37	< LoQ
	2	36.71	0.10	0.68	0.07	0.50	1.04	43.77	0.54	< LoQ
	3	4.11	0.90	6.08	0.66	4.50	0.86	69.23	2.64	< LoQ
	4	23.00	0.16	1.09	0.12	0.80	1.08	18.00	0.35	< LoQ
	5	5.51	0.67	4.54	0.49	3.36	0.78	55.80	1.06	< LoQ
	6	45.95	0.08	0.54	0.06	0.40	1.63	20.80	0.30	< LoQ
	7	17.22	0.21	1.45	0.16	1.07	0.55	18.52	0.27	< LoQ
	8	17.88	0.21	1.40	0.15	1.03	2.20	111.30	0.46	< LoQ
	9	8.90	0.42	2.81	0.30	2.08	0.58	32.41	0.79	< LoQ
	10	16.31	0.23	1.53	0.17	1.13	0.37	28.65	0.46	< LoQ
	11	43.32	0.09	0.58	0.06	0.43	2.01	33.34	0.57	< LoQ
	12	32.02	0.12	0.78	0.08	0.58	1.78	26.77	0.49	< LoQ
	13	34.70	0.11	0.72	0.08	0.53	1.16	37.88	0.44	< LoQ
	14	27.81	0.13	0.90	0.10	0.67	1.44	35.35	0.40	< LoQ
	15	44.14	0.08	0.57	0.06	0.42	5.19	70.68	0.22	< LoQ
	16	21.29	0.17	1.17	0.13	0.87	1.32	52.76	0.28	< LoQ
	17	42.59	0.09	0.59	0.06	0.43	2.42	25.41	0.34	< LoQ
	18	29.10	0.13	0.86	0.09	0.64	0.43	15.10	0.52	< LoQ
New Island	19	82.10	0.05	0.30	0.03	0.23	1.44	35.82	0.52	< LoQ
	20	73.34	0.05	0.34	0.04	0.25	1.04	42.91	0.72	< LoQ
	21	7.40	0.50	3.38	0.36	2.50	0.53	47.48	3.95	< LoQ
	22	89.60	0.04	0.28	0.03	0.21	1.69	44.28	0.79	< LoQ
	23	120.66	0.03	0.21	0.02	0.15	2.08	38.45	0.60	< LoQ
	24	30.51	0.12	0.82	0.09	0.61	1.82	32.65	1.44	< LoQ
	25	71.29	0.05	0.35	0.04	0.26	1.81	37.55	0.58	< LoQ
	26	22.58	0.16	1.11	0.12	0.82	1.00	39.36	1.21	< LoQ
	27	36.79	0.10	0.68	0.07	0.50	1.87	24.56	0.61	< LoQ
	28	127.72	0.03	0.20	0.02	0.14	1.37	35.10	0.46	0.71
	29	47.03	0.08	0.53	0.06	0.39	3.03	43.31	0.90	< LoQ
	30	50.74	0.07	0.49	0.05	0.36	0.89	38.83	0.44	< LoQ
	31	52.79	0.07	0.47	0.05	0.35	2.28	26.18	0.56	< LoQ
	32	17.06	0.22	1.47	0.16	1.08	1.39	69.56	0.72	< LoQ
	33	142.43	0.03	0.18	0.02	0.13	1.77	39.86	0.55	0.88
	34	24.89	0.15	1.00	0.11	0.74	1.12	44.45	0.80	< LoQ
	35	120.47	0.03	0.21	0.02	0.15	2.01	23.22	0.56	< LoQ
	36	190.28	0.02	0.13	0.01	0.10	1.32	19.64	0.48	< LoQ
	37	37.81	0.10	0.66	0.07	0.49	3.09	62.53	1.46	< LoQ
	38	41.68	0.09	0.60	0.06	0.44	1.98	36.97	2.24	0.74

Table A4 Review of trace elements concentrations (mg/kg; mean ± SD, range) in blood from 3 species of albatrosses from this study (Falkland Islands) and other studies in the Southern Ocean. Data since 1998. All the existing literature was reviewed up to date (October 2019).

Trace elements	Black-browed albatross *Thalassarche melanophris*				Wandering albatross *Diomedea exulans*						Grey-headed albatross *Thalassarche chrysostoma*	
	Colonies				Colonies						Colony	
	Falkland Islands		Bird Island		South Georgia		Crozet Island		Marion Island		South Georgia	
	Number of samples	mean ± SD (range)	Number of samples	mean ± SD (range)	Number of samples	mean ± SD (range)	Number of samples	mean ± SD (range)	Number of samples	mean ± SD (range)	Number of samples	mean ± SD (range)
Arsenic (As)	38	2.56 ± 0.71 (1.64 – 4.83)		0.37 ± 0.27			80	0.70 ± 0.40 (min – 2.30)				
Cadmium (Cd)	7	0.06 ± 0.08 (0.01 – 0.24)		0.09 ± 0.18			143	0.07 ± 0.03 (min – 0.22)	8	32.19 ± 13.01 (11.06 – 53.38)		
Cobalt (Co)	3	0.02 ± 0.02 (0.01 – 0.05)										
Copper (Cu)	37	1.62 ± 0.30 (1.24 – 2.88)		0.82 ± 0.58			165	1.20 ± 0.30 (0.50 – 4.10)				
Mercury (Hg)	38	1.55 ± 0.91 (0.37 – 5.19)	16	4.38 ± 1.10 (2.49 – 6.12)	28	9.57 ± 4.29 (3.69 – 19.91)	169	7.70 ± 3.60 (2.00 – 18.70)			15	6.57 ± 1.11 (5.35 – 8.77)
Manganese (Mn)	19	0.18 ± 0.19 (0.07 – 0.88)		0.15 ± 0.13								
Selenium (Se)	38	39.32 ± 18.89 (15.10 – 111.30)		102.34 ± 85.05			165	77.10 ± 33.00 (13.60 – 216.00)				
Strontium (Sr)	38	0.79 ± 0.73 (0.22 – 3.96)										
Vanadium (V)	3	0.78 ± 0.09 (0.71 – 0.88)		0.14 ± 0.13								
Sources	Present study		Anderson et al., 2019, 2010		Tavares et al., 2013		Carravieri et al., 2014		Summers et al., 2014		Anderson et al., 2009	

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CHAPTER 7

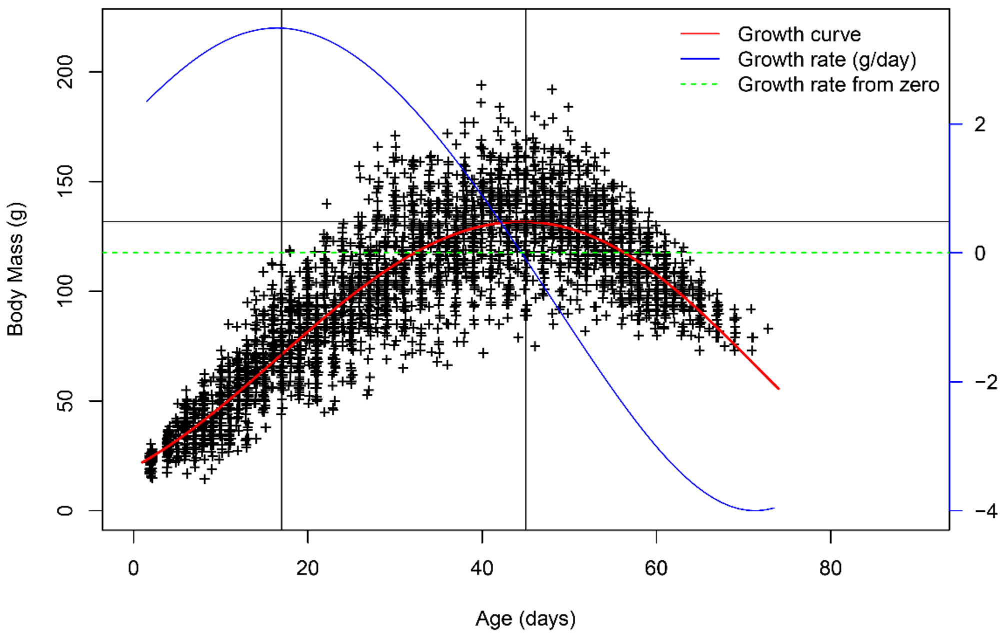

Fig. D1 Growth curve of body mass and their fitted model Bulwer’s petrel at Desertas Island, Madeira Archipelago, Portugal.

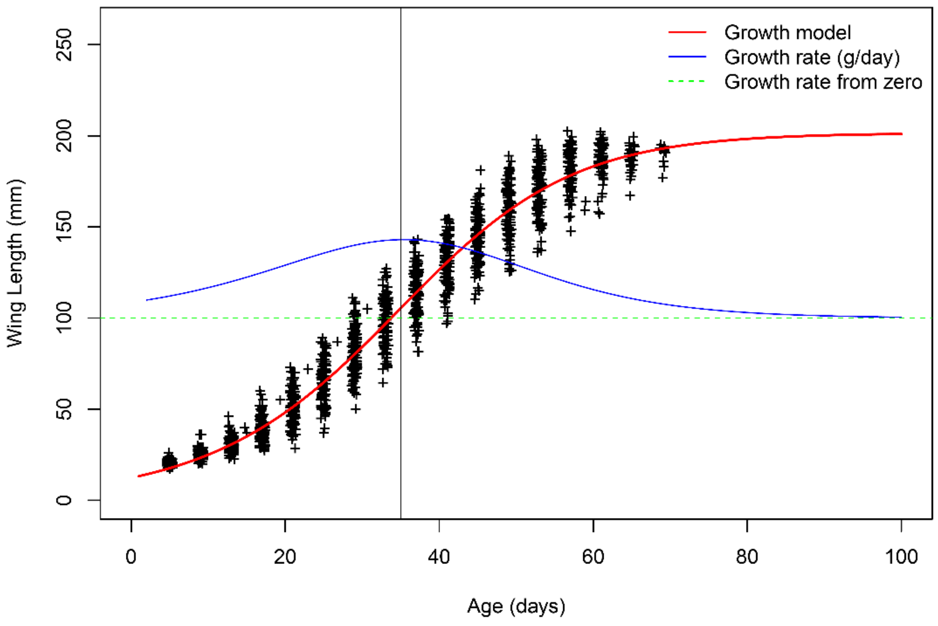

Fig. D2 Growth curve of wing length and their fitted model for Bulwer’s petrel at Desertas Island, Madeira Archipelago, Portugal.

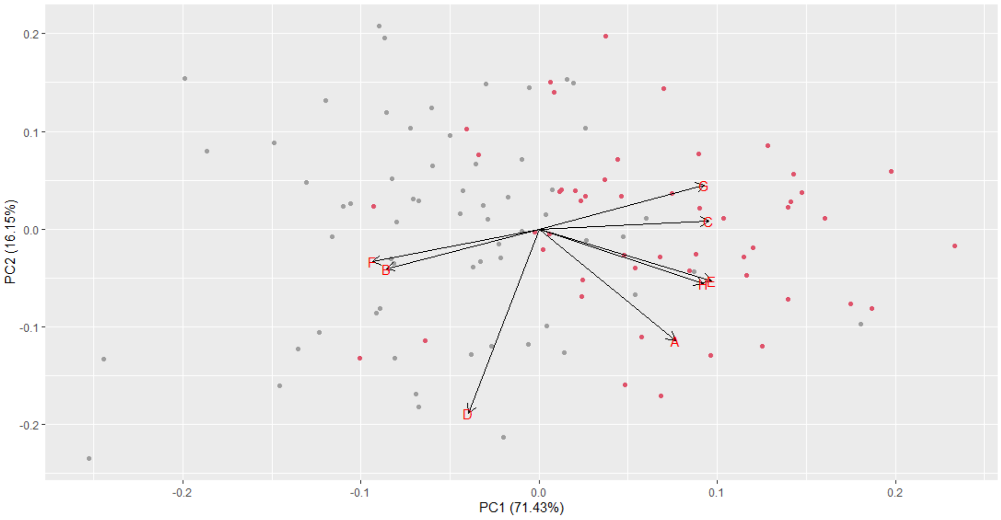

Fig. D3 Biplot of individual scores and parameters loadings on the two principal axes (PC1 and PC2), obtained from a principal component analysis (PCA) on curve relating body weight to age in chicks of Bulwer's petrel, from the Atlantic Ocean. Colour legend: grey: chicks from 2016; red: chicks from 2018. Letters: A, Peak mass W_{max} (g); B, Age of chick reaches its peak mass (days); C, Maximum growth rate (g d-1); D, Age at which chick reaches its maximum growth rate (days); E, Average growth rate (g d-1); F, Age at which chick reaches its 90 grams (days); G, Body mass of chick at 20 days old (g); H, Growth rate of chick at 20 days old (g d-1).

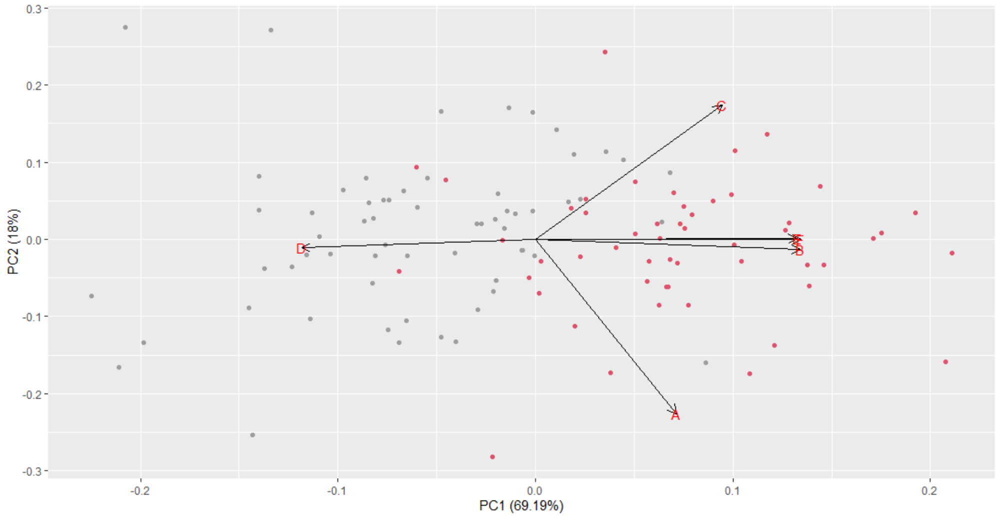

Fig. D4 Biplot of individual scores extracted by principal component analysis (PCA) and element loadings on the two principal axes (PC1 and PC2), on the curve relating wing length to age in chicks of Bulwer's petrel, from the Atlantic Ocean. Colour legend: grey: chicks from 2016; red: chicks from 2018. Letters: A, Peak length of wing (mm); B, Wing length at 60 days old (mm); C, Maximum growth rate of wing length (mm d^{-1}); D, Age at which the chick reaches its maximum growth rate of wing length (mm); E, Growth rate of chick at 20 days old (mm d^{-1}); F, Average growth rate of wing length (mm d^{-1}).