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# Mercury as an indicator of foraging ecology but not the breeding hormone prolactin in seabirds

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ABSTRACT

Marine predators are frequently exposed to contaminants through diet, and thus contaminants like mercury have the potential to be used as tracers of foraging ecology. Mercury's neurotoxic and endocrine-disrupting effects can have far-ranging consequences for both individuals and populations, and thus mercury concentrations could also be indicative of wildlife health. Because blood samples are relatively non-invasive and easy to obtain in seabird colonies, we investigated whether blood-based mercury concentrations were representative of foraging ecology and breeding hormone concentrations in seabirds. Blood-based mercury carbon and nitrogen stable isotopes, and the reproductive hormone, prolactin, were sampled from two seabird species that exhibit different foraging strategies in Western Australia: Great-winged Petrels (*Pterodroma macroptera*) are pelagic squid-specialists whose populations are under-studied; Flesh-footed Shearwaters (*Ardenna carneipes*) are coastal foragers that associate with fishing vessels, and are a species listed as Vulnerable in Western Australia. Mercury was six times higher in Great-winged Petrels (geometric mean ± SE: 3.360 ± 0.180 μg $g^{-1}$ ww, n = 15) than Flesh-footed Shearwaters (0.554 ± 0.109 μg $g^{-1}$ ww, n = 12). There was a significant difference in $\delta^{15}N$ between species, and within-species variation in $\delta^{13}C$ mirrored variation in mercury concentrations, supporting the view that foraging ecology plays a central role in mercury exposure. Furthermore, Great-winged Petrels' mercury concentrations are among the highest reported in seabirds. However, no relationship between mercury and prolactin concentrations was detected. Overall, these results demonstrate that mercury can be used as a foraging ecology tracer in these populations but may not be a good indicator of seabirds' breeding hormones like prolactin, though mercury may affect other aspects of reproduction that we did not measure. These results may aid in future assessment of population trends in these, and other, species.

## 1. Introduction

Exposure to contaminants like mercury varies between environments and species due to differences in habitat types, diets, and physiology. Therefore, mercury concentrations have the potential to be used as dietary tracers within and between habitats (e.g. Peterson et al., 2017). Mercury tracers in the marine environment are especially useful because dynamic oceanographic processes often make the discernment of marine predators' foraging ecologies difficult (Weimerskirch, 2007). Through atmospheric and oceanic processes, mercury becomes available to marine biota: inorganic mercury originates from industrial emissions from coal combustion and from natural sources (e.g. volcanoes; Pacyna et al., 2010; Driscoll et al., 2013; Lamborg et al., 2014; Giang et al., 2015), and then deposits into the ocean where microbes transform it in biochemical reactions (Hammerschmidt and Fitzgerald, 2004; Blum et al., 2013). Mercury enters marine foodwebs as the methylated form, monomethyl-mercury (MeHg), which is synthesized in sediments, and throughout the water column (Sunderland et al., 2009; Hollweg et al., 2010; Blum et al., 2013). Once absorbed, MeHg is difficult for organisms to depurate, and it therefore accumulates in tissues, and biomagnifies with increasing size and trophic position (Bank et al., 2007; Cai et al., 2007; Kojadinovic et al., 2007), and predators that forage at high trophic levels, particularly on organisms from the mesopelagic layer (~400–1000 m deep; Blum et al., 2013), exhibit elevated MeHg concentrations (Monteiro et al., 1996; Peterson et al., 2015). Long-lived predators that forage at high trophic levels are thus

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potentially exposed to large mercury concentrations over their lifetimes. Because MeHg synthesis varies between habitats due to differential input of inorganic mercury sources (Mason et al., 2012), and because mercury concentrations vary with trophic position (Campbell et al., 2005), mercury can be used to differentiate foraging ecologies (Peterson et al., 2017).

In addition to being a tracer of foraging ecology, the well-documented adverse effects of mercury on wildlife could enable mercury to be used as an indicator of wildlife health. MeHg has a high affinity for adrenal and reproductive organs and hormones, and these associations disrupt the hypothalamus-pituitaryadrenal and hypothalamus-pituitary-gonadal axes, thereby altering or inhibiting hormone synthesis and changing behaviors (Tan et al., 2009). Consequently, complex relationships between mercury and hormone concentrations have been observed (Table 1; Oliveira et al., 2006; Tartu et al., 2013). For example male, but not female, birds had negative relationships between hormones and mercury concentrations (Tartu et al., 2013, 2015a,b). Sex-based differences were also observed in an Arctic seabird, the Black-legged Kittiwake (*Rissa tridactyla*): males had a negative correlation between mercury and the hormone prolactin, and mercury concentrations were higher in males that successfully raised two chicks than males that successfully raised only one chick (Tartu et al., 2015b). These variable and non-linear interactions between mercury and reproductive hormones and behaviors among species and between sexes suggest that the relationships between mercury, foraging ecology, and breeding physiology require more attention.

Seabirds are apex predators that can act as sentinels of ocean health because they integrate resources (e.g. diet) across spatial scales ranging from tens to thousands of kilometers (Piatt et al., 2007; Young et al., 2010), and seabirds are generally long-lived with potential exposure to high concentrations of MeHg over their lifetimes (Burger and Gochfeld, 2004; Elliott and Elliott, 2013). Therefore, it is critical to consider the utility of mercury as a state indicator of foraging ecology and reproductive physiology in these seabird sentinels. To better understand these relationships, mercury concentrations were measured in conjunction with concentrations of the pituitary hormone prolactin. Prolactin is a hormone involved in the expression and maintenance of parental behaviors in birds (Buntin et al., 1991; Vleck et al., 2000; Smiley and Adkins-Regan, 2018). Prolactin is especially important for seabird species that undergo long incubation shifts because one parent fasts on the nest while their mate forages at-sea for up to several weeks at a time, and prolactin induces the fasting parent to remain on the nest (e.g. Cherel et al., 1994). Prolactin concentrations are thus expected to be the highest during the incubation period (Angelier et al., 2016). Due to these relationships, and mercury's role as an endocrine disruptor, we predicted that there would be a negative relationship between mercury and prolactin concentrations in seabirds, thus allowing mercury to be used as an indicator of wildlife health.

We measured total mercury (THg) and prolactin concentrations and carbon and nitrogen stable isotopes in Great-winged Petrels (*Pterodroma macroptera*) and Flesh-footed Shearwaters (*Ardenna carneipes*) that breed in south Western Australia during the summer and winter, respectively. Stable isotopes provide information about marine foraging habitat ($\delta^{13}$C, inshore vs offshore; Graham et al., 2010) and trophic position ($\delta^{15}$N; Hobson, 1993). Great-winged Petrels mainly forage on mesopelagic squid (Falla, 1934; Schramm, 1986; Marchant and Higgins, 1990; Ridoux, 1994; Cooper and Klages, 2009), and have prolonged incubation shifts (up to 17 days; Imber, 1976), indicating that they forage in pelagic regions far from the breeding colony. Conversely, Flesh-footed Shearwaters forage nearshore on lower trophic position fishes like sardines (Powell, 2009) and fishing boat offal. This latter foraging strategy has led to high incidences of fisheries by-catch (Dunlop, 2008; Thalman et al., 2009; Lavers, 2015). Recent population surveys have observed that the Western Australia population of Flesh-footed Shearwaters is declining (Lavers, 2015). These factors have led to Flesh-footed Shearwaters being listed as a "vulnerable" species by the Western Australia government (Western Australia Government, 2015). The measurement of mercury and its relation to breeding physiology could provide information about potential factors that affect productivity and population trends in these species. Therefore, our objectives were to: 1) measure blood THg concentrations to establish baseline body burdens, because blood THg concentrations represent diet-based mercury (Monteiro and Furness, 2001) and are representative of THg concentrations in other tissues (Eagles-Smith et al., 2008); 2) assess the utility of THg concentrations as indicators of foraging ecology between species because contaminants in seabirds are derived from diet; and 3) assess the utility of THg concentrations as indicators of wildlife health with concurrent measurements of the breeding hormone prolactin.

## 2. Methods

### 2.1. Sampling locations and study species

Blood samples were collected from Great-winged Petrels and Flesh-footed Shearwaters during the mid-late-incubation breeding phase (Warham, 1956; Powell et al., 2007) at Breaksea Island and Shelter Island in July and December 2015, respectively (Table 2). Samples were collected opportunistically throughout the day; though prolactin concentrations can vary diurnally, prolactin concentrations in our study species likely did not vary enough to be biologically meaningful because 1) prolactin concentrations remain elevated throughout the incubation phase (Angelier et al., 2016); and 2) both study species are nocturnal and sleep in dark burrows during the day, and may not be affected by light level changes – this could be functionally similar to Antarctic species that experience 24-hour daylight but whose prolactin levels do not change diurnally (e.g. Vleck and Van Hook, 2002). Breaksea Island (35.0642°S, 118.0577°E; 100 ha) is located in eastern King George Sound, 12 km offshore of Albany, Western Australia and has a breeding population of < 100 pairs of Great-winged Petrels (Marchant and Higgins, 1990; MEG, pers obs). Shelter (Muttonbird) Island (35.0515°S, 117.6935°E; 2.7 ha) is located 130 m offshore of Torbay, Western Australia and has a breeding population of approximately 200 pairs of Flesh-footed Shearwaters (Lavers, 2015).

Great-winged Petrels and Flesh-footed Shearwaters are Procellariiform seabird species that share similarities in most aspects of breeding that may otherwise create confounding factors when studying the breeding hormone prolactin and mercury concentrations. Breeding strategies are similar: both species lay one egg per year, and like other single-clutch species, it is unlikely that they re-lay if the egg is lost (Hindwood and Serventy, 1941; Marshall and Serventy, 1956); and both male and female parents incubate the egg and raise the chick. Their breeding strategies differ in season (in Western Australia, Great-winged Petrels breed April-September and Flesh-footed Shearwaters breed November-April; Warham, 1956; Powell et al., 2007) and in incubation shift lengths (Great-winged Petrels' incubation shifts average 17 days long during the 55-day incubation period; Flesh-footed Shearwaters' incubation shifts last 1–8 days over the 54-day incubation period; Imber, 1976; Powell et al., 2007). While one parent incubates, the other parent forages at-sea, requiring that the incubating parent fasts. Prolactin remains elevated during incubation, regardless of shift length or breeding failure (Angelier et al., 2016). Inter-specific differences in incubation shift length therefore should not cause inter-specific differences in prolactin concentrations. Because female seabirds depurate contaminants like mercury into their eggs, females' mercury concentrations could be affected if their first clutch fails and they lay a second egg into which additional contaminants are depurated. However, inter-specific differences in mercury concentrations are unlikely to be affected by a second clutch for two reasons. First, both species only lay one egg per clutch, and females of both species would potentially depurate the same relative amount of mercury into their second eggs. Second, as mentioned above, a re-lay after egg loss in these species is

**Table 1**
Summary of correlational field studies in which effects of blood THg were tested on reproductive parameters (sexual and parental hormones, breeding outputs) in free-living adult seabirds. Studies are listed in order of increasing THg concentration (μg $g^{-1}$ ww). Either mean or median of sample population is presented, and THg concentrations are grouped into categories tested, if this distinction was provided in the literature. n = sample size; in some cases, only sample size for whole study was given.

| Species | Summary statistic type Group | THg concentration (μg $g^{-1}$ ww) (n) | Tissue type | Reproductive parameter | Correlation with Hg | Reference |
|---|---|---|---|---|---|---|
| Cassin's Auklet (*Ptychoramphus aleuticus*) | Mean ± SE, chick-feeders: | 0.13 ± 0.02[a] (24) | Whole blood | Breeding stage (pre-lay, incubation, or chick-feeding) | No | Hipfner et al. (2011) |
| Black-legged Kittiwake (*Rissa tridactyla*) | Median, males that raised one chick:<br>females that raised one chick:<br>males that raised two chicks:<br>females that raised two chicks: | 0.25[a]<br>0.17[a]<br>0.2[a]<br>0.17[a]<br>(173) | Red blood cells | Prolactin concentration<br>Breeding success (number of eggs hatched; number of chicks successfully raised) | Yes, but only males<br>Yes, but only males | Tartu et al. (2015b) |
| Blue-footed Booby (*Sula nebouxii*) | Mean, males:<br>females: | 0.29[a]<br>0.20[a]<br>(243) | Whole blood | Breeding status (laying or not laying an egg)<br>Number of eggs<br>Number of chicks | No<br>No<br>No | Lerma et al. (2016) |
| Black-legged Kittiwake (*Rissa tridactyla*) | Median, breeding females:<br>non-breeding females:<br>breeding males:<br>non-breeding males: | 0.34[a] (40)<br>0.40[a] (26)<br>0.40[a] (48)<br>0.42[a] (42) | Red blood cells | Breeding probability<br>Lutenizing hormone concentration (in birds that skipped breeding)<br>Egg lay-date<br>Clutch size<br>Breeding success | Yes<br>negative in males, positive in females<br>No<br>No<br>No | Tartu et al. (2013) |
| Rhinoceros Auklet (*Cerorhinca monocerata*) | Mean ± SE, chick-feeders: | 0.4 ± 0.02[a] (25) | Whole blood | Breeding stage (pre-lay, incubation, or chick-feeding) | Yes | Hipfner et al. (2011) |
| Snow Petrel (*Pagodroma nivea*) | Mean ± SD, both sexes: | 0.4 ± 0.2[a] (49) | Red blood cells | Stress-induced prolactin concentration<br>Egg neglect | Yes, but only males<br>Yes, but only males | Tartu et al. (2015a) |
| Black-legged Kittiwake (*Rissa tridactyla*) | Mean ± SD, pre-lay females, 2008:<br>pre-lay females, 2009:<br>pre-lay males, 2008:<br>pre-lay males, 2009: | 0.42 ± 0.09[a]<br>0.42 ± 0.09[a]<br>0.43 ± 0.09[a]<br>0.49 ± 0.12[a]<br>(105) | Red blood cells | Breeding probability, current year<br>Probability of successfully raising one or two chicks in following year | Yes<br>No | Goutte et al. (2015) |
| South Polar Skua (*Catharacta maccormicki*) | Mean ± SE: | 0.5 ± 0.04[a] (76) | Red blood cells | Breeding probability<br>Breeding success in following year<br>Probability of successfully raising two chicks in following year | No<br>Yes<br>No | Goutte et al. (2014b) |
| Flesh-footed Shearwater (*Ardenna carneipes*) | Median ± SE: | 0.625 ± 0.109 | Red blood cells | Prolactin concentration<br>Egg volume | No<br>No | This study |
| Snow Petrel (*Pagodroma nivea*) | Mean ± SD, females:<br>males: | 0.76 ± 0.21[a] (29)<br>0.47 ± 0.19[a] (16) | Red blood cells | Lutenizing hormone concentration<br>GnRH-induced Lutenizing hormone concentration | Yes, but only in birds < 23 y/o<br>No | Tartu et al. (2014) |
| Leach's Storm-Petrel (*Oceanodroma leucorhoa*) | Mean ± SD: | 0.9 ± 0.4 (90)[b] | Whole blood | Egg lay-date<br>Egg volume<br>Hatch rate<br>Chick growth<br>Fledging rate | No<br>No<br>No<br>No<br>No | Pollet et al. (2017) |
| Wandering Albatross (*Diomedea exulans*) | Mean ± SD: | 1.6 ± 0.8[a] (1 6 9) | Red blood cells | Breeding status (breeding vs non-breeding) | No | Carravieri et al. (2014) |
| Brown Skua (*Catharacta lonnbergi*) | Mean ± SE: | 1.7 ± 0.05[a] (68) | Red blood cells | Breeding probability<br>Breeding success in following year<br>Probability of successfully raising two chicks in following year | No<br>Yes<br>Yes | Goutte et al. (2014b) |
| Wandering Albatross | Mean ± SD, females:<br>males: | 2.3 ± 0.1[a] (57)<br>1.3 ± 0.6[a] (90) | Red blood cells | Breeding probability in following four years<br>Hatching probability in following four years<br>Fledging probability in following four years | Yes<br>Yes<br>Yes | Goutte et al. (2014a) |
| Great-winged Petrel (*Pterodroma macroptera*) | Median ± SE: | 3.670 ± 0.180 (15) | Red blood cells | Prolactin concentration<br>Egg volume | No<br>No | This study |

[a] Dry weight values reported in literature were converted to wet weight values by multiplying THg concentrations by 0.21, which assumed a 79% moisture content in blood (Eagles-Smith et al., 2008).
[b] Mean taken from five years of Hg concentrations.

unlikely.

Because Great-winged Petrels and Flesh-footed Shearwaters are similar in size (mean ± SD mass, Great-winged Petrel: 512.8 ± 49.6 g, n = 20; Flesh-footed Shearwater: 633.8 ± 64.0 g, n = 16), inter-specific differences in mercury concentrations would likely result from diet-based differences and not from body size differences. However, these species have different foraging strategies and diets that could result in different mercury concentrations. Great-winged Petrels are

**Table 2**
Sample sizes for each analysis of blood samples (molecular sex determination, mercury, stable isotope, prolactin), and blood sample dates of Flesh-footed Shearwaters and Great-winged Petrels in Western Australia.

| Species | Date of blood sampling | Molecular sex determination | Mercury analysis | Stable isotope analysis | Prolactin analysis |
|---|---|---|---|---|---|
| Flesh-footed Shearwater | 14–18 Dec 2015 | 12 | 12 | 11 | 15 |
| Great-winged Petrel | 9–11 July 2015 | 12 | 15 | 14 | 14 |

nocturnal foragers that feed on mesopelagic squid that migrate to the sea surface at night (Falla, 1934; Schramm, 1986; Marchant and Higgins, 1990; Ridoux, 1994; Cooper and Klages, 2009). Flesh-footed Shearwaters are diurnal, shallow divers that feed on schooling fish like Australian pilchards (*Sardinops sagax*; Powell, 2009). Because mercury bioaccumulates with increasing trophic position, these different prey items could contribute to inter-specific differences in mercury concentrations (Lavoie et al., 2010).

Birds were captured by hand from their nesting burrows. Approximately 1 mL of blood was sampled from the brachial vein by a 25G needle and plastic syringe, transferred into polypropylene Eppendorf tubes (Eppendorf North America, Hauppauge, New York, USA), and kept cold for 6–8 h. Blood was centrifuged for 1 min at 2200X g and then separated into plasma and red blood cell factions by pipette. Plasma for prolactin analysis and red blood cells for mercury, stable isotope analyses and molecular sex determination were stored in Eppendorf tubes; all samples were stored at −20 °C until analyses. Some blood samples yielded a small amount of blood, so that some individuals did not have enough sample material that could be divided into sufficient amounts of blood for all analyses, resulting in varying sample sizes between mercury, stable isotope, and prolactin analyses (Table 2).

### 2.2. Molecular sex determination

Molecular sex determination was used to identify female and male petrels and shearwaters at the University of Tennessee School of Veterinary Medicine. Briefly, red blood cells were digested with lysis buffer and proteinase K; DNA was isolated via ethanol precipitation; and sex-specific markers ($CHD_W$ and $CHD_Z$ via P2 and P8 primers) were amplified and visualized with modified PCR protocols (Boutette et al., 2002). The sexes of 12 out of 14 Great-winged Petrels and all 12 Flesh-footed Shearwaters were determined molecularly. The sexes of two Great-winged Petrels could not be determined because those samples resulted in ambiguous products with which we did not want to make a sex determination.

### 2.3. Mercury analysis

Blood mercury concentrations reflect mercury from recent diet and the redistribution of mercury among tissues (Monteiro and Furness, 2001; Evers et al., 2005). Red blood cells were analyzed for THg concentrations at the University of California Santa Cruz. Blood was analyzed for THg because avian blood mercury concentrations are composed of nearly all methylmercury (Rimmer et al., 2005). Frozen blood samples were thawed at room temperature. Liquid blood was pipetted into quartz sample boats, and samples were weighed to achieve a mass that ranged 0.02–0.03 g to the nearest $10^{-5}$ g with a Sartorius microbalance (Brinkman Instruments, Inc., Westbury, New York, USA). Samples were analyzed for THg content by thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (DMA-80, Milestone, Shelton, Connecticut, USA), according to U.S. E.P.A. Method 7473 (2007). Quality assurance/quality control procedures included analysis of a method blank every ten samples; analysis of two standard reference materials (SRM 320R-Channel sediment, and SRM 414-plankton, European Commission Community Bureau of Reference, Belgium); and a duplicate sample. Minimum detection limits were defined as three times the standard deviation of blank samples (Pollet et al., 2017); the minimum detection limit was $1.70 \times 10^{-4}$ μg $g^{-1}$ ww.

### 2.4. Stable isotope analyses

Red blood cells were analyzed for $\delta^{13}C$ and $\delta^{15}N$ at the Light Stable Isotope Lab at the University of California Santa Cruz. Red blood cells represent diet integrated into blood over the previous 1–2 months prior to sampling, thus representing recent isotopic discrimination (Hobson and Clark, 1993; Hahn et al., 2012). The ratio of C:N in the sample can also be used as a proxy for dietary lipids (Post et al., 2007). Briefly, red blood cells were dried for 48 h and weighed into tin capsules to achieve a mass of 0.7–0.9 mg, to the nearest $10^{-6}$ g with a Sartorius microbalance (Brinkman Instruments, Inc., Westbury, New York, USA). Samples were then analyzed with an EA 1108 Carlo Erba Elemental Analyzer coupled with a ThermoFinnigan Delta Plus XP mass spectrometer (Thermo Fisher Scientific). Stable isotope ratios are expressed in standard delta ($\delta$) notation in parts per thousand (‰) as relative to international standards Vienna Pee Dee Belemnite for carbon and air for nitrogen as: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1]$, where R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Acetanilide was used as an internal standard, and the SD for $\delta^{13}C$ was 0.15‰ and the SD for $\delta^{15}N$ was 0.17‰. An in-house standard (Pugel; bovine gelatin) was used to calculate the average experimental precision for isotope samples with the mean of the SD among all isotope runs: $\delta^{13}C = 0.29$‰; $\delta^{15}N = 0.36$‰.

### 2.5. Prolactin analysis

Plasma samples were analyzed for prolactin concentrations following (Chastel et al., 2005; Tartu et al., 2015b) at Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique, France. Briefly, plasma prolactin concentrations were determined by heterologous radioimmunoassay. Pooled samples of petrels and of shearwaters each produced a separate species-specific dose response curve that paralleled chicken prolactin standard curves (AFP 4444B, Dr. Parlow, N.H.P.P. Harbor-UCLA Medical Center, Torrance, California, USA). The parallelism observed between the petrel and shearwater curves and the chicken curve indicated that the concentration-dependent binding dynamics of the petrel and shearwater prolactin with the antibody were similar to the binding dynamics of the chicken prolactin, and thus that radioimmunoassay could be used to assess relative levels of plasma prolactin in Great-winged Petrels and Flesh-footed Shearwaters. The intra-assay coefficient of variation was 10.9% and 7.07%, Great-winged Petrels and Flesh-footed Shearwaters, respectively (n = 4 duplicates for each species).

### 2.6. Statistical analyses

Non-parametric Mann-Whitney tests were used to examine differences in stable isotope values and THg concentrations between species, and differences in prolactin and THg concentrations between sexes; due to the uneven sample sizes of sex in Flesh-footed Shearwaters (11 females and one male), sex-based differences were only tested in Great-winged Petrels. To assess whether THg was directly correlated with stable isotopes and prolactin, non-parametric Spearman's rho

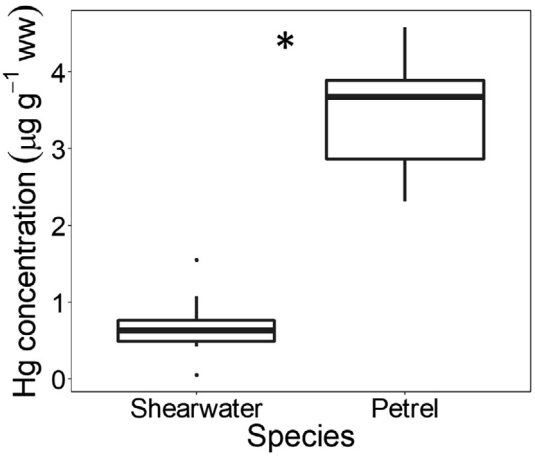


**Fig. 1.** Total red blood cell mercury ($\mu g\,g^{-1}$) concentrations of Flesh-footed Shearwaters and Great-winged Petrels sampled during the incubation period in Western Australia in 2015. *indicates $p < 0.05$.

correlation coefficients were calculated. All analyses were conducted in the program R (R Core Team, 2016, version 3.3.2). Mercury data are presented as geometric mean ± SE and stable isotope and prolactin data are presented as median ± SE.

## 3. Results

### 3.1. Total mercury (THg)

THg was significantly higher in Great-winged Petrels (geometric mean ± SE: 3.360 ± 0.180 $\mu g\,g^{-1}$ ww, n = 15) than Flesh-footed Shearwaters (0.554 ± 0.109 $\mu g\,g^{-1}$ ww, n = 12; Mann-Whitney *U* test: W = 0, $p < 0.001$; Fig. 1).

THg was not different between male (geometric mean ± SE: 2.312 ± 0.23 $\mu g\,g^{-1}$ ww, n = 5) and female Great-winged Petrels (2.175 ± 0.27 $\mu g\,g^{-1}$ ww, n = 7; Mann-Whitney *U* test: W = 7, p = 0.11). Flesh-footed Shearwater THg concentrations were within the range of THg concentrations of other seabird species, but Great-winged Petrel THg concentrations were much higher (Fig. 2).

### 3.2. Foraging ecology

Great-winged Petrels had significantly more enriched $^{15}N$ values (14.3 ± 0.1‰, n = 14) than Flesh-footed Shearwaters (12.0 ± 0.2‰, n = 11; Mann-Whitney *U* test: W = 0, $p < 0.0001$). There was more variation in $\delta^{13}C$ values in Great-winged Petrels (SD = 0.27), whereas the distribution of $\delta^{13}C$ in Flesh-footed Shearwaters was tighter (SD = 0.14; Fig. 3), though $\delta^{13}C$ was not different between species (Mann-Whitney *U* test: W = 64.5, p = 0.512). $\delta^{13}C$ and $\delta^{15}N$ values were not different between male and female Great-winged Petrels ($\delta^{13}C$ males, median ± SE: −18.9 ± 0.1‰, n = 5; $\delta^{13}C$ females: −18.9 ± 0.1‰, n = 7; Mann-Whitney *U* test: W = 17, p = 1.0; $\delta^{15}N$ males, 14.4 ± 0.1‰, n = 5; $\delta^{15}N$ females: 14.2 ± 0.1‰, n = 7; Mann-Whitney *U* test: W = 14, p = 0.64). Great-winged Petrels (3.1 ± 0.1‰, n = 14) had significantly higher ratios of carbon to nitrogen than Flesh-footed Shearwaters (3.0 ± 0.0‰, n = 11; Mann-Whitney *U* test: U = 30, p = 0.007). There were no relationships between $\delta^{13}C$, $\delta^{15}N$, or C:N and mercury (Great-winged Petrels: $\delta^{13}C$:THg, $r_s = 0.11$, p = 0.70; $\delta^{15}N$:THg, $r_s = 0.02$, p = 0.94; C:N ratio:THg, $r_s = 0.26$, p = 0.37; Flesh-footed Shearwaters: $\delta^{13}C$:THg, Spearman's correlation coefficient, $r_s = -0.37$, p = 0.27; $\delta^{15}N$:THg, $r_s = -0.31$, p = 0.36; C:N ratio:THg, $r_s = -0.01$, p = 0.98) or prolactin concentrations (Great-winged Petrels: $\delta^{13}C$:prolactin, $r_s = 0.02$, p = 0.95; $\delta^{15}N$:prolactin, $r_s = -0.14$, p = 0.64; C:N ratio:prolactin, $r_s = -0.14$, p = 0.46; Flesh-footed Shearwaters: $\delta^{13}C$:prolatin, Spearman's correlation coefficient, $r_s = -0.37$, p = 0.26; $\delta^{15}N$:prolactin, $r_s = -0.33$, p = 0.33; C:N ratio:prolactin, $r_s = -0.15$, p = 0.66).

### 3.3. Breeding ecology

Prolactin concentrations varied widely in Flesh-footed Shearwaters (SD = 19.0) but were more tightly distributed in Great-winged Petrels (SD = 13.6; Fig. 4). Prolactin concentrations were significantly greater in female Great-winged Petrels (41.8 ± 4.5 $ng\,mL^{-1}$, n = 7) than males (23.7 ± 3.0 $ng\,mL^{-1}$, n = 5; Mann-Whitney *U* test: W = 90, p = 0.005). There was no relationship between THg and prolactin concentrations in either species (Great-winged Petrels: $r_s = -0.30$, p = 0.30; Flesh-footed Shearwaters: Spearman's correlation coefficient, $r_s = -0.29$, p = 0.39). There were also no relationships between THg and prolactin when assessed in male (Spearman's correlation coefficient, $r_s = -0.10$, p = 0.95) and female (Spearman's correlation coefficient, $r_s = -0.14$, p = 0.78) Great-winged Petrels separately.

THg concentrations for Great-winged Petrels and Flesh-footed Shearwaters were within the range of THg values measured in other studies reporting negative correlations between THg and reproductive parameters (Table 1).

## 4. Discussion

Mercury concentrations and stable isotopes were significantly different between Great-winged Petrels and Flesh-footed Shearwaters, demonstrating that mercury can be used as a tracer of foraging ecology. Differences in foraging ecology were distinct: Great-winged Petrels foraged on prey that were more enriched in $^{15}N$ than Flesh-footed Shearwaters, and variation in $\delta^{13}C$ isotope values indicated that within species, Flesh-footed Shearwaters foraged in either similar habitats to each other or in different areas that had similar $\delta^{13}C$, but Great-winged Petrels foraged in habitats that were more variable (Graham et al., 2010). Furthermore, the within-species variation in THg mirrored the within-species variation in $\delta^{13}C$. No relationship was detected between prolactin concentrations and THg concentrations in either species, but small sample sizes may have masked any relationships. These results suggest that mercury concentrations may not be good indicators of hormone concentrations in these species. Given the established negative relationships between mercury and health, including neurotoxicity, developmental and reproductive impairment, and mortality (Grandjean et al., 1997; Ceccatelli et al., 2010; Frederick and Jayasena, 2011), the baselines that we established for these species highlight that contaminant exposure in many organisms, especially top predators, is likely complex, and the effects may not be apparent during a short sampling period.

### 4.1. THg reflects foraging ecology

Great-winged Petrels had significantly higher THg than Flesh-footed Shearwaters; a difference that was likely driven by inter-specific differences in foraging ecology. Flesh-footed Shearwaters had a small range of $\delta^{13}C$ that mirrored a small range in THg, indicating that within this species, foraging locations and diet are more similar than the large ranges observed in Great-winged Petrels. The small $\delta^{13}C$ range also supports observations that this species forages exclusively nearshore (Powell, 2009; Lavers et al., 2018). Flesh-footed Shearwaters are shallow divers that hunt their prey underwater, and rely heavily on Australian pilchards (*Sardinops sagax*) in Western Australia, a shallow-dwelling schooling fish, and other low trophic level prey (Gould et al., 1997). This piscivorous diet is unique to Western Australia because Flesh-footed Shearwaters in other populations eat mostly squid (Lavers et al., 2014). Flesh-footed Shearwaters also frequently associate with fishing vessels in Western Australia (Lavers, 2015) and throughout the Pacific Ocean (Thalman et al., 2009). Conversely, Great-winged Petrels had more variable $\delta^{13}C$ and THg. Slightly more variable $\delta^{13}C$ values of

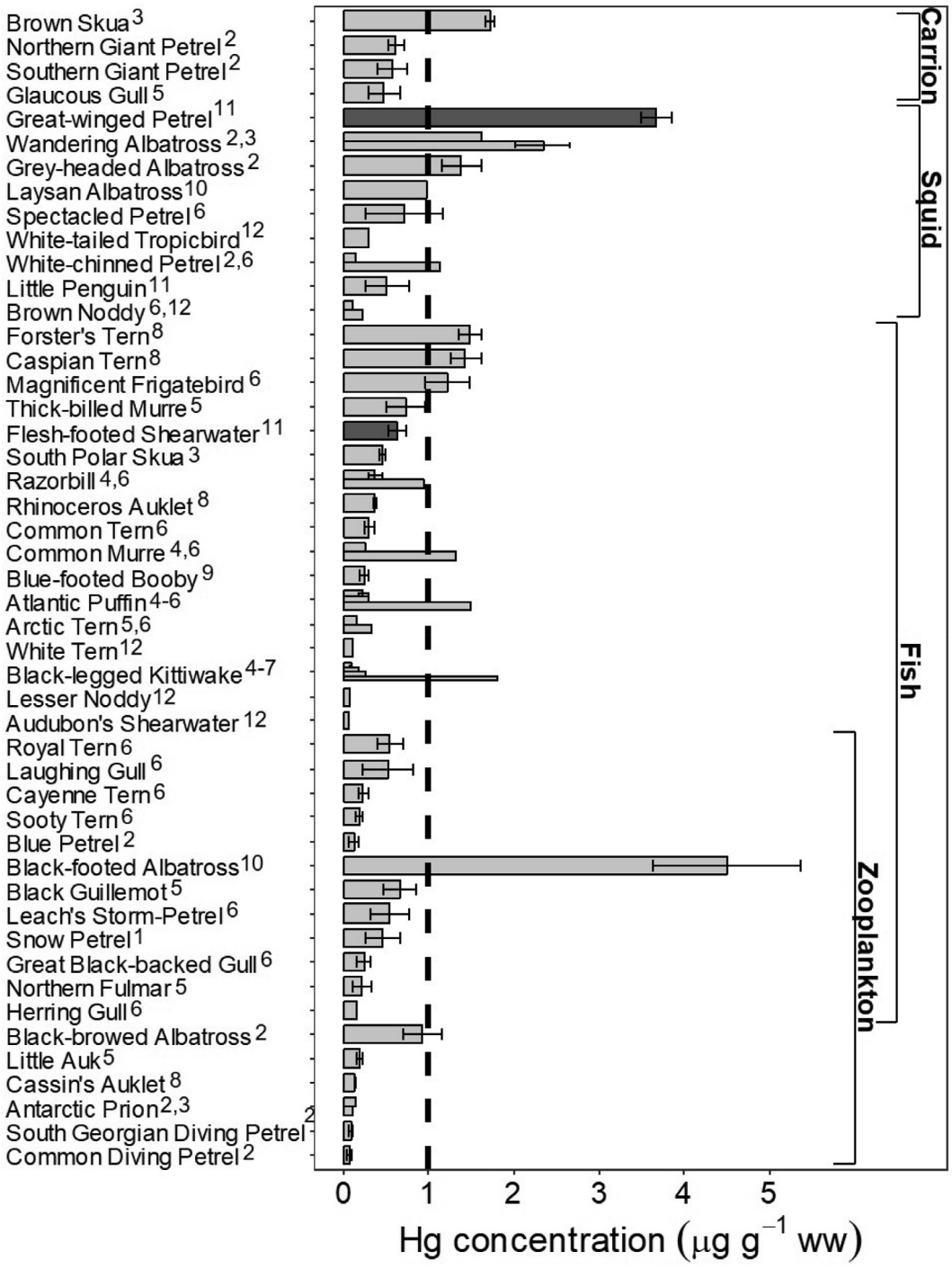


**Fig. 2.** Total blood Hg concentrations (μg $g^{-1}$ ww) of adult seabirds from other studies, grouped by general diet category (see Sources, below). Due to regional variations in Hg concentrations in some species (e.g. Carravieri et al., 2014), species are grouped into general ocean regions, with some species' values reported from multiple studies from different regions (superscript numbers: 1 = west Antarctica; 2 = southern Atlantic Ocean; 3 = southern Indian Ocean; 4 = eastern Atlantic Ocean; 5 = North Atlantic Ocean; 6 = western Atlantic Ocean; 7 = North Sea; 8 = eastern Pacific Ocean; 9 = Gulf of California, México; 10 = central Pacific Ocean; 11 = Great Australian Bight; 12 = Indian Ocean). Values are reported as mean ± SD or median ± SE (SD or SE represented as error bars, when available from literature). Dark grey bars correspond to Flesh-footed Shearwaters and Great-winged Petrels from the current study. Dashed line corresponds to the general toxicity benchmark of 1.0 μg $g^{-1}$, above which, general health, physiology, behavior, and reproduction tend to be affected by mercury (Ackerman et al., 2016b). Dry weight values of whole blood and red blood cells reported in literature were converted to wet weight values by multiplying THg concentrations by 0.21, which assumed a 79% moisture content in whole blood (Eagles-Smith et al., 2008). Diet categories are based on information provided in the source study; if no diet information was available, additional studies were used to assign species to general diet categories (see Sources, below). This list is not exhaustive, and the aim is to present the THg concentrations of Great-winged Petrels and Flesh-footed Shearwaters within a context of global seabird THg concentrations.

Sources for Fig. 2 (listed in alphabetical order by species' common name):

**Antarctic Prion** (*Pachyptila desolata*): Anderson et al. 2009, Fromant et al. 2016; **Arctic Tern** (*Sterna paradisaea*): Bond and Diamond 2009, Burnham et al. 2018; **Atlantic Puffin** (*Fratercula arctica*): Goodale et al. 2008, Bond and Diamond 2009, Fort et al. 2015, Burnham et al. 2018; **Audubon's Shearwater** (*Puffinus lherminieri*): Catry et al. 2008; **Black-browed Albatross** (*Thalassarche melanophrys*): Anderson et al. 2009, and diet source: Croxall and Prince 1980; **Black-footed Albatross** (*Phoebastria nigripes*): Finkelstein et al. 2007, and diet source: Conners et al. 2018; **Black-legged Kittiwake** (*Rissa tridactyla*): Lavoie et al. 2010, Tartu et al. 2013, 2015b, Goutte et al. 2015, Burnham et al. 2018; **Black Guillemot** (*Cepphus grylle*): Burnham et al. 2018, and diet source: Hobson 1993; **Blue-footed Booby**

(*Sula nebouxii*): Lerma et al. 2016; **Blue Petrel** (*Halobaena caerulea*): Anderson et al. 2009, and diet source: Bocher et al. 2003; **Brown Noddy** (*Anous stolidus*): Catry et al. 2008, Sebastiano et al. 2017; **Brown Skua** (*Catharacta lonnbergi*): Goutte et al. 2014b; **Caspian Tern** (*Hydroprogne caspia*): Eagles-Smith et al. 2008, and diet source: Evans et al. 2011; **Cassin's Auklet** (*Ptychoramphus aleuticus*): Hipfner et al. 2011; **Cayenne Tern** (*Thalasseus sandvicensis*): Sebastiano et al. 2017; **Common Diving Petrel** (*Pelecanoides urinatrix*): Anderson et al. 2009, and diet source: Bocher et al. 2003; **Common Murre** (*Uria aalge*): Bond and Diamond 2009, Fort et al. 2015; **Common Tern**: Goodale et al. 2008, Bond and Diamond 2009; **Flesh-footed Shearwater**: this study; **Forster's Tern** (*Sterna forsteri*): Eagles-Smith et al. 2008, and diet source: Ackerman et al. 2016a; **Glaucous Gull** (*Larus hyperboreus*): Burnham et al. 2018; **Great-winged Petrel**: this study; **Great Black-backed Gull** (*Larus marinus*): Goodale et al. 2008, Lavoie et al. 2010; **Grey-headed Albatross** (*Thalassarche chrysostoma*): Anderson et al. 2009; **American Herring Gull** (*Larus smithsonianus*): Goodale et al. 2008, Lavoie et al. 2010; **Laughing Gull** (*Leucophaeus atricilla*): Sebastiano et al. 2017; **Laysan Albatross** (*Phoebastria immutabilis*): Finkelstein et al. 2006, and diet source: Conners et al., 2018; **Leach's Storm-petrel** (*Oceanodroma leucorhoa*): Goodale et al. 2008, Bond and Diamond 2009, Pollet et al. 2017; **Lesser Noddy** (*Anous tenuirostris*): Catry et al. 2008; **Little Auk** (*Alle alle*): Fort et al. 2014, Burnham et al. 2018; **Little Penguin** (*Eudyptula minor*): Finger et al. 2016; **Magnificent Frigatebird** (*Fregata magnificens*): Sebastiano et al. 2016; **Northern Fulmar** (*Fulmarus glacialis*): Burnham et al. 2018, and diet source: Hobson 1993; **Northern Giant Petrel** (*Macronectes halli*): González-Solís et al. 2002, Anderson et al. 2009, and diet source: Croxall and Prince 1980; **Razorbill** (*Alca torda*): Goodale et al. 2008, Bond and Diamond 2009, Lavoie et al. 2010, Fort et al. 2015; **Rhinoceros Auklet** (*Cerorhinca monocerata*): Hipfner et al. 2011; **Royal Tern** (*Thalassues maximus*): Sebastiano et al. 2017; **Snow Petrel** (*Pagodroma nivea*): Tartu et al. 2014, 2015a; **Sooty Tern** (*Onychoprion fuscatus*): Sebastiano et al. 2017; **Southern Giant Petrel** (*Macronectes giganteus*): González-Solís et al. 2002, Anderson et al. 2009, and diet source: Bocher et al. 2003; **South Georgian Diving Petrel** (*Pelecanoides georgicus*): Anderson et al. 2009; **South Polar Skua** (*Catharacta maccormicki*): Goutte et al. 2014b; **Spectacled Petrel** (*Procellaria conspicillata*): Carvalho et al. 2013; **Thick-billed Murre** (*Uria lomvia*): Burnham et al. 2018; **Wandering Albatross** (*Diomedea exulans*): Anderson et al. 2009, Tavares et al. 2013, Carravieri et al. 2014; **White-chinned Petrel** (*Procellaria aequinoctialis*): Anderson et al. 2009, Carvalho et al. 2013; **White-tailed Tropicbird** (*Phaethon lepturus*): Catry et al. 2008; **White Tern** (*Gygis alba*): Catry et al. 2008.

Great-winged Petrels suggested that this species foraged both on the continental shelf and offshore, similar to foraging observations of this species in other regions (Imber, 1973; Camphuysen, 2007). Great-winged Petrels are surface-feeders that mainly eat mesopelagic squid (Falla, 1934; Schramm, 1986; Marchant and Higgins, 1990; Cooper and Klages, 2009; Ridoux, 1994). Great-winged Petrels had significantly more enriched $\delta^{15}$N than Flesh-footed Shearwaters, suggesting that these petrels fed on higher trophic level prey like squid (Navarro et al., 2013), compared with the pilchards that Western Australian Flesh-footed Shearwaters eat (Gould et al., 1997; Lavers et al., 2014). However, it is also possible that inter-specific differences in $\delta^{15}$N could be due to varying baseline $\delta^{15}$N between habitats (Jardine et al., 2006). Great-winged Petrels also had significantly higher C:N ratios than Flesh-footed Shearwaters, indicating a larger proportion of lipids in their diet (Post et al., 2007). Taken together, the similarities in variation between THg and stable isotopes demonstrate that THg can be used as a tracer of foraging ecology. Greater THg variation could suggest more varied diet and foraging locations.

These inter-specific foraging differences are paralleled by these species' mercury concentrations. Mercury is patchily distributed in the marine environment because rates of anthropogenic mercury deposition to the ocean vary regionally (Lamborg et al., 2014) and rates of in situ methylation of mercury also vary with the carbon cycle throughout the water column (Mason et al., 2012). Therefore, the habitats in which Great-winged Petrels and Flesh-footed Shearwaters foraged in southern Australia likely varied in both point sources of mercury, and bacteria that methylate mercury throughout the water column. In Western

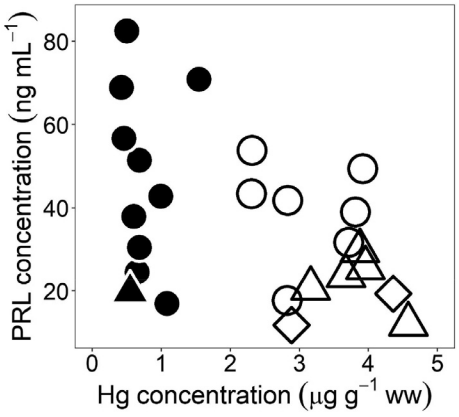


**Fig. 4.** Scatterplot of total red blood cell Hg concentration (μg g$^{-1}$) and plasma prolactin concentration (ng mL$^{-1}$) in female (circles) and male (triangles) Flesh-footed Shearwaters (filled symbols) and Great-winged Petrels (open symbols) sampled during the incubation period in Western Australia in 2015. Open diamonds depict undetermined sex.

Australia, there are only historical point sources of inorganic Hg in industrial municipalities, including a fertilizer plant in Albany (the closest city to our study colonies; Jackson et al., 1986) and agricultural, mining, shipping, and dredging activities (Environmental Protection Authority, 2007; Australian Government, 2012). Therefore, terrestrial

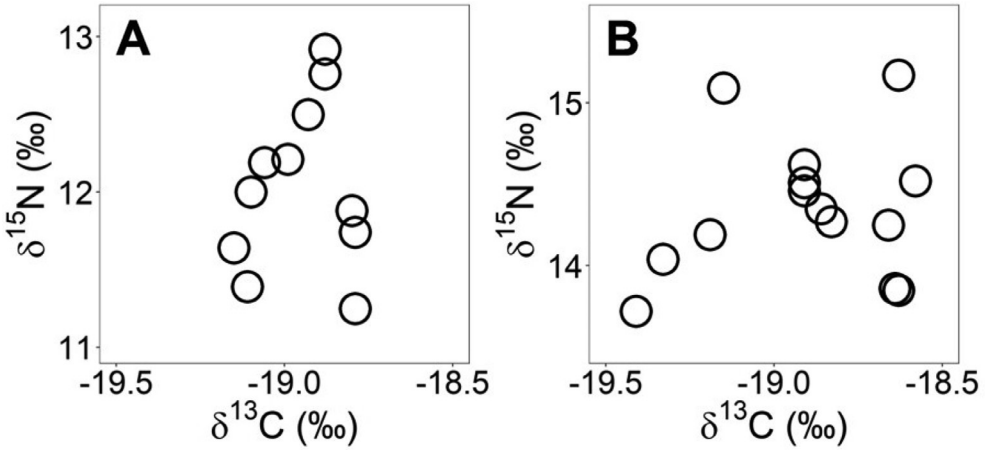


**Fig. 3.** Scatterplots of $\delta^{13}$C (‰) and $\delta^{15}$N (‰) values of incubating (A) Flesh-footed Shearwater and (B) Great-winged Petrel red blood cells, sampled in Western Australia in 2015. Two Great-winged Petrels had very similar stable isotope values at (−18.6, 13.9) resulting in an overlap between these points.

point sources of inorganic Hg from coastal Australia are likely limited in this region. In-situ methylation of mercury is a more likely source to the marine foodwebs in which seabirds in southern Australia forage. Biological and oceanographic factors contribute to these MeHg distributions. For example, because MeHg is thought to be mainly synthesized by bacteria, the distribution of MeHg is dependent on population sizes of sulfate-reducing bacteria (which change seasonally; Hammerschmidt and Fitzgerald, 2004), the availability of inorganic Hg (Hammerschmidt et al., 2004; Hammerschmidt and Bowman, 2012), and the availability of organic matter, because Hg methylation requires a substrate such as sediment (Hammerschmidt and Fitzgerald, 2004; Hollweg et al., 2010) or particles and dissolved organic matter throughout the water column (Hammerschmidt and Bowman, 2012). Additionally, inorganic Hg is remineralized from sinking organic matter, and this remineralization process also produces MeHg (Sunderland et al., 2009). Therefore, each of these processes could potentially influence MeHg exposure to predators that forage at different spatial and temporal scales.

Due to inter-specific differences in diet, and seasonal variations in the amount of organic matter, bacteria population sizes, and ocean currents in southern Australia, it is possible that diet items of summer-foraging Flesh-footed Shearwaters and winter-foraging Great-winged Petrels are exposed to differing amounts of MeHg. The prey of Great-winged Petrels include nocturnal vertically migrating mesopelagic fishes and high trophic level squid (Imber, 1973; Navarro et al., 2013) that may have high MeHg concentrations (Monteiro et al., 1996; Anderson et al., 2009). High rates of mercury remineralization occur at depth in the mesopelagic layer (Sunderland et al., 2009) where remineralization in general is greatest (Fitzgerald et al., 2007), giving rise to higher MeHg concentrations and therefore higher MeHg availability to predators via plankton grazing and subsequent biomagnification at these depths (Monteiro et al., 1996). The lower trophic level pilchards along the continental shelf on which Flesh-footed Shearwaters forage extensively may subsequently contain much smaller MeHg concentrations (e.g. Finger et al., 2017). Seasonal oceanographic differences may also affect productivity, which may then affect MeHg availability. For example, seasonal changes in oceanic and atmospheric currents can induce upwelling, which bring nutrients to surface waters that enhance primary productivity and organic matter production. Primary productivity enhances food availability (Polovina et al., 2001; Mannocci et al., 2014) and increases the amount of organic matter available as a substrate for inorganic Hg methylation (Sunderland et al., 2009). During the summer, Flesh-footed Shearwaters foraged in nearshore waters (Powell, 2009) that are influenced by a weak Leeuwin Current and continuous southerly winds that enable localized upwelling on the continental shelf, so primary productivity is generally high (Middleton and Cirano, 2002; Hanson et al., 2005). Conversely, in the winter, Great-winged Petrels foraged both inshore and offshore, where the Leeuwin Current is strong and there is high regional eddy activity, but primary production is generally low due to reduced light attenuation (Hanson et al., 2005). Therefore, seasonal changes in primary productivity may not be a good predictor of MeHg exposure in the ocean near south Western Australia, and inter-specific differences in diets may be more informative (e.g. Anderson et al., 2009).

### 4.2. Thg does not reflect prolactin concentrations

No relationships were detected between THg and prolactin concentrations in either Great-winged Petrels or Flesh-footed Shearwaters, suggesting that mercury concentrations may not be a good indicator of hormones in these species. Given that the Great-winged Petrels' THg levels were high, this result was unexpected, though we acknowledge that our small sample size may not have been large enough to detect an effect. There was also little variation in prolactin concentrations in Great-winged Petrels, possibly indicating that prolactin may not be a sensitive endpoint that reflects Hg toxicity. Several factors may explain these results. First, it is possible that Hg affected breeding physiology parameters that were not measured in this study. For example, prolactin concentrations have been positively associated with overall breeding success (Angelier et al., 2016). Because Hg affected chick growth and survival and breeding success in other studies (Burgess and Meyer, 2008; Tartu et al., 2013, 2014; Goutte et al., 2014a,b), long-term measurements of these parameters over the course of the breeding season could provide information on temporal trends of Hg in relation to reproductive effort and success. Given changes in seabirds' diet and thus THg exposure throughout the breeding season (Lavoie et al., 2014; Lerma et al., 2016), and studying additional reproductive parameters (e.g. hatch success; chick growth; fledging success) would be helpful to assess the potential for negative THg impacts over a longer time. Second, THg may have interacted with other hormones and compounds, thereby masking any direct relationship with prolactin. Mercury has an affinity for sulfhydryl groups, which can disrupt antioxidant activity of enzymes like glutathione (Rooney, 2007). For example, female Wandering Albatrosses (*Diomedea exulans*) exhibited a positive relationship between blood Hg concentrations and oxidative damage (Costantini et al., 2014). The hormones testosterone and estradiol modulate metabolism of glutathione in the liver and kidneys, which regulates retention and excretion of mercury in these tissues (Hirayama et al., 1987; Malagutti et al., 2009). Mercury also binds to luteinizing hormone, which may have disrupted the hypothalamus within the hypothalamus-pituitary-gonadal axis in another seabird, Black-legged Kittiwakes (Tartu et al., 2013). Prolactin secretion is also affected by other hormones: the antagonist actions of dopamine also regulate prolactin secretion, and thus prolactin may be under control of multiple factors that may be difficult to disentangle (Freeman et al., 2000). Because of the non-linear interactions between many hormones and compounds, it is likely that a direct correlation between mercury and hormones like prolactin may be difficult to observe. Third, the threshold of contaminant concentration required to provoke a relationship with prolactin could be much higher than the THg concentrations observed in the current study; however, studies that observed relationships between THg and prolactin had much lower THg concentrations (Tartu et al., 2013, 2015b; Table 1). Similarly, comparable or higher THg concentrations were observed in other studies that had non-significant results between THg and breeding behaviors (Tartu et al., 2013; Carravieri et al., 2014; Pollet et al., 2017; Table 1). Because mercury methylation is high in the mesopelagic zone (Sunderland et al., 2009), organisms that forage on mesopelagic prey may have evolved to tolerate high concentrations of MeHg (Thompson, 1996). For example, some species consume foods containing selenium, which detoxify MeHg in the liver, and may enable a greater tolerance of elevated THg concentrations (Ikemoto et al., 2004; Campbell et al., 2005). However, with a global increase in anthropogenic Hg into the atmosphere and ocean (Lamborg et al., 2014), organisms not adapted to elevated concentrations may be more at risk to mercury toxicity because they do not have well-developed MeHg detoxification mechanisms (e.g. Thompson, 1996). Taken together, these results suggest that interactions between THg and reproductive hormones and behaviors are not linear and may be dose-dependent.

Prolactin concentrations were significantly higher in female Great-winged Petrels than males, as observed in Procellariiformes and other seabirds (Angelier et al., 2016; Masked Boobies, *Sula dactylatra*, Red-footed Boobies, *S. sula*, and Red-tailed Tropicbirds, *Phaeton rubricauda*, Lormée et al., 2000; Snow Petrels, Tartu et al., 2015a). Sex-related differences in parental care are associated with variation in prolactin concentrations, where the sex that invests more parental effort also has higher prolactin concentrations (Van Roo et al., 2003), inducing behaviors like longer incubation shifts and longer foraging trips than their mates (Lormée et al., 2000). The significantly higher prolactin concentrations in female Great-winged Petrels could indicate that females invest more effort into incubation than males. Indeed, high prolactin concentrations are necessary to maintain breeding effort throughout the

breeding season because females already invest considerable internal resources into the egg. Despite the high prolactin concentrations in female Great-winged Petrels, however, no relationship was detected with THg. Although male Great-winged Petrels also did not exhibit a relationship between prolactin and THg, negative relationships between THg and prolactin were observed in males, but not females, of two other seabird species (Black-legged Kittiwakes; Tartu et al., 2015b; Snow Petrels, *Pagodroma nivea*; Tartu et al., 2015a). During the breeding season, female birds dispose of Hg in their eggs (Monteiro and Furness, 2001; Ackerman et al., 2016a), which may explain some sex-based THg differences observed in other studies. If female Great-winged Petrels had high THg during late-incubation, it suggests that exposure to MeHg via their diet remained high, even after transferring Hg to their eggs. Thus, THg may be consistently elevated in Flesh-footed Shearwaters and Great-winged Petrels, but they have developed a high tolerance and can cope with elevated THg during the breeding season.

#### 4.2.1. THg toxicity

THg concentrations we observed in Great-winged Petrels and Flesh-footed Shearwaters reflect a range of THg that has been associated with observed adverse toxicity effects in other bird species (Ackerman et al., 2016b). For example, THg can impair individuals' health and physiology when concentrations are as low as $0.2\,\mu g\,g^{-1}$ ww (Custer et al., 2000). Although all sampled Flesh-footed Shearwaters had THg concentrations greater than $0.2\,\mu g\,g^{-1}$ ww, birds did not exhibit sub-lethal effects (e.g. visual signs of illness such as lethargy, or physical impairment) during sampling. Because THg concentrations as small as $0.2\,\mu g\,g^{-1}$ ww have altered gene expression in Double-crested Cormorants (*Phalacrocorax auritus*; Gibson et al., 2014), increased egg neglect in Black-legged Kittiwakes (Tartu et al., 2013), and decreased current and future reproduction in Common Loons (*Gavia immer*; Burgess and Meyer, 2008) and South Polar Skuas (*Stercorarius maccormicki*; Goutte et al., 2014b), it is possible that Flesh-footed Shearwaters may experience genetic or behavioral effects that were not measured in this study. It is also possible that consumption of foods laden with selenium promote detoxification below levels that manifest in negative effects of Hg toxicity (Campbell et al., 2005; Eagles-Smith et al., 2009).

Mean THg concentrations observed in Great-winged Petrels corresponded to severe reproductive impairment in several waterbird species, including decreased reproduction and reproductive failure in Common Loons (Barr, 1986; Burgess and Meyer, 2008; Evers et al., 2008), and embryonic deformities in Forster's Terns (*Sterna forsteri*; Herring et al., 2010). The highest observed THg in Great-winged Petrels from Breaksea Island was $4.6\,\mu g\,g^{-1}$ ww, which corresponded to reduced reproduction in Common Loons (Burgess and Meyer, 2008) and reduced antioxidant activity in Surf Scoters (*Melanitta perspicillata*) that increased oxidative stress (Hoffman et al., 1998), which in turn may affect reproduction (Catoni et al., 2008). While these concentrations were not lethal, it is possible that these high THg concentrations may cause cellular changes in Great-winged Petrels that we did not measure or observe, and that may be harmful over the long-term in these long-lived species.

Although our study focused on blood-based THg concentrations during the breeding season, it is interesting to note that feathers sampled from Great-winged Petrels and Flesh-footed Shearwaters at other colonies (Great-winged Petrel: Kerguelen Island, Atlantic Ocean, Carravieri et al., 2014; Marion Island, Indian Ocean, Becker et al., 2016; Flesh-footed Shearwater: Lord Howe Island, Australia, and New Zealand, Bond and Lavers, 2011; Raison, 2018), plus a closely-related species, the Grey-faced Petrel (Lyver et al., 2017) exhibited moderate to very high feather Hg concentrations (mean concentrations ranged 4–36 $\mu g\,g^{-1}$ dw) that were molted during both the breeding and non-breeding season. Additionally, Great-winged Petrels from this study had high heavy metal (e.g. Pb, Se, Zn) feather concentrations (Philpot et al., 2019), indicating that these birds are also exposed to other toxic metals. These data suggest that both species may maintain similar foraging ecologies and diets throughout the year that continuously expose them to elevated Hg.

### 4.3. Conclusions

Great-winged Petrels in Western Australia had six times more THg than sympatric Flesh-footed Shearwaters. The large inter-specific difference in THg is likely due to differences in foraging ecology, where variation in THg reflected variation in stable isotopes. Thus, mercury may be a useful tracer of foraging ecology in these species, and other seabirds (Peterson et al., 2017). However, we did not detect relationships between THg concentrations and the breeding hormone prolactin, suggesting that mercury is not a good state indicator of hormones in these seabird species. Because other studies have detected variable and non-linear relationships between mercury and other aspects of breeding (Table 1), we recommend that multiple measure of breeding be assessed in combination with mercury as part of a larger comprehensive approach to assess seabirds' interactions with their environment. These types of monitoring efforts are especially important because many seabirds are long-lived, and subtle adverse effects may be more harmful over the long-term than the parameters that we measured during the incubation period of one breeding season. For example, adverse effects of contaminants on seabirds may be more exacerbated during years of low prey availability (Golet et al., 2002). The link between foraging ecology and mercury is a good indicator of seabirds' response to their environment, but further study is needed to connect mercury to reproductive parameters and provide a better monitoring system of both individuals and populations.

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## Author contributions

MEG and JLL conceived the study. MEG and JLL collected field data. MEG, OC, and SK conducted laboratory analyses. CL provided equipment and supplies for mercury analyses and contributed to the interpretation of mercury results. MEG wrote the manuscript. All authors provided critical feedback on the manuscript.

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