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Exposure to mercury and polychlorinated biphenyls affects the thyroid function of an Australian seabird (*Ardenna carneipes*)

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ABSTRACT

As top predators in the marine environment, seabirds can be exposed to high levels of persistent pollutants that can bioaccumulate and biomagnify making these predators excellent indicators of ecosystem health. Commonly found in the marine environment, mercury (Hg) and polychlorinated biphenyls (PCBs) are known to interfere with the thyroid system in wildlife. This study quantified PCBs and Hg concentrations and investigated the relationship with thyroxine (T4) and triiodothyronine (T3) levels, in fledgling and adult sable shearwaters (*Ardenna carneipes*). Hg and PCBs were measured in feathers and red blood cells, respectively. The results indicate Hg and PCBs were more abundant in adult shearwaters than in fledglings. Negative associations were found between Hg/PCB body burdens and circulating thyroid hormone concentrations in both age categories. However, some of these correlations were not statistically significant. This study presents an empirical dataset of these contaminants and the thyroid function of adult and juvenile birds. This is a significant step towards better understanding the threat posed by Hg and PCBs to the health of seabirds.

1. Introduction

Chemical pollutants, including heavy metals and persistent organic pollutants (POPs), are a serious global concern for marine ecosystems due to documented harmful effects on wildlife and ecosystem health (Ali et al., 2019; Gilmour et al., 2019; Walker et al., 2012).These contaminants originate primarily from mostly anthropogenic activities, including mining, industrial processes, fossil fuel combustion, urban wastewater, stormwater discharge, and agricultural runoff (Bradl, 2005). Once released into the aquatic environment, they can undergo oceanic currents and long-range atmospheric transport, allowing them to reach even remote marine environments far from their emission sources (Corsolini et al., 2011; AMAP, 1997, 1998). Notably, several studies have reported the presence and accumulation of these pollutants in Arctic ecosystems, where they are deposited through long-distance transport mechanisms and bioaccumulate in top trophic-level organisms, including seabirds (Burkow and Kallenborn, 2000; Corsolini, 2009; Wild et al., 2022). These findings highlight the importance of understanding global pollutant distribution patterns and their sources.

Polychlorinated biphenyls (PCBs) are of particular concern due to their deleterious effects on living organisms (Gilmour et al., 2019). These pollutants are among the most prevalent chemicals in marine food webs due to their persistent, lipophilic, and bioaccumulative nature, and the potential to magnify through the trophic chain (Alava et al., 2018). Persistent organic pollutants (POPs) such as PCBs are organic compounds that are lipophilic and resistant to environmental degradation. Their nonpolar nature allows them to bioaccumulate and dissolve in adipose tissues making them particularly threatening for organisms at the top of the food chain. Despite having been mostly banned worldwide since the late-1970s (UNEP, 2019), PCBs are ubiquitous and are still in detectable concentrations in the marine environment (Anh et al., 2021; Gong et al., 2017; Wang et al., 2021). Their ability to biomagnify through the trophic chain increases the risks of exposure to predators such as seabirds. PCBs, and mainly their metabolites, are known to competitively displace T4 from transthyretin (TTR) binding, a protein that acts as a main transport protein for T4, thereby affecting circulating T4 (Purkey et al., 2004). In addition contaminants can interfere with the deiodinase enzymes and therefore interfere with in the conversion of T4

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to T3 (Köhrle and Frädrich, 2022; Thomas et al., 2024; Weis et al., 2001).

Unlike PCBs, methylmercury (MeHg) occurs naturally, and thus anthropogenic Hg has been added onto this natural background (Lamborg et al., 2016). The biological effects of Hg in aquatic environments are strongly dependent on the chemical species present, with methylated forms being highly toxic and bioaccumulative (Ullrich et al., 2001). The predominant microbiological transformation from inorganic and hydrophilic Hg to the lipophilic state makes Hg more prone to biomagnification in aquatic food chains (Hansen and Danscher, 1997). MeHg interferes with selenium availability, a vital micronutrient in organisms. Selenium is an integral component of selenoproteins including iodothyronine deiodinases (D1, D2, and D3) which are involved in both the activation (T4 to T3) and the deactivation of thyroid hormones (Darras et al., 2006). For both Hg and PCBs, adverse toxicological effects induced by acute and chronic exposure to organisms have been well documented and there is growing interest in their ability to act as potent endocrine disruptors with an adverse impact on thyroid homeostasis (Nøst et al., 2012; McNabb, 2007; Nugegoda and Kibria, 2017; Whitney and Cristol 2017, Sonne et al., 2020).

Marine birds are particularly sensitive and vulnerable to environmental pollutants and climate change (Grant et al., 2024; Keogan et al., 2018). Seabirds are increasingly used as sentinels of marine ecosystem health because of their wide distribution and position in the trophic chain where they integrate information from the bottom to the top of the food web (Chen et al., 2021; Hazen et al., 2019). As top predators, seabirds could enable monitoring of pollutants since they are long-lived species and over time, can bioaccumulate and biomagnify high body burdens of contaminants (Bond and Lavers, 2011, 2020; Gilmour et al., 2019; Philpot et al., 2019a) depending on where they forage. In birds, the health of the thyroid hormone system is essential for their survival (McNabb, 2007; Ruuskanen et al., 2016, 2021). Thyroid hormones (THs) regulate vital functions in birds such as development and growth, seasonal physiology including the regulation of their metabolism and other key physiological functions including gonadal maturation (Darras, 2019; Hsu et al., 2022; McNabb, 2007), moulting, coloration, and feather regeneration (Pati and Pathak, 1986; Vézina et al., 2009), and are part of the hormonal mechanism underlying migration (Dardente et al., 2014). Pollutants can interfere with thyroid homeostasis through many mechanisms of action, i.e. at the receptor level, in binding to transport proteins, in cellular uptake mechanisms or in modifying the metabolism of THs (Boas et al., 2006). Both PCBs and MeHg are known to interfere with normal thyroid function by disrupting thyroxine (T4) and triiodothyronine (T3) metabolism, by interfering with the binding of THs to receptors or transport proteins.

The study location Lord Howe Island, New South Wales, 600 km directly east of Port Macquarie, is home to the world's largest population of sable shearwaters and considered a pristine environment. However, It has been suggested that increased mercury and organic pollutant body burdens in the sable shearwaters inhabiting Lord Howe Island has played some role in the decline of their individual and population health (Lavers, et al., 2014; Bond and Lavers, 2011). Endocrine function, which is prone to disruption following exposure to mercury and a variety of organic pollutants (Zhu et al., 2000), has not been reported in shearwaters or any other Australian seabird. Hence, the study of thyroid hormone levels in relation to mercury and organic pollutant body burdens in these birds was warranted.

This study aimed to: (a) examine the body burden of PCBs and Hg in the blood and feathers of adult and fledgling Sable Shearwaters *Ardenna carneipes* (Bond and Lavers, 2024) from Lord Howe Island, Australia; and (b) assess whether concentrations of these contaminants were correlated with thyroid hormone levels in the blood. We also aimed to evaluate seasonal variability in the body burdens of these toxicants in the shearwaters.

2. Materials and methods

2.1. Study sites, and sample collection

Sable shearwaters are medium-sized, pelagic seabirds with an adult body mass of 550–750 g. This study was conducted in the Clear Place (31.528°S, 159.079°E) and Ned's Beach (31.515°S, 159.061°E) colonies on Lord Howe Island, New South Wales, where 83 adult and fledgling (80–90 days old) shearwaters were captured by hand under an Animal Ethics Permit from the University of Tasmania (A0013836), Lord Howe Island Board Research Permit (06/16), and New South Wales Scientific Licence (SL100169). Data were collected across two years (2017 and 2019), and four distinct seasons, as detailed in Table S1. A map illustrating the study area and the migratory patterns of sable shearwaters from Lord Howe Island is available in Howell et al., (2012);), showing the distribution of individuals tracked from Lord Howe Island.

Samples were collected from sable shearwaters following sampling detailed in Lavers et al. (2019). Briefly, 1 mL of blood was collected from the brachial vein using a 25-gauge butterfly needle coupled with a 3 mL syringe. The blood was immediately transferred into a cold Eppendorf tube and placed on ice for no longer than 1 h. Blood samples were then centrifuged for 3 min at 2200 rpm at 4°C and the plasma was collected into a new tube for further ELISA. Red blood cells (pellets) were stored for PCB analysis, since the small volumes of plasma samples were required for the hormone assays using ELISAs. Both plasma and blood cells were stored in a −20°C freezer. After blood sample collection, four feathers were also collected from the upper breast of each bird and stored in dry, sterile paper envelopes. Breast feathers were selected for trace metal analysis as they are considered the best indicators of endogenous whole-body metal burdens in seabirds (Bond and Lavers, 2011) representing exogenous trace metal input (Szumiło-Pilarska et al., 2017; Zabala et al., 2019).

2.2. Mercury analysis

We measured mercury concentration in feathers as per Paritte and Kelly (2009) and (Philpot et al., 2019b) with minor modifications. Briefly, feathers were washed in Petri dishes in chloroform: methanol mixed solution (2:1) to remove external contamination, and the Petri dishes were placed in an orbital shaker for 24 h before being washed 5 times with Milli-Q water (Paritte and Kelly, 2009). Afterward, feathers were dried, cut as close to midvein as possible, and approximately 15–25 mg) were weighed, and placed in acid-washed (5 % HNO_3) glass tubes before acid digestion.

For the digestion, 1 mL of 70 % HNO_3 was added, and the tube was covered with an acid-washed glass marble and placed on a hotplate at 70 °C. After 24 h, the hotplate was cooled, and the samples made up to 10 mL with milli-Q water and filtered with a 0.45 μm syringe filter. Samples were further diluted with milli-Q water containing 1 % of HCl to a total of 10 mL. Samples were then analysed for total Hg by ICP-MS using an Agilent 7700x. The attached sample loader was an Agilent ASX-520 series autosampler (Agilent Mass Hunter). All samples were analysed in helium mode with a 0.12 s/mass integration time. For quality assurance and quality control, standard reference material ERM-DB001 human hair (trace elements) was prepared, duplicated, and analysed alongside each sampling round. The calculated mean experimental recovery efficiency was 97 %, 94 %, and 99 % respectively.

2.3. PCBs extraction and analysis

For PCBs, extraction and analysis in red blood cells (RBCs) was conducted using a scaled-down version of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) PCB and pesticide analysis procedure, optimised and validated for small volumes and matrix type, particularly the dense matrix in the RBCs. In brief, individual QuEChERS anhydrous salt mixture pouches, each containing 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of sodium citrate dihydrate and 0.5 g of sodium hydrogen citrate sesquihydrate were purchased from Agilent Technologies. Two separate PCB standard solutions and the chemical components utilised in preparing QuEChERS internal standard (QIS) and system monitoring compound (SMC) solutions - both utilised in monitoring extraction efficiency and gas chromatograph (GC) injection performance - were purchased from ULTRA Scientific. The two PCB standard solutions, containing target congeners PCB-8, −18, −28, −44, −52, −66, −77, −101, −105, −118, −126, −128, −138, −153, −170, −180, −187, −195, −206 and −209 at 100 mg/L in an acetone matrix and PCB-169 at 100 mg/L in a hexane matrix were combined and used in preparing 0.01, 0.1 and 1.0 mg/L standards in 1 % acetic acid in Acetonitrile. Triphenyl phosphate, tributyl phosphate, 4-chloro-3-nitrobenzotrifluoride, and 2,4,5,6-tetrachloro-m-xylene solutions, each at 1000 mg/L in an acetone matrix were combined and used in preparing a 20 mg/L QIS solution in 1 % acetic acid. A solution comprising 1,4-dichlorobenzene-d4, naphthalene-d8, acenapthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12, each at 2000 mg/L in a dichloromethane matrix was combined with a 1-bromo-2-nitrobenzene solution at 1000 mg/L in an acetone matrix in preparing a 4 mg/L SMC solution in toluene. Sample extraction and analysis was conducted at the Australian National Measurement Institute. Agilent 7890B Gas chromatography with 7000 C tandem-mass spectrometry (GC-MS) was used to quantify PCBs.

The method was validated at the National Measurement Institute, Australia (NMIA) which is an accredited laboratory under the National Association of Testing Authorities (NATA) of Australia. using chicken blood due to ethical constraints, on sample numbers and blood volumes extracted from the shearwaters. Ethical permits allowed only 1 mL of blood to be sampled of which all serum was used for analysis of thyroid hormones, leaving only the RBCs for PCB analysis. The extraction method is based on solvents and from our prior experience in similar analysis, we believe that for the purpose of extraction efficiency and the measurement of residues, both bird blood types will behave similarly. The validation protocol included spiking 5 concentration levels of known amounts of PCBs in seven replicates, achieving recoveries of 45–117 percent. The measurement uncertainty for similar chemical residue testing methods range between ± 10–25 percent. Based on this the recoveries were carried out at spike levels of 2.5 μg/L, 5 μg/L, 10 μg/L, 50 μg/L and 100 μg/L respectively. Results obtained for the positive samples were reported after correcting for their percentage recovery within the relevant analytical batch. QA/QC for PCB Analysis of the shearwater samples: Calibration curves for each PCB congener exhibited high R^2 values (>0.99). Recovery rates for spiked shearwater samples ranged from 45 % to 112 %, with a decline in recovery observed at lower concentrations and for higher-chlorinated congeners (e.g., PCB-206 and PCB-209). Precision, measured by relative standard deviation (RSD), was < 20 % for most congeners, with the lowest RSD values observed at higher concentrations, ensuring the method's accuracy and consistency.

2.4. Thyroid hormone analysis

The concentrations of T4 and T3 were determined in shearwater blood plasma samples using commercially available competitive enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (CUSABIO, Wuhan, China). All reagents and blood plasma samples were brought to room temperature for 30 min prior to use. Samples were then diluted 30 × with phosphate-buffered saline (PBS; 0.01 M, pH 7.4) and 50 μL of the sample. PBS (blank) or standard were loaded in triplicate to a pre-coated 96-well microtiter plate containing an antibody specific to either T4 or T3 as the T4 and T3 antigens associated with the samples and standards added to each well compete with those associated with added biotin-conjugated T4 and T3 for the fixed number of antibody binding sites. The wells were then washed to remove the biotin-conjugated T4 and T3.

Avidin-conjugated horseradish peroxidase (HRP) was subsequently added to each well to bind to biotin-conjugated T4 and T3. The wells were washed once more to remove unbound avidin-conjugated HRP, and a substrate solution was then added. The remaining avidin-conjugated HRP and substrate solution interact to produce a colorimetric reaction measured at 450 nm using a UV/vis microplate spectrophotometer (POLARstar Omega, BMG LABTECH) within 5 min of the addition of the stopping solution. T4 and T3 levels were calculated by a log-log linear regression and presented in ng/mL.

2.5. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics, version 29 with a significance level of 0.05. In evaluating whether individual variables (PCB, Hg, T4, and T3 levels) varied significantly with the age of the sable shearwaters, a Welch's analysis of variance (ANOVA) was conducted in conjunction with a Games-Howell post hoc test. The normality of each dependent variable was determined separately in SPSS using the Shapiro-Wilk test. Relationships between variable pairs within each sample group were evaluated using Pearson correlation coefficients.

3. Results

3.1. Mercury levels in Sable Shearwater breast feathers

Mean (± SE) Hg concentrations in breast feathers of adults and fledglings varied across the sampling seasons of 2017 and 2019, and between age groups (Fig. 1). In 2017, the mean concentrations were 2.05 ± 0.18 mg/kg for fledglings and 4.04 ± 0.40 mg/kg for adults. In 2019, these values rose to 2.12 ± 0.92 mg/kg for fledglings and 5.69 ± 0.97 mg/kg for adults. Overall, there were no significant differences in mercury levels in both fledgelings and adults across seasons (2017–2019). In 2017, fledglings had significantly lower concentrations than adults, with a mean difference of −1.95 mg/kg; 95 % CI: −3.16 to −0.75; $p < 0.001$. In 2019, no significant differences were observed between ages, but fledglings from 2017 had significantly lower levels compared to adults from 2019, with a mean difference of −3.60 mg/kg; 95 % CI: −6.41 to −0.78; $p = 0.01$. Although adults in 2019 tended to have higher mercury levels than fledglings, this difference was not statistically significant.

3.2. PCBs in shearwater red blood cells

The detection frequency of targeted PCB congeners in the blood cells of adult sable shearwaters sampled from 2017 to 2019 exhibited notable variations across seasons and age groups (Fig. 2) indicating the percentage of samples positive for specific congeners during these years. Congeners PCB-66, PCB-118, PCB-138, and PCB-153 were consistently identified and showed elevated levels across all seasons and age categories compared to other congeners, although PCB-66 had lower detection frequencies than the others. The histograms further elucidated the variability of PCB congeners over time, revealing that PCB-118, PCB-138, and PCB-153 consistently exhibited high frequencies of detection, particularly in adults, with greater detection rates than in fledglings. Notably, PCB-153 was especially prevalent among adults in 2017, while other congeners, such as PCB-206 and PCB-209, displayed lower frequencies of detection throughout the study period, with PCB-209 detected only in 2019 and higher in fledglings than in adults.

3.3. Thyroid hormones in shearwater blood plasma

The results of T4 and T3 analysis across the two breeding seasons on Lord Howe Island are depicted in Fig. 3. For T4, a significant difference was detected between the mean concentration in fledglings and adults sampled in 2017 (mean difference = −549.80 ng/mL; 95 % CI: −684.18 to −415.42; $p < 0.001$), fledglings in 2017 and 2019 (mean difference =

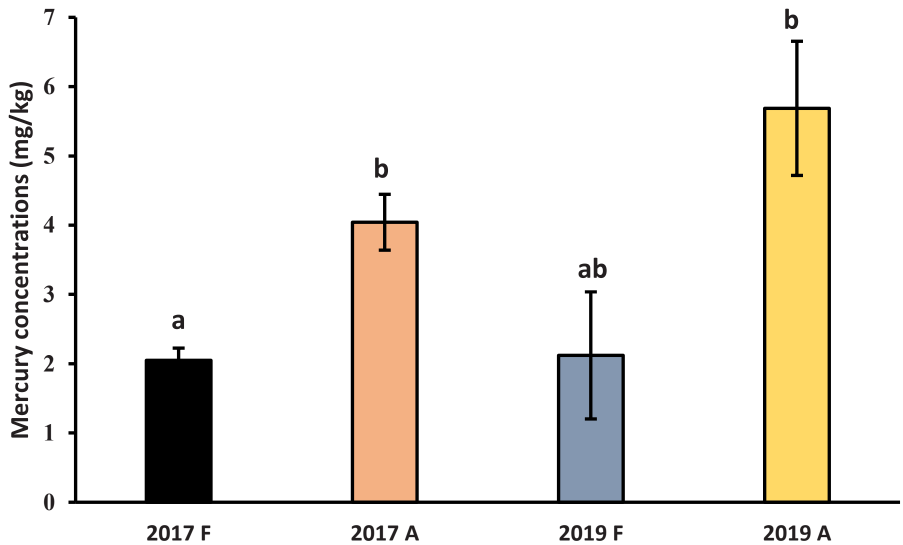

Fig. 1. Mean concentrations of mercury (mg/kg dry weight) (A) in breast feathers of sable shearwater (*Ardenna carneipes*) sampled from fledglings (F) and adults (A) in 2017 and 2019. The letters 'a', 'b', and 'ab' above the bar columns indicate statistically significant differences, whereas the same letters represent no significant differences (p > 0.05).

−792.68 ng/mL; 95 % CI: −1376.25 to −209.11; p = 0.007), as well as between the 2017 fledglings and the 2019 adults (mean difference = −242.47 ng/mL; 95 % CI: −369.16 to −115.78; p < 0.001).

Regarding T3, a significant difference was observed between the 2017 adults and the 2019 fledglings (mean difference = 0.56 ng/mL; 95 % CI: 0.11–1.00; p = 0.009). No statistically significant difference was observed in the T3 levels of adult shearwaters when comparing samples from 2017 to those from 2019. However, there was a statistically significant variation in the T3 levels between fledglings sampled in 2017 and those in 2019, with a mean difference of 1.46 ng/mL (95 % CI: 0.48–2.43; p = 0.002). No statistically significant difference was found between the T3 levels in adult shearwaters from 2017 compared to 2019.

3.4. Associations between mercury and thyroxine

Mercury concentrations in breast feathers were negatively associated with T4 concentrations in blood plasma obtained from Sable Shearwaters in all four sampling rounds (Figs. 4, 5, S1 and S2). However, this relationship was statistically significant (p = 0.008) only in the 2017 adults while the correlations were not statistically significant in 2017and 2019 fledglings or in 2019 adults.

3.5. Associations between mercury and triiodothyronine

Mercury concentrations in breast feathers were negatively correlated with T3 concentrations in blood plasma obtained from fledgling sable shearwaters sampled in 2017 and 2019 (Figs. 4 and 5). The negative correlation was statistically significant (p = 0.038) in the 2017 fledglings. However, these relationships were not statistically significant in 2017 and 2019 fledglings and in 2017 and 2019 adults Contrastingly, a positive correlation (r = 0.091) was observed between these two variables in samples obtained from 2017 adults (Fig. 4) and 2019 fledglings (Fig. S1) though not statistically significant.

3.6. Associations between polychlorinated biphenyls and thyroxine

The concentrations of ∑PCB in blood cells were negatively associated with T4 concentrations in blood plasma obtained from Sable Shearwaters in all five sampling rounds in adults and fledglings. and 2017 respectively; Figs. 4–5 and Figs. S1-S2). However, none of these relationships were statistically significant.

3.7. Associations between Polychlorinated biphenyls and triiodothyronine

Similar to the correlation between ∑PCB and T4, the ∑PCB concentrations were negatively associated with T3 concentrations in all four sampling rounds (Figs. 4–5 and Figs. S1-S2). However, the only statistically significant correlation was for 2017 fledglings (Fig. 5).

4. Discussion

To our knowledge, this is the first study to quantify PCBs within blood cells fraction of whole blood of the sable shearwater, or any other Australian seabird, and presents a novel approach to quantify PCBs using the QuEChERS technique. Our results also provide the first data on circulating thyroid hormone concentrations in Australian seabirds and assess mercury and PCB accumulation in relation to their effects on bird endocrine health. In this study, the highest mercury levels were detected in the adult sable shearwaters; however, showed statistical differences only the 2017 fledglings (Fig. 1). Mercury concentrations have previously been studied in adult and fledgling sable shearwaters (Bond and Lavers, 2011; Lavers et al., 2014a). These authors found that Hg in feathers was higher in adults in 2011 than in our 2017 and 2019 adults, while the fledglings in 2014 had Hg levels similar to those of our 2017 fledglings. However, the 2019 fledglings in our study had significantly higher levels of Hg in breast feathers than in 2011 (Lavers et. al., 2014) or in 2017 fledglings in our study. This suggests mercury concentrations vary annually in this species, likely in response to changes in diet and exposure to plastics, which contain metals and other contaminants (Kojadinovic et al., 2007; Lavers et al., 2014b; Turner and Filella, 2021).

The hypothesised effect level for mercury in bird feathers as a reflection of their body burdens is 5 mg/kg (Burger, 1993; Burger and Gochfeld, 2000) and feather mercury concentrations in all birds included in this study are below this limit. The mercury concentrations we report in fledgling able shearwaters (2.05 mg/kg and 2.12 mg/kg) are similar to those reported in fledglings sampled in 2011 (2.40 mg/kg; Lavers et al., 2014a). However, the lead (Pb)concentrations detected in this study are much lower than those previously reported for the same species from the same site (11.221 mg/kg; Bond and Lavers, 2011). This finding agrees with observations made by Bond and Lavers (2020) who demonstrated a long-term decline in Pb of around −0.3 % per year based

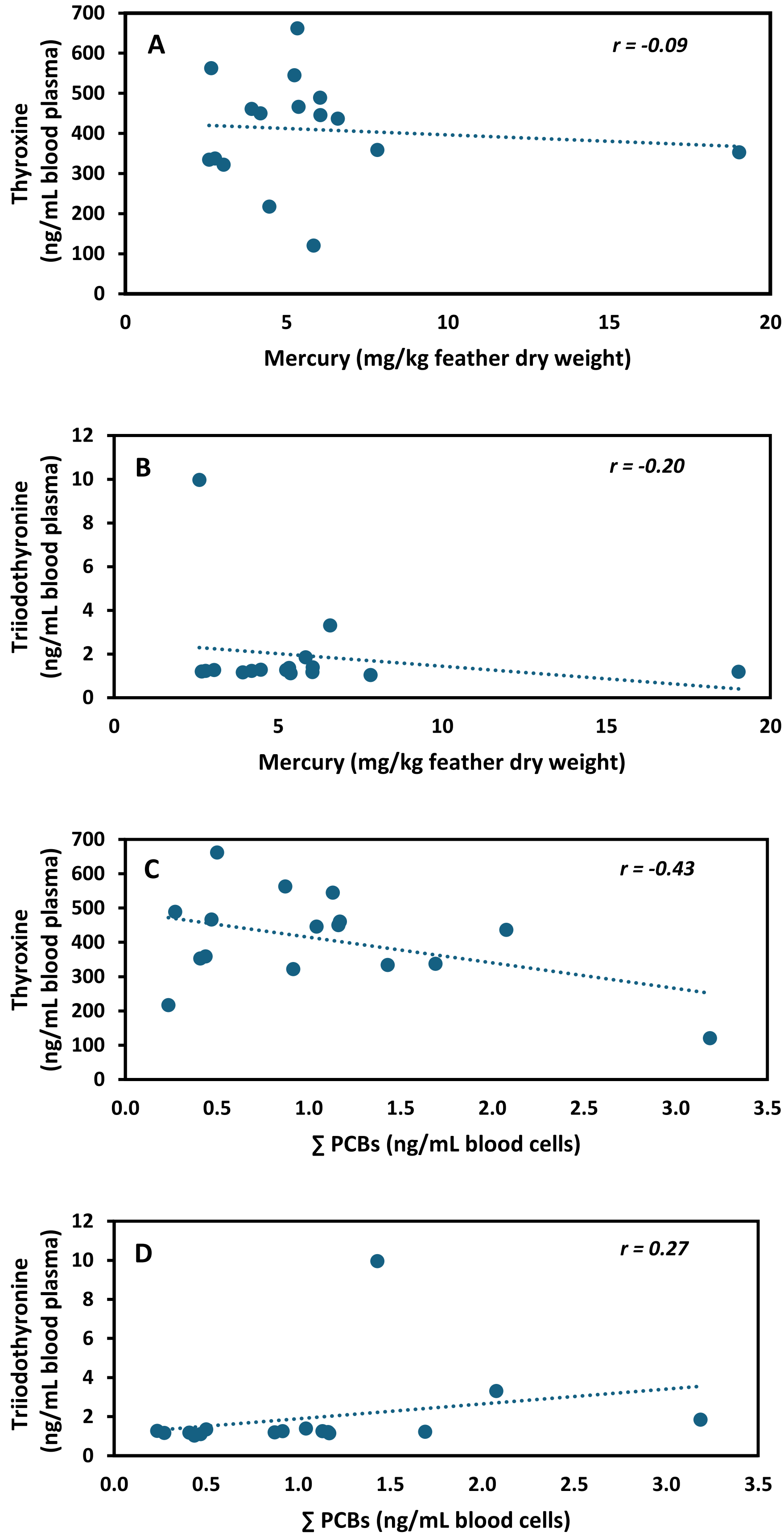

Fig. 2. Frequency of detection (reported as a percentage) of each target PCB congener in the blood cells of fledglings (F) and adults (A) Sable Shearwaters (*Ardenna carneipes*) collected in 2017 and 2019.

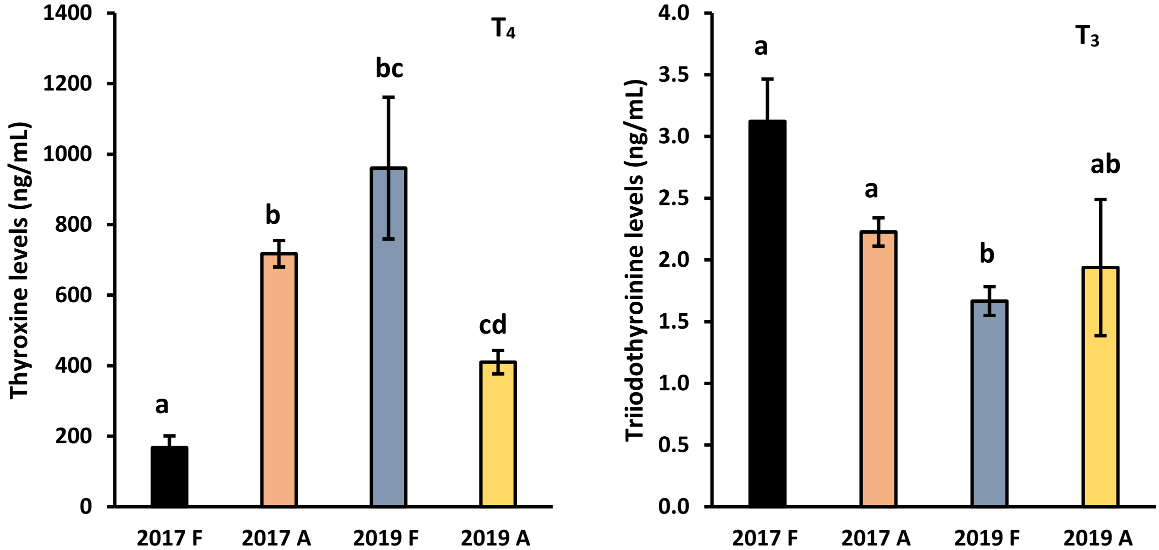

Fig. 3. Mean levels of thyroxine (T_4) and triiodothyronine (T_3) in the blood plasma of fledgling (F) and adult (A) sable shearwaters *(Ardenna carneipes)* sampled during 2017 and 2019. The letters 'a', 'b', 'ab', 'bc', and 'cd' above the bar columns indicate statistically significant differences, whereas the same letters represent no significant differences ($p > 0.05$).

on the analysis of hundreds of sable shearwater feathers from museum skins collected during 1900–2011.

For PCBs, a significantly higher congener detection frequency and mean ΣPCB concentrations were more abundant in adult Sable Shearwaters than in fledgelings. This would indicate that the age of birds (at least up until the point of sexual maturation) is an important factor influencing circulating PCB concentrations, as older birds bioaccumulate more PCBs over time. Adults accumulate significantly higher levels of PCBs than fledglings due to different exposure and consequent levels of bioaccumulation but also metabolic differences. In fledgelings, primary exposure is through parental feeding since fledgeling are exposed to reduced areas. Accumulated PCBs are then further reduced through growth and higher metabolic rates relatively to their body size compared to reduced metabolic excretion and cumulative exposure further elevate PCB levels in adults (Campioni et al., 2024). The reasons for the variability in individual PCB congeners in fledglings and adults in 2017 and 2019 are unknown but could be due to many factors including the changing of the fingerprint in the PCBs the shearwaters were exposed to, most likely through their diet. There are no studies that have evaluated the relationship between individual PCB congeners and thyroid function in birds hence it is not possible to conclude the effect of this variability.

At the time of writing, there is no empirical data on PCBs in sable shearwater blood cells. However, the mean concentrations of ΣPCB quantified in blood cells of this species were significantly lower than those previously quantified in the liver, fat, and muscle tissue of other seabird species (Braune et al., 2014; Buckman et al., 2004; Fromant et al., 2016). This was expected as PCBs are highly hydrophobic and lipophilic, and thus have a higher affinity for lipid-rich tissues within organisms where they would deposit. The high detection rates of PCB-118, PCB-138, and PCB-153 in adult sable shearwaters suggest ongoing exposure, as higher degrees of chlorination within PCB compounds are indicative of localised and more recent sources (Brown et al., 2015), raising health concerns for these birds. Although specific studies on the health effects of these congeners are lacking for birds, they are known to bioaccumulate significantly in fish-eating species due to their hydrophobic nature and longer elimination half-lives (Antoniadou et al., 2007; Brown et al., 2018). Exposure to PCB-118 and 153 have also been linked with reproductive developmental issues and gene and protein expression in mammals (Chu et al., 1995; Krogenæs et al., 2014; Tremoen et al., 2014), so it is unsurprising that altered protein expression has recently been detected in sable shearwater fledgelings (de Jersey et al., in review). Importantly, these congeners (PCB-118, PCB-138, PCB-153 and others) are able to persist in fatty tissues and biomagnify through the food web, resulting in higher concentrations in top predators, so this is particularly relevant for long-lived seabirds like shearwaters. Such trends raise significant environmental concerns regarding ecosystem health and potential risks to wildlife (Antoniadou et al., 2007)

Circulating thyroid hormone concentrations have not to date been quantified in an Australian seabird species, and the results of this study are therefore important as they provide baseline data for reference in future studies in avian ecotoxicology. This lack of baseline data has been a gap in understanding the thyroid physiology of Australian seabirds, which is critical for monitoring environmental impacts on these species. This study, and in line to previous bird studies, revealed that T4 (Thyroxine) concentrations were consistently higher than T3 (triiodothyronine)concentrations in in both fledgling and adult seabirds sampled in 2017 and 2019.

This is expected, as T4 concentrations are higher than T3 in birds, and in many other species, primarily due to the physiological roles and characteristics of these two thyroid hormones. T4 is the precursor or inactive form of the hormone, circulating in larger concentrations in the blood and having a longer half time allowing a steady supply and in enough quantities that can be converted in the active form T3 when needed (McNabb, 2007). The contrasting abundances of T4 and T3 within the Sable Shearwaters sampled during this study are consistent with those previously quantified in Black-legged Kittiwake (*Rissa tridactyla*) (Blévin et al., 2017).

4.1. Correlations between mercury and thyroid hormones

The negative associations between mercury and thyroid hormone (T4 and T3) concentrations quantified within fledgling sable shearwaters were expected. As previously stated, mercury has a strong affinity for selenium, which is an integral part of the iodothyronine deiodinase enzymes responsible for converting T4 to T3 in organisms (Raymond and Ralston, 2020; Soldin et al., 2008). The formation of mercuric selenides limits the availability of selenium for recruitment in the synthesis of these enzymes and thus, a correlation between decreased circulating abundances of T3 and an elevated mercury body burden is expected. In particular, the statistically significant negative relationship between

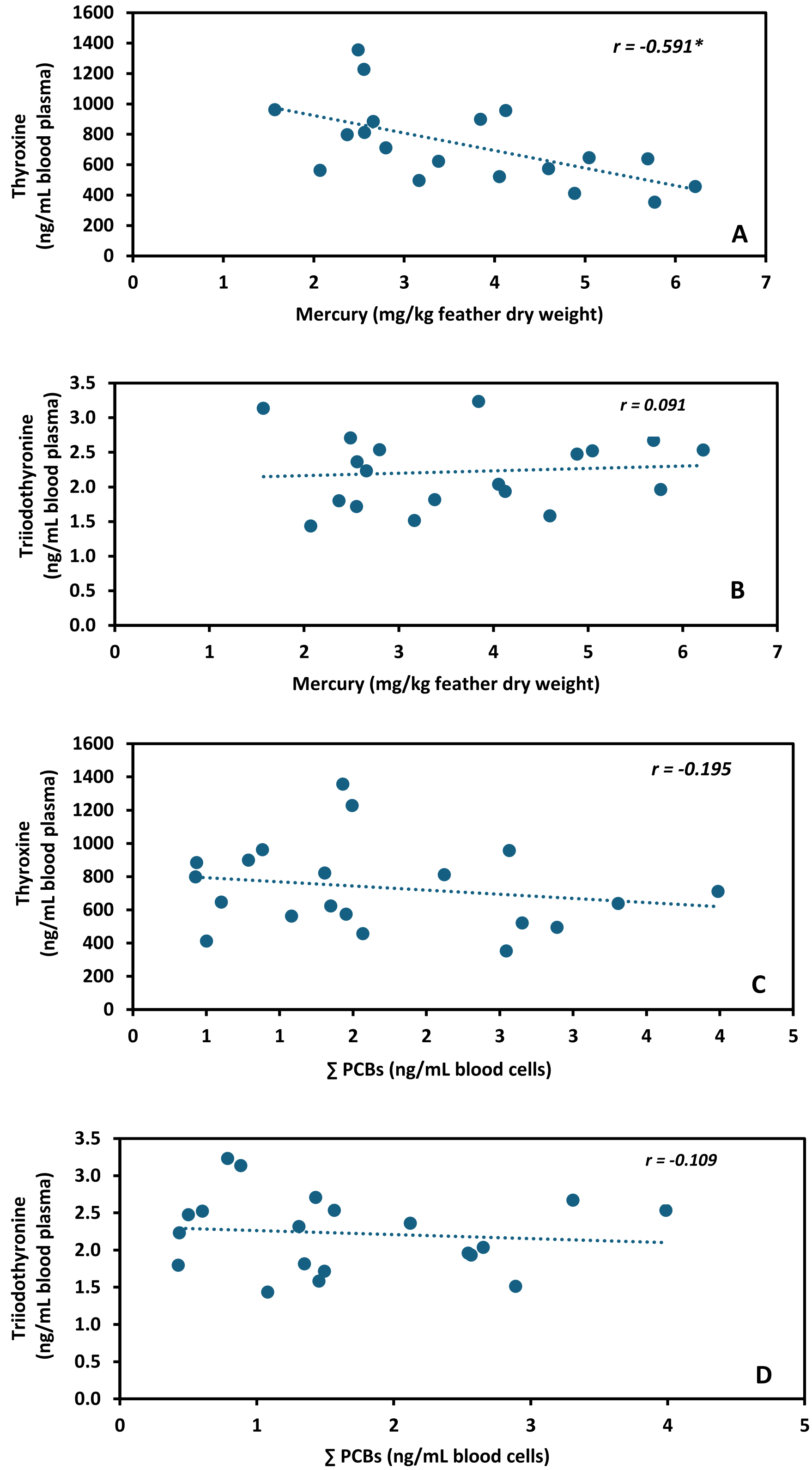

Fig. 4. Relationship between mean T4 and mercury levels (n = 19; A), T3 and mercury levels (n = 19; B), T4 and ∑PCB levels (n = 20; C) and T3 and ∑PCB levels (n = 20; D) in adult sable shearwaters sampled summer 2017. Pearson's correlation coefficient (*r*).

mercury and T3 concentrations within fledgling sable shearwaters sampled in 2017 serves to support this notion. Furthermore, a similar relationship has previously been found in a separate bird species, such as Tree Swallows (*Tachycineta bicolor*) (Wada et al., 2009) and thick-billed murres (*Uria lomvia*)(Esparza et al., 2022). Unexpectedly, a positive correlation was observed between mercury and T3 concentrations within adult sable shearwaters. However, it was weakly positive and not significant.

Interestingly, the negative relationship observed between mean mercury and T4 concentrations from adult sable shearwaters were

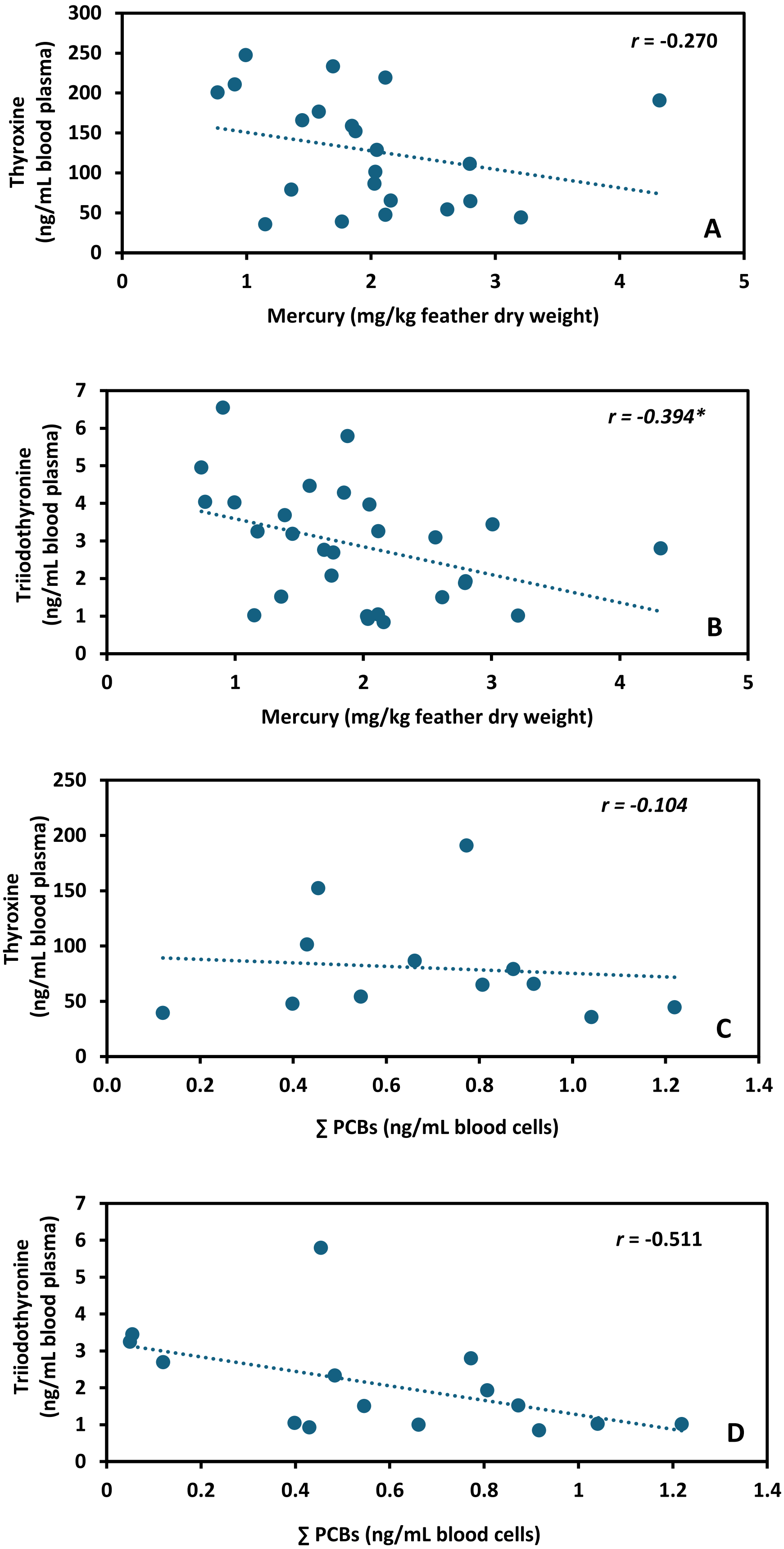

Fig. 5. Relationships between mean T_4 and mercury levels (n = 22; A), T_3 and mercury levels (n = 28; B), T_4 and ∑PCB levels (n = 12; C) and T_3 and ∑PCB levels (n = 15; D) in fledgling sable shearwaters sampled in 2017. Pearson's correlation coefficient (*r*).

statistically significant. It has been suggested that methylmercury affects the anterior pituitary gland within organisms, specifically by interfering with the production of Thyroid-Stimulating Hormone (TSH) (Soldin et al., 2008). As TSH-thyroid gland interactions play a central role in T4 production within organisms, suppressed TSH production because of methylmercury-facilitated interference, would in theory coincide with reduced circulating T4 concentrations. Reductions in circulating T4 concentrations have previously been observed in association with elevated mercury body burdens within tree swallow species (Wada et al., 2009). Monitoring circulating TSH and T4 concentrations alongside mercury body burdens in this population in future studies would facilitate a better understanding of the potential impacts on thyroid homeostasis facilitated by mercury-TSH interactions.

4.2. Correlations between PCBs and thyroid hormones

PCBs in red blood cells of fledglings and adults varied between seasons with the level increasing in higher PCB concentrations in adults observed in 2017 than in 2019 while the highest levels were in 2019 fledglings, almost triple that of 2017 fledglings (Fig. 2). PCBs are thought to be concentrated in marine sediments (Deng et al., 2020) therefore the PCB concentrations in the food fish around Lord Howe Island would not be expected to change. Unlike Hg, PCBs are relatively less mobile in oceanic currents (Figueiredo et al., 2020; Joiris et al., 1995; Lamborg et al., 2014; Lohmann et al., 2012; Protasowicki et al., 1999) and this result highlights the importance of understanding mobility of pollutants and thus their bioaccumulation through the food chain and impacts on top predators. Our study confirms that there is bioaccumulation of PCBs up food chains in the coastal area around Lord Howe Island, indicating this legacy persistent organic pollutant may still be bioavailable despite its use being banned in Australia in 1975 (Lyn, 2013).

The negative relationships found between ΣPCB and thyroid hormone (T4 and T3) concentrations in fledgling and adult sable shearwaters were expected. Similar relationships have been evaluated in other predatory birds, including Cooper's Hawk (*Accipiter cooperii*) and American Kestrels (*Falco sparverius*) (Brogan et al., 2017; Smits et al., 2002). As previously stated, PCBs are structurally and chemically similar to T4 and consequently exhibit a high affinity for transthyretin (one of the major T4 transport proteins in birds; McNabb, 2007; Ucán-Marin et al., 2010). It is possible that the competitive displacement of T4 because of PCB-transthyretin interactions resulted in reduced circulating T4 (and in turn T3) concentrations within the sable shearwaters sampled during this study.

PCBs have also been associated with increases in the glucuronidation and subsequent biliary excretion of T4 within organisms (Martin et al., 2012; Schuur et al., 1998). This may have served as an additional mechanism through which circulating T4 (and in turn T3) concentrations were reduced within the blood of sable shearwaters sampled. Interestingly, PCB-118, one of the most abundant and frequently detected congeners within sable shearwater samples during this study, has previously been observed to induce dramatic increases in the biliary excretion of T4 (Martin et al., 2012). Monitoring the activity of the enzyme responsible for the catalysis of glucuronidation, uridine diphosphoglucuronosyl transferase in relation to circulating concentrations of PCB-118 within sable shearwaters on Lord Howe Island would be useful in providing greater insight into the effect of PCB accumulation on thyroid homeostasis within seabirds.

4.3. Study limitations

There were important limitations to our ability to draw general conclusions from our results. First, the relatively small sample sizes (Table S1) available for analysis during this study may have been a limiting factor in this regard and the utilization of larger sample sizes in future studies should therefore be considered.

Second, we were unable to include Hg and PCBs data from environmental sites where animals were sampled. However, Hg is well known to circulate in ocean currents (Figueiredo et al., 2020; Lamborg et al., 2014) and sable shearwaters are migratory. Hence, the body burdens of Hg in adults are unlikely to reflect the local environment around Lord Howe Island, and fledglings would reflect Hg from a combination of maternal transfer and food. There was seasonal variability in Hg concentrations in breast feathers of fledglings, with higher concentrations in 2019 than in 2017. These inter-annual changes are likely influenced by the variability in the Hg content of the shearwaters' diet which is primarily comprised of squid (Gould et al., 1997).

We were unable to capture seasonal variability of the T3 and T4 levels and therefore this study may overlook critical hormonal dynamics during other life stages. Thyroid hormones fluctuate significantly throughout adult life stages, including during migration, breeding, and molting (Otsuka et al., 2004; Schmidt, 2002). Addressing these limitations through more frequent monitoring would provide a more complete understanding of thyroid hormone regulation and how it is affected by its surrounding environment.

5. Conclusions

To our knowledge, this is the first study, globally, in which PCBs have been quantified exclusively within the blood cell fraction of whole blood of marine wildlife, and the first in Australia to quantify a persistent organic pollutant within samples collected from living seabirds. This is also the first study in Australia to quantify circulating thyroid hormone concentrations and to address the accumulation of mercury and PCBs in relation to their effects on endocrine health within a migratory seabird species. Our results suggest thyroid function is likely to be negatively affected in sable shearwaters due to the bioaccumulation of mercury and PCBs. This is particularly concerning given the sampling site, Lord Howe Island, is a remote, UNESCO World Heritage-listed site that should be less affected by anthropogenic activity.

CRediT authorship contribution statement

Jason Lu: Methodology, Formal analysis, Data curation. **Foord Chantel:** Methodology, Formal analysis, Data curation. **Stephen Raison:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Miranda Ana:** Writing – review & editing, Investigation, Data curation. **Dayanthi Nugegoda:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Damien Nzabanita:** Writing – review & editing, Data curation. **Lavers Jennifer:** Writing – review & editing, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Prof. Dayanthi Nugegoda reports financial support was provided by Seaworld Research and Rescue Foundation Incorporated. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118501.

Data availability

Data will be made available on request.

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