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Title:
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Date:
2024-04-01

Citation:
Nzabanita, D., Shen, H., Grist, S., Lewis, P. J., Hampton, J. O., Firestone, S. M., Hufschmid, J. & Nugegoda, D. (2024). Exposure to Persistent Organic Pollutants in Australian Waterbirds. Environmental Toxicology and Chemistry, 43 (4), pp.736-747. https://doi.org/10.1002/etc.5804.

Persistent Link:
https://hdl.handle.net/11343/351098

Environmental Toxicology

Exposure to Persistent Organic Pollutants in Australian Waterbirds

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Abstract: There is growing worldwide recognition of the threat posed by persistent organic pollutants (POPs) to wildlife populations. We aimed to measure exposure levels to POPs in a Southern Hemisphere aquatic waterbird species, the nomadic gray teal (*Anas gracilis*), which is found across Australia. We collected wings from 39 ducks harvested by recreational hunters at two sites (one coastal, one inland) in Victoria, southeastern Australia, in 2021. We examined three groups of POPs: nine congeners of polychlorinated biphenyls (PCBs), 13 organochlorine pesticides (OCPs), and 12 polycyclic aromatic hydrocarbons (PAHs). The PCBs, OCPs, and PAHs were detected at quantifiable levels in 13%, 72%, and 100% of birds, respectively. Of the congeners we tested for in PCBs, OCPs, and PAHs, 33%, 38%, and 100% were detected at quantifiable levels, respectively. The highest levels of exposure to POPs that we found were to the PAH benzo[*b*]fluoranthene, occurring at a concentration range of 1.78 to 161.05 ng/g wet weight. There were some trends detected relating to differences between geographical sites, with higher levels of several PAHs at the coastal versus inland site. There were several strong, positive associations among PAHs found. We discuss potential sources for the POPs detected, including industrial and agricultural sources, and the likely role of large-scale forest fires in PAH levels. Our results confirm that while Australian waterbirds are exposed to a variety of POPs, exposure levels are currently relatively low. Additional future investigations are required to further characterize POPs within Australian waterbird species. *Environ Toxicol Chem* 2024;43:736–747. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Avian toxicity; Environmental contamination; Wetlands; Wildlife toxicology

INTRODUCTION

Persistent organic pollutants (POPs) are toxic organic (carbon-based) chemicals that bioaccumulate and are environmentally persistent and able to undergo long-range atmospheric transport far from emission sources. Through long-range atmospheric transport, POPs have accumulated in higher latitudes and remote regions (Ma et al., 2011; Wang et al., 2019). The toxic and bioaccumulative effects of POPs on ecosystems are a global contamination issue. In recognition of these risks to human health and the environment, the Stockholm Convention on Persistent Organic Pollutants was adopted on May 22, 2001, and entered into force May 17, 2004, as a binding instrument for implementing international action beginning with 12 POPs, or "the dirty dozen" (United Nations Environment Programme [UNEP], 2009). Australia became a party of the Convention in 2004 (Australian Government, 2020).

Among the initial 12 POPs, legacy POPs such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), like dichlorodiphenyltrichloroethane (DDT), are the most widely studied in wildlife, having been investigated for several decades (Keswani et al., 2022; Olsen et al., 1980; Ross et al., 2000). For example, in eastern Australia, OCPs were detected in the wings of waterfowl harvested for human consumption in the 1970s, and DDT was found at levels exceeding those recommended for human consumption, which was a "maximum residue limit" of 7 ppm at the time of the study (Olsen et al., 1980). In another study, straw-necked ibis (*Threskiornis spinicollis*) chicks

This article includes online-only Supporting Information.

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Published online 12 December 2023 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5804

died from poisoning with chlorpyrifos (a chlorinated pesticide with similar properties to the OCPs) in the 1990s in inland eastern Australia, although its source was not identified (Kingsford & Norman, 2002).

Polycyclic aromatic hydrocarbons (PAHs) are another important group of persistent chemicals but are not defined as POPs under the Stockholm Convention (UNEP, 2009). They can originate from both anthropogenic and natural sources, including from the incomplete combustion of organic matter (e.g., from the burning of fossil fuels) or from forest fires (McCready et al., 2000; Nguyen et al., 2014). As a result, these chemicals are generally abundant in aquatic environments, where they can be ingested by aquatic species (Idowu et al., 2019), including waterbirds; however, levels remain a significant knowledge gap in contemporary Australia.

Yet, despite there being little research in the field of POPs or legacy contaminants in Australian waterbirds, most recently, per- and polyfluoroalkyl substances (PFAS) have become the focus within Australia because of the human health risk posed by consuming contaminated bird tissues (Death et al., 2021; Sharp et al., 2021; Szabo et al., 2021, 2022). Ducks and other waterbirds are considered to be important bioindicators for several reasons (Kushlan, 1993; Zhang & Ma, 2011), including their unique foraging habits and capacity for long-distance migration (McEvoy et al., 2017). Because of their high trophic positions in many aquatic ecosystems, waterbirds are seen to be particularly valuable for assessing chemical contamination and ecosystem health (Løseth et al., 2019; Luo et al., 2009). Accordingly, many studies have used waterbirds as local bioindicators for certain contaminants in specific environments. Waterbirds are also considered to be species of high ecological importance because of the multiple ecosystem services they provide (Green & Elmberg, 2014), as well as being valued as game species (Moloney et al., 2022). Given their popularity for human consumption globally, ducks are also used as good indicators of environmental contamination relevant to human health (Warenik-Bany et al., 2019).

Waterbirds face an imperiled future in many countries through anthropogenic threats including harmful chemical pollutants (Jackson et al., 2019; Wang et al., 2022). Concerningly, the populations of eastern Australian waterbirds have declined significantly over the past four decades (Brandis et al., 2021; Kingsford et al., 2020). The role that pollutants play for many Australian waterbirds is unclear, with the field of ecotoxicology having been underrecognized in Australia until recently (Death et al., 2019). Well-known toxicants such as lead can cause intermittent mass mortality events in at-risk species (Harper & Hindmarsh, 1990; Koh & Harper, 1988). However, contaminants can also cause more subtle effects that are important over long timescales and at the population level, such as changed reproductive behavior and reduced offspring performance (Goutte et al., 2014). In wild birds, high POP concentrations have been linked to processes including oxidative stress, wing feather asymmetry, and endocrine disruption (Hao et al., 2021). Waterbird species occupying high trophic positions in aquatic food chains may be particularly threatened by POPs, given their potential for biomagnification (Goerke et al., 2004).

To our knowledge, there have been no published studies on the body burdens of POPs, outside of PFAS, in Australian waterbirds. However, coastal ecosystems and wetlands are known to be especially threatened by POPs because of their bioaccumulative behavior (Girones et al., 2021). Given the importance of waterbirds to aquatic ecosystems, and the potential implication to human health, this is an important knowledge gap. The aim of the present study was to determine contamination levels for legacy POPs including PCBs and OCPs, as well as the persistent PAHs, using adipose (fat) samples from a nomadic duck species harvested in southeastern Australia. The specific objectives were (1) to establish baseline exposure levels to three groups of POPs for a previously unstudied duck species, (2) to compare the concentrations found in male and female birds to examine the influence of sex on exposure in this species, (3) to compare the POP burdens in birds from an inland versus coastal site to better understand different source inputs, and (4) to examine the possible sources of these POPs from their congener profiles.

MATERIALS AND METHODS

Study areas and species

Gray teal (*Anas gracilis*; Figure 1) is a common duck species in Australasia, inhabiting water bodies across the Australian continent, from coastal wetlands to ephemeral inland wetlands (Gentilli & Bekle, 1983). They exhibit nomadic movement patterns (Roshier et al., 2008), moving >2000 km per year (Roshier et al., 2006), and have clutch sizes of approximately 10 eggs. They have also been one of the main species harvested by recreational hunters in southeastern Australia for decades (Halse et al., 1993; Menkhorst et al., 2019; Moloney et al., 2022).

We accessed hunter-donated wing samples as per past studies to use wing-collection surveys (Myatt & Krementz, 2007; Nzabanita et al., 2023; Wickson et al., 1992). This was a nonrandomized sampling method. In total, samples from 39 ducks were collected in May 2021 as part of official duck season hunting by government game hunting enforcement officers and provided through the Game Management Authority after being sexed and aged (Rogers et al., 2019). Wings were collected from two wetlands: Black Swamp in northern Victoria ($n = 20$: 11 male and nine female), which we designated our "inland" site, and Dowd Morass, in coastal eastern Victoria ($n = 19$: eight male and 11 female), which we designated our "coastal" site (Figure 2). Black Swamp is a small (~1.4 km^2) ephemeral wetland described as a shallow freshwater marsh (Fitzsimons et al., 2004) and is surrounded by agricultural land and approximately 55 km from the nearest regional city (Albury-Wodonga, human population ~100 000). Dowd Morass is a larger site (~15 km^2) and is a permanent wetland making up part of the Gippsland Lakes Ramsar site (Boon et al., 2008). It is only approximately 17 km from the ocean and approximately 12 km from the nearest large regional town (Sale, human population ~14 000).

FIGURE 1: Our study species, the gray teal (*Anas gracilis*) in its habitat in southeastern mainland Australia. Photo: Nick Foster.

Sample collection

The sex and age of each bird were recorded in the field by government staff and determined via wing plumage analysis as per standard methods (Rogers et al., 2019). Whole-wing samples were then stored at −20 °C before being thawed and dissected for adipose tissue. Adipose tissue was collected from each wing via the following procedure: Feathers were firstly plucked from the wings, then the skin and adipose fatty tissue were gently removed using a sterile scalpel blade and steel forceps. All dissected fat tissue was then placed in separate LLG® polypropylene transport tubes (Lab Logistics Group, Meckenheim, Germany) and stored at −80 °C until analysis.

Standards and reagents

Individual, mixed analytical standards for PCBs, OCPs, and PAHs were purchased from Accustandard® (New Haven, CT). The chemical abbreviations for all target analytes are summarized in Supporting Information, Table S1. Mixed standards included nine PCB congeners (congener calibration mix 27: PCB-28, -52, -101, -105, -118, -138, -156, -180, -209), 13 OCPs (α-hexachlorocyclohexane [HCH], β-HCH, δ-HCH, γ-HCH [lindane], heptachlor exo-epoxide, *trans*-chlordane, *cis*-chlordane, endosulfan sulfate, endosulfan I [alpha], endrin ketone, p,p′-DDT, p,p′-dichlorodiphenyldichloroethylene [DDE], and p,p′-dichlorodiphenyldichlorethane [DDD]), and 16 PAHs (acenaphthylene, acenaphthene, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*j*+*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a*,*h*]anthracene, fluorene, fluoranthene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene, and pyrene).

Sample extraction

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was used to measure all chemicals as per methods outlined in Shen et al. (2020). Individual samples of adipose tissue were thawed, cut into as small pieces as possible, and weighed (between 0.5 and 1 g). Samples were homogenized with a mortar and pestle and placed in a 15-ml Falcon™ tube (Corning, Corning, NY) with 2 ml of Milli-Q water, 2 ml of acetonitrile, and 2 g of a prepared salt mixture containing 1 g of sodium chloride, 4.6 g of magnesium sulfate monohydrate, 0.5 g of sodium hydrogen citrate sesquihydrate, and 1.4 g of dehydrated sodium citrate. After that, the homogenized samples were shaken by hand for 90 s and vortexed for 120 s. Then, the cocktail samples were centrifuged for 20 min at a speed of 4000 rpm. After centrifugation, 1 ml of supernatant was poured into a 2-ml Eppendorf® tube (Eppendorf, Hamburg, Germany), then 0.3 g of mixed salt, composed of 1 g of $MgSO_4$, 0.4 g of octadecylsilane, 0.4 g of activated magnesium silicate (Florisil®; Sigma-Aldrich, Sofia, Bulgaria), and 0.4 g of primary–secondary amine, were added (Buah-Kwofie & Humphries, 2019). Subsequently, the supernatant of each sample was transferred into a Supel™ QuE PSA/C18 tube (Sigma-Aldrich).

For quality control, three calibration standard solutions (PAH-600-1; Agilent Technologies), including 16 types of PAHs and four types of isotopically labeled internal standards (Sigma-Aldrich), were prepared and measured with each PAH (Supporting Information, Table S1).

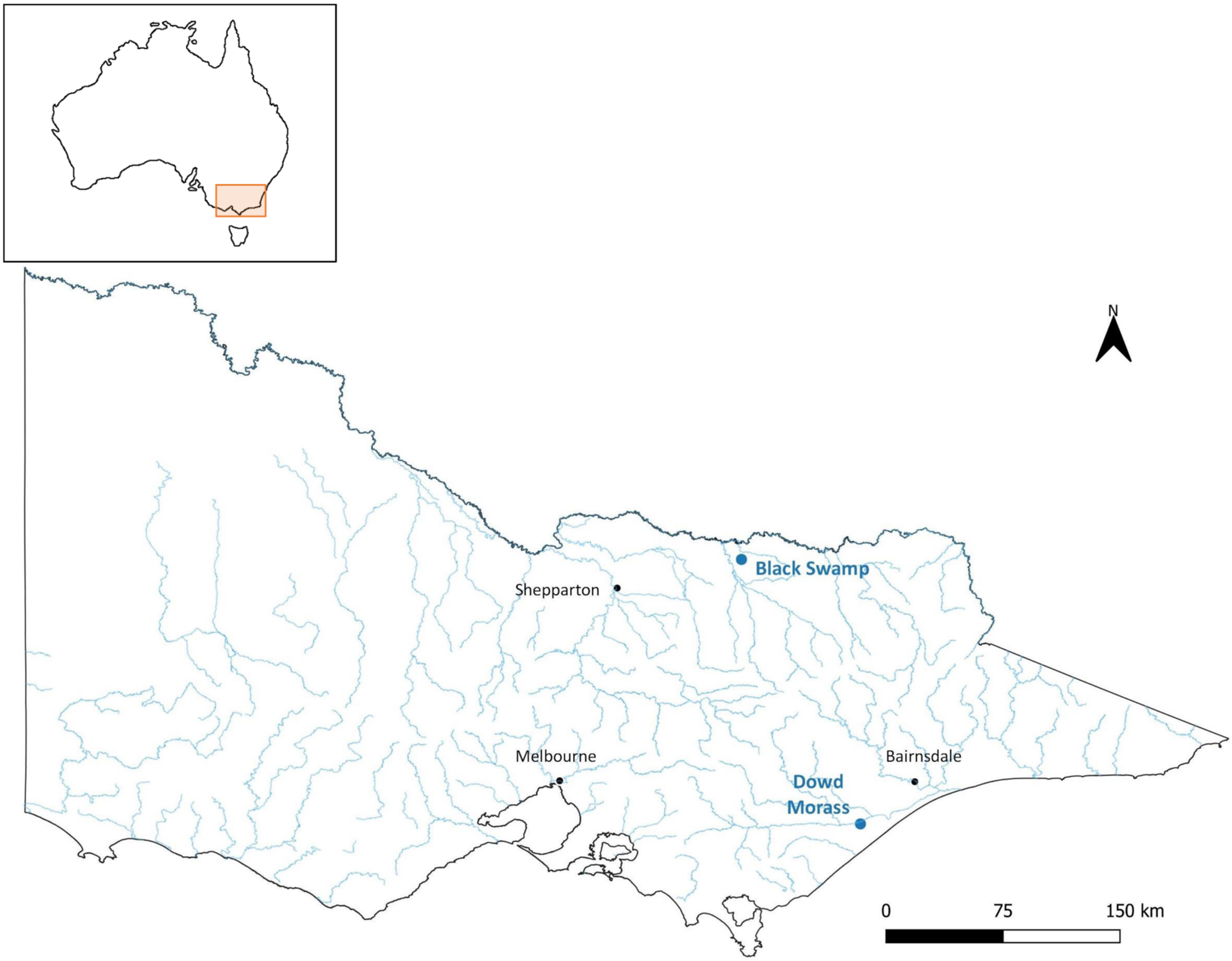

FIGURE 2: Our two field sites in Victoria, southeastern mainland Australia—inland, Black Swamp; coastal, Dowd Morass—where we collected wings from harvested gray teal (*Anas gracilis*).

Instrumental analysis and quantitation

Analyses of target PCBs and OCPs were performed using an Agilent 7000C gas chromatograph coupled to a triple quadrupole mass spectrometer, as per methods outlined in Lewis et al. (2020). Briefly, the instrument used a DB-5MS column (30 m × 250 µm internal diameter, 0.25 µm film thickness) and a 2-mm dimpled, single taper, splitless ultra inert liner. Injections were 2 µl using pulsed splitless mode, and the initial inlet temperature was 90 °C for 0.1 min before ramping to 325 °C at 900 °C/min. Initial oven temperature was 50 °C for 1 min, ramped to 320 °C at 25 °C/min, with a hold time of 4 min. Helium was the carrier gas, with a flow rate of 1.4 ml/min for 14 min before increasing at 100 ml/min to 4 ml/min, with total run time 15.8 min. Temperature of the transfer line was 280 °C, and ion source temperature was 280 °C, with each quadrupole 150 °C.

Analyses of target PAHs were undertaken on the same instrument based on Shen et al. (2020). Briefly, Agilent DB-5MS columns were paired first with 5 m long (325 °C) and second with 25 m (350 °C). The multimode was operated in solvent removal mode with injection at 60 °C and a ramp rate of 900 °C/min at 300 °C. The minimum oven temperature was 50 °C, while maximum was 325 °C. The run time was 17.2 min. Injections were 1 µl using pulsed spitless inlet mode. The injection pulse pressure was 50 psi for 1 min, and purge flow to split vent was 50 ml/min at 3 min. The injection speed was 2 µVs, while filling speed was 2 µVs. The ion source was electron impact with 350 °C and electron energy of 70 eV. The scan range mass was 40 to 450 AMU.

Calibration standard solutions with levels of 1 µg/L, 10 µg/L, 50 µg/L, 100 µg/L, 500 µg/L, and 1000 µg/L for PAHs and 1 µg/L, 10 µg/L, 50 µg/L, 100 µg/L, and 2000 µg/L for PCBs and OCPs were prepared. The levels of internal standards were 10 µg/L. Quality control (QC) samples for PCBs/OCPs and PAHs were prepared based on Shen et al. (2020). Specifically, three random tissues were homogenized together as one QC sample. Six QC samples were respectively mixed with calibration standards (PAHs and PCBs/OCPs) at three levels: 1 µg/L, 10 µg/L, and 300 µg/L (for PAHs)/100 µg/L (for PCBs and OCPs). Three replicates of each QC sample were measured three times at the beginning, middle, and end of sample measurement sequences.

All values below the limit of detection (LOD) were marked as nondetects, while all values between the LOD and the limit of quantification (LOQ) were marked as detected but too low to be quantified (<LOQ). Concentrations were measured in nanograms per gram wet weight, as per convention (Power et al., 2021).

Statistical analysis

The R statistical package, Ver 4.3.1 (2023), was used to determine descriptive statistics, plot histograms, and investigate the skew of POP concentrations.

Many values were <LOQ for certain POPs. To investigate associations between POP concentrations and two variables, site (inland vs. coastal) and sex, censored regression was undertaken using Tobit models (Hampton, Cobb, et al., 2023) for left-censored data at the respective LOQ, using the "vglm" package in R (Yee et al., 2015). The Akaike information criterion (AIC) was used to ascertain whether there was any association with either or both of the covariates (Akaike, 1998), and bootstrapped 95% confidence intervals (CIs) were estimated using the boot package in R (Canty & Ripley, 2016; Fieberg et al., 2020). All POPs were found to be severely positively skewed, so they were log-transformed prior to inclusion in Tobit regression models. Therefore, the outputs (coefficients and their bootstrapped CIs) were interpreted as exponentiated and multiplicative increases compared to the reference categories (the inland site and female animals, for site and sex, respectively). Residual value plots were examined to assess whether model assumptions (normal distribution of residuals and homoscedasticity) were met. Where >50% of the samples had POP concentrations <LOQ, logistic regression was performed instead, using the generalized linear model function in R, based on a dichotomized outcome variable of >LOQ or not. Again, the AIC was used for model selection, and bootstrapped 95% CIs were estimated. Interpretation of the outputs of the logistic regression models when exponentiated are as multiplicative increases in the odds of samples being >LOQ compared to the reference categories.

RESULTS

PCBs and OCPs

We detected PCBs in 23% (9/39) of birds, with levels exceeding the LOQ in 13% (5/39) of birds. Of the nine PCB congeners we tested for, only three (PCB-138, PCB-156, and PCB-180; 33%) were detected at quantifiable levels, found in 3% (1/39), 10% (4/39), and 1% (3/39) of birds, respectively (Table 1). Their distributions (median, range; nanograms per gram) were 3.22 (3.22–3.22), 7.23 (1.89–22.32), and 0.93 (0.93–0.93), respectively. We detected OCPs in 85% ($n = 33$) of birds, with levels exceeding the LOQ in 72% ($n = 28$) of birds. We tested for 13 OCPs, but only four (31%) of these were detected at quantifiable levels: endosulfan sulfate, endrin ketone, p,p′-DDD, and p,p′-DDE, found in 13% (5/39), 3% (1/39), 10% (4/39), and 67% (26/39) of birds, respectively (Table 2). Their distributions (median, range; nanograms per gram) were 1.66 (1.45–2.06), 1.04 (1.04–1.04), 1.84 (1.45–4.06), and 4.06 (0.40–29.87), respectively.

TABLE 1: Concentrations of nine polychlorinated biphenyl (PCB) congeners (ng/g ww; median, range) in adipose tissue from 39 gray teal (*Anas gracilis*) sampled at two sites (inland, Black Swamp; coastal, Dowd Morass) in southeastern mainland Australia in 2021

PCB congener	No. detected	No. quantified	Median	Range
PCB-28	0	0	NA	NA
PCB-52	0	0	NA	NA
PCB-101	0	0	NA	NA
PCB-105	0	0	NA	NA
PCB-118	3	0	NA	NA
PCB-138	6	1	3.22	NA ($n = 1$)
PCB-156	4	4	7.23	1.89–22.32
PCB-180	1	1	0.93	NA ($n = 1$)
PCB-209	0	0	NA	NA
%PCBs	23	13		

ND = not detected; %PCB = polychlorinated biphenyl; NA = not applicable.

PAHs

We detected PAHs at quantifiable levels in 100% ($n = 39$) of birds. We tested for 16 PAHs, and all were detected at quantifiable levels in at least one bird (Table 3). The values for each PAH type (median, range; nanograms per gram) were as follows: acenaphthylene, 1.31 (0.52–14.03); acenaphthene, 5.41 (0.96–96.55); anthracene, 1.15 (0.41–28.93); benz[*a*]anthracene, 1.98 (1.92–2.08); benzo[*a*]pyrene, 6.60 (0.72–64.27); benzo[*j*+*k*]fluoranthene, 7.34 (1.64–63.77); benzo[*b*]fluoranthene, 19.71 (1.78–161.05); benzo[*ghi*]perylene, 0.40 (0.07–2.38); chrysene, 1.44 (1.03–1.85); dibenz[*a*,*h*]anthracene,

TABLE 2: Concentrations of 13 organochlorine pesticides (ng/g ww; median, range) in adipose tissue from 39 gray teal (*Anas gracilis*) sampled at two sites (inland, Black Swamp; coastal, Dowd Morass) in southeastern mainland Australia in 2021

OCP congener	No. detected	No. quantified	Median	Range
Cis-chlordane	1	0	NA	NA
Endosulfan I (alpha)	0	0	NA	NA
Endosulfan sulfate	9	5	1.66	1.45–2.06
Endrin ketone	12	1	1.04	NA ($n = 1$)
HCH-alpha	0	0	NA	NA
HCH-beta	0	0	NA	NA
HCH-delta	0	0	NA	NA
HCH-gamma	0	0	NA	NA
Heptachlor	0	0	NA	NA
p,p′-DDD	6	4	1.84	1.45–4.06
p,p′-DDE	33	26	4.06	0.40–29.87
p,p′-DDT	0	0	NA	NA
Trans-chlordane	0	0	NA	NA
%OCPs	84.6	71.8		

NA = not applicable; ND = not detected; %OCP = organochlorine pesticide; NA = not applicable; HCH = hexachlorocyclohexane; DDD = dichlorodiphenyldichlorethane; DDE = dichlorodiphenyldichloroethylene; DDT = dichlorodiphenyltrichloroethane.

TABLE 3: Concentrations of 16 polycyclic aromatic hydrocarbons (ng/g ww; median, range) from adipose tissue from 39 gray teal (*Anas gracilis*) collected at two sites (inland, Black Swamp; coastal, Dowd Morass) in southeastern mainland Australia in 2021

PAH	No. detected	No. quantified	Median	Range
Acenaphthylene	37	19	1.31	0.52–14.03
Acenaphthene	39	39	5.41	0.96–96.55
Anthracene	38	25	1.15	0.41–28.93
Benz[*a*]anthracene	39	4	1.98	1.92–2.08
Benzo[*a*]pyrene	32	30	6.60	0.72–64.27
Benzo[*j*+*k*]fluoranthene	32	30	7.34	1.64–63.77
Benzo[*b*]fluoranthene	36	33	19.71	1.78–161.05
Benzo[*ghi*]perylene	39	39	0.40	0.07–2.38
Chrysene	39	2	1.44	1.03–1.85
Dibenz[*a*,*h*]anthracene	39	36	4.20	1.05–25.38
Fluorene	39	3	2.87	2.66–63.52
Fluoranthene	39	34	0.92	0.27–27.05
Indeno[1,2,3-*cd*]pyrene	21	6	1.16	0.99–1.93
Naphthalene	38	38	3.11	0.54–45.46
Phenanthrene	39	31	7.25	1.16–138.25
Pyrene	39	22	2.68	1.10–27.47
%PAHs	100	100		

PAH = polycyclic aromatic hydrocarbon.

4.20 (1.05–25.38); fluorene, 2.87 (2.66–63.52); fluoranthene, 0.92 (0.27–27.05); indeno[1,2,3-*cd*]pyrene, 1.16 (0.99–1.93); naphthalene, 3.11 (0.54–45.46); phenanthrene, 7.25 (1.16–138.25); and pyrene, 2.68 (1.10–27.47).

The effect of location and sex

Because of the small number of birds with quantifiable PCB or OCP levels, associations between exposure, sex, and location could only be explored for p,p′-DDE. The inland site had levels of p,p′-DDE that were three times higher than those of the coastal site (95% CI 0.91–11.9 times higher), and females had levels of p,p′-DDE that were 4.7 times higher than those of males (95% CI 1.37–15.6; Table 4). For PAHs, location had the strongest impact on anthracene and phenanthrene, with ducks from the coastal site returning concentrations that were over three times higher than those from Black Swamp. Similarly, ducks from the coastal site had more than twice the concentration of fluoranthene. Including sex improved the model fit for several of the pollutants studied. Females tended to have higher concentrations of acenaphthene, benzo[*a*]pyrene, benzo [*b*]fluoranthene, benzo[*j*+*k*]fluoranthene, and dibenz[*a*,*h*]anthracene, while males tended to have higher levels of benzo [*ghi*]perylene, naphthalene, and pyrene (Table 4).

PAH correlations

There were several strong, positive associations between PAHs found (Figures 3 and 4), especially at the inland site. At the inland site, the Spearman correlation profiles demonstrated strong correlations with (1) benzo[*a*]pyrene and benzo[*j*+*k*]fluoranthene ($r_s = 1.00$), and (2) phenanthrene and anthracene ($r_s = 0.88$; Figure 3). At the coastal site, PAH correlation patterns were broadly similar. Here, the Spearman correlation profiles demonstrated strong correlations with (1) benzo[*a*] pyrene and benzo[*j*+*k*]fluoranthene ($r_s = 0.93$), and (2) phenanthrene and anthracene ($r_s = 0.92$; Figure 4). At the coastal site, benzo[*b*]fluoranthene had a negative correlation with phenanthrene ($r_s = -0.35$).

DISCUSSION

The present study provides the first published assessment of exposure to legacy POPs in Australian aquatic waterbirds. The majority of PCBs (67%) and OCPs (69%) were below the method quantification limit in the wildlife species and geographical sites we assessed. In comparison, exposure to almost all PAHs was found to be widespread. This baseline assessment is especially important in a neglected global region for ecotoxicology research in the context of increasing global POP contamination issues (Bao et al., 2012).

Detection of PCBs was generally very low. One exception was the congener PCB-156, which suggests there may be a local, legacy source of PCBs to the environment in Victoria, which requires further investigation. Generally, OCPs had a low detection rate, but DDE exposure was still relatively high and consistent with worldwide literature, indicating that DDT breakdown products are still a global issue (Molina et al., 2021). In addition, there has been recent discussion of the dangerous nature of ongoing poorly related use of pesticides in Australia (Schneider, 2021). For example, there is ongoing use of the OCP dicofol, which is chemically related to DDT; and DDT is one of the intermediates used in its production (Weaver et al., 2012). For PAHs, the highest concentrations found were for benzo[*b*]fluoranthene, occurring at a median concentration of 19.71 (1.78–161.05) ng/g wet weight across both sites. Despite limited research on the toxicity of PAHs to animals and humans, this level of exposure is not expected to have marked adverse impacts on animal health (Deutsch-Wenzel et al., 1983; Kim et al., 2013; Ross et al., 1995).

Geographical differences between the two sites are worth exploring. Dowd Morass is a wetland surrounded by nearby small towns and more human activity (Boon et al., 2015) than the inland site, Black Swamp. The higher levels of the OCP p,p′-DDE detected at the inland site, when compared to the coastal site, likely reflect historical use of OCPs for irrigated crop agriculture in the Murray-Darling basin of inland southeastern Australia (Arthington, 1996), whereas the generally higher levels of PAHs at the coastal site may reflect higher numbers of motorized vehicles in the immediate vicinity, from both road traffic and the presence of motorboats. Globally, in excess of 90% of PAHs discharged into the environment are believed to derive from incomplete combustion sources (Howsam & Jones, 1998). For example, the major source of benzo[*ghi*]perylene to the environment is typically petrol-powered vehicles, while the release of phenanthrene to the environment comes primarily from wood combustion and coal (Neilson, 1998). In 2019 to 2020, eastern Victoria was affected by an extremely large-scale bushfire, referred to as "the Australian megafire" (Wintle et al., 2020). This fire occurred very close to our costal site but much farther from

TABLE 4: Censored regression model with coefficients, exponentiated coefficients (interpret multiplicatively for named logged dependent variable compared to reference value), and bootstrapped 95% confidence intervals for associations between location, sex, and concentrations of various persistent organic pollutants detected in the adipose tissue of gray teal (*Anas gracilis*) harvested in 2021 in Victoria, southeastern mainland Australia

POP	Class	Metric	coef	exp(coef)	2.50%	97.50%
Acenaphthylene	PAH	Intercept	−0.405	0.667	0.235	1.714
		Location (coastal)[a]	0.724	2.062	0.545	10.00
		AIC	56.78439			
		r^2	0.023232			
Acenaphthene	PAH	Intercept	1.676	5.343	2.595	10.392
		Location (coastal)[a]	0.455	1.576	0.786	3.403
		Sex (male)[b]	−0.116	0.891	0.434	1.993
		AIC	122.7214			
		r^2	0.05004			
Anthracene	PAH	Intercept	−1.024	0.359	0.165	0.649
		Location (coastal)[a,c]	1.286	3.617	1.333	10.331
		AIC	115.464			
		r^2	0.15616			
Benzo[*a*]pyrene	PAH	Intercept	1.71	5.527	2.599	10.329
		Sex (male)[b]	−1.07	0.343	0.104	1.097
		AIC	143.9428			
		r^2	0.067081			
Benzo[*b*]fluoranthene	PAH	Intercept	2.802	16.474	7.712	35.191
		Location (coastal)[a]	−0.008	0.992	0.42	2.342
		Sex (male)[b]	−0.270	0.763	0.323	1.803
		AIC	139.3461			
		r^2	0.008562			
Benzo[*j* + *k*]fluoranthene	PAH	Intercept	1.812	6.12	3.338	10.339
		Sex (male)[b]	−0.605	0.546	0.207	1.416
		AIC	128.9886			
		r^2	0.023359			
Benzo[*ghi*]perylene	PAH	Intercept	−1.143	0.319	0.238	0.424
		Sex (male)[b],[c]	0.62	1.859	1.106	3.124
		AIC	99.81057			
		r^2	0.128992			
Dibenz[*a*,*h*]anthracene	PAH	Intercept	1.729	5.638	3.292	9.064
		Location (coastal)[a]	−0.425	0.654	0.386	1.163
		Sex (male)[b]	−0.426	0.653	0.343	1.106
		AIC	110.1134			
		r^2	0.100277			
Fluoranthene	PAH	Intercept	−0.581	0.559	0.367	0.85
		Location (coastal)[a]	0.793	2.21	1.04	4.922
		AIC	123.7931			
		r^2	0.111256			
Naphthalene	PAH	Intercept	0.966	2.627	1.319	5.489
		Location (coastal)[a]	0.197	1.218	0.623	2.277
		Sex (male)[b]	0.228	1.256	0.664	2.399
		AIC	116.2787			
		r^2	0.020585			
Phenanthrene	PAH	Intercept	0.973	2.645	1.441	4.691
		Location (coastal)[a]	1.1	3.005	0.963	8.38
		AIC	137.6675			
		r^2	0.148472			
Pyrene	PAH	Intercept	−0.109	0.897	0.271	2.115
		Location (coastal)[a]	0.258	1.294	0.453	3.989
		Sex (male)[b]	0.4	1.492	0.537	4.387
		AIC	110.5671			
		r^2	0.007669			
p,p′-DDE	OCP	Intercept	1.296	3.653	1.227	9.771
		Location (coastal)[a]	−1.113	0.329	0.084	1.093
		Sex (male)[b],[c]	−1.55	0.212	0.064	0.73
		AIC	133.9364			
		r^2	0.181237			

[a]Compared to a reference category of ducks from the inland site.
[b]Compared to a reference category of female ducks.
[c]Predictor variable where 95% confidence interval does not include 1.
POP = persistent organic pollutant; coef = coefficient; OCP = organochlorine pesticide; PAH = polycyclic aromatic hydrocarbon; AIC = Akaike's information criterion; DDE = dichlorodiphenyldichloroethylene.

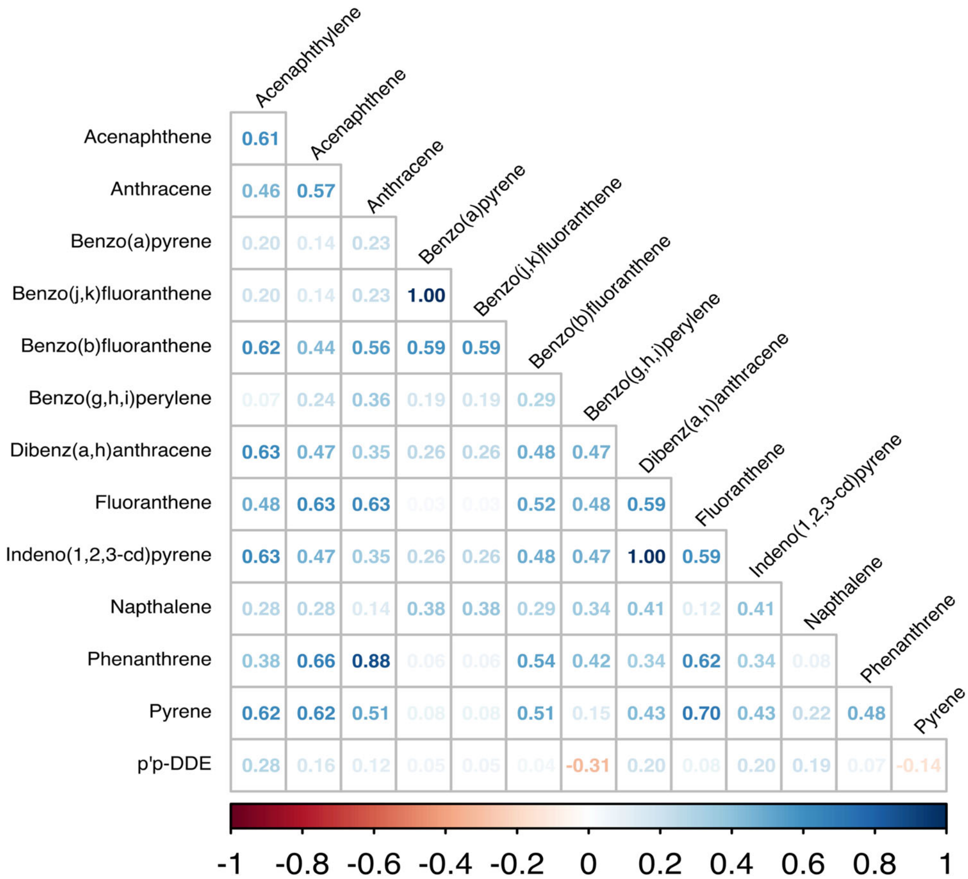

FIGURE 3: Spearman correlations for polycyclic aromatic hydrocarbons detected in the adipose tissue of gray teal (*Anas gracilis*) harvested in 2021 from an inland site in Victoria, southeastern mainland Australia. DDE = dichlorodiphenyldichloroethylene.

our inland site (Geary et al., 2022). It is possible that this event may have produced PAHs now being detected in the tissues of wildlife inhabiting the same geographical area.

At the inland site, the strong correlations observed between several PAHs can be regarded as likely indicators that these chemicals arose from the same type of source, likely fuel combustion, because none of these PAHs degrade to one another (Mishra et al., 2014; Rachna & Shanker, 2019). At the coastal site, PAH correlation patterns were broadly similar, although not as strong. This difference likely reflects different sources influential in inland versus coastal areas, for example, the presence of motorboats in use near the coastal site, compared to farm machinery, solid waste, or coal-fired power plants that may be closer to the inland site. Fluoranthene had generally stronger correlations at the coastal compared to the inland site, suggesting the presence of a local source near the coastal site, because its production is affiliated with incineration, oil burning, and coal or wood burning (Khalili et al., 1995). There were several negative, weak correlations between PAHs at the coastal site. Notably, benzo[*b*]fluoranthene was broadly negatively correlated with a number of PAHs, including phenanthrene ($r_s = -0.35$; Figure 4). This chemical enters the environment mostly from diesel exhaust (Guo et al., 2020), which is consistent with increased motor vehicle activity in the immediate proximity of the coastal site.

There were mixed and weak effects of sex for several PAHs. Although we were only able to test for differences between sexes for one PCB or OCP (p,p′-DDE), the significantly higher concentrations of this OCP detected in female ducks do not have an obvious explanation and could be the focus of further investigation.

At the time of writing, there were no published studies on PAHs in Australian duck species with which to compare our results. However, international waterbird studies permit some comparisons. For example, the anthracene levels we report (Table 3) are similar to those found in the blood of common loons (*Gavia immer*, 2.03 ± 0.17 and 4.82 ± 0.36 ng/g) from 2011 and 2012 samples, respectively, in coastal Louisiana, USA (Paruk et al., 2014). In comparison, we found a median concentration of 1.15 ng/g in adipose tissue across both of our sites (Table 3). A recent paper from Spain (Dulsat-Masvidal et al., 2023) quantified six PAHs in the blood of greater flamingos (*Phoenicopterus roseus*): naphthalene (range 1.29–50.3 ng/ml), pyrene (0.18–333 ng/ml), fluoranthene (0.13–41.1 ng/ml), acenaphthylene (0.66–23.7 ng/ml), benz[*a*]anthracene (1.95–2.27 ng/ml), and benzo[*ghi*]perylene (2.24–2.24 ng/ml). These studies assessed blood levels, which typically show lower concentrations of POPs when compared to concentrations found in adipose tissues, because of the lipophilic nature of these chemicals, resulting in their tendency to accumulate in the fatty tissues of organisms (Jackson et al., 2017). Therefore, comparatively, exposure levels found in Australian species are likely to be substantially lower than those in species studied in Europe

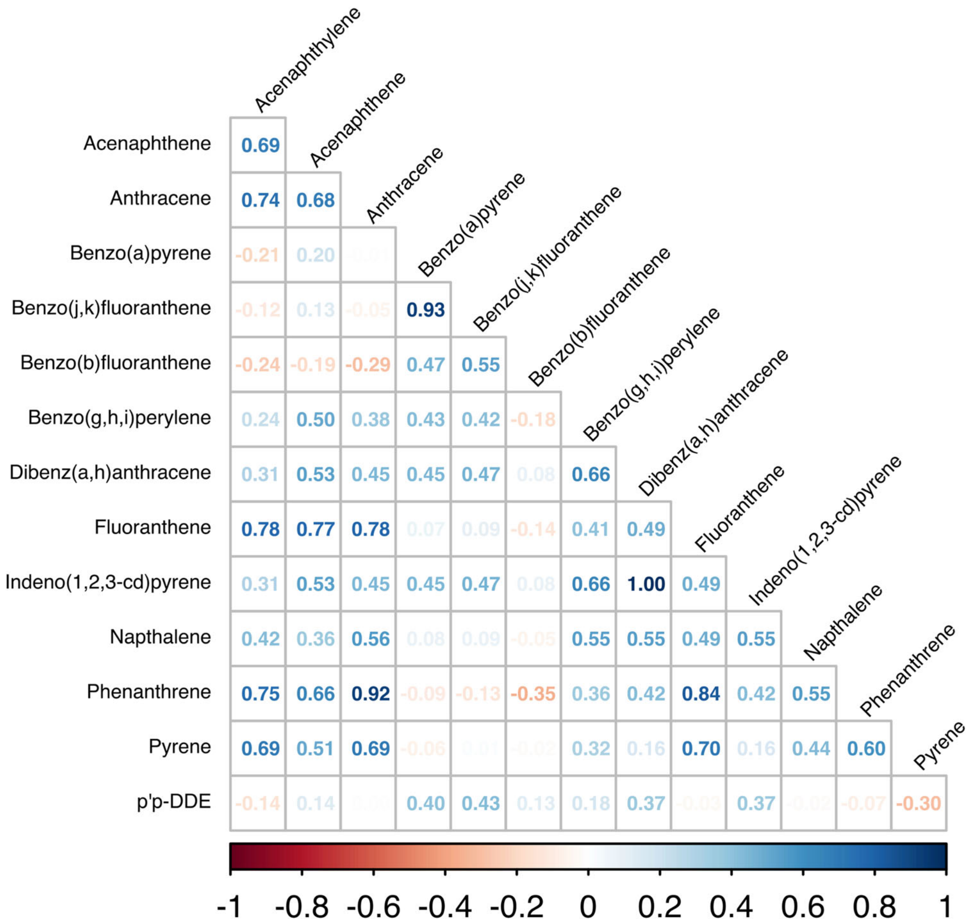

FIGURE 4: Spearman correlations for polycyclic aromatic hydrocarbons detected in the adipose tissue of gray teal (*Anas gracilis*) harvested in 2021 from a coastal site in Victoria, southeastern mainland Australia. DDE = dichlorodiphenyldichloroethylene.

and North America, which is due largely to their distance from the major urban centers and manufacturing regions of the Northern Hemisphere.

There were important limitations to our ability to extrapolate from our results. First, we looked at a single species of duck. Second, we used a nonrandomized sampling method—our sampling was heavily influenced by the preferred hunting locations of volunteering hunters. A random sample of animals may well yield differing results. For example, sublethal exposure to harmful contaminants in wild birds may conceivably increase the risk of mortality through processes such as being killed by hunters (Hampton, Lohr, et al., 2023). Sampling of hunter-collected animals will always be opportunistic rather than random, whereas live capture programs could be used for the collection of other sample types (e.g., blood; Franson et al., 2004) and tend to be less biased because trapping effort can be standardized across a study area (Descalzo et al., 2021). Third, we looked at only two sites, both of which were confined to eastern Victoria. Fourth, our sample size was relatively small (<50). Finally, we did not look at environmental contamination levels of POPs in soil or water. Nonetheless, we feel that our results are likely to be indicative of current exposure to POPs in nomadic waterbirds inhabiting southeastern mainland Australia.

Our preliminary results will be useful to guide future investigations looking at biomarkers of stress in duck species or exposure to humans and transfer through the food chain (e.g., to human game meat consumers; Warenik-Bany et al., 2019) or other wildlife species that may be exposed at similar geographical sites. We suggest that gray teal may act as a good indicator species in Australia, given their nomadic nature, use of nearly all geographical regions, and relative ease of sampling through opportunistic collection of tissue during recreational hunting. Future studies may consider looking at wetlands/sites near more densely populated areas for comparison. Finally, it would be interesting to explore exposure to other contaminant classes (e.g., heavy metals) in birds showing exposure to POPs, given the physiological importance of exposure to multiple contaminants (Fong-McMaster et al., 2020; Saaristo et al., 2018).

Our study suggests that further investigations are required into exposure to POPs in Australian wildlife species. We met the aims of our study by quantifying exposure to PCBs, OCPs, and PAHs in a useful indicator species for Australian ecosystems. These are important results because of the ecological implications of higher levels of PAH contamination, as well as human health effects for consumers of game ducks.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5804.

Acknowledgments—The authors declare no conflicts of interest. Funding was received from the Cybec Foundation and RMIT University. We acknowledge the Taungurung and Gunaikurnai peoples as the traditional owners of the lands and waters on which this research was conducted. For access to samples and photographs, we acknowledge the contribution of S. Toop, J. Flesch, and N. Foster. Because no live animals were used, animal ethics committee approval was not required. Open access publishing facilitated by RMIT University, as part of the Wiley - RMIT University agreement via the Council of Australian University Librarians.

Disclaimer—The authors declare no conflict of interest.

Author Contributions Statement—**Damien Nzabanita:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Validation; Visualization; Writing—original draft; Writing—review & editing. **Hao Shen:** Investigation; Methodology; Writing—review & editing. **Stephen Grist:** Methodology; Validation. **Phoebe J. Lewis:** Data curation; Formal analysis; Methodology; Writing—review & editing. **Jordan O. Hampton:** Supervision; Visualization; Writing—review & editing. **Simon M. Firestone:** Formal analysis; Writing—review & editing. **Jasmin Hufschmid:** Data curation; Formal analysis; Supervision; Visualization; Writing—review & editing. **Dayanthi Nugegoda:** Conceptualization; Funding acquisition; Project administration; Supervision; Writing—review & editing.

Data Availability Statement—Data are available from the corresponding author (s3362989@student.rmit.edu.au).

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