SPATIAL TRENDS OF PERSISTENT ORGANIC POLLUTANT LEVELS IN AUSTRALIAN WHITE IBIS EGGS

Ridoutt C[1], Kingsford R[2], Roach A[3], Yates A[4*], Mamahit G[4]

[1]NSW Department of Primary Industries, Woodbridge Road, Menangle, Sydney, Australia; [2]School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia; [3]Ecotoxicology and Environmental Contaminants Section, NSW Office of Environment and Heritage, PO Box A290, Sydney, Australia, [4]National Measurement Institute, Riverside Corporate Park, 105 Delhi Road, North Ryde, Sydney, Australia.

Introduction

The Australian white ibis (*Threskiornis molucca*) is prevalent throughout mainland Eastern Australia in both rural and urban habitats. The natural diet of the Australian white ibis is mainly aquatic and terrestrial invertebrates, but as opportunistic feeders in urban areas such as parks and landfill sites, they will supplement their diet with waste food scraps, paper and plastics[1, 2]. This scavenging behaviour makes it a useful sentinel species not only to study the level of persistent organic pollutants (POPs) in a specific habitat but also to investigate the potential variation in POP levels across different habitat locations. The use of avian eggs for biomonitoring has been previously established as a suitable indicator of environmental contamination [3, 4]. This study sampled Australian white ibis eggs from three categories of habitat indicative of different degrees of urbanisation, and to our knowledge represents the most comprehensive survey to date in Australia. The eggs were analysed for polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), dioxin-like polychlorinated biphenyls (dl-PCBs) and polybrominated diphenyl ethers (PBDEs). Patterns in egg traits (egg weight, volume, content, width and shell weight) were also studied in relation to habitat location for potential correlation to observed POP levels present.

Materials and methods

Egg Collection

Eleven separate Australian white ibis nesting sites were sampled across southeastern Australia. These sites were categorised as urban, peri-urban or inland, dependent on human population density within a 35km radius of the nesting site location. Sampling was carried out at the start of the breeding event to obtain eggs in the earliest stage of development. At each site, a minimum of 19 different nests were surveyed along half the colony perimeter by choosing nests perpendicular to a random points along a transect. A single egg was removed from nests where at least 3 eggs were present and euthanized on site by freezing to -20°C. Egg weight, length and width were all recorded in situ. A total of 219 eggs were sampled; after thawing to ambient temperature in the laboratory, the eggs were initially wiped clean with methanol to remove potential contamination from residual dirt or faeces. Additional physical parameters (volume, content, shell weight and shell thickness) were also measured prior to extraction.

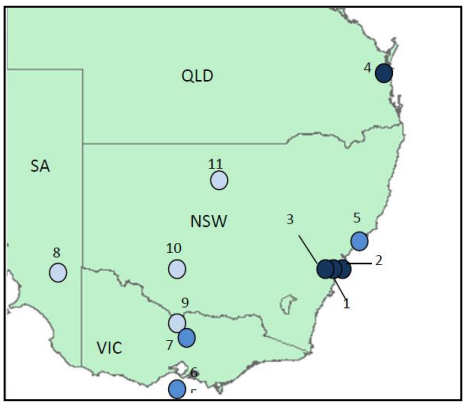


Figure 1 – Colony Sampling Locations

1) Lake Gillawarna, Bankstown 2) Royal Botanic Gardens, Sydney 3) Lake Annan, Camden 4) Murrarie, Brisbane 5) Hunter Wetland, Newcastle 6) Mud Islands, Queenscliffe 7) Reedy Swamp, Shepparton 8) Watchels Lagoon 9) Reed Beds South, Mathoura 10) Lowbidgee 11) Macquarie Marshes

Urban (dark blue circles), peri-urban (medium blue circles) and inland sites (light blue circles).

Sample Extraction
A random sub-sample of 33 eggs (3 per site) was taken for chemical analysis to determine levels of PCDD/Fs, dl-PCBs and PBDEs. Each egg sample was broken into separate solvent rinsed jars then spiked with isotopically labelled 13C surrogate solutions of PCDD/Fs (15) dl- PCBs (12) and PBDEs (15). The lipid component of the egg matrix was extracted using a combination of 10M hydrochloric acid (HCl) to denature proteins and break down the matrix structure together with a hexane/dichloromethane (3:1) solvent mixture. The samples were left to digest overnight at ambient temperature, tumbled for two hours then centrifuged at a speed of 250 RPM for 20 minutes. The organic phase (containing extracted lipids) was decanted and filtered through clean anhydrous sodium sulphate. The extraction process was then repeated twice more with additional portions of solvent. These solvent extracts were combined and concentrated to constant weight using a Büchi Syncore evaporation unit. The resultant lipid was dissolved in hexane and 50% of the extract used to determine PCDD/F, dl-PCB and PBDE content. Of the remaining extract, 25% was used to determine organochlorine pesticide levels (not discussed in this work) and 25% was retained as reserve.

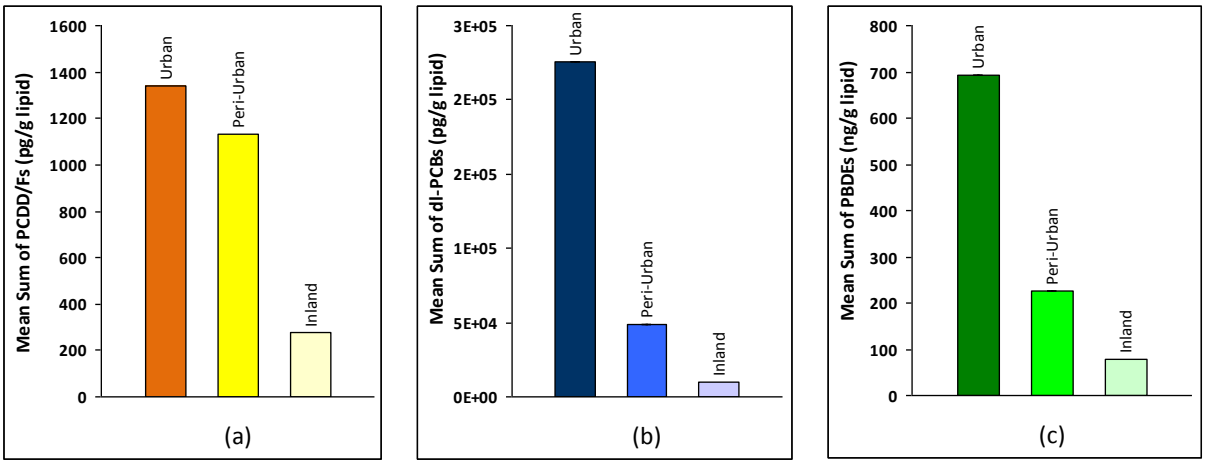
Sample Clean-Up
The 50% sample extract was partitioned in a separating funnel using concentrated sulfuric acid (H_2SO_4), and repeated until the acid layer was colourless. The resulting hexane extracts were washed with Milli-Q water and dried through pre-cleaned anhydrous sodium sulfate. The extracts were exchanged into dichloromethane and passed through a multi column Gel Permeation Chromatography (GPC) system to remove high molecular weight biomolecules co-extracted from the sample. The samples were then re-exchanged back into hexane for subsequent clean-up using a Fluid Management Systems Power-Prep to separate PCDD/Fs and non-ortho dl-PCBs from PBDEs and mono-ortho dl-PCBs as two discretely eluted fractions. Both fractions were subsequently concentrated to near dryness and isotopically labelled 13C recovery standard solutions of PCDD/Fs (2) dl- PCBs (4) and PBDEs (4) added.

Analysis
Sample extracts were analysed using an Agilent 6890 / Waters AutoSpec Premier HRGC/HRMS instrument which met all the performance requirements of USEPA methods 1613, 1668 and 1614. A DB-5 (J & W Scientific) GC column (60m × 0.25mm × 0.25µm) was used as the primary analytical column for PCDD/Fs and dl-PCBs together with a DB-Dioxin (J & W Scientific)capillary column (60m × 0.25mm × 0.15µm) for congener confirmations where necessary. A DB-5 column (10m × 0.1 mm ×0.1µm) was used for PBDE analysis. The individual congeners were identified using the GC retention time and ion abundance ratios with reference to surrogate standards and quantified by isotope dilution. Congeners below the instrumental LOD were considered to be not detected (ND) [3] and were assigned a value of zero when calculating means and standard deviations. Data from two samples were excluded due to PCCD/F and dl-PCB surrogate recoveries below 25%; the associated PBDE data for these samples were also removed from the data set.

Results
There was clear gradient of contamination observed with highest mean PCDD/F, dl-PCB and PBDE levels at urban sites compared to peri-urban and inland sites (Figs. 2a-c).

Persistent organic pollutant levels
In terms of PCDD/F congener profiles, it was noted that for both urban and peri-urban sites the predominant congeners were 1,2,3,4,6,7,8-HpCDD (12-17%) and 1,2,3,4,6,7,8,9-OCDD (61-73%). This corresponds to congener patterns previously observed in Australia soils [5]. At inland sites 2,3,4,7,8-PeCDF (44%) dominated these two dioxin congeners; this can be ascribed to one egg sampled at Macquarie Marshes which showed an unusually elevated 2,3,4,7,8-PeCDF contamination pattern which effectively skewed the data. Mean sum PCDD/Fs were highest at urban sites (1350 pg/g lw) compared to peri-urban (1130 pg/g lw) and inland sites (275 pg/g lw).The dioxin: furan ratios were dominated by PCDD congeners in both urban and peri-urban sites; in fact, the dioxin concentrations observed in this study were higher than most comparable international studies, in contrast with the furan results which are relatively low internationally. Furans are usually observed to dominate profiles, indicating potentially different pollution sources in Australia.

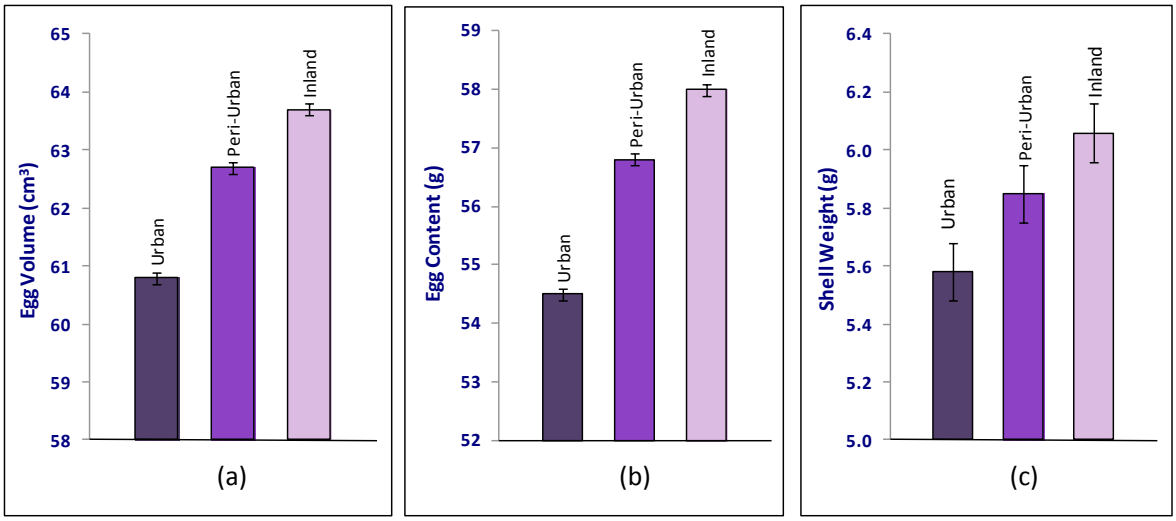
Figure 2 – Variation of (a) Mean Sum of 2378-substituted PCDD/F Congeners (b) Mean Sum of dl-PCB Congeners (c) Mean Sum of PBDEs by site location.



The dl-PCB congener profiles were similar across all three site categories, suggesting a common source of contamination. The profiles were dominated by mono-ortho dl-PCBs, mainly PCB 118 (55-60%), PCB 105 (20%) and PCB 156 (10%). Again, this corresponds to congener patterns previously observed in Australia soils[5]. The absolute levels of dl-PCBs detected were one to two orders of magnitude greater than PCDD/Fs present; Mean sum dl-PCBs were highest at urban sites (2.25×10^5 pg/g lw) compared to peri-urban (0.49×10^5 pg/g lw) and inland sites (1.02×10^4 pg/g lw).

PBDE congener profiles were broadly similar across all three sites, although there was relatively more contribution from BDE 209 in inland areas. The dominant congeners observed were BDE 209 (40-60%) > BDE 99 (7-14%) > BDE 47 (7-10%) > BDE 153 (10%) > BDE 100 (5%). Mean sum PBDE concentrations were highest at urban sites (691 ng/g lw) followed by peri-urban (226 ng/g lw) and inland sites (79 ng/g lw).

Figure 3 – Variation of (a) Mean egg volume (b) content and (c) egg shell weight for Australian white ibis eggs by site location..



Physical Egg Parameters
A clear trend was observed for physical egg parameters (excluding egg length and shell thickness) to be significantly lower at urban sites compared to either peri-urban or inland sites (Fig. 3).

Discussion
The differing levels of POPs with site location may be attributed to exposure to contaminants in more highly populated areas, potentially occurring when parental birds foraged in and around landfill sites or industrial areas. This contaminant trend is also prevalent in soils in Australia, with higher PCDD/F in urban areas, compared to agricultural and remote areas[5]. One egg from Macquarie Marshes had a significantly different congener pattern to other samples from the same site and was dominated by 2,3,7,8-PeCDF, which has a toxic equivalent factor for birds at least twice that for mammals. This observation suggests that the parent may have foraged at an alternative location with a high furan levels compared to other birds at the marsh site. The clear differences observed in egg contaminant levels are probably a function of increased urbanisation in different locations and reflects their environmental exposure; this suggests that the Australian white ibis may be a suitable sentinel species for monitoring POP contamination.

Risk assessments based on avian toxic equivalents identified that both PCDD/F and dl-PCB levels were below international thresholds, although PBDE levels may carry a low risk of reproductive impairment and cumulative effects may also negatively impact on reproduction. The observed decrease in physical egg parameters appears correlated with the corresponding increase in POP concentration observed in this study. Researchers have investigated the negative impacts of POPs on avian reproductive success [5] and although this observation has not been proved as a causative link, it merits further investigation.

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