POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN EGGS FROM GANNETS, GOLDEN EAGLES AND MERLINS

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Introduction

PAHs are primarily released to the environment from point and diffuse natural and anthropogenic sources[1,2] including crude oil spills[3]. PAHs can be ingested by birds during feeding, grooming and respiration and they can have toxic (particularly embryotoxic) effects[4], including reduced embryo survival or cellular and developmental abnormalities. However, there appear to be few data on PAH uptake by free-living terrestrial vertebrates. This may be because vertebrates have well developed mixed-function oxidase systems that facilitate efficient metabolism and excretion of some PAHs[5,6,7], although large aromatic hydrocarbons remain difficult to excrete[7,8]. The presence of detectable PAHs residues and metabolites in the body tissues and eggs of some biota[9] indicates that exposure does occur. PAHs in birds are often associated with lipids, bioaccumulation tending to occur not only in the livers but also in eggs where residues can be two or three orders of magnitude higher than in livers[10]. Thus, eggs are good potential biomonitors for PAH contamination in the environment.

The aim of this pilot study was to determine if there was evidence of widespread exposure of predatory birds to PAHs in Britain, as detected by measurement of egg concentrations. We compared concentrations of PAHs in gannet (*Morus bassanus*), golden eagles (*Aquila chrysaetos*) and merlin (*Falco columbarius*) eggs which were collected as part of the UK Predatory Bird Monitoring Scheme.

Materials and Methods

Twenty fresh gannet eggs (half from Ailsa Craig in the Irish Sea, half from Bass Rock in the North Sea), 13 failed golden eagle eggs from eyries across Scotland, and 7 failed merlin eggs from North East England and Scotland were analysed. A sub-sample of each egg was spiked with deuterated PAHs, ground with sand, dried with anhydrous sodium sulphate, and cold extracted in 50 ml hexane. 10 ml of the extract were used to determine gravimetrically the lipid content. A 200 µl aliquot of the remaining extract was cleaned-up with 1g of pre-treated alumina (4 h at 800°C) that had been deactivated with 5 % deionised water (w/w). The clean extract was then injected into the GC-MS (Agilent, Wokingham, UK) using a Programmable Temperature Vaporising (PTV) inlet. The PAHs were separated on an Agilent 30m HP5-MS column (0.25 mm internal diameter, 0.25 µm film thickness) and the carrier gas was helium (constant 2 ml min^{-1} flow). The oven temperature was: isothermal at 50°C for 5 min, 15°C min^{-1} to 200°C, isothermal at 200°C for 5 min, 5°C min^{-1} to 250°C, 10°C min^{-1} to 300°C, and isothermal at 300°C for 10 min. The mass spectrometer was operated in single-ion (SIM) mode with Electron Mass Ionisation (70 eV). For quality control assurance purposes, a blank, a chicken egg and a chicken egg spiked with a know concentration of deuterated PAHs were analysed with each batch of samples. The concentration of each compound was calculated using the isotope dilution method.

Statistical analysis - data distributions were skewed and the assumptions of parametric statistical tests were not met. Therefore, summary data on PAH concentrations are expressed as medians and inter-species comparisons were made using the non-parametric Kruskal-Wallis test. When more than one alkylated isomer was detected, the average was calculated (the number of isomers is presented in

parentheses) and this average was considered to be one PAH. We assigned a value of zero to undetectable concentrations.

Results and discussion

A suite of 52 PAHs, including two to seven ring parent PAHs and alkylated compounds, were analysed and reduced to 37 PAHs after grouping of the alkylated compounds. All PAHs were detected in one or more samples except naphthalene which was not detected at all. This study, contrasts with reported concentrations of naphthalene in coastal nesting birds[3]. We believe that relatively high detection limits and low and recovery were responsible for the absence of naphthalene in this study.

Eleven PAHs were detected in ≥50% of the eggs and most (>90%) contained four or more compounds (Fig. 1). These results suggest that the occurrence of PAHs in eggs is extensive, despite the high metabolic capacity of birds. The most frequently detected PAHs were the larger compounds, namely those with five rings (benzo[ghi]fluoranthene and cyclo[cd]pyrene, detected in 90% of the eggs) and four rings (present in ≥50% of the samples).

All the PAHs that were quantified were detected in at least one gannet egg and one golden eagle egg except for naphthalene and dibenzothiophene (not detected in either species) and perylene (not detected in golden eagles). In contrast only 25 (out of 37) PAHs were detected in the merlin eggs. The PAHs that occurred in the highest concentrations (average concentration ≥ 1 ng/g lipid wt) are depicted in Figure 2. There were between 16 and 18 such PAHs detected in the gannet and golden eagle eggs but only nine in merlin eggs (Fig. 2). These results suggest that merlins bioaccumulate fewer and lower concentrations of PAHs in their eggs than gannets and golden eagles.

The most abundant PAHs (concentrations >20 ng/g lipid wt) were phenanthrene (gannets) and methylnaphthalenes (golden eagles and merlins). A further nine and six PAHs were present in concentrations ≥ 5 ng/g lipid wt in golden eagles and gannets, respectively; of these, fluoranthene and dimethylnaphthalenes were common to both species (Fig. 2). In merlin eggs, no other PAHs (apart from the methylnaphthalenes) were present in concentrations ≥ 5 ng/g lipid wt, suggesting that the methylnaphthalenes dominate the PAH profile in merlins. The most abundant PAHs in gannet eggs (≥ 5 ng/g lipid wt) included three PAHs with 3 rings, one with 4 rings and three alkylated compounds; in golden eagles, they included one PAH with 3 rings, two alkylated PAHs, four PAHs with 4 rings, one PAH with 5 rings and two PAHs with 6 rings (Fig. 2). Therefore, gannet eggs were characterised by small PAHs and by alkylated compounds, whereas golden eagle eggs contained a wider range and larger PAHs than the other two species. Our results suggest that different species either accumulate different numbers of PAHs (perhaps because of differences in metabolic capacity) and/or that they are exposed to different sources of PAHs, reflecting their different dietary preferences.

The sum of the medians of the concentrations of all PAHs in the three species ranged between 94.1 and 128 ng/g lipid wt (Fig. 3), with no significant difference between species (Kruskall-Wallis statistic = 0.15, P>0.05, DF=2). Although, there is limited information on the embryotoxicity of individual PAHs, one study[4] assessed the embryotoxicity of 24 PAHs injected into chicken eggs. The most toxic compounds were benzo[k]fluoranthene, dibenz[a,h]anthracene and benzo[a]anthracene (LD50s of 14, 39 and 79 ng/g wet wt respectively). In our analysis, lipid wt concentrations of these compounds were greatest in golden eagle eggs (Fig. 2) and the equivalent median wet wt concentrations were 0.03 ng/g at most, some two-three orders of magnitude below the reported LD50s. Maximum wet wt concentrations in any sample were at least five fold lower than the LD50 values. Doses of approximately 36 ng/g benzo[*a*]pyrene and 270 ng/g chrysene cause significant reduction in embryonic growth and increased incidence of abnormal chicks when applied externally to the eggshell of mallard (*Anas platyrhynchos*) eggs[11]. Maximum wet wt concentrations of these compounds in samples in the present study were at least ten fold lower. Thus, although these compounds were not

present in concentrations that cause embryotoxic effects, they were widely distributed and were found in > 40% of the eggs analysed (Fig. 1).

The sum of the median concentrations for the 16 Priority Pollutant PAHs (included in the US Environmental Protection Agency's list of priority compounds for monitoring of pollution in the environment) in gannet, golden eagle and merlin eggs were 47.5, 22.6 and 31.6 ng/g lipid wt, respectively (Fig 3). These 16 PAHs of concern represented 20 to 37% of the sum of total PAHs. A previous study[3] reported approximately equivalent concentrations of the 16 PAHs in herring gull, cormorant, shag and chough eggs (29, 71, 228 and 30.6 ng/g lipid, respectively). A comparison between our results and this study[3] appears to indicate a broad similarity of PAHs accumulation across a range of bird species.

Total PAH concentration suggests that the measured egg residues may be the result of background levels of exposure to diffuse PAH sources. The eggs from species exposed to large point sources, such as species nesting and feeding on the estuaries of industrially contaminated rivers or alongside busy roads and motorways, might be expected to have higher concentrations of PAHs than those measured in this study. As far as can be judged from the available toxicity data, the concentrations of individual compounds in the eggs analysed in the gannet, golden eagle and merlin eggs are unlikely to have embryotoxic effects. However, there is evidence that simultaneous exposure to multiple PAHs can result in additive toxicity[12] and concurrent exposure to PAHs and other organic pollutants, such as PCBs, may also result in enhanced toxicity[13]. Thus, comparison of residues in eggs with experimental doses of single PAHs may under-estimate likely toxicity.

Overall, this pilot study has provided preliminary data on PAH concentrations in the eggs of three species of predatory bird in Britain and has shown that eggs could be used as biomonitors of background concentrations of PAHs. This work also revealed that low levels of PAHs can be detected in bird species with very different feeding habits. Despite the differences in feeding habits and geographical distribution of gannets, golden eagles and merlins, there were no significant differences in the total PAH concentrations in their eggs. However, the data appear to suggest that differences between species may occur at the individual PAHs level. A more detailed analysis of the data is required to highlight a petrogenic or pyrogenic PAH signature in the birds.

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Figure 1. Percentage of eggs in which each PAH was detected.

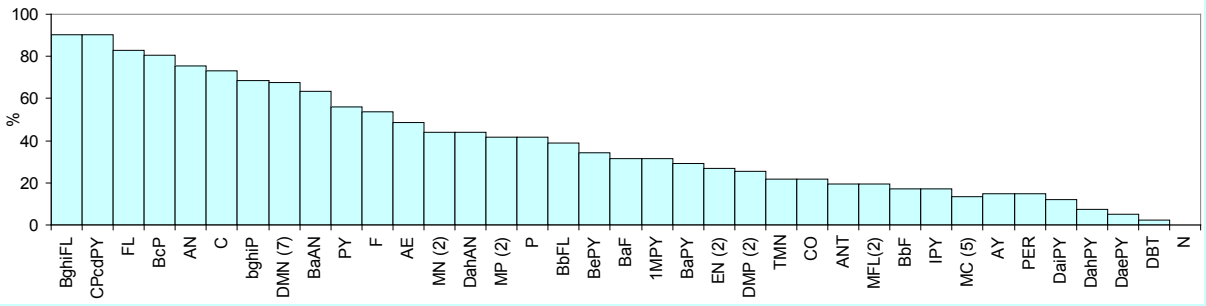
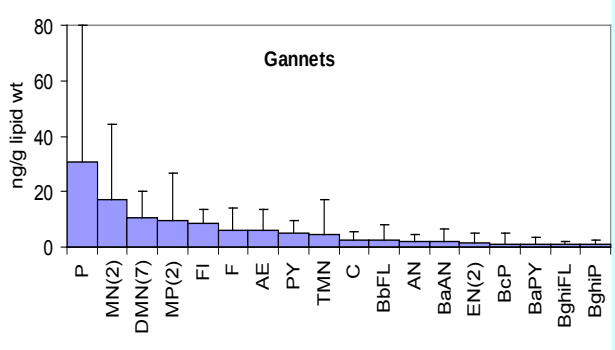
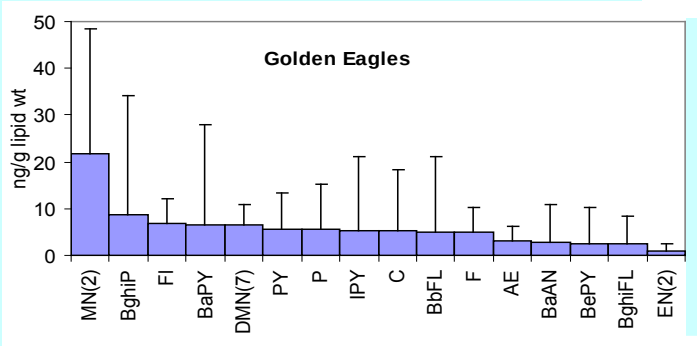


Figure 2. Average (ng/g lipd wt) and standard deviation of PAHs in gannet, golden eagle and merlin eggs.



Key for Figure 1 and 2: BghiFL-Benzo[ghi]fluoranthene; CpcdPY-Cyclopenta[cd]pyrene; FL-Fluoranthene; BcP-Benzo[c]phenanthrene; AN–Anthracene; C-Chrysene; BghiP-Benzo[ghi]perylene; DMN(7)-Dimethylnaphthalenes (7); BaAN-Benz[a]anthracene; PY- Pyrene; F-Fluorene, AE-Acenaphthene; MN(2)-Methylnaphthalenes (2) DahAN-Dibenz[ah]anthracene; MP(2)- Methylphenanthrenes (2); P-Phenanthrene; BbFL-Benzo[b & j & k]fluoranthene; 2MFL-2-Methylfluoranthene; BePY- Benzo[e]pyrene; BaF-Benzo[a]fluorene; 1MPY-1-Methylpyrene; BaPY-Benzo[a]pyrene; EN(2)- Ethylnaphthalenes (2); DMP(2)-Dimethylphenanthrenes (2); TMN-2,3,5-Trimethylnaphthalene; CO-Coronene; ANT-Anthanthrene; MFL-Methylfluoranthenes (2) ;BbF-Benzo[b]fluorene; IPY- ideno[1,2,3-cd]pyrene; MC(5)-Methylchrysene (5); AY-Acenaphthylene; PER-Perylene; DaiPY- Dibenzo[a,i]pyrene; DahPY-Dibenzo[a,h]pyrene; DaePY-Dibenzo[a,e]pyrene; DBT-Dibenzothiophene; N-Naphthalene



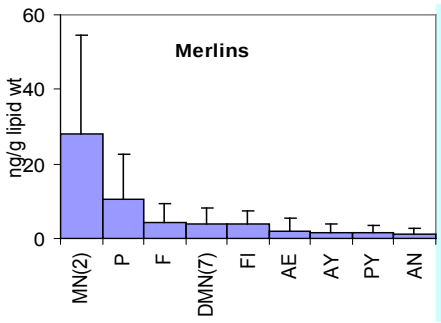


Figure 3. Median (and inter-quartile range) sum of total and the 16 priority pollutant PAH concentrations in the eggs of gannets in golden eagle and merlins. Number of samples shown in parentheses