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Polycyclic aromatic hydrocarbons (PAHs) in seabird eggs in Ireland

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ABSTRACT

Seabird eggs are considered a favourable matrix for monitoring marine pollutants and are widely used as higher trophic level indicators. Concentrations of Polycyclic aromatic hydrocarbons (Σ15PAH) were determined in the eggs of four piscivorous seabirds in Ireland from multiple colonies for the first time, Common Guillemot *Uria aalge*, Northern Gannet *Morus bassanus,* Common Tern *Sterna hirundo* and Arctic Tern *S. paradisaea.* PAH concentrations were generally lower than levels detected in eggs from other seabird studies and considerably lower than concentrations associated with no adverse effect in the eggs of domestic avian species. This study indicates potential site and species differences in PAH concentrations. Baseline data of PAHs in a range of seabird species from this study may provide an important reference point should a major pollution event occur in European waters, such as an oil spill.

Polycyclic aromatic hydrocarbons (PAHs) are a widespread group of environmental contaminants that can originate from both natural sources such as forest fires as well as anthropogenic sources such as the burning of fossil fuels and from oil spills (WHO, 1998). PAHs are ubiquitous in marine environments and have been recorded in water, sediment, fish, benthic invertebrates, seabirds, and marine mammals (Honda and Suzuki, 2020). Birds have been shown to bioaccumualte PAHs in their tissues with detectable concentrations reported in the livers, kidneys, lungs, brain, eggs, muscle, blood and faeces of various bird species (Dhananjayan and Muralidharan, 2013; González-Gómez et al., 2020; Pereira et al., 2009; Pérez et al., 2008; Provencher et al., 2020; Waszak et al., 2021). The effects of PAHs on aquatic ecosystems, including birds, is a concern due to their known toxic and bioaccumulative effects (Beyer et al., 2016; Meador et al., 1995).

The PAH Benzo(*a*)pyrene is a well-known mutagen and carcinogen (Phillips, 1983) while Benzo(*k*)fluoranthene has been shown to be toxic to chick embryos (Brunström et al., 1990). Birds are capable of quickly metabolising PAHs (Broman et al., 1990). However, PAHs have been shown to bioaccumulate in wild bird eggs (Kwok et al., 2013; Pereira et al., 2009) and concentrations present in eggs can be up to three orders of magnitude higher than in the liver (Malcolm and Shore, 2003). Birds may be exposed through a diet of PAH-contaminated food or directly through the respiratory uptake of atmospheric PAHs (Brunström et al., 1990). Seabirds are thought to be sensitive to PAHs as a result of their exposure to marine oil spills (Vidal et al., 2011). The Common Guillemot *Uria aalge*, Northern Gannet *Morus bassanus,* Common Tern *Sterna hirundo* and Arctic Tern *S. paradisaea* are seabirds that feed predominantly on fish (Ainley et al., 2020; Green, 2017; Lewis et al., 2003). During the egg formation period lipophilic persistent pollutants ingested by the adult female are passed into the developing egg with lipid reserves that are essential for the development of the embryo (Speake et al., 1998). Common Guillemot (hereafter Guillemots), Common and Arctic Terns have been shown to be income breeders, using recently derived nutrients to form their eggs (Bond and Diamond, 2010). Recent evidence also suggests that Northern Gannets (hereafter Gannets) are also income breeders (Power et al., 2021b). Consequently, bird eggs may have potential as biomonitors of PAH contamination in the local marine environment (Pereira et al., 2009).

In May 2017, two Guillemot eggs and two Gannet eggs were collected from Great Saltee Island in the St. George's Channel, off the southeast coast of County Wexford, Ireland (Fig. 1). In June 2018, six Common Tern eggs were collected from inner Galway Bay on the west coast of Ireland and two Arctic Tern eggs were collected from the Inishkea Islands, situated in the north-east Atlantic Ocean off the west coast of Ireland (Fig. 1). All samples in this study were collected under license from the Irish National Parks and Wildlife Service as part of a

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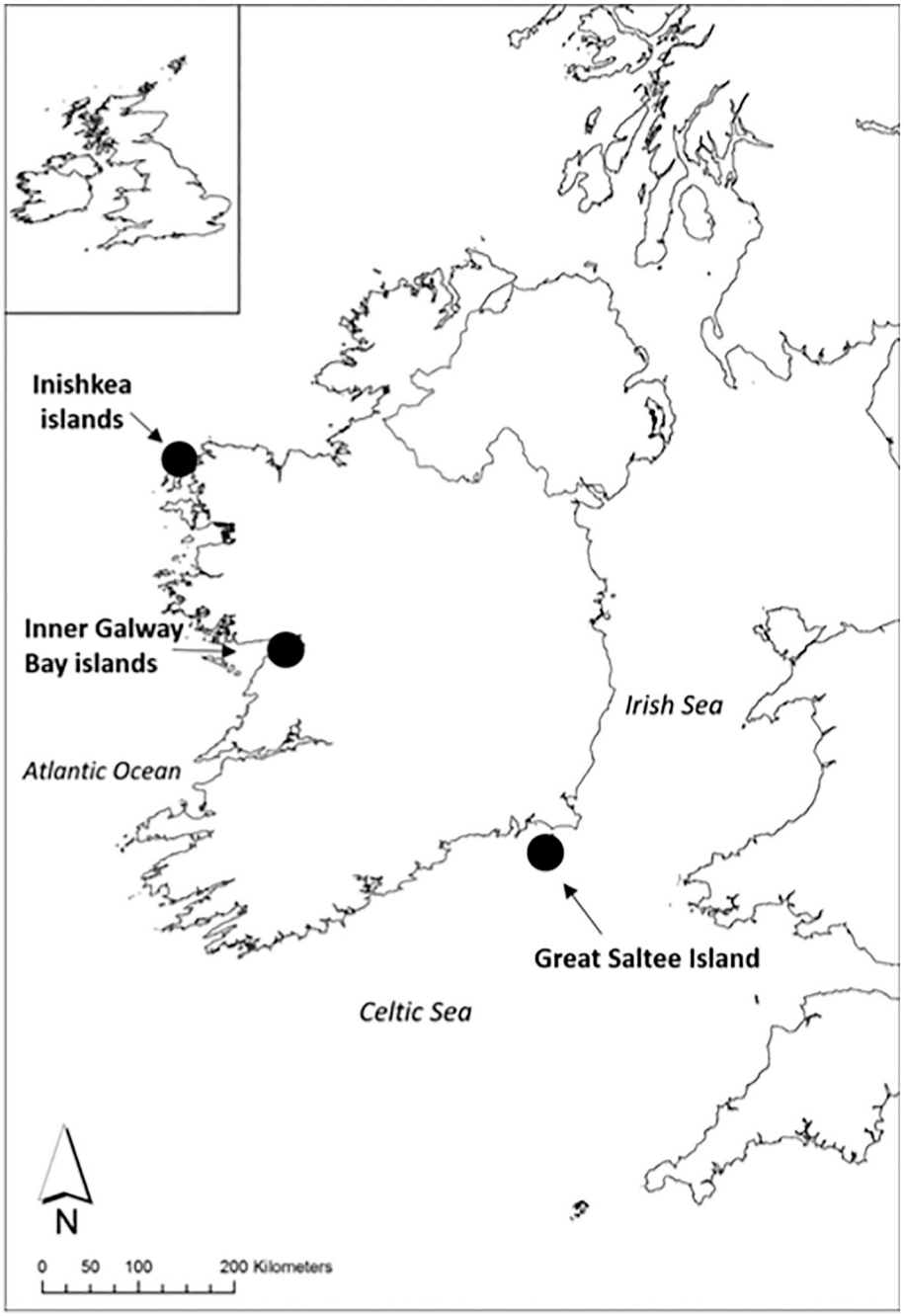

Fig. 1. Location of sampled seabird colonies.

larger project to identify toxic contaminants present in seabird eggs (see Power et al., 2021a–c). Samples were collected in accordance with the Oslo and Paris commissions (OSPAR) Joint Assessment and Monitoring Programme guidelines (JAMP; OSPAR, 2014). Only freshly laid eggs were collected in this study as the contaminant concentrations in the egg can increase as the embryo develops (Drouillard et al., 2003). To ensure that only fresh eggs were collected, eggs were placed in a small container of water (per OSPAR guidelines) to check if the eggs had been laid recently, as fresh eggs sink in water (JAMP; OSPAR, 2014). Each egg collected originated from a different breeding pair. Fresh eggs were wrapped carefully in aluminium foil, placed in a sealed bag and stored. Each individual egg was labelled and the time, date and exact location (GPS coordinates) were recorded. On return from sampling locations, eggs were immediately refrigerated on the day of sampling and frozen the following day at −20 °C.

Sample analysis was conducted in the Marine Institute laboratory (Co. Galway, Ireland), a state agency with a track record of successful participation in QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) proficiency exercises for the analysis of pollutants. Egg contents (yolk and albumen) were homogenised using an Ultra-Turrax® (IKA T25, Germany). Samples were placed into solvent washed (*n*-hexane) jars. Sample jars were then frozen at −20 °C until chemical analysis. Egg samples were extracted using the Smedes' lipid extraction technique (i.e. 'Total' Lipid) (Smedes and Askland, 1999). All lipid concentrations were determined gravimetrically. Column chromatography, using alumina and silica to perform clean-up, was completed prior to analysis to remove lipid. All samples were spiked with internal standards (100 mg of $^{C}13$ isotopically labelled internal standards for each individual PAH compound). An Agilent 7890B gas chromatograph (GC) coupled with a 5977 a mass

Table 1
PAH concentrations and stable isotope ratios of carbon and nitrogen (‰) in seabird eggs in Ireland (ng/g ww). ND = not detected.

	Guillemot (n = 2)		Gannet (n = 2)		Arctic Tern (n = 2)		Common Tern n = 6 Mean (range)
	Great Saltee Island		Great Saltee Island		Inishkea Islands		Galway Bay
δ^{13}C corrected	−16.7	−16.6	−17.8	−19.07	−19.1	−19.5	−19.9 (−18.3 to −23.9)
δ^{15}N	13.8	13.9	15.3	13.9	12.1	10.9	13.7 (12.8–14.7)
Acenaphthylene	0.09	0.07	0.04	0.03	0.09	0.09	0.06 (0.05–0.08)
Acenaphthene	0.02	0.01	ND	0.001	0.12	0.1	0.1 (0.09–0.13)
Fluorene	0.02	0.03	0.03	0.04	0.16	0.1	0.11 (0.1–0.12)
Phenanthrene	0.88	0.88	0.86	0.93	0.56	0.43	0.34 (0.3–0.38)
Anthracene	0.29	0.24	0.03	0.03	ND	0.04	0.01 (0–0.04)
Fluoranthene	0.23	0.21	0.27	0.15	0.18	0.14	0.11 (0.09–0.13)
Pyrene	0.18	0.15	0.15	0.09	0.26	0.26	0.14 (0.12–0.17)
Chrysene	0.05	0.03	0.08	0.03	0.06	0.14	0.1 (0–0.51)
Benzo(*a*)anthracene	0.09	0.05	0.1	0.05	ND	ND	ND
Benzo(*b*)fluoranthene	0.04	0.01	0.02	0.04	0.01	0.01	0.002 (0–0.004)
Benzo(*k*)fluoranthene	0.1	0.07	0.07	0.07	0.05	0.04	0.01 (0–0.04)
Benzo(*a*)pyrene	ND	ND	ND	ND	0.01	0.01	0.01 (0.005–0.03)
Dibenz(*ah*)anthracene	0.01	0.003	0.005	0.004	0.01	0.01	0.005 (0–0.008)
Indeno(1,2,3-C,D)pyrene	0.07	0.06	ND	0.001	ND	ND	0.0008 (0–0.005)
Benzo(*g,h,i*)perylene	0.01	0.01	0.003	0.003	0.01	0.01	0.0006 (0–0.004)
Σ15PAH	2.08	1.82	1.67	1.47	1.5	1.37	1.005 (0.8–1.4)

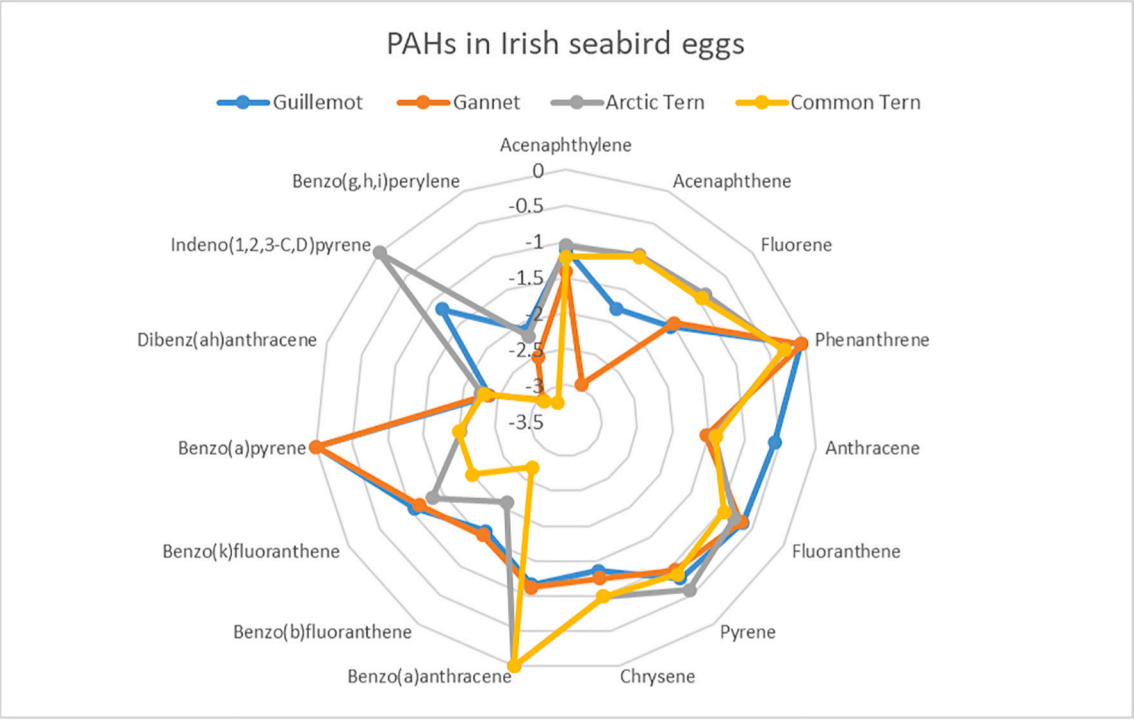

Fig. 2. Radar plot showing differing patterns of PAHs between seabird species in this study. Log transformed data was used in order to best capture the pattern across all contaminants.

selective detector (MSD) was used for PAH analysis. An Agilent J&W Select PAH (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness) GC capillary column was used. The oven temperature programme had an initial temperature of 100 °C increased to 320 °C at 23 °C/min and held for 10 min. Oven temperature programming was used to achieve resolution of analyte peaks. Single ion monitoring (SIM) mode was used for the quantification of analytes. The analysis of all calibration standards and samples in SIM mode allowed for increased specificity and sensitivity. The carrier gas used was helium at a constant flow rate of 1.3 mL/min. The GC–MS was operated under the positive electron ionization (EI) mode at 70 eV. A comprehensive analytical quality assurance programme underpinned the sampling and laboratory analyses. Blanks, a certified reference material (CRM) and a laboratory reference material (LRM) were included in each batch of samples as quality controls. A chicken *Gallus gallus* egg sample (FAPAS) was used as the CRM. Egg homogenate from Great Black-backed Gull *Larus marinus*, that was collected from another study, was used as an LRM as no suitable seabird egg reference materials are currently commercially available. Concentrations for PAHs are presented on a wet weight (ww) basis. Stable isotope ratio analysis of eggs was also used in this study to investigate the trophic position and foraging niche of adult female seabirds. δ^{13}C values can help determine carbon source, indicative of dietary niche, while δ^{15}N value increase with trophic level (Bond and Jones, 2009). Egg samples were homogenised and freeze dried. The isotopic

composition of organic carbon and nitrogen was then measured in all seabird egg samples by Iso-Analytical Limited (Crewe United Kingdom) using Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS). Variation in lipid content can confound interpretations of diet as lipids are depleted in $\delta^{13}C$ compared with protein (Elliott et al., 2014). $\delta^{13}C$ values were corrected using a lipid normalisation equation for aquatic bird eggs (Elliott et al., 2014). Further details on sample preparation and analysis can be found in Power et al., 2021a–c.

PAH concentrations between species in this study were broadly similar with only 2 ng separating the sample with the highest Σ15PAH concentration (Guillemot, 2.08 ng/g) from the sample with lowest concentration (Common Tern, 0.8 ng/g) (Table 1). The profile of PAHs differed somewhat between species (Fig. 2). The concentration of acenaphthene and fluorene was up to two orders of magnitude higher in terns compared to Guillemots and Gannets while anthracene was a couple of orders of magnitude higher in Guillemots compared to other species. Gannet and Guillemot eggs were sampled at the same time and location and patterns differed between species (Fig. 2). However, the sample size is very small and variability of pollutant concentrations in seabird species is high. Dietary differences of adult female seabirds between sites and species may explain contrasting patterns in PAH concentrations and patterns. Stable isotope ratio values in this study show that different species sampled in this study had different diets ($\delta^{13}C$) and fed at different trophic levels ($\delta^{15}N$) (Table 1). These isotopic niche differences, but with a larger sample size, have been demonstrated in previous studies (Power et al., 2021a–c). Additionally, contrasting PAH concentrations may be a result of PAHs being metabolised differently between species (Waszak et al., 2021).

Σ15PAH concentrations in Guillemot egg from this study (2.08, 1.82 ng/g ww) were higher than Σ16PAH levels detected in eggs of the closely related Brünnich's Guillemot *Uria lomvia* from pristine sites in Norway (mean of 0.4 and 0.3 ng/g ww) (Norwegian Polar Institute, 2010). Mean concentrations of phenanthrene, fluoranthene and pyrene detected in this study in Common Terns (0.34, 0.11 and 0.14 ng/g ww respectively) are lower than mean concentrations detected in Common Tern eggs from the Netherlands (4, 1 and 3 ng/g ww respectively) (Stronkhorst et al., 1993). PAH levels in Gannets eggs in this study were generally lower than levels detected in Gannet eggs from Bass Rock and Ailsa Craig in Scotland (Pereira et al., 2009). For example, Phenanthrene concentrations in Gannet eggs in this study (0.86 and 0.93 ng/g ww) were lower than levels detected in Ailsa Craig (mean 2.14, range ND – 6.392 ng/g ww). Phenanthrene accounted for the highest concentration of any PAH in all species in this study. PAH concentrations are within range, generally lower, than concentrations found in eggs of Herring Gulls *L. argentatus*, Cormorants *Phalacrocorax carbo* and Shag *P. aristotelis* in Britain (Shore et al., 1999). Shore et al. (1999) and Pereira et al. (2009) conclude that PAH levels detected in seabird eggs are unlikely to have embryotoxic effects. Similarly, Σ15PAH concentrations in this study were marginally higher than Σ16PAH reported in eggs from Common Eider *Somateria mollissima* (lower trophic level) and Shag but much lower than Herring Gull (7.63 ng/g ww) (Huber et al., 2015).

PAH levels are also considerably lower than concentrations associated with no adverse effect in the eggs of domestic avian species (Brunström et al., 1990, 1991). These preliminary findings suggest that while PAHs were detected in seabird eggs the concentrations are low, most likely an accumulation of background environmental levels, it is unlikely to cause embryotoxic effects. Therefore, income breeding seabird species in Ireland are at low risk to the negative impacts of PAHs. However, previous studies of persistent organic pollutants (POPs) in seabird eggs in Ireland have shown elevated concentrations in eggs from the Dublin coast, near Ireland's industrial capital city, compared to other coastal areas in the country (Power et al., 2021a–c; Wilson and Earley, 1986). No site from the Dublin coast was sampled in this study and it is likely that PAH concentrations are higher in seabird eggs from this region. Ireland has less of an industrial history than many countries in Europe and there have been no significant PAH contamination events close to seabird breeding areas in recent years which may explain the low concentrations detected in this study.

However, it has been shown that concentrations of PAHs can significantly increase in bird eggs, to potentially toxic levels, after a pollution event such as a forest fire or oil spill (Vidal et al., 2011). Previous seabird studies have highlighted the value of conducting baseline research on PAH concentrations, Provencher et al. (2020) determined background levels of PAHs in seabird tissues from the Atlantic/Arctic Oceans in areas where shipping activity is relatively low, which can then be used for future comparisons following expected increases in shipping and oil and gas activities in the region.

Baseline data of PAHs in a range of seabird species from this study may provide an important reference point should a major pollution event occur in Irish or European waters, such as an oil spill.

CRediT authorship contribution statement

Philip White: conceptualization, methodology, review & editing, supervision **Brendan McHugh:** conceptualization, methodology, review & editing, supervision **Simon Berrow:** conceptualization, review & editing, supervision **Stephen Newton:** conceptualization, review & editing, supervision **Evin McGovern:** conceptualization, review & editing, supervision **Sinéad Murphy:** conceptualization, review & editing, supervision **Denis Crowley:** validation, investigation **Ian O'Connor:** conceptualization, review & editing, supervision **Aaron McKeown:** investigation.

Declaration of competing interest

The authors 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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