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Legacy and emerging contaminants in common guillemot *Uria aalge* eggs in Ireland and Wales

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ABSTRACT

Guillemot eggs from multiple Irish colonies and one Welsh colony were analysed for legacy pollutants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and other organochlorine compounds (OCs), as well as metals. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were measured in eggs to understand the influence of diet on contaminant levels detected. Wide-scope target and suspect screening techniques were used on a single guillemot egg, providing novel information on contaminants of emerging concern. Stable isotope ratio analysis showed that guillemots from Great Saltee Island and Lambay Island (Ireland's east coast) had a similar carbon source ($\delta^{13}C$) and fed at similar trophic levels ($\delta^{15}N$), pollutant levels were higher in eggs from Lambay Island near Dublin, Ireland's industrialised capital city. Guillemot eggs from Aughris Head (Atlantic west coast of Ireland), and Skomer Island (Wales) had differing isotopic niches to other colonies. Egg samples from Aughris Head had the lowest levels of pollutants in this study (with the exception of mercury) and amongst the lowest levels reported worldwide. In contrast, Skomer Island had the highest level of pollutants with higher concentrations of Σ16PCB, Σ6PBDE and HCB than Irish colonies, most likely a result of its proximity to historically industrial areas. Levels of PCBs, *p,p'* –DDE and mercury in guillemot eggs have decreased over time according to this study, in concurrence with worldwide trends. Levels of pollutants in guillemot eggs, in this study, fall below existing thresholds for adverse effects in other species, with the exception of mercury.

1. Introduction

The common guillemot *Uria aalge* (also known as common murre) is a colonial nesting, piscivorous seabird (Zador et al., 2009). Common guillemots (hereafter guillemots) have a circumpolar distribution and are the most abundant seabird in Britain and Ireland (JNCC, 2020; Mitchell et al., 2004). They feed on micronekton prey items (mainly 6–10 cm in length) including fish such as sandeels *Ammodytes* spp., clupeids, and gadoids. (Ainley et al., 2020; Anderson et al., 2014; Swennen and Duiven, 1977). Guillemots may winter in the same region as the breeding colony but migration behaviors can differ between colonies (Ainley et al., 2020). Guillemots breed on cliffs, rocky offshore stacks and islands, and colonies are widespread along the coastline of Ireland and Britain (Balmer et al., 2013). Guillemots return to their respective breeding colonies approximately two months before the first eggs are laid (Harris et al., 2010), typically in the month of May (JNCC, 2021). The mean foraging range of a guillemot from the breeding colony is generally <40 km (Ainley et al., 2020). The egg formation period is 14–15 days (Birkhead and Del Nevo, 1987) and it has been shown that guillemots use recently derived nutrients to form eggs (income breeding) (Bond and Diamond, 2010). Therefore, it is thought that the contaminant burden in guillemot eggs mainly reflects that of recently consumed prey. 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). Legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and other organochlorine compounds (OCs), as well as metals, have been shown to

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exhibit a wide range of toxic properties in seabirds (AMAP, 2003; Gore et al., 2015; Scheuhammer, 1987; Walker et al., 2012; Walsh, 1990; WHO, 2012). Aside from the well-known historical residues of legacy pollutants, emerging contaminants in the marine environment are also of concern (Tornero and Hanke, 2017).

Guillemot diet can have significant spatial and temporal variation (Anderson et al., 2014). Therefore, diet and foraging behavior of guillemots are important considerations when making comparisons of contaminant burdens between different colonies and when looking at long-term trends in contaminants from a single colony. Stable isotope ratio analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of an egg can provide insight into maternal seabird diet (Hobson et al., 1994) during the egg formation period. $\delta^{13}C$ values can help determine carbon source, indicative of dietary niche, while $\delta^{15}N$ values increase with trophic level (Bond and Jones, 2009).

Guillemots feed at a relatively high trophic level and can be effective monitoring units for detecting geographic and temporal patterns of contaminants in marine food webs (Day et al., 2006). The International Council for the Exploration of the Sea (ICES) and OSPAR recommend guillemot eggs for the monitoring of contaminants (ICES, 2003, 1995; OSPAR, 2018, 2014) and numerous studies across Europe and North America have shown guillemot eggs to be a favorable accumulative bioindicator of persistent pollutants (Day et al., 2006; Jörundsdóttir et al., 2009). A long-term monitoring scheme using guillemot eggs has been in place in the Baltic Sea since 1969 helping Sweden fulfill requirements of the European Union's (EU) Marine Strategy Framework Directive (MSFD), the ultimate goal of which is to help member states achieve good environmental status (GES) (Holmström et al., 2005; Miller et al., 2014). To the authors' knowledge there are no recent studies of pollutants in guillemot eggs from either Ireland or Wales, though some historical studies have been undertaken (Moore and Tatton, 1965; Parslow, 1973). Data on contaminants in seabird eggs in Ireland are scant and this research is part of a larger investigation to determine the feasibility of using seabird eggs as a higher trophic level indicator of pollutants in Ireland. The primary aim of this research was to provide contemporary baseline data on the levels of persistent pollutants from multiple guillemot colonies in Ireland, and one from Wales. Bulk stable isotope ratio analysis was used to examine possible differences in diet between colonies. The potential impact of legacy pollutants, as well as emerging pollutants, on guillemot eggs was also examined.

2. Methods

2.1. Sampling locations

Lambay Island is a 250 ha island located in the Irish Sea, off the north coast of County Dublin, Ireland (Fig S1) (Newton et al., 2015). Dublin city is Ireland's capital and largest city, and has an industrial history (Glennon et al., 2014). Great Saltee Island is an 89 ha island located in the St. George's Channel, off the southeast coast of County Wexford, Ireland (Fig S1) (Newton et al., 2015), approximately 158 km south of Lambay Island. The St. George's Channel separates the Irish and Celtic Seas and is located off the southeast coast of Ireland and the southwest coast of Britain. Skomer Island in Wales is a 292 ha island which is also located in the St. George's channel approximately 98 km southeast from Great Saltee Island. Aughris Head is located on the Atlantic west coast of Ireland, in Sligo Bay.

2.2. Sample collection

Samples from Ireland were collected under licence from the Irish National Parks and Wildlife Service, and in accordance with OSPAR Joint Assessment and Monitoring Programme guidelines (OSPAR, 2014). Samples from Skomer Island were collected under licence from Natural Resources Wales. Only freshly laid eggs were collected in this study as the contaminant concentrations in an egg can increase as the embryo develops (Drouillard et al., 2003). Where possible, eggs were collected early in the breeding season as replacement eggs can have different pollutant burdens to first laid eggs (Bignert et al., 1995). On the 6^{th} and 7^{th} of May 2017, 20 guillemot eggs were collected from Great Saltee Island. On the 13^{th} of May, 20 guillemot eggs were collected from Lambay Island. Twenty guillemot eggs were collected from Skomer Island by the University of Sheffield on the 15^{th} and 17^{th} of May 2018. Sample collection took place later in the guillemot breeding season, 2^{nd} of June 2018, on the Aughris head site due to difficulties in accessing guillemot colonies. As a result, it is likely that replacement eggs were collected (n = 20). Replacement eggs are laid if the original egg is lost due to predation, bad weather or other circumstances. Further details on sample collection and analytical methods used can be found in the supplementary materials.

2.3. Sample preparation

All eggs were later thawed so the contents of each egg could be homogenised and subsampled according to analysis type. Egg contents (yolk and albumen) were homogenised using an Ultra-Turrax® (IKA T25, Germany). Samples were divided into four sub-samples and placed in two acid washed containers for metals and mercury analysis, respectively, and two solvent washed (*n*-hexane) jars for the analysis of POPs and stable isotope ratio analysis. Sample jars were then frozen at −20 °C until chemical analysis. Sub-samples for metals and stable isotope ratio analysis were freeze dried (Labconco: Freeze Dryer – Model Freezone & Bulk Tray Drier (12L), USA).

2.4. Contaminant analysis

2.4.1. Quality assurance

A comprehensive analytical quality assurance programme underpinned the sampling and laboratory analyses. Reagent blanks, a certified reference material (CRM) and a laboratory reference material (LRM) were included in each batch of samples as quality controls. Egg homogenate from great black-backed gull *Larus marinus* (collected from another study) was used as an LRM as no suitable seabird egg reference materials are currently commercially available. Fish tissue (NIST, 1947; Lake Michigan Fish Tissue) was used as CRM for analysis of POPs and bivalve tissue (Freeze-Dried, SRM 2976) for metals and mercury analysis. Further details on quality assurance can be found in the supplementary materials.

2.4.2. PCBs, PBDEs and OCs analysis

Eggs were analysed for a suite of 16 PCB congeners (18, 28, 31, 52, 44, 101, 105, 149, 118, 105, 138, 153, 156, 170, 180, 209). The Σ7PCB, also known as the ICES-7, refers to the sum of seven individual PCB congeners (28, 52, 101, 118, 138, 153, 180) that are widely used as indicators of PCB contamination in the marine environment. Eggs were analysed for 6 PBDE congeners (28, 47, 99, 100, 153, 154) and 17 OCs (Hexachlorobutadiene (HCBD), hexachlorobenzene (HCB), α-HCH, γ –HCH, β-HCH, heptachlor, heptachlor epoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane, transnonachlor, *o,p*' –DDE, *p,p*' –DDE, *o,p*' –DDT, *o'p*' –DDD, *p,p*' –DDD, *p,p*' -DDT). ΣHCH refers to the sum of α-HCH, γ –HCH and β-HCH. ΣCHL refers to the sum of heptachlor, heptachlor epoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane and transnonachlor. ΣDDT refers to the sum of *o,p*' –DDE, *p,p*' –DDE, *o,p*' –DDT, *o'p*' –DDD, *p,p*' –DDD and *p,p*' -DDT. Egg samples were extracted using the Smedes' lipid extraction technique (Smedes and Askland, 1999). All lipid concentrations were determined gravimetrically. Column chromatography, using alumina and silica, was completed prior to analysis to remove lipid. All samples were spiked with internal standards (100 mg of a stock solution of ^{13}C isotopically labelled internal standards for PCBs, PBDEs and OCs). An Agilent 6890 gas chromatograph (GC) coupled to a 5973 N mass spectrometric detector (MSD) with a 30 m

DB5-MS column was used for these analyses.

2.4.3. Metal analysis

All samples were analysed for 14 metals (arsenic, cadmium, chromium, copper, lead, nickel, silver, zinc, aluminium, cobalt, iron, manganese, selenium and vanadium). Concentrated nitric acid (4 ml) and hydrogen peroxide (4 ml) were added to approximately 0.2g freeze-dried egg homogenate, which was then digested in a laboratory microwave oven (CEM Mars Xpress). After cooling, samples were diluted to 50 ml with deionised water. Metal concentrations were determined by ICP-MS (Agilent 7700x with High Matrix Introduction (HMI) system).

2.4.4. Mercury analysis

Concentrated nitric acid (4 ml) was added to 0.6–0.8 g of egg homogenate, which was then digested in a laboratory microwave oven (CEM Mars Xpress). After cooling, potassium permanganate was added until the purple colour of the solution stabilised. Sufficient hydroxylamine sulphate/sodium chloride solution was added to neutralise the excess potassium permanganate and potassium dichromate was added as a preservative. The solution was diluted to 100 ml with deionised water. Following reduction of the samples with tin (II) chloride, mercury concentrations were determined by Cold Vapour Atomic Fluorescence Spectroscopy (CV-AFS) using a PSA Millennium Merlin Analyser.

2.4.5. Contaminants of emerging concern - wide-scope target and suspect screening

In collaboration with NORMAN (Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances) one freeze dried egg-sample from Aughris Head, Sligo was screened for several thousand organic pollutants and their transformation products by wide-scope target and suspect screening techniques. Only one sample was analysed due to preliminary nature and expense of the emergent contaminant analysis..

An accelerated solvent extraction (ASE) and solid phase extraction (SPE) was undertaken prior to analysis. Analysis was conducted in the Environmental Institute (EI) in the Slovak Republic. Target screening for approximately 2400 substances was carried using liquid chromatography-high-resolution mass spectrometry (LC-HR-MS), liquid chromatography with tandem mass spectrometry (LC-MS-MS) and gas chromatography atmospheric pressure chemical ionisation high resolution mass spectrometry (GC-APCI-HR-MS).

In addition, suspect screening of >65,000 compounds in the sample including semi-quantification was carried out using LC-HR-MS and GC-APCI-HR-MS. In the absence of toxic thresholds for birds, concentration data were referenced against predicted no-effect concentration (PNEC) from the NORMAN Database (NORMAN, 2021) and Environmental Quality Standards (EQS) values for fish (EQS Directive, 2013/39/EU) for completion of an assessment of potential risk posed by the measured contaminant levels.

2.4.6. Stable isotope ratio analysis

Bulk stable isotope ratio analysis of eggs was used to investigate the indicative trophic position and foraging niche of adult female guillemots (Fig. 1). Egg samples were homogenised and freeze-dried and the isotopic composition of organic carbon and nitrogen was then measured in 80 guillemot 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 δ^{13}C compared with protein (Elliott et al., 2014). δ^{13}C values were corrected using a lipid normalisation equation for aquatic bird eggs (Elliott et al., 2014).

2.4.7. Statistical analysis

Concentrations of POPs are presented on a wet weight (ww) and lipid weight (lw) basis in ng/g. Metals are presented in mg/kg ww and dry weight (dw). Concentrations below the limit of detection (LoD) were assigned a value of half the LoD (Pereira et al., 2009). Statistical analysis was performed using Graphpad Prism 9. Shapiro-Wilk tests were used to assess normality, due to small sample sizes. Differences between four colonies were assessed using ordinary one-way ANOVA with Tukey's multiple comparison post-hoc test for parametric data, and Kruskal Wallis test with Dunn's multiple comparison post-hoc test for nonparametric data. Direct comparisons between eggs from Great Saltee Island and Lambay Island were tested using Mann-Whitney *U* test for non-parametric data and unpaired *t*-test for parametric data.

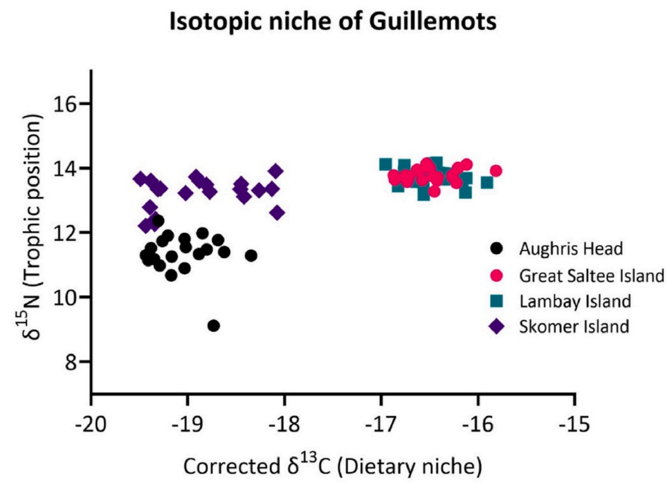

Fig. 1. Nitrogen (δ^{15}N) and carbon (δ^{13}C) isotopic niches, (δ^{13}C, corrected for lipid using method from (Elliott et al., 2014) of guillemot eggs from Aughris Head (n = 20), Great Saltee Island (n = 20), Lambay Island (n = 20) and Skomer Island (n = 20).

3. Results and discussion

3.1. Site differences and stable isotope ratio analysis

Eggs were collected from four colonies in this study; care must be taken when interpreting differences in contaminant burdens in a higher trophic level predator between multiple sites, especially without inter-annual data. For example, differing feeding strategies, diet, condition and age of adult bird may all impact the concentration of pollutants found in a guillemot egg. Stable isotope ratio analysis showed no significant difference between eggs from Great Saltee Island and Lambay Island (Fig. 1), off the east coast of Ireland, indicating that adult female guillemots from both sites have similar dietary composition (δ^{13}C) and based on bulk nitrogen isotope ratios they fed at similar trophic levels (δ^{15}N). Significant dietary differences were detected between Great Saltee Island and Lambay Island with Aughris Head, west coast, and Skomer Island, Wales. Guillemots from Great Saltee Island and Lambay Island were both sampled in 2017, only one week apart, and given the similar carbon and nitrogen isotopic niches of both groups they can be most easily compared.

3.2. Great Saltee Island and Lambay Island

Fluvial transport of contaminants into the Irish Sea and the presence of large cities with industrial history, such as Dublin and Liverpool, are likely to be significant contributors to contamination on the east coast of Ireland, where Great Saltee and Lambay Islands are located. Inputs of pollutants into the Irish Sea from the Bristol Channel combined with the generally northward flow of the Irish Sea (Dabrowski et al., 2010) may also contribute to pollutants detected in the region. Significantly higher concentrations of Σ16PCB, Σ6PBDE (lw) were found in guillemot eggs from Lambay Island compared to Great Saltee Island (Fig S2). Dublin generally has higher levels of pollution than other parts of Ireland due to its industrial history. Pollutant levels in the eggs of two piscivorous bird species from coastal colonies, cormorant *Phalacrocorax carbo* and shag *P. aristotelis* eggs, were elevated in eggs from Dublin Bay compared to other parts of Ireland, including Great Saltee Island (Wilson and Earley, 1986). Within the current study, there was no difference determined in levels of HCB*,* ΣHCH ΣCHL and ΣDDT and total mercury between the two sites. A study comparing pollutant levels in gannet *Morus bassanus* eggs between the same two sites (Great Saltee and Lambay Island) from the same year (2017) followed a similar pattern to this study with higher levels of Σ16PCB and Σ6PBDE in eggs from Lambay island and similar levels OC compounds between the two sites (Power et al., 2021b).

3.3. Aughris Head

Guillemots typically lay their eggs in May (JNCC, 2021), and egg-laying is highly synchronous (Hatchwell, 1991). Guillemots are single-brooded and will lay a replacement egg in the event the first-laid egg is lost, a replacement egg is laid approximately 15 days after the loss; Hedgren, 1980). Eggs from Aughris Head were collected on the 2nd of June 2018 and are likely to be replacement eggs. Bignert et al. (1995) showed that replacement eggs had significantly higher levels of DDT and PCBs than first-laid eggs. Guillemots can regulate their reproductive effort by varying egg size or the amount of resources allocated to the egg (Birkhead and Harris, 1985), and guillemots may use more stored reserves from body fat to form the replacement egg as significant energy has already been expended forming the first-laid egg (Bignert and Helander, 2015). However, despite being replacement eggs, guillemot eggs from Aughris Head in this study had significantly lower levels of Σ16PCBs, Σ6PBDE, HCB and ΣDDT than all other sites. This gives a strong indication of the contrasting levels of pollution in the Irish Sea and St. George's Channel compared to Aughris Head, a relatively pristine site on the Irish Atlantic coast. In contrast, levels of mercury in guillemot eggs were significantly higher in the Aughris head eggs compared to all other sites (Fig S3). A study of pollutants in common terns *Sterna hirundo* and arctic terns *S. paradisaea* in Ireland showed significantly higher levels of POPs (ΣPCBs, ΣPBDE) between Dublin Bay and west coast colonies but similar levels of mercury (Power et al., 2021a). It is possible that first-laid eggs from Aughris Head have similar levels of mercury to first-laid eggs from Great Saltee, Lambay and Skomer Island, like the aforementioned terns. However, several studies show that mercury levels decrease with laying order (Ackerman et al., 2016; Ou et al., 2015) and it has been shown that mercury levels in the closely related Brünnich's guillemot *Uria lomvia* are decreased in replacement eggs, while levels of PCBs and OCs are increased (Braune et al., 2018).

Mercury and lipophilic organic pollutants are transferred into the egg in different ways which may explain contrasting patterns. Mercury binds with proteins primarily found in albumen while lipophilic POPs are found primarily in the yolk (Blundell and Jenkins, 1977; Drouillard et al., 2003). Braune et al. (2018) suggest that mercury concentrations in first-laid eggs of Brünnich's guillemot are derived from a mixture of stored reserves and recently derived nutrients while the albumen in replacement eggs is formed entirely from local nutrients. They suggest that reduced mercury levels were detected in replacement eggs in their study as mercury pollution close to breeding grounds was lower than their wintering grounds. Therefore, elevated concentrations in eggs from Aughris Head may be a result of other factors such as dietary niche or colony location. Stable isotope ratio analysis shows that guillemot eggs from Aughris head had the lowest bulk $\delta^{15}N$ of all groups indicating they may be feeding on lower trophic level prey (Fig. 1). A study of stable isotopes in feathers from common terns suggested that high exposure to mercury resulted from feeding on prey at relatively low trophic levels (Nisbet et al., 2002). Mercury concentrations also tend to be higher in areas with elevated suspended particulate matter in high-flow conditions as mercury has a high affinity for particles (Lawson et al., 2001). Aughris Head is the only site in this study that is not an offshore island and is a headland in outer Sligo Bay. Sligo Town is situated in the inner bay where the Garravogue river enters the sea. Further research is needed to determine the cause of elevated mercury concentrations in eggs from Aughris Head. Mercury aside, levels of persistent pollutants in Sligo Bay appear to be lower than other sites in this study which is most likely as a result of its location in area with low industrial activity compared to the Irish Sea and St. George's Channel.

3.4. Skomer Island

Guillemots from Skomer Island appear to be feeding on prey items of a less pelagic nature than birds on Great Saltee Island and Lambay Island but at a similar trophic level. The isotopic niche is similar to that of birds from Aughris Head but generally at a higher trophic level with a small degree of overlap (Fig. 1). Contaminant levels from Skomer Island were amongst the highest recorded in this study with significantly higher levels of Σ16PCB (ww and lw), Σ6PBDE (ww and lw), mercury (excluding Aughris Head) and HCB (lw) than all other sites (Fig. 2). Historical studies of pollutants in seabird eggs indicates lower levels in Irish colonies compared to Britain (Wilson and Earley, 1986). However, information on pollutants in seabird eggs on the west coast of Britain is scant compared to other areas of the British coastline, such as the North Sea. Although Dublin has an industrial history (Glennon et al., 2014) it is not to the same scale and extent as the west coast of Britain. Fluvial transport of pollutants from the Severn Estuary into the Bristol Channel, where Skomer Island is located, is likely to be a significant contributor to the elevated pollutant levels detected in guillemot eggs from the island. The Severn Estuary is the confluence of several rivers, such as the River Severn, the River Wye, the River Usk and the River Avon. Smelters, incinerators, chemical plants (including PCB manufacture), coal and steel industry, paper mills, chemical and pharmaceutical manufacturers, sewage from large towns and cities (Bristol, Gloucester, Newport and Cardiff), agricultural runoff all contribute to the pollutant load in the estuary (Langston et al., 2010).

3.5. Comparisons with other studies and thresholds

Using PCB-153 as a marker for total ΣPCB, mean levels in this study for all sites were lower than levels detected in guillemot eggs from the Baltic Sea in 2003 (mean PCB-153: 2500 ng/g lw) (Jörundsdóttir et al., 2009). PCB-153 levels from all sites in this study were higher than other colonies in Europe outside of the Baltic Sea, including comparatively pristine areas, such as Faroe Islands, Iceland and Norway (Jörundsdóttir et al., 2009). Mean PCB-153 concentrations in Aughris Head (mean: 593 ng/g lw), the less polluted site in this study, were close to upper limits of range in guillemot eggs from Vestmannaeyjar, Iceland (mean: 370, range: 180–750 ng/g lw) and Hjelmsøya, Norway (mean: 360, range: 210–680 ng/g lw) (Jörundsdóttir et al., 2009). Similarly, PBDE levels in this study for all sites were much lower than concentrations detected in guillemot eggs from the Baltic Sea (Jörundsdóttir et al., 2009). Mean BDE-47 concentrations from Skomer Island, the highest reported in this study (27 ng/g lw), were lower than concentrations reported from the Baltic Sea in Sweden (mean BDE-47: 120 ng/g lw) and similar to concentrations in less polluted colonies outside of the Baltic Sea (Jörundsdóttir et al., 2009). HCB levels in this study (Table 1) were generally lower than levels detected in Europe (Jörundsdóttir et al., 2009) and very similar to levels detected in guillemot eggs in Alaska from 2010 (39.6 ng/g ww) (Kalia et al., 2021). Similar to PCBs and PBDEs, HCH levels in this study were much lower than in the Baltic Sea and similar to other European sites and Alaskan colonies (Jörundsdóttir, 2009; Kalia et al., 2021), with eggs from Aughris Head amongst the lowest levels in Europe. Concentrations of chlordanes, such as heptachlor-epoxide, and oxychlordane in guillemot eggs from Aughris Head (mean: 1.9, 4.4 ng/g ww respectively, the lowest in this study), were very similar to concentrations from Alaskan colonies (mean 1.8, 4.4 ng/g ww respectively) (Kalia et al., 2021). *p,p'* –DDE levels in

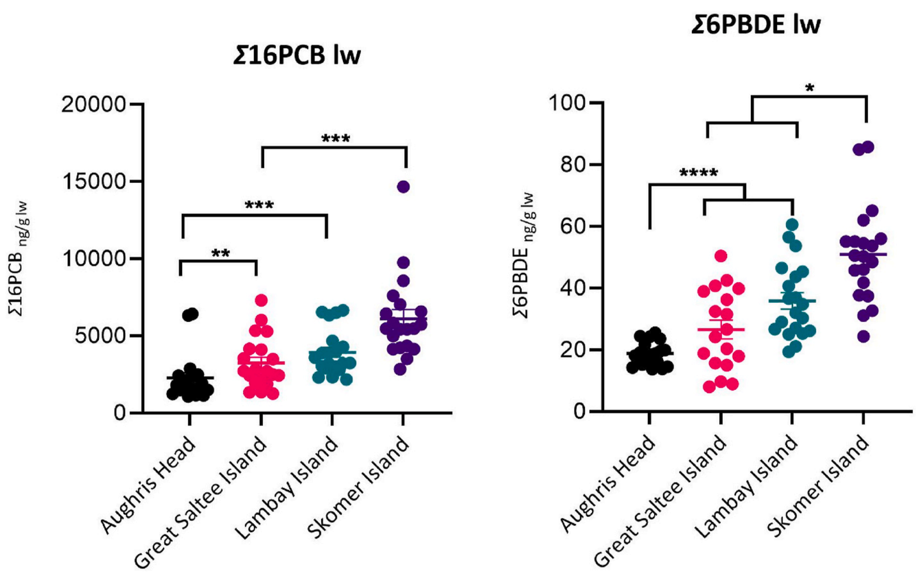

Fig. 2. Higher Σ16PCB and Σ6PBDE concentrations (ng/g lw) were observed in guillemot eggs in Skomer Island (n = 20) compared to Great Saltee Island (n = 20), Lambay Island (n = 20) and Aughris Head (n = 20). Statistical significance was tested by a Kruskal Wallis test (Σ16PCB, non parametric data) with Dunn's multiple comparison post-hoc test and an ordinary one-way ANOVA with Tukey's multiple comparison test (Σ6PBDE parametric data). Error bars = SEM and ns = not significant i.e. $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$ *** = $P \leq 0.001$. Comparisons with other studies and thresholds.

Table 1
Mean, standard error of the mean (SEM), range of % lipid, stable isotope ratios of carbon and nitrogen (‰), selected POPs (ng/g) and mercury (mg/kg) for guillemot eggs from Great Saltee Island (n = 20), Lambay Island (n = 20), Aughris Head (n = 20) and Skomer Island (n = 20).

Stable Isotopes	Great Saltee Island (n = 20)		Lambay Island (n = 20)		Aughris Head (n = 20)		Skomer Island (n = 20)	
	Mean (SEM) range		Mean (SEM) range		Mean (SEM) range		Mean (SEM) range	
% lipid	18.9 (1.18)		14.8 (0.61)		17 (0.57)		14.1 (0.65)	
	13.5–32		11.7–22.5		12.9–22.6		6.4–19.3	
$\delta^{15}N$	13.8 (0.05)		13.7 (0.06)		11.3 (0.14)		13.2 (0.11)	
	13.3–14.1		13.2–14.2		9.1–12.4		12.2–13.9	
$\delta^{13}C$ corrected	−16.5 (0.06)		−16.4 (0.06)		−19 (0.07)		−18.9 (0.11)	
	−16.9 to −15.8		−17 to −15.9		−19.4 to −18.3		−19.5 to −18.1	
POPs	ww	lw	ww	lw	ww	lw	ww	lw
PCB-153	150 (14.8)	833 (87)	159 (10.9)	1107 (95.3)	124 (20.7)	729 (108)	257 (44.5)	1787 (235)
	63.1–293	334–1693	87.5–265	557–2055	51.9–480	289–2208	98.7–1023	629–5473
Σ16PCB	535 (48.1)	3000 (300)	561 (34.1)	3930 (321)	385 (60.2)	2273 (325)	862 (109)	6115 (572)
	257–1029	1262–6032	331–859.2	2174–6657	181–1397	1081–6417	447–2743	2848–14,677
Σ7PCB	419 (37.3)	2340 (228)	439 (29.3)	3070 (264)	314 (50.4)	1851 (272)	671 (95.9)	4742 (510)
	192–766	1016–4491	246–693	1718–5372	145–1167	874–5360	316–2329	2012–12,464
BDE-47	3.4 (0.4)	17.9 (2.5)	2.9 (0.4)	19.5 (2.5)	1.8 (0.09)	10.6 (0.68)	3.8 (0.28)	27 (1.71)
	0.7–16.3	5–62	0.8–7.4	6–45	1.2–2.8	7–17	1.5–7.1	14–40
Σ6PBDE	6.3 (0.5)	33.6 (2.61)	5.4 (0.5)	35.9 (2.61)	3.2 (0.12)	18.9 (0.84)	7.1 (0.57)	50.9 (3.43)
	1.3–21.9	8–111	2.7–11.1	19–61	2.3–4.4	14–26	3.5–15.1	24–86
HCBD	0.49 (0.15)	2.67 (0.82)	0.4 (0.07)	3 (0.55)	0.6 (0.05)	3.4 (0.31)	1.3 (0.11)	9.8 (0.72)
	0 - 2.1	0–13	0 - 1.0	0–8	0.1–1	1–6	0.9–3.1	6–18
HCB	33.2 (1.02)	183 (7.03)	27.1 (0.97)	188 (8.57)	24.1 (1.41)	143 (7.94)	31.6 (2.55)	232 (9.7)
	25.6–62	160–336	19.9–34	105–278	14.3–38	87–217	20.3–68.7	115–517
ΣHCH	2.86 (0.19)	15.5 (0.85)	3.8 (0.24)	26.4 (2.29)	1.8 (0.1)	10.8 (0.65)	4.1 (0.22)	30.3 (2.15)
	1.7–4.8	10–24	1.8–6.6	14–54	1.1–2.7	6–16	2.5–6.5	16–59
ΣCHL	37.8 (9.87)	176 (44.7)	13.1 (0.74)	90.5 (6.09)	6.9 (0.72)	39.5 (4.14)	20.1 (1.97)	146 (15.2)
	4.2–145	24–770	9.2–17.4	55–114	2.9–11.8	19–69	7.4–36.8	63–277
p,p' -DDE	166 (7.9)	915 (49.7)	143 (8.36)	993 (67.3)	71.6 (5.81)	423 (34.7)	122 (8.39)	894 (64.4)
	107–329	722–1928	93–234	519–1815	30.2–123	201–760	69.4–216	456–1627
ΣDDT	168 (8.01)	923 (49.6)	145 (8.33)	1003 (67.1)	73.4 (5.84)	433 (34.8)	126 (8.3)	925 (64.6)
	108–332	729–1936	96–236	533–1819	31.8–126	211–772	74.1–219	487–1649
Metals	ww	dw	ww	dw	ww	dw	ww	dw
Hg	(0.01)	1 (0.06)	0.3 (0.02)	1 (0.06)	0.59 (0.04)	2.03 (0.18)	0.38 (0.01)	1.32 (0.06)
	0–0.4	0–1	0.1–0.4	0–2	0.3–1	1–4	0.3–0.5	1–2

Skomer Island (the highest in this study) were between 10 and 20 times lower than concentrations in guillemot eggs from the Baltic Sea (Bignert et al., 2008; Jörundsdóttir et al, 2006, 2009). *p,p'* –DDE levels in guillemot eggs from Aughris Head were lower than values reported from pristine European colonies outside of the Baltic Sea (Jörundsdóttir et al., 2009).

Total mercury levels in this study (Table 1) were marginally higher, within range of other studies in Europe (including the Baltic Sea) and North America (Day et al., 2006). In general, all sites in this study had much lower levels of pollutants in eggs than those recorded in the Baltic Sea. Pollutant levels from Irish sea colonies were higher than relatively pristine colonies across Europe and North America while concentrations in guillemot eggs from Aughris Head (excluding mercury) were amongst the lowest levels reported worldwide, despite being replacement eggs. To the author's knowledge there are no ecotoxicological thresholds specific to guillemot eggs. Levels of persistent pollutants (PCBs, PBDEs, HCB, heptachlor epoxide, DDT, selenium) in guillemot eggs fall below existing ecotoxicological thresholds for adverse effects in other bird species, summarized in Power et al. (2021) and Viñas et al. (2020) with the exception of mercury in eggs from Aughris Head. Shore et al. (2011) suggest that reproductive success may be impaired in most bird species with mercury concentrations of ≥0.6 mg/kg in the egg, a threshold exceeded by 10 out of 20 eggs from Aughris Head in this study. This may be an indication that replacement eggs could potentially be more vulnerable to the harmful impact of pollutants.

3.6. Historical declines of legacy pollutants

There is no contemporary information on pollutant levels in guillemots from either Ireland or Wales, historical baseline data are summarized in Table S6. Concentrations of *p,p'* -DDE in guillemot eggs on Great Saltee Island from 1964 ranged from 1300 to 2100 ng/g ww which is higher than levels detected in the current study (mean: 166, range: 107–329 ng/g ww). Similarly, in Skomer Island, DDE levels have fallen from 10,680 ng/g lw in 1971/1972 to 915 ng/lw (mean *p,p'* -DDE) and ΣPCB levels from 56,000 to 5474 ng/g lw (mean). Similarly, mercury levels in guillemot eggs from Skomer Island from 1972 were much higher than contemporary levels reported in this study (4.61 and 1.33 mg/kg dw respectively). Lower present-day levels of legacy pollutants are a reflection of bans and restrictions placed on certain contaminants in the intervening years. Due to their high toxicity and their persistent and bioaccumulative (PBT) properties, the production and use of many POPs have been phased out through various worldwide legislative instruments such as the Stockholm Convention on POPs, a legally binding international environmental treaty, that aims to ban, eliminate or restrict the production and use of POPs. This has been generally successful, leading to downward trends of legacy pollutants in European marine environments (OSPAR, 2010).

3.7. Contaminants of emerging concern

Eleven contaminants in total were quantifiable from the wide-scope target screening analysis for the selected sample from Aughris Head (Table 2). Twelve compounds were tentatively identified and semi-quantified in suspect screening analysis (Table 2).

MSFD reporting by Member States tends to focus on legacy and already regulated chemicals such as metals, PCBs and DDT (Grandjean et al., 2011; Sobek et al., 2016), often with little consideration of the myriad range of other pollutants that can be present in the aquatic environment (Tornero et al., 2019). It is recommended that emerging contaminants should be an integral part of contaminant monitoring systems (Zampoukas et al., 2014).

Per/polyfluoroalkyl substances (PFAS), widely used as surfactants and repellents, are perhaps the most well represented CECs in seabird egg studies (Glüge et al., 2020). Guillemots have been shown to accumulate high concentrations of (PFAS) compounds such as Perfluorooctanesulfonic acid (PFOS) (Holmström et al., 2005; Houde et al., 2006). The estimated concentration of PFOS detected in the single egg from Aughris Head (28.9 ng/g ww) (Table 2) is similar, a little higher, to levels detected in a study of guillemots in 2002 from other countries such as Iceland (mean: 16 ng/g ww) and the Faroe Islands (mean: 15 ng/g ww) but lower than concentrations detected in the Norwegian Sea from Norway (85 ng/g ww) and the Baltic Sea from Sweden (400 ng/g ww) (Löfstrand et al., 2008). A PNEC of approximately 1000 ng/g ww for PFOS in the eggs of avian top predators (Newsted et al., 2005) is much higher that the concentration detected in this study. Similarly, Polycyclic aromatic hydrocarbons (PAHs) are also reasonably well studied in seabird eggs. PAHs can originate from both natural sources such as forest fires as well as anthropogenic sources such as the burning of fossil fuels and oil spills (WHO, 1998). The concentration of naphthalene in the egg from Aughris Head is higher than levels detected in Brünnich's guillemot from Norway and herring gull *L. argentatus* and cormorant from Britain (Norwegian Polar Institute, 2010; Shore et al., 1999). Higher levels of naphthalene were detected in shag eggs from Britain (mean 183 ng/g lw), the authors conclude that PAH levels detected in shag eggs are unlikely to have embryotoxic effects (Shore et al., 1999).

Table 2
Selected results from wide-scope target screening analysis and semi-quantitative results of suspect screening analysis in ng/g ww. PNEC as per NORMAN database. * = EQS.

Wide-scope target screening analysis Compound	Compound use	PNEC	Concentration ng/g ww
3-Hydroxycotinine	Metabolite of nicotine	41.2	10.7
2, 4- Dinitrophenol- (DNP)	Used in the manufacture of dyes and wood preservatives and as a pesticide	43	2.2
Perfluorohexanoix acid (PFHxA)	Perfluorinated alkyl surfactants (PFAS) are highly persistent compounds that are widely utilised as surfactants and repellents	50.6	4.4
Perfluorooctanesulfonic acid (PFOS)		**9.1***	**29**
4-Formyl-antipyrine	Pharmaceutical	51	6.5
Antipyrine- 4-Acetamido	Metabolite of the widely used analgesic metamizole	16,385	41.9
Benzophenone-4	Ingredient in some sunscreens	15.3	4.6
Methylparaben	Anti-fungal agent used in cosmetics and personal-care products	199	16.1
Salicylic acid	Active metabolite of aspirin and widely used in skin-care products	64,113	6.88
Dinoterb	Herbicide and a rodenticide	54.9	1.98
Naphthalene	Polycyclic aromatic hydrocarbon (PAH), derived from coal tar	377	12.1
Semi-quantitative results of suspect screening analysis Compound	**Classification**		
8-Hydroxychinolin	Industrial pollutants	21	3.2
Nonanedioic acid		4561	14
Erucamide		0.74	17
Dipropyl phthalate		270	0.8
Pentaethylene glycol		2272	288
3-*tert*-Butyladipic acid		212	85
-β,21-dihydroxypregn-5-en-20-one 21-acetate		220	40
Hexanedioic acid		608	21
PEMA (2-Phenyl-2-ethylmalonamid)	Pharmaceuticals	16	134
Alprostadil		74	25
4-Acetamidosalicylic acid		46	0.46
Norethandrolone	Androgen and anabolic steroid	113	12

In respect of lesser monitored compounds suspect screening for approximately 65,0000 organic compounds detected, erucamide, which is commonly used in the plastic manufacturing industry, exceeded the PNEC from the NORMAN Database (NORMAN, 2021). However, unlike legacy pollutants there are few thresholds for emerging contaminants in birds and information on the toxicity of erucamide is scant, and it is not currently considered a threat to biota (Hazardous Substances Data Bank, 2021). Dinitrophenol-2-4- (DNP) is known to have a range of uses ranging from photographic applications though to use as a fertiliser and in dyes and explosives, additionally it is illegally used as a diet pill; captive birds treated with DNP having been shown to have reduced lifespan (Stier et al., 2021). It is difficult to put results of many detected compounds into context with other seabird egg studies, or other biota, due to the lack of published studies available. While the occurrence of compounds ranging from industrial pollutants though to pharmaceutical residues in bird eggs is potentially worrying, the scarcity of avian specific thresholds towards such compounds challenges the completion of ecotoxicological assessment in seabird species. Further screening work on high trophic level organisms will provide an important tool for identifying substances that are not well considered on current target lists. These results may be a useful reference point as research on CECs in seabirds and marine biota develops. Additionally, legacy CECs, as well as legacy pollutants, could be analysed in prey items of seabird species to provide more detailed information on pollutants in marine environments.

4. Conclusions

This study provides a comprehensive overview of legacy pollutants in guillemot eggs from three Irish colonies and one island colony from Wales. Pollutant levels generally were higher in Skomer Island (Wales), followed by Lambay Island and Great Satlee Island. Samples from Aughris Head on Ireland's Atlantic coast had the lowest levels of pollutants in this study with the exception of mercury. Levels of PCBs, *p,p'* –DDE and mercury in guillemot eggs have decreased over time according to this study. Levels of persistent pollutants in guillemot eggs fall below most existing thresholds for adverse effects in other species, with the exception of mercury. This study provides novel information on CECs in guillemot eggs demonstrating the possibility of using screening approaches in seabird eggs for identifying potential new contaminants of concern. Few data exist on contaminants in seabird eggs in Ireland and this study may help inform future contaminant monitoring programmes for seabirds.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.131161.

Author contributions

Philip White: conceptualization, methodology, review & editing, supervision. **Brendan McHugh:** conceptualization, methodology, review & editing, supervision. **Simon Berrow:** conceptualization, review & editing, supervision. **Moira Schlingermann:** investigation. **Cillian Gately:** investigation. **Marissa Tannian:** investigation. **Stephen Newton:** conceptualization, review & editing, supervision. **Evin McGovern:** conceptualization, review & editing, supervision. **Sinéad Murphy:** conceptualization, review & editing, supervision. **Denis Crowley:** validation, investigation. **Linda O'Hea:** investigation. **Brian Boyle:** investigation. **Ian O'Connor:** conceptualization, review & editing, supervision.

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